Exposure assessment of annatto colouring principles bixin and norbixin (E 160b) when used as food additives

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Abstract

Following a request for technical assistance, EFSA carried out an exposure assessment of the colouring principles bixin and norbixin of the food colour annatto extracts (E 160b), from its use as a food additive, taking into account new proposed uses and use levels. In 2016, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted a scientific opinion on the safety of annatto extracts (E 160b) as a food additive. In that opinion, the Panel concluded that the toxicological database was sufficient to derive an acceptable daily intake (ADI) of 6 mg bixin/kg body weight (bw) per day and an ADI of 0.3 mg norbixin/kg bw per day. Dietary exposure for annatto (E 160b), bixin- and norbixin-based annatto extracts was estimated taking into account the maximum permitted levels (MPLs) as set in the Annex II to Regulation (EC) No 1333/2008, use levels provided by food industry as well as proposed use levels from an applicant for an extension of use. Exposure estimates for bixin were below the ADI for all population groups and for all refined exposure scenarios, including the proposed extension of use. For norbixin, exceedance was observed for the extension of use at the 95th percentile for some population groups. In the current estimates, the dietary exposure for bixin does not exceed the ADI. For norbixin, dietary exposure exceeds the ADI at the high level (95th percentile) for toddlers and children.

Keywords: annatto extracts, E 160b, bixin, norbixin, exposure assessment

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Summary

Following a request from the European Commission, the European Food Safety Authority (EFSA) was asked to deliver a statement on the exposure assessment of the colouring principles bixin and norbixin of the food colour annatto extracts (E 160b) from its use as a food additive, taking into account new proposed uses and use levels.

In 2016, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted a scientific opinion on the safety of annatto extracts (E 160b) as a food additive, in which the Panel concluded that the exposure estimates for bixin were below the acceptable daily intake (ADI) of 6 mg/kg body weight (bw) per day for all population groups and for all refined exposure scenarios, including for the extension of use proposed by an applicant. For norbixin, an ADI of 0.3 mg/kg bw per day was derived. The ADI was exceeded in some population groups at the 95th percentile, when considering the proposed extension of use.

Following the 2016 opinion, new uses and use levels were proposed by the applicant. The present statement provides an exposure assessment for the colouring principles bixin and norbixin of the food colour annatto extracts (E 160b), according to these latest proposed uses and use levels.

On the basis of the current updated assessment, EFSA concluded that exposure to bixin would not exceed the ADI of 6 mg bixin/kg bw per day derived by the ANS Panel in its re-evaluation of the safety of annatto extracts (E 160b) as a food additive (EFSA ANS Panel, 2016). The estimated exposure to norbixin would be above the ADI of 0.3 mg norbixin/kg bw per day at the high level (95th percentile) for toddlers and children.

Moreover, the applicant proposed to raise the accepted natural level for norbixin in the bixin-based annatto extract from 2.5% to 5%. In the case that bixin is used at the proposed use levels and that bixin and norbixin are both used in the same food categories, this would result in an additional exposure to norbixin from 0.026 to 0.052 mg/kg bw per day (considering the refined exposure assessment scenario bixin-based annatto extracts, 95th percentile in toddlers).
Updated exposure assessment to annatto extracts (E 160b)

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

1.1.1. Background

The use of food additives is regulated under the European Parliament and Council Regulation (EC) No 1333/2008 on food additives.\(^1\) Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market and used in foods under the conditions of use specified therein. Moreover, food additives shall comply with the specifications as referred to in Article 14 of that Regulation and laid down in Commission Regulation (EU) No 231/2012\(^2\).

On 24 August 2016, the European Food Safety Authority (EFSA) published a scientific opinion on the safety of annatto extracts (E 160b) as a food additive (EFSA ANS Panel, 2016). This opinion covers not only the re-evaluation of the safety of E 160b Annatto, Bixin, Norbixin in accordance with Regulation (EC) No 1333/2008 and the programme for the re-evaluation of food additives set up under Regulation (EU) No 257/2010\(^3\), but also deals with the evaluation of a request for amendment of the current specifications for E 160b Annatto, Bixin, Norbixin in Commission Regulation (EU) No 231/2012 and the extension of use of E 160b to additional food categories, submitted by the Natural Food Colours Association (NATCOL). The opinion includes an estimation of the exposure to the colouring principles present in annatto extracts (bixin and norbixin), from the use of these extracts as a food additive.

Since the publication of EFSA’s opinion on annatto extracts, some changes were introduced to the requested uses and use levels of bixin-based and norbixin-based annatto extracts, which are expected to have an impact on the exposure estimates calculated by EFSA. Consequently, the Commission has decided to consult EFSA on this matter.

