Consumer exposures to anthocyanins from colour additives, colouring foodstuffs and from natural occurrence in foods

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\textbf{ABSTRACT}

Anthocyanins are responsible for the red/blue colour of grapes, currants, and other fruits and vegetables. They may also be extracted for use as colour additives (E163) or concentrated for use as colouring foods. Consumer exposures have been assessed using data on natural occurrence, use levels and frequencies from food manufacturers and European food consumption data. Intakes from natural occurrence can be up to 4 mg kg bw\textsuperscript{−1} day\textsuperscript{−1} at the mean and up to 17 mg kg bw\textsuperscript{−1} day\textsuperscript{−1} for children who are high level consumers of red/black berries and small fruits. High-level intakes for children from food colour and colouring food applications lie in the range 0.3–6.3 mg kg bw\textsuperscript{−1} day\textsuperscript{−1} and for adults at 0.6–2.8 mg kg bw\textsuperscript{−1} day\textsuperscript{−1}. Exposures from food colour use and colouring foods separately or combined are therefore lower than those from natural occurrence in foods.

\textbf{Introduction}

Anthocyanins are responsible for the red/blue colour of grapes, currants, and other fruits and vegetables and are potentially one of the most important naturally occurring antioxidants (Zafra-Stone et al. 2007; Pérez-Jiménez et al. 2011). Their consumption has been linked to risk reduction in some human illness including hypertension (Cassidy et al. 2011) and possibly cancer (Zamora-Ros et al. 2011).

Anthocyanins (E163) prepared by physical means from fruits and vegetables are authorised food colouring substances in the European Union (EU) with an MPL of 200 mg kg\textsuperscript{−1} food in fruit-flavoured breakfast cereals (Commission Regulation (EU) No. 1129/2011). Anthocyanins (E163) are also permitted at ‘quantum satis’ in all other foods except for a few in which the use of food additives is specifically prohibited or restricted. In addition to the use of anthocyanins (E163) as such, there is limited use of anthocyanin lakes in some panned confectionery and edible ices.

In April 2013 the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) published a detailed Scientific Opinion on the revaluation of anthocyanins (E163) as a food additive (EFSA 2013) and from its natural occurrence in foods. Information about typical and maximum usage of anthocyanins in a range of food categories was provided to EFSA by the Natural Colours Association (NATCOL) following a data call. These data were used as the basis for a set of input data for use in the ANS exposure assessment.

Although NATCOL had provided a range of use levels corresponding to different colour tones, the ANS Panel used only the highest reported level associated with the most intense colours for use in the exposure assessment. For categories such as noodles, soup, processed meat, processed fish and dietary foods, where NATCOL had no information about use, the panel invoked ‘quantum satis (QS) rules’ to provide a value (EFSA 2013, Annex A). The QS rules adopt either an analytical value, the MPL value from the Codex Committee on Food Additives (CCFA) (if either of these is available) or extrapolate a value. If many values are available for other colours in the same food category, then the highest of these will be used. Some food categories are not included in the EFSA Comprehensive European Food Consumption Database and therefore were not taken into account in the ANS assessment.

Exposure to anthocyanins from food additive use was estimated using the food additives intake model (FAIM), available on the EFSA website (EFSAa). The age group with the highest intakes was children aged 3–9 years with high-level intakes up to 7.8 mg kg bw\textsuperscript{−1} day\textsuperscript{−1} in Belgium. The highest contributors to average intake were processed
meat, flavoured drinks, dehydrated milk, and processed fruit and vegetables. Two of these categories have regulatory restrictions such as ‘only red marbled cheese’ that were not taken into account in the assessment.

The panel also considered the typical exposure to anthocyanins naturally present in the diet from foods such as fruits and vegetables using food consumption data from Irish food consumption surveys only. For Irish adults, mean intakes were reported to be 8.6 mg day\(^{-1}\) (0.1 mg kg bw\(^{-1}\) day\(^{-1}\)) and intakes at the 97.5th percentile were 46.5 mg day\(^{-1}\) (0.6 mg kg bw\(^{-1}\) day\(^{-1}\)). For Irish children, mean intakes were reported to be 7.9 mg day\(^{-1}\) (0.3 mg kg bw\(^{-1}\) day\(^{-1}\)) and intakes at the 97.5th percentile were 53.7 mg day\(^{-1}\) (2.1 mg kg bw\(^{-1}\) day\(^{-1}\)) for the total population.

The ANS Panel concluded that exposure estimates to anthocyanins via the regular diet are lower than the highest exposure estimates from the use of anthocyanins as food additive and so the maximum permitted levels of anthocyanins in grape skin and blackcurrant extracts might need to be revised. The panel also noted that there were no data to estimate exposure to anthocyanins from colouring foods.

The EFSA FAIM model is known to produce conservative results which can produce exposure estimates that are two to three times higher the values produced by exposure models based on the same use levels but using raw data from food consumption surveys (EFSA 2014a). Furthermore, there have been revisions made to the ANS Panel exposure modelling approach that includes scenarios taking into account consumer loyalty (see, for example, the EFSA ANS Panel Opinion on the re-evaluation of Indigo Carmine, E132; EFSA 2014b). EFSA has also recently released a more detailed version of the European Comprehensive Food Consumption Database (EFSAb). NATCOL has investigated use levels for anthocyanins as colouring foods and the rates of occurrence of colour additive and colouring food applications.

