SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of anthocyanins (E 163) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

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ABSTRACT

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion re-evaluating the safety of anthocyanins (E 163). The Panel concluded that the currently available toxicological database was inadequate to establish a numerical ADI for anthocyanins. For anthocyanins extracted from edible fruits and vegetables by aqueous processes, changes in composition would not be expected. The Panel concluded that provided exposure from use as a food additive was comparable to that from the diet the underlying conclusion in the 1975 SCF opinion that such food additives derived from natural sources would still apply. The majority of data are on aqueous grape skin extract (GSKE) and blackcurrant extracts and the Panel considers that exposures estimated from current uses and use levels these extracts are unlikely to be of safety concern. The Panel recommends that the specifications for E 163 should be modified to reflect this conclusion. For anthocyanins extracted from other sources and/or using non-aqueous extraction methods the absence of characterisation does not allow verification that this conclusion in the 1975 SCF opinion could be applied. The Panel noted that for some extracts it had proven possible to assess a group based on toxicological and compositional data on representative samples across the range of extracts. The Panel concluded that refined exposure estimates of anthocyanins used as a food additive were higher than dietary intakes and that these did not include intakes from colouring foods. Therefore the Panel would recommend that appropriate characterisation and toxicological data should be required to permit a further re-evaluation of anthocyanins including comparative data on anthocyanins (E 163) produced by aqueous extraction.

KEY WORDS

Anthocyanins, E 163, food colour.

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[1] On request from the European Commission, Question No EFSA-Q-2011-00349, adopted on 13 March 2013.

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SUMMARY

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to provide a scientific opinion re-evaluating the safety of anthocyanins (E 163) when used as a food additive.

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

Anthocyanins (E 163) are authorised as food additives in the EU, and have been previously evaluated by JECFA in 1982 and the SCF in 1975. JECFA has established an ADI of 2.5 mg/kg bw/day for anthocyanins from grape skin, while the SCF has not derived an ADI for anthocyanins.

Anthocyanins represent a very large group of water-soluble plant pigments. Anthocyanins are obtained by extraction from the natural strains of vegetables and edible fruits.

Anthocyanins are distributed in various fruits, and several fruit extracts are used as synonyms for anthocyanins. The extracts mentioned most often are grape skin extract (GSKE) (containing glucosides of the anthocyanins peonidin, malvidin, delphinidin and petunidin) and blackcurrant extract (containing the colouring matters cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and delphinidin 3-glucoside). However, in the EU specifications it is not indicated which fruits or vegetables can be used to obtain the food additive anthocyanins. In addition, the composition and identity of anthocyanins which may be present in the food additive E 163 is not specified. These compounds are normally present in food as glycosides (anthocyanins).

No JECFA specifications are available for specific anthocyanins, only for GSKE and blackcurrant extract, which contain more than one anthocyanins. The specifications of the European Commission for anthocyanins and of JECFA for GSKE and blackcurrant extract differ. Limits for mercury and cadmium are included in the EU specifications, but not in those of JECFA

Limited data on stability, reaction and fate in food were available. No formal method for the analysis of anthocyanins in food appears to have been adopted.

Studies on the toxicokinetics and toxicological properties of anthocyanins have mainly used fruit extracts, which contain several anthocyanins. Therefore, based on these studies, conclusions cannot be drawn for specific anthocyanins, but may be made for anthocyanins in general. Since anthocyanins used as the food additive E 163 are poorly defined, it is not clear whether the substances used in the various studies are relevant for assessment of the specific E 163 anthocyanins.

Studies in rats have revealed that the amount of absorption was low (< 2 %). After oral administration, a maximum plasma concentration of anthocyanins was reached after 15-120 minutes, depending on the aglycone and sugar moieties of the anthocyanins. Higher absorption levels (up to 37 %) have been reported in *in situ* experiments in anesthetised rats.

In rats as well as pigs, anthocyanins can be methylated or conjugated with glucuronic acid or sulphate and aglycone, have also been reported. However, delphinidin anthocyanins were not metabolized to any measurable extent.

Anthocyanins are excreted unchanged and as metabolites. Those with either a di- or tri-saccharide attached to them were primarily excreted unchanged in the urine. Plasma clearance rates are influenced by both the aglycone (delphinidin > cyanidin > petunidin = peonidin > malvidin) and the sugar moiety (galactoside > glucoside > arabinoside). Urinary excretion in rats and pigs is only 0.04-0.58 % of the ingested amount.

Limited studies in humans showed that only a small portion of orally ingested anthocyanins was absorbed (<1 %). Maximum plasma levels were reached within 2 hours of consumption. In humans, glucuronic acid conjugates, sulphate conjugates and methylated metabolites were found in both plasma and urine, together with oxidized derivatives. About 68 % of absorbed anthocyanins was reported to be metabolized, and excretion occurred mainly as a monoglucuronide.

The majority of anthocyanins ingested are excreted in the faeces. The elimination of plasma anthocyanins appeared to follow first-order kinetics in humans.

In guinea pigs and dogs, no short-term or subchronic toxic effects were observed at anthocyanins doses up to 3 g/kg and 15 % of grape-skin extract respectively in the diet. In addition, in rats fed an unspecified anthocyanins extract at levels up to 6 g/day or grape seed extract (GSE) or GSKE at dietary levels up to 2.5 % (1780 mg/kg bw/day in males and 2150 mg/kg bw/day in females) for a period of 90 days, no relevant treatment-related adverse effects were observed. It is not possible to convert the reported NOAEL for GSE and GSKE into a NOAEL for anthocyanins since the anthocyanins contents of GSE and GSKE were not further defined. In this case, the value reported by JECFA of 3 % anthocyanins content in GSKE (and assuming the same level in GSE), would result in a dose equivalent to 53 mg anthocyanins/kg bw/day for males and 64 mg anthocyanin/ kg bw/day for females.

In a 2-generation reproduction study with anthocyanins from GSKE, no effects were observed on reproductive performance or pup viability at dietary levels up to 15 % (equivalent to 225 mg anthocyanins/kg bw/day based on an assumed 3 % anthocyanins content in GSKE) anthocyanins. However, in both the F_1 and F_2 rats, body weight was reduced in the 15 % group. This was also observed in the F_2 pups in the 7.5 % (equivalent to 112 mg anthocyanins/kg bw/day based on an assumed 3 % anthocyanins content in GSKE) group; however, this was marginal and related to a reduced food intake. In addition, a decrease in organ weights of the liver, adrenal and thyroid (without histological effects) occurred in the 15 % group of the F_1 rats. The ADI established by JECFA was based on this study with 7.5 % GSKE in the diet (equivalent to 7500 mg/kg bw) considered as the NOAEL. Since GSKE contains approximately 3 % anthocyanins, this level was correlated to a NOAEL of 225 mg/kg bw/day for anthocyanins. This level was converted into an estimated ADI of 0-2.5 mg/kg bw/day for anthocyanins.

There are no indications that anthocyanins glycosides from currants, blueberries or elderberries induce developmental effects in rats, mice or rabbits at dose levels up to 9 g/kg bw.

Several anthocyanidins and anthocyanins (cyanidin, delphinidin, GSE and GSKE were negative in bacterial mutagenicity tests, with and without metabolic activation. Also *in vitro* Comet assays in mammalian cells did not result in increased DNA strand breaks when exposed to 0.1-100 μg/mL (GSE) or 1-10 μM (delphinidin, malvidin, pelargonidin and peonidin). However, in another study, doses of ≥ 50 μM (~ 17.5 μg/mL), delphinidin, cyanidin, malvidin, pelargonidin and peonidin did induce a slight, but significant increase in strand breaks in HT29 cells. Pelargonidin (doses ≤ 2 μM) was found non-genotoxic in a micronucleus test in HL-60 cells.

Overall, in most *in vitro* assays anthocyanins, tested at low doses, were not genotoxic. Some evidence of genotoxicity was provided by a single *in vitro* study using pure anthocyanidins. However *in vivo* a negative guideline bone marrow micronucleus test at a limit dose was considered to exclude *in vivo* genotoxicity of GSE and GSKE.

Due to a lack of data, no conclusion can be drawn with respect to long-term toxicity or carcinogenicity of anthocyanins.

The Panel noted that the specification for anthocyanins and the information available on their manufacture do not allow identification of the specific anthocyanins nor their overall composition in the material used as the food additive E 163. The Panel noted that there is no information on the range

of anthocyanins composition within the food additive and it is therefore not possible to determine the extent to which the available toxicological data are relevant.

Furthermore, the Panel noted that the variety of sources for anthocyanins and lack of information on manufacturing process do not allow identification and quantification of minor components. The Panel considered that minor components could be present at different ratios than in the normal consumption of foods and might therefore exert different biological effects. The Panel also noted that the importance of data covering the range of compositions arising from different manufacturing methods was illustrated in the opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on Rosemary extracts (EFSA, 2008b). The recent opinion of the Scientific Committee entitled 'Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements' (EFSA, 2009) highlights principles which should be considered in the risk assessment of any botanical preparations including extracts. The Panel considered that the absence of characterisation does not allow verification of the underlying presumption of safety on food additives derived from natural sources in the 1975 SCF opinion.

In a refined exposure estimation performed with the EFSA Comprehensive European Food Consumption Database, using the maximum reported use levels, the mean exposure to anthocyanins (E 163) range from 1.5 to 4.0 mg/kg bw/day for toddlers (high level exposure 3.2-6.9 mg/kg bw/day), from 1.5 to 4.7 mg/kg bw/day for children (high level exposure 2.7-7.8 mg/kg bw/day) and from 1.0 to 2.5 mg/kg bw/day for adolescents (high level exposure 1.6-3.9 mg/kg bw/day). The mean estimated exposures for adults and the elderly are respectively from 0.7 to 1.9 mg/kg bw/day (high level exposure 1.1-3.8 mg/kg bw/day) and from 0.5 to 1.1 mg/kg bw/day (high level exposure 0.9-2.3 mg/kg bw/day). Compared to these exposure estimates, the estimated exposure to anthocyanins from the regular diet is very low (at the mean about 0.1 mg/kg bw/day for adults and 0.3 mg/kg bw/day for children, values at the 97th percentiles are 0.6 mg/kg bw/day and 2.1 mg/kg bw/day respectively).

The Panel concluded that the currently available toxicological database was inadequate to establish a numerical ADI for anthocyanins.

For anthocyanins extracted from edible fruits and vegetables by aqueous processes, the Panel would not expect of changes in composition. The Panel concluded in principle that provided exposure from use of food colours was comparable to that from the diet the underlying conclusion of safety on food additives derived from natural sources in the 1975 SCF opinion would still apply.

Using a weight of evidence evaluation of toxicological and general exposure data, the Panel concluded that aqueous grape skin and blackcurrant extracts are unlikely to be of safety concern. The Panel recommends that the specifications for E 163 should be modified to reflect the conclusions on these two sources.

With the exception of aqueous grape skin and blackcurrant extracts, the Panel considered that the absence of characterisation does not allow verification of the applicability of the conclusion of safety of food additives derived from natural sources in the 1975 SCF opinion for anthocyanins extracted from other sources and/or using non-aqueous extraction methods.

The Panel concluded that the following information is required to permit an adequate risk evaluation for derivation of an ADI for anthocyanins (E 163) as food additive;

- Definition of the sources from which it is extracted
- Method of extraction
- Qualitative and quantitative chemical characterisation of the extracts including minor components

Data on toxicokinetics, subchronic toxicity, genotoxicity, reproductive and developmental toxicity and chronic toxicity/carcinogenicity for an appropriate number of extracts covering the range of sources and current manufacturing methods for each source.

The Panel considered that this data package would also need to include comparative data on anthocyanins (E 163) produced by aqueous extraction. The Panel noted that using target read across it has proven possible to perform a risk assessment for a group of extracts (e.g. rosemary extracts) based on compositional and toxicological data on representative samples. This approach was currently not applicable on anthocyanins.

The Panel concluded that refined exposure estimates of anthocyanins from their use as a food additive (E 163), albeit conservative, were higher than estimated intakes from the regular diet and that these did not include intakes from colouring foods. The general principle about safety of food additives derived from natural sources does not apply.

The Panel recommends that appropriate chemical characterisation and toxicological data are required to permit a further re-evaluation of anthocyanins.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[5] of the European Parliament and of the Council on food additives requires that food additives are subject to a safety evaluation by the European Food Safety Authority (EFSA) before they are permitted for use in the European Union. In addition, it is foreseen that food additives must be kept under continuous observation and must be re-evaluated by the EFSA.

For this purpose, a programme for the re-evaluation of food additives that were already permitted in the European Union before 20 January 2009 has been set up under the Regulation (EU) No 257/2010[6]. This Regulation also foresees that food additives are re-evaluated whenever necessary in light of changing conditions of use and new scientific information. For efficiency and practical purposes, the re-evaluation should, as far as possible, be conducted by group of food additives according to the main functional class to which they belong.

The order of priorities for the re-evaluation of the currently approved food additives should be set on the basis of the following criteria: the time since the last evaluation of a food additive by the Scientific Committee on Food (SCF) or by EFSA, the availability of new scientific evidence, the extent of use of a food additive in food and the human exposure to the food additive taking also into account the outcome of the Report from the Commission on Dietary Food Additive Intake in the EU[7] of 2001. The report "Food additives in Europe 2000[8]" submitted by the Nordic Council of Ministers to the Commission, provides additional information for the prioritisation of additives for re-evaluation. As colours were among the first additives to be evaluated, these food additives should be re-evaluated with the highest priority.

In 2003, the Commission already requested EFSA to start a systematic re-evaluation of authorised food additives. However, as a result of the adoption of Regulation (EU) 257/2010 the 2003 Terms of Reference are replaced by those below.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The Commission asks the European Food Safety Authority to re-evaluate the safety of food additives already permitted in the Union before 2009 and to issue scientific opinions on these additives, taking especially into account the priorities, procedure and deadlines that are enshrined in the Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme for the re-evaluation of approved food additives in accordance with the Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives

[5] Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives, OJ L 354, 31.12.2008, p. 16.

[6] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up the program for the re-evaluation of approved food additives in accordance with Regulation (EC) No 1333/2008 of the European Parliament and of the Council on food additives, OJ L 80, 26.03.2010, p.19.

[7] Report from the Commission on Dietary Food Additive Intake in the European Union, Brussels, 01.10.2001, COM (2001) 542 final.

[8] Food Additives in Europe 2000, Status of safety assessments of food additives presently permitted in the EU, Nordic Council of Ministers. TemaNord 2002:560.

ASSESSMENT

1. Introduction

The present opinion deals with the re-evaluation of the safety of anthocyanins (E 163) when used as a food additive.

Anthocyanins (E 163) are authorised as food additives in the EU and have previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1982 and the EU Scientific Committee for Food (SCF) in 1975 and 1997. JECFA has established an Acceptable Daily Intake (ADI) of 2.5 mg/kg bw for anthocyanins from grape skin, while the SCF has not derived an ADI for anthocyanins.

The TemaNord review compiled literature data prior to 1999 (TemaNord, 2002). Papers published in the period from 1999 until September 2008 were retrieved searching on compound name and CAS Registry Number in the following databases: Medline, Toxline, Toxcenter and ChemAbs. Besides a search on anthocyanins (in general) and its CAS Registry Number, also searches for the main anthocyanins aglycones cyanidin, peonidin, malvidin, delphinidin, petunidin and pelargonidin and their respective CAS Registry Numbers were performed.

Several publications were found describing beneficial effects of anthocyanins or flavonoids in general (i.e. beneficial effects on cholesterol and lipoprotein parameters, antitumor activity and antioxidant status). However, beneficial effects are not relevant for the risk evaluation on the use of anthocyanins as a food additive and these studies have therefore not been evaluated. Anthocyanins (E 163) refers to the food additive which are not sufficiently characterized and, may contain both the glycosides (anthocyanins) and the aglycones (anthocyanidins). When the term "anthocyanins" without the E number in parentheses is used in this opinion it refers only to the glycosides in accordance with its proper chemical meaning.

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

2. Technical data

2.1. Identity of the substance

The anthocyanins/anthocyanidins class consists of some 500 or more compounds, of which the most common are cyanidin, peonidin, malvidin, delphinidin, petunidin and pelargonidin (see Figure 1 and table for structural formulae of these most common constituents). The structural variations of anthocyanins are due to differences in the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of the sugar moiety attached to the phenolic (aglycone) molecule and the position of the attachment, as well as the nature and number of aliphatic or aromatic acids attached to the sugars. Anthocyanins most frequently occur as 3-monosides, 3-biosides and 3-triosides as well as 3,5-diglycosides and more rarely 3,7-diglycosides associated with the sugars glucose < rhamnose < galactose < xylose < arabinose (JECFA, 1982; McGhie and Walton, 2007)

The basic structure of the flavylium cation of the anthocyanins is shown in Figure 1 and the individual anthocyanins and the anthocyanidins are listed below.