1.1.2. Terms of Reference

In accordance with Article 31 of Regulation (EC) 178/2002\(^4\), the European Commission requests EFSA to provide technical assistance as regards the estimation of the exposure to the annatto colouring principles bixin and norbixin, when used as food additives. In particular, EFSA is requested to carry out a new estimation of the exposure to bixin and norbixin on the basis of the provided new information on proposed uses and maximum use levels. This should also include an estimation of the contribution of the use of bixin-based annatto extracts to the exposure to norbixin, linked to the natural presence of norbixin in those extracts (up to 5%, according to the applicant NATCOL).

1.2. Additional information

1.2.1. EFSA re-evaluation of the safety of annatto extracts (E 160b) as a food additive

In 2016, the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) completed the re-evaluation of the safety of annatto extracts (E 160b) as a food additive. A request for the extension of use of bixin-based and norbixin-based annatto extracts (E 160b) to additional food categories, submitted by the NATCOL was also evaluated by the ANS Panel at the same time (EFSA ANS Panel, 2016). Having reviewed the available toxicological database, the Panel concluded that an acceptable daily intake (ADI) of 6 mg/kg body weight (bw) per day could be derived for bixin, whereas an ADI of 0.3 mg/kg bw per day was established for norbixin.

Based on the reported use levels provided by the industry, the Panel concluded that exposure estimates were below the ADI for both bixin and norbixin for all population groups and for all refined exposure scenarios. Considering the extension of use for the additional 16 food categories, all refined exposure estimates for bixin remained below the ADI of 6 mg/kg bw per day for all populations. For norbixin, the ADI of 0.3 mg/kg bw per day was instead exceeded in the brand-loyal scenario at the

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\(^1\) OJ L 354, 31.12.2008, p. 16.
\(^2\) OJ L 83, 22.3.2012, p. 1.
\(^3\) OJ L 80, 26.3.2010, p. 19.
\(^4\) Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002.
95th percentile for infants (in one country), toddlers (in three countries) and children (in one country). However, the Panel noted that this exceedance resulted from the overestimation of the contribution from at least one food category (i.e. unripened cheese).

2. Data and methodologies

2.1. Data

EFSA was provided with newly submitted proposed uses and proposed maximum use levels submitted by an applicant to the European Commission (Documentation provided to EFSA n.1).

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database5) was used to estimate the dietary exposure.

The Mintel’s Global New Products Database (GNPD) is an online resource listing food products and compulsory ingredient information that should be included in labelling. This database was used to verify the use of annatto (E 160b), both bixin and norbixin-based annatto extracts in food products.

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA Scientific Committee, 2009) and following the relevant existing Guidances from the EFSA Scientific Committee.

Dietary exposure to the colouring principles bixin and norbixin of annatto extracts (E 160b) from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the proposed levels submitted to EFSA. Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Proposed uses and use levels

Table 1 summarises foods that are currently authorised to contain annatto (E 160b) and the corresponding maximum permitted levels (MPLs) and foods that are proposed to be authorised for containing bixin-based and norbixin-based annatto extracts (E 160b) and the corresponding proposed maximum levels.

Table 1: Current uses and MPLs for annatto (E 160b) and proposed uses and maximum use levels for bixin-based and norbixin-based annatto extracts (E 160b) (in mg/L or mg/kg)

| Food category number | Food category name | Restrictions/exceptions | Maximum permitted level (mg/L or mg/kg) for annatto | Proposed maximum level (mg/L or mg/kg) for bixin | Proposed maximum level (mg/L or mg/kg) for norbixin |
|----------------------|--------------------|-------------------------|----------------------------------------------------|-------------------------------------------------|---------------------------------------------------|
| 01.4                 | Flavoured fermented milk products including heat-treated products |                         | 10                                                 | 10                                              | 6                                                 |
| 01.7.2               | Ripened cheese     | Only ripened orange, yellow and broken-white cheese, and red and green pesto cheese | 15                                                 | 15                                              | 15                                                 |
| 01.7.2               | Ripened cheese     | Only red Leicester cheese | 50                                                 | –                                               | 50                                                 |
| 01.7.2               | Ripened cheese     | Only Mimolette cheese   | 35                                                 | –                                               | 35                                                 |
| 01.7.3               | Edible cheese rind |                         | 20                                                 | 20                                              | 20                                                 |
| 01.7.5               | Processed cheese   |                         | 15                                                 | 15                                              | 8                                                  |

