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Safety of annatto E and the exposure to the annatto colouring principles bixin and norbixin (E 160b) when used as a food additive

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Abstract

The Scientific Panel on Food Additives and Flavouring (FAF) provides a scientific opinion on the safety evaluation of annatto E and an exposure assessment of the annatto colouring principles bixin and norbixin (E 160b) when used 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. The Panel was not able to conclude on the safety of annatto E. 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, the use levels provided by food industry as well as the 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 2017 EFSA statement related to the exposure assessment of colouring principles bixin and norbixin considering new proposed uses and use levels, the dietary exposure for bixin did not exceed the ADI. For norbixin, dietary exposure exceeded the ADI at the high level (95th percentile) for toddlers and children. In the current opinion, the Panel concluded that food colour annatto E does not raise concern for genotoxicity. Accordingly, the ADIs established in 2016 for the two colouring principles bixin and norbixin can be applied also to the annatto E. For bixin, none of the exposure estimates exceeded the ADI of 6 mg/kg bw per day. For norbixin, the ADI was reached at the high level (p95) for toddlers in the refined exposure assessment scenarios in one country. Considering the uncertainties and the very likely overestimation of the exposure, the Panel concluded that the level of exposure does not raise a health concern.

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Summary

Following a request from the European Commission to the European Food Safety Authority (EFSA), the Scientific Panel on Food Additives and Flavouring (FAF) carried out a safety evaluation of annatto E and an exposure assessment of the annatto colouring principles bixin and norbixin (E 160b) when used as a food additive, taking into account new proposed uses and use levels.

Annatto, Bixin, Norbixin (E 160b) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.

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. The Panel was not able to conclude on the safety of annatto E. 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, the use levels provided by food industry as well as the 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.

Since the ANS Panel (EFSA ANS Panel, 2016) was not able to conclude on the safety of annatto E (aqueous-processed bixin) due to the equivocal mutagenicity results obtained with an *in vivo* comet assay, the applicant decided to generate new mutagenicity data, reviewed in the current opinion.

In a new *in vivo* comet assay submitted by the applicant, annatto E did not induce DNA damage in the liver and stomach of rats after oral treatment. The results of this study allow to clarify the concern on the mutagenicity of the annatto E raised by the comet assay evaluated in the previous EFSA opinion and to conclude that the food colour annatto E does not raise concern for genotoxicity. Accordingly, the ADIs established in 2016 for the two colouring principles bixin and norbixin can be applied also to the annatto E.

Following the 2016 opinion, new use and use levels were proposed by the applicant. The 2017 EFSA statement provided an updated exposure assessment for the colouring principles bixin and norbixin of the food colour annatto extracts (E 160b), according to these proposed uses and use levels. In these exposure estimates, the dietary exposure for bixin did not exceed the ADI. For norbixin, dietary exposure exceeded the ADI at the high level (95th percentile) for toddlers and children.

Based on the outcome of the 2017 exposure assessment, the applicant has revised again the proposed uses and use levels for bixin-based and norbixin-based annatto extracts, in the form of three alternative scenarios. These three scenarios differ in the proposed maximum and normal use levels for bixin-based and norbixin-based annatto extracts for food categories 01.4 Flavoured fermented milk products including heat-treated products, 05.2 Other confectionery including breath refreshing microsweets, 12.5 Soups and broths and 16 Desserts excluding products covered in categories 1, 3 and 4. All other remaining requested uses and use levels for bixin and norbixin are the same for all three scenarios, and are the same as those considered by EFSA in the 2017 exposure assessment.

For bixin, none of the exposure estimates in the *maximum level exposure assessment scenario* or the *refined exposure assessment scenarios* exceeded the ADI of 6 mg/kg bw per day.

For norbixin, exposure estimates in the *maximum level exposure assessment scenario* reached the ADI of 0.3 mg/kg bw per day in the three scenarios proposed by the applicant at the high level (p95) for toddlers and children. The ADI was also reached at the p95 for toddlers in the three scenarios of the *brand-loyal* and *non-brand-loyal refined exposure assessment scenario* in one country. Considering the uncertainties and the very likely overestimation of the exposure, the Panel concluded that the level of exposure does not raise a health concern.

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1. Introduction

The present opinion deals with the safety of annatto E and on the exposure to the annatto colouring principles bixin and norbixin (E 160b) when used as a food additive.

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 231/2012².

On 21 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, but it 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.

EFSA was not able to conclude on the safety of annatto E (aqueous-processed bixin) due to the equivocal mutagenicity results obtained with the *in vivo* comet assay. Therefore, the applicant decided to generate new mutagenicity data.

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 were expected to have an impact on the exposure estimates calculated by EFSA. Therefore, the Commission requested EFSA in 2017 to carry out a new exposure assessment, which was published on 10 August 2017 (EFSA, Tard, 2017).

In the 2017 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. Based on the outcome of this exposure assessment, the applicant has revised the proposed uses and use levels for bixin-based and norbixin-based annatto extracts, in the form of three alternative scenarios. These three scenarios differ in the proposed maximum and normal use levels for bixin-based and norbixin-based annatto extracts for food categories 01.4 Flavoured fermented milk products including heat-treated products, 05.2 Other confectionery including breath refreshing microsweets, 12.5 Soups and broths and 16 Desserts excluding products covered in categories 1, 3 and 4. All other remaining requested uses and use levels for bixin and norbixin are the same for all three scenarios, and are the same as those considered by EFSA in the 2017 exposure assessment.

Consequently, the Commission has decided to consult EFSA on this matter.

1.1.2. Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002³, the European Commission requests the European Food Safety Authority (EFSA) to provide a scientific opinion in relation to new mutagenicity data for annatto E (aqueous-processed bixin) as well as new use and use level data for bixin-based and norbixin-based annatto extracts. In particular, EFSA is requested to carry out a scientific evaluation of the new mutagenicity data for annatto E and to indicate whether it is now

¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16–33.

² Commission Regulation (EU) No 231/2012 laying down specification for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

³ 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, p. 1–24.

possible to conclude on the safety of this bixin-based extract. In addition, EFSA is requested to carry out a new exposure assessment for bixin and norbixin on the basis of the new uses and use levels for bixin-based and norbixin-based annatto extracts, in the form of three alternative scenarios.