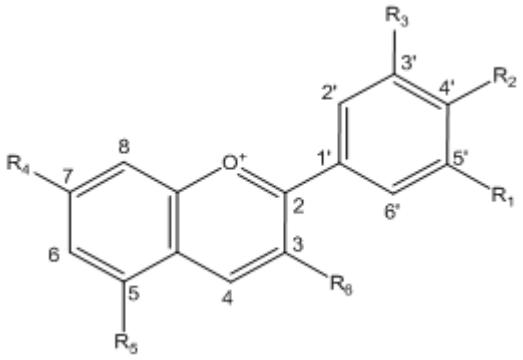

Figure 1: Generic structural formula of the anthocyanins and anthocyanidins (adapted from McGhie and Walton, 2007).

Table 1: Chemical structure and names of most common individual anthocyanidins and anthocyanins

Anthocyanidins	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**
Cyanidin:	OH	OH	H	OH	OH	OH
Peonidin:	OCH_3	OH	H	OH	OH	OH
Malvidin:	OCH_3	OH	OCH_3	OH	OH	OH
Delphinidin:	OH	OH	OH	OH	OH	OH
Petunidin:	OCH_3	OH	OH	OH	OH	OH
Pelargonidin:	H	OH	H	OH	OH	OH
Anthocyanins: anthocyanidin-3-O-glucoside	as above	as above	as above	as above	as above	O-glycosyl

There is limited information on the types and percentages of individual anthocyanins and anthocyanidins neither in anthocyanins (E 163) nor in various natural sources.

The anthocyanins pigments present in grape skin extract (GSKE) consist of diglucosides, monoglucosides, acylated monoglucosides, and acylated diglucosides of peonidin, malvidin, cyanidin, petunidin and delphinidin (JECFA, 1982). The main colouring principles of blackcurrant extract are cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside, delphinidin 3-glucoside (JECFA, 2006)

Several synonyms of anthocyanins are in use in published literature, including grape anthocyanins, GSKE, enocianina, enocianina concentrate, oenin and oenocyanin. In addition, each anthocyanins compound has its own synonyms (ChemIDplus advanced, via internet, 2008). Anthocyanins have a CAS Registry Number of 11029-12-2.

The chemical name (according to Commission Regulation (EU) No 231/2012[9]) and formula, CAS and EINECS numbers, log P_{ow}, solubility in ethanol and water, and molecular weights of the principal components are given below (EC, 1994; ChemID plus advanced, via internet, 2008; JECFA, 2006).

[9] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1-295.

Cyanidin is the chloride salt of 3,3',4',5,7-pentahydroxy-flavylium and its chemical formula is $C_{15}H_{11}O_6Cl$, its CAS Registry Number is 525-58-5 and its EINECS number is 208-438-6. No log P_{ow} was found. Cyanidin 3-rutinoside and cyanidin 3-glucoside are soluble in ethanol and water and the molecular weight of cyanidin chloride is 322.6 g/mol.

Peonidin is the chloride salt of 3,4',5,7-tetrahydroxy-3'-methoxyflavylium and its chemical formula is $C_{16}H_{13}O_6Cl$, its CAS Registry Number is 134-01-0 and its EINECS number is 205-125-6. No log P_{ow} was found, nor was information available on solubility in ethanol, but it is soluble in water and the molecular weight of peonidin chloride is 336.7 g/mol.

Malvidin is the chloride salt of 3,4',5,7-tetrahydroxy-3',5'-dimethoxyflavylium and its chemical formula is $C_{17}H_{15}O_7Cl$, its CAS Registry Number is 643-84-5 and its EINECS number is 211-403-8. Log P_{ow} was reported to be 2.33. No information was available on solubility in ethanol, but it is soluble in water and the molecular weight of malvidin chloride is 366.7 g/mol.

Delphinidin is the chloride salt of 3,5,7-trihydroxy-2-(3,4,5,trihydroxyphenyl)-1-benzopyrylium and its chemical formula is $C_{15}H_{11}O_7Cl$, its CAS Registry Number is 528-53-0 and its EINECS number is 208-437-0. Log P_{ow} was reported to be 2.14; delphinidin 3-rutinoside and delphinidin 3-glucoside are soluble in ethanol and water and the molecular weight of delphinidin chloride is 338.7 g/mol.

Petunidin is the chloride salt of 3,3',4',5,7-pentahydroxy-5'-methoxyflavylium and its chemical formula is $C_{16}H_{13}O_7Cl$, its CAS Registry Number is 1429-30-7 and its EINECS number is 215-849-4. No log P_{ow} was found. No information was available on solubility in ethanol, but it is soluble in water and the molecular weight of petunidin chloride is 352.7 g/mol.

Pelargonidin is the chloride salt of 3,5,7-trihydroxy-2-(4-hydroxyphenyl)-1-benzopyrylium and its chemical formula is $C_{15}H_{11}O_5Cl$, its CAS Registry Number is 134-04-3 and its EINECS number is 205-127-7. Log P_{ow}, was reported to be 2.68. No information was available on solubility in ethanol and water and the molecular weight of pelargonidin chloride is 306.7 g/mol.

2.2. Specifications

Specifications for anthocyanins have been defined in the Commission Regulation (EU) No 231/2012 and JECFA (2006) (Table 2). These specifications do not include information on the types and percentages of individual anthocyanins and anthocyanidins in anthocyanins (E163).

Anthocyanins contain common components of the source material, namely anthocyanins, organic acids, tannins, sugars, minerals etc., but not necessarily in the same proportions as found in the source material (Commission Regulation (EU) No 231/2012).

Table 2: Specifications for purity for anthocyanins according to Commission Regulation (EU) No 231/2012 and GSKE and blackcurrant extract according to JECFA (2006)

Purity	**Commission Regulation (EU) No 231/2012**	**JECFA (2006)**	
Compound	**Anthocyanins**	**Grape skin extract**	**Blackcurrant extract**
Peak absorbance at pH 3		525 nm	520 nm
Solvent residues			
methanol	≤ 50 mg/kg		
ethanol	≤ 200 mg/kg		
Sulphur dioxide	≤ 1000 mg/kg per % pigment	≤ 0.005 %[1]	≤ 50 mg/kg[1]
Arsenic	≤ 3 mg/kg		
Lead	≤ 2 mg/kg	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg		
Cadmium	≤ 1 mg/kg		

[1] Per colour unit

According to Commission Regulation (EU) No 231/2012, the above purity criteria also apply to the raw material from which the aluminium lake is produced. In addition, under neutral conditions, the aluminium lake should contain no more than 0.5 % HCl-insoluble material and no more than 0.2 % ether-extractable material. There are no additional specification requirements for the aluminium lake (Commission Regulation (EU) No 231/2012).

According to the origin of these compounds (e.g. obtained from grape skin), the Panel noted that data on pesticides and mycotoxins contaminations of anthocyanins may be relevant for the specifications.

The Panel noted that the aluminium lake of the food additive could add to the daily intake of aluminium for which a Tolerable Weekly Intake (TWI) of 1 mg aluminium/kg bw/week has been established (EFSA, 2008a) and that therefore specifications for the maximum level of aluminium in the lakes may be required. According to the Natural Food Colours Association (NATCOL) use of anthocyanins lake is limited to panned confectionery products, compressed confectionery products and edible ices.

The Panel noted that the sulphur dioxide levels allowed in the different specifications could not be directly compared due to the difference in the units used.

2.3. Manufacturing process

Anthocyanins are obtained by extraction with sulphited water, acidified water, carbon dioxide, methanol or ethanol from the natural strains of vegetables and edible fruits (Commission Regulation (EU) No 231/2012). Blackcurrant extract is obtained from blackcurrant pomace by aqueous extraction, and GSKE extract is obtained by aqueous extraction of grape skin or marc, after the juice has been expressed from it. Most of the extracted sugars are fermented to alcohol and practically all the alcohol is removed during the concentration of fermented extract by vacuum evaporation. During the extraction process, sulphur dioxide is used and residual sulphur dioxide may be present (JECFA, 2006).

According to NATCOL anthocyanins (E 163) extracts are derived from a water soluble fraction of edible fruits or vegetables. The extraction process for E 163 products is generally very similar and applies to all 'common' sources: grape, black carrot, purple sweet potato, etc. The object of anthocyanins processing is to remove some or all of the saccharides/polysaccharides whilst retaining the polyphenol fraction (which contains the anthocyanins).

Anthocyanins from grape skins are extracted with water containing sulphur dioxide either in a batch or a continuous counter-flow process. The sulphur dioxide facilitates the solubilisation of anthocyanins due to the transient formation of complexes which are more water soluble. After extraction is completed the insoluble plant material is removed by filtration. The supernatant contains, in addition to anthocyanins, carbohydrates, which are fermented to yield ethanol. Subsequently the product is heated under vacuum to recover the ethanol, concentrate the product and release most of the sulphur dioxide. If the resulting extract is still too dilute, it may be further processed to increase its content of anthocyanins. Due to the fermentation step ethanol and other alcohols may also be present.

An extraction process, similar to the one described for grape skin extract, is applied to other source materials. Hot water (without sulphur dioxide) is normally employed as the extraction solvent though in some cases aqueous ethanol may be used. Solubilisation of anthocyanins will be facilitated by acids that are either naturally present in foods (e.g. citric acid) or intentionally added and moderate heating. Spent source material can be removed by filtration, fine materials and macromolecules such as polysaccharides by membrane filtration. Further concentration is achieved by selective adsorption of anthocyanins using a suitable resin.

2.4. Methods of analysis in food

Kong et al. (2003) have reviewed the most recent analytical techniques concerning anthocyanins isolation and identification. The described methods to analyze the content of anthocyanins in various foods included Liquid Chromatography - Mass Spectrometry (LC-MS) using an Electro Spray Ionization (ESI) or atmospheric pressure chemical ionization interface (APCI), matrix-assisted laser desorption/ionization mass spectrometry (MALDI–MS) or Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI–TOF–MS), capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) (Kong et al., 2003).

2.5. Reaction and fate in food

Anthocyanins are reactive compounds and degrade readily, or react with other constituents in food to form colourless or brown compounds. Scotter and Castle (2004) have reviewed the chemical interactions and the fate of additives in foodstuffs. Anthocyanins readily degrade or react in food systems to form complex reaction products, and therefore use in processed foods leads invariably to a mixture of products in addition to the parent anthocyanins. The most important observations described are that the rate of anthocyanin degradation and the intensity and stability of anthocyanin food additives are influenced by several factors. These include pH, structure, concentration, copigmentation and metal complexing, as well as temperature, light, oxygen, acetaldehyde, ascorbic acid, sugars and their degradation products, sulphur dioxide, amino acids and catechins. When low pH conditions are maintained, anthocyanins are relatively stable.

In the presence of iron or copper ions and oxygen, oxidation of ascorbic acid to dehydroascorbic acid is accompanied by the formation of hydrogen peroxide, which in turn can oxidize anthocyanins to colourless malvones. Under anaerobic conditions, anthocyanins and ascorbic acid may react via condensation-type mechanisms.

Anthocyanins form adducts with bisulphite ion ($S_2O_5^-$). The bisulphite ion adds to position 2 or 4 of the flavylium nucleus, discolouring the pigment and simultaneously conferring 'high' heat stability on the glycosidic bond (Scotter and Castle, 2004, Scotter, 2011).

In addition, Hubbermann et al. (2006) showed that different compounds in foods can act as copigments for anthocyanins enhancing their colour, while others show no effect or even reduce the colour. However, copigmentation does not necessarily result in stabilization of colour over time, but may lead to fast discoloration during storage (Hubbermann et al., 2006).

2.6. Case of need and proposed uses

Maximum Permitted Levels (MPLs) of anthocyanins (E 163) have been defined in Commission Regulation (EU) No 1129/2011[10] on food additives for use in foodstuffs.

Currently, anthocyanins (E 163) prepared by physical means from fruits and vegetables are authorised food colouring substances in the EU with a MPL of 200 mg/kg food in fruit-flavoured breakfast cereals (alone or in combination with cochineal (E 120) and beetroot red (E 162) (Table 3). Furthermore, anthocyanins (E 163) are permitted at *quantum satis* in all other foods except for a few in which the use of food additives is specifically prohibited or restricted to other food additives than anthocyanins (E 163) (Commission Regulation (EU) No 1129/2011).

More information on current use levels was made available to the Panel for several food categories in finished products (Table 4).

In addition to the use levels of anthocyanins (E 163) as such, Tennant (2007a, b) has reported the use levels for aluminium lakes of anthocyanins (E 163). According to these reports, the usage of anthocyanins (E 163) lakes is very limited and restricted to panned confectionery and edible ices.

Table 3: MPLs of anthocyanins (E 163) in foods according to the Commission Regulation (EU) No 1129/2011

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
1.4	**Flavoured fermented milk products including heat treated products**		
1.5	**Dehydrated milk as defined by Directive 2001/114/EC**	except unflavoured products	*quantum satis*
1.6.3	**Other creams**	only flavoured creams	*quantum satis*
1.7.1	**Unripened cheese excluding products falling in category 16**	only flavoured unripened cheese	*quantum satis*
1.7.2	**Ripened cheese**	only Red marbled cheese	*quantum satis*
1.7.3	**Edible cheese rind**		*quantum satis*
1.7.4	**Whey cheese**		*quantum satis*
1.7.5	**Processed cheese**	only flavoured processed cheese	*quantum satis*
1.7.6	**Cheese products (excluding products falling in category 16)**	only flavoured unripened products	*quantum satis*
1.7.6	**Cheese products (excluding products falling in category 16)**	only Red marbled products	*quantum satis*
1.8	**Dairy analogues, including beverage whiteners**		*quantum satis*
3	**Edible ices**		*quantum satis*
4.2.1	**Dried fruit and vegetables**	only preserves of red fruit	*quantum satis*
4.2.2	**Fruit and vegetables in vinegar, oil, or brine**	only preserves of red fruit	*quantum satis*
4.2.2	**Fruit and vegetables in vinegar, oil, or brine**	only vegetables (excluding olives)	*quantum satis*
4.2.3	**Canned or bottled fruit and vegetables**	only preserves of red fruit	*quantum satis*

[10] Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council establishing a Union list of food additives. The Panel noted that the Commission Regulation (EU) No 1129/2011 of 11 November 2011 will enter into force on June, 1st 2013 but confirms the approved uses of anthocyanins as food additive as described in previous Directives still active until end of May 2013: Council Directive No 94/36/EC of 30 June 1994 on colours for use in foodstuffs.

