SCIENTIFIC OPINION

Scientific Opinion on the re-evaluation of beetroot red (E 162) as a food additive[1]

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

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Beetroot red (E 162) is a natural colour containing a number of pigments, all belonging to the class known as betalains. The main colouring principle consists of a number of betacyanins. The Panel noted that the specification for the content of red colour (expressed as betanin) in beetroot red, as not less than 0.4%, may give rise to some confusion, given the number of different forms of beetroot red that may be on the market, including simple extracts, refined extracts and spray–dried powders. The Panel considered that revision of the current specification to reflect betanin content on a dried solids basis could be appropriate. The Panel noted that toxicological studies carried out on material conforming to the specifications for beetroot red are limited in number. Acute and short-term toxicity studies are too limited to draw conclusions on these endpoints. The genotoxic potential of beetroot red could not be evaluated based on the available data. There are only limited or inadequate studies available on the chronic toxicity and carcinogenicity of beetroot red and therefore the Panel could not conclude on these endpoints. No adequate studies on reproduction and developmental toxicity were available. The Panel concluded that the currently available toxicological database was inadequate to establish an acceptable daily intake (ADI) for beetroot red as defined by the specifications set for the food additive E 162. However, the colouring principles in E 162 are natural dietary constituents having a long history of food consumption. In addition, the betanin exposure resulting from the use of beetroot red (E 162) as food additive is in the same range as the exposure to the betanin from the regular diet. Therefore, the Panel concluded that, at the reported use levels, beetroot red (E 162) is not of safety concern as regards its current use as a food additive.

KEY WORDS

beetroot red, betanin, E 162, food colour

[1] On request from the European Commission, Question No EFSA-Q-2011-00350, adopted on 17 November 2015.

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SUMMARY

Following a request from the European Commission (EC), the Panel on Food Additives and Nutrient Sources added to Food (ANS) was asked to deliver a scientific opinion re-evaluating the safety of beetroot red (E 162) 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 has become available since then, and data available following a European Food Safety Authority (EFSA) public call for data.[4] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of beetroot red (E 162), which covered the period up to the end of 2014. A further update has been performed by the Panel.

Beetroot red (E 162) is a natural colour authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.[5] It has been previously evaluated by the Scientific Committee for Food (SCF) in 1975 and in relation to special medical purposes for young children in 1996 (SCF, 1975, 1997a). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated beetroot red in 1974, 1978, 1982 and 1987 (JECFA 1975, 1978, 1982, 1987, 1988). A previous JECFA temporary Acceptable Daily Intake (ADI) 'not specified' was withdrawn in 1982 due to the fact that information on metabolism and long-term toxicity that had been requested by JECFA was still not available. Neither the SCF nor JECFA have currently specified an ADI.

Beetroot red is a natural colour obtained from the roots of natural strains of red beets (*Beta vulgaris* L. var. *ruba*). The most commonly used synonyms for beetroot red in the published literature are Beet Red, Betanin, INS No. 162.

Beetroot red (E 162) contains a number of different pigments, all belonging to the general class known as betalains. The main colouring principle consists of a number of betacyanins (red), of which betanin accounts for 75–95% and isobetanin (epimer of betanin) 15–45%; a range of other betacyanins can also be detected, amounting up to 20% of the total content of betacyanins. Vulgaxanthin I (25–70%) and vulgaxanthin II (5–15%) are present in the betaxanthin (yellow) as well as several degradation products of betalains (light brown). Besides, the colour pigments beetroot red contains sugars, salts and/or proteins naturally occurring in red beets.

A number of different beetroot red products may be marketed, ranging from press juices or aqueous extracts of shredded roots to more concentrated or refined forms including pastes, powders and other solid forms. The Panel noted that the current re-evaluation does only refer to beetroot red prepared by pressing crushed beet as pressed juice or by aqueous extraction, in accordance with the definition of the Commission Regulation (EU) No 231/2012 and not to preparations manufactured by solvent extraction with methanol or ethanol.

Specifications for beetroot red have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). EC specifications including purity criteria for beetroot red define not less than 0.4% of the commercial material must be betanin pigment. The remaining 99.6% is accounted for by sugars, salts and proteins naturally occurring in red beets, and a small amount of other pigments belonging to the class of betalains and but this is not further specified. According to industry (NATCOL, 2012), non-colouring substances present in beetroot red are sugars, proteins, minerals, organic acids, vitamins, sterols, purines and phenolic compounds. The Panel noted that the reported content of betanin in beetroot to be around 0.4%. The Panel further noted that the specification for the content of

[4] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[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.

red colour (expressed as betanin) in beetroot red, as not less than 0.4%, may give rise to some confusion, given the number of different forms of beetroot red that may be on the market, including simple extracts, refined extracts and spray–dried powders. The Panel also noted that some forms of beetroot red designated as colouring foodstuffs rather than as food colours may contain more than 0.4% colouring matter. The Panel considered that revision of the current specification to reflect betanin content on a dried solids basis could be appropriate.

The specifications for beetroot red include a maximum level for nitrate of $\leq$ 2 g nitrate anion/g of red colour, given the relatively high content of nitrate in beetroot. The Panel considered a nitrate limit important in minimising the contribution to nitrate intake from this source as a food additive. This should be evaluated together with dietary exposure and use as a colouring food when evaluating the total intake of nitrate by the population.

There are no validated methods for the analysis of beetroot red (or betanin) in foods that may be used for official purposes.

The toxicological studies are described variously as using (1) red beet juice, (2) beetroot red, (3) betalains, (4) betanin, (5) beetroot red extracts, (6) freeze-dried beetroot, (7) beet red powder. All of these products inevitably contained betanin pigment. However, the Panel considered that only those (relatively few) studies carried out with a test material containing not less than 0.4% betanin are relevant for the assessment of the safety of the food additive beetroot red (E 162). It is unclear which of the above substances most closely resembles the commercial food colour and whether testing of purified betanin provides sufficient insight into the biological and toxicological behaviour of the commercial product.

The betacyanin pigments from beetroot red are metabolised or degraded *in vitro* by chopped tissue preparations from the stomach, small and large intestine, indicating that some breakdown of pigments is likely to occur in the gastrointestinal tract following oral administration of beetroot red. Studies in humans, supported by animal studies, have however shown that the betalain pigments present in beetroot are absorbed in an intact form to a limited extent (approximately 3% of the dose in rats and less than 1% of the dose in humans) after oral administration and are not metabolised further in the body, as demonstrated by the excretion of betanin, isobetanin and other betacyanin pigments at low levels in urine. Information from intravenous and intraperitoneal administration of beetroot extracts in rats demonstrated that intact pigments were extensively excreted unchanged in the urine. In humans, ingestion of beetroot can produce red urine ('beeturia') in some individuals. It has been suggested that beeturia is more a function of an individual's physiological constitution than a phenomenon under direct polymorphic genetic control.

The Panel noted that toxicological studies carried out on material conforming to the specifications for beetroot red are limited in number. However, there was no evidence of adverse effects in a range of studies conducted with poorly defined material and/or judged to be of limited relevance for the assessment of beetroot red (E 162).

The genotoxic potential of beetroot red (E 162) cannot be evaluated based on the available data.

There are only limited or inadequate studies available on the carcinogenicity of beetroot red and therefore the Panel could not conclude on the chronic toxicity and carcinogenicity of beetroot red.

No adequate studies on reproduction and developmental toxicity were available.

There is no indication of intolerance or allergenicity of beetroot red in the available literature.

Exposure assessments of food additives under re-evaluation are carried out by the ANS Panel based on (1) maximum permitted levels (MPLs) set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*). It was not possible to carry out a scenario based on the MPLs set out

in EU legislation, as, for all food categories, beetroot red (E 162) is authorised according to *quantum satis* (QS). However, maximum levels of the available data were used to provide a conservative estimate scenario (noted as the *maximum level exposure assessment* scenario). With regard to the refined exposure assessment scenario, only reported use levels were made available by industry. The Panel considers that the refined exposure assessment approach results in more realistic long-term exposure estimates because of the underlying assumptions and the concentration data used.

Reported use levels were all provided in mg betanin/kg food. Usages notes were also added by NATCOL (NATCOL, 2015) which could mention the percentages of food items per food category in which the food additive (E 162) or the colouring food (CFS) is used. These percentages were not taken into account as they should have been used to reduce the number of time the additive E 162 is used. This is not possible in the current modelling. Meanwhile, the reported use levels provided by NATCOL are the correct ones when the food additive E 162 is used. Therefore, the scenarios presented in the current opinion assume that irrespective of whether the food additive or colouring food is used, all the betanin is coming from the food additive E 162.

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of E 162.

Using the *maximum level exposure assessment scenario*, mean exposure to beetroot red (E 162) from its use as a food additive ranged from 0.1 mg/kg bw/day for the elderly to 2.1 mg/kg bw/day in toddlers, whereas the high exposure using this scenario ranged from 0.3 mg/kg bw/day for the elderly to 3.6 mg/kg bw/day in children. Using the *refined brand-loyal assessment exposure scenario*, mean exposure to beetroot red (E 162) from its use as a food additive ranged from 0.1 mg/kg bw/day in adults and the elderly to 1.6 mg/kg bw/day in toddlers. The high exposure to beetroot red (E 162) using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 2.8 mg/kg bw/day in toddlers. Using the *refined non-brand-loyal assessment exposure scenario*, mean exposure to beetroot red (E 162) from its use as food additive ranged from 0.05 mg/kg bw/day for the elderly to 1.0 mg/kg bw/day in toddlers. The high exposure to beetroot red (E 162) from its use as food additive using this scenario ranged from 0.1 mg/kg bw/day for the elderly to 1.8 mg/kg bw/day in infants. Overall, the lowest exposure to beetroot red (E 162) was estimated for the elderly, whereas the highest exposure to beetroot red (E 162) was calculated for toddlers in all scenarios. The food categories that, at the individual level, had the highest contribution to the total individual exposure to beetroot red (E 162) were breakfast cereals, fine bakery wares, soups and broths, and flavoured drinks.

Mean intakes of betanin from the regular diet for consumers only are in the range of the mean estimated exposure from the use of the food additive itself (Table 4, non-brand loyal consumer scenario).

The Panel concluded that the currently available toxicological database was inadequate to establish an ADI for beetroot red as defined by the specifications set for the food additive E 162. 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 colouring principles in E 162 are natural dietary constituents having a long history of food consumption. In addition, the betanin exposure resulting from the use of beetroot red (E 162) as food additives is in the same range as the exposure to the betanin from the regular diet. Therefore, the Panel concluded that, at the reported use levels, beetroot red (E 162) is not of safety concern as regards its current use as a food additive.

The Panel recommended that:

- revision of the current specification to reflect betanin content on a dried solids basis could be appropriate;

- limits for mycotoxin contamination may be relevant for the specifications of beetroot red (E 162);
- maximum limits for the toxic elements (arsenic, lead, mercury and cadmium) present as impurities and nitrates in the EC specification for beetroot red (E 162) should be revised in order to ensure that beetroot red (E 162) as a food additive will not be a significant source of exposure;
- EU Regulation should include the specification for solubility as given in the JECFA specification.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Regulation (EC) No 1333/2008[6] 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 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.[7] 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[8] of 2001. The report 'Food additives in Europe 2000[9]' 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 a highest priority.

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

[6] 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. 16–33.

[7] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme 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, 26.3.2010, p. 19–27.

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

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

ASSESSMENTS

1. INTRODUCTION

The present opinion deals with the re-evaluation of the safety of beetroot red (E 162) when used as a food additive.

Beetroot red (E 162) is a natural colour authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008.[10] It has previously been evaluated by the EU Scientific Committee for Food (SCF) in 1975 and in relation to food for special medical purposes for young children in 1996 (SCF, 1975, 1997a). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated beetroot red (E 162) in 1974, 1978, 1982 and 1987 (JECFA 1975, 1978, 1982, 1987, 1988). The Nordic Council of Ministers released a report that took into account the literature published on beetroot red up to the year 2000 (TemaNord, 2002).

The Panel on Food Additives and Nutrient Sources added to Food (ANS) was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then, and data available following European Food Safety Authority (EFSA) public call for data.[11] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation. To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of beetroot red (E 162) which covered the period up to the end of 2014. A further update has been performed by the Panel.

2. TECHNICAL DATA

2.1. Identity of the substance

Beetroot red (E 162) is obtained from the roots of strains of red beets (*Beta vulgaris* L. var. *ruba*) by pressing crushed beet as pressed juice or by aqueous extraction of shredded beetroots and subsequent enrichment in the active principle. Beetroot red (E 162) contains a number of pigments, belonging to the general class of the betalain. The main colouring principle consists of betacyanins (red), of which betanin accounts for 75–95%. Minor amounts of betaxanthin (yellow) and degradation products of betalains (light brown) may be present (Commission Regulation (EU) No 231/2012[12]).

According to industry (NATCOL, 2012), 'the typical colour content of beetroot red (expressed as betanin) available on the European market is 0.5–1.2% on the dry weight basis (red beet juices concentrates and powders)'.

The Panel noted that some aqueous beet extracts may be considered as colouring foodstuffs rather than as food colours and that this issue is currently a matter for discussion between the Commission and the Member States. From the information provided to the Commission, red beet (vegetable) contains 0.35–1.6% betacyanins (expressed on dry weight basis). The colouring food extract available on the market contains 0.5–2% of betacyanins. The content which would be permitted according to the Guidelines is still under discussion and would depend on the reference values for the pigment content in the source material.

The food additive preparation should contain at least 0.4% of betanin and in reality the content could go up to 10%, so there is a certain overlap with the colouring foods. However, in case of E 162 the

[10] 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.

[11] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

[12] 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, pp. 1–295.

product could be refined to remove most of the sugars, salts and proteins. The Panel agreed that this would be desirable.

Industry provided information on other betacyanins than betanin present in beetroot red (E 162): isobetanin (C-2 epimer of betanin, position C-2 being market with an 'S*' in the 2,3-dihydro-2,6-pyridinedicarboxylic acid moiety shown in Figure 1) (15–45%); 17-decarboxy-betanin (5–20%); 15-decarboxy-betanin (4–15%) and a range of other betacyanins can also be detected (NATCOL, 2012).

According to industry (NATCOL, 2012), vulgaxanthin I (25–70%) and vulgaxanthin II (5–15%) are present in the betaxanthin (yellow) as well as several degradation products of betalains (light brown). Some of these degradation products are *γ*-aminobutyric acid-bx, betalamic acid, brown compounds (including Maillard derivatives), decarboxylated betanin, *cyclo*-dopa, *cyclo*-dopa-5-O-glucoside, dopamine-bx (miraxanthine V), indicaxanthin (proline-bx), isoindicaxanthin (proline-isobx), 4-methylpyridine-2,6-dicarboxylic acid.

As a complex mixture, beetroot red (E 162) does not have a defined molecular weight. Betanin, the main colouring principle, has an empirical formula of $C_{24}H_{26}N_2O_{13}$ and a molecular weight of 550.48 g/mol. EINECS No. 231-628-5 and CAS Registry No. 7659-95-2.

The systematic name for betanin is:

- (*S*-(*R*',*R*')-4-(2-(2-carboxy-5-(beta-D-glucopyranosyloxy)-2,3-dihydro-6-hydroxy-1*H*-indol-1-yl)ethenyl)-2,3-dihydro-2,6-pyridinedicarboxylic acid.

The structural formula of betanin is shown in Figure 1.