These developments make it possible to produce revised estimates of exposure to anthocyanins from natural sources, food additive use and colouring food applications that are directly comparable between countries. This report provides a new evaluation of anthocyanins exposure based on EFSA food consumption data, levels of anthocyanins in foods published in the EFSA Opinion and using up-to-date ANS Panel methodology. Information about the rates of occurrence of anthocyanins in foods is also taken into account.

**Materials and methods**

**Levels of anthocyanins in foods**

**Natural occurrence**

Levels of anthocyanins are based on those published in the 2013 EFSA Opinion on anthocyanins which were taken from Wu et al. (2006). Where more than one variety of a fruit or vegetable has been reported, the average was used in exposure calculations. The Wu et al. data have been supplemented by data from the Phenol Explorer Database of Polyphenols in Food (Phenol Explorer), Boulton (2001) and Netzel et al. (2001), which contain additional information about anthocyanins levels in beverages. The combined data used for exposure modelling are provided in Table 1.

| Fruit                  | Mean | Reference    | Vegetables     | Mean | Reference |
|------------------------|------|--------------|----------------|------|-----------|
| 1. Apple               | 53   | Wu et al.    | 1. Black bean  | 445  | Wu et al. |
| 2. Blackberry          | 2725 | Wu et al.    | 2. Eggplant    | 857  | Wu et al. |
| 3. Blueberry           | 4363 | Wu et al.    | 3. Red cabbage | 3220 | Wu et al. |
| 4. Cherry, sweet       | 1220 | Wu et al.    | 4. Red leaf lettuce | 22   | Wu et al. |
| 5. Chokeberry          | 14 800 | Wu et al. | 5. Red onion | 485  | Wu et al. |
| 6. Cranberry           | 140  | Wu et al.    | 6. Red radish  | 1001 | Wu et al. |
| 7. Currant             | 2444 | Wu et al.    | 7. Small red bean | 67   | Wu et al. |
| 8. Elderberry          | 13 750 | Wu et al. |            |      |           |
| 9. Gooseberry          | 37   | Wu et al.    |                |      |           |
| 10. Grape              | 168  | Wu et al.    |                |      |           |
| 11. Nectarine          | 68   | Wu et al.    |                |      |           |
| 12. Peach              | 48   | Wu et al.    |                |      |           |
| 13. Plum               | 718  | Wu et al.    |                |      |           |
| 14. Raspberry          | 3896 | Wu et al.    |                |      |           |
| 15. Strawberry         | 315  | Wu et al.    |                |      |           |
| Bilberry               | 2990 | Phenol explorer |            |      |           |
| Fruit jam              | 155  | Phenol explorer |            |      |           |
| Fig, whole, fresh      | 24   | Phenol explorer |            |      |           |
| Cranberry juice        | 98   | Milbury et al. |            |      |           |
| Blackcurrant juice     | 765  | Netzel et al. |            |      |           |

Note: Wu et al. data are numbered according to the EFSA Opinion.
Use levels for food colour additive E163
Use levels data for anthocyanins E163 were based on those provided by NATCOL to EFSA and published in the 2013 ANS report on anthocyanins (EFSA 2013). Where NATCOL had not provided a use level the value proposed by EFSA based on QS rules was not used since these were based on the use of completely different colour additives. No account was taken of differences in colour index and so the use levels proposed would not necessarily produce a desirable colour tone. Instead, values for anthocyanin use in similar food matrices have been applied to those categories where data were missing.

Anthocyanins usage data were matched with food codes at Level 3 in the EFSA FoodEx system. A conservative approach has been followed whereby any food within the broader FoodEx Level 2 category that could be coloured red was assumed to be coloured with anthocyanins. The colour additive usage data provide as a modelling input table are provided in the supplemental data online.

The data in the supplemental data online, Part A, include the typical and maximum values reported by NATCOL. Typical values relate to the most common colour tones used for products which frequently require a pink tone (for strawberry, for example). Maximum values relate to more intense colour tones that are less frequently used (as in some deep red aniseed flavoured confectionery).

Use levels for colouring foodstuffs (CFS) applications
The 2013 ANS report on anthocyanins did not contain any information on colouring foods because the assessment predated European Commission Guidance on this subject (European Commission 2013). Colouring foods differ from food colours in that the colouring principle is obtained by concentration and not selective extraction. NATCOL has contacted its members who produce anthocyanins are colour extract (E163) and concentrate (CFS) and they report that the colour level in a given food is identical. The choice between E163 and CFS use is made by the food manufacture based on the foods physical properties and other factors. As a consequence the concentration of anthocyanins is the same as those reported in the supplemental data online, Part A regardless of whether E163 or CFS is being used.