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
4.2.4.1	**Fruit and vegetable preparations excluding compote**	only mostarda di frutta	*quantum satis*
4.2.4.1	**Fruit and vegetable preparations excluding compote**	only preserves of red fruit	*quantum satis*
4.2.5.2	**Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC**	except chestnut puree	*quantum satis*
4.2.5.3	**Other similar fruit or vegetable spreads**	except *crème de pruneaux*	
5.2	**Other confectionery including breath refreshening microsweets**		*quantum satis*
5.3	**Chewing gum**		*quantum satis*
5.4	**Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4**		*quantum satis*
6.3	**Breakfast cereals**	only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals	*quantum satis*
6.3	**Breakfast cereals**	only fruit flavoured breakfast cereals	200
6.5	**Noodles**		*quantum satis*
6.6	**Batters**		*quantum satis*
6.7	**Pre-cooked or processed cereals**		*quantum satis*
7.2	**Fine bakery wares**		*quantum satis*
8.2.3	**Casings and coatings and decorations for meat**	except edible external coating of pasturmas	*quantum satis*
9.2.	**Processed fish and fishery products including mollusks and crustaceans**	only surimi and similar products and salmon substitutes.	*quantum satis*
9.2.	**Processed fish and fishery products including mollusks and crustaceans**	only fish paste and crustacean paste	*quantum satis*
9.2.	**Processed fish and fishery products including mollusks and crustaceans**	only precooked crustacean	*quantum satis*
9.2.	**Processed fish and fishery products including mollusks and crustaceans**	only smoked fish	*quantum satis*
9.3	**Fish roe**	except Sturgeons' eggs (Caviar)	*quantum satis*
12.2.2	**Seasonings and condiments**	only seasonings, for example curry powder, tandoori	*quantum satis*
12.4	**Mustard**		*quantum satis*
12.5	**Soups and broths**		*quantum satis*
12.6	**Sauces**	excluding tomato-based sauces	*quantum satis*
12.7	**Salads and savoury based sandwich spreads**		*quantum satis*
12.9	**Protein products, excluding products covered in category 1.8**		*quantum satis*
13.2	**Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)**		*quantum satis*

Category number	Foods	restrictions/exception	Maximum level (mg/L or mg/kg as appropriate)
13.3	**Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)**		*quantum satis*
13.4	**Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009**		*quantum satis*
14.1.4	**Flavoured drinks**	excluding chocolate milk; malt products	*quantum satis*
14.2.3	**Cider and perry**	excluding *cidre bouché*	*quantum satis*
14.2.4	**Fruit wine and made wine**		*quantum satis*
14.2.5	**Mead**		*quantum satis*
14.2.6	**Spirit drinks as defined in Regulation (EC) No 110/2008**	except: spirit drinks as defined in article 5(1) and sales denominations listed in Annex II, paragraphs 1-14 of Regulation 110/2008 and spirits (preceded by the name of the fruit) obtained by maceration and distillation, London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà.	*quantum satis*
14.2.7.1	**Aromatised wines**	Except *americano, bitter vino*	
14.2.7.2	**Aromatised wine-based drinks**	except *bitter soda, sangria, claria, zurra*	*quantum satis*
14.2.7.3	**Aromatised wine-product cocktails**		*quantum satis*
14.2.8	**Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol**		*quantum satis*
15.1	**Potato-, cereal-, flour- or starch-based snacks**		*quantum satis*
15.2	**Processed nuts**		*quantum satis*
16	**Desserts excluding products covered in category 1, 3 and 4**		*quantum satis*
17.1	**Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms**		*quantum satis*
17.2	**Food supplements supplied in a liquid form**		*quantum satis*
17.3	**Food supplements supplied in a syrup-type or chewable form**		*quantum satis*

2.6.1. Reported use levels of anthocyanins (E 163)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. For those additives where no MPL is set and which are authorised as *quantum satis*, information on actual use levels is required. In the framework of Regulation (EC) No 1333/2008 on food additives and of Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call for scientific data on anthocyanins (E 163) including present use and use patterns (i.e. which food categories and subcategories, proportion of

food within categories/subcategories in which it is used, actual use levels (typical and maximum use levels), especially for those uses which are only limited by *quantum satis*).

2.6.1.1. Summarised data on reported use levels in foods from industries and other sources

Table 4 provides data on the use levels of anthocyanins (E 163) in foods as reported by industries. It also shows the levels used for the refined exposure assessment identified by the Panel and based on data for several food categories in finished products reported by industries or from the rules followed to deal with *quantum satis* (QS) authorisation, as indicated in Appendix A, Figures 1 and 2.

Table 4: Summary of levels used in the refined exposure assessment (mg/kg or mg/L)

Matching FAIM foodcodes	Category number	Food category name	MPL	NATCOL		QS rules	Levels used for calculations
				typical	max		
1.4 - Flavoured fermented milk products including heat treated products	**1.4**	**Flavoured fermented milk products including heat treated products**		50	100		100
1.5 - Dehydrated milk as defined by Directive 2001/114/EC	**1.5**	**Dehydrated milk as defined by Directive 2001/114/EC**	*QS*	50	100		100
1.6 - Cream	**1.6.3**	**Other creams**	*QS*	50	100		100
1.7.1 - Unripened cheese (excl cat 16)	**1.7.1**	**Unripened cheese excluding products falling in category 16**	*QS*	50	100		100
1.7.2 - Ripened cheese	**1.7.2**	**Ripened cheese**	*QS*	50	100		100
	1.7.3	**Edible cheese rind**	*QS*	50	100		Not available in FAIM
1.7.4 - Whey cheese	**1.7.4**	**Whey cheese**	*QS*	50	100		100
1.7.5 - Processed cheese	**1.7.5**	**Processed cheese**	*QS*	50	100		100
	1.7.6	**Cheese products (excluding products falling in category 16)**	*QS*	50	100		Not available in FAIM
1.8 - Dairy analogues, including beverage whiteners	**1.8**	**Dairy analogues, including beverage whiteners**	*QS*	50	100		100
3 - Edible ices	**3**	**Edible ices**	*QS*	50	130		130
4.2 - Processed fruit and vegetables	**4.2.1**	**Dried fruit and vegetables**	*QS*	80	120		120
	4.2.2	**Fruit and vegetables in vinegar, oil, or brine**	*QS*	80	120		
	4.2.2	**Fruit and vegetables in vinegar, oil, or brine**	*QS*	50	80		
	4.2.3	**Canned or bottled fruit and vegetables**	*QS*	80	120		
	4.2.4.1	**Fruit and vegetable preparations excluding compote**	*QS*	80	120		
	4.2.4.1	**Fruit and vegetable preparations excluding compote**	*QS*	80	120		
	4.2.5.2	**Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EEC**	*QS*	100	100		
	4.2.5.3	**Other similar fruit or vegetable spreads**		100	100		
5.2 - Other confectionery including breath refreshening microsweets	**5.2**	**Other confectionery including breath refreshening microsweets**	*QS*	50	80		80
5.3 - Chewing gum	**5.3**	**Chewing gum**	*QS*	50	80		80
	5.4	**Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4**	*QS*	200	300		Not available in FAIM
6.3 - Breakfast cereals	**6.3**	**Breakfast cereals**	*QS*	100	120		120
	6.3	**Breakfast cereals**	200	100	120		

Matching FAIM foodcodes	Category number	Food category name	MPL	NATCOL		QS rules	Levels used for calculations
				typical	max		
6.5 - Noodles	**6.5**	**Noodles**	*QS*			510	510*
	6.6	**Batters**	*QS*				Not available in FAIM
	6.7	**Pre-cooked or processed cereals**	*QS*				Not available in FAIM
7.2 - Fine bakery wares	**7.2**	**Fine bakery wares**	*QS*	40	70		70
8.2 - Processed meat	**8.2.3**	**Casings and coatings and decorations for meat**	*QS*			500	500
9.2 - Processed fish and fishery products including mollusks and crustaceans	**9.2.**	**Processed fish and fishery products including mollusks and crustaceans**	*QS*			500	500
	9.2.	**Processed fish and fishery products including mollusks and crustaceans**	*QS*				
	9.2.	**Processed fish and fishery products including mollusks and crustaceans**	*QS*				
	9.2.	**Processed fish and fishery products including mollusks and crustaceans**	*QS*				
9.3 - Fish roe	**9.3**	**Fish roe**	*QS*			300	300
12.2 - Herbs, spices, seasonings	**12.2.2**	**Seasonings and condiments**	*QS*	50	90		90
12.4 - Mustard	**12.4**	**Mustard**	*QS*			300	300
12.5 - Soups and broths	**12.5**	**Soups and broths**	*QS*			50	50
12.6 - Sauces	**12.6**	**Sauces**	*QS*	50	90		90
12.7 - Salads and savoury based sandwich spreads	**12.7**	**Salads and savoury based sandwich spreads**	*QS*	50	90		90
12.9 - Protein products, excluding products covered in category 1.8	**12.9**	**Protein products, excluding products covered in category 1.8**	*QS*			100	100
13.2 - Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding 13.1.5)	**13.2**	**Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)**	*QS*			50	50
13.3 - Dietary foods for weight control diets intended to replace total daily food intake or an individual meal	**13.3**	**Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)**	*QS*			50	50
13.4 - Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009[4]	**13.4**	**Foods suitable for people intolerant to gluten as defined by Regulation (EC) 41/2009**	*QS*			50	50
14.1 - Non-alcoholic beverages	**14.1.4**	**Flavoured drinks**	*QS*	25	120		120
14.2 - Alcoholic beverages, including alcohol-free and low-alcohol counterparts	**14.2.3**	**Cider and perry**	*QS*	60	60		60
	14.2.4	**Fruit wine and made wine**	*QS*	60	60		
	14.2.5	**Mead**	*QS*	60	60		
	14.2.6	**Spirit drinks as defined in Regulation (EC) No 110/2008**	*QS*	60	60		
	14.2.7.1	**Aromatised wines**		60	60		
	14.2.7.2	**Aromatised wine-based drinks**	*QS*	60	60		
	14.2.7.3	**Aromatised wine-product cocktails**	*QS*	60	60		

Matching FAIM foodcodes	Category number	Food category name	MPL	NATCOL		QS rules	Levels used for calculations
				typical	max		
	14.2.8	**Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15 % of alcohol**	*QS*	60	60		
15.1 - Potato-, cereal-, flour- or starch-based snacks	**15.1**	**Potato-, cereal-, flour- or starch-based snacks**	*QS*	150	300		300
15.2 - Processed nuts	**15.2**	**Processed nuts**	*QS*	150	300		300
16 - Desserts excluding products covered in category 1, 3 and 4	**16**	**Desserts excluding products covered in category 1, 3 and 4**	*QS*	50	100		100
17 - Food supplements as defined in Directive 2002/46/EC[5] excluding food supplements for infants and young children	**17.1**	**Food supplements supplied in a solid form including capsules and tablets and similar forms excluding chewable forms**	*QS*			300	300
	17.2	**Food supplements supplied in a liquid form**	*QS*			300	
	17.3	**Food supplements supplied in a syrup-type or chewable form**	*QS*			300	

* it was assumed that not all noodles are coloured and that 10% could be coloured with anthocyanins

Some food categories are not referenced in the EFSA Comprehensive European Food Consumption Database and therefore could not be taken into account in the present estimate. These food groups are described below (referenced in ascending order of the food category codes); it results in minor under-estimations considering the food additive assessed is a food colour:

- 1.7.3: Edible cheese rind: This food category is not available in the FoodEx nomenclature, but the ripened cheese food category is already included in the assessment.

- 1.7.6: Cheese products: This food category is not available in the FoodEx nomenclature.

- 5.4: Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4: This category covers any confectionery product generally used for decorating and filling any foodstuff e.g. fine bakery wares, edible ices, candy and confections. This food category is not available in the FoodEx nomenclature, but the possible foodstuffs that may be filled or decorated are included in the assessment (e.g. fine bakery ware).

- 6.6: Batters: as for decorations and coatings, batters represent a part of a composite food and are not referenced in the FoodEx nomenclature.

- 6.7: Pre-cooked or processed cereals: No information on the processed status (cooked, treated…) of cereals is available in the FoodEx nomenclature.

Restrictions and exceptions mentioned in the legislation (Table 3) couldn't be taken into consideration with the food nomenclature available in the Comprehensive database. Therefore the whole food category was considered for the exposure estimates. This results in an over-estimation. For noodles (category number 6.5), it was assumed that only a percentage of noodles (10 %) can be coloured in purple/red to avoid a too high overestimation of exposure due to this food.

Table 4 provides data on the use levels of anthocyanins (E 163) in foods as reported by industries. The Panel noted that there is a considerable discrepancy between data on anthocyanins usage reported by NATCOL and those originally reported by CIAA in 2009. Following exchanges with CIAA in 2011 it was deduced that the original data reported by CIAA referred to colour preparations rather than to the colouring principle. For this reason the data reported by CIAA were not used in the exposure assessment.

2.7. Information on existing authorisations and evaluations

Anthocyanins have been evaluated previously by the SCF in 1975 and 1997 and by JECFA in 1982.

In its evaluation of 1982, JECFA concluded that toxicological studies with anthocyanins were limited, and have been carried out with mixtures extracted from a variety of fruits. Diets containing 15 % of a GSKE preparation resulted in a decreased organ weight of the liver, adrenal and thyroid in rats, and 7.5 % (equivalent to 7500 mg/kg bw/day) was considered the No-Observed-Adverse-Effect Level (NOAEL) (based on the reproduction study of Cox and Babish (1978a). Since GSKE contains approximately 3 % anthocyanins, this correlates to a NOAEL of 225 mg/kg bw/day for anthocyanins. This level was converted into an estimated ADI of 0-2.5 mg/kg bw/day for anthocyanins (JECFA, 1982).

The SCF decided that anthocyanins, prepared from natural foods by physical processes, could be accepted for use as colouring matter in food without further investigation, due to the fact that anthocyanins are constituents of food and are derived from coloured natural foods by purely physical processes. In the same report the SCF stressed that the use of food additives derived from natural sources are only acceptable as food additives provided the quantities ingested from this use do not differ substantially from the amounts likely to be ingested as a result of the normal consumption of the foods in which they occur naturally (SCF, 1975). In 1997 the SCF issued an opinion permitting the use of anthocyanins in Foods for special medical purposes for children at levels up to 20 mg/L[11].

Anthocyanins were also evaluated by TemaNord in 2002, who recognised a need for further exposure assessment.

2.8. Exposure assessment

2.8.1. Food consumption data used for exposure assessment

In 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been built from existing national information on food consumption at a detailed level. Competent authorities in the European countries provided EFSA with data on the level of food consumption by the individual consumer from the most recent national dietary survey in their country (cf. Guidance of EFSA 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a).

Overall, the food consumption data gathered at EFSA were collected by different methodologies and thus direct country-to-country comparison should be made with caution.

For calculation of chronic exposure, intake statistics have been calculated based on individual average consumption over the total survey period excluding surveys with only one day per subject. High level consumption was only calculated for those foods and population groups were the sample size was sufficiently large to allow calculation of the 95th percentile (EFSA, 2011a). The Panel estimated chronic exposure for the following population groups: toddlers, children, adolescents, adults and the elderly. Calculations were performed using individual body weights.

Thus, for the present assessment, food consumption data were available from 26 different dietary surveys carried out in 17 different European countries as mentioned in Table 5.

[11] http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_41.pdf).

Table 5: Population groups considered for the exposure estimates of anthocyanins (E 163)

Population	Age range	Countries with food consumption surveys covering more than one day
Toddlers	from 12 up to and including 35 months of age	Bulgaria, Finland, Germany, Netherlands
Children[12]	from 36 months up to and including 9 years of age	Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden
Adolescents	from 10 up to and including 17 years of age	Belgium, Cyprus, Czech Republic, Denmark, France, Germany, Italy, Latvia, Spain, Sweden
Adults	from 18 up to and including 64 years of age	Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Spain, Sweden, UK
The elderly[12]	Older than 65 years	Belgium, Denmark, Finland, France, Germany, Hungary, Italy

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from FoodEx classification system has been linked to the Food Classification System as presented in the Commission Regulation (EU) No 1129/2011, part D, to perform exposure estimates.

2.8.2. Exposure to anthocyanins (E 163) from its use as food additive

Exposure to anthocyanins (E 163) from its use as food additive has been calculated by using data on maximum reported use levels as listed in Table 4 including data following the rules for QS regulations, both combined with national consumption data for the five population groups (Table 6).

High level exposure (typically 95th percentile of consumers only) was calculated by adding the 95th percentile of exposure from one food group (i.e. the one having the highest value) to the mean exposure resulting from the consumption of all other food groups. This is based on the assumption that an individual might be a high level consumer of one food category and would be an average consumer of the others. This approach has been tested several times by the Panel in re-evaluation of food colours and has shown reasonable correlation with high level total intakes when using the raw food individual consumption data. Therefore, this approach was preferred for the calculations based on maximum reported use levels in order to avoid excessively conservative estimates. However, the Panel noted that its estimates should be considered as being conservative as it is assumed that all processed foods contain the colour added at the maximum reported use levels.

Exposure was estimated using the food additives intake model (FAIM), available on the EFSA website[13].

Table 6 summarises the estimated exposure to anthocyanins (E 163) from its use as food additive of all five population groups.

[12] The terms "children" and "the elderly" correspond respectively to "other children" and the combination of "elderly" and "very elderly" in the Guidance of EFSA on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011b).

[13] (http://www.efsa.europa.eu/en/topics/topic/additives.htm).