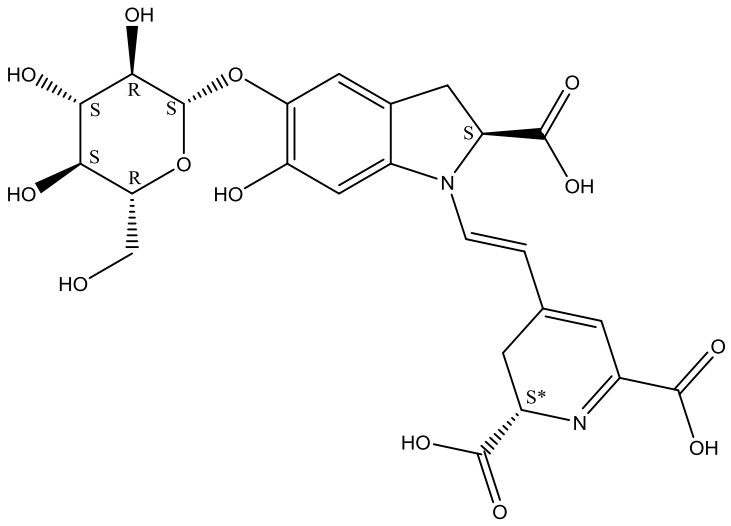
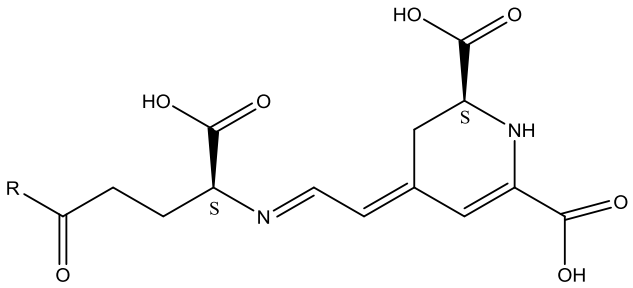


Figure 1: Structural formula of betanin

The structural formula of vulgaxanthins I and II is shown in Figure 2



R = NH_2 vulgaxanthin I
R = OH vulgaxanthin II

Figure 2: Structural formula of vulgaxanthin

Beetroot red appears as red or dark red liquid, paste, powder or solid. According to JECFA specifications beetroot red (E 162) is soluble in or miscible with water but insoluble in or immiscible with ethanol (JECFA, 2006). The Panel noted that the products soluble in ethanol described in the literature (Delgado-Vargas et al., 2000) do not comply with the EC definition and JECFA specifications.

The most commonly used synonyms for beetroot red are Beet Red, Betanin, INS No. 162.

According to Commission Regulation (EU) No 231/2012, besides the colour pigments, the juice or extract contains sugars, salts and/or proteins naturally occurring in red beets. The solution may be concentrated and some products may be refined in order to remove most of the sugar, salts and proteins.

According to industry (NATCOL, 2012), 'non-colouring substances present in beetroot red are sugars, proteins, minerals, organic acids, vitamins, sterols, purines and phenolic compounds. The main flavouring compounds in beetroot red are geosmin (trans-1,10-dimethyl-trans-(9)-decalol) responsible for the flavour of the red beet and 3-sec-butyl-2-methoxypyrazine in a small quantity responsible for the earthy note'. In addition to water, the typical average composition for non-fermented red beet was provided (Megard, 1993): carbohydrates (52%) [glucose (4%), fructose (10%), sucrose (38%)], proteins (8%), fat (0.05%), minerals (5.5%).

The Panel noted that beetroot contains relatively high levels of nitrate (2400 mg/kg, Hotchkiss et al., 1992) compared with a number of other vegetables, and that specifications for a maximum level of nitrate in beetroot red (E 162) have therefore been established (see section 2.2). According to industry (NATCOL, 2012), the nitrate content was < 1 mg/kg in the analysis of three beetroot red (E 162) batches.

2.2. Specifications

Specifications for beetroot red have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006) (Table 1).

Table 1: Specifications for assay and purity of beetroot red according to Commission Regulation (EU) No 231/2012 and JECFA (JECFA, 2006)

	Commission Regulation (EU) No 231/2012	**JECFA (2006)**
Assay	Content of red colour (expressed as betanin) is not less than 0.4%	Content of red colour (expressed as betanin) is not less than 0.4%
Purity		
Nitrate	≤ 2 g nitrate anion/g of red colour (as calculated from assay)	≤ 2 g nitrate anion/g of red colour (as calculated from assay)

	Commission Regulation (EU) No 231/2012	JECFA (2006)
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	–
Cadmium	≤ 1 mg/kg	–

According to industry (NATCOL, 2012), the content of betacyanins depends on the manufacturing process and 'the typical colour content of beetroot red (expressed as betanin) available on the European market is 0.5–1.2% on the dry weight basis (red beet juices concentrates and powders)'.

The Panel noted that some forms of beetroot red designated as colouring foodstuffs rather than as food colours may contain more than 0.4% colouring matter. According to industry (NATCOL, 2015), 'the current E 162 specification gives a minimum value of 0.4% which results in a range of concentrations of betanin being included. It does not allow the distinction between colouring food and colour additive. Indeed, 0.4% min. betanin is a very low level for this additive. Moreover, the specification does not specify if it is expressed on fresh or dry weight basis. Today the products that contain 0.4% to 1.5% on a dry weight basis can still be considered as colouring foods complying with the colouring foods guidance notes of the European Commission dated 29/11/2013'.

Because beetroot red is manufactured and sold in various forms, such as liquids, pastes or solids, the Panel considered that revision of the current specification to reflect betanin content on a dried solids basis could be more appropriate.

The Panel also noted the importance of maintaining the nitrate level in beetroot red to < 2 g nitrate anion/g of red colour, in order to minimise the contribution from this source to total intake of nitrate by the population. The Panel noted that beetroot red may potentially contain pesticide residues, but that this aspect is covered by Regulation (EC) No 396/2005.[13]

The Panel further noted that mycotoxins could be present in the material used for the production of beetroot red (E 162). The Panel considers that limits for mycotoxin contamination may be relevant for the specifications of beetroot red (E 162).

The Panel noted that, according to the EU specifications for beetroot red, impurities of the toxic elements arsenic, lead, mercury and cadmium are accepted up to a concentration of 3, 2, 1 and 1 mg/kg respectively. Contamination at these levels could have a significant impact on the exposure to these metals, for which the exposures are already close to the health-based guidance values established by EFSA (EFSA, 2009; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009, 2010, 2012). The Panel considered that the maximum limits for the toxic elements (arsenic, lead, mercury and cadmium) present as impurities in the EC specification for beetroot red (E 162) should be revised to ensure that beetroot red (E 162) as a food additive will not be a significant source of exposure to these toxic elements in foods.

The Panel noted that the EU Regulation differs from the JECFA specification in not including the specification for solubility.

2.3. Manufacturing process

According to industry (NATCOL, 2012), the most widespread product is red beet juice concentrate that is obtained from typical juice processes using mechanical extraction and physical processes. Natural strains of red beets (*Beta vulgaris* L. var *rubra*) are crushed and pressed. Subsequent concentration and/or other processes such as membranes filtration and clarification can be used, if

[13] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive No 91/414/EEC. OJ L 70, 16 March 2005.

necessary. The resulting product can be pulverised by an industrial drying process. Fibres and non-water soluble polysaccharides are removed from the product.

In the case of enrichment, the juice undergoes other processes such as chromatography or purification by membranes and the resulting product is enriched in colouring principle and in proteins whereas other constituents such as sugars (glucose, fructose, sucrose) are significantly reduced.

The traditional process to extract the pigments from red beets (Beta vulgaris L) is the use of water, however, food grade citric acid can be used for the purpose of extraction of the pigments as a processing aid (NATCOL, 2012). According to Delgado-Vargas et al. (2000), methanol or ethanol solutions can be used to complete the extraction. According to NATCOL (2012), 'at the present time, these solvents are only used on a laboratory scale. However, products obtained by solvent extraction exist on the market but currently in limited volumes'.

Food grade acids (e.g. citric, lactic, L-ascorbic) may be added as pH controlling agents and stabilisers and carriers (e.g. maltodextrin) may be added as aids for manufacturing dry powders (NATCOL, 2007).

2.4. Methods of analysis in food

No method has been reported specifically for the analysis of beetroot red (or betanin) in food.

A method for the electrophoretic separation or the quantitative analysis of red beet pigments (betacyanins) was described by Powrie and Fennema (1963) and by Von Elbe et al. (1972).

Schwartz and Von Elbe (1980) described a method for the quantitative determination of individual betacyanins by high performance liquid chromatography.

Saguy et al. (1978) described a computer-aided method for determining all major beet pigments (betanin, vulgaxanthin I, betalamic acid) and browning substances, from the visible spectrum of the mixture.

Cohen and Saguy (1982) described a method for the determination of betanin and vulgaxanthin I in beet powder using a tristimulus colorimeter.

Methods for analysis of the main betacyanins, betanin and isobetanin and the corresponding aglycones from extracts of *Beta vulgaris* were described, using capillary zone electrophoresis (CZE) (Stuppner and Egger, 1996) or high performance liquid chromatography (HPLC) (Pourrat et al., 1988).

There are no validated methods for the analysis of beetroot red (or betanin) in foods that might be used for official purposes.

2.5. Reaction and fate in food

In beetroot red, the betacyanins exhibit only fair stability to heat and light and therefore they are principally recommended for use in minimally processed packaged products (Emerton, 2008). Betacyanins are stable under a wide range of pH and food-processing conditions, but prone to oxidation, thus heating in the presence of air at neutral pH causes irreversible breakdown to brown products (Scotter and Castle, 2004). The betanin content of beetroot extracts undergoes progressive degradation which is increased by higher pH, temperatures and water activity; consequently storage conditions will affect the colour in commercial products (JECFA, 2006). Betanin may be enzymatically hydrolysed to the corresponding aglycone betanidine and glucose. In the presence of acids, betanin is transformed into its isomer, isobetanin, and further to yellow betalamic acid products, containing an open ring system, and finally to brown products. In alkaline medium, betanin is transformed into a red-violet pigment which decomposes into colourless products (Scotter and Castle, 2004).

According to industry (NATCOL, 2012), degradation of the pigments may occur by heat, acid, base, β-glucosidase and oxidation. Light brown degradation products of betalains are formed during further degradation, including Maillard reaction, which leads to a complex non-defined mixture of brown degradation products. Spray-dried beetroot red contains more pigment degradation products due to the increased temperature applied during the concentration process than the freeze- and air-dried products. The increased temperature as well as acidic condition during the manufacturing process leads to isomerisation, dehydrogenation and decarboxylation resulting in an increase of degradation products. When fermentation or acid is used during the manufacturing process, the content of betanin decreases due to the break of the glycosidic bond.

2.6. Case of need and proposed uses

Maximum levels of beetroot red (E 162) have been defined in Annex II to Regulation (EC) No 1333/2008[14] on food additives. These levels are referred by the Panel as Maximum Permitted Levels (MPLs) in this document.

Currently, beetroot red (E 162) is authorised as a food additive in the EU at *quantum satis* (QS) in the categories as listed in table 2 apart from breakfast cereals. Beetroot red (E 162) is included in the Group II of food colours authorised at QS.

Table 2 summarises foods that are permitted to contain beetroot red (E 162) and the corresponding maximum levels as set by Annex II to Regulation (EC) No 1333/2008.

Table 2: Maximum levels of beetroot red (E 162) in foods according to the Annex II to Regulation (EC) No 1333/2008

FCS category number (a)	FCS Food category	E-Number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
01.4	Flavoured fermented milk products including heat-treated products	Group II		Quantum satis
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Group II	Except unflavoured products	Quantum satis
01.6.3	Other creams	Group II	Only flavoured creams	Quantum satis
01.7.1	Unripened cheese excluding products falling in category 16	Group II	Only flavoured unripened cheese	Quantum satis
01.7.3	Edible cheese rind	Group II		Quantum satis
01.7.4	Whey cheese	Group II		Quantum satis
01.7.5	Processed cheese	Group II	Only flavoured processed cheese	Quantum satis
01.7.6	Cheese products (excluding products falling in category 16)	Group II	Only flavoured unripened products	Quantum satis
01.8	Dairy analogues, including beverage whiteners	Group II		Quantum satis
03	Edible ices	Group II		Quantum satis
04.2.1	Dried fruit and vegetables	E 162	Only preserves of red fruit	Quantum satis
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 162	Only preserves of red fruit	Quantum satis
04.2.2	Fruit and vegetables in vinegar, oil or brine	E 162	Only vegetables (excluding olives)	Quantum satis
04.2.3	Canned or bottled fruit and vegetables	E 162	Only vegetables (excluding olives)	Quantum satis

[14] 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.

FCS category number (a)	FCS Food category	E-Number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
04.2.4.1	Fruit and vegetable preparations excluding compote	Group II	Only *mostarda di frutta*	Quantum satis
04.2.4.1	Fruit and vegetable preparations excluding compote	E 162	Only vegetables (excluding olives)	Quantum satis
04.2.4.1	Fruit and vegetable preparations excluding compote	E 162	Only seaweed based fish roe analogues	Quantum satis
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	E 162	Except chestnut puree	Quantum satis
04.2.5.3	Other similar fruit or vegetable spreads	Group II	Except crème de pruneaux	
05.2	Other confectionery including breath refreshening microsweets	Group II		Quantum satis
05.3	Chewing gum	Group II		Quantum satis
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4	Group II		Quantum satis
06.3	Breakfast cereals	Group II	Only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals	Quantum satis
06.3	Breakfast cereals	E 162	Only fruit flavoured breakfast cereals	200
06.5	Noodles	Group II		Quantum satis
06.6	Batters	Group II		Quantum satis
06.7	Pre-cooked or processed cereals	Group II		Quantum satis
07.2	Fine bakery wares	Group II		Quantum satis
08.2	Meat preparations as defined by Regulation (EC) No 853/2004	E 162	Only merguez type products, salsicha fresca, butifarra fresca, longaniza fresca and chorizo fresco	Quantum satis
08.3.1	Non-heat-treated meat products	E 162	Only sausages	Quantum satis
08.3.2	Heat-treated meat products	E 162	Only sausages, patés and terrines	Quantum satis
08.3.3	Casings and coatings and decorations for meat	Group II	Except edible external coating of *pasturmas*	Quantum satis
09.2	Processed fish and fishery products including molluscs and crustaceans	Group II	Only surimi and similar products and salmon substitutes.	Quantum satis
09.2	Processed fish and fishery products including molluscs and crustaceans	E 162	Only precooked crustacean	Quantum satis
09.2	Processed fish and fishery products including molluscs and crustaceans	E 162	Only fish paste and crustacean paste	Quantum satis
09.3	Fish roe	Group II	Except Sturgeons' eggs (Caviar)	Quantum satis
12.2.2	Seasonings and condiments	Group II	Only seasonings, for example curry powder, tandoori	Quantum satis
12.4	Mustard	Group II		Quantum satis
12.5	Soups and broths	Group II		Quantum satis

FCS category number (a)	FCS Food category	E-Number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
12.6	Sauces	Group II	Excluding tomato-based sauces	Quantum satis
12.7	Salads and savoury based sandwich spreads	Group II		Quantum satis
12.9	Protein products, excluding products covered in category 1.8	Group II		Quantum satis
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)	Group II		Quantum satis
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)	Group II		Quantum satis
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009	Group II		Quantum satis
14.1.4	Flavoured drinks	Group II	Excluding chocolate milk and malt products	Quantum satis
14.2.3	Cider and perry	Group II	Excluding *cidre bouché*	Quantum satis
14.2.4	Fruit wine and made wine	Group II	Excluding *wino owocowe markowe*	Quantum satis
14.2.5	Mead	Group II		Quantum satis
14.2.6	Spirit drinks as defined in Regulation (EC) No 110/2008	Group II	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, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	Quantum satis
14.2.7.3	Aromatised wine-product cocktails	Group II		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	Group II		Quantum satis
15.1	Potato-, cereal-, flour- or starch-based snacks	Group II		Quantum satis
15.2	Processed nuts	Group II		Quantum satis
16	Desserts excluding products covered in categories 1, 3 and 4	Group II		Quantum satis
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms	Group II		Quantum satis
17.2	Food supplements supplied in a liquid form	Group II		Quantum satis

FCS category number (a)	FCS Food category	E-Number/ Group	Restrictions/exceptions	Maximum level (mg/l or mg/kg as appropriate)
17.3	Food supplements supplied in a syrup-type or chewable form	Group II		Quantum satis

(a): FCS, Food Categorisation System (food nomenclature) presented in the Annex II to Regulation (EC) No 1333/2008.