Food additive (E163) and colouring foods occurrence data
The Mintel Global New Products Database (GNPD) gathers data on new product launches and provides an insight into current trends of food additive and ingredient use over recent years and across EU member states but may exclude some products that have not been subject to a recent relaunch (Mintel). The GNPD has been searched over the period 2010–15 to identify the total number of products launched in European countries containing anthocyanins food colour (E163) or colouring foods uses at the highest level of sub-categorisation available. The GNPD database was searched for all EU countries and all food/beverage categories using two search criteria:

- For anthocyanin extracts (E163): one or more of grape colour extract, blueberry extract, black radish extract, cabbage extract, radish extract, red radish extract, sweet potato extract or anthocyanins in the ingredients.
- For anthocyanin colouring foods (CFS): one or more of black gooseberry concentrate, black grape concentrate, radish concentrate, purple carrot extract, elderberry juice concentrate, red fruit concentrate or black carrot extract in the ingredients.

The total number of product launches for each food sub-category in the Mintel data base in each country that contained E163 (see the supplemental data online, Part B1) or CFS (see Part B2) was divided by the total number of product launches for each category (see Part B3) to derive the percentage occurrence of E163 or CFS as an ingredient on product launch labels (see Parts B4 and B5 respectively). For some European countries no products containing E163 or CFS in some food categories were identified. Overall occurrence rates were similar for E163 (1.6%) and CFS (1.0%) applications. Patterns of use within food categories were also similar suggesting a large degree of interchangeability.

For food categories that contained anthocyanins, the majority (84% for E163 and 91% for CFS) had occurrence rates of less than 10% containing anthocyanins. About 15% (E163) or 9% (CFS) of categories had occurrence rates between 10% and 50% containing anthocyanins. These were categories such as non-alcoholic beverages and confectionery where food colours are most likely to be used. In a small number of cases the frequency of occurrence appeared to be much higher; up to 100% in some cases. These were limited to situations where there were relatively few product releases so the so that if these were all or mostly containing anthocyanins then the proportion appeared to be very high. To maintain a conservative approach, the highest occurrence rate for each food category in any European country was identified and this value was used in the exposure estimates (see the columns headed...
‘E163 Occ’ or ‘CFS Occ’ in the supplemental data online, Part A).

In the period between 2005 and 2015, the ratio of anthocyanins used in colouring foods to those used in food additives (E163) steadily increased from 31% to 42% suggesting a gradual shift from food colour use to CFS.

**Modelling consumer exposures to anthocyanins**

Each national survey in the EFSA Comprehensive European Food Consumption Database has been extracted into a new dietary exposure model based on the methodology of the EFSA Food Additive Intake Model (FAIM) in which the population average and consumers P95 intake from each food category is calculated (Tennant 2016). A high-level total intake for each food category is estimated by adding the highest P95 value from any group to the sum of the population averages for all other foods. The highest high level total intake from any food is taken as the high intake for each population group.

The EFSA ANS Panel has introduced a refinement to their exposure assessment approach in some recent Opinions (see, for example, the re-evaluation of Scientific Opinions on indigo carmine; EFSA 2014b). This introduces new scenarios in addition to using just the MPL or maximum level of actual usage data as provided in the FAIM model by making use of supplied mean or typical use levels. The new approach takes account of the potential for brand loyalty in two additional scenarios:

- Brand-loyal consumer scenario: intakes are estimated by combining food consumption with the maximum of the maximum reported use levels or the maximum of the analytical results for the main contributing food category at the individual level, using the mean of the typical reported use levels or the mean of analytical results for the remaining food categories.

- Non-brand-loyal consumer scenario: it is assumed that the population is exposed long-term to the food additive present at the mean reported use/analytical levels in food. This exposure estimate is calculated using the mean of the typical reported use levels or the mean of analytical results for all food categories.

Exposure models can therefore be used with usage data in three scenarios (Table 2). Where typical or average use levels are not available, then the model will return the same result for all three scenarios. In the case of anthocyanins from natural sources, only one scenario is reported that represents average levels of anthocyanins in all food categories. For anthocyanins from food additive (E163) and colouring food (CFS) uses it is possible to apply the maximum and typical use levels provided in the supplemental data online, Part A. In addition, occurrence estimates based on the maximum observed frequency of occurrence in any EU country can be applied to the model to create a more refined estimate of exposure. Occurrence data are not used in scenario A. When included, occurrence data are applied in scenario B to average intakes only and do not apply to high-level intake for the ‘loyal’ consumer. Occurrence data are applied to all data in scenario C when they are included in the model.

Food consumption data from Level 3 of the 2015 EFSA Comprehensive European Food Consumption Database were extracted into a set of linked Microsoft Excel workbooks. The linked workbooks create a program that automatically calculates the levels of intake in mg kg bw⁻¹ day⁻¹ for an additive or other food component for each food category in each age category in each country, when data on use levels are entered (Tennant 2016). The result are reported in standard templates (see the supplemental data online, C1–C4) and summarised in standard tables (Tables 3–6).