1.2. Information on existing authorisations and evaluations

Annatto, Bixin, Norbixin (E 160b) is authorised as a food additive in the European Union (EU) in accordance with Annex II to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.⁴

In 1974, JECFA established a temporary acceptable daily intake (ADI) of 1.25 mg/kg body weight (bw) for the sum of bixin and norbixin (expressed as bixin) (JECFA, 1975), which was endorsed by the EU SCF in 1975 (SCF, 1975).

In 1978, the SCF assessed the results of pharmacokinetic and metabolism studies of annatto extracts in rats following short- and long-time exposure and reviewed the results of acute metabolic studies in man. The Committee allocated an ADI of 0–2.5 mg/kg bw per day for annatto extracts containing 2.6% carotenoids expressed as bixin, equivalent to 0–0.065 mg/kg bw per day of carotenoids, expressed as bixin (SCF, 1979). At its 26th meeting in 1982, JECFA allocated an ADI of 0–0.065 mg/kg bw 'in terms of the carotenoid content expressed as bixin' (JECFA, 1982). The ADIs of the SCF (1979) and JECFA (1982) were both based on a long-term rat study performed with well-defined annatto extracts, which varied in total bixin content from 0.2% to 2.6%.

Within the food colour legislation of the EU (Commission Regulation (EU) No 231/2012⁵ laying down specifications for food additives), three different annatto extracts are described: (i) solvent-extracted bixin and norbixin (E160b(i)); (ii) alkali-extracted annatto (E160b(ii)); and (iii) oil-extracted annatto (E160b(iii)).

In 2006, JECFA agreed upon two separate ADIs for bixin- and norbixin-containing annatto extracts: an ADI of 12 mg/kg bw for bixin (92% bixin pure) and a group ADI of 0.6 mg/kg bw for norbixin (91.6% norbixin pure) and its sodium and potassium salts (JECFA, 2007).

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 Natural Food Colours Association (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 ADI of 6 mg/kg 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 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).

In 2017, EFSA updated the exposure assessment of annatto extracts (E 160b) based on the reported use levels provided by industry used in the 2016 opinion (EFSA ANS Panel, 2016) and on new proposed uses and 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 (in one survey) and children (in one survey).

⁴ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) no 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p 1.

⁵ Commission Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1.

2. Data and methodologies

Data

The Panel on Food Additives and Flavourings (FAF) was provided with a newly submitted dossier including new mutagenicity data for annatto E and proposed uses and use levels for bixin-based and norbixin-based annatto extracts.

The EFSA Comprehensive European Food Consumption Database (Comprehensive Database⁶) 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 are 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.

Methodologies

This opinion was formulated following the principles described in the EFSA Guidance on transparency with regard to scientific aspects of risk assessment (EFSA Scientific Committee, 2009).

Dietary exposure to annatto (E 160b), both bixin and norbixin-based annatto extracts from its use as a food additive was estimated combining food consumption data available within the EFSA Comprehensive European Food Consumption Database with the maximum levels according to Annex II to Regulation (EC) No 1333/2008⁷ and the proposed use levels submitted to EFSA. Different scenarios were used to calculate exposure (see Section 3.4.1). Uncertainties on the exposure assessment were identified and discussed.

3. Assessment

3.1. Genotoxicity data

3.1.1. Identity of the substance

Currently, specifications for the food colour E 160b (Annatto, Bixin, Norbixin) are defined in Regulation (EU) No 231/2012 (Table 1). In the previous opinion of the former ANS Panel (EFSA ANS Panel, 2016), it was recommended to amend these specifications so to align with those prepared by JECFA for Annatto B, C, F and G (Table 2).

The material tested in the comet assay and defined as annatto E in this opinion is identified as an annatto derivative (Biocon Bixin Powder) complying with JECFA specifications for aqueous-processed bixin (annatto E) (JECFA, 2007). It is described as an orange brown coarse powder containing approximately 30% bixin as a colouring matter.

The applicant informed that the original batch from the first study was expired therefore a new batch of annatto E was used. The new batch of annatto E (batch no. M612815) was obtained from the same manufacturer (Biocon del Peru) and is equivalent to the test item used in the first study. It is representative for the product (annatto E) on the market and complies with the JECFA specifications for aqueous-processed bixin.

⁶ Available online: <http://www.efsa.europa.eu/en/food-consumption/comprehensive-database>

⁷ Commission Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

3.1.2. Specifications

Table 1: Specifications for E 160b according to Commission Regulation (EU) No 231/2012^(a)

Compound	Solvent-extracted bixin and norbixin (E 160b(i))	Alkali-extracted annatto (E 160b(ii))	Oil-extracted annatto (E 160b(iii))
Assay	Content of bixin powders not less than 75% total carotenoids calculated as bixin. Content of norbixin powders not less than 25% total carotenoids calculated as norbixin	Contains not less than 0.1% of total carotenoids expressed as norbixin	Contains not less than 0.1% of total carotenoids expressed as bixin
Purity			
<i>Solvent residues</i>			
Acetone	≤ 50 mg/kg, singly or in combination	–	–
Methanol		–	–
Hexane		–	–
Dichloromethane	≤ 10 mg/kg	–	–
<i>Heavy metals</i>			
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	≤ 1 mg/kg	≤ 1 mg/kg
Cadmium	≤ 1 mg/kg	≤ 1 mg/kg	≤ 1 mg/kg

(a): in the Regulation, bixin and norbixin are also identified as 6'-methylhydrogen-9'-[*cis* or *trans*]-6,6'-diapocarotene-6,6'-dioate and 9'-[*cis* or *trans*]-6,6'-diapocarotene-6,6'-dioic acid, respectively.