5 Available online: http://www.efsa.europa.eu/en/food-consumption/comprehensive-database
| Food category number | Food category name                                      | Restrictions/exceptions                                                                 | Maximum permitted level (mg/L or mg/kg) for annatto | Proposed maximum level (mg/L or mg/kg) for bixin | Proposed maximum level (mg/L or mg/kg) for norbixin |
|----------------------|--------------------------------------------------------|------------------------------------------------------------------------------------------|------------------------------------------------------|-----------------------------------------------|--------------------------------------------------|
| 01.7.6               | Cheese products (excluding products falling in category 16) | Only ripened orange, yellow and broken-white products                                   | 15                                                   | –                                             | 8                                                |
| 02.1                 | Fats and oils essentially free from water (excluding anhydrous milkfat) | Only fats                                                                               | 10                                                   | 10                                            | –                                                |
| 02.2.2               | Other fat and oil emulsions including spreads as defined by Council Regulation (EC) No 1234/2007 and liquid emulsions | Excluding reduced fat butter                                                             | 10                                                   | 10                                            | –                                                |
| 03                   | Edible ices                                            |                                                                                         | 20                                                   | –                                             | 20                                               |
| 04.2.5.2             | Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC |                                                                                         | NP                                                   | 20                                            | 20                                               |
| 04.2.5.3             | Other similar fruit or vegetable spreads               | Except crème de pruneaux                                                                 | NP                                                   | 20                                            | 20                                               |
| 04.2.6               | Processed potato products                              | Only dried potato granules and flakes                                                   | NP                                                   | 10                                            | 10                                               |
| 05.2                 | Other confectionery including breath refreshing microwafers |                                                                                         | NP                                                   | 30                                            | 30                                               |
| 05.4                 | Decorations, coatings and fillings, except fruit-based fillings covered by category 4.2.4 | Only decorations and coatings                                                            | 20                                                   | 80                                            | 20                                               |
| 06.3                 | Breakfast cereals                                      | Only extruded puffed and or fruit flavoured breakfast cereals                           | 25                                                   | –                                             | 20                                               |
| 06.5                 | Noodles                                                |                                                                                         | NP                                                   | 20                                            | 20                                               |
| 06.6                 | Batters                                                | Only batters for coating                                                                 | 20                                                   | 50                                            | 50                                               |
| 07.2                 | Fine bakery wares                                      |                                                                                         | 10                                                   | –                                             | 10                                               |
| 08.2                 | Meat preparations as defined by Regulation (EC) No 853/2004 | Only breakfast sausages with a minimum cereal content of 6%, burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat (in these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance) | NP                                                   | 20                                            | 20                                               |
According to the request from European Commission, in the food categories in which the use of both bixin and norbixin is proposed, these two colouring principles could be used:

- singly and therefore never exceeding their specific/individual maximum level, or
- in combination, never exceeding the highest maximum level; in any case, the amount of norbixin used should not exceed the maximum level for norbixin (e.g. in FC 01.7.5 Processed cheese, the total amount of bixin + norbixin cannot exceed 15 mg/kg (the maximum level of bixin, which is the highest of the two individual maximum levels of bixin and norbixin), however, the amount of norbixin used cannot exceed the maximum of norbixin, which is 8 mg/kg).

| Food category number | Food category name | Restrictions/exceptions | Maximum permitted level (mg/L or mg/kg) for annatto | Proposed maximum level (mg/L or mg/kg) for bixin | Proposed maximum level (mg/L or mg/kg) for norbixin |
|----------------------|-------------------|-------------------------|-----------------------------------------------|----------------------------------|----------------------------------|
| 08.3.1 | Non-heat-treated meat products | Only chorizo sausage, salchichón, pasturmas and sobrasada | NP | 20 | 20 |
| 08.3.2 | Heat-treated meat products | Only sausages, patés, terrines and luncheon meat | NP | 20 | 20 |
| 08.3.3 | Casings and coatings and decorations for meat | | 20 | 50 | 50 |
| 09.2 | Processed fish and fishery products including molluscs and crustaceans | Only smoked fish | 10 | 10 | 10 |
| 09.2 | Processed fish and fishery products including molluscs and crustaceans | Only surimi and similar products and salmon substitutes | NP | 30 | 30 |
| 12.5 | Soups and broths | | NP | 15 | 15 |
| 12.6 | Sauces | Including pickles, relishes, chutney and picalilli; excluding tomato-based sauces | NP | 30 | 30 |
| 14.1.4 | Flavoured drinks | | NP | 20 | – |
| 14.2.6 | Spirit drinks as defined in Regulation (EC) No 110/2008 | Only liqueurs | 10 | 10 | – |
| 14.2.8 | Other alcoholic drinks including mixtures of alcoholic drinks with non-alcoholic drinks and spirits with less than 15% of alcohol | Only alcoholic drinks with less than 15% of alcohol | 10 | – | 10 |
| 15.1 | Potato-, cereal-, flour- or starch-based snacks | | 10(a) | 20 | 20 |
| 15.1 | | | 20(b) | | |
| 15.2 | Processed nuts | | 10(c) | 10 | 10 |
| 16 | Desserts excluding products covered in categories 1, 3 and 4 | | 10 | 10 | 10 |

NP: currently not permitted; –: not requested.