**Table 2. Methods/scenarios used in additive exposure modelling.**

| Method/scenario | Additive usage assumptions |
|-----------------|---------------------------|
| A ‘MPL’         | Maximum-use levels in all food categories. Corresponds to the EFSA ANS Panel maximum scenario |
| B ‘Loyal’       | Maximum level in the highest intake category, typical use levels in all others. Corresponds to the EFSA ANS Panel ‘Brand-loyal’ scenario |
| C ‘Typical’     | Typical-use level in all categories. Corresponds to the EFSA ANS Panel ‘non-brand loyal’ scenario |

**Table 3. Potential intakes of anthocyanins from natural occurrence in food (mg kg bw⁻¹ day⁻¹).**

| Population average intake | Total high-level intake |
|---------------------------|-------------------------|
| Infants                   |                         |
| Minimum                   | 0.11                    | 0.15                      |
| Maximum                   | 2.72                    | 16.07                     |
| Toddlers                  |                         |
| Minimum                   | 0.18                    | 0.19                      |
| Maximum                   | 4.00                    | 17.12                     |
| Other children            |                         |
| Minimum                   | 0.09                    | 0.36                      |
| Maximum                   | 2.38                    | 12.95                     |
| Adolescents               |                         |
| Minimum                   | 0.05                    | 0.16                      |
| Maximum                   | 0.58                    | 3.21                      |
| Adults                    |                         |
| Minimum                   | 0.11                    | 0.52                      |
| Maximum                   | 0.95                    | 8.90                      |
| Elderly                   |                         |
| Minimum                   | 0.16                    | 0.18                      |
| Maximum                   | 1.61                    | 8.25                      |
| Very elderly              |                         |
| Minimum                   | 0.16                    | 0.22                      |
| Maximum                   | 0.93                    | 2.46                      |
Table 4. Potential intakes of anthocyanins from food colour (E163) and colouring food use (mg kg bw\(^\text{−1}\) day\(^\text{−1}\)).

| Population average intake | Total high-level intake Method A | Method B | Method C |
|---------------------------|---------------------------------|----------|----------|
| Infants                   | Minimum 0.10                    | 0.27     | 0.14     | 0.14     |
|                           | Maximum 0.43                    | 2.19     | 1.94     | 1.55     |
| Toddlers                  | Minimum 0.30                    | 0.59     | 0.30     | 0.00     |
|                           | Maximum 1.33                    | 7.43     | 6.85     | 2.38     |
| Other children            | Minimum 0.23                    | 0.69     | 0.49     | 0.00     |
|                           | Maximum 1.08                    | 5.68     | 4.80     | 2.27     |
| Adolescents               | Minimum 0.12                    | 0.30     | 0.15     | 0.15     |
|                           | Maximum 0.51                    | 3.42     | 3.06     | 1.01     |
| Adults                    | Minimum 0.06                    | 0.52     | 0.39     | 0.20     |
|                           | Maximum 0.31                    | 2.22     | 2.04     | 1.67     |
| Elderly                   | Minimum 0.05                    | 0.20     | 0.15     | 0.10     |
|                           | Maximum 0.29                    | 1.37     | 1.24     | 0.76     |
| Very elderly              | Minimum 0.05                    | 0.16     | 0.08     | 0.08     |
|                           | Maximum 0.29                    | 1.77     | 1.59     | 0.61     |

Table 5. Potential intakes of anthocyanins from food colour (E163) use after taking occurrence into account (mg kg bw\(^\text{−1}\) day\(^\text{−1}\)).

| Population average intake | Total high level intake Method A | Method B | Method C |
|---------------------------|---------------------------------|----------|----------|
| Infants                   | Minimum 0.00                    | 0.27     | 0.00     | 0.00     |
|                           | Maximum 0.03                    | 2.19     | 1.72     | 0.14     |
| Toddlers                  | Minimum 0.03                    | 0.59     | 0.03     | 0.00     |
|                           | Maximum 0.11                    | 7.43     | 6.28     | 0.31     |
| Other children            | Minimum 0.02                    | 0.69     | 0.32     | 0.00     |
|                           | Maximum 0.09                    | 5.68     | 4.25     | 0.26     |
| Adolescents               | Minimum 0.01                    | 0.30     | 0.01     | 0.01     |
|                           | Maximum 0.05                    | 3.42     | 2.78     | 0.13     |
| Adults                    | Minimum 0.00                    | 0.52     | 0.28     | 0.02     |
|                           | Maximum 0.03                    | 2.22     | 1.84     | 0.59     |
| Elderly                   | Minimum 0.00                    | 0.20     | 0.02     | 0.01     |
|                           | Maximum 0.02                    | 1.37     | 1.07     | 0.04     |
| Very elderly              | Minimum 0.00                    | 0.16     | 0.00     | 0.00     |
|                           | Maximum 0.02                    | 1.77     | 1.36     | 0.05     |

Table 6. Potential intakes of anthocyanins from colouring food (CFS) use after taking occurrence into account (mg kg bw\(^\text{−1}\) day\(^\text{−1}\)).

| Population average intake | Total high level intake Method A | Method B | Method C |
|---------------------------|---------------------------------|----------|----------|
| Infants                   | Minimum 0.00                    | 0.27     | 0.00     | 0.00     |
|                           | Maximum 0.02                    | 2.19     | 1.72     | 0.10     |
| Toddlers                  | Minimum 0.02                    | 0.59     | 0.02     | 0.00     |
|                           | Maximum 0.07                    | 5.68     | 4.24     | 0.20     |
| Other children            | Minimum 0.01                    | 0.69     | 0.32     | 0.00     |
|                           | Maximum 0.03                    | 3.42     | 2.77     | 0.09     |
| Adolescents               | Minimum 0.01                    | 0.30     | 0.01     | 0.01     |
|                           | Maximum 0.03                    | 3.42     | 2.77     | 0.09     |
| Adults                    | Minimum 0.00                    | 0.52     | 0.28     | 0.01     |
|                           | Maximum 0.02                    | 2.22     | 1.84     | 0.17     |
| Elderly                   | Minimum 0.00                    | 0.20     | 0.01     | 0.00     |
|                           | Maximum 0.01                    | 1.37     | 1.06     | 0.03     |
| Very elderly              | Minimum 0.00                    | 0.16     | 0.00     | 0.00     |
|                           | Maximum 0.01                    | 1.77     | 1.35     | 0.03     |