Table 2: Specifications for annatto extracts according to JECFA

Compound	Aqueous-processed bixin (annatto E) (JECFA, 2007)	Solvent-extracted bixin (annatto B) (JECFA, 2015)	Solvent-extracted norbixin (annatto C) (JECFA, 2015)	Alkali-processed norbixin, acid-precipitated (annatto F) (JECFA, 2007)	Alkali-processed norbixin, not acid-precipitated (annatto G) (JECFA, 2007)
Definition	Contains several coloured components; the major colouring principle is <i>cis</i> -bixin, a minor colouring principle is <i>trans</i> -bixin; thermal degradation products of bixin may also be present as a result of processing	Contains several coloured components; the major colouring principle is <i>cis</i> -bixin, a minor colouring principle is <i>trans</i> -bixin; thermal degradation products of bixin may also be present as a result of processing	Contains several coloured components; the major colouring principle is <i>cis</i> -norbixin, a minor colouring principle is <i>trans</i> -norbixin; thermal degradation products of norbixin may also be present as a result of processing	Contains several coloured components; the major colouring principle is <i>cis</i> -norbixin, a minor colouring principle is <i>trans</i> -norbixin; thermal degradation products of norbixin may also be present as a result of processing	Contains several coloured components; the major colouring principle is <i>cis</i> -norbixin, a minor colouring principle is <i>trans</i> -norbixin; thermal degradation products of norbixin may also be present as a result of processing
Assay	≥ 25% colouring matter (expressed as bixin)	≥ 85% colouring matter (expressed as bixin)	≥ 85% colouring matter (expressed as norbixin)	≥ 35% colouring matter (expressed as norbixin)	≥ 15% colouring matter (expressed as norbixin)
Description	Dark red-brown to red-purple powder				
Solubility	Insoluble in water, slightly soluble in ethanol		Soluble in alkaline water, slightly soluble in ethanol		

Compound	Aqueous-processed bixin (annatto E) (JECFA, 2007)	Solvent-extracted bixin (annatto B) (JECFA, 2015)	Solvent-extracted norbixin (annatto C) (JECFA, 2015)	Alkali-processed norbixin, acid-precipitated (annatto F) (JECFA, 2007)	Alkali-processed norbixin, not acid-precipitated (annatto G) (JECFA, 2007)
Purity					
<i>Residual solvents</i>					
Ethanol	–	≤ 50 mg/kg, singly or in combination	≤ 50 mg/kg, singly or in combination	–	–
Iso-propyl alcohol	–			–	–
Ethyl acetate	–			–	–
Acetone	–	≤ 30 mg/kg	≤ 30 mg/kg	–	–
Methanol	–	≤ 50 mg/kg	≤ 50 mg/kg	–	–
Hexane	–	≤ 25 mg/kg	≤ 25 mg/kg	–	–
Norbixin	≤ 7% of total colouring matters	≤ 2.5% of total colouring matters	–	–	–
<i>Heavy metals</i>					
Arsenic	≤ 3 mg/kg	≤ 3 mg/kg	≤ 3 mg/kg	≤ 3 mg/kg	≤ 3 mg/kg
Lead	≤ 2 mg/kg	≤ 2 mg/kg	≤ 2 mg/kg	≤ 2 mg/kg	≤ 2 mg/kg
Mercury	≤ 1 mg/kg	≤ 1 mg/kg	≤ 1 mg/kg	≤ 1 mg/kg	≤ 1 mg/kg

3.1.3. Alkaline comet assay

Annatto E was given to Sprague Dawley rats by gavage at dose levels of 500, 1,000 and 2,000 mg/kg bw at two occasions. The assay was conducted in compliance with OECD Guideline 489 and with the principle of Good Laboratory Practise (GLP).

Only males were used in the main experiments as no differences in toxicity between the sexes has been reported previously. The test article was administered to rats using corn oil as vehicle at two occasions: 0 h (day 1) and 21 h (day 2). The positive control was ethyl methanesulfonate (150 mg/kg). The vehicle control and the three dose groups included six animals per group; the positive control group consisted of three animals. At the end of the main experiment, animals were sacrificed and liver and stomach were sampled at necropsy (at day 2, 24 h).

No clinical signs of toxicity were observed in any animal following treatments with vehicle, annatto E at any dose level or the positive control. No notable effect of treatment on body weights was observed.

Liver and stomach cells from animals treated with annatto E at all doses exhibited tail intensities and tail moments that were similar to the concurrent vehicle control group and that fell within the laboratory's historical vehicle control data. There were no statistically significant increases in tail intensity for any of the groups receiving the test article, compared to the concurrent vehicle control. There was no dose-related increase in the percentage of hedgehogs in liver or stomach cells following treatment with annatto E.

Both the vehicle control and the positive control data reported in this study were within laboratory's historical data.

It is concluded that, under the conditions of this comet assay, annatto E did not induce DNA strand breaks in the liver or stomach of rats when tested up to 2000 mg/kg bw per day.

3.2. Authorised uses and use levels

Maximum levels of annatto (E 160b) have been defined in Annex II to Regulation (EC) No 1333/2008⁸ on food additives, as amended. In this document, these levels are named maximum permitted levels (MPLs).

⁸ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

Currently, annatto (E 160b) is an authorised food additive in the EU with MPLs ranging from 10 to 50 mg/kg in 22 food categories.

Table 3 summarises foods that are currently permitted to contain annatto (E 160b) and the corresponding MPLs as set by Annex II to Regulation (EC) No 1333/2008, as well as the proposed uses and maximum use levels for bixin-based and norbixin-based annatto extracts (E 160b) from the applicant. For four food categories: 01.4, 05.2, 12.5 and 16, the applicant proposed three different scenarios. The Panel noted that, for bixin-based annatto extracts, scenarios 2 and 3 are similar.

Compared to the previous opinion (EFSA, Tard, 2017), the maximum proposed use level for the FC 01.4 is now increased for bixin-based extracts (15 instead of 10 mg/kg) and decreased for norbixin-based extracts (4 instead of 6 mg/kg). For the FC 05.2, the maximum proposed use level for bixin-based remain the same while for norbixin-based extract, one scenario proposes a maximum use level of 25 mg/kg (instead of 30 mg/kg in 2017). The current proposed maximum use level for bixin-based extract for FC 12.5 is the same as in 2017 (15 mg/kg) while the one for the norbixin-based extracts is lower (10 instead of 15 mg/kg). Finally, for the FC 16, the proposed maximum level for bixin-based extract is higher for two of the scenario (15 instead of 10 mg/kg) and the proposed maximum level for the norbixin-based extracts is lower in the same two scenarios (7.5 instead of 10 mg/kg).