(a): Excluding extruded or expanded savoury snack products.
(b): Only extruded or expanded savoury snack products.
(c): Only savoury-coated nuts.
In the current exposure assessment, considering that bixin and norbixin have different ADIs, EFSA decided to estimate exposure to each separately using their individual maximum permitted levels and proposed levels from the request on the extension of use.

According to the information submitted by the applicant, bixin-based annatto extracts are proposed to be used in 26 food categories and norbixin-based annatto extracts are proposed to be used in 29 food categories. Some of these food categories are new proposed uses.

For both bixin- and norbixin-based annatto extracts, 11 are food categories in which annatto extracts are not currently authorised, e.g. jams, jellies and marmalades and similar products (FC 04.2.5.2 and 04.5.2.3), other confectionary (FC 05.2), meat preparations (FC 08.2), heat-treated and non-heat-treated meat products (FC 08.3.1 and 08.3.2), some with restrictions. Another food category (flavoured drinks, FC 14.1.4) is also proposed to be authorised for containing bixin-based annatto extracts.

For four already authorised uses for both bixin- and norbixin-based annatto extracts, the new proposed use levels are higher than the current MPL: for decorations and coatings (FC 05.4) only for bixin, batters (FC 06.6), casings and coatings and decorations for meat (FC 08.3.3) and for potato-, cereal-, flour- or starch-based snacks (FC 15.1) (Table 1).

3.2. Reported use levels of annatto (E 160b)

Most food additives in the European Union (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 quantum satis (QS).

In the framework of Regulation (EC) No 1333/2008 on food additives and of Commission Regulation (EU) No 257/2010 regarding the re-evaluation of approved food additives, EFSA issued a public call6 for occurrence data (usage level and/or concentration data) on annatto (E 160b). In response to this public call, both types of data on annatto (E 160b) were submitted to EFSA by industry and Member States, respectively.

These data were used to re-evaluate the safety of annatto extracts (E 160b) as a food additive in the 2016 scientific opinion (EFSA ANS Panel, 2016).

For the purpose of this technical assistance, for food categories currently authorised for use, EFSA took into account the reported use levels which were used in the previous scientific opinion. This allows to perform more refined exposure estimates.

Appendix A provides the concentration levels of bixin and norbixin used in the refined exposure estimates.

3.3. Food consumption data used for exposure assessment

3.3.1. 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 added in the Comprehensive database were also taken into account in this assessment.7

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 subjects’ underreporting 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.

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

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6 Call for food additives usage level and/or concentration data in food and beverages intended for human consumption. Published 27 March 2013. Available online: http://www.efsa.europa.eu/en/dataclosed/call/130327
7 Available online: http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm
assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 2).

### Table 2: Population groups considered for the exposure estimates of bixin and norbixin

| Population | Age range | Countries with food consumption surveys covering more than 1 day |
|------------|-----------|---------------------------------------------------------------|
| Infants    | From more than 12 weeks 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 categorisation system (FCS) as presented in Annex II of Regulation (EC) No 1333/2008, part D, to perform exposure estimates. In practice, the FoodEx food codes were matched to the FCS food categories.

#### 3.3.2. Food categories considered for the exposure assessment of bixin-based and norbixin-based annatto extracts (E 160b)

The food categories in which the use of both colouring principles bixin and norbixin of the food additive annatto extracts (E 160b) is proposed were selected from the nomenclature of the EFSA Comprehensive Database (FoodEx classification system), at the most detailed level possible (up to FoodEx Level 4) (EFSA, 2011b).

Some food categories or their restrictions/exceptions are not referenced in the EFSA Comprehensive Database and could therefore not be taken into account in the present estimate. This was the case for 3 food categories and may have resulted in an underestimation of the exposure. The food categories which were not taken into account are described below (in ascending order of the FCS codes):

- 01.7.3 Edible cheese rind,
- 08.2 Meat preparations as defined by Regulation (EC) No 853/2004, only breakfast sausages with a minimum cereal content of 6%, burger meat with a minimum vegetable and/or cereal content of 4% mixed within the meat (in these products, the meat is minced in such a way so that the muscle and fat tissue are completely dispersed, so that fibre makes an emulsion with the fat, giving those products their typical appearance),
- 08.3.3 Casings and coatings and decorations for meat.