Table 6: Summary of anticipated exposure to anthocyanins (E 163) from its use as food additive using maximum reported use levels in five population groups (mg/kg bw/day)

	Toddlers (12-35 months)	Children (3-9 years)	Adolescents (10-17 years)	Adults (18-64 years)	The elderly (>65 years)
Mean	1.5-4.0	1.5-4.7	1.0-2.5	0.7-1.9	0.5-1.1
High level[14]	3.2-6.9	2.7-7.8	1.6-3.9	1.1-3.4	0.9-2.3

2.8.3. Main food categories contributing to exposure of anthocyanins (E 163) using maximum reported use levels

Table 7: Main food categories contributing to exposure to anthocyanins (E 163) using maximum reported use levels (> 5 % to the total mean exposure) and number of surveys in which each food category is contributing

		Toddlers	Children	Adolescents	Adults	The elderly
Category number	**Foods**	**% contribution to total exposure (Number of Surveys)***				
1.4	Flavoured fermented milk products including heat treated products	9-22 (3)	6-19 (8)	5-6 (3)	9 (1)	5 (1)
1.5	Dehydrated milk as defined by Directive 2001/114/EC		26 (1)			
1.7.1	Unripened cheese (excl cat 16)		7 (1)	7 (1)	8 (1)	7 (1)
1.7.2	Ripened cheese	10 (1)	6 (1)	7 (1)	5-6 (2)	6-7 (2)
3	Edible ices	7 (1)	5-7 (4)	5 (1)		
4.2	Processed fruit and vegetables	13-51 (4)	7-25 (15)	6-25 (11)	5-28 (14)	12-29 (7)
6.3	Breakfast cereals		7 (1)		5 (1)	
7.2	Fine bakery wares	14 (1)	6-12 (6)	6-9 (4)	6 (2)	5-6 (2)
8.2	Processed meat	10-32 (4)	13-37 (15)	14-49 (12)	17-49 (15)	19-49 (7)
9.2	Processed fish and fishery products including mollusks and crustaceans		7-10 (2)	5 (1)		
12.5	Soups and broths		9 (1)	8 (1)	9 (1)	9 (1)
12.7	Salads and savoury based sandwich spreads				7 (1)	
14.1.4.1	Flavoured drinks with sugar	9-17 (3)	7-33 (14)	6-44 (11)	6-26 (13)	7-17 (3)
14.1.4.2	Flavoured drinks with sweeteners		5-7 (3)	7-25 (4)	5-31 (6)	
14.2	Alcoholic beverages, including alcohol-free and low-alcohol counterparts				5-24 (14)	6-28 (7)

[14] Typically 95th percentile of consumers only.

		Toddlers	Children	Adolescents	Adults	The elderly
Category number	**Foods**	**% contribution to total exposure (Number of Surveys)***				
15.1	Potato-, cereal-, flour- or starch-based snacks	8 (1)		5-7 (2)		
16	Desserts excluding products covered in category 1, 3 and 4	7 (1)	6 (1)			

* Total number of surveys may be greater than total number of countries as listed in Table 4, as some countries submitted more than one survey for a specific age range.

2.8.4. Exposure via the regular diet

The Panel also considered the typical exposure to anthocyanins from the regular diet as relevant for the assessment, as anthocyanins (E 163) are naturally present in foods such as fruits, vegetables, chocolate, tea, wine etc. In a study performed in the USA, over 100 common foods were screened (using chromatographic analyses), and 24 of them were found to contain anthocyanins (E 163) (Wu et al., 2006a). These included fruits (apple, blackberry, blueberry, chokeberry, cranberry, black and red current, elderberry, gooseberry, grapes, nectarines, peaches, plums, raspberry, and strawberry), vegetables (black beans, small red beans, cabbages, eggplant, lettuces, onions and red radishes) and nuts (pistachios). Concentrations of total anthocyanins (E 163) varied considerably from 0.7 to 1480 mg/100 g of fresh weight in gooseberry ('Careless' variety) and chokeberry, respectively. Not only does the concentration vary, but the specific anthocyanins (E 163) present in foods are also quite different. Sugar moieties and acylation patterns vary from food to food, probably due to the specific anthocyanins (E 163) compound profile present in a certain food source. Using these values, the intake of anthocyanins (E 163) in the US using the NHANES 2001-2002 data was estimated at 12.5 mg/person/day.

In addition the Panel obtained data on natural concentrations of anthocyanins (E 163) in seven fruits and vegetables (Discussion paper on anthocyanins (E 163) reply of NATCOL to EFSA 17 Nov 2011), showing similar concentrations as reported in Wu et al. (2006a). Only the reported concentration in grapes (130-270 mg/100 g) was considerably higher than the value presented in Wu et al. (37 mg/100 g).

On the basis of all these natural concentrations, the intake of anthocyanins (E 163) from foods as consumed and in those used in home-made recipes was further assessed using data from Irish adults (1379 adults aged 18-64 years) and children (594 children aged 5-12 years) (Harrington et al., 2001; IUNA, 2005). For adults, mean intakes were found to be 8.6 mg/day (0.1 mg/kg bw/day) and intakes at the 97.5th percentile were 46.5 mg/day (0.6 mg/kg bw/day) for the total population. For children, mean intakes were found to be 7.9 mg/day (0.3 mg/kg bw/day) and intakes at the 97.5th percentile were 53.7 mg/day (2.1 mg/kg bw/day) for the total population. The estimated intake of naturally occurring anthocyanins (E 163) is lower than the refined exposure estimates from its use as a food additive.

2.8.5. Exposure via food supplements

The Panel noted that no data was available on the concentrations of anthocyanins (E 163) in food supplements.

2.9. Uncertainty analysis

Uncertainties in the exposure assessment of anthocyanins have been previously discussed in the present opinion. According to the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised below:

Table 8: Qualitative evaluation of influence of uncertainties

Sources of uncertainties	Direction *
Consumption data: different methodologies / representativeness / under reporting / misreporting / no portion size standard	+/-
Extrapolation from food consumption survey of few days to estimate chronic exposure	+
Linkage between reported use levels and food items in the consumption database: uncertainties on which precise types of food the use levels refer.	+/-
Occurrence data: maximum reported use levels within a food category	+
Exposure model: uncertainty in possible national differences in use levels of food categories, data set not fully representative of foods on the EU market, exposure calculations based on the maximum reported use levels (no use of typical use levels when available)	+

* + = uncertainty with potential to cause over-estimation of exposure; - = uncertainty with potential to cause underestimation of exposure.

3. Biological and toxicological data

Anthocyanins were previously evaluated by JECFA, the SCF and TemaNord. The present opinion briefly reports the major studies evaluated in these opinions with a more detailed description of any relevant new data identified in the scientific literature.

The Panel considered the biological and toxicological data from studies on the food additive anthocyanins (E 163) itself and related preparations of anthocyanins and anthocyanidins including other major components (tannins) of the food additive E 163 to be relevant for this opinion.

3.1. Absorption, distribution, metabolism and excretion

JECFA (1982) describes several studies on the absorption, distribution metabolism and excretion of anthocyanins. The available literature often does not provide sufficient details on the material tested to identify whether aglycones, conjugates or mixtures were used therefore in such circumstances the term anthocyanins/anthocyanidins has been used.

Anthocyanins/anthocyanidins (principally delphinidin) extracted from Concord grapes were administered to rats by either gavage (100 mg/animal) or by subcutaneous injection (50 mg/animal), and the urine was analysed for unchanged anthocyanins by an HCl-acid red test (Horwitt, 1933). Anthocyanins were detected in the urine of rats administered anthocyanins by the subcutaneous route but not by gavage. In studies in dogs where anthocyanins (500 mg) were administered by gastric fistula, no urinary colouration was demonstrated.

However, in rabbits, 1-2 % of an oral dose of anthocyanins (500 mg/animal) was present in the urine as the unchanged pigment. It should be noted that the analytical method used in this study would only detect unchanged anthocyanins (Scheline, 1978). If the anthocyanins were transformed into colourless pseudobases or pale anhydrolases prior to absorption and excretion, they would not be detected (Kuhnau, 1976).

The absence of pigmented urine in individuals ingesting anthocyanins-containing foods, coupled with the apparent lack of metabolism of anthocyanins, has been interpreted as showing that gastrointestinal absorption of these compounds does not occur (Clark and Mackay, 1950).

When anthocyanins/anthocyanidins were absorbed (or injected i.p. or i.v.) in rodents, they accumulated primarily in the kidney, skin, liver, heart and lung, whereas elimination of the compound occurred primarily via the kidney and bile (Lietti and Forni, 1976).

More data evaluated by TemaNord show that anthocyanins can be absorbed in humans and that only a small percentage of the dose was excreted unchanged in the urine. Since hydrolysis of conjugates was not attempted in these studies, actual absorption might be higher than indicated by this study (Lapidot et al., 1998).

Some data on metabolism of anthocyanins are described by JECFA (1982) and are cited below.

Metabolism of anthocyanins was limited and may be restricted to the activity of the intestinal bacterial flora Kuhnau, 1976). The metabolites of anthocyanins have not been identified. However, the insensitivity of the assay techniques used for measuring unmetabolised anthocyanins may result in a significant underestimate of the degree of absorption and metabolism of the anthocyanins.

Studies in rats have shown that some anthocyanins (notably pelargonidin, delphinidin, malvidin) were subject to degradation by intestinal bacteria. *p*-Hydroxyphenyl-lactic acid was detected in the urine of rats following the oral administration of a 3',3-diglycoside of pelargonidin (Griffiths and Smith, 1972a and b).

The presence of two unidentified metabolites in the urine of rats, after treatment by gavage with 100 mg of delphinidin/animal, has also been reported. Rats gavaged with a 3',5'-diglycoside of malvidin had three unidentified metabolites present in the urine (Scheline, 1978). These studies suggest that some of the metabolites of anthocyanins (aglycones) can be absorbed.

Metabolism of anthocyanins may occur by ring fission and/or glycoside hydrolysis of the anthocyanins (Parkinson & Brown, 1981). Cyanidin, the most widespread anthocyanins, has not been shown to be attacked by intestinal bacteria (Scheline, 1968; Griffiths and Smith, 1972a).

Loss of colour of anthocyanins after incubation with rat caecal cell extracts has been reported. In contrast, anthocyanins extracts incubated with human faecal suspensions for 2-3 days remained unchanged (as measured by a reduction in suspension colour) (Haveland-Smith, 1981).

TemaNord (2002) considered that the absence of studies with pure anthocyanins had been due to their complicated chemistry as they can exist in several stable chemical forms at different pH values.

3.1.1. New literature

3.1.1.1. Rats

Following oral administration of purified delphinidin 3-*O*-*β*-rutinoside (D3R), cyanidin 3-*O*-*β*-rutinoside, or cyanidin 3-*O*-*β*-glucoside (C3G) (800 μmol/kg bw) to male Wistar rats (13/group), all three anthocyanins were detected in the plasma with *C*max values of 580 ± 410, 850 ± 120 and 840 ± 190 nmol/L respectively, 0.5-2 hours after administration (Matsumoto et al., 2001). In addition, two metabolites of C3G were detected in plasma.

Male Wistar rats had free access to a cyanidin 3-glucoside and malvidin 3-glucoside-containing diet (200 g blackberry powder/kg) for 8 hours/day for 8 days (no food available during the rest of the day) (Felgines et al., 2002). Background levels of anthocyanins did not increase in plasma at any of the sampling times. Anthocyanins were recovered in urine as the intact glycosidic forms. Neither aglycones nor other conjugates were detected. Peonidin-3-glucoside was present in urine, which could have resulted from hepatic methylation at the 3'hydroxyl moiety of cyanidin-3-glucoside. Urinary recovery of cyanidin-3-glucoside in either intact or methylated forms was 0.26 % of the ingested amount, whereas that of malvidin-3-glucoside (M-3-G) was 0.67 %. Low amounts of cyanidin 3-glucoside and malvidin 3-glucoside, as well as free cyanidin, were recovered in caecal contents.

Gastric absorption of anthocyanins was compared after *in situ* gastric administration of individual purified anthocyanins (cyanidin 3-glucoside, cyanidin 3-galactoside, cyanidin 3-rutinoside and malvidin 3-glucoside at a concentration of 14 μmol/L), blackberry anthocyanins (14 and 750 μmol/L)

or bilberry anthocyanins (88 µmol/L) for 30 minutes in anaesthetised male Wistar rats with ligated pylorus and gastroesophageal sphincter (Talavera et al., 2003). About 25 % of anthocyanins monoglycosides (glucoside or galactoside) was absorbed from the stomach, whereas absorption of cyanidin 3-rutinoside was lower. Bilberry anthocyanins were also efficiently absorbed, but absorption varied greatly (19–37 %) according to the anthocyanins structure; delphinidin glycosides were the most absorbed. The percentage of cyanidin 3-glucoside absorption was lower when the higher concentration of blackberry anthocyanins (750 µmol/L) was injected into the gastric lumen than after administration of the lower concentration (14 µmol/L). After administration of this high concentration, blackberry anthocyanins were observed in plasma from gastric vein and aorta, but neither aglycones nor metabolites were detected. Analysis of bile samples revealed that cyanidin 3-glucoside appeared in bile after as little as 20 minutes. Peonidin-3-glucoside (the methylated form of cyanidin 3-glucoside), as well as unknown anthocyanins metabolites, were also observed in bile. Although cyanidin 3-glucoside, peonidin-3-glucoside and unknown anthocyanins metabolites were detectable in bile by High-Performance Liquid Chromatography (HPLC) with ultraviolet (UV) detection, levels were too low to be quantifiable.

When the intestine of anaesthetized male Wistar rats was perfused for 45 minutes with various anthocyanins (purified anthocyanins glycosides (9.2 nmol/min), blackberry (9 nmol/min) or bilberry (45.2 nmol/min) anthocyanins) in a physiological buffer, a high proportion of anthocyanins glycosides was absorbed through the small intestine (Talavera et al., 2004). The rate of absorption was influenced by the chemical structure of the anthocyanins and varied from 10.7 % (malvidin 3-glucoside) to 22.4 % (cyanidin 3-glucoside). Regardless of the anthocyanins perfused, only intact glycosides were recovered in the intestinal lumen. After perfusion of a high amount of blackberry anthocyanins (600 nmol/min), cyanidin 3-glucoside was recovered in urine and plasma from the aorta and mesenteric vein. Methylated and/or glucuronidated derivatives were also identified. Analysis of bile samples revealed that cyanidin 3-glucoside and its methylated derivatives (peonidin 3-glucoside + peonidin glucuronide) quickly appeared in the bile.

Rats (n=36) were administered by gavage 10 mL/kg bw of an anthocyanins extract from purple sweet potato (containing 27 mg/mL anthocyanins) (Harada et al., 2004). Only two of the eight major anthocyanins present in the anthocyanins extract (cyanidin-3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-*β*-D-glucopyranosyl)-*β*-D-glucopyranoside)-5-*O*-*β*-D-glucopyranoside) and peonidin-3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-*β*-D-gluco-pyranosyl)-*β*-D-glucopyranoside)-5-*O*-*β*-D-glucopyranoside) were detected in plasma and urine. The peak plasma concentration of anthocyanins was reached five minutes after administration and the anthocyanins were rapidly excreted in urine (100 % after 180 minutes).

Anthocyanins metabolism and distribution to the digestive organs (stomach, jejunum) and liver, as well as to the brain and kidney was studied in male Wistar rats fed with a blackberry anthocyanins enriched diet (15 g blackberry/kg diet) for 15 days (Talavera et al., 2005). In the stomach only native blackberry anthocyanins (cyanidin-3-*O*-glucoside and cyanidin-3-*O*-pentose) were found, while in other organs (jejunum, liver and kidney) both native and methylated anthocyanins and conjugated anthocyanidins (cyanidin and peonidin monoglucuronides) were identified. Proportions of anthocyanins derivatives differed between organs, with the liver having the highest proportion of methylated forms. Jejunum and plasma also contained aglycone forms. Total anthocyanins content (blackberry anthocyanins and peonidin-3-*O*-glucoside) reached 68.6 ± 5.8, 605 ± 71, 3.27 ± 1.13, 0.38 ± 0.04 and 0.25 ± 0.05 nmol/g of tissue in stomach, jejunum, kidney, liver and brain, respectively (n = 6). The urinary excretion of total anthocyanins was low (0.19 ± 0.02 % of the ingested amount).

Cyanidin-3-*O*-*β*-D-glucopyranoside (Cy3G, 100 mg/kg bw) was orally administered to male Wistar rats (Ichiyanagi et al., 2005). The plasma concentration of cyanidin-3-*O*-*β*-D-glucopyranoside reached its maximum at 15 minutes after the ingestion. Two metabolites (both monomethylated cyanidin-3-*O*-*β*-D-glucopyranoside) reached maximum plasma levels of 5 nM and 21 nM at 15 and 30 minutes, respectively, whereas two other metabolites (glucuronides of cyanidin and methylated cyanidin, respectively) reached maximum plasma levels of 8.5 nM and 20 nM at 60 and 120 minutes, respectively.

Male Wistar rats were fed approximately 2.8 µmol anthocyanins/day in a red orange anthocyanins enriched diet containing cyanidin-3-glucoside and an acylated derivative, cyanidin-3-(600-malonyl)-glucoside, for 12 days (Felgines et al., 2006). The absorption of these anthocyanins was also studied in both the stomach and intestine using *in situ* anaesthetized rat models (~ 22 µmol/L red orange juice anthocyanins). The red orange anthocyanins, cyanidin-3-glucoside and cyanidin-3-(600-malonyl)-glucoside, as well as their respective methylated derivatives, were recovered in urine after red orange juice intake. The 24-hour urinary excretion of total anthocyanins was low (0.081 ± 0.009 % of the ingested amount). However, a high proportion (~ 20 %) of red orange anthocyanins was absorbed from the stomach in the *in situ* model. More cyanidin-3-(600-malonyl)-glucoside than cyanidin-3-glucoside was absorbed in the intestine.