Table 3: Current uses and MPLs of annatto (E 160b) in foods according to the Annex II to Regulation (EC) No 1333/2008, and proposed uses and maximum use levels for bixin-based and norbixin-base annatto extracts (E 160b) (in mg/L or mg/kg as appropriate)

Food category number	Food category name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate) for annatto	Normal/Mean use level (mg/L or mg/kg as appropriate) for bixin	Proposed maximum level (mg/L or mg/kg as appropriate) for bixin	Normal/Mean use level (mg/L or mg/kg as appropriate) for norbixin	Proposed maximum level (mg/L or mg/kg as appropriate) for norbixin
01.4	Flavoured fermented milk products including heat-treated products		10	Scenario 1/2/3: 8	Scenario 1/2/3:15	Scenario 1/2/3: 4	Scenario 1/2/3: 4
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 <i>red Leicester</i> cheese	50		–		50
01.7.2	Ripened cheese	Only <i>Mimolette</i> cheese	35		–		35
01.7.3	Edible cheese rind		20		20		20
01.7.5	Processed cheese		15		15		8
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

Food category number	Food category name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate) for annatto	Normal/Mean use level (mg/L or mg/kg as appropriate) for bixin	Proposed maximum level (mg/L or mg/kg as appropriate) for bixin	Normal/Mean use level (mg/L or mg/kg as appropriate) for norbixin			Proposed maximum level (mg/L or mg/kg as appropriate) for norbixin			
						Sc.1:	Sc.2:	Sc.3:	Sc.1:	Sc.2:	Sc.3:	
04.2.5.2	Jam, jellies and marmalades and sweetened chestnut purée as defined by Directive 2001/113/EC	Except chestnut purée	NP		20							20
04.2.5.3	Other similar fruit or vegetables spreads	Except <i>crème de pruneaux</i>	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 microsweets		NP	Scenario 1/2/3: 30	Scenario 1/2/3: 30	Sc.1: 30	Sc.2: 30	Sc.3: 25	Sc.1: 30	Sc.2: 30	Sc.3: 25	
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

Food category number	Food category name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate) for annatto	Normal/Mean use level (mg/L or mg/kg as appropriate) for bixin	Proposed maximum level (mg/L or mg/kg as appropriate) for bixin	Normal/Mean use level (mg/L or mg/kg as appropriate) for norbixin	Proposed maximum level (mg/L or mg/kg as appropriate) for norbixin
08.2	Meat preparations as defined by Regulation (EC) N°853/2004	Only <i>breakfast sausages</i> with a minimum cereal content of 6%, <i>burger meat</i> 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
08.3.1	Non-heat-treated meat products	Only chorizo sausage, salchichon, pasturmas and sobrasada	NP		20		20
08.3.2	Heat-treated meat products	Only sausages, pâtés, terrines and <i>luncheon meat</i>	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

Food category number	Food category name	Restrictions/ exceptions	MPL (mg/L or mg/kg as appropriate) for annatto	Normal/Mean use level (mg/L or mg/kg as appropriate) for bixin	Proposed maximum level (mg/L or mg/kg as appropriate) for bixin			Normal/Mean use level (mg/L or mg/kg as appropriate) for norbixin			Proposed maximum level (mg/L or mg/kg as appropriate) for norbixin		
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	Scenario 1/2/3: 15	Scenario 1/2/3:15			Scenario 1/2/3: 10			Scenario 1/2/3: 10		
12.6	Sauces	Including pickles, relishes, chutney and piccalilli; 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		
			20 ^(b)										
15.2	Processed nuts		10 ^(c)		10						10		
16	Desserts excluding products covered in categories 1, 3 and 4		10	Scenarios 1/2/3: 5	Sc.1: 10	Sc.2: 15	Sc.3: 15	Scenarios 1/2/3: 7.5			Sc.1: 10	Sc.2: 7.5	Sc.3: 7.5

MPL: maximum permitted level; 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.

The maximum levels mentioned above apply to the quantities of the colouring principles bixin and norbixin contained in the bixin-based and norbixin-based annatto extracts.

In the food categories in which the use of both bixin and norbixin is requested, these two colouring principles could be used:

- singly and not exceeding their specific/individual maximum permitted level or
- or when in combination, the sum of both should not exceed the higher maximum permitted level of the two compounds.

3.3. Exposure data

3.3.1. Reported use levels or data on analytical levels of annatto (E 160b)

Most food additives in the EU are authorised at a specific MPL. However, a food additive may be used at a lower level than the MPL. Therefore, information on actual use levels is required for performing a more realistic exposure assessment.

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 call⁹ 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 opinion, 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.

3.3.2. Summarised data extracted from the Mintel's Global New Products Database

The Mintel's GNPD is an online database which monitors new introductions of packaged goods in the market worldwide. It contains information of over 2.9 million food and beverage products of which more than 1,000,000 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.¹⁰

For the purpose of this Scientific Opinion, Mintel's GNPD¹¹ was used for checking the labelling of food and beverages products and food supplements for annatto (E 160b) within the EU's food market as the database contains the compulsory ingredient information on the label.

According to Mintel's GNPD, annatto (E 160b) was labelled on almost 6600 products between January 2013 and December 2018.¹² The food categories in which foods are mostly labelled with annatto (E 160b) is 'Hard Cheese & Semi-Hard Cheese', 'Dairy Based Ice Cream & Frozen Yogurt' and 'Sandwiches/Wraps'.

Appendix B lists the percentage of the food products labelled with annatto (E 160b) out of the total number of food products per food subcategories according to the Mintel's GNPD food classification. The percentages ranged from less than 0.1% in many food subcategories to 14.4% in Mintel's GNPD food subcategory 'Hard Cheese & Semi-Hard Cheese'. The average percentage of foods labelled to contain annatto (E 160b) was 1.4%.

⁹ 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>

¹⁰ Missing Bulgaria, Cyprus, Estonia, Latvia, Lithuania, Luxembourg, Malta and Slovenia.

¹¹ <http://www.gnpd.com/sinatra/home/> accessed on 17/12/2018.

¹² In the 2017 EFSA Statement, almost 3,000 products were found to be labelled with annatto (E 160b) between 2012 and 2017 while more than the double were found in the search done for the current assessment. This is due to the review and reclassification by Mintel GNPD of all colour additive ingredients. Much more products are found to be labelled now with the food additive in the Mintel categories of: Hard Cheese & Semi-Hard Cheese, Potato Snacks, Soft Cheese & Semi-Soft Cheese, Cakes, Pastries & Sweet Goods, Pizzas, Prepared Meals, Pastry Dishes.

3.3.3. Food consumption data used for exposure assessment

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)). Consumption surveys added in the Comprehensive database in 2015 were also taken into account in this assessment.¹³

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 includes the currently best available food consumption data across Europe.

Food consumption data from the following population groups were used for the exposure assessment: infants, toddlers, children, adolescents, adults and the elderly. For the present assessment, food consumption data were available from 33 different dietary surveys carried out in 19 European countries (Table 4).