For the food category 06.3 Breakfast cereals, only extruded puffed and or fruit-flavoured breakfast cereals, the restrictions/exceptions which apply to the use of annatto extracts (E 160b) could not be taken into account, and therefore the whole food category was considered in the exposure assessment. This may have resulted in an overestimation of the exposure.

For the food category 06.6 Batters, only batters for coating, information was given to EFSA that under this food category, industry would plan to add annatto to breadcrumbs. Breadcrumbs are referenced in the EFSA Comprehensive Database and were therefore taken into account in the present estimate.
For the food category 08.3.1 Non-heated –treated meat products, only chorizo sausage, salchichón, pasturmas and sobrasada, only chorizo is a food item available in the EFSA Comprehensive database; salchichón, pasturmas and sobrasada could not be taken into account.

For the food category 09.2 Processed fish and fishery products including molluscs and crustaceans, only smoked fish, the restrictions/exceptions which apply to the use of annatto extracts (E 160b) could not be taken into account, and therefore processed fishes which are usually smoked where taken into account, i.e. herring, salmon, trout, anchovy, mackerel, sardine and pilchard. This may have resulted in an overestimation of the exposure.

For the food category 14.1.4 Flavoured drinks, no restrictions/exceptions apply to the use of annatto extracts (E 160b) which could therefore be used in all flavoured drinks. There is no possibility to differentiate the coloured one from the non-coloured flavoured drinks in the EFSA Comprehensive database, thus the entire food category was taken into account. Taking into account all flavoured drinks may have resulted in an overestimation of the exposure.

For the food category 01.4 Flavoured fermented milk products, including heat-treated product, no restrictions/exceptions apply to the use of annatto extracts (E 160b) neither and thus, it could be used in all flavoured fermented milk products. There is no possibility to differentiate the coloured one from the non-coloured fermented milk products in the EFSA Comprehensive database, thus the entire food category was taken into account. This may have resulted in an overestimation of the exposure.

Other refinements considering the restrictions/exceptions as proposed in the request for technical assistance were applied.

Therefore, for the bixin exposure assessment scenario, 23 food categories were taken into account. For the norbixin exposure assessment scenario, 26 food categories were taken into account (Appendix A).

3.4. Summarised data extracted from the Mintel’s Global New Products Database

The Mintel GNPD is an online database which monitors product introductions in consumer packaged goods markets worldwide. It contains information of over 2 million food and beverage products of which more than 900,000 are or have been available on the European food market. Mintel started covering EU’s food markets in 1996, currently having 20 out of its 28 member countries and Norway presented in the Mintel GNPD.

For the purpose of this statement, the Mintel GNPD was used for knowing which are the current labelling of products containing annatto (E 160b), both bixin and norbixin-based annatto extracts within the EU's food products as the Mintel GNPD shows the compulsory ingredient information presented in the labelling of products.

According to Mintel, annatto (E 160b) is labelled on almost 3,000 products. The main categories containing annatto are cheeses, desserts and flavoured fermented milk-products, fine bakery wares.

Appendix F presents the percentage of the food products labelled with annatto (E 160b) between 2012 and 2017, out of the total number of food products per food subcategories according to the Mintel GNPD food classification.

It can be noted from the Mintel database (Appendix F), many products of fine bakery wares (‘cakes, pastries, and sweet goods’, ‘pastry dishes’, ‘snacks/cereals/energy bars’) are labelled with annatto according to Mintel GNPD. On the other side, few products of breakfast cereals (‘cold cereals’) are currently labelled with annatto according to Mintel GNPD. Dessert (‘shelf-stable dessert’, ‘chilled dessert’) and snacks (‘corn-based snacks’, ‘rice snacks’, wheat and other grain-based snacks) are labelled with annatto according to Mintel GNPD but do not appear as main contributors to dietary exposure to annatto, bixin or norbixin-based annatto extracts.

3.5. Exposure estimates

3.5.1. Exposure to bixin-based and norbixin-based annatto extracts (E 160b) as food additives

EFSA estimated chronic exposure to the annatto colouring principles bixin and norbixin when used as food additives for the following population groups: infants, toddlers, children, adolescents, adults...
and the elderly. Dietary exposure was calculated by multiplying both annatto colouring principles bixin and norbixin concentrations for each food category (Appendix A) 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. Dietary surveys with only 1 day per subject were excluded as they are considered as not adequate to assess repeated exposure.

This was carried out for all individuals per survey and per population group, resulting in distributions of individual exposure per survey and population group (Table 2). On the basis of these distributions, the mean and 95th percentile of exposure were calculated per survey and per population group. The 95th percentile of exposure was only calculated for those population groups where the sample size was sufficiently large to allow this calculation (EFSA, 2011a). Therefore, in the present assessment, the 95th percentile of exposure for infants from Italy and for toddlers from Belgium, Italy and Spain were not included.