2.7. Reported use levels or data on analytical levels of beetroot red (E 162) in food

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. Therefore, information on actual use levels is required for performing a more realistic exposure assessment, especially for those food additives for which no MPL is set and which are authorised according to QS.

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010[15] regarding the re-evaluation of approved food additives, EFSA issued a public call[16] for scientific data on beetroot red (E 162).

In response to this public call, updated information on the actual use levels of beetroot red (E 162) in foods was made available to EFSA by industry. No analytical data on the concentration of beetroot red (E 162) in foods were made available by the Member States. According to the Regulation (EC) No 1333/2008, the maximum levels for colours set out in Annex II shall apply to the quantities of colouring principle contained in the colouring preparation unless otherwise stated.

2.7.1. Summarised data on reported use levels in foods provided by industry

Industry provided EFSA with data on use levels of beetroot red (E 162) in foods for 53 out of the 58 food categories in which beetroot red (E 162) is authorised.

Updated information on the actual use levels of beetroot red (E 162) in foods was made available to EFSA by FoodDrinkEurope (FDE, formerly CIAA) and Natural Food Colour Association (NATCOL).

A report was joined to the usage levels provided by NATCOL (2015). This report mentioned that 'beetroot pigments can be added to food as a food colour additive comprised of beetroot extracts (E 162) or as beetroot juice used as a colouring food (CFS)'. In both case, levels were expressed as mg betanin/kg food. Besides the levels provided per food categories, usages notes were added by NATCOL. These notes could mention the percentages of food items per food category in which the food additive (E 162) or the colouring food (CFS) is used. These percentages were not taken into account as they should have been used to reduce the number of time the additive E 162 is used. This is not possible in the current modelling. Meanwhile, the reported use levels provided by NATCOL are the correct ones when the food additive E 162 is used. Therefore, the scenarios presented in the current opinion assume that irrespective of whether the food additive or colouring food is used, all the betanin is coming from the food additive E 162. For some food categories, NATCOL stated that use is very limited or that there is currently no usage for the food category. When the latter was specified, the food category was not taken into account in the present exposure assessment.

Appendix A provides data on the use levels of beetroot red (E 162) in foods as reported by industry. Usage notes provided by NATCOL are also reported as received.

[15] Commission Regulation (EU) No 257/2010 of 25 March 2010 setting up a programme 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, 26.3.2010, p. 19.

[16] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 7 December 2006. Available online: http://www.efsa.europa.eu/en/dataclosed/call/afc061208

2.8. Information on existing authorisations and evaluations

Beetroot red (E 162) is authorised as a food additive in the EU under Annex II of Regulation 1333/2008 food additives for use in foodstuffs.

Beetroot red was evaluated by the SCF in 1975 and in relation to special medical purposes for young children in 1996 (SCF, 1975, 1997a), as well as by JECFA in 1974, 1978, 1982 and 1987 (JECFA 1975, 1978, 1982, 1987, 1988). At the 26th JECFA Meeting in 1982, the temporary ADI 'not specified' was withdrawn due to the fact that information on metabolism and long-term toxicity that had been requested at its 18th and 22nd meetings was still not available (JECFA, 1974 and 1978). In 1988, the JECFA report concluded that 'when the concentrate is used to enhance the colour of beet products, it could be considered as food. If, on the other hand, the concentrate is used more generally as a colourant, careful specifications need to be established. Because nitrate is a component of beet red, it is necessary to ensure that levels of nitrate do not exceed the specifications. Under these conditions beet red could be used according to good manufacturing practice with an ADI 'not specified', keeping in mind the need to limit the nitrate content of foods produced for infants and young children'. JECFA also mentioned 'Previous Committees had considered beet red together with its major colour component, betanin. This Committee decided that it would be appropriate to evaluate these food colours separately and pointed out that, for the compound betanin, insufficient data were available to establish an ADI, since the information available to the Committee did not meet currently accepted standards' (JECFA, 1988).

Neither the SCF nor JECFA have currently specified a numerical ADI.

An additional evaluation can be found in a report released by the Nordic Council of Ministers (TemaNord, 2002) who have taken into account the literature published until 2000.

Beet juice (as vegetable juice) and beet powder (dehydrated beets) are exempt from certification and permanently listed for food use in the USA. Vegetable juice 'may be safely used for the colouring of foods generally, in amounts consistent with good manufacturing practice, except that it may not be used to colour foods for which standards of identity have been promulgated under section 401 of the act, unless the use of added colour is authorised by such standards' (FDA, 2009).

2.9. Exposure assessment

2.9.1. Food consumption data used for exposure assessment

2.9.1.1. EFSA Comprehensive European Food Consumption Database

Since 2010, the EFSA Comprehensive European Food Consumption Database (Comprehensive Database) has been populated with national data on food consumption at a detailed level. Competent authorities in the European countries provide 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 on the 'Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment' (EFSA, 2011a). New consumption surveys recently[17] added in the Comprehensive database were also taken into account in this assessment.[18]

The food consumption data gathered by EFSA were collected by different methodologies and thus direct country-to-country comparisons should be interpreted with caution. Depending on the food category and the level of detail used for exposure calculations, uncertainties could be introduced owing to possible under-reporting by subjects and/or misreporting of the consumption amounts. Nevertheless, the EFSA Comprehensive Database represents the best available source of food consumption data across Europe at present.

[17] http://www.efsa.europa.eu/en/press/news/150428.htm
[18] Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm

Food consumption data from the following population groups: infants, toddlers, children, adolescents, adults and the elderly were used for the exposure assessment. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Table 3: Population groups considered for the exposure estimates of beetroot red (E 162)

Population	Age range	Countries with food consumption surveys covering more than one day
Infants	From 4 months up to and including 11 months of age	Bulgaria, Denmark, Finland, Germany, Italy, UK
Toddlers	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children [(a)]	From 36 months up to and including 9 years of age	Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Latvia, Netherlands, Spain, Sweden, UK
Adolescents	From 10 years up to and including 17 years of age	Austria, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Italy, Latvia, Spain, Sweden, UK
Adults	From 18 years up to and including 64 years of age	Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Netherlands, Romania, Spain, Sweden, UK
The elderly [(a)]	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Sweden, UK

(a): The terms 'children' and 'the elderly' correspond, respectively, to 'other children' and the merge 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, 2011a).

Consumption records were codified according to the FoodEx classification system (EFSA, 2011b). Nomenclature from the FoodEx classification system has been linked to the Food Classification System (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates.

2.9.1.2. Food categories selected for the exposure assessment of beetroot red (E 162)

The food categories in which the use of beetroot red (E 162) is authorised were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system food codes), at the most detailed level possible (up to FoodEx level 4) (EFSA, 2011b).

Some food categories are not referenced in the EFSA Comprehensive Database, therefore no consumption data are available for them. They were not taken into account in the present estimate. This may have resulted in an underestimation of the exposure. The food categories that were not taken into account are described below (in ascending order of the FCS code):

- 01.6.3. Other creams, only flavoured creams
- 01.7.3. Edible cheese rind
- 01.7.6. Cheese products (excluding products falling in category 16)
- 04.2.3. Canned or bottled fruit and vegetables, only vegetables (excluding olives): no information available on the packaging in FoodEx
- 04.2.4.1. Fruit and vegetable preparations excluding compote, only *mostarda di frutta*
- 04.2.4.1. Fruit and vegetable preparations excluding compote, only seaweed-based fish roe analogues

- 05.4. Decorations, coatings and fillings, except fruit-based fillings covered by category 04.2.4
- 06.6. Batters
- 06.7. Pre-cooked or processed cereals
- 08.3.3. Casings and coatings and decorations for meat
- 14.2.4. Fruit wine and made wine
- 14.2.5. Mead
- 14.2.7.3. Aromatised wine-product cocktails

For the following food categories, the restrictions which apply to the use of beetroot red (E 162), could not be taken into account, and therefore the whole food category was considered for the exposure estimates. This results in an overestimation of the exposure:

- 01.5. Dehydrated milk as defined by Directive 2001/114/EC, except unflavoured products
- 01.7.1. Unripened cheese excluding products falling in category 16, only flavoured unripened cheese
- 01.7.5. Processed cheese, only flavoured processed cheese
- 04.2.5.2. Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC, except chestnut purée
- 04.2.5.3. Other similar fruit or vegetable spreads, except crème de pruneaux
- 9.3. Fish roe, except sturgeons' eggs (caviar)
- 14.2.3. Cider and perry, excluding *cidre bouché*
- 17.1./17.2./17.3. Food supplements: it was not possible to differentiate solid, liquid or syrup-type, or chewable forms of food supplements within FoodEx codes therefore all were assigned the maximum level provided for one of the three food categories.

Overall, 13 food categories were not taken into account in the exposure assessment because they are not referenced in the EFSA Comprehensive Database, therefore no consumption data are available. 10 food categories were included in the exposure assessment without considering the restrictions as set in Annex II to Regulation No 1333/2008. For eight food categories, no usage data were provided to EFSA or industry reported 'no current usage' of the food additive, therefore they were not included in the exposure estimates. In the current exposure estimates, 20 food categories out of 58 are not taken into account for one or both reason described above (no FoodEx code, no reported use levels) (see Appendix B).

2.9.2. Exposure to beetroot red (E 162) from its use as a food additive

The Panel estimated chronic exposure to beetroot red (E 162) expressed as betanin. Dietary exposure was calculated by multiplying beetroot red (E 162) concentrations reported in Appendix B for each food category with their respective consumption amount per kilogram of body weight for each individual in the Comprehensive Database. The exposure per food category was subsequently added to derive an individual total exposure per day. These exposure estimates were averaged over the number of survey days, resulting in an individual average exposure per day for the survey period. Surveys with only one day per subject were excluded as considered as not adequate to assess chronic dietary exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual average exposure per survey and population group. Based on these distributions, the mean and 95th percentile of exposures were calculated per survey for the total population and per

population group. High percentile exposure was only calculated for those population groups where the sample size was sufficiently large to allow calculation of the 95th percentile of exposure (EFSA, 2011a). Therefore, in this assessment, high levels of exposure for toddlers from Belgium, Italy and Spain were not included. Thus, for this assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 3).

Two exposure scenarios are defined and carried out by the ANS Panel regarding the concentration data of food additives used: (1) maximum levels provided to EFSA (defined as the *maximum level exposure assessment scenario*); (2) the reported use levels (defined as the *refined exposure assessment scenario*). These two scenarios are discussed in detail below.

2.9.2.1. Maximum level exposure assessment scenario

The regulatory maximum level exposure assessment scenario is based on the MPLs as set in Annex II to Regulation (EC) No 1333/2008. As beetroot red (E 162) are authorised according to QS in almost all food categories, a 'maximum level exposure assessment' scenario was estimated based on the maximum reported use levels as provided by industry, as described in the EFSA Conceptual framework (EFSA ANS Panel, 2014).

The exposure estimates derived following this scenario should be considered as the most conservative, as this scenario assumes that a consumer will be continuously (over a life-time) exposed to beetroot red (E 162) present in food at the maximum reported use levels.

2.9.2.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on use levels reported by industry. This exposure scenario can consider only food categories where these data were available to the Panel.

Appendix B summarises the concentration levels of beetroot red (E 162) used in the refined exposure assessment scenario. Based on the available dataset, the Panel calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It is assumed that a consumer is exposed long-term to the beetroot red (E 162) present at the maximum reported use for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum reported use for the main contributing food category at the individual level.
  - Using the mean of the typical reported use for the remaining food categories.
- The non-brand-loyal consumer scenario: It is assumed that a consumer is exposed long-term to beetroot red (E 162) present at the mean reported use in food. This exposure estimate is calculated using the mean of the typical reported use levels for all food categories.

2.9.2.3. Anticipated exposure to beetroot red (E 162)

Table 4 summarises the estimated exposure to beetroot red (E 162) from their use as food additives in five population groups (Table 3) according to the different exposure scenario's (section 2.9.2.2). Detailed results per population group and survey are presented in Appendix C.

Table 4: Summary of anticipated exposure to beetroot red (E 162) from their use as food additives in the maximum level exposure assessment scenario and in the refined exposure scenarios, in five population groups (minimum–maximum across the dietary surveys in mg betanin/kg bw/day)

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Maximum level exposure assessment scenario						
• Mean	0.2–0.6	0.6–2.1	0.6–1.8	0.3–0.9	0.2–0.5	0.1–0.6
• High level (95th percentile)	0.7–2.8	1.8–3.5	1.3–3.6	0.6–1.8	0.4–1.3	0.3–1.4
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.2–0.5	0.5–1.6	0.5–1.3	0.2–0.6	0.1–0.4	0.1–0.5
• High level (95th percentile)	0.7–2.2	1.4–2.8	1.0–2.6	0.5–1.3	0.3–1.0	0.2–1.2
Non-brand-loyal scenario						
• Mean	0.1–0.4	0.3–1.0	0.2–0.9	0.1–0.4	0.1–0.3	0.05–0.3
• High level (95th percentile)	0.3–1.8	0.7–1.7	0.5–1.7	0.3–0.9	0.2–0.8	0.1–0.9

2.9.3. Main food categories contributing to exposure to beetroot red (E 162) using the maximum level exposure assessment scenario

Table 5: Main food categories contributing to exposure to beetroot red (E 162) using maximum usage levels (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [a]					
01.4	Flavoured fermented milk products including heat-treated products	6.3–24.2 (5)	8.9–38.6 (9)	5.9–31.9 (14)	5.8–26.3 (9)	5.6–12.4 (13)	6.8–10.4 (10)
01.5	Dehydrated milk as defined by Directive 2001/114/EC	9.6 (1)		21.7 (1)			
01.7.1	Unripened cheese excluding products falling in category 16	14.1 (1)	7.4 (1)	6.1 (1)	7.6 (1)	11.3 (1)	11.0 (1)
01.7.5	Processed cheese	8.3 (1)	5.5 (1)				
03	Edible ices	–	5.5 (1)	5.1–9.3 (8)	5.2–9.5 (5)	5.9–7.0 (2)	6.0 (1)
04.2	Processed fruit and vegetables	18.6 (1)					5.7–9.1 (3)
05.2	Other confectionery including breath freshening microsweets	–		13.6 (1)	6.1–23.4 (2)	5.3 (1)	

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
06.3	Breakfast cereals	32.2–70.0 (4)	9.1–52.7 (6)	5.0–20.5 (14)	5.9–25.0 (14)	5.5–39.6 (9)	5.4–59.8 (9)
06.5	Noodles	–			6.7 (1)		
07.2	Fine bakery wares	8.1–75.2 (3)	5.4–62.0 (9)	5.8–57.8 (17)	5.2–46.5 (16)	8.6–43.4 (17)	8.9–47.4 (14)
08.3	Meat products	8.4 (1)	5.2–12.2 (4)	5.3–9.6 (10)	5.7–8.7 (10)	5.0–25.3 (11)	5.6–23.9 (5)
09.2	Processed fish and fishery products including molluscs and crustaceans		5.1 (1)	5.1–8.3 (2)			
12.5	Soups and broths	5.1–77.3 (2)	5.2–17.6 (4)	6.8–28.6 (5)	5.9–27.7 (6)	7.3–36.9 (8)	9.5–37.8 (8)
14.1.4	Flavoured drinks	13.0 (1)	6.8–24.1 (7)	5.5–30.3 (17)	8.4–42.6 (17)	5.1–34.5 (16)	6.1–24.5 (7)
15.1	Potato–, cereal–, flour– or starch–based snacks	6.8–8.4 (2)	5.3–11.3 (4)	5.3–10.3 (8)	5.8–13.6 (10)	5.2–14.5 (5)	
15.2	Processed nuts					7.1 (1)	6.3 (1)
16	Desserts excluding products covered in categories 1, 3 and 4	9.0 (1)	5.5–11.6 (5)	5.1–9.3 (4)	5.6–7.0 (2)	5.9–6.1 (2)	6.7–9.3 (3)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