Results

Anthocyanins from natural occurrence in foods

The modelling results are provided in the supplemental data online, C1, and summarised in Table 3. Average intakes range from less than 0.1 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) up to 4.0 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) for toddlers in Finland and high-level intake ranged up to 17 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) for UK toddlers and infants. The main sources of high-level intakes were the consumption of berries and small fruit of several varieties by children. For adults, red wine was frequently a significant contributor to exposure. The highest anthocyanins intakes were observed in Bulgaria, Finland, Germany and the UK. Adult intakes were influenced by several food categories associated with high consumption but few consumers. When these were removed the highest intakes were 5.7 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) in Finland. There were notable differences between countries indicating differences in consumption patterns, particularly of red or black berries and small fruit. It would not therefore be realistic to take one country to represent the whole of Europe.

Anthocyanins from food colour use (E163)

Data from the supplemental data online, Part A were matched with FoodEx Level 3 codes and installed into the modelling system. In the first round of assessment occurrence data were not used and so it was assumed that all foods in every category where anthocyanins were used would always contain anthocyanins at the typical or maximum levels in Part A. The modelling results are provided in the supplemental data online, C2, and summarised in Table 4. Average intakes ranged from less than 0.1 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) up to 1.3 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) for toddlers in Belgium and high-level intake ranged up to 7.4 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) for Finnish toddlers based on maximum use levels (scenario A) throughout. The main sources of high-level intakes were non-alcoholic beverages. For adults, cider also appeared to be a significant potential source of intake for the relatively small proportion of consumers in Ireland and the UK.

When the loyal consumer scenario was considered (scenario B), high-level intakes fell slightly to up to 6.9 mg kg bw\(^\text{−1}\) day\(^\text{−1}\) because the background intake from other foods was reduced. In situations where strong consumer loyalty was not present (scenario C), then a high level consumer would be assumed to on average consume products containing typical use levels, and high-level intakes would fall to below 2.4 mg kg bw\(^\text{−1}\) day\(^\text{−1}\).

The model was rerun after including the occurrence data for food colour uses (E163) listed under 'E163
Occ’ in the supplemental data online, Part A. Occurrence data were applied to average intakes in scenarios B and C and to P95 intakes in scenario C only. The modelling results are provided in the supplemental data online, C3, and summarised in Table 5. Average intakes range from less than 0.1 mg kg bw$^{-1}$ day$^{-1}$ up to 0.11 mg kg bw$^{-1}$ day$^{-1}$ for toddlers in the Netherlands and high-level intakes under scenario A remained the same. When the loyal consumer scenario was considered (scenario B) with occurrence data applied to average intakes from other foods, high-level intakes fell slightly to up to 6.3 mg kg bw$^{-1}$ day$^{-1}$. In situations where strong consumer loyalty was not present (scenario C) and occurrence data were applied to all intake values, then high-level intakes would fall to below 0.6 mg kg bw$^{-1}$ day$^{-1}$ for a small number of adult cider consumers in Ireland and the UK. For other consumers, the main sources of high-level intakes were non-alcoholic beverages and flavoured fermented desserts (yoghurt).

**Anthocyanins from colouring food use (CFS)**

Use levels for anthocyanins as colouring foods and for colour additive use are identical and so potential intakes, before taking occurrence data into account, are the same (Table 4). When the model was rerun for colouring foods (CFS) using occurrence data for colouring food uses (CFS) listed under ‘CFS Occ’ in the supplemental data online, Part A, the results were similar to but slightly lower than the equivalent results for additive use (see the supplemental data online, C4, and summarised in Table 6). When the loyal consumer scenario was considered (scenario B) high-level intakes fell slightly to up to 6.3 mg kg bw$^{-1}$ day$^{-1}$. In situations where strong consumer loyalty was not present (scenario C) high-level intakes would fall to below 0.2 mg kg bw$^{-1}$ day$^{-1}$ for toddler consumers of flavoured fermented desserts in the Netherlands. For other consumers, the main sources of high-level intakes were non-alcoholic beverages and flavoured fermented desserts (yoghurt) and cider.

**Total anthocyanins from colour additive and colouring food use (CFS)**

Total intakes of anthocyanins from colour additive and colouring food uses can be estimated by adding the frequencies of occurrence for E163 and CFS use together (listed under ‘Total Occ’ in the supplemental data online, Part A). The results were similar to but slightly higher than the equivalent results for additive-only use (see the supplemental data online, C5, and summarised in Table 7). When the loyal consumer scenario was considered (scenario B) high-level intakes remained at 6.3 mg kg bw$^{-1}$ day$^{-1}$. In situations where strong consumer loyalty was not present (scenario C) high-level intakes would fall to below 0.8 mg kg bw$^{-1}$ day$^{-1}$ for adult cider consumers in Ireland and the UK.