Exposure assessment to bixin-based and norbixin-based annatto extracts (E 160b) was carried out by the EFSA based on two different sets of concentration data: (1) proposed maximum uses levels as provided by the applicant (defined as the maximum level exposure assessment scenario); and (2) proposed maximum uses levels as provided by the applicant and reported use levels from industry (defined as the refined exposure assessment scenario). These two scenarios are discussed in detail below.

3.5.1.1. Maximum level exposure assessment scenario

The maximum level exposure assessment scenario is based on the proposed uses and maximum use levels as provided by the applicant (Appendix A).

EFSA considers the exposure estimates derived following this scenario as the most conservative as it is assumed that the population groups would be exposed to bixin-based and norbixin-based annatto extracts (E 160b) present in food at the maximum proposed use levels over a longer period of time.

3.5.1.2. Refined exposure assessment scenario

The refined exposure assessment scenario is based on the proposed uses and maximum use levels as provided by the applicant and use levels reported by industry, as used in the 2016 ANS Panel scientific opinion, for previously authorised use.

Appendix A summarises the concentration levels of bixin-based and norbixin-based annatto extracts (E 160b) used in the refined exposure assessment scenario. Based on the available data set, EFSA calculated two refined exposure estimates based on different model populations:

- The brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to bixin-based and norbixin-based annatto extracts (E 160b) present at the maximum proposed/ reported use for one food category. This exposure estimate is calculated as follows:
  - Combining food consumption with the maximum use levels proposed or maximum of the reported use levels for the main contributing food category at the individual level.
  - Using the maximum use levels proposed or the mean of the typical reported use levels for the remaining food categories.

- The non-brand-loyal consumer scenario: It was assumed that a consumer is exposed long-term to bixin-based and norbixin-based annatto extracts (E 160b) present at the mean reported use for foods already regulated and at the maximum use levels proposed for the extension of use. This exposure estimate is calculated using maximum use levels proposed or the mean of the typical reported use levels for all food categories.

3.5.2. Dietary exposure of bixin-based and norbixin-based annatto extracts (E 160b)

Table 3 summarises the estimated exposure to the colouring principles bixin and norbixin from the use of annatto extracts (E 160b) as a food additive in six population groups (Table 2).

Detailed results per population group and survey are presented in Appendix B (maximum scenario) and Appendices D.1 and D.2 (refined scenario, respectively, for bixin and norbixin-based annatto extracts).
For the maximum exposure assessment scenario, mean exposure to bixin-based annatto extract ranged from 0.01 mg/kg bw per day in infants and the elderly to 0.52 mg/kg bw per day in toddlers. The 95th percentile of exposure to bixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 1.03 mg/kg bw per day in toddlers.

The 95th percentile of exposure to norbixin-based annatto extract ranged from 0.01 mg/kg bw per day in infants and the elderly to 0.26 mg/kg bw per day in toddlers. The 95th percentile of exposure to norbixin-based annatto extract ranged from 0.01 mg/kg bw per day in the elderly to 0.36 mg/kg bw per day in toddlers. For the non-brand-loyal scenario, mean exposure to norbixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 0.41 mg/kg bw per day in toddlers.

For the refined exposure assessment, brand-loyal scenario, mean exposure to bixin-based annatto extract ranged from 0.01 mg/kg bw per day in infants and the elderly to 0.51 mg/kg bw per day in toddlers. The 95th percentile of exposure to bixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 1.03 mg/kg bw per day in toddlers. For the non-brand-loyal scenario, mean exposure to bixin-based annatto extract ranged from 0.01 mg/kg bw per day in infants and the elderly to 0.50 mg/kg bw per day in toddlers. The 95th percentile of exposure to bixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 0.41 mg/kg bw per day in toddlers.

For the refined exposure assessment, brand-loyal scenario, mean exposure to norbixin-based annatto extract ranged from 0.01 mg/kg bw per day in the elderly to 0.24 mg/kg bw per day in toddlers. The 95th percentile of exposure to norbixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 0.36 mg/kg bw per day in toddlers. For the non-brand-loyal scenario, mean exposure to norbixin-based annatto extract ranged from 0.01 mg/kg bw per day in infants, adults and the elderly to 0.23 mg/kg bw per day in toddlers. The 95th percentile of exposure to norbixin-based annatto extract ranged from 0.03 mg/kg bw per day in the elderly to 0.34 mg/kg bw per day in toddlers.