2.9.4. Main food categories contributing to exposure to beetroot red (E 162) using the refined exposure assessment scenario

Table 6: Main food categories contributing to exposure to beetroot red (E 162) using the brand–loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
01.4	Flavoured fermented milk products including heat-treated products	5.4–-25.1 (5)	7.7–43.8 (9)	6.9–41.0 (11)	8.5–29.0 (6)	5.1–11.1 (10)	5.2–11.8 (9)
01.5	Dehydrated milk as defined by Directive 2001/114/EC	6.1 (1)	–	22.3 (1)			
01.7.1	Unripened cheese excluding products falling in category 16	13.4 (1)	6.5 (1)	–	6.3 (1)	9.7 (1)	9.6 (1)
01.7.5	Processed cheese	8.3 (1)	5.4–7.2 (2)	–	–		
03	Edible ices	–	–	5.5–6.5 (3)	5.2–8.0 (2)	5.5 (1)	
04.2	Processed fruit and vegetables	16.7 (1)	–				6.9 (1)

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
05.2	Other confectionery including breath freshening microsweets	–	–	13.7 (1)	5.3–26.7 (2)		
06.3	Breakfast cereals	37.1–75.4 (4)	5.2–58.5 (7)	5.4–25.8 (14)	6.7–29.1 (14)	5.4–49.7 (11)	5.6–71.6 (9)
06.5	Noodles	–	–	5.2 (1)	8.6 (1)	–	
07.2	Fine bakery wares	5.7–79.0 (3)	9.3–70.9 (8)	13.1–69.7 (16)	18.2–58.0 (15)	6.5–53.6 (17)	5.6–53.7 (14)
08.3	Processed meat	–	6.9 (1)	5.3–5.6 (2)	5.2–5.4 (2)	7.5–22.9 (3)	6.1–20.7 (3)
09.2	Processed fish and fishery products including molluscs and crustaceans	–	5.1 (1)	8.7 (1)	–	–	–
12.5	Soups and broths	6.2–77.3 (2)	5.9–24.2 (5)	5.4–36.6 (8)	5.6–36.4 (7)	9.6–45.6 (8)	12.0–47.9 (8)
12.9	Protein products, excluding products covered in category 1.8	–	–	–	–		5.1 (1)
14.1.4	Flavoured drinks	10.8 (1)	5.7–27.7 (6)	5.2–36.4 (14)	5.4–53.3 (17)	6.1–42.2 (15)	5.5–24.4 (5)
15.1	Potato-, cereal-, flour- or starch-based snacks	6.4–7.3 (2)	6.1–9.9 (3)	5.2–11.0 (6)	5.1–12.6 (9)	5.5–16.6 (3)	5.0 (1)
15.2	Processed nuts	–	–		–	8.2 (1)	7.1 (1)
16	Desserts excluding products covered in categories 1, 3 and 4	7.6 (1)	5.4–10 (3)	5.5–7.2 (2)	–		5.9–7.3 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

Table 7: Main food categories contributing to exposure to beetroot red (E 162) using the non-brand-loyal refined exposure scenario (> 5% to the total mean exposure) and number of surveys in which each food category is contributing

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
01.4	Flavoured fermented milk products including heat-treated products	7.0–14.4 (3)	5.5–29.8 (9)	6.6–26.6 (11)	6.4–21.0 (6)	5.0–10.3 (9)	5.2–7.2 (5)
01.5	Dehydrated milk as defined by Directive 2001/114/EC	8.0 (1)		25.5 (1)	–		
01.7.1	Unripened cheese excluding products falling in category 16	13.9 (1)	7.5 (1)	8.4 (1)	11.0 (1)	15.5 (1)	5.7–14.2 (3)
01.7.5	Processed cheese	9.1 (1)	10.0–12.7 (2)	–	–	–	6.0 (1)

FCS category number	FCS Food category	Infants	Toddlers	Children	Adolescents	Adults	The elderly
		Range of % contribution to the total exposure (number of surveys) [(a)]					
03	Edible ices	–		5.2–7.6 (5)	7.9 (1)	5.6 (1)	–
04.2	Processed fruit and vegetables	13.8 (1)	5.2 (1)			–	5.1–8.7 (3)
05.2	Other confectionery including breath freshening microsweets			11.9 (1)	6.0–22.4 (2)	–	
06.3	Breakfast cereals	41.9–76.7 (4)	5.8–66.9 (8)	5.5–32.2 (17)	5.5–39.7 (17)	5.0–54.1 (17)	5.3–73.8 (11)
06.5	Noodles	–	–	5.7 (1)	10.3 (1)		
07.2	Fine bakery wares	7.9–69.8 (2)	8.4–56.5 (8)	11.9–53.2 (16)	13.3–38.7 (15)	5.1–36.0 (17)	9.2–35.8 (13)
08.3	Processed meat	–	7.4 (1)	5.9–6.7 (4)	5.1–6.8 (5)	5.8–20.7 (5)	6.7–19.7 (2)
09.2	Processed fish and fishery products including molluscs and crustaceans	–	5.4–6.4 (3)	8.0–13.3 (2)	5.7–5.8 (3)	–	–
12.5	Soups and broths	8.8–85.0 (2)	9.2–36.1 (5)	5.3–47.6 (10)	5.0–47.4 (10)	7.9–57.6 (9)	6.7–58.4 (9)
12.6	Sauces	–	–		5.1–6.0 (4)	5.0–5.9 (4)	5.7 (1)
12.9	Protein products, excluding products covered in category 1.8	–	–	–	–		7.7 (1)
14.1.4	Flavoured drinks	7.6 (1)	5.2–18.2 (6)	5.4–23.7 (14)	8.6–32.3 (16)	6.5–27.1 (14)	5.5–20.7 (4)
15.1	Potato-, cereal-, flour- or starch-based snacks	5.9–11.6 (2)	7.4–15.4 (3)	5.1–11.0 (11)	5.0–14.5 (11)	5.0–12.8 (6)	
15.2	Processed nuts	–	–	–	5.3 (1)	5.1–13.4 (5)	5.6–12.0 (2)
16	Desserts excluding products covered in categories 1, 3 and 4	5.2 (1)	8.4–9.5 (2)	5.8–7.7 (3)	5.5 (1)	–	5.4–5.9 (2)

(a): The total number of surveys may be greater than the total number of countries as listed in Table 3, as some countries submitted more than one survey for a specific population.

2.9.5. Uncertainty analysis

Uncertainties in the exposure assessment of beetroot red (E 162) have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2006), the following sources of uncertainties have been considered and summarised in Table 8.

Table 8: Qualitative evaluation of influence of uncertainties on the dietary exposure estimate

Sources of uncertainties	Direction [(a)]
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/–
Use of data from food consumption survey of a few days to estimate long-term (chronic) exposure for high percentiles (95th percentile)	+
Correspondence of reported use levels to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/–
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n=11)	–
Food categories included in the exposure assessment: concentration data not available for certain food categories which could not be included in the exposure estimates (n=5)	–
Reported use levels: - levels considered applicable for all items within the entire food category, - levels (in mg betanin/kg food) considered to come from the food colour E 162	 + +
Maximum exposure assessment scenario: food categories authorised at the maximum reported use levels	+
Refined exposure assessment scenarios: exposure calculations based on the maximum or mean levels (reported use from industries)	+/–
Uncertainty in possible national differences in use levels of food categories	+/–

(a): +, uncertainty with potential to cause over-estimation of exposure; –, uncertainty with potential to cause underestimation of exposure.

Overall, the Panel considered that the uncertainties identified would result in an over-estimation of the real exposure to beetroot red (E 162) as a food additive in European countries.

2.9.6. Exposure via the regular diet

According to Spórna-Kucab (2015) and Attia-Gamila et al. (2013), the content of betanin of fresh red beet juice was 370 mg/100 g and 380 mg/100 g respectively. According to Sturzoiu (2011), 'Betanin content in beetroot varies from 100 mg/100 g fresh product to 16–38 mg/100 g dried vegetable product'. No intake was estimated from dried vegetable products as the food item is not available in the EFSA Comprehensive Database.

Table 9 describes the consumption of beetroot (in g/person per day) from both the vegetable and the juice (all population and consumers only) and the corresponding intake of betanin (in mg/kg bw/day) using the concentration factor presented above.

Table 9: Consumption of beetroot (vegetable and juice) (all population, consumers only) and resulting consumption of betanin in the five population groups

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Consumption of beetroot (vegetables and juice) (g/person per day)						
• All population						
○ Mean	0.06	0.4	0.8	1.5	2.2	2.4
○ p95	0.0[(a)]	0.8	0.0[(a)]	0.0[(a)]	4.9	8.2
• Consumers only						
○ Mean	2.4	6.5	17.8	33.2	28.9	28.2
○ p95	7.1	27.8	100.0	187.5	150.0	120.0
○ % consumers	2.7	5.8	4.6	4.6	7.7	8.5

	Infants (4–11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Consumption of betanins (mg/kg bw/day)						
• All population						
○ Mean	0.01	0.03	0.04	0.03	0.03	0.04
○ p95	0.00[(a)]	0.06	0.00[(a)]	0.00[(a)]	0.07	0.11
• Consumers only						
○ Mean	0.3	0.5	0.8	0.7	0.4	0.4
○ p95	0.7	2.5	4.1	3.6	2.2	1.8

(a): Less than 5% of the population reported consumption of the foods in which beetroot is naturally occurring, resulting in an exposure level of 0 at the 95th percentile in some surveys.

Mean intakes of betanin from the regular diet for consumers only are in the range of the mean estimated exposure from the use of the food additive itself (Table 4, non-brand loyal consumer scenario).

3. BIOLOGICAL AND TOXICOLOGICAL DATA

The Panel was not provided with a newly submitted dossier and based its evaluation on previous evaluations, additional literature that has become available since then, and data available following an EFSA public call for data.[19] The Panel noted that not all of the original studies on which previous evaluations were based were available for this re-evaluation.

To assist in identifying any emerging issue or any information relevant for the risk assessment, EFSA outsourced a contract to deliver an updated literature review on toxicological endpoints, dietary exposure and occurrence levels of beetroot red (E 162), which covered the period up to the end of 2014. A further update has been performed by the Panel.

The toxicological studies are described variously as using (1) red beet juice; (2) beetroot red; (3) betalains; (4) betanin; (5) beetroot red extracts; (6) freeze-dried beetroot, (7) beet red powder. The Panel considered that only those studies carried out with a test material containing not less than 0.4% betanin are relevant for the assessment of the safety of the food additive beetroot red (E 162). Information regarding the characterisation of the material tested and the consequent relevance of each study for the assessment of beetroot red has been included in the following sections.

3.1. Absorption, distribution, metabolism and excretion

3.1.1. *In vitro* studies

The degradation of betanin by gastrointestinal tissue was investigated *in vitro* (Krantz et al., 1980). The material tested was a 4.5 mM (2477.16 mg/L) solution of betanin in phosphate buffer, prepared from 'beetroot powder' containing approximately 1% betanin, 1.5% nitrate, 10% citric acid, 10% ascorbic acid, 5% lactose and 72.5% calcium stearate. The Panel noted that the material tested met the specifications for beetroot red, E 162. Chopped tissue preparations of stomach, small intestine or colon were incubated with 2.25 µmol betanin for 24 hours at 37°C (no further experimental details provided), and the extent of metabolism of betanin determined by measuring residual betanin concentrations in supernatants as absorbance at 535 nm in comparison with standard betanin solutions. Seventy, 35 or 60% respectively of added betanin was metabolised/degraded by suspensions of chopped stomach, small intestine or colon. In contrast, almost no metabolism/degradation of betanin occurred when the colour was incubated with suspensions of intestinal or caecal contents (Krantz et al., 1980). The compound was similarly not metabolised/degraded in an isolated perfused rat liver

[19] Call for scientific data on food colours to support re-evaluation of all food colours authorised under the EU legislation. Published: 8 December 2006. Available from: http://www.efsa.europa.eu/en/dataclosed/call/afc061208.htm

system (Krantz et al., 1980). The same authors demonstrated the stability of betanin in other biological samples by adding known amounts of betanin to samples of blood, urine and faeces *in vitro* and measuring the extractable pigment. In all cases, there was 70–75% recovery of betanin from these samples (no further details provided) (Krantz et al. 1980).

Reynoso and co-workers (1999) examined the metabolism/degradation of betalain pigments extracted from a cactaceous fruit (garambullo) by chopped tissue preparations of stomach, small intestine or large intestine, using a similar experimental design as that used by Krantz et al. (Reynoso et al., 1999). The pigment extract contained 0.23% betacyanins, identified as indicaxanthin, phyllocactin, betanin and isobetanin. The Panel noted that the pigments present in cactaceous fruit are also present in beetroot but the material tested did not meet the specifications laid down for beetroot red (E 162). The authors reported 26–29% degradation of the pigments in the presence of chopped tissue preparations from the large intestine, 20–26% in the presence of chopped tissue from small intestine and 24–29% in the presence of chopped tissue from the stomach (Reynoso et al., 1999).

Watts et al. (1993) analysed a solution of beet red powder using HPLC, and identified two compounds absorbing at 536 nm, betacyanin I and betacyanin II (Watts et al., 1993). The beet red powder was a commercial preparation containing 0.375% betacyanins. Based on mass spectroscopic analysis, the authors concluded that betacyanin I was either betanin or isobetanin, whereas betacyanin II was provisionally identified as the diglucoside of betanidin, the aglycone common to all betacyanins (Watts et al., 1993). As part of the same study, the authors examined the effect of acidic conditions on the stability of the betacyanins, by adjusting a solution of the powder in water to pH 2.0 or 4.7 with hydrochloric acid. Both betacyanins were degraded over time in the presence of acid, with an associated colour change from red to yellow. Watts and co-workers also examined the absorption of betacyanins from beet red powder solution using isolated perfused rat jejunum preparations (Watts et al., 1993). Twenty-seven mg of beet red powder was added to the luminal perfusate as a solution in water and absorption was assessed by HPLC of the vascular perfusate. No betacyanins were detected in the jejunal vascular perfusate over the time course of 60 min; the level of the 2 betacyanins in the luminal fluid fell to 80% of the initially administered dose over the same period. Using an isolated perfused rat liver system, the same authors also confirmed that betanin was not metabolised by the liver and that its biliary secretion was not detectable (Watts et al., 1993).

More recently, the stability of betacyanins and betaxanthins obtained as preparations from either fresh foods or manufactured products of cactus pear fruit and red beet was assessed and compared with the digestive stability of purified pigments, using simulated oral, gastric and small intestinal digestion models (Tesoriere et al., 2008). The food preparations variously contained betanin, isobetanin, indicaxanthin and vulgaxanthin. A minor loss of indicaxanthin, in the simulated gastric environment only, and a decrease of vulgaxanthin I in all simulated digestion models were observed, which was not affected by the food matrix. In contrast, the food matrix prevented decay of betanin and isobetanin in the gastric-like environment. Loss of betacyanins, either purified or food-derived, was observed during the small intestinal phase of digestion. The authors suggested that digestive stability influences the bioavailability of dietary betaxanthins, whereas additional factors, relevant to the food matrix and style of processing, affect the stability of betacyanins (Tesoriere et al., 2008).

3.1.2. Animal studies

As demonstrated by the *in vitro* studies described above, beetroot pigments are unstable at low pH, and consequently, the acidic environment of the fasting stomach promotes the rapid degradation of beetroot red.

Betanin from beetroot powder was reported to be poorly absorbed from the gastrointestinal tract of 5–8 rats after single oral administration (by gavage) of 4.5 µmol betanin, equivalent to approximately 12.5 mg betanin/kg bw (Krantz et al., 1980). The material administered was identical to that described for the *in vitro* studies of Krantz et al. described above (section 3.1.1), and the Panel noted that the material tested met the specifications for beetroot red, E 162. Approximately 3% of the oral dose appeared in urine with a similar percentage detected as unchanged compound in faeces.