Eight male F344 rats per group received control diet or chokeberry-, bilberry- and grape-enriched diets for 14 weeks containing 3.85 g cyanidin-3-galactoside equivalents/kg diet (He et al., 2006). In the urine, the total concentration of intact anthocyanins and methylated derivatives ranged from 17.4 (bilberry) to 52.6 (chokeberry) nmol/L. Less than 0.05 % of the ingested anthocyanins was excreted in urine within 6 hours. The type and number of anthocyanins glycosylations affected the absorption. All anthocyanins 3,5-diglucosides from GSKE were consistently excreted in the urine in higher proportion than their corresponding monoglucosides. Although the authors concluded that there was better absorption of anthocyanins diglucosides than their corresponding monoglucosides, this could also result from higher degradation or metabolism of monoglucosides compared to the diglucosides.

A bilberry extract containing 15 different anthocyanins was orally administered to male Wistar rats at a dose of 400 mg/kg bw bilberry extract (153.2 mg/kg bw as anthocyanins) (Ichiyanagi et al., 2006). All the anthocyanins except peonidin 3-*O*-*α*-L-arabinoside were detectable in the blood plasma. The plasma concentration of anthocyanins as a whole reached a maximum level of 1.2 µM 15 minutes after oral administration. Uptake and decay profiles of each anthocyanin in the plasma were similar for all anthocyanins, except a few had their maximum plasma level after 30 minutes. For anthocyanins with the same aglycone, the plasma level 15 min after oral administration was galactoside > glucoside > arabinoside. Plasma clearance of anthocyanins after intravenous administration showed that arabinoside disappeared more rapidly than glucoside and galactoside. On the other hand, when anthocyanins carrying the same sugar moiety were compared, the half life of plasma anthocyanins was in the following order: delphinidin > cyanidin > petunidin = peonidin > malvidin. The bioavailability of anthocyanins was in the range of 0.61-1.82 % and was 0.93 % for the anthocyanins mixture.

Delphinidin-3-rutinoside was administered to male Wistar rats either orally at 152 µmol/kg bw or intravenously at 7.62 µmol/kg bw (Matsumoto et al., 2006). Delphinidin-3-rutinoside was primarily absorbed in the blood and excreted into urine unmetabolised with a T_{max} of 26.3 ± 7.5 minutes and a C_{max} of 0.285 ± 0.071 µmol/L. Small amounts of the metabolite 4'-*O*-methyl-delphinidin-3-rutinoside were detected in the plasma, but neither anthocyanidin (the relevant aglycone) nor glucurono- or sulpho-conjugates were detected. In the 8-hour period after intake, 795 ± 375 and 12.3 ± 2.91 nmol of delphinidin-3-rutinoside and 4'-*O*-methyldelphinidin-3-rutinoside were excreted in the urine, respectively. Oral administration of delphinidin-3-rutinoside resulted in a bioavailability of 0.49 ± 0.06 %. Analysis of delphinidin-3-rutinoside plasma concentrations in bile cannulated rats revealed that, in the 8-hour period after intake, the intact delphinidin-3-rutinoside excretion ratio in bile was 11 % of the excretion ratio of 4'-*O*-methyl-delphinidin-3-rutinoside; 1.91 ± 0.35 nmol versus 17.4 ± 8.67 nmol, respectively. The results indicate that delphinidin-3-rutinoside might be metabolised differently from cyanidin-3-glucoside and pelargonidin-3-glucoside.

After administration of 50 mg/kg bw pelargonidin by oral gavage to male Sprague-Dawley rats, pelargonidin was found in plasma (El Mohsen et al., 2006). The main structurally related pelargonidin metabolite identified in plasma and urine was pelargonidin glucuronide. Furthermore, p-hydroxybenzoic acid, a ring fission product of pelargonidin, was detected in plasma and urine samples obtained at 2 and 18 hours after ingestion. At 2 hours post-gavage, pelargonidin glucuronide was the major metabolite detected in kidney and liver, with levels reaching 0.5 and 0.15 nmol pelargonidin equivalents/g tissue, respectively. Brain and lung tissues contained detectable levels of the aglycone,

with the glucuronide also present in the lungs. Other tissues, including spleen and heart, did not contain detectable levels of pelargonidin or ensuing metabolites. At 18 hours post-gavage, tissue analyses did not reveal detectable levels of the aglycone nor of pelargonidin glucuronides. The overall uptake of the administered pelargonidin was 18 % after 2 hours, while the majority of the detected levels was located in the stomach. Amounts recovered dropped to 1.2 % only 18 hours post-gavage, with the urine and faecal content constituting almost 90 % of the total recovered pelargonidin (~ 43 and 47 % of dose in urine and faeces, respectively).

The gastric and intestinal absorption of pelargonidin 3-glucoside (Pg 3-glc) was studied in *in situ* models in anaesthetised male Wistar rats at concentrations of 12 μM (Felgines et al., 2007). A high proportion of Pg 3-glc was rapidly absorbed from both the stomach (23 %) and small intestine (24 %). Its metabolism was further studied by feeding rats during 8 days with a diet enriched in freeze-dried strawberries (rats consumed ~ 3.85 g strawberry powder/day). Only low amounts of total anthocyanins were recovered in 24-hour urine (0.163 ± 0.013 % of ingested anthocyanins; n = 8). Similar proportions of intact glycosides (about 53 %) and glucuronidated metabolites (about 47 %) were found. Based on the comparison with earlier studies, the authors concluded that pelargonidin 3-glucoside was glucuronidated to a larger extent than cyanidin 3-glucoside.

3.1.1.2. Rabbits

Nielsen and co-workers (2003) dosed Watanabe heritable hyperlipidemic (WHHL) rabbits and healthy normolipidemic female volunteers (human results described in the human section) with black currant juice containing anthocyanins to study absorption and excretion. A number of anthocyanins were identified (delphinidin 3-O-glucoside (Dp-3-glc), delphidin3-O-rutinoside (Dp-3-rut), cyanidin 3-O-glucoside (Cy-3-glc), cyaniding 3-O-rutinoside (Cy-3-rut), cyanidin 3,5-O-diglucoside (Cy-3,5-diglc) and pelargonidin 3-O-glucoside (Pg-3-glc). In rabbits controls received 6 mL of aqueous citric acid pH 3.5/kg bw and treatment groups received either 1) a black currant juice concentrate pH 3.5 (6 mL, containing 21 mg of Dp-3-glc, 58 mg of Dp-3-rut, 10 mg of Cy-3-glc, and 28 mg of Cy- 3-rut per kg of bw, mean anthocyanins intake) 182 mg/animal), 2) a 63 % pure anthocyanins fraction isolated from black currants dissolved in aqueous citric acid pH 3.5 (6 mL containing 63 mg of Dp-3-glc, 45 mg of Dp-3-rut, 22 mg of Cy-3-glc, and 34 mg of Cy-3-rut per kg of bw, mean anthocyanin intake) 256 mg/animal) or 3) a 79 % pure anthocyanins fraction containing two black currant Dp-3-gly in aqueous citric acid pH 3.5 (6 mL containing 8 mg of Dp-3-glc and 45 mg of Dp-3-rut per kg of bw, mean anthocyanins intake) 81 mg/animal). The remaining 37 and 21 % of the anthocyanins fractions used in the latter groups consisted mainly of monosaccharide derivates and other similar low molecular weight compounds originating from the black currant source. The human results are presented in section 3.1.1.4 on humans.

Peak plasma concentrations of all anthocyanins in rabbits receiving juice alone were observed between 15 and 60 minutes after intubation. The baseline plasma samples collected prior to dosage contained anthocyanins. The dose-adjusted absorption (AUCdose) for the rabbits dosed with black currant juice was significantly larger than for the two groups dosed with fractions of purified black currant anthocyanins or purified delphinidin glycosides dissolved in aqueous citric acid which was attributed to a food matrix effect. The urinary excretion within the first 4 hours and absorption of anthocyanins from black currant juice was 0.035 %.A larger proportion of the anthocyanin rutinosides was absorbed than the corresponding glucosides, whereas the structure of the aglycone did not influence absorption and excretion.

Nielsen and co-workers (2005) investigated the effect of black currant anthocyanins on atherosclerosis in Watanabe Heritable Hyperlipidemic rabbits. Four groups of rabbits received either 1) 100 g standard diet, 2) 100 g standard diet containing an anthocyanins fraction purified from black currant juice (100.3 ± 12.8 mg pure anthocyanins/100 g diet), 3) 100 g standard diet and black currant juice (58 mg anthocyanins/100 mL) ad libitum instead of drinking water and 4) 100 g/day standard diet supplemented with 0.5 w/w % probucol as a positive control. The anthocyanins fraction dose was chosen to result in similar total anthocyanins intake as in the juice group, based on a pilot study

investigating the palatability of the juice. The anthocyanin content in the anthocyanins fraction was 35 g/100 g and the total phenol content was 47 g/100 g.

Urine samples for anthocyanin analyses were obtained from eight animals per group before the start of treatment and at 1, 2, 3, 5, 7, 9, 13 and 16 weeks of treatment. Collection continued for 4 hours following administration of test diets. A longer collection time was impossible due to rapid degradation of the anthocyanins in the collection trays. Urinary excretion of anthocyanins was only observed in the groups dosed with anthocyanins, and was significantly higher in the juice group (8.8 ± 6.8 %) than in the anthocyanins fraction group (3.9 ± 7.0 %). Although this was probably explained by free access to the juice, whereas the feed was provided at initiation of the 4 hours urine collection, the authors considered that the possibility of lower absorption from solid rather than liquid sources could not be excluded. There was significantly lower fluid intake in the juice group attributed to its low palatability but animals receive the same amount of anthocyanins as the anthocyanins fraction group, equivalent to 47 to 48 mg/kg bw/day.

3.1.1.3. Pigs

Weanling pigs (7.9 ± 1.7 kg) were fed a freeze-dried powder of marionberry, containing cyanidin-3-glucoside (Cy-3-glc, 78 %), cyanidin-3-rutinoside (Cy-3-rutin, 20 %), pelargonidin-3-glucoside (Pg-3-glc, 0.4 %), and unknown acylated cyanidin-based anthocyanins (UACy, 1.5 %), by stomach tube at a dose of 74.2 ± 11.6 mg total anthocyanins/kg bw (Wu et al., 2004). In the urine, the four original anthocyanins and 11 metabolites (mainly glucuronidated and/or methylated forms of the original anthocyanins) were identified and quantified. Total urinary recovery of the anthocyanins plus their related metabolites was 0.087 ± 0.034 % for Cy-3-glc, 0.084 ± 0.026 % for Cy-3-rutin, 0.583 ± 0.229 % for Pg-3-glc and 0.036 ± 0.011 % for UACy. For the individual anthocyanins, the amount of Cy-3-rutin metabolites recovered was lower than the administered intact Cy-3-rutin, whereas the amounts of metabolites from Cy-3-glc and Pg-3-glc in the urine were much higher than their original forms. In pig plasma, Cy-3-glc, Cy-3-rutin and a trace of another metabolite (cyanidin monoglucuronide) were detected. The plasma concentration:dose ratio of Cy-3-rutin was greater than that of Cy-3-glc. Different aglycones and/or sugar moieties appear to influence the absorption and metabolism of anthocyanins. Cy-3-glc and Cy-3-rutin had similar apparent excretion rates relative to dose, whereas Pg-3-glc had a much higher total urinary excretion than cyanidin-based anthocyanins. Most of Cy-3-glc and Pg-3-glc were excreted in the form of metabolites, whereas most of the Cy-3-rutin was excreted unchanged. Urinary recovery of the acylated anthocyanins was lower than that of non-acylated anthocyanins.

Weanling pigs were fed, in a single meal, a freeze-dried powder of chokeberry, black currant or elderberry at single doses of 229, 140 or 228 μmol total anthocyanins/kg bw, respectively (Wu et al., 2005). Delphinidin anthocyanins were not metabolized to any measurable extent. Cyanidin anthocyanins were metabolized via methylation and glucuronidation as well as by formation of both derivatives on the same anthocyanins molecule. Anthocyanins with either a di- or tri-saccharide attached to them were excreted in the urine primarily as the intact form. Over 80 % of the anthocyanins compounds containing rutinose or sambubiose were excreted in urine as the intact molecule. The limited metabolism of these anthocyanins that did occur, was via methylation. Anthocyanin monoglycosides other than the glucosides were metabolised via methylation and/or glucuronide formation. Only a small amount of monoglucuronide was formed.

Five weanling pigs were fed freeze-dried black raspberry powder containing 50.5 ± 3.7 mg/kg bw of total anthocyanins by oral administration (Wu et al., 2006b). After 4 hours, recoveries of total anthocyanins were 41.7 ± 4.9 %. Anthocyanins were recovered primarily in the ileum, caecum and colon. The recovery of cyanidin within the gastrointestinal (GI) tract varied according to the different sugar moieties, with sambubiose recovery being greater than that of sambubiose-rhamnose and rutinose, which were much greater than glucose. Recovery of anthocyanins within the GI tract was linearly associated with urinary anthocyanins recovery suggesting that stability within the GI tract and not decreased absorption, accounts for the increased recovery. Complex anthocyanins containing di- or

triglycosides disappeared more slowly in the GI tract than simple anthocyanins such as a monoglycoside.

3.1.1.4. Humans

Malvidin-3-glucoside (M-3-G) was found in plasma and urine of six non-smoking male volunteers after ingestion of 500 mL red wine, dealcoholized red wine or red grape juice (containing 136.9 ± 1.3, 115.4 ± 1.3 and 233.6 ± 0.5 mg/L M-3-G , respectively) (Bub et al., 2001). After consumption of red wine, a mean maximum of 1.38 ± 0.36 nM was detected after 20 minutes. The mean and maximum plasma concentrations of M-3-G, after consumption of dealcoholized red wine and red grape juice, were reached within 90 and 180 minutes, respectively. The aglycone, sulphate or glucuronate conjugates of M-3-G were not detected in plasma and urine. M-3-G was poorly absorbed after a single ingestion of all the beverages studied. Similar to the plasma data, urine showed the highest concentrations of M-3-G in samples collected during the first 3 hours. The total amount of M-3-G excreted in urine during the first 6 hours after ingestion was less than 0.03 % of the ingested amount. Twenty four hours after ingestion of beverages, M-3-G was not detected in the urine samples, indicating a rapid excretion of M-3-G. The aglycone malvidin was not detected in urine.

Four healthy elderly women consumed 720 mg total anthocyanins (mainly cyanidin 3-sambubioside and cyanidin 3-glucoside) contained in 12 g elderberry extract (EBX) dissolved in 500 ml water (Cao et al., 2001). Anthocyanins were detected as glycosides in plasma and urine. The maximum plasma concentration of total anthocyanins varied from 55.3 to 168.3 nmol/L, with an average of 97.4 nmol/L, and was reached within 71.3 minutes. The elimination of plasma anthocyanins appeared to follow first-order kinetics. The elimination half-life of plasma total anthocyanins was calculated to be 132.6 minutes. Most anthocyanins compounds were excreted in urine during the first 4 hours. The excretion rate of total anthocyanins was 77 µg/hour during the first 4 hours and 13 µg/hour during the second 4 hours.

A mixture of 6.24 µmol (3.58 mg) black currant anthocyanins, (consisting of 2.75 µmol (1.68 mg) of D3R, 2.08 µmol (1.24 mg) of cyanidin 3-*O*-*β*-rutinoside (C3R), 1.04 µmol (0.488 mg) of delphinidin 3-*O*-*β*-glucoside (D3G) and 0.37 µmol (0.165 mg) of (C3G)/kg bw)) was orally ingested by eight volunteers (Matsumoto et al., 2001). D3R, C3R, D3G, and C3G were detected in the plasma and urine. Faeces were not analysed. The plasma C_{max} values were 73.4 ± 35.0, 46.3 ± 22.5, 22.7 ± 12.4 and 5.0 ± 3.7 nmol/L, respectively, 1.25 - 1.75 hours after intake, and the cumulative excretion of the four compounds in urine in the period 0-8 hours after intake, was 0.11 ± 0.05 % of the dose ingested.