Table 4: Population groups considered for the exposure estimates of bixin-based and norbixin-based annatto extracts (E 160b)

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 ^(a)	From 12 months up to and including 35 months of age	Belgium, Bulgaria, Denmark, Finland, Germany, Italy, Netherlands, Spain, UK
Children ^(b)	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, Netherlands, 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 ^(b)	From 65 years of age and older	Austria, Belgium, Denmark, Finland, France, Germany, Hungary, Ireland, Italy, Romania, Netherlands, Sweden, UK

(a): The term 'toddlers' in the EFSA Comprehensive Database corresponds to 'young children' in Regulations (EC) No 1333/2008 and (EU) No 609/2013.

(b): 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.

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 authorised and proposed were selected from the nomenclature of

¹³ Available online: <http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm>

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 three food categories (Appendixes C and D) 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, salchichon, pasturmas and *sobrasada*, only chorizo is a food item available in the EFSA Comprehensive database; salchichon, 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 were 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 a scientific opinion 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 (Appendixes C and D).

3.4. Exposure estimates

3.4.1. Exposure to bixin-based and norbixin-base annatto extracts (E 160b) from their use as a food additive

The Panel estimated the chronic dietary exposure to the annatto colouring principles bixin and norbixin (E 160b) when used as a food additive 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 C and D) with their respective consumption amount per kilogram 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 4). 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 with a sufficiently large sample size (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 estimated.

Exposure assessment to bixin-based and norbixin-based annatto extracts (E 160b) was carried out by the FAF Panel based on two different sets of concentration data: (1) proposed maximum use levels as provided by the applicant (defined as the *maximum level exposure assessment scenario*); and (2) proposed maximum use 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.

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 (Appendixes C and D).

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

Refined exposure assessment scenario

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

Appendixes C and D summarise 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, the Panel calculated two refined exposure estimates based on two 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 level 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 'normal/mean' use levels proposed or the mean of 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 'normal/mean' 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.

Dietary exposure to bixin-based and norbixin-based annatto extracts (E 160b)

Tables 5, 6 and 7 summarise the estimated exposure to bixin-based and norbixin-based annatto extracts (E 160b) from its use as a food additive in six population groups (Table 4) according to the different exposure scenarios. Detailed results per population group and survey are presented in Appendixes E, G, I, K and M.

Table 5: Summary of dietary exposure to bixin-based and norbixin-base annatto extracts (E 160b) from its use as a food additive in the maximum level exposure assessment scenario and in the refined exposure scenarios, **according to levels defined in the scenario 1**, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Bixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.01–0.08	0.08–0.56	0.06–0.46	0.05–0.32	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.38	0.33–1.06	0.15–0.97	0.13–0.64	0.07–0.43	0.04–0.19
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.01–0.08	0.07–0.53	0.05–0.43	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.31–1.03	0.15–0.93	0.13–0.63	0.07–0.42	0.04–0.19
Non-brand-loyal scenario						
• Mean	0.01–0.08	0.06–0.50	0.05–0.42	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.20–1.03	0.15–0.89	0.13–0.63	0.06–0.42	0.03–0.19
Norbixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.02–0.07	0.07–0.22	0.06–0.18	0.03–0.09	0.02–0.06	0.01–0.07
• 95th percentile	0.07–0.24	0.16–0.35	0.13–0.33	0.06–0.18	0.05–0.14	0.03–0.15
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.02–0.06	0.06–0.21	0.06–0.16	0.02–0.08	0.02–0.06	0.01–0.06
• 95th percentile	0.07–0.22	0.15–0.33	0.12–0.29	0.05–0.17	0.04–0.13	0.03–0.14
Non-brand-loyal scenario						
• Mean	0.01–0.05	0.05–0.20	0.05–0.15	0.02–0.08	0.01–0.05	0.01–0.05
• 95th percentile	0.05–0.17	0.12–0.32	0.10–0.28	0.04–0.17	0.04–0.12	0.03–0.11

Table 6: Summary of dietary exposure to bixin-based and norbixin-base annatto extracts (E 160b) from its use as a food additive in the maximum level exposure assessment scenario and in the refined exposure scenarios, **according to levels defined in the scenario 2**, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Bixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.01–0.08	0.08–0.57	0.06–0.46	0.05–0.32	0.02–0.16	0.01–0.08
• 95th percentile	0.05–0.40	0.33–1.07	0.15–0.99	0.13–0.65	0.07–0.43	0.04–0.20
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.01–0.08	0.08–0.54	0.05–0.43	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.31–1.03	0.15–0.93	0.13–0.63	0.07–0.42	0.04–0.19
Non-brand-loyal scenario						
• Mean	0.01–0.08	0.06–0.50	0.05–0.42	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.20–1.03	0.15–0.89	0.13–0.63	0.06–0.42	0.03–0.19

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Norbixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.02–0.06	0.06–0.22	0.06–0.17	0.03–0.09	0.02–0.06	0.01–0.07
• 95th percentile	0.07–0.23	0.16–0.34	0.13–0.32	0.05–0.18	0.05–0.14	0.03–0.15
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.02–0.06	0.06–0.20	0.06–0.16	0.02–0.08	0.02–0.06	0.01–0.06
• 95th percentile	0.07–0.22	0.15–0.33	0.12–0.29	0.05–0.17	0.04–0.13	0.03–0.14
Non-brand-loyal scenario						
• Mean	0.01–0.05	0.05–0.20	0.05–0.15	0.02–0.08	0.01–0.05	0.01–0.05
• 95th percentile	0.05–0.17	0.12–0.32	0.10–0.28	0.04–0.17	0.04–0.12	0.03–0.11

Table 7: Summary of dietary exposure to bixin-based and norbixin-base annatto extracts (E 160b) from its use as a food additive in the maximum level exposure assessment scenario and in the refined exposure scenarios, **according to levels defined in the scenario 3**, in six population groups (minimum–maximum across the dietary surveys in mg/kg bw per day)