### Table 7. Potential intakes of anthocyanins from E163 and colouring food use after taking combined E 63 and CFS occurrence into account (mg kg bw$^{-1}$ day$^{-1}$).

| Population | Method A | Method B | Method C |
|------------|----------|----------|----------|
| Infants    | Minimum  | 0.01     | 0.27     | 0.01     |
|            | Maximum  | 0.05     | 2.19     | 1.73     | 0.24     |
| Toddlers   | Minimum  | 0.05     | 0.59     | 0.05     |
|            | Maximum  | 0.18     | 7.43     | 6.32     | 0.53     |
| Other children | Minimum | 0.03     | 0.69     | 0.33     | 0.00     |
|            | Maximum  | 0.16     | 5.68     | 4.29     | 0.46     |
| Adolescents| Minimum  | 0.02     | 0.30     | 0.02     |
|            | Maximum  | 0.08     | 3.42     | 2.79     | 0.21     |
| Adults     | Minimum  | 0.01     | 0.02     | 0.03     |
|            | Maximum  | 0.04     | 2.22     | 1.85     | 0.76     |
| Elderly    | Minimum  | 0.01     | 0.20     | 0.03     |
|            | Maximum  | 0.03     | 1.37     | 1.08     | 0.07     |
| Very elderly| Minimum | 0.01     | 0.16     | 0.01     |
|           | Maximum  | 0.03     | 1.77     | 1.37     | 0.07     |

### Uncertainty analysis

#### Model uncertainties

Estimates of exposure to anthocyanins from natural occurrence and the use of plant extracts (E163) and concentrates (CFS) are subject to considerable uncertainties. The exposure model is based on the EFSA FAIM model which is designed to be a screening tool and so will always tend to overestimate intakes. EFSA has reported that used in this way the model will tend to overestimate intakes two- to three-fold (EFSA 2014a). The model used in this report differs from the FAIM model in that it is based in data from the 2015 release of the EFSA Comprehensive European Food Consumption Database and applies the more recent EFSA ANS Panel approach to address consumer loyalty.

#### Parameter uncertainties

Natural occurrence of anthocyanins is related to the species and variety of the fruit, the level of ripeness before picking, duration of storage and the degree of processing of fruit-based beverages. However, once average anthocyanin concentrations in foods have been established (Table 1) these can be linked to consumption data relating to the same foods to determine potential intakes.

Levels of anthocyanins related to food colour or colouring food use in beverages and other foods are subject to additional uncertainty because they will relate to the amount added, which will not necessarily...
correspond to the maximum amount reported, and to the proportion of products that contain the additive. For scenario A estimates data on maximum use levels only have been applied (see the supplemental data online, Part A) and so it is assumed that all foods in which anthocyanins are permitted contain the maximum concentration at all times. Since not all foods are coloured and those that are do not necessarily contain anthocyanins, this can result in significant overestimation of true intakes.

Additive and colouring foods manufacturers have been able to provide more detailed information about the necessary use levels for different food types (see the supplementary data online, Part A). In addition data from the Mintel GNPD can be used to indicate the proportion of beverages within given categories that actually contain anthocyanins from food additive or colouring foods (see the supplemental data online, B). The information about actual use levels and frequency of occurrence can be used to develop more realistic scenarios of anthocyanin exposure from food additive and colouring food uses. Mintel GNPD data do not represent overall usage but only products launched during the previous 5 years. They therefore do not give an absolute indication of usage but provide an indication, which if used cautiously, can be used to moderate exposure data. The highest reported occurrence rate in any country was used to describe occurrence across the whole EU in an effort to maintain a conservative approach. This can introduce considerable uncertainties when there were limited numbers of product releases in some countries. Germany was included in this part of the assessment although national labelling regulations mean that food additive or colouring foods information may not necessarily recorded on the label. European labelling regulations do not require that alcoholic beverages include E163 or CFS use on labels and so such data may be unreliable.

The use of actual use levels and frequency of occurrence must be moderated against the possibility that a high volume consumer has a strong loyalty to a particular product that contains added anthocyanins at the maximum concentration. For the majority of food types, anthocyanins as food additives or colouring foods have relatively low market penetration and so the possibility of the ‘loyal consumer’ scenario B is therefore considerably reduced.

Uncertainty also affects the food consumption data contained in the EFSA Comprehensive database. Data from different countries have been collected using different methods and often cover relatively short survey periods. This introduces the possibility of extrapolating relatively short exposures to represent exposures in the longer-term. Some surveys also include very small numbers of subjects for certain foods and these should be excluded if reliable estimates of upper percentiles are to be made. There are also problems arising from the food categorisation system which can lead to foods being categorised differently in different surveys. Furthermore, the FoodEx categorisation system does not correspond well to all use categories corresponding to authorised anthocyanins use as a food additive.

Uncertainty tables

Various authorities have proposed methods for addressing uncertainties in dietary intake assessments (EFSA 2006; WHO/FAO 2008). One of the most useful is through the use of a semi-quantitative uncertainty table (Table 8). The result of this analysis is that overall the uncertainties associated with this assessment are likely to cause an overestimation of exposure. This is because where uncertainties have been identified, they have been addressed by following a conservative approach.