Approximately 95% of orally administered betanin was not recovered in either urine or faeces, as indicated by the loss of the characteristic betanin absorbance at 535 nm, suggesting that extensive biotransformation of the compound occurred in the gastrointestinal tract. In contrast, following intravenous administration of 4.5 µmol betanin the urinary excretion of betanin was 88 + 6.7% (n=3), 4 h after administration. Red colouration of the urine was seen within 3 min of administration, showing rapid excretion, and the estimated half-life of betanin in plasma was estimated to be 32 min (Krantz et al., 1980).

In a study in which beet red powder (67 mg/kg bw as a solution in saline) was administered to four male Wistar rats as an intravenous (i.v.) bolus, the half-life of betanin in plasma was found to be around 20 min with urinary recovery of 80% of the dose (Watts et al., 1993). The material administered was identical to that described for the *in vitro* studies of Watts et al. (1993) described above (section 3.1.1); the Panel noted that the material tested could be considered to meet the specifications for beetroot red, E 162. The renal clearance rate of betanin was reported in the same study to be similar to its plasma clearance. This study corroborated the findings of an earlier albeit limited study that reported that at least 75% of betanin was recovered in the urine of rats that had received an i.v. injection of 5–10 mg betanin/kg bw (Watson et al., 1963; Watson, 1964). Watts et al, (1993) also reported that following oral administration of beet red powder (1000 mg in distilled water by gavage) to six rats, betacyanins I and II were virtually undetectable in the stomach lumen after 6 hours, and were not detectable in blood; trace quantities (approximately 0.6% of administered dose) of both betacyanins were detected in urine.

The toxicokinetics of betalain pigments extracted from a cactaceous fruit (garambullo) were evaluated in male and female Wistar rats (Reynoso et al., 1999). The material administered was identical to that described for the *in vitro* studies of Reynoso et al. described above (section 3.1.1); the Panel noted that the pigments present in cactaceous fruit are also present in beetroot but that the material tested did not meet the specifications laid down for beetroot red (E 162). The aqueous pigment extract was administered by gavage at dose levels of 0.5, 2.5 and 5.0 g/kg bw and urine collected for determination of excreted pigments. The authors reported that approximately 2% of the administered dose was excreted in the urine, phyllocactin, betanin and isobetanin being specifically identified by HPLC. At dose levels of 2.5 and 5.0 g/kg, the urine was red and at 0.5 g/kg, the colour was orange.

3.1.3. Human studies

Ingestion of beetroot can produce red urine ('beeturia') in some individuals, suggesting that betanin and/or other betacyanins are absorbed from the gastrointestinal tract followed by urinary excretion (Watson et al., 1963; Watts et al., 1993; Mitchell, 2001). In a review, Mitchell (2001) concluded that beeturia is primarily a function of an individual's physiological constitution rather than being a phenomenon under direct polymorphic genetic control. Beetroot pigments are non-enzymatically decomposed in the stomach and the colon (Dressman et al., 1990; Watts et al., 1993; Watson et al., 1963; Eastwood and Nyhlin, 1995). Among the factors influencing this decomposition are the acidity of the stomach and the presence of oxalic acid and ascorbic acid (Eastwood and Nyhlin, 1995; Mitchell, 2001).

Watts et al. (1993) studied absorption of betanin from a liquidised beetroot preparation administered orally to 100 volunteers of both sexes. The administered beetroot preparation contained 20, 40 or 60 mg of betacyanins, each dose being administered sequentially to each subject on separate occasions. The authors reported that the 0–8 hour urinary recovery of betacyanin ranged from 0.06 to 0.64% of the administered dose. Excretion was rapid, betacyanins being detected in the urine within 30 minutes of ingestion of the beetroot preparation. Subsequently, Kanner et al. (2001) showed that betanin and isobetanin bioavailability in four volunteers after oral ingestion of 300 mL beetroot juice was 0.5–0.9 % of the ingested betacyanin dose.

The plasma kinetics and urinary excretion of betalains were studied in eight healthy volunteers after a single ingestion of 500 g cactus pear fruit pulp, which provided 28 and 16 mg indicaxanthin and betanin respectively (Tesoriere et al., 2004). Betanin and indicaxanthin reached their maximum

plasma concentrations 3 hours after the fruit meal and declined according to first-order kinetics. The half-life of betanin (approximately 1 hour) was shorter than that of indicaxanthin (approximately 2.5 hours). Both compounds had disappeared from plasma by 12 hours after intake. The urinary excretion of indicaxanthin and betanin over 12 hours represented 76% and 3.7% respectively, of the ingested compounds. The Panel noted that this study provides additional confirmation of the absorption and excretion of betanin, and that the pigments present in cactaceous fruit are also present in beetroot, thus the study is of relevance in the assessment of beetroot red (E 162).

Six healthy, non-smoking females aged 23–24 years ingested 500 mL red beet juice (containing 362.7 mg betalains) and urinary samples were collected for up to 11 hours after ingestion, with a final sample taken 24 hours after dosing. After a one-week wash out period to allow for control measurements, the same volunteers were given 500 mL tap water and urinary samples were collected as in the first study (Frank et al., 2005; Netzel et al., 2005). Renal excretion of betalains was determined spectrophotometrically and quantified as betanin-equivalents. The identity of individual compounds was confirmed by high-performance liquid chromatography coupled with diode-array detection (HPLC-DAD) and liquid chromatography/mass spectrometry (LC/MS) respectively. The authors noted that, for mass spectrometric analyses providing unambiguous pigment identification, removal of co-eluting colourless phenolics was a prerequisite (Frank et al., 2005). The amount of intact betalains (betanin and isobetanin) recovered in urine was 0.28% of the administered dose. The overall half-life after oral dosing was approximately 7.5 hours. The authors of these studies noted that the extent of absorption of the betacyanins is unknown. (Frank et al., 2005). They suggested that consideration of the pH-dependent ionisation of betalains and interactions between betalains and biomolecules may help in the interpretation of bioavailability data. The authors also concluded that renal clearance is a minor route of systemic elimination for these compounds and suggested that other pathways of betacyanin elimination may exist (Frank et al., 2005; Netzel et al., 2005). The Panel noted that the material tested met the specifications for beetroot red (E 162).

A limited study in three human volunteers receiving i.v. injections of betanin showed that up to 75% of the pigment were recovered essentially unchanged in the urine (Watson, 1964). The Panel noted that i.v. administration in this study bypassed the acidic conditions of the stomach following oral administration.

3.1.4. Conclusion on the ADME of beetroot red

The betacyanin pigments are metabolised or degraded *in vitro* by chopped tissue preparations from the stomach, small and large intestine, indicating that some breakdown is likely to occur in the gastrointestinal tract following oral administration of beetroot red. Studies in humans, supported by animal studies, have however shown that the betalain pigments present in beetroot are absorbed in an intact form to a limited extent after oral administration and are not metabolised further in the body, as demonstrated by the excretion of betanin, isobetanin and other betacyanin pigments at low levels in urine. Urinary excretion is very variable in humans, and the fraction of the administered dose excreted in the urine in a range of studies has been reported to range from less than 0.1 to 0.6%, although a figure of 3.7% has been reported for betanin from a cactus pear fruit pulp preparation. A similar low excretion of betacyanin pigments has been demonstrated in rats. These data indicate that in human, if absorbed the pigments were not metabolised but were rapidly excreted via the kidneys. This interpretation is supported by evidence from rats wherein intravenous or intraperitoneal injection of beetroot extracts demonstrated a high renal clearance (Mitchell, 2001). Absorption of the intact betacyanin pigments is dependent on a number of factors such as the intestinal milieu and the presence of associated substances including oxalic acid and ascorbic acid in the betacyanin-containing preparation. Ultimately absorption and bioavailability of betacyanin pigments is likely to be highly dependent on the pH-dependent ionisation of these compounds. The fate of metabolites or degradation products of the betacyanins in the gastrointestinal tract is not known.

3.2. Toxicological data

3.2.1. Acute oral toxicity

JECFA (1988) noted that no mortality occurred in a study in which rats were given high oral doses of beetroot red (Druckrey, 1959). This unpublished study, submitted directly to JECFA was not available to the Panel, and there is no information on the doses administered or the material tested. JECFA also reported a study in which single doses of a beetroot extract containing 1% betanin and 1.5% nitrate as well as other components were injected intravenously into anaesthetised rats. The authors reported a transient increase in blood pressure and heart rate, the effect of 0.9 µmol betanin being similar to that of 2 µmol adrenalin, and considered that betanin was the most likely to be responsible for these effects (Kranz et al., 1980).

Reynoso et al., 1999 evaluated the toxicological and toxicokinetic effects of betalain pigments from a cactaceous fruit (garambullo) in male and female Wistar rats. In this study, a yeast-fermented garambulla extract containing 0.23% (w/w) betacyanins was produced in which indicaxanthin, phyllocactin, betanin and isobetanin were identified but not individually quantified. A single dose of an aqueous solution of the garambullo pigments (to provide 0, 0.5, 2.5, 5.0 g of extract/kg bw, respectively) was administered by oral gavage to groups of four to six Wistar rats of each sex and the animals were observed for 15 days. Even at the highest dose, the pigments did not affect weight gain and did not induce any gross morphological changes (Reynoso et al., 1999). The Panel noted that a number of the pigments present in cactaceous fruit are also present in beetroot but that the material tested did not meet the specifications laid down for beetroot red (E 162). The Panel considered therefore that the study was of limited relevance for the assessment of the safety of beetroot red (E 162).

No other acute toxicity studies have been published since these previous evaluations.

3.2.2. Short-term and subchronic toxicity

A two-week administration of betanin in the drinking water at 2.5 mg/100 mL (corresponding to approximately 3.75 mg/kg bw/day) was described not to cause any overt toxicity in mice (Kapadia et al., 2003); however, no data were presented by the authors to corroborate their statement. The Panel considered that this study was of limited relevance for the assessment of the safety of beetroot red.

The JECFA (1988) evaluation reported one short-term toxicity study. Groups of 6 males and 6 females of Sprague–Dawley adult albino rats were fed beetroot red preparations containing 2000 mg/kg betalains in the diet for 7 days. This concentration of pigment was approximately 50–100 times the amount of pigment normally required to colour food (von Elbe and Schwartz, 1981) and is equivalent to approximately 200 mg pigment/kg bw/day. Two different formulations of beet pigments were used as the source of betalains, a spray-dried powder prepared from beet juice after fermentation and consisting of a pigment content of 1.96% on a dry weight basis, and a gel filtrate consisting of 50% pigment. There were no significant differences in body weight gain, food intake, animal behaviour or gross pathological features in any of the treatment groups relative to controls or between the two pigment formulations (von Elbe and Schwartz, 1981). The Panel noted that the betalain pigments present were not further identified. The Panel also noted that the formulations were prepared from fermented beetroot juice and that the identity of the substance or substances formed as a result of the fermentation was not well defined. The Panel therefore considered that this study was of limited relevance for the assessment of the safety of beetroot red.

Administration of beetroot red colour (not further specified) at 150 mg/kg bw/day in oil, 5 days per week, for 3 weeks to 12 male rats resulted in death of 2 animals during the first 2 weeks of the study (Holmberg, 1980). It is not known whether these deaths were treatment-related, and the method of administration of the beetroot red was not specified, although it was assumed in BIBRA (1991) to have been carried out by oral intubation. No effects on growth or haemoglobin levels were observed, but decreased activity of liver lactate dehydrogenase was reported. The Panel noted the lack of

information regarding the nature of the material tested and considered that this study was of limited relevance for the assessment of the safety of beetroot red.

Kujawaska and co-workers (2009) exposed male Wistar rats (8 animals/group) to beetroot juice by gavage (8 mL/kg bw/day) for 28 days; the exposure corresponded to 53 mg/kg bw/day for betacyanins (mainly betanin) and to 26 mg/kg bw per day for betaxanthins (mainly vulgaxanthin) (Kujawaska et al., 2009). The authors examined the effect of beetroot juice treatment on liver glutathione content, the activities of liver superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, and the levels of plasma protein carbonyl content was examined. No statistically significant differences in these parameters were observed between beetroot juice-treated animals and the corresponding control animals. Furthermore, there was no difference in the level of liver microsomal thiobarbituric acid reactive substances (used as an indicator of lipid peroxidation) between beetroot juice-treated animals and control animals. Subsequent *in vitro* treatment of the microsomes with Fe^{2+} and ascorbate to generate reactive oxygen species resulted in microsomal lipid peroxidation, with no difference in the degree of peroxidation in the microsomes from beetroot juice-treated animals compared with the microsomes from the control animals (Kujawaska et al., 2009).The Panel noted that the beetroot juice tested in this studies meets the specifications laid down for beetroot red (E 162).

In a study by Lee and co-workers (Lee et al., 2005), several fractions were prepared using HPLC from crude aqueous and 95% ethanol extracts of root tissue of red and high-pigment strains of beet (*Beta vulgaris* L.) and were tested in post-weanling male Sprague–Dawley rats. The animals were fed control diets, diets containing 10 mg/kg aqueous high-pigment beet extract fraction IV or diets containing 150 mg/kg aqueous high beetroot fraction I for 2 months. Body weights were measured at intervals of 2–3 days. After 2 months, animals were sacrificed and portions of liver, proximal small intestine, colon, kidney and lungs were sampled and assayed for quinone reductase and glutathione-S-transferase activities. No significant differences were observed in average weight gains. No statistical differences were observed in the various tissue levels of quinone reductase and glutathione-S-transferase in the different treatment groups (Lee et al., 2005). The Panel considered that the materials tested were likely to contain a high proportion of betanin.

3.2.3. Genotoxicity

Genotoxicity studies were carried out with different, often poorly defined beetroot red formulations, and with beetroot pigments. The Panel noted that tests on raw beetroot extracts provide limited possibilities to detect genotoxic components present in low amounts, because of the dilution effect and the consequent low doses tested.

Beetroot red (spray-dried powder prepared from beet juice after fermentation and consisting of a betalain pigment content of 1.96% on a dry weight basis) was tested at concentrations ranging from 500 µg to 2500 µg/plate in the Ames test using 5 strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, TA1538), with or without S9 metabolic activation (von Elbe and Schwartz, 1981) in two independent assays. In parallel positive and negative control were performed. No increase in revertants was seen at any concentration in any of the strains tested. The Panel noted that the betalain pigments present in the test material were not further identified and that the identity of the substance or substances formed as a result of the fermentation was not well defined. The Panel also noted that the protocol was limited (no TA 102 strain, top dose limited to 2500 µg/plate and due to centrifugation of the solutions, the concentrations tested were not certified).

Beetroot red (stated to conform to food specifications, no further details provided) was reported not to induce DNA damage in a liquid *rec*-assay in *Bacillus subtilis* at a concentration of 0.5 mg/mL with or without metabolic activation by rat liver S9 (from rat livers induced by phenobarbitone only) or rat intestinal microbial extract preparations (Haveland-Smith, 1981). In the same study, no mutagenic activity of beetroot red was detected in fluctuation assays using *Escherichia coli WP2 uvrA* and *Salmonella typhimurium* TA 1538 strains at only one concentration of 0.5 mg/mL, with or without the same metabolic activation systems used in the *rec*-assay (Haveland-Smith, 1981). The Panel noted that the identity of the substance was not well defined and that the protocols were very limited (only 2

strains: Escherichia coli WP2 uvrA and Salmonella typhimurium TA 1538 strains, only one dose of 0.5 µg/mL without justification and S9 from rat liver induced only by phenobarbitone).

An additional study investigated whether the metabolic activation (S9) of urine from rats fed for 26 weeks with a beet diet which also contained aflatoxin B_1 (AFB_1) caused a statistically significant increase in the number of revertants in *S. typhimurium* strain TA98, compared to the number of revertants caused by the urine of rats fed a basal diet plus AFB_1 (Boyd et al., 1982). Weanling male Fischer rats were fed diets containing 25% (w/w) freeze-dried ground beets (*Beta vulgaris L*) with or without AFB_1 in the diet for 26 weeks. The effects of urine from rats fed the beet diet, either without metabolic activation (S9) or without AFB_1, on the number of revertants were not statistically different compared to the effect of urine from the control animals. The authors of the study interpreted this effect as an indicator that the beet diet enhanced the production of AFB1 metabolites. The Panel considered this study not relevant for the assessment of the genotoxicity of beetroot.