	Infants (12 weeks to 11 months)	Toddlers (12–35 months)	Children (3–9 years)	Adolescents (10–17 years)	Adults (18–64 years)	The elderly (≥ 65 years)
Bixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.01–0.08	0.08–0.57	0.06–0.46	0.05–0.32	0.02–0.16	0.01–0.08
• 95th percentile	0.05–0.40	0.33–1.07	0.15–0.99	0.13–0.65	0.07–0.43	0.04–0.20
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.01–0.08	0.08–0.54	0.05–0.43	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.31–1.03	0.15–0.93	0.13–0.63	0.07–0.42	0.04–0.19
Non-brand-loyal scenario						
• Mean	0.01–0.08	0.06–0.50	0.05–0.42	0.04–0.30	0.02–0.15	0.01–0.08
• 95th percentile	0.05–0.36	0.20–1.02	0.15–0.89	0.13–0.63	0.06–0.42	0.03–0.19
Norbixin-based annatto extracts (E 160b)						
Maximum level exposure assessment scenario						
• Mean	0.02–0.06	0.06–0.21	0.06–0.17	0.03–0.09	0.02–0.06	0.01–0.07
• 95th percentile	0.07–0.23	0.15–0.34	0.13–0.31	0.05–0.18	0.05–0.14	0.03–0.15
Refined estimated exposure assessment scenario						
Brand-loyal scenario						
• Mean	0.02–0.06	0.06–0.20	0.06–0.15	0.02–0.08	0.02–0.06	0.01–0.06
• 95th percentile	0.07–0.22	0.14–0.31	0.12–0.29	0.05–0.17	0.04–0.13	0.03–0.14
Non-brand-loyal scenario						
• Mean	0.01–0.05	0.05–0.19	0.05–0.15	0.02–0.08	0.01–0.05	0.01–0.05
• 95th percentile	0.05–0.17	0.12–0.31	0.10–0.28	0.04–0.17	0.04–0.12	0.03–0.11

For bixin-based extracts, and according to the three scenarios, mean exposure estimates are all below the ADI of 6 mg/kg bw per day. At the maximum level exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.57 mg/kg bw per day and at the 95th percentile between 0.04 and 1.07 mg/kg bw per day. At the refined brand-loyal estimated exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.54 mg/kg bw per day and at the 95th percentile between 0.04 and 1.03 mg/kg bw per day. At the refined non-brand-loyal estimated exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.5 mg/kg bw per day and at the 95th percentile between 0.03 and 1.03 mg/kg bw per day.

For norbixin-based extracts, and according to the three scenarios, at the maximum level exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.22 mg/kg bw per day and at the 95th percentile between 0.03 and 0.35 mg/kg bw per day. At the refined brand-loyal estimated exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.21 mg/kg bw per day and at the 95th percentile between 0.03 and 0.33 mg/kg bw per day. At the refined non-brand-loyal estimated exposure assessment scenario, exposure estimates range at the mean between 0.01 and 0.2 mg/kg bw per day and at the 95th percentile between 0.03 and 0.31 mg/kg bw per day.

Main food categories contributing to exposure to bixin-based annatto extracts (E 160b) using the maximum level exposure assessment scenario

Scenario 1: From the *maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates for infants and toddlers were flavoured fermented milk products and flavoured drinks. For children, added to these two food categories, soups and broths are also a main contributing food category. For adolescents, adults and the elderly, the main contributing food categories were flavoured drinks and soups and broths (Appendix F).

Scenarios 2 and 3: From the *maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates for infants, toddlers, children and adolescents were flavoured fermented milk products and flavoured drinks. For adults and the elderly, the main contributing food categories were flavoured drinks and soups and broths (Appendix H).

Main food categories contributing to exposure to bixin-based annatto extracts (E 160b) using the refined exposure assessment scenario

Scenario 1: From the *refined estimated exposure scenario, brand-loyal scenario*, the main contributing food categories to the total mean exposure estimates were flavoured fermented milk products and flavoured drinks for infants and toddlers; and flavoured fermented milk products, flavoured drinks and soups and broths for children. For adolescents, adults and the elderly, they were flavoured drinks and soups and broths. In the *non-brand-loyal scenario*, the main contributing food categories were flavoured fermented milk products and flavoured drinks for infants and toddlers. For children, adolescents, adults and the elderly, they were flavoured drinks and soups and broths (Appendix F).

Scenarios 2 and 3: From the *refined estimated exposure scenario, brand-loyal scenario*, the main contributing food categories to the total mean exposure estimates were flavoured fermented milk products and flavoured drinks for infants and toddlers; and flavoured fermented milk products, flavoured drinks and soups and broths for children. For adolescents, adults and the elderly, they were flavoured drinks and soups and broths. In the *non-brand-loyal scenario*, the main contributing food categories were the same (Appendix H).

Main food categories contributing to exposure to norbixin-based annatto extracts (E 160b) using the maximum level exposure assessment scenario

Scenarios 1, 2 and 3: From the *maximum level exposure assessment scenario*, the main contributing food categories to the total mean exposure estimates for infants were breakfast cereals, fine bakery wares and soups and broths; for toddlers, they were breakfast cereals and fine bakery wares; for children, they were fine bakery wares and soups and broths; for adolescents, they were fine bakery wares and meat products; for adults, they were fine bakery wares, meat products and soups and broths; for the elderly, they were breakfast cereals, fine bakery wares, meat products and soups and broths (Appendices J, L and N).

Main food categories contributing to exposure to norbixin-based annatto extracts (E 160b) using the refined exposure assessment scenario

Scenario 1: From the *refined estimated exposure scenario*, for both *brand-loyal* and *non-brand-loyal scenarios*, the contributing food categories to the total mean exposure estimates are for infants breakfast

cereals, fine bakery wares and soups and broths; for toddlers, they were breakfast cereals and fine bakery wares; for children, they were fine bakery wares and soups and broths; for adolescents and adults, they were fine bakery wares, meat products and soups and broths; for the elderly, they were breakfast cereals, fine bakery wares, meat products and soups and broths (Appendix J).

Scenario 2: For the *brand-loyal scenario*, the main contributing food categories to the total mean exposure estimates for infants were breakfast cereals, fine bakery wares and soups and broths; for toddlers, they were breakfast cereals and fine bakery wares; for children, they were fine bakery wares and soups and broths; for adolescents, they were fine bakery wares, meat products and soups and broths; for adults, they were fine bakery wares, meat products, soups and broths and breakfast cereals; for the elderly, they were breakfast cereals, fine bakery wares, meat products and soups and broths. For the *non-brand-loyal scenario*, the main contributing food categories to the total mean exposure estimates for infants were breakfast cereals, fine bakery wares and soups and broths; for toddlers, they were breakfast cereals, fine bakery wares, flavoured fermented milk products and meat products; for children, they were fine bakery wares and soups and broths; for adolescents, they were fine bakery wares, meat products and soups and broths; for adults, they were fine bakery wares, meat products, soups and broths and sauces; for the elderly, they were breakfast cereals, fine bakery wares, meat products and soups and broths (Appendix J).