### Table 3: Summary of dietary exposure to the colouring principles bixin and norbixin from the use of annatto extracts (E 160b) as food additives in the maximum and refined level exposure assessment scenario, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

| Population Group | Bixin-based Annatto Extracts: Maximum Exposure Assessment Scenario | Bixin-based Annatto Extracts: Refined Exposure Assessment Scenario | Norbixin-based Annatto Extracts: Maximum Exposure Assessment Scenario | Norbixin-based Annatto Extracts: Exposure Assessment Scenario |
|------------------|---------------------------------------------------------------------|------------------------------------------------------------------|---------------------------------------------------------------------|------------------------------------------------------------------|
|                  | Mean                                                                | 95th Percentile                                                 | Mean                                                                | 95th Percentile                                                 |
| **Infants**      | 0.01-0.08                                                           | 0.05-0.38                                                      | 0.02-0.07                                                           | 0.07-0.26                                                      |
| **(12-35 months)** | 0.06-0.51                                                           | 0.15-0.42                                                      | 0.04-0.30                                                           | 0.06-0.42                                                      |
| **Children**     | 0.05-0.42                                                           | 0.15-0.91                                                      | 0.04-0.30                                                           | 0.06-0.42                                                      |
| **(3-9 years)**  | 0.04-0.30                                                           | 0.13-0.63                                                      | 0.02-0.15                                                           | 0.03-0.19                                                      |
| **Adolescents**  | 0.02-0.15                                                           | 0.06-0.42                                                      | 0.01-0.08                                                           | 0.03-0.19                                                      |
| **(10-17 years)**| 0.01-0.08                                                           | 0.06-0.42                                                      | 0.01-0.08                                                           | 0.03-0.19                                                      |
| **Adults**       | 0.02-0.07                                                           | 0.05-0.24                                                      | 0.14-0.39                                                           | 0.16-0.41                                                      |
| **(18-64 years)**| 0.06-0.24                                                           | 0.06-0.51                                                      | 0.01-0.07                                                           | 0.04-0.15                                                      |
| **The Elderly**  | 0.07-0.24                                                           | 0.15-0.91                                                      | 0.07-0.20                                                           | 0.03-0.10                                                      |
| **(≥ 65 years)** | 0.14-0.39                                                           | 0.13-0.63                                                      | 0.06-0.42                                                           | 0.07-0.26                                                      |

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3.5.3. Main food categories contributing to exposure to bixin and norbixin from the use of annatto extracts (E 160b)

For the maximum exposure assessment scenario, the main contributing food category to the total mean exposure estimates to bixin-based annatto extract were flavoured drinks for all population groups. Additionally, flavoured fermented milk products for infants and toddlers, and soups and broths for adults and the elderly can be noted as second most contributing food categories (Processed fruit and vegetables is the main contributing food category for one survey in the infant population) (see Appendix C for more details).

For the maximum exposure assessment scenario, the main contributing food categories to the total mean exposure estimates to norbixin-based annatto extract were fine bakery wares for all population groups together with breakfast cereals and soups and broths for infants; breakfast cereal for toddlers, soups and broths for children and adolescents; meat products and soups and broths for adults; and breakfast cereal, meat products and soups and broths for the elderly (see Appendix C for more details).

For the refined exposure assessment scenario, the main contributing food categories to the total mean exposure estimates to bixin-based annatto extract, brand-loyal and non-brand-loyal scenarios were flavoured drinks for all population groups. Other main contributing food categories were flavoured fermented milk products for infants and toddlers, and soups and broths for infants, adults and the elderly (Processed fruit and vegetables is the main contributing food category for one survey in the infant population) (see Appendix E.1 for more details). Comparing to the previous request of extension of use for bixin (EFSA ANS Panel, 2016), ripened cheese and other confectionary are not any longer main contributors in brand-loyal or non-brand-loyal scenarios.

For the refined exposure assessment scenario, the main contributing food categories to the total mean exposure estimates to norbixin-based annatto extract, brand-loyal and non-brand-loyal scenarios were fine bakery wares for all population groups. Other main contributing food categories were breakfast cereals for infants, meat products for adults and the elderly, and soups and broths for infants, children, adolescents, adults and the elderly (see Appendix E.2 for more details). Comparing to the previous request of extension of use for norbixin (EFSA ANS Panel, 2016), flavoured fermented milk products, unripened cheese and sauces are not any longer major contributors in brand-loyal or non-brand-loyal scenarios.