An extensive number of synthetic and natural food additives including beet red were tested in a bacterial mutagenicity assay using *Salmonella typhimurium* strains TA92, TA94, TA98 TA100, TA1535 and TA1537, with and without S9 metabolic activation (Ishidate et al., 1984). The assay with metabolic activation used a 20 min pre-incubation protocol. At a level of 50 mg/plate, in TA 100 only, beetroot red (no further details but classed as a food additive from 'natural sources') induced 251 revertants with metabolic activation and 237 revertants without metabolic activation, compared to 90 and 116 revertants in respective controls (Ishidate et al., 1984). The response in the absence of S9 was dose-related, but only 2 dose levels were employed in the presence of S9, both showing an increase in revertants compared with controls, but with no evidence of a dose response. The authors concluded that beetroot red had a weak mutagenic potential with and without metabolic activation. The Panel noted that insufficient details were provided in this publication to judge the validity of the study, and also noted that the study used very high concentrations of beetroot red (5–75 mg/plate) above the maximum recommended dose level, and that no information was provided on bacterial toxicity. The Panel noted also that the identity of the substance was not well defined and that no positive controls were tested in parallel.

Beet red did not induce chromosomal aberrations in Chinese hamster fibroblast cells (CHL) in culture, examined at 24 and 48 hours after a continuous treatment at concentrations up to 8 mg/mL which induced about 50% growth cell inhibition (Ishidate et al., 1984). Only 100 metaphases/concentration were observed. The Panel noted that no metabolic activation system and that no short-time treatment followed by a recovery period was used in these studies.

The clastogenicity and sister chromatid exchange (SCE)-inducing potential of beetroot colour was investigated in human peripheral lymphocytes from three volunteers *in vitro* (Conforti-Froes et al., 1990). No details were provided in the publication regarding the material tested, described as betanin, other than that the major yellow pigments vulgaxanthin I and vulgaxanthin II had been removed from the powder before testing, and that two preparations of the material were tested. The Panel noted that the only information regarding specifications was that one of the preparations contained nitrates at an undefined level. The powder was tested at concentrations of 4.3, 8.6 or 17.2 µg/mL, using a 72-hour incubation period; only 100 cells for chromosomal aberrations and 30 cells for SCE were examined per culture. The authors reported an increase in chromosome aberrations in two out of three lymphocyte samples each from three healthy male donors, in the presence of the highest concentration of the betanin without nitrate. Some degree of cytotoxicity was reported at this dose (approximately 30% inhibition of mitotic index compared with controls). This form of the colour preparation (without nitrate) also showed an increase in SCEs at the highest dose tested, whereas a weaker response was seen in the presence of nitrate (Conforti-Froes et al, 1990). The Panel noted that this study was poorly reported (e.g. no indication of the types of chromosomal aberrations observed and on the statistical significance of the effect reported). In addition, the protocol was limited (only 100 cells for chromosomal aberrations and 30 cells for SCE were examined per culture, only one sampling time, *viz.* 72 hours, appropriate for SCE analysis but too late for the detection of structural chromosomal

aberrations). Therefore, the Panel considered the validity of the results of this study cannot be assessed.

Arimoto-Kobayashi et al. (2005) investigated the photo-mutagenicity of beetroot red (*Beta vulgaris*) and its photodegradation products. Cultures of *Salmonella typhimurium* TA98, TA100 and TA102 were mixed with 0.001, 0.01, 0.1, 1 or 10 mg/mL beetroot red and were irradiated with UV (ultraviolet) light at 25 °C for 30 minutes with continuous shaking. The dose of UVA was 1.25 ± 0.21 J/cm^2. 8-Methoxypsolarene was used as positive photomutagenic control. Aliquots were poured into plates containing soft agar and revertant colonies were scored after incubation for 48 hours at 37 °C. The Panel noted the unusual protocol of this study, which entails the irradiation of UV sensitive tester strains (TA98 and TA100). In another experiment, beetroot red solutions (0.001, 0.01, 0.1, 1 or 10 mg/mL) were irradiated with UVA (ultraviolet A) for 4 hours at 37 °C with continuous mixing. Thereafter, aliquots were assayed using the pre-incubation method of the Ames test, with or without metabolic activation. No photomutagenicity was observed for beetroot red under both experimental conditions tested (Arimoto-Kobayashi et al., 2005). The Panel noted that the identity of the substance was not well defined and that the protocol was limited (only 3 strains).

In the same publication, induction of chromosome aberrations and micronuclei by beetroot red powder, described as betanin, was investigated *in vivo* in rat bone marrow (Conforti-Froes et al, 1990). Wistar rats (4–8 animals per test group and 10 control animals) were treated orally (method not stated but presumed to be by gavage) with either 0.2 mg/kg bw/day or 50 mg/kg bw/day beetroot red powder for 7 days and sacrificed on day 8, 90 minutes after administration of colchicine. The same two beetroot preparations that were used for the *in vitro* studies were administered *in vivo*. Cyclophosphamide (50 mg/kg bw for 24 hours) was used as a positive control. The Panel noted that the number of animals in the latter group was not specified and that no results for the positive control treatment were shown or discussed. The authors state that there was no significant increase in the frequency of micronuclei based on the following frequency of micronucleated bone marrow cells: control, 36.5; betanin at 0.2 mg/kg bw dose, range 23.3–40.4; betanin at 50 mg/kg bw dose, range 41.4–46.2. For the micronucleus assay, the Panel noted that no information was provided on the type of cell analysed (polychromatic or the whole erythrocytes), nor on the number of polychromatic erythrocytes analysed, and the ratio between polychromatic and normochromatic erythrocytes was not given. The Panel finally considered that the administration of colchicine in studies examining induction of micronuclei is not appropriate due to the fact that colchicine is inducing, *per se*, micronuclei. For the chromosomal aberration arm of the study, the authors reported that no statistically significant increases in the number of cells bearing aberrations compared to the negative control were observed. However, the Panel noted that the protocol is limited for the number of animals used and no description of chromosomal aberrations found. Moreover, the doses tested, calibrated on the acceptable daily intake of another food colour, were lower than recommended by current guidelines. Overall, the results of this study cannot be used for risk assessment. Following the exposure of male Wistar rats (average body weight 240 g, 8 animals/group) to beetroot juice by gavage (8 mL/kg bw/day) for 28 days, which corresponded to 53 mg/kg bw/day for betacyanins (mainly betanin) and to 26 mg/kg bw per day for betaxanthins (mainly vulgaxanthin), there was no evidence of DNA damage, as based on the alkaline Comet assay on whole blood leukocytes (Kujawaska et al., 2009). The Panel noted that this study was designed to investigate the potential protective effect of beetroot red against chemically induced oxidative stress, rather than to assess beetroot red genotoxicity. Therefore the Panel considered that the relevance of this study for risk assessment is limited, if any.

In conclusion, the results of two genotoxicity studies on beetroot red indicate that one or more of the components of the pigment may have weak mutagenic and clastogenic potential *in vitro* but the validity of the studies could not be adequately assessed. Therefore, no conclusions could be drawn from these studies. The results of one *in vivo* study on betanin pigment suggest that any *in vitro* genotoxic potential is not manifest *in vivo* but the relevant study was considered to be not adequate by the Panel. Overall, the Panel concluded that the genotoxic potential of beetroot red (E 162) cannot be evaluated based on the available data.

3.2.4. Chronic toxicity and carcinogenicity

In a long-term study, a group of 32 male and female rats were given 50–78 mg betanin/kg bw/day in drinking water throughout their lives, to provide a total dose of 17 g per animal (Druckrey, 1959, as cited by the JECFA, 1988, and not available to the Panel). A group of 56 rats acted as controls. The mean life span was 845 days and the last animal died at 1220 days. In the test group one intraperitoneal sarcoma and one mammary fibroadenoma occurred, compared with two sarcomas and two fibroadenomata in the controls (Druckrey, 1959). However, JECFA (1988) considered that since no details were provided no conclusions could be drawn from the study regarding the carcinogenicity of beetroot red. The Panel agreed with this conclusion, noting that the number of animals used in the study was lower than that required by current test guidelines and that a very limited range of organ and tissues was investigated.

Initiation/promotion studies

Kapadia et al., (2003), investigated in mice the potential of betanin to modulate the development of tumours in three different experimental models for initiation–promotion. The effect of betanin was tested in each of the following three models: (i) skin tumour initiation with 7,12-dimethylbenz(a)anthracene and promotion with ultraviolet light-B; (ii) skin tumour initiation with (±)-(E)-4-methyl-2-[(E)-hydroxyamino]-5-nitro-6-methoxy-3-hexanamide and promotion with 12-*O*-tetradecanoylphorbol-13-acetate; (iii) liver tumour initiation with N-nitrosodiethylamine and promotion with phenobarbital. In all three models, the oral co-administration of betanin at 2.5 mg/100 mL drinking water (corresponding to approximately 3.75 mg/kg bw/day) with the tumour promoter for 20 weeks caused a significant inhibition of both the incidence and multiplicity of the tumours. The authors furthermore stated that betanin on its own did not induce the formation of any tumours. The Panel considered that this study was of limited relevance in the assessment of the carcinogenicity of beetroot red (E 162).

Other authors reported on the anti-carcinogenic effects of natural pigments from beetroot on the two-stage carcinogenicity of mouse pulmonary tumours (induced by 4-nitroquinoline-N-oxide as an initiator and glycerol as a promoter) and hepatic tumours (induced by N-nitrosodiethylamine as an initiator and Phenobarbital as a promoter) (Konoshima and Takasaki, 2003, only abstract available).

A study investigated whether diets containing 25% (w/w) freeze-dried ground beets (*Beta vulgaris L*) enhanced liver tumorigenesis induced by 0.1 mg aflatoxin (AF)B_1/kg bw per day (Boyd et al., 1982). Weanling male Fischer rats were fed freeze-dried ground beets with or without AFB_1 in the diet for 26 weeks. Following this period, the rats were then maintained on a basal diet without AFB_1 for 16 weeks. The authors of the study reported that the diet supplemented with 25% freeze-dried ground beet significantly increased relative liver weight in AFB_1-treated animals and the number of AFB_1-induced tumours. Control ground beet containing diet did not cause an increase in liver weight or liver tumour incidence. The Panel considered that this study was of little relevance in the assessment of the carcinogenicity of beetroot red (E 162).

The Panel noted that only limited or inadequate studies are available on the carcinogenicity of beetroot red and therefore could not conclude on the chronic toxicity and carcinogenicity of beetroot red.

3.2.5. Reproductive and developmental toxicity

No adequate studies on reproductive and developmental toxicity are available on beetroot red. The JECFA (1988) evaluation reported a two generation study in rats in which 32 rats received betanin in drinking water, a total of 17 g/animal over their lifetime (Druckrey, 1959). The JECFA evaluation provides no further details regarding the reproductive aspects of beetroot red, and as the study report was not available to the Panel, no conclusions can be drawn regarding reproductive toxicity of beetroot red.

3.2.6. Allergenicity, hypersensitivity and intolerance

In a case report of a woman suffering from an anaphylactic shock associated with beeturia, the authors concluded that anaphylaxis could not be attributed to a hypersensitivity reaction to beetroot extract (Luke and Watson, 1963).

One limited *in vitro* study reported a decreased IgE production by rat spleen lymphocytes exposed to µM concentrations (1 and 10) of betanin of unknown specification (Kuramoto et al., 1996).

Considering the widespread consumption of beetroot red and the absence of reports on allergic and intolerance reactions, the Panel concluded that the food additive beetroot red (E 162) would not represent a safety concern as regards allergy and immunotoxicity.

3.2.7. Other studies

In an *in vitro* study, human chronic myeloid leukaemia K562 cells were exposed to 10, 20, 40 and 80 µM betanin isolated (purity unknown) from fruits of the cactus *Opuntia ficus-indica* for 12, 24 or 48 hours. After 24 and 48 hours of exposure, cell death by apoptosis was observed at all concentrations with a statistically significant decrease ($p<0.05$) in cell growth (Sreekanth et al., 2007).

In another study, female Sprague–Dawley rats (6–11 animals/group) were partially hepatectomised (PH) and after 24 hours were given a single intragastric dose of either a) fermented betacyanin solution (50 mg/kg bw), b) pure betanin (50 mg/kg bw) c) degraded betanin (50 mg/kg bw) followed by a diet containing 0.05% phenobarbital for 8 months to test the ability of these compounds to initiate carcinogenesis (Schwartz et al., 1983). Another group of animals was exposed to a diet containing 2000 mg betacyanins/kg food for 4 days prior to PH and for 4 days after PH followed by a diet containing 0.05% phenobarbital for 8 months to test the ability of betacyanins to initiate carcinogenesis. A further group of animals previously initiated with N-nitrosodiethylamine was given a betacyanin solution (100 mg/L), described by the authors to be equivalent to 3.5 mg/rat/day) to determine the ability of betacyanins to promote carcinogenesis after initiation relative to control and phenobarbitone-treated rats. After 6 months (promotion studies) or 8 months (initiation studies), the livers were examined histologically and histochemically for gamma-glutamyl transpeptidase positive enzyme altered foci. In these medium-term assays, there was no evidence that any of the betalain preparations initiated or promoted hepatocarcinogenesis (Schwartz et al., 1983). The Panel noted that this study included pure betanin as test material, and could therefore be considered to be of relevance in the assessment of beetroot red, but that the number of animals per group was very small and the study was of limited duration.

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 of the original studies on which previous evaluations were based were available for this re-evaluation. In addition, no relevant data were identified from the updated literature search commissioned by EFSA.

Beetroot red (E 162) is authorised as a food additive in the EU in accordance with Annex II to Regulation (EC) No 1333/2008,[20] and previously evaluated by the SCF in 1975 and in relation to special medical purposes for young children in 1996 (SCF, 1975, 1997a). JECFA evaluated beetroot red in 1974, 1978, 1982 and 1987 (JECFA 1975, 1978, 1982, 1987, 1988). Neither body has established a numerical ADI. In its latest evaluation, JECFA noted that 'Previous Committees had considered beet red together with its major colour component, betanin. This Committee decided that it would be appropriate to evaluate these food colours separately and pointed out that, for the compound betanin, insufficient data were available to establish an ADI, since the information available to the

[20] 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.

Committee did not meet currently accepted standards' (JECFA, 1988). JECFA further concluded that 'when the [beet red] concentrate is used to enhance the colour of beet products, it could be considered as food. If, on the other hand, the concentrate is used more generally as a colourant, careful specifications need to be established. Because nitrate is a component of beet red, it is necessary to ensure that levels of nitrate do not exceed the specifications. Under these conditions beet red could be used according to good manufacturing practice with an ADI 'not specified', keeping in mind the need to limit the nitrate content of foods produced for infants and young children'.

Beetroot red is a natural colour obtained from the roots of natural strains of red beets (*Beta vulgaris* L. var. *ruba*). Beetroot red is a complex natural product of variable chemical composition. The colour (and presumably chemical composition) can vary considerably according to variety, cultivar, crop and age of beet. The method of manufacture will also impact on composition. Beetroot red (E 162) contains a number of different pigments, all belonging to the general class known as betalains. The main colouring principle consists of a number of betacyanins (red), of which betanin accounts for 75–95% and isobetanin (epimer of betanin) 15–45%; a range of other betacyanins can also be detected, amounting to up to 20% of the total content of betacyanins (NATCOL, 2012). According to industry (NATCOL, 2012), vulgaxanthin I (25–70%) and vulgaxanthin II (5–15%) are present in the betaxanthin (yellow) as well as several degradation products of betalains (light brown). Besides the colour pigments beetroot red contains sugars, salts, and/or proteins naturally occurring in red beets.