Scenario 3: For both the *brand-loyal* and *non-brand-loyal scenario*, the main contributing food categories to the total mean exposure estimates for infants were breakfast cereals, fine bakery wares and soups and broths; for toddlers, they were breakfast cereals and fine bakery wares; for children, they were fine bakery wares and soups and broths; for adolescents, they were fine bakery wares, meat products and soups and broths; for adults, they were fine bakery wares, meat products and soups and broths; for the elderly, they were breakfast cereals, fine bakery wares, meat products and soups and broths (Appendix L).

Uncertainty analysis

Uncertainties in the exposure assessment of bixin-based and norbixin-based annatto extracts (E 160b) 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 8.

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

Sources of uncertainties	Direction ^(a)
Consumption data: different methodologies/representativeness/underreporting/misreporting/no portion size standard	+/-
Methodology used to estimate high percentiles (95th) long-term (chronic) exposure based on data from food consumption surveys covering only a few days	+
Correspondence of reported use levels and analytical data to the food items in the EFSA Comprehensive Food Consumption Database: uncertainties to which types of food the levels refer to	+/-
Uncertainty in possible national differences in use levels of food categories	+/-
Concentration data: - use levels considered applicable to all foods within the entire food category, whereas on average 1.4% of the foods, belonging to food categories with foods labelled with additive, was labelled with the additive	+
Food categories selected for the exposure assessment: exclusion of food categories due to missing FoodEx linkage (n=3 for both for bixin and norbixin-based annatto extracts)	-
Food categories selected for the exposure assessment: inclusion of food categories without considering the restriction/exception (n=3 for both for bixin and norbixin-based annatto extracts)	+
Maximum level exposure assessment scenario: - exposure calculations based on the maximum proposed use levels	+
Refined exposure assessment scenarios: - exposure calculations based on the maximum or mean levels (reported use from industries or maximum proposed use levels)	+/-

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

Considering that exposure estimates are based on proposed use levels and on reported use levels for food categories which are already authorised and for which no changes in the currently MPLs are proposed, almost all foods in which annatto is authorised are taken into account in the exposure estimates; only the food for which no consumption data are available in the EFSA Comprehensive database are missing.

The Panel noted that information from Mintel's GNPD (Appendix B) indicated that some subcategories are labelled to contain annatto (E 160b) while the corresponding food categories from the Regulation (EC) No 1333/2008 are not authorised to contain the food additive (not in the Table 1, e.g. eggs and egg products, cream, Vitamins & Dietary Supplements, chocolate tablets, etc.).

The Panel noted that the main contributing food categories to the total mean exposure seem to have a low percentage of foods labelled with annatto (e.g. FC 14.1.4 Flavoured drinks which in Mintel are 'Fruit/Flavoured Still Drinks', 'Beverage Concentrates', 'Flavoured Milk'; FC 12.5 Soups and broths which in Mintel are 'Dry Soup', 'Wet Soup'; FC 06.3 Breakfast cereals which in Mintel are 'Cold Cereals', 'Snack/Cereal/Energy Bars'), only the FC 07.2 Fine bakery wares have a higher percentage of foods labelled with the additive ('Pastry dishes', 'Pizzas'). See Appendix B.

The Panel further noted that the percentage of foods per subcategory labelled to contain annatto (E 160b) was maximally about 14% in the food subcategory Hard Cheese & Semi-Hard Cheese (out of almost 10,000 products in total) (Appendix B), with an average percentage of 1.4% among all subcategories. In the exposure assessment, it was assumed that 100% of the foods belonging to an authorised food category contained the additive.

Given the observation abovementioned and based on the assumption that the food additive will not be used in all food categories at the proposed use levels, the Panel considered that overall the uncertainties identified would result in an overestimation of the exposure to bixin-based and norbixin-based annatto extracts (E 160b) as a food additive in European countries considered in the EFSA European database for the maximum level exposure scenario.

Considering the methodology used in the exposure assessment which take into account that all foods from the diet of each person contain the food additives, also the refined scenarios would in general result in an overestimation of exposure.

3.5. Discussion

This opinion covers the assessment of new genotoxicity data provided to EFSA for the substance annatto E jointly with a revised exposure assessment taking into account new proposed uses and use levels.

New genotoxicity data have been generated as a follow-up of the previous re-evaluation of the food colour Annatto (E 160b) for which some concerns with respect to the mutagenicity of annatto E could not be ruled out by the former ANS Panel (EFSA ANS Panel, 2016).

These data consist of a new *in vivo* comet assay conducted in compliance with OECD Guideline 489 and with the GLP principle. In this assay, rats were treated at two occasions at an interval of 21 hours up to the maximum concentration recommended by OECD for non-toxic substances (2,000 mg/kg bw per day) and liver and stomach were analysed for the occurrence of DNA damage.

In the animals treated with annatto E at all doses, the parameters of DNA damage (tail intensity and tail moment) were similar to the concurrent vehicle control group and fell within the laboratory's historical vehicle control data.

The results of this study show that annatto E does not induce DNA damage *in vivo* and are sufficient to rule out the concern regarding the potential mutagenicity of this food colour.

To assess the dietary exposure to bixin-based and norbixin-based annatto extracts (E 160b) from its use as a food additive, the exposure was calculated based on the proposed use levels and reported use levels according to three different scenarios proposed by the applicant.

For bixin, none of the exposure estimates in the *maximum level exposure assessment scenario* or the *refined exposure assessment scenarios* exceeded the ADI of 6 mg/kg bw per day.

For norbixin, exposure estimates in the *maximum level exposure assessment scenario* reached the ADI of 0.3 mg/kg bw per day in the three scenarios proposed by the applicant at the high level (p95) for toddlers and children. The ADI was also reached at the p95 for toddlers in the three scenarios of the *brand-loyal* and *non-brand-loyal refined exposure assessment*. The high level was observed in one country, the Netherlands. The Panel noted that the main food category contributing for this population of toddlers is flavoured fermented milk products including heat-treated products. It has been indicated previously that the whole food category was taken into account while most probably not all foods would

contain the food additive. This is confirmed by Mintel GNPD which shows a low percentage of foods within this food category currently labelled with annatto (E 160b). Considering the uncertainties and the very likely overestimation of the exposure, the level of exposure is likely to be below the ADI.