Discussion

In the 2013 ANS Panel Opinion on anthocyanins, potential intakes of from natural sources were based on published data from one source (Wu et al. 2006) although the actual values used in the exposure model were not provided in the opinion. Wu et al. did not include data on beverages such as fruit juices and wine and so this could have led to an underestimation. The exposure estimate was based on children and adults in one country only (Ireland). For Irish adults, average intakes were reported to be 0.1 and 0.6 mg kg bw⁻¹ day⁻¹ at the 97.5th percentile. For Irish children, mean intakes

Table 8. Semi-quantitative evaluation of influence of uncertainties.

| Sources of uncertainties                                                                 | Magnitude and direction* |
|------------------------------------------------------------------------------------------|--------------------------|
| Exposure model: based on EFSA FAIM                                                       | +                        |
| Consumption data: different methodologies/representativeness/under-reporting/ no portion size standard | –/+                      |
| Extrapolation from food consumption survey of a few days to estimate chronic exposure     | +                        |
| Variation in actual anthocyanin additive/colouring food usage between countries, seasons, etc. | –/+                      |
| Application of the highest occurrence data from any country for use in all European Union countries | ++                       |
| Variability of naturally occurring anthocyanin levels                                    | –/+                      |
| Poor correspondence between categorisation systems                                       | –/+                      |
| Overall effect                                                                           | ++                       |

Notes: *Key to direction and magnitude: +, ++, +++ = uncertainty likely to cause a small, medium or large overestimation of exposure; and –, –, – = uncertainty likely to cause a small, medium or large underestimation of exposure.
were reported to be 0.3 mg kg bw\(^{-1}\) day\(^{-1}\) and intakes at the 97.5th percentile were 2.1 mg kg bw\(^{-1}\) day\(^{-1}\).

In the current exposure model additional data on anthocyanins in beverages was included (Table 1). Intakes were estimated for five age groups for all EU countries for which data are available in the EFSA Comprehensive data base. For all European adults average intakes ranged from 0.2 to 1.6 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from 0.2 to 8.9 mg kg bw\(^{-1}\) day\(^{-1}\). For European children average intakes ranged from 0.1 to 4.0 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from 0.2 to 17.1 mg kg bw\(^{-1}\) day\(^{-1}\). No data are available for Irish children in the EFSA comprehensive database. For Irish adults, average intakes were 0.1–0.2 and 1.1–1.8 mg kg bw\(^{-1}\) day\(^{-1}\) at the 97.5th percentile. Irish adults were in the lowest quartile for anthocyanins intakes for European Consumers. This factor, together with the absence of data on beverages might explain why the estimated intakes of anthocyanins from natural sources were relatively low in the EFSA Opinion. That estimate was also based on the raw data from individual food consumptions surveys which would be expected to give lower results that a deterministic model such as FAIM (EFSA 2014a).

The 2013 ANS Opinion on anthocyanins was based on maximum colour additive use levels provided by EFSA supplemented with additional data derived from other colour additives and extrapolated using the QS rules. The results gave average intakes for children of 1.5–4.7 mg kg bw\(^{-1}\) day\(^{-1}\) at the mean rising to 2.7–7.8 mg kg bw\(^{-1}\) day\(^{-1}\) at the upper levels. For adults average intakes ranged from 0.5 to 1.9 mg kg bw\(^{-1}\) day\(^{-1}\) and up to 0.9–3.4 mg kg bw\(^{-1}\) day\(^{-1}\) at the upper levels. The proportion of products containing anthocyanins as colour additives or colouring foods was not taken into consideration.

In the current exposure model all of the data provided by NATCOL was used including typical usage following the ANS Panel’s recently published approach for ‘loyal’ and ‘non-loyal’ consumers (scenarios B and C). Where NATCOL had been unable to provide usage data, values were extrapolated from use of anthocyanins on foods with a similar matrix. Use levels (concentrations in food) were identical for food colour additive (E163) and colouring food (CFS) applications. For European children average intakes ranged from 0.1 to 1.3 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from 0.1 to 6.9 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from 0.1 to 2.4 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers. For European adults average intakes ranged from 0.1 to 0.3 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from 0.1 to 2.0 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from 0.1 to 1.7 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers.

When frequency of occurrence of use of E163 was taken into account, children’s average intakes ranged from < 0.1 to 0.1 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from < 0.1 to 6.3 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from < 0.1 to 0.3 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers. For adults average intakes were < 0.1 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from < 0.1 to 1.8 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from < 0.1 to 0.6 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers.

When frequency of occurrence of use of CFS was taken into account, children’s average intakes were < 0.1 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes ranged from < 0.1 to 6.3 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from < 0.1 to 0.2 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers. For adults average intakes were < 0.1 mg kg bw\(^{-1}\) day\(^{-1}\) and high-level intakes from < 0.1 to 1.9 mg kg bw\(^{-1}\) day\(^{-1}\) for loyal consumers and from < 0.1 to 0.2 mg kg bw\(^{-1}\) day\(^{-1}\) for non-loyal consumers.