A number of different beetroot red products may be marketed, ranging from press juices or aqueous extracts of shredded roots to more concentrated or refined forms including pastes, powders and other solid forms (NATCOL, 2012). The Panel noted that the current re-evaluation does only refer to beetroot red prepared by pressing crushed beet as pressed juice or by aqueous extraction, in accordance with the definition of the Commission Regulation (EU) No 231/2012 and not to preparations manufactured by solvent extraction with methanol or ethanol.

Specifications for beetroot red (E 162) have been defined in Commission Regulation (EU) No 231/2012 and by JECFA (2006). EC specifications including purity criteria for beetroot red define not less than 0.4% of the commercial material must be betanin pigment. The remaining 99.6% is accounted for by sugars, salts and proteins naturally occurring in red beets, and a small amount of other pigments belonging to the class of betalains but this is not further specified. According to industry (NATCOL, 2012), non-colouring substances present in beetroot red are sugars, proteins, minerals, organic acids, vitamins, sterols, purines and phenolic compounds. The Panel noted that the reported content of betanin in beetroot to be around 0.4%. The Panel further noted that the specification for the content of red colour (expressed as betanin) in beetroot red, as not less than 0.4%, may give rise to some confusion, given the number of different forms of beetroot red that may be on the market, including simple extracts, refined extracts and spray-dried powders. The Panel also noted that some forms of beetroot red designated as colouring foodstuffs rather than as food colours may contain more than 0.4% colouring matter. The Panel considered that revision of the current specification to reflect betanin content on a dried solids basis could be appropriate.

The specifications for beetroot red (E 162) include a maximum level for nitrate of $\leq$ 2 g nitrate anion/g of red colour, given the relatively high content of nitrate in beetroot. The Panel considered a nitrate limit important in minimising the contribution to nitrate intake from this source as a food additive. This should be evaluated together with dietary exposure and use as a colouring food when evaluating the total intake of nitrate by the population.

There are no validated methods for the analysis of beetroot red (or betanin) in foods that may be used for official purposes.

Toxicokinetic and toxicological studies have been carried out among others on (1) red beet juice, (2) beetroot red, (3) betalains, (4) betanin, (5) beetroot red extracts, (6) freeze-dried beetroot, (7) beet red powder. All of these products will contain betanin pigment. However, the Panel considered that only those (relatively few) studies carried out with a test material containing not less than 0.4% betanin are relevant for the assessment of the safety of the food additive beetroot red (E 162). The Panel

additionally noted that it is unclear which of the above substances most closely resemble the commercial food colour and whether testing of purified betanin provides sufficient insight into the biological and toxicological behaviour of the commercial product.

The betacyanin pigments from beetroot red are metabolised or degraded *in vitro* by chopped tissue preparations from the stomach, small and large intestine, indicating that some breakdown of pigments is likely to occur in the gastrointestinal tract following oral administration of beetroot red. Studies in humans, supported by animal studies, have however shown that the betalain pigments present in beetroot are absorbed in an intact form to a limited extent (approximately 3% of the dose in rats and less than 1% of the dose in humans) after oral administration and are not metabolised further in the body, as demonstrated by the excretion of betanin, isobetanin and other betacyanin pigments at low levels in urine. Information from intravenous and intraperitoneal administration of beetroot extracts in rats demonstrated that intact pigments were extensively excreted unchanged in the urine. In humans, ingestion of beetroot can produce red urine ('beeturia') in some individuals. It has been suggested that beeturia is more a function of an individual's physiological constitution than a phenomenon under direct polymorphic genetic control (Mitchell, 2001).

The Panel noted that toxicological studies carried out on material conforming to the specifications for beetroot red are limited in number. However, there was no evidence of adverse effects in a range of studies conducted with poorly defined material and/or judged to be of limited relevance for the assessment of beetroot red (E 162).

The genotoxic potential of beetroot red (E 162) cannot be evaluated based on the available data.

There are only limited or inadequate studies available on the carcinogenicity of beetroot red and therefore the Panel could not conclude on the chronic toxicity and carcinogenicity of beetroot red.

No adequate studies on reproduction and developmental toxicity were available.

There is no indication of intolerance or allergenicity of beetroot red in the available literature.

Exposure assessments of food additives under re-evaluation are carried out by the ANS Panel based on (1) MPLs set down in the EU legislation (defined as the *regulatory maximum level exposure assessment scenario*) and (2) usage or analytical data (defined as the *refined exposure assessment scenario*). It was not possible to carry out a scenario based on the MPLs set out in EU legislation, as, for all food categories, beetroot red (E 162) is authorised according to QS. However, maximum levels of the available data were used to provide a conservative estimate scenario (noted as the *maximum level exposure assessment* scenario). With regard to the refined exposure assessment scenario, only reported use levels were made available by industry. The Panel considers that the refined exposure assessment approach results in more realistic long-term exposure estimates because of the underlying assumptions and the concentration data used.

Reported use levels were all provided in mg betanin/kg food. Usages notes were also added by NATCOL (NATCOL, 2015) which could mention the percentages of food items per food category in which the food additive (E 162) or the colouring food (CFS) is used. These percentages were not taken into account as they should have been used to reduce the number of time the additive E 162 is used. This is not possible in the current modelling. Meanwhile, the reported use levels provided by NATCOL are the correct ones when the food additive E 162 is used. Therefore, the scenarios presented in the current opinion assume that irrespective of whether the food additive or colouring food is used, all the betanin is coming from the food additive E 162.

The Panel noted that the refined exposure estimates will not cover future changes in the level of use of E 162.

Using the *maximum level exposure assessment scenario*, mean exposure to beetroot red (E 162) from its use as a food additive ranged from 0.1 mg/kg bw/day for the elderly to 2.1 mg/kg bw/day in

toddlers, whereas the high exposure using this scenario ranged from 0.3 mg/kg bw/day for the elderly to 3.6 mg/kg bw/day in children. Using the *refined brand-loyal assessment exposure scenario*, mean exposure to beetroot red (E 162) from its use as a food additive ranged from 0.1 mg/kg bw/day in adults and the elderly to 1.6 mg/kg bw/day in toddlers. The high exposure to beetroot red (E 162) using this scenario ranged from 0.2 mg/kg bw/day in the elderly to 2.8 mg/kg bw/day in toddlers. Using the *refined non-brand-loyal assessment exposure scenario*, mean exposure to beetroot red (E 162) from its use as food additive ranged from 0.05 mg/kg bw/day for the elderly to 1.0 mg/kg bw/day in toddlers. The high exposure to beetroot red (E 162) from its use as food additive using this scenario ranged from 0.1 mg/kg bw/day for the elderly to 1.8 mg/kg bw/day in infants. Overall, the lowest exposure to beetroot red (E 162) was estimated for the elderly, whereas the highest exposure to beetroot red (E 162) was calculated for toddlers in all scenarios. The food categories that, at the individual level, had the highest contribution to the total individual exposure to beetroot red (E 162) were breakfast cereals, fine bakery wares, soups and broths and flavoured drinks.

Mean intakes of betanin from the regular diet for consumers only are in the range of the mean estimated exposure from the use of the food additive itself (Table 4, non-brand loyal consumer scenario).

CONCLUSIONS

The Panel concluded that the currently available toxicological database was inadequate to establish an ADI for beetroot red as defined by the specifications set for the food additive E 162. 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 colouring principles in E 162 are natural dietary constituents having a long history of food consumption. In addition, the betanin exposure resulting from the use of beetroot red (E 162) as food additive is in the same range as the exposure to the betanin from the regular diet. Therefore, the Panel concluded that, at the reported use levels, beetroot red (E 162) is not of safety concern as regards its current use as a food additive.

RECOMMENDATIONS

- The Panel noted that the specification for the content of red colour (expressed as betanin) in beetroot red, as not less than 0.4%, may give rise to some confusion, given the number of different forms of beetroot red that may be on the market, including simple extracts, refined extracts and spray-dried powders. The Panel recommended that revision of the current specification to reflect betanin content on a dried solids basis could be appropriate.

- The Panel recommended that the maximum limits for the toxic elements (arsenic, lead, mercury and cadmium) present as impurities and nitrates in the EC specification for beetroot red (E 162) should be revised in order to ensure that beetroot red (E 162) as a food additive will not be a significant source of exposure.

- The Panel further noted that mycotoxins could be present in the material used for the production of beetroot red (E 162). The Panel recommended that limits for mycotoxin contamination may be relevant for the specifications of beetroot red (E 162).

- The Panel recommended that the EU Regulation should include the specification for solubility as given in the JECFA specification.

DOCUMENTATION PROVIDED TO EFSA

1. Pre-evaluation document prepared by the Dutch National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands, October 2008.
2. NATCOL (Natural Food Colours Association). Reply to EFSA: Re-evaluation of food colours: call for data (7.12.06). Beetroot red, Betanin. E 162. NATCOL Submission: March 2007
3. CIAA (Confederation of the Food and Drink Industries of the EU). Exercise on occurrence data – EFSA re-evaluation of some food colours. CIAA submission: December 2009.
4. CIAA (Confederation of the Food and Drink Industries of the EU). Personal communication from CIAA on usage levels of beetroot red, June and November 2010.
5. NATCOL (Natural Food Colours Association). Personal communication from NATCOL on the specification of beetroot red, November and December 2010.
6. NATCOL (Natural Food Colours Association). Personal communication from NATCOL on the specification of beetroot red, February 2011.
7. NATCOL (Natural Food Colours Association). Personal communication from NATCOL on the usage levels of beetroot red, February 2011.
8. CIAA (Confederation of the Food and Drink Industries of the EU). Personal communication from CIAA on the usage levels of beetroot red, April and June 2011.
9. NATCOL (Natural Food Colours Association). Personal communication from NATCOL on the manufacturing process, specification, degradation and toxicological properties of beetroot red, September 2012.
10. NATCOL (Natural Food Colours Association). Personal communication from NATCOL on the specifications of beetroot red E 162 and usage categories and use levels for beetroot red (E 162) and beetroot juice as a colouring food, January 2015.
11. FDE (Food and Drink Europe). Personal communication from FDE on usage categories and use levels for beetroot red (E 162), February 2015.

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APPENDICES

A. SUMMARY OF REPORTED USE LEVELS (MG BETANIN/KG) OF BEETROOT RED (E 162) PROVIDED BY INDUSTRY

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Reported use levels		Information provided by
				Typical	Maximum	
01.4	Flavoured fermented milk products including heat-treated products		QS	25	75	NATCOL
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Except unflavoured products	QS	20*	35	NATCOL (Very limited use)
01.6.3	Other creams	Only flavoured creams	QS	20*	35	NATCOL (Very limited use)
01.7.1	Unripened cheese excluding products falling in category 16	Only flavoured unripened cheese	QS	20	35	NATCOL (Very limited use)
01.7.3	Edible cheese rind		QS	15*	25	NATCOL (Very limited use)
01.7.4	Whey cheese		QS		20	NATCOL (No current usage)
01.7.5	Processed cheese	Only flavoured processed cheese	QS	30	30	NATCOL (Very limited use)
01.7.6	Cheese products (excluding products falling in category 16)	Only flavoured unripened products	QS	15	75	NATCOL (Very limited use)
01.8	Dairy analogues, including beverage whiteners		QS		35	NATCOL (No current usage)
03	Edible ices		QS	25	75	NATCOL
04.2.1	Dried fruit and vegetables	Only preserves of red fruit	QS	60*	100	NATCOL (Very limited use)
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only preserves of red fruit	QS	60*	100	NATCOL (Very limited use)
04.2.3	Canned or bottled fruit and vegetables	Only vegetables (excluding olives)	QS	60*	100	NATCOL (Very limited use)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only vegetables (excluding olives)	QS		50	NATCOL (No current usage)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only *mostarda di frutta*	QS	125	125	NATCOL
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	Except chestnut puree	QS	30	70	NATCOL
04.2.5.3	Other similar fruit or vegetable spreads	Except *crème de pruneaux*	QS		30	NATCOL (No current usage)

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Reported use levels		Information provided by
				Typical	Maximum	
05.2	Other confectionery including breath refreshening microsweets		QS	30	75	NATCOL
				4	25	FDE (not representative of the EU market)
05.3	Chewing gum		QS	15	60	NATCOL
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		QS	50	50	NATCOL
				10	21	FDE
06.3	Breakfast cereals	Only fruit flavoured breakfast cereals	200	150*	200	NATCOL (Very limited use)
		Only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals	QS	150*	200	NATCOL (Very limited use)
06.5	Noodles		QS	150*	200	NATCOL (Very limited use)
06.6	Batters		QS	150*	200	NATCOL (Very limited use)
06.7	Pre-cooked or processed cereals		QS	150*	200	NATCOL (Very limited use)
07.2	Fine bakery wares		QS	50	150	NATCOL
08.3.1	Non-heat-treated processed meat	Only sausages	QS	20	30	NATCOL (Very limited use)
08.3.2	Heat-treated processed meat	Only sausages, patés and terrines	QS	15	60	NATCOL (Very limited use)
08.3.3	Casings and coatings and decorations for meat	Except edible external coating of pasturmas	QS	33*	60	NATCOL (Very limited use)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only fish paste and crustacean paste	QS	100*	150	NATCOL (Very limited use)
09.3	Processed fish and fishery products including molluscs and crustaceans	Except Sturgeons' eggs (Caviar)	QS	10*	20	NATCOL (Very limited use)
12.2.2	Seasonings and condiments	Only seasonings, for example curry powder, tandoori	QS	25	25	NATCOL (Very limited use)
12.4	Mustard		QS	10*	20	NATCOL (Very limited use)
12.5	Soups and broths		QS	75	75	NATCOL
12.6	Sauces	Excluding tomato-based sauces	QS	25	25	NATCOL
12.7	Salads and savoury based sandwich spreads		QS	10*	20	NATCOL

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Reported use levels		Information provided by
				Typical	Maximum	
12.9	Protein products, excluding products covered in category 1.8		QS	150*	200	NATCOL (Very limited use)
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	10	30	NATCOL
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	10	10	NATCOL (Very limited use.)
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009		QS	150*	200	NATCOL (Very limited use)
14.1.4	Flavoured drinks	Excluding chocolate milk and malt products	QS	10	30	NATCOL (very limited use)
			QS	2.35	2.6	FDE (for milk based fruit flavoured drinks)
14.2.3	Cider and perry	Excluding *cidre bouché*	QS	8*	15	NATCOL (Very limited use)
14.2.4	Fruit wine and made wine	Excluding *wino owocowe markowe*	QS	15*	30	NATCOL (Very limited use)
14.2.5	Mead		QS	15*	30	NATCOL (Very limited use)
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, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	QS	15*	30	NATCOL (Very limited use)
14.2.7.1	Aromatised wines	Except *Americano, bitter vino*	QS	15*	30	NATCOL (Very limited use)
14.2.7.2	Aromatised wine-based drinks	Except *bitter soda, sangria, claria, zurra*	QS	15*	30	NATCOL (Very limited use)
14.2.7.3	Aromatised wine-product cocktails		QS	15*	30	NATCOL (Very limited use)

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Reported use levels		Information provided by
				Typical	Maximum	
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	15*	30	NATCOL (Very limited use)
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	100	200	NATCOL (Very limited use)
15.2	Processed nuts		QS	150	200	NATCOL (Very limited use)
16	Desserts excluding products covered in categories 1, 3 and 4		QS	25	75	NATCOL
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		QS	15*	30	NATCOL (Very limited use)
17.2	Food supplements supplied in a liquid form		QS	8*	15	NATCOL (Very limited use)
17.3	Food supplements supplied in a syrup-type or chewable form		QS	8*	15	NATCOL (Very limited use)

* Industry provided minimum and maximum levels, but no typical levels. Here are calculated levels as the mean of max and min levels.