The absorption and metabolism of anthocyanins was studied in four elderly women given 12 g EBX (720 mg total anthocyanins), and six elderly women given 189 g lowbush blueberry (BB) (690 mg total anthocyanins) (Wu et al., 2002). The two major anthocyanins in EBX, cyanidin-3-glucoside and cyanidin-3-sambubioside, as well as four metabolites (peonidin 3-glucoside, peonidin 3-sambubioside, peonidin monoglucuronide, and cyanidin- 3-glucoside monoglucuronide) were identified in urine within 4 hours of consumption. Total EBX anthocyanins excretion was 554 ± 90 µg (0.077 % of intake in 4 hours). In five out of six women who ate BB, urine samples contained anthocyanins, which were identified as the original forms based upon comparisons to the BB food sample, which contained 24 different anthocyanins. The total urinary excretion during the first 6 hours was 23.2 ± 10.9 µg (0.004 % of intake in 6 hours). In these women, plasma anthocyanins levels were below detection limits in 2 mL plasma samples. The Panel noted that some of the EBX data reported appeared to be the same as that described in the paper by Cao and co-workers in 2001.

After an overnight fast, five male subjects were given a high-fat meal with a freeze-dried blueberry powder containing 25 individual anthocyanins (containing 1.16 g total anthocyanins) (Mazza et al., 2002). Nineteen of the 25 anthocyanins present in the blueberries were detected in human blood serum. Concentrations of 6.63 ± 1.35, 9.58 ± 2.05, 12.10 ± 2.82 and 13.09 ± 2.74 ng/mL total anthocyanins were detected in serum 1, 2, 3 and 4 hours after ingestion, respectively. Thus, after 3 hours, only 0.002 - 0.003 % of the ingested amount of anthocyanins was present in human serum.

Six healthy volunteers (3/sex) were fed 200 g strawberries (providing 179 µmol pelargonidin-3-glucoside) (Felgines et al., 2003). Urine samples were collected before and after the meal. In addition to pelargonidin-3-glucoside, five anthocyanins metabolites were identified in urine: three monoglucuronides of pelargonidin, one sulphoconjugate of pelargonidin and pelargonidin itself. Total urinary excretion of strawberry anthocyanins metabolites corresponded to 1.80 ± 0.29 % (mean ± SEM, *n* = 6) of pelargonidin-3-glucoside ingested. More than 80 % of this excretion was related to a monoglucuronide. Four hours after the meal, more than two-thirds of anthocyanins metabolites had been excreted, although urinary excretion of the metabolites continued until the end of the 24-hour experiment.

After an overnight fast, six healthy volunteers drank a purple sweet potato beverage, containing 311 mg anthocyanins (Harada et al., 2004). The plasma concentration of measured anthocyanins in humans (cyanidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-*β*-*D*-glucopyranosyl)- *β*-*D*-glucopyranoside)-5-*O*- *β*-D-glucopyranoside) and peonidin 3-*O*-(2-*O*-(6-*O*-(*E*)-caffeoyl-*β*-*D*-glucopyranosyl)- *β*-*D*-glucopyranoside)-5-*O*- *β*-*D*-gluco-pyranoside)) reached a maximum 90 minutes after ingestion, and the recovery in urine was estimated at 0.01-0.03 %.

Volunteers consumed approximately 20 g chokeberry extract containing 1.3 g cyanidin 3-glycosides (899 mg cyanidin 3-galactoside, 321 mg cyanidin 3-arabinoside, 51 mg cyanidin 3-xyloside and 50 mg cyaniding 3-glucoside) (Kay et al., 2004). At least ten individual anthocyanins metabolites were observed in the urine and serum. Average concentrations of anthocyanins and anthocyanins metabolites in the urine reached levels of 17.9 µmol/L (range 14.9-20.9 µmol/L) within 5 hours post-consumption and cyanidin 3-galactoside and metabolized derivatives of cyanidin 3-galactoside persisted in 22-24 hours urine samples at levels of 11.1 - 13.0 µmol/L[15]. In addition, average total levels of anthocyanins and anthocyanins metabolites detected in the serum of 591.7 (range 197.3-986.1) nmol/L were observed within 2 hours post-consumption. Cyanidin 3-galactoside accounted for 55.4 % (9.9 µmol/L, range 7.2 -12.6 µmol/L) and 66 % (390.6 nmol/L, range 119.4 - 661.9 nmol/L) of the detected anthocyanins in the urine and serum samples, respectively. The metabolites were identified as glucuronide conjugates, as well as methylated and oxidised derivatives of cyanidin 3-galactoside and cyanidin glucuronide.

Five healthy volunteers (two males, three females) consumed 200 g of blackberries (960 µmol of anthocyanins; *i.e.* cyanidin 3-glucoside) (Felgines et al., 2005). In addition to native cyanidin 3-glucoside, several other anthocyanins metabolites were identified in the urine including methylated glycosides, glucuronides of anthocyanidins and anthocyanins, a sulphoconjugate of cyanidin, and anthocyanidins. Total urinary excretion of blackberry anthocyanins metabolites was 0.160 ± 0.02 % of the amount of anthocyanins ingested. Monoglucuronides of anthocyanidins represented > 60 % of this excretion. Urinary excretion of anthocyanins was maximal between 2 and 4 hours after the meal, but continued during the 24 hours of the experiment.

Eleven volunteers were fed 45 g of freeze-dried black raspberries (BRB) (containing 1.44 g total anthocyanins) daily for 7 days (Stoner et al., 2005). The main anthocyanin in BRB is cyanidin-3-rutinoside (0.963 g/dose). Maximum concentrations of anthocyanins in plasma occurred at 1 to 2 hours after consumption and maximum quantities in urine appeared between 0 and 4 hours. Overall, less than 1 % of anthocyanins were absorbed and excreted in urine. None of the pharmacokinetic parameters in plasma changed significantly between days 1 and 7. Parameters on day 7 for cyanidin-3-rutinoside were: C_{max}: 24.82 ± 9.08 ng/mL, T_{max}: 1.45 ± 0.52 hours, and mean t½ = 2.20 hours.

Three volunteers were administered a 721 mg oral dose of cyanidin 3-glycosides from chokeberry extract (Kay et al., 2005). The cumulative concentration of total anthocyanins (parent and metabolites) detected in the serum within 7 hours was 376.65 ± 16.20 (nmol x hour)/L (area under the

[15] There is a discrepancy in the 22-24 hours urine values mentioned in the abstract (11.1-13.0 nmol/L) and the discussion (0.011-0.013 nmol/L). Based on the other values and units reported in the paper, it is assumed that the unit mentioned in the abstract is a typing error.

concentration time curve), reaching a maximum concentration (96.08 ± 6.04 nmol/L) within 2.8 hours. The parent anthocyanins represented only 32 % (120.63 ± 2.85 (nmol x hour)/L)) of the total anthocyanins detected with 68 % (256.02 ± 5.23 (nmol x hour) identified as conjugated metabolites. Additionally, the total urinary excretion of anthocyanins over 24 hours was 1071.54 ± 375.46 µg, reaching a maximal rate of excretion (202.74 ± 85.06 µg/hour) at 3.72 ± 0.83 hour. Parallel to the serum data, only 32.5 % (347.85 ± 60.61 µg) of the anthocyanins excreted in the urine (total 24 hours) were the parent compounds with 67.5 % (723.69 ± 92.59 µg) occurring as conjugated metabolites. The metabolites were identified as glucuronidated and methylated derivatives of the parent cyanidin 3-glycosides.

Black raspberry anthocyanins were administered daily for one week at high doses (2.69 ± 0.085 g/day, in 45 g of freeze-dried black raspberries) to ten healthy males (Tian et al., 2006). Anthocyanins were excreted in urine in intact forms and metabolised into methylated derivatives. The urinary excretion of anthocyanins reached a maximum concentration (1.09 ± 1.08 nmol/L) 4-8 hours after black raspberry ingestion. As compared to the anthocyanins distribution in black raspberries, where cyanidin-3-rutinoside is the main anthocyanins present, in urine, cyanidin 3-xylosylrutinoside was detected at a higher concentration than that of cyanidin-3-rutinoside.

Eleven healthy volunteers consumed 200 mL cranberry juice containing 12 different anthocyanins (650.8 µg total anthocyanins) (Ohnishi et al., 2006). Eight anthocyanins were detected in urine and the main six were quantified. Maximum urinary levels were reached between 3 and 6 hours after ingestion. In total, the recovery of anthocyanins in urine over 24 hours was approximately 5 % of the amount consumed. Up to 80 % of the anthocyanins administered was excreted within 6 hours after consumption. More than 90 % of the anthocyanins excreted was in the form of cyanidin galactoside and peonidin arabinoside.

Twelve healthy volunteers were dosed with 7 mL/kg bw açai pulp (972 ± 27 mg/kg anthocyanins) and clarified açai juice (531 ± 0.2 mg/L anthocyanins) after a 72-hours washout phase (diet low in antioxidants) and an overnight fast (Mertens-Talcott et al., 2008). Plasma was repeatedly sampled for 12 hours and urine for 24 hours after consumption. For pulp and juice, maximal plasma concentrations of anthocyanins were 2321 and 1138 ng/L at times of 2.2 and 2 hours, half-lives were 6.56 and 3 hours and AUC_{last} values were 8568 and 3314 ng*hours/L.

Volunteers were fed in a random order, four portions ranging between 100–400 g of fresh strawberries (containing 57.1 ± 19.8 µmol total anthocyanins/100g fresh weight), as part of a standard breakfast (Hollands et al., 2008). Fresh strawberries contained pelargonidin-3-glucoside as the major anthocyanins, with smaller amounts of cyanidin-3-glucoside and pelargonidin-3-rutinoside. Anthocyanins were detected in the urine of all volunteers for all doses, predominantly as pelargonidin glucuronide and sulphate metabolites. There was a strong, linear relationship between oral dose and anthocyanins excretion, which indicated that on average, every additional unit of dose caused 0.0166 units of excretion. Within individuals, dose-excretion data fitted a linear regression model.

Six healthy adults per sex consumed 100, 200 or 400 g of pureed strawberries, delivering 15, 30 or 60 mmol anthocyanins (mainly pelargonidin 3-glucoside), respectively (Carkeet et al., 2008). Pelargonidin 3-glucoside, and three of its metabolites (detected as monoglucuronides) were excreted in urine after ingestion. The amount of the anthocyanins dose excreted in 24 hours after ingestion of 400 g strawberry puree was 37.4 ± 4.8 nmol for pelargonidin 3-glucoside and 91.4 ± 12.4, 960.6 ± 96.1 and 73.4 ± 7.8 nmol for the three metabolites, respectively. Maximum output of urinary anthocyanins occurred at 2 hours after treatment, and 90 % of the recovered anthocyanins were collected by 10 hours. Total urinary anthocyanins excretion increased linearly with the ingested dose.

As described in the section on rabbits Nielsen and co-workers (2003) dosed Watanabe heritable hyperlipidemic (WHHL) rabbits and healthy normolipidemic female volunteers with black currant juice containing anthocyanins to study absorption and excretion. A number of anthocyanins were identified (delphinidin 3-O-glucoside (Dp-3-glc), delphidin3-O-rutinoside (Dp-3-rut), cyanidin 3-O-

glucoside (Cy-3-glc), cyaniding 3-O-rutinoside (Cy-3-rut), cyanidin 3,5-O-diglucoside (Cy-3,5-diglc) and pelargonidin 3-O-glucoside (Pg-3-glc). The human volunteers received either 1) black currant juice similar to the one used in the rabbit study was used with sugar added to 16.7 w/w %. The dose of the undiluted juice was 4.4 g containing 7.5 mg of Dp-3-glc, 6.4 mg of Dp-3-rut, 0.61 mg of Cy- 3-glc, and 5.2 mg of Cy-3-rut per kg bw, mean anthocyanins intake 1239 mg) 2) 2.7 g of juice containing 4.6 mg of Dp-3-glc , 3.4 mg of Dp-3-rut, 0.38 mg of Cy- 3-glc, and 3.2 mg of Cy-3-rut per kg bw, mean anthocyanins intake 716 mg) or 3) the same dose as group 2 together with a rice cake (mean anthocyanins intake 746 mg). Peak plasma concentrations of all anthocyanins receiving juice alone were observed between 0 to 90 minutes but the co-administration of a rice cake retarded this to between 45 and 150 min. The baseline plasma samples collected prior to dosage contained anthocyanins. No significant differences were detected between the three human treatment groups. The urinary excretion within the first 4 hours and absorption of anthocyanins from black currant juice was 0.072 %. A larger proportion of the anthocyanin rutinosides was absorbed than the corresponding glucosides, whereas the structure of the aglycone did not influence absorption and excretion.

Summary of ADME data

Studies on the toxicokinetics and toxicological properties of anthocyanins have mainly used fruit extracts, which contain several anthocyanins. Therefore, based on these studies, conclusions cannot be drawn for specific anthocyanins, but may be made for anthocyanins in general. Since anthocyanins used as the food additive E 163 are poorly defined, it is not clear whether the substances used in the various studies are relevant for assessment of the specific E 163 anthocyanins.

Given the fact only a few % of anthocyanins can be found in the urine the absorption is assumed to be very low. The elimination of plasma anthocyanins appeared to follow first-order kinetics in humans. Anthocyanins are excreted rapidly via urine and excretion is almost complete after 24 hours. In general, less than 1 % of the ingested dose is excreted via urine, although a urinary excretion of 5 % was reported in one study.

Studies in rats have revealed that the amount of absorption was low (< 2 %). After oral administration, a maximum plasma concentration of anthocyanins was reached after 15-120 minutes, depending on the aglycone and sugar moieties of the anthocyanin. Higher absorption levels (up to 37 %) have been reported in *in situ* experiments in anesthetised rats.

In rats as well as pigs, anthocyanins can be methylated or conjugated with glucuronic acid or sulphate and excretion of these metabolites and their aglycones in urine have been reported. However, delphinidin anthocyanins were not metabolized to any measurable extent.

Anthocyanins are excreted unchanged and as metabolites. Those with either a di- or tri-saccharide attached to them were excreted in the urine primarily unchanged. Plasma clearance rates are influenced by both the aglycone (delphinidin > cyanidin > petunidin = peonidin > malvidin) and the sugar moiety (galactoside > glucoside > arabinoside). Urinary excretion in rats and pigs is only 0.04-0.58 % of the ingested amount.

Limited studies in humans showed that only a small portion of orally ingested anthocyanins was absorbed (<1 %). Maximum plasma levels were reached within 2 hours of consumption. In humans, glucuronide conjugates, sulphate conjugates and methylated metabolites were found in both plasma and urine, together with oxidized derivatives. About 68 % of absorbed anthocyanins was reported to be metabolized, and urinary excretion occurred mainly as a monoglucuronide.

3.2. Toxicological data

3.2.1. Acute oral toxicity

Acute oral toxicity has been assessed in mice, rats and rabbits. An anthocyanins mixture extracted from currants, blueberries and elderberries containing cyanidin, petunidin and delphinidin was studied (Pourrat et al., 1967). In mice, the LD_{50} value was determined to be 25 000 mg/kg bw in mice and 20 000 mg/kg bw in rats. No adverse effect was noted on blood pressure in rabbits that were orally administered 6 g/kg bw anthocyanins glycosides.

No new literature has been published on acute oral toxicity.

3.2.2. Short-term and subchronic toxicity

JECFA (1982) described three short-term or subchronic studies with anthocyanins. In two of the studies the anthocyanins compound was not further specified.

No adverse effects were reported when guinea pigs received 3 g/day of anthocyanins extract (alcoholic extract of fermented blueberries juice in presence of yeasts) for 15 days followed by a washout period of one month (Pourrat et al., 1967).

Weanling Wistar rats (10/sex/group) were treated with anthocyanins extract (alcoholic extract of fermented blueberries juice in presence of yeasts) at levels equivalent to 0, 1.2 or 3 g/day for a period of 90 days, then sacrificed (6/sex/group) or maintained without treatment for 1 month (4/sex/group) (Pourrat et al., 1967). Normal behaviour was noted in all animals and no differences were observed between the test animals and controls in survival, growth, hematology parameters or histopathology of the principal tissues at the termination of the study or after the reversibility period.

Beagle dogs (4/sex/group) received 0, 7.5 or 15 % of grape skin extracts (GSKE) (approximately 2.4 % anthocyanins (not further specified) by weight) in the diet for 90 days (Cox and Babish, 1978b). No differences were noted between control and treated animals in body weights, growth, survival, clinical chemistries (haematology, biochemistry or urinalysis), organ weights or pathological lesions (gross or microscopic) at doses up to 15 % of the diet.