In addition, the applicant proposed to raise the accepted natural level for norbixin in the bixin-based annatto extract from 2.5% to 5%. The Panel noted that when this suggestion is implemented, the foods to which this bixin-based annatto is added should still comply with the legislation with respect to the MPLs for bixin and norbixin.

Compared to the previous estimates, the maximum use level proposed for norbixin-based annatto extracts for FC 01.4 Flavoured fermented milk products including heat-treated products decreased from 6 to 4 mg/kg and for FC 12.5 Soups and broths from 15 to 10 mg/kg. This explains mainly the decrease in Belgium children exposure to norbixin-based annatto extracts which does not longer exceed the ADI.

Not all food categories could be included in the assessment owing to specific restrictions/exceptions regarding products not referenced in the FoodEx classification. This may have resulted in an underestimation of exposure to both bixin-based and norbixin-based annatto extracts (E 160b). On the other hand, one food category was included without considering specific restrictions/exceptions (breakfast cereals) and two others (flavoured drinks, flavoured fermented milk products) which do not formally have restrictions, but it is clear that not all foods within those food categories will be coloured with annatto, such as clear flavoured drinks (seven up/sprite) or colas. This may have overestimated the exposure. In total, 23 and 26 food categories were taken into account in the present exposure estimates for bixin-based and norbixin-based annatto extracts (E 160b), respectively.

Added to that, approximately 92% of the food products labelled with annatto (E 160b) in the Mintel's GNPD belonged to food subcategories that were considered in the exposure assessment (Appendix B).

The Panel considered that the exposure estimates in all exposure scenarios should result in overestimates of the exposure to bixin-based and norbixin-based annatto extracts (E 160b) from its use as a food additive according to Annex II to Regulation (EC) No 1333/2008.

The Panel noted that these exposure estimates are based on information provided on the proposed use levels of bixin-based and norbixin-based annatto extracts (E 160b) and are therefore theoretical exposure estimates.

The Panel noted that there are no substantial differences in exposure between the three scenarios proposed by the applicant and the previous exposure assessments calculated by EFSA ANS Panel (2016). This is due to the fact that the changes in use levels proposed by the applicant for the three scenarios are not on the main food categories contributing to the overall exposure. Therefore, no major impact on the current estimates is observed.

4. Conclusions

In a new *in vivo* comet assay submitted by the applicant, annatto E did not induce DNA damage in the liver and stomach of rats after oral treatment. The results of this study allow to clarify the concern on the mutagenicity of the annatto E raised by the comet assay evaluated in the previous EFSA opinion and to conclude that the food colour annatto E does not raise concern for genotoxicity. Accordingly, the ADIs established in 2016 for the two colouring principles bixin and norbixin can be applied also to the annatto E.

For bixin, none of the exposure estimates in the *maximum level exposure assessment scenario* or the *refined exposure assessment scenarios* exceeded the ADI of 6 mg/kg bw per day.

For norbixin, exposure estimates in the *maximum level exposure assessment scenario* reached the ADI of 0.3 mg/kg bw per day in the three scenarios proposed by the applicant at the high level (p95) for toddlers and children. The ADI was also reached at the p95 for toddlers in the three scenarios of the *brand-loyal* and *non-brand-loyal refined exposure assessment scenario* in one country. Considering the uncertainties and the very likely overestimation of the exposure, the Panel concluded that the level of exposure does not raise a health concern.

Documentation provided to EFSA

- 1) Proposed uses and maximum uses for bixin-based and nobixin-nased annatto extracts. Submitted by the European Commission on 01/08/2018.

- 2) Final report Annatto E: Rat Alkaline Comet Assay. Covance Laboratories Ltd. Covance study number 8361014, report issue on 13 July 2017. Submitted by the European Commission on 01/08/2018.

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Abbreviations

ADI	acceptable daily intake
ANS	EFSA Scientific Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
FAF	Food Additives and Flavourings
FCS	food categorisation system
JECFA	Joint FAO/WHO Expert committee on Food Additives
MPL	Maximum permitted level
NATCOL	Natural Food Colours Association
OECD	Organisation for Economic Co-operation and Development

Appendix A – Summary of the proposed use levels (mg/kg or mg/L as appropriate) of Annatto, Bixin, Norbixin (E 160b) provided by industry

Appendix B – Number and percentage of food products labelled with Annatto, Bixin, Norbixin (E 160b) out of the total number of food products present in the Mintel GNPD per food subcategory between 2013 and 2018

Appendix C – Concentration levels of bixin-based annatto extracts (E 160b) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

Appendix D – Concentration levels of norbixin-based annatto extracts (E 160b) used in the exposure assessment scenarios (mg/kg or mL/kg as appropriate)

Appendix E – Summary of total estimated exposure of bixin-based annatto extracts (E 160b) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day) – Scenario 1

Appendix F – Main food categories contributing to exposure to bixin-based annatto extracts (E 160b) from its use as a food additive using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure) – Scenario 1

Appendix G – Summary of total estimated exposure of bixin-based annatto extracts (E 160b) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day) – Scenario 2

Appendix H – Main food categories contributing to exposure to bixin-based annatto extracts (E 160b) from its use as a food additive using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure) – Scenario 2

Appendix I – Summary of total estimated exposure of norbixin-based annatto extracts (E 160b) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day) – Scenario 1

Appendix J – Main food categories contributing to exposure to norbixin-based annatto extracts (E 160b) from its use as a food additive using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure) – Scenario 1

Appendix K – Summary of total estimated exposure of norbixin-based annatto extracts (E 160b) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day) – Scenario 2

Appendix L – Main food categories contributing to exposure to norbixin-based annatto extracts (E 160b) from its use as a food additive using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure) – Scenario 2

Appendix M – Summary of total estimated exposure of norbixin-based annatto extracts (E 160b) from its use as a food additive for the maximum level exposure scenario and the refined exposure assessment scenarios per population group and survey: mean and 95th percentile (mg/kg bw per day) – Scenario 3

Appendix N – Main food categories contributing to exposure to norbixin-based annatto extracts (E 160b) from its use as a food additive using the maximum level exposure assessment scenario and the refined exposure assessment scenarios (> 5% to the total mean exposure) – Scenario 3

Appendices A–N can be found in the online version of this output ('Supporting information' section)