B. CONCENTRATION LEVELS OF BEETROOT RED (E 162) USED IN THE REFINED EXPOSURE SCENARIOS (MG BETANIN/KG OR ML BETANIN/KG AS APPROPRIATE)

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Concentration levels used in the exposure assessment		Data sources / comments
				Mean	Maximum	
01.4	Flavoured fermented milk products including heat-treated products		QS	25	75	
01.5	Dehydrated milk as defined by Directive 2001/114/EC	Except unflavoured products	QS	20	35	
01.6.3	Other creams	Only flavoured creams	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.1	Unripened cheese excluding products falling in category 16	Only flavoured unripened cheese	QS	20	35	
01.7.3	Edible cheese rind		QS	–	–	Not taken into account (no corresponding FoodEx code)
01.7.4	Whey cheese		QS	–	–	Not taken into account (reported by NATCOL as 'no current usage')
01.7.5	Processed cheese	Only flavoured processed cheese	QS	30	30	
01.7.6	Cheese products (excluding products falling in category 16)	Only flavoured unripened products	QS	–	–	Not taken into account (no corresponding FoodEx code)
01.8	Dairy analogues, including beverage whiteners		QS	–	–	Not taken into account (reported by NATCOL as 'no current usage')
03	Edible ices		QS	25	75	
04.2.1	Dried fruit and vegetables	Only preserves of red fruit	QS	60	100	
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only preserves of red fruit	QS	60	100	
04.2.2	Fruit and vegetables in vinegar, oil, or brine	Only vegetables (excluding olives)	QS	–	–	Not taken into account (no usage data available)
04.2.3	Canned or bottled fruit and vegetables	Only vegetables (excluding olives)	QS	–	–	Not taken into account (no corresponding FoodEx code)
04.2.4.1	Fruit and vegetable preparations excluding compote	Only mostarda di frutta	QS	–	–	Not taken into account (no corresponding FoodEx code)

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Concentration levels used in the exposure assessment		Data sources / comments
				Mean	Maximum	
04.2.4.1	Fruit and vegetable preparations excluding compote	Only vegetables (excluding olives)	QS	–	–	Not taken into account (reported by NATCOL as 'no current usage')
04.2.4.1	Fruit and vegetable preparations excluding compote	Only seaweed based fish roe analogues	QS	–	–	Not taken into account (no corresponding FoodEx code/no usage data available)
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut puree as defined by Directive 2001/113/EC	Except chestnut puree	QS	30	70	
04.2.5.3	Other similar fruit or vegetable spreads	Except crème de pruneaux		–	–	Not taken into account (reported by NATCOL as 'no current usage')
05.2	Other confectionery including breath refreshening microsweets		QS	30	75	
05.3	Chewing gum		QS	15	60	
05.4	Decorations, coatings and fillings, except fruit based fillings covered by category 4.2.4		QS	–	–	Not taken into account (no corresponding FoodEx code)
06.3	Breakfast cereals	Only breakfast cereals other than extruded, puffed and/or fruit flavoured breakfast cereals	QS	150	200	
06.3	Breakfast cereals	Only fruit flavoured breakfast cereals	200	150	200	
06.5	Noodles		QS	150	200	
06.6	Batters		QS	–	–	Not taken into account (no corresponding FoodEx code)
06.7	Pre-cooked or processed cereals		QS	–	–	Not taken into account (no corresponding FoodEx code)
07.2	Fine bakery wares		QS	50	150	

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Concentration levels used in the exposure assessment		Data sources / comments
				Mean	Maximum	
08.2	Meat preparations as defined by Regulation (EC) No 853/2004 (M42)	Only merguez type products, salsicha fresca, butifarra fresca, longaniza fresca and chorizo fresco	QS	–	–	Not taken into account (no usage data available)
08.3.1	Non-heat-treated meat products (M42)	Only sausages	QS	20	60	
08.3.2	Heat-treated meat products (M42)	Only sausages, patés and terrines	QS	15	60	
08.3.3	Casings and coatings and decorations for meat (M42)	Except edible external coating of pasturmas	QS	–	–	Not taken into account (no corresponding FoodEx code)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only surimi and similar products and salmon substitutes.	QS	100	150	
09.2	Processed fish and fishery products including molluscs and crustaceans	Only precooked crustacean	QS	–	–	Not taken into account (no usage data available)
09.2	Processed fish and fishery products including molluscs and crustaceans	Only fish paste and crustacean paste	QS	100	150	
09.3	Fish roe	Except Sturgeons' eggs (Caviar)	QS	10	20	
12.2.2	Seasonings and condiments	Only seasonings, for example curry powder, tandoori	QS	25	25	
12.4	Mustard		QS	10	20	
12.5	Soups and broths		QS	75	75	
12.6	Sauces	Excluding tomato-based sauces	QS	25	25	
12.7	Salads and savoury based sandwich spreads		QS	10	20	
12.9	Protein products, excluding products covered in category 1.8		QS	150	200	
13.2	Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		QS	10	30	

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Concentration levels used in the exposure assessment		Data sources / comments
				Mean	Maximum	
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	10	10	
13.4	Foods suitable for people intolerant to gluten as defined by Regulation (EC) No 41/2009		QS	150	200	
14.1.4	Flavoured drinks	Excluding chocolate milk and malt products	QS	10	30	
14.2.3	Cider and perry	Excluding cidre bouché	QS	8	15	
14.2.4	Fruit wine and made wine	Excluding wino owocowe markowe	QS	–	–	Not taken into account (no corresponding FoodEx code)
14.2.5	Mead		QS	–	–	Not taken into account (no corresponding FoodEx code)
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, Geist (with the name of the fruit or the raw material used), London Gin, Sambuca, Maraschino, Marrasquino or Maraskino and Mistrà	QS	15	30	
14.2.7.3	Aromatised wine-product cocktails		QS	15	30	Not taken into account (no corresponding FoodEx code)
14.2.8	Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol		QS	15	30	
15.1	Potato-, cereal-, flour- or starch-based snacks		QS	100	200	
15.2	Processed nuts		QS	150	200	

FCS category number	FCS Food category	Restrictions/exceptions	MPL	Concentration levels used in the exposure assessment		Data sources / comments
				Mean	Maximum	
16	Desserts excluding products covered in categories 1, 3 and 4		QS	25	75	
17.1	Food supplements supplied in a solid form including capsules and tablets and similar forms, excluding chewable forms		QS	15	30	
17.2	Food supplements supplied in a liquid form		QS	15	30	
17.3	Food supplements supplied in a syrup-type or chewable form		QS	15	30	

C. SUMMARY OF TOTAL ESTIMATED EXPOSURE OF BEETROOT RED (E 162) FROM THEIR USE AS FOOD ADDITIVES FOR THE MAXIMUM LEVEL EXPOSURE SCENARIO AND THE REFINED EXPOSURE ASSESSMENT SCENARIOS PER POPULATION GROUP AND SURVEY: MEAN AND HIGH LEVEL (MG/KG BW/DAY)

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Infants							
Bulgaria (NUTRICHILD)	659	0.2	0.9	0.2	0.9	0.1	0.3
Germany (VELS)	159	0.6	2.8	0.5	2.2	0.4	1.8
Denmark (IAT 2006 07)	826	0.6	1.5	0.5	1.3	0.3	0.8
Finland (DIPP 2001 2009)	500	0.3	0.7	0.3	0.7	0.2	0.5
United Kingdom (DNSIYC 2011)	1366	0.6	1.7	0.5	1.4	0.4	1.1
Italy (INRAN SCAI 2005 06)	12	0.2		0.2		0.2	
Toddlers							
Belgium (Regional Flanders)	36	2.1		1.6		1.0	
Bulgaria (NUTRICHILD)	428	0.7	1.8	0.6	1.5	0.3	0.7
Germany (VELS)	348	1.5	3.1	1.1	2.3	0.7	1.5
Denmark (IAT 2006 07)	917	1.0	1.9	0.7	1.4	0.5	0.9
Spain (enKid)	17	1.0		0.8		0.4	
Finland (DIPP 2001 2009)	500	0.9	2.1	0.8	1.7	0.5	1.1
United Kingdom (NDNS-RollingProgrammeYears1–3)	185	1.3	2.7	1.0	2.0	0.7	1.7
United Kingdom (DNSIYC 2011)	1314	1.2	2.5	0.9	1.9	0.7	1.6
Italy (INRAN SCAI 2005 06)	36	0.6		0.5		0.3	
Netherlands (VCP kids)	322	1.7	3.5	1.3	2.8	0.7	1.5
Children							
Austria (ASNS Children)	128	1.0	1.9	0.7	1.3	0.5	1.0
Belgium (Regional Flanders)	625	1.8	3.6	1.3	2.6	0.9	1.7
Bulgaria (NUTRICHILD)	433	0.9	2.0	0.7	1.6	0.3	0.7
Czech Republic (SISP04)	389	1.0	2.4	0.7	1.6	0.4	0.9
Germany (EsKiMo)	835	0.8	1.6	0.6	1.2	0.4	0.8
Germany (VELS)	293	1.5	2.7	1.0	1.9	0.6	1.2
Denmark (DANSDA 2005–08)	298	0.7	1.4	0.5	1.0	0.3	0.6
Spain (enKid)	156	0.9	2.1	0.6	1.4	0.4	0.9
Spain (NUT INK05)	399	0.9	1.8	0.6	1.3	0.4	0.9
Finland (DIPP 2001 2009)	750	0.8	1.6	0.6	1.2	0.4	0.7
France (INCA2)	482	1.1	2.0	0.8	1.5	0.5	0.9
United Kingdom (NDNS-RollingProgrammeYears1–3)	651	1.1	2.1	0.8	1.5	0.6	1.2
Greece (Regional Crete)	838	1.0	2.0	0.8	1.5	0.5	1.1
Italy (INRAN SCAI 2005 06)	193	0.6	1.3	0.5	1.0	0.2	0.5
Latvia (EFSA TEST)	187	1.1	2.3	0.9	1.8	0.7	1.4
Netherlands (VCP kids)	957	1.6	3.2	1.1	2.4	0.6	1.3

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Netherlands (VCPBasis AVL2007 2010)	447	1.4	2.5	1.0	1.8	0.6	1.1
Sweden (NFA)	1473	1.5	2.9	1.0	1.8	0.7	1.3
Adolescents							
Austria (ASNS Children)	237	0.6	1.2	0.4	0.9	0.3	0.6
Belgium (Diet National 2004)	576	0.7	1.3	0.5	1.0	0.3	0.7
Cyprus (Childhealth)	303	0.3	0.6	0.2	0.5	0.1	0.3
Czech Republic (SISP04)	298	0.7	1.6	0.5	1.1	0.3	0.6
Germany (National Nutrition Survey II)	1011	0.5	1.2	0.4	1.0	0.2	0.6
Germany (EsKiMo)	393	0.6	1.3	0.5	0.9	0.3	0.6
Denmark (DANSDA 2005–08)	377	0.4	0.9	0.3	0.6	0.2	0.4
Spain (AESAN FIAB)	86	0.4	0.9	0.3	0.7	0.1	0.3
Spain (enKid)	209	0.5	1.2	0.4	0.9	0.2	0.5
Spain (NUT INK05)	651	0.5	1.0	0.4	0.8	0.2	0.5
Finland (NWSSP07 08)	306	0.3	0.8	0.3	0.6	0.1	0.3
France (INCA2)	973	0.5	1.1	0.4	0.9	0.2	0.5
United Kingdom (NDNS-RollingProgramme Years1–3)	666	0.6	1.3	0.4	0.9	0.3	0.7
Italy (INRAN SCAI 2005 06)	247	0.3	0.8	0.2	0.6	0.1	0.3
Latvia (EFSA TEST)	453	0.7	1.5	0.5	1.2	0.4	0.9
Netherlands (VCPBasis AVL2007 2010)	1142	0.9	1.8	0.6	1.3	0.4	0.8
Sweden (NFA)	1018	0.8	1.6	0.6	1.1	0.4	0.7
Adults							
Austria (ASNS Adults)	308	0.4	1.0	0.3	0.8	0.2	0.6
Belgium (Diet National 2004)	1292	0.5	1.0	0.4	0.8	0.3	0.6
Czech Republic (SISP04)	1666	0.3	0.8	0.2	0.6	0.1	0.3
Germany (National Nutrition Survey II)	10419	0.4	0.9	0.3	0.7	0.2	0.5
Denmark (DANSDA 2005–08)	1739	0.2	0.5	0.2	0.4	0.1	0.2
Spain (AESAN)	410	0.3	0.7	0.2	0.5	0.1	0.3
Spain (AESAN FIAB)	981	0.2	0.6	0.2	0.5	0.1	0.2
Finland (FINDIET2012)	1295	0.5	1.3	0.4	1.0	0.3	0.8
France (INCA2)	2276	0.3	0.7	0.2	0.5	0.1	0.3
United Kingdom (NDNS-RollingProgrammeYears1–3)	1266	0.3	0.7	0.3	0.6	0.2	0.4
Hungary (National Repr Surv)	1074	0.2	0.5	0.1	0.4	0.1	0.2
Ireland (NANS 2012)	1274	0.4	0.9	0.3	0.7	0.2	0.6
Italy (INRAN SCAI 2005 06)	2313	0.2	0.4	0.1	0.3	0.1	0.2
Latvia (EFSA TEST)	1271	0.4	0.9	0.3	0.7	0.2	0.6
Netherlands (VCPBasis AVL2007 2010)	2057	0.5	1.1	0.4	0.8	0.2	0.5

	Number of subjects	Maximum level scenario		Brand-loyal scenario		Non-brand-loyal scenario	
		Mean	High level	Mean	High level	Mean	High level
Romania (Dieta Pilot Adults)	1254	0.2	0.5	0.2	0.4	0.1	0.3
Sweden (Riksmaten 2010)	1430	0.4	0.8	0.3	0.6	0.2	0.4
The elderly							
Austria (ASNS Adults)	92	0.4	0.8	0.3	0.7	0.2	0.5
Belgium (Diet National 2004)	1215	0.4	0.9	0.3	0.7	0.3	0.6
Germany (National Nutrition Survey II)	2496	0.3	0.8	0.3	0.6	0.2	0.5
Denmark (DANSDA 2005–08)	286	0.2	0.4	0.1	0.3	0.1	0.2
Finland (FINDIET2012)	413	0.6	1.4	0.5	1.2	0.3	0.9
France (INCA2)	348	0.2	0.5	0.2	0.5	0.1	0.2
United Kingdom (NDNS-RollingProgrammeYears1–3)	305	0.4	0.8	0.3	0.6	0.2	0.5
Hungary (National Repr Surv)	286	0.1	0.4	0.1	0.3	0.1	0.1
Ireland (NANS 2012)	226	0.4	1.1	0.4	0.9	0.3	0.7
Italy (INRAN SCAI 2005 06)	518	0.1	0.3	0.1	0.2	0.0	0.1
Netherlands (VCPBasis AVL2007 2010)	173	0.4	0.8	0.3	0.7	0.2	0.5
Netherlands (VCP-Elderly)	739	0.4	0.9	0.3	0.7	0.2	0.6
Romania (Dieta Pilot Adults)	128	0.2	0.5	0.2	0.4	0.1	0.4
Sweden (Riksmaten 2010)	367	0.4	0.7	0.3	0.6	0.2	0.4

GLOSSARY AND ABBREVIATIONS

ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
AFB_1	aflatoxin B_1
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
BIBRA	British Industrial Biological Research Association
bw	body weight
CAS	Chemical Abstracts Service
CIAA	Confederation of the Food and Drink Industries of the EU, now Food Drink Europe
CONTAM	EFSA Panel on Contaminants in the Food Chain
EC	European Commission
EINECS	European Inventory of Existing Commercial chemical Substances
FAO	Food and Agriculture Organization of the United Nations
FCS	Food Categorisation System
FDA	US Food and Drug Administration
FDE	FoodDrinkEurope
HPLC-DAD	high-performance liquid chromatography with diode-array detection
HPLC	high-performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC/MS	liquid chromatography/mass spectrometry
MPL	maximum permitted use level
NATCOL	Natural Food Colours Association
QS	quantum satis
SCE	sister chromatid exchange
SCF	Scientific Committee on Food
UVA	ultraviolet A (ranges from 400 to 320 nm)
WHO	World Health Organization