One additional publication was found regarding the subchronic oral toxicity of grape seed (GSE) and GSKE (Bentivegna and Whitney, 2002). Sprague-Dawley rats (20/sex/group) were fed diets containing GSE (concentrations of 0.63, 1.25 and 2.5 % (w/w) of diet) or GSKE (2.5 % (w/w) of diet) for 90 days. The study was compliant with US Food and Drug Administration (FDA) Good Laboratory Practice (GLP). No biologically relevant effects were observed on mortality, body weight, ophthalmic examination, haematology or clinical chemistry. Six female rats of the 2.5 % GSE dose group showed mild head-tilt at the last two (weekly) observations; however, this was not observed in males. In addition, the observation was only apparent when the animal was on an open, flat surface; animals in their cages appeared normal. It was considered of doubtful relationship to treatment. Male rats receiving 2.5 % GSE or GSKE had a small (10 %) but statistically significant increase in food intake from day 7 to the end of the study. In males of the 1.25 % dose group, statistical increases in food intake were also observed, but at irregular intervals. Female rats treated with 2.5 % GSKE showed a decreased absolute and relative heart weight. The authors considered this was not relevant, since it was not observed in males, there was no dose response amongst GSE treated groups and no corresponding histopathology was found. In 11/20 male rats of the 2.5 % GSKE dose group, an increased occurrence of a common renal cortical inflammation was observed. The study authors considered this was a component of the frequently occurring nephropathy in male rats and therefore was not treatment-related. The NOAEL for both GSE and GSKE were considered to be 2.5 % (mean time-weighted daily doses equivalent to approximately 1780 GSE or GSKE mg/kg bw/day in males and 2150 mg GSE or GSKE/kg bw/day in females). It was not possible to convert the NOAEL for GSE and GSKE into a NOAEL for anthocyanins since the anthocyanins contents of GSE and GSKE were not further defined. However the Panel considered that using the value reported by JECFA of 3

% anthocyanins content in GSKE (and assuming the same level in GSE), these would be equivalent to 53 mg anthocyanins/kg bw/day for males and 64 mg anthocyanins/kg bw/day for females

3.2.3. Genotoxicity

Three mutagenicity studies are described by JECFA.

Cyanidin and delphinidin were inactive in the Ames assay system using five different strains of *Salmonella typhimurium* (TA1535, TA100, TA1537, TA1538 and TA98) with and without activation (Brown and Dietrich, 1979).

Cyanidin was not mutagenic when examined in the Ames assay using *Salmonella typhimurium* strain TA98 with and without metabolic activation (arochlor 1254 induced rat liver S-9 fraction) (MacGregor and Jurd, 1978). Structure-activity testing of a large group of flavonols for mutagenic response in this assay system indicated that compounds of flavylium class, to which anthocyanins belong, were inactive.

Anthocyanins (compounds not specified) was tested in both the Ames test using *Salmonella typhimurium* TA1538 for mutagenicity and in another *in vitro* test employing *Escherichia coli* WP2 for induction of DNA damage (Haveland-Smith, 1981). In both assay procedures with or without metabolic activation (using either rat caecal extracts or rat liver microsomes) anthocyanins were not found to induce any response. Negative findings were also reported for the anthocyanins in a gene conversion assay using *Saccharomyces cerevisiae* D4.

Also in another study, mentioned by TemaNord, some anthocyanins (not further specified) have been adequately tested in bacterial mutagenicity tests and found to be inactive (Sweeny et al., 1981).

Several additional studies were found regarding the genotoxicity of anthocyanins.

The genotoxicity of an alcohol-free hydro-alcoholic (GSKE) obtained from red grapes *Vitis labrusca* (Isabel varietal) was tested in different bioassays, at doses from 0.1–100 μg/mL using the preincubation method under 100 microl extract/plate (Aiub et al., 2004). Using *Salmonella typhimurium* strains TA97, TA98, TA100 and TA102, no mutagenicity was detected for all tested concentrations in the presence or absence of metabolic activation with S9 fraction from rat liver. Nevertheless, cytotoxicity was observed for TA97 and TA102 with metabolic activation at concentrations above 1 μg/mL and 10 μg/mL, respectively, and without metabolic activation at concentrations above 0.1 μg/mL, and for TA100 with metabolic activation at 100 μg/mL. The measurement of *β*-galactosidase induction in the SOS-chromotest was positive for *Escherichia coli* PQ37 at 100 μg/mL in the presence of metabolic activation. Using Balb/c 3T3 fibroblasts, DNA strand breaks induction by GSE was also investigated by the Comet assay, and no significant difference was detected for treated and non-treated cells for 60 minutes at doses from 0.1–100 μg/mL.

In the Comet assay in human adenocarcinoma HT29 cells, none of the anthocyanidins tested (delphinidin, cyanidin, malvidin, pelargonidin and peonidin) showed an effect on DNA integrity at 1 μM but a slight, but significant increase in DNA strand breaks was found at concentrations of 10 μM for cyanidin and at doses of 50 μM and above for all other anthocyanidins tested (Habermeyer et al., 2005). All of these analogues were able to compete with ethidium bromide for the intercalation into calf thymus DNA and to replace the minor groove binder Hoechst 33258. These data indicate substantial affinity to double-stranded DNA, which might contribute at least to the DNA strand breaking effect of anthocyanidins at higher concentrations (≥ 50 μM). In the same study, delphinin and cyanidin were found to be powerful inhibitors of topoisomerases I and II at 2.5 micromolar and above. The Panel noted that the inhibition of topoisomerase activity may imply a clastogenic potential, which is generally acknowledged to be thresholded. Pelargonidin was tested for anti-genotoxicity in HL-60 cells at concentrations from 0.0625-2 μM for 4 hours. Pelargonidin (≤ 2 μM) was non-genotoxic in the cytokinesis block micronucleus test. In addition, no significant effects were found in a Comet assay

(Abraham et al., 2007). The Panel noted that only low doses of pelargonidin were applied in this antimutagenicity study, which is considered of limited relevance for the assessment of genotoxicity

GSE and GSKE were tested for *in vivo* clastogenic activity and/or induction of chromosomal loss the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in Crl:CD-11(ICR) BR mouse bone marrow (Erexson, 2003). The assay was conducted in accordance with US FDA GLP Recommendation. Five young adult male mice per dose and time point were dosed by gavage with 500, 1000 and 2000 mg/kg bw of GSE or GSKE. For both GSE and GSKE, no statistically significant increase in micronucleated polychromatic erythrocytes was observed at any dose level or harvest time point (24 or 48 hours). GSE induced cytotoxicity (decreased polychromatic erythrocyte to normochromatic erythrocyte ratio) at the 2000 mg/kg dose level for the 48-hour harvest time point, confirming that the test article reached the target bone marrow in toxicologically significant amounts.

Overall, in most *in vitro* assays anthocyanins, tested at low doses, were not genotoxic. Some evidence of genotoxicity, was provided by a single *in vitro* study using pure anthocyanidins. However *in vivo* a negative guideline bone marrow micronucleus test at a limit dose was considered to exclude *in vivo* genotoxicity of GSE and GSKE.

3.2.4. Chronic toxicity and carcinogenicity

No studies on chronic toxicity and/or carcinogenicity were reported in any of the previous evaluations. No new studies on chronic toxicity and/or carcinogenicity are available.

3.2.5. Reproductive and developmental toxicity

A 2-generation reproduction study in Sprague-Dawley rats was described in the evaluation by JECFA in 1982. A grape-skin extract preparation was prepared by spray-drying the liquid form of the extract after addition of maltodextrose as a carrier. The preparation contained approximately 3 % anthocyanins. The test groups received dietary levels of 7.5 % or 15 % of the grape-skin extract throughout the study (equivalent to 0.22 and 0.45 % anthocyanins or 112 and 225 mg anthocyanins/kg bw/day, respectively). There were two concurrent control groups, one receiving the basal diet, the other receiving a diet containing 9 % of the maltodextrin used as the carrier for the GSKE preparation. The F_2 generation (10/litter, after being culled at 4 days) was maintained for 21 days post-partum and then autopsied. No differences in reproductive performance or indices including pup viability were apparent between control and dosed groups. At the higher dose level, both rats from F_1 and F_2 pups exhibited lower body weights than the concurrent controls. Body weights of the F_2 pups in the 7.5 % group were also marginally depressed. However, this was accompanied by a concomitant decrease in food intake resulting from both decreased calorific density and decreased palatability. No compound-related effects were found on haematological and blood chemistry parameters or urinalyses in the F_1 group at week 6 and at termination (week 18) of the studies. However, a decrease in mean and relative liver and thyroid and mean adrenal organ weights occurred in the 15 % group of the F_1 rats and was ascribed to decreased caloric consumption or underfeeding. There were no corresponding compound-related histopathological effects (Cox and Babish, 1978a).

Anthocyanins (an extract from currants, blueberries and elderberries) were reported not to be a developmental toxicant in rats, mice or rabbits when given at dose levels of 1.5, 3 or 9 g/kg bw/day over three successive generations (Pourrat et al., 1967 as cited by JECFA).

No new literature has been published on reproductive or developmental toxicity.

3.2.6. Other studies

In addition, JECFA (1982) describes some effects of anthocyanins on enzymes and biochemical parameters.

Both pelargonidin and delphinidin have been shown to inhibit aldoreductase in the lens of rats (Varma and Kinoshita, 1976). In other studies, anthocyanin-3-monoglycosides (namely petunidin-, delphinidin- and malvidin-) extracted from grapes were found to increase the activity of α-glucan phosphorylase and glutamic acid decarboxylase, but to inhibit glycerol dehydrogenase, malate dehydrogenase and hexokinase (Carpenter et al., 1967).

Other studies have shown that anthocyanins are capable of chelating ions such as copper (Somaatmadja et al., 1964) and iodide (Moudgal et al., 1958). The iodide ion was observed *in vitro* to form a stable complex with the anthocyanins (Moudgal et al., 1958).

TemaNord (2002) concludes, without giving further details, that several studies indicate that anthocyanins can inhibit various enzymes *in vitro* (Gibb et al., 1987; Galvez et al., 1995).

The Panel noted that there are publications on the beneficial effects of anthocyanins in the literature, however the Panel considered that these were not relevant for the risk assessment of anthocyanins (E 163) as a food additive.

4. Discussion

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

Anthocyanins (E 163) are authorised as food additives in the EU, and have been previously evaluated by JECFA in 1982 and the SCF in 1975. JECFA has established an ADI of 2.5 mg/kg bw/day for anthocyanins from grape skin extracts, while the SCF has not derived an ADI for anthocyanins.

Anthocyanins are distributed in various fruits, and several fruit extracts are used as synonyms for anthocyanins. The extracts mentioned most often are GSKE (containing glucosides of the anthocyanidins peonidin, malvidin, delphinidin and petunidin) and blackcurrant extract (containing the colouring matters cyanidin 3-rutinoside, delphinidin 3-rutinoside, cyanidin 3-glucoside and delphinidin 3-glucoside). However, in the EU specifications it is not indicated which fruits or vegetables can be used to obtain the food additive anthocyanins (E 163) nor are extraction methods specified or described. In addition, the composition and identity of anthocyanins which may be present in the food additive E 163 are not specified. These compounds are normally present in food as glycosides (anthocyanins). No JECFA specifications are available for specific anthocyanins, only for GSKE and blackcurrant extract, which contain more than one anthocyanins. The specifications of the European Commission for anthocyanins and of JECFA for GSKE and blackcurrant extract differ. Limits for mercury and cadmium are included in the EU specifications, but not in those of JECFA

Limited data on stability, reaction and fate in food were available. No formal method for the analysis of anthocyanins in food appears to have been adopted.

Studies in rats have revealed that the amount of absorption was low (< 2 %). Urinary excretion in rats and pigs is only 0.04-0.58 % of the ingested amount.

After oral administration to rats, a maximum plasma concentration of anthocyanins was reached after 15-120 minutes, depending on the aglycone and sugar moieties of the anthocyanins. Higher absorption levels (up to 37 %) have been reported in *in situ* experiments in anesthetised rats.

In rats as well as pigs, anthocyanins can be methylated or conjugated with glucuronic acid or sulphate and excretion in urine of these metabolites and aglycone have been reported. However, delphinidin anthocyanins were not metabolized to any measurable extent. Anthocyanins with either a di- or tri-saccharide attached to them were primarily excreted unchanged in the urine. Plasma clearance rates

are influenced by both the aglycone (delphinidin > cyanidin > petunidin = peonidin > malvidin) and the sugar moiety (galactoside > glucoside > arabinoside).

Limited studies in humans showed that only a small portion of orally ingested anthocyanins was absorbed (<1 %). The majority of anthocyanins ingested are excreted in faeces. Maximum plasma levels were reached within 2 hours of consumption. In humans, glucuronic acid conjugates, sulphate conjugates and methylated metabolites were found in both plasma and urine, together with oxidized derivatives. The elimination of plasma anthocyanins appeared to follow first-order kinetics in humans. Anthocyanins are excreted rapidly via urine and excretion is almost complete after 24 hours. In general, less than 1 % of the ingested dose is excreted via urine, although a urinary excretion of 5 % was reported in one study. About 68 % of absorbed anthocyanins were metabolized, and excretion occurred mainly as the respective monoglucuronides.

In guinea pigs and dogs, no short-term or subchronic toxic effects were observed at anthocyanins doses up to 3 g/kg and 15 % of grape-skin extract in the diet, respectively. In addition, in rats fed an unspecified anthocyanins extract at levels up to 6 g/day or GSE or GSKE at dietary levels up to 2.5 % (1780 mg/kg bw/day in males and 2150 mg/kg bw/day in females) for a period of 90 days, no relevant treatment-related adverse effects were observed. It is not possible to convert the reported NOAEL for GSE and GSKE into a NOAEL for anthocyanins since the anthocyanins contents of GSE and GSKE were not further defined. In this case, the value reported by JECFA of 3 % anthocyanins content in GSKE (and assuming the same level in GSE), would result in a dose equivalent to 53 mg anthocyanins/kg bw/day for males and 64 mg anthocyanin/ kg bw/day for females. However, the Panel is of the opinion that these figures cannot be used to calculate a margin of safety (MOS) due to the unclear composition of the different forms of anthocyanins (glycosides of cyanidin, peonidin, malvidin, delphinidin and petunidin) in the colour preparation.

In a 2-generation reproduction study with anthocyanins from GSKE, no effects were observed on reproductive performance or pup viability at dietary levels up to 15 % (equivalent to 225 mg anthocyanins/kg bw/day based on an assumed 3 % anthocyanins content in GSKE) anthocyanins. However, in both the F_1 and F_2 rats, body weight was reduced in the 15 % group. This was also observed in the F_2 pups in the 7.5 % (equivalent to 112 mg anthocyanins/kg bw/day based on an assumed 3 % anthocyanins content in GSKE) group; however, this was marginal and related to a reduced food intake. In addition, a decrease in organ weights of the liver, adrenal and thyroid (without histological effects) occurred in the 15 % group of the F_1 rats. The ADI established by JECFA was based on this study with 7.5 % GSKE in the diet (equivalent to 7500 mg/kg bw) considered as the NOAEL. Since GSKE contains approximately 3 % anthocyanins, this level was correlated to a NOAEL of 225 mg/kg bw/day for anthocyanins. This level was converted into an estimated ADI of 0-2.5 mg/kg bw/day for anthocyanins.

There are no indications that anthocyanins glycosides from currants, blueberries or elderberries induce developmental toxicity in rats, mice or rabbits at dose levels up to 9 g/kg bw/day.

Several anthocyanidins and anthocyanins (cyanidin, delphinidin, GSE and GSKE) were negative in bacterial mutagenicity tests, with and without metabolic activation. Also *in vitro* Comet assays in mammalian cells did not result in increased DNA strand breaks when exposed to 0.1-100 µg/mL (GSE) or 1-10 µM (delphinidin, malvidin, pelargonidin and peonidin). However, in another study, doses of ≥ 50 µM (~ 17.5 µg/mL), delphinidin, cyanidin, malvidin, pelargonidin and peonidin did induce a slight, but significant increase in strand breaks in HT29 cells. In the same study, cyanidin and delphinin at ≥ 2.5 micromolar inhibited topoisomerase activity.

Overall, in most *in vitro* assays anthocyanins, tested at low doses, were not genotoxic. Some evidence of genotoxicity, was provided by a single *in vitro* study using pure anthocyanidins. However a negative guideline bone marrow micronucleus test at a limit dose was considered to exclude *in vivo* genotoxicity of GSE and GSKE.

Due to a lack of data, no conclusion can be drawn with respect to long-term toxicity or carcinogenesis of anthocyanins.