Appendices can be found in the online version of this output ('Supporting information’ section): https://doi.org/10.2903/j.efsa.2017.4966

3.5.4. Uncertainty analysis

Uncertainties in the exposure assessment of the annatto colouring principles bixin and norbixin have been discussed above. In accordance with the guidance provided in the EFSA opinion related to uncertainties in dietary exposure assessment (EFSA, 2007), the following sources of uncertainties have been considered and summarised in Table 4.

| 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 proposed 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 = 3 for both bixin and norbixin scenario) | -            |
| Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n = 1 for both bixin and norbixin scenario) | +            |
| Food categories selected for the exposure assessment: inclusion of food categories while most probably not all foods belonging to the food category will contain bixin or norbixin (e.g. flavoured fermented milk products, breakfast cereal, fine bakery wares, flavoured drinks, smoked fish) | +            |
Overall, considering all the uncertainties summarised above, these should result in an overestimation of the exposure to the annatto colouring principles bixin and norbixin for maximum exposure assessment scenarios, as the proposed use levels are the maximum levels and most likely, all foods will not contain annatto extracts at the maximum proposed use levels (see Section 3.3.2).

4. Conclusions

In 2016, the ANS Panel completed the re-evaluation of the use of annatto extracts (E 160b) as a food additive, establishing two different ADIs for the two colouring principles bixin and norbixin, of 6 mg/kg bw and 0.3 mg/kg bw, respectively. Exposure estimates for bixin-based annatto extract were below the ADI for all population groups and for all refined exposure scenarios, including for the extension of use. For norbixin-based annatto extract, exceedance was observed for the extension of use at the 95th percentile for some population groups. Main food categories contributing to the total mean exposure to norbixin in the brand-loyal scenario were unripened cheese, soups and broths, and fine bakery wares for infants, children and adolescents, unripened cheese, fine bakery wares and flavoured fermented milk products for toddlers, unripened cheese, soups and broths, and sauces for adults, and unripened cheese and soups and broths for the elderly.

Having carried out an updated estimation of the exposure to bixin-based annatto extract and norbixin-based annatto extract on the basis of newly provided information on proposed uses and maximum use levels, EFSA concluded that the estimated exposure to bixin-based annatto extract does not exceed the ADI of 6 mg bixin/kg bw in any of the scenarios carried out.

The estimated exposure to norbixin-based annatto extract at the proposed uses and maximum use levels would exceed the ADI of 0.3 mg norbixin/kg bw per day at the high level (95th percentile) for toddlers and children. EFSA did not identify brand loyalty to a specific food category and therefore it was considered that the non-brand-loyal scenario covering the general population was the more appropriate and realistic scenario for risk characterisation because it is assumed that the population would probably be exposed long-term to the food additive present at the mean reported use in processed food. Thus, in the refined non-brand-loyal exposure assessment scenario, the estimated exposure to norbixin-based annatto extract would exceed the ADI of 0.3 mg norbixin/kg bw per day only at the high level (95th percentile) for toddlers (in one survey (the Netherlands)) and children (in one survey (Belgium)).

Moreover, the applicant proposed to raise the accepted natural level for norbixin in the bixin-based annatto extract from 2.5% to 5%. In the case that bixin is used at the proposed use levels and that bixin and norbixin are both used in the same food categories, this would result in an additional exposure to norbixin from 0.026 to 0.052 mg/kg bw per day (considering the refined exposure assessment scenario bixin-based annatto extracts, 95th percentile in toddlers).

Documentation provided to EFSA

1) Proposed uses and maximum uses for bixin-based and norbixin-based annatto extracts. Submitted to EFSA by the European Commission on 2 March 2017.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| ADI          | acceptable daily intake |
| ANS          | EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food |
| bw           | body weight |
| FCS          | food categorisation system |
| MPL          | maximum permitted level |
| NATCOL       | Natural Food Colours Association |
| QS           | * quantum satis* |
Appendix A – Proposed uses and use levels of annatto (E 160b), bixin-based and norbixin-based taken into account in the maximum and refined exposure scenario (mg/kg or mL/kg as appropriate)

Appendix B – Summary of total estimated exposure of annatto (E 160b), maximum scenario, bixin-based and norbixin-based, from their use as food additives per population group and survey: mean and high level (mg/kg bw per day)

Appendix C – Main food categories contributing to exposure to annatto (E 160b), maximum scenario, bixin-based and norbixin-based

Appendix D – Summary of total estimated exposure of annatto (E 160b), refined scenario, bixin-based and norbixin-based, from their use as food additives per population group and survey: mean and high level (mg/kg bw per day)

Appendix E – Main food categories contributing to exposure to annatto (E 160b), refined scenario, bixin-based and norbixin-based

Appendix F – Number and percentage of food products labelled with annatto (E 160b) out of the total number of food products present in the Mintel GNPD per food sub-category between 2012 and 2017

Appendix A–F can be found in the online version of this output (‘Supporting information’ section): https://doi.org/10.2903/j.efsa.2017.4966