It is difficult to state whether long-term consumer loyalty would be a factor in determining exposure to anthocyanins from colour additive of colouring food applications. The true situation probably lies between one extreme where there is no consumer loyalty to the other of 100% loyalty to a particular product. As a consequence real high-level intakes for children form food colour and colouring food applications probably lay in the range 0.3–6.3 mg kg bw\(^{-1}\) day\(^{-1}\) and for adults between 0.6 and 2.8 mg kg bw\(^{-1}\) day\(^{-1}\). The upper values are similar to those presented in the EFSA Opinion. However, these values would be achieved only if high level consumers always chose a product containing anthocyanins. For the majority of consumers for the majority of the time, high intakes would be represented by scenario C taking average use levels and frequency of occurrence into account and fall below 1.0 mg kg bw\(^{-1}\) day\(^{-1}\).

**Conclusions**

The 2013 EFSA ANS Panel Opinion on anthocyanins underestimates intakes of anthocyanins from their normal presence in foods because it appears to exclude beverages and focuses on one population who have amongst the lowest intakes in Europe. Adult consumers in Denmark, Finland and Germany have up to four times the intake of anthocyanins when compared to Irish adults. Intakes from natural occurrence can be
up to 4 mg kg$^{-1}$ day$^{-1}$ at the mean and up to 17 mg kg$^{-1}$ day$^{-1}$ for children who are high level consumers of red/black berries and small fruits.

Intakes from food colour uses reported in the ANS Opinion provide only values at the extreme where a high level consumer always chooses products that contain the highest levels of anthocyanins as a food colour. Real intakes reflect the range of use levels reflecting different colour tones and the proportion of foods in a given category that contains the additive. Actual intakes of anthocyanins from food colour (E163) or colouring food (CFS) uses are very similar. Real high-level intakes for children from food colour and colouring food applications lie in the range 0.3–6.3 mg kg$^{-1}$ day$^{-1}$ and for adults between 0.6 and 2.8 mg kg$^{-1}$ day$^{-1}$.

It can be concluded that high level exposure estimates to anthocyanins from food colour additive (E163) or colouring food (CFS) applications are lower than the highest exposure estimates from the occurrence of anthocyanins in the regular diet.

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**References**

Boulton R. 2001. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. Am J Enol Vitic. 52:2.

Cassidy A, O’Reilly EJ, Kay C, Sampson L, Franz F, Forman J, Curhan G, Rimm E. 2011. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nut. 93:338–347.

EFSAa Food Additives web page including FAIM model. [cited 2015 Sep]. Available from: http://www.efsa.europa.eu/en/topics/topic/additives

EFSAb Comprehensive European Food Consumption Database. [cited 2015 Sep]. Available from: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb

European Commission. 2013. Guidance notes on the classification of food extracts with colouring properties; [cited 2015 Sep]. Available from: http://ec.europa.eu/food/food/fAEF/additives/guidance_en.htm

[EFSA] European Food Safety Authority. 2006. Guidance of the Scientific Committee on a request from EFSA related to uncertainties in dietary exposure assessment. EFSA J. 438:2–54.

[EFSA] European Food Safety Authority. 2013. EFSA panel on food Additives and Nutrient Sources added to food (ANS); scientific opinion on the re-evaluation of anthocyanins (E 163) as a food additive. EFSA J. 11:3145.

[EFSA] European Food Safety Authority. 2014a. Food Additives Intake Model (FAIM): comments received from stakeholders and EFSA’s views. EFSA supporting publication:EN–566. Available from: http://www.efsa.europa.eu/publications

[EFSA] European Food Safety Authority. 2014b. EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food). Scientific opinion on the re-evaluation of Indigo Carmine (E 132) as a food additive. EFSA J. 12:3768, 51 pp.

MINTEL Global New Products Database. [cited 2015 Aug]. Available from: http://www.gnpd.com/sinatra/anonymous_frontpage/

Netzel M, Strass G, Janssen M, Bitsch I, Bitsch R. 2001. Bioactive anthocyanins detected in human urine after ingestion of blackcurrant juice. J Environ Pathology Toxicol Oncol. 20:7–95.

Pérez-Jiménez J, Fezèu L, Touvier M, Arnault N, Manach C, Hercberg S, Galan P, Scalbert A. 2011. Dietary intake of 337 polyphenols in French adults. Am J Clin Nut. 93:1220–1228.

Phenol Explorer Database of Polyphenols in Food. [Cited 2015 Sep]. Available from: http://www.phenol-explorer.eu

Tennant D. 2016. A comprehensive European dietary exposure model. Food Addit Contam. doi:10.1080/19440049.2016.1166898.

[WHO/IPCS] World Health Organization/International Program on Chemical Safety. 2008. Uncertainty and data quality in exposure assessment. Part 1: Guidance on characterizing and communicating uncertainty in exposure assessment. Geneva: World Health Organisation.

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. 2006. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 54:4069–4075.

Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson J, Bagchi D. 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. Mol Nutr Food Res. 51:675–683.

Zamora-Ros R, Knaze V, Luján-Barroso L, Slimani N, Romieu I, Touillaud M, Kaaks R, Teucher B, Mattiello A, Grioni S, et al. 2011. Estimation of the intake of anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Br J Nutr. 106:1090–1099.