Studies on the toxicokinetics and toxicological properties of anthocyanins have mainly used aqueous fruit extracts, which contain several anthocyanins. Therefore, based on these studies, conclusions cannot be drawn for specific anthocyanins, but may be made for anthocyanins in general. The Panel noted that the specification for anthocyanins and the information available on their manufacture do not allow identification of the specific anthocyanins nor their overall composition in the material used as the food additive E 163. The Panel noted that there is no information on the anthocyanins composition within the food additive and it is therefore not possible to determine the extent to which the available toxicological data are relevant.

Furthermore, the Panel noted that the variety of sources for anthocyanins and lack of information on manufacturing process do not allow identification and quantification of minor components. The Panel considered that if these minor components were selectively extracted there could be alterations in the biological effects of the extract. The Panel also noted that the importance of data covering the range of compositions arising from different manufacturing methods was illustrated in the opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on Rosemary extracts (EFSA, 2008b). The recent opinion of the Scientific Committee entitled '*Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements*' (EFSA, 2009) highlights principles which should be considered in the risk assessment of any botanical preparations including extracts.

The Panel concluded that the currently available toxicological database was inadequate to establish a numerical ADI for anthocyanins as defined by the specifications set for the food additive E 163. However, the Panel concurred with SCF opinion that for *colours for which an ADI cannot be established……. exceptions might be made in the case of compounds which are in fact constituents of food and derived from coloured natural foods by purely physical process* (SCF, 1975).

The Panel noted that toxicological information available from grape-skin extracts and from blackcurrant extracts, including genotoxicity tests, short-term, sub-chronic and reproduction toxicity studies, does not show adverse effects overall.

In a refined exposure estimation performed with the EFSA Comprehensive European Food Consumption Database, using the maximum reported use levels (based on the amount of anthocyanins as colouring principle[16] of the food additive without further specification), the mean exposure to anthocyanins (E 163) range from 1.5 to 4.0 mg/kg bw/day for toddlers (high level exposure 3.2-6.9 mg/kg bw/day), from 1.5 to 4.7 mg/kg bw/day for children (high level exposure 2.7-7.8 mg/kg bw/day) and from 1.0 to 2.5 mg/kg bw/day for adolescents (high level exposure 1.6-3.9 mg/kg bw/day). The mean estimated exposures for adults and the elderly are respectively from 0.7 to 1.9 mg/kg bw/day (high level exposure 1.1-3.8 mg/kg bw/day) and from 0.5 to 1.1 mg/kg bw/day (high level exposure 0.9-2.3 mg/kg bw/day). Compared to the refined exposure estimates, the estimated exposure to anthocyanins from the regular diet is low (at the mean about 0.1 mg/kg bw /day for adults and 0.3 mg/kg bw/day for children, values at the 97th percentiles are 0.6 mg/kg bw/day and 2.1 mg/kg bw/day respectively). The Panel noted that there were no data to estimate exposure to anthocyanins from colouring foods.

Thus taking into account the overall information available for this re-evaluation and the fact that grape-skin and blackcurrant extracts are widely consumed as edible foods, the Panel considered that the use of grape-skin and blackcurrant aqueous extracts as food additives are unlikely to be of safety concern.

[16] The Panel use the term colouring principle for food ingredients used to colour food.

The Panel also noted that exposure to anthocyanins via the regular diet results in highest intakes for the total population of approximately 1 mg/kg bw/day for adults and 2 mg/kg bw/day for children. Exposure estimates to anthocyanins via the regular diet result in lower than the highest exposure estimates from the use of anthocyanins as food additive amounting to 2.7 to 7.8 mg/kg bw/day, therefore to be in line with the former SCF statement (SCF, 1975) the Panel suggested that the maximum permitted levels of anthocyanins in grape-skin and blackcurrant extracts might need to be revised accordingly.

The Panel also recommend that the specifications for E 163 should be modified to qualitatively and quantitatively define these two sources (grape skin and blackcurrant) including anthocyanins and anthocyanidins and other components

The Panel noted that the consumption of the food additive E 163 may lead to intake of sulphur dioxide. However, the available data do not allow estimate of this intake and assessment of this exposure.

According to the origin of these compounds (e.g. obtained from grape skin), the Panel noted that data on pesticides and mycotoxins contaminations of anthocyanins may be relevant for the specifications, The Panel considered that the absence of characterisation of extracts from other production sources and using non-aqueous extraction methods did not allow conclusions on their safety. The Panel is aware that the application of those extraction methods to obtain anthocyanins from food matrices of other origin that gape skin and blackcurrant extracts can lead to markedly different composition compared to the aqueous extracts.

CONCLUSIONS

The Panel concluded that the currently available toxicological database was inadequate to establish a numerical ADI for anthocyanins.

The Panel concluded in principle that provided exposure from use of food colours was comparable to that from the diet the underlying conclusion of safety on food additives derived from natural sources in the 1975 SCF opinion would still apply.

Using a weight of evidence evaluation of toxicological and general exposure data, the Panel concluded that aqueous grape skin and blackcurrant extracts are unlikely to be of safety concern. The Panel recommends that the specifications for E 163 should be modified to reflect the conclusions on these two sources.

With the exception of aqueous grape skin and blackcurrant extracts, the Panel considered that the absence of characterisation does not allow verification of the applicability of the conclusion of safety of food additives derived from natural sources in the 1975 SCF opinion for anthocyanins extracted from other sources and/or using non-aqueous extraction methods.

The Panel concluded that the following information is required to permit an adequate risk evaluation for derivation of an ADI for anthocyanins (E 163) as food additive;

- Definition of the sources from which it is extracted
- Method of extraction
- Qualitative and quantitative chemical characterisation of the extracts including minor components
- Data on toxicokinetics, subchronic toxicity, genotoxicity, reproductive and developmental toxicity and chronic toxicity/carcinogenicity for an appropriate number of extracts covering the range of sources and current manufacturing methods for each source.

The Panel considered that this data package would also need to include comparative data on anthocyanins (E 163) produced by aqueous extraction. The Panel noted that using target read across it has proven possible to perform a risk assessment for a group of extracts (e.g. rosemary extracts) based on compositional and toxicological data on representative samples. This approach was currently not applicable on anthocyanins.

The Panel concluded that refined exposure estimates of anthocyanins from their use as a food additive (E 163), albeit conservative, were higher than estimated intakes from the regular diet and that these did not include intakes from colouring foods. The general principle about safety of food additives derived from natural sources does not apply.

The Panel recommends that appropriate chemical characterisation and toxicological data are required to permit a further re-evaluation of anthocyanins.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands, December 2008.

2. NATCOL contribution concerning use levels of anthocyanins, 3 April 2012.

3. NATCOL contribution concerning the manufacturing, chemical and colouring principle of anthocyanins, 17 November 2011.

4. CIAA response concerning further clarification on usage data of natural food additives 25 May 2011.

5. 8. CIAA response on behalf of stakeholder's contribution concerning an EFSA request on usage data of natural colours, 18 April 2011.

6. NATCOL contribution concerning the manufacturing and colouring principle of anthocyanins, 21 February 2011.

7. NATCOL contribution concerning use levels of anthocyanins, 14 February 2011.

8. NATCOL contribution concerning the extraction and colouring principle of anthocyanins, 7 September 2010.

9. CIAA submission of occurrence data, 14 December 2009.

10. NATCOL dossier on anthocyanins E 163, in response to the re-evaluation of Food Colours: 30 March 2007.

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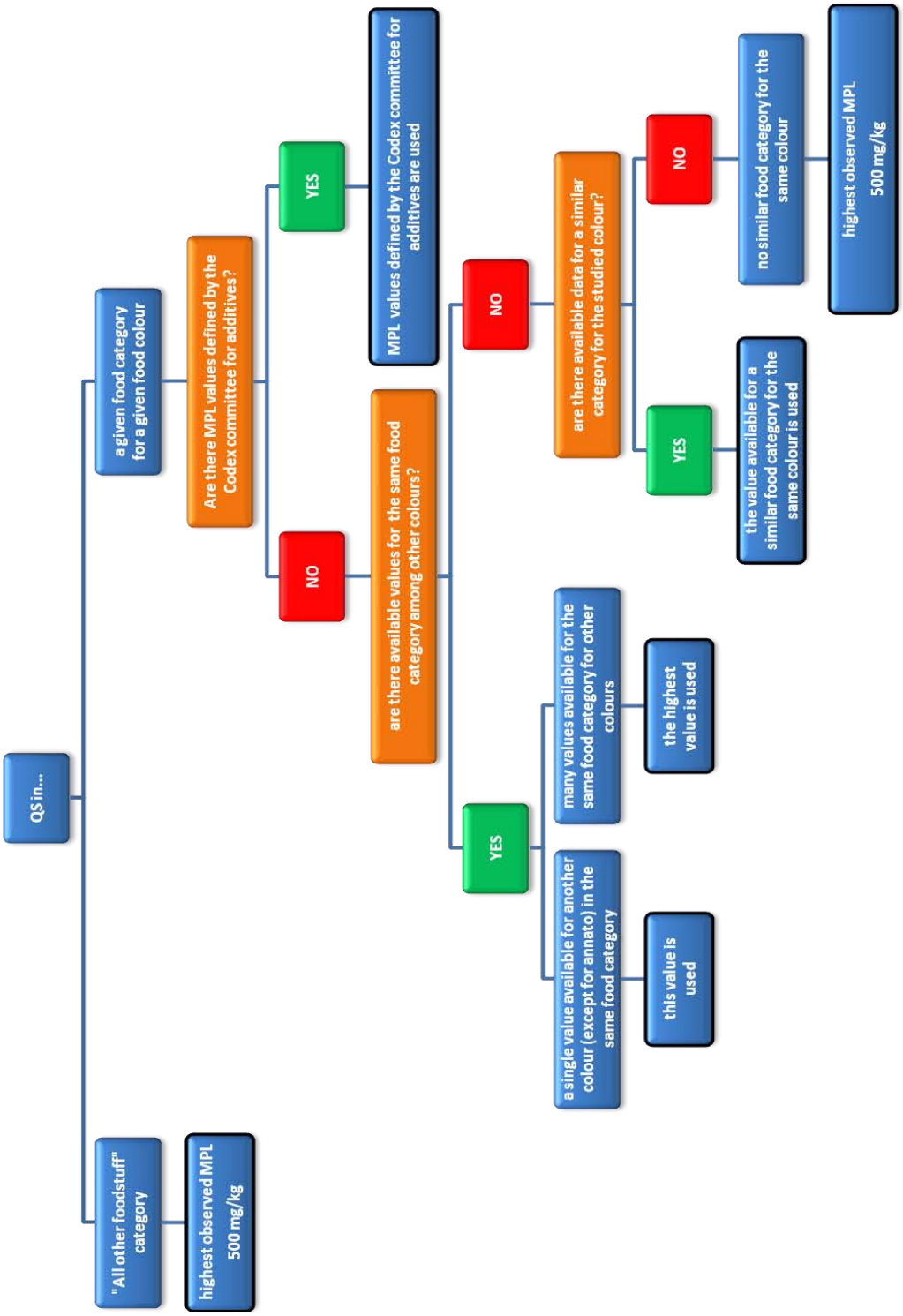
APPENDICES

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

Figure 1: Rules defined by the Panel to deal with usage data or observed analytical data for all regulated food additives to be re-evaluated and procedures for estimating intakes using these rules.

Figure 2: Rules defined by the Panel to deal with *quantum satis* (QS) authorisation



B. SUMMARY OF TOTAL ESTIMATED EXPOSURE (USING MPLS AND USE LEVELS) PER AGE CLASS AND SURVEY*: MEAN AND HIGH LEVEL (MG/KG BW/DAY)

	Use levels	
	Mean	**High level**
Toddlers		
Bulgaria (Nutrichild)	1.5	3.7
Finland (DIPP)	2.0	3.2
Germany (Donald 2006_2008)	2.3	4.0
The Netherlands (VCP_Kids)	4.0	6.9
Children		
Belgium (Regional_Flanders)	4.7	7.8
Bulgaria (Nutrichild)	2.0	4.2
Czech Republic (SISP04)	2.4	5.4
Denmark (Danish Dietary Survey)	2.4	3.7
Finland (DIPP)	2.6	6.0
Finland (STRIP)	3.6	5.6
France (INCA 2)	2.6	4.1
Germany (Donald 2006_2008)	3.0	5.2
Greece (Regional_Crete)	2.4	3.9
Italy (INRAN_SCAI_2005_06)	1.5	2.7
Latvia (EFSA_TEST)	2.4	4.7
The Netherlands (VCP_Kids)	3.6	5.8
Spain (enKid)	2.6	4.3
Spain (Nut_Ink05)	2.3	3.6
Sweden (NFA)	4.1	5.8
Adolescents		
Belgium (Diet_National_2004)	1.9	3.8
Cyprus (Childhealth)	1.0	2.0
Czech Republic (SISP04)	2.0	3.9
Denmark (Danish Dietary Survey)	1.7	2.9
France (INCA 2)	1.3	2.1
Germany (National_Nutrition_Survey_II)	1.4	3.5
Italy (INRAN_SCAI_2005_06)	1.0	1.7
Latvia (EFSA_TEST)	1.7	2.8
Spain (AESAN_FIAB)	1.0	1.6
Spain (enKid)	1.7	3.1
Spain (Nut_Ink05)	1.5	2.4
Sweden (NFA)	2.5	3.7

	Use levels	
	Mean	**High level**
Adults		
Belgium (Diet_National_2004)	1.5	3.4
Czech Republic (SISP04)	1.4	2.8
Denmark (Danish_Dietary_Survey)	1.1	2.1
Finland (FINDIET_2007)	0.9	2.2
France (INCA2)	1.0	1.7
Germany (National_Nutrition_Survey_II)	1.2	3.1
Hungary (National_Repr_Surv)	1.0	1.8
Ireland (NSIFCS)	1.5	2.6
Italy (INRAN_SCAI_2005_06)	0.7	1.1
Latvia (EFSA_TEST)	1.1	1.9
The Netherlands (DNFCS_2003)	1.9	3.3
Spain (AESAN)	0.9	2.2
Spain (AESAN_FIAB)	0.8	1.8
Sweden (Riksmaten_1997_98)	1.5	2.3
United Kingdom (NDNS)	1.2	2.0
The elderly		
Belgium (Diet_National_2004)	1.1	2.3
Denmark (Danish_Dietary_Survey)	0.9	1.6
Finland (FINDIET_2007)	0.6	1.3
France (INCA2)	0.9	1.2
Germany (National_Nutrition_Survey_II)	1.0	2.2
Hungary (National_Repr_Surv)	0.8	1.5
Italy (INRAN_SCAI_2005_06)	0.5	0.9

* The different methodologies of European dietary surveys included in the EFSA Comprehensive Database are fully described in the Guidance on the use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011a). A summary is available p.11, Table 1 of the guidance.

GLOSSARY / ABBREVIATIONS

ADI	Acceptable Daily Intake
AFC	Scientific Panel on Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	Scientific Panel on Food Additives and Nutrient Sources added to Food
Aluminium lakes	Aluminium lakes are produced by the absorption of water soluble dyes onto a hydrated aluminium substrate rendering the colour insoluble in water. The end product is coloured either by dispersion of the lake into the product or by coating onto the surface of the product
APCI	Atmospheric pressure chemical ionization interface
AUC_{last}	Area under the curve
BB	Lowbush blueberry
BCA	Black currant Anthocyanins
BRB	Black raspberries
CAS	Chemical Abstracts Service
C3G	Cyanidin 3-*O*-*β*-glucoside
C3R	Cyanidin 3-*O*-*β*-rutinoside
CZE	Capillary zone electrophoresis
D3G	Delphinidin 3-*O*-*β*-glucoside
D3R	Delphinidin 3-*O*-*β*-rutinoside
EBX	Elderberry extract
EFSA	European Food Safety Authority
EINECS	European Inventory of Existing Commercial Chemical Substances
ESI	Electro Spray Ionization
FDA	US Food and Drug Administration
GI	Gastrointestinal
GLP	Good Laboratory Practice
GSE	Grape seed extract
GSKE	Grape skin extract
HPLC	High-Performance Liquid Chromatography

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
MALDI-MS	Matrix-assisted laser desorption/ionization mass spectrometry
MALDI-TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
M-3-G	Malvidin-3-glucoside
MEKC	Micellar electrokinetic chromatography
MOS	Margin of safety
NATCOL	Natural Food Food Colours Association
NOAEL	No-Observed-Adverse-Effect Level
PCE	Polychromatic erythrocyte
SCF	Scientific Committee for Food
TWI	Tolerable Weekly Intake
UV	Ultraviolet
WHHL	Watanabe heritable hyperlipidemic