Abstract

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) of EFSA was requested to deliver a scientific opinion on the implications for human health of the flavouring substance 4',5,7-trihydroxyflavanone or naringenin [FL-no: 16.132], in the Flavouring Group Evaluation 410 (FGE.410), according to Regulation (EC) No 1331/2008 of the European Parliament and of the Council. The substance occurs naturally in grapefruits, oranges and tomatoes. It is intended to be used as a flavouring substance with flavour-modifying properties in specific categories of food. Information on specifications and manufacturing of [FL-no: 16.132] were considered adequate; however, data on stability in food are incomplete. The Panel noted that the available genotoxicity studies have significant shortcomings and are insufficient to conclude on the genotoxic potential of naringenin. Therefore, [FL-no: 16.132] cannot be evaluated through the Procedure. Additionally, the Panel noted that inhibition of CYP 450 by [FL-no: 16.132] has been clearly demonstrated in animal species in vivo which implies that the substance may interact with the metabolism and elimination of medicines and no convincing information is available that this does not pose a risk to humans at the estimated levels of exposure. To continue with the safety assessment of [FL-no: 16.132], a bacterial gene mutation assay and an in vitro micronucleus assay (according to OECD guidelines 471, 487 and GLP) are required. Even if these studies do not indicate a genotoxic potential, additional toxicological data are needed to finalise the evaluation.

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1. Introduction

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

The use of flavouring in food is regulated under Regulation (EC) No 1334/2008\(^1\) of the European Parliament and Council of 16 December 2008 on flavourings and certain food ingredients with the flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

Regulation (EC) No 1331/2008\(^2\) establishing a common authorization procedure on food additives, food enzymes and food flavourings applies for the evaluation and approval of new flavouring substances.

An application for the substance 4’,5,7-trihydroxyflavanone (CASnr 67604-48-2) has been submitted to the Commission for authorisation as a new flavouring substance. The application has now been considered valid by the Commission.

1.1.1. Terms of Reference

In order for the Commission to be able to consider its inclusion of this substance in the Union list of flavourings and source materials (Annex I of Regulation (EC) No 1334/2008), EFSA should carry out a safety assessment of this substance.

The European Commission requests the European Food Safety Authority to carry out a safety assessment on 4’,5,7-trihydroxyflavanone as a new flavouring substance in accordance with Regulation (EC) No 1331/2008.

1.2. Interpretation of the Terms of Reference

The present scientific opinion FGE.410 covers the safety assessment of the following flavouring substance: 4’,5,7-trihydroxyflavanone [FL-no: 16.132], also reported as naringenin. For sake of consistency, only the trivial name will be used in this opinion. The substance will be evaluated as a flavouring substance with modifying properties\(^3\) in line with Regulation (EC) No 1334/2008.

2. Data and methodologies

The present evaluation is based on data on naringenin [FL-no: 16.132] provided by the applicant in a dossier submitted in support of its application for authorisation as a new flavouring substance.

The estimation of the dietary intake of naringenin will be based on use and use levels submitted by the applicant for the following categories of food and beverages: 1.7, 3.0, 5.1, 6.3, 7.2, 8.2, 12.5, 14.1c, 14.2.1 and 15.1. (see Appendix B).

The safety assessment of naringenin [FL-no: 16.132] (CASrn 67604-48-2) will be carried out by EFSA in accordance with the procedure as lined out in the EFSA scientific opinion ‘Guidance on the data required for the risk assessment of flavourings to be used in or on foods’ (EFSA CEF Panel, 2010a) and the technical report of EFSA ‘Proposed template to be used in drafting scientific opinions on flavouring substances (explanatory notes for guidance included)’ (EFSA, 2012).

The Procedure for the safety evaluation of the flavouring substance is given in Appendix A (see also Cramer et al., 1978).

3. Assessment

3.1. Identity of the substance

4’,5,7-Trihydroxyflavanone has been allocated the FLAVIS number [FL-no: 16.132]. The trivial name of the flavouring substance is naringenin. For the sake of consistency, this will be used in this opinion.

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\(^1\) Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34–50.

\(^2\) Regulation (EC) No 1331/2008 of the European Parliament and of the Council of 16 December 2008 establishing a common authorisation procedure for food additives, food enzymes and food flavourings. OJ L 354, 31.12.2008, p. 1–6.

\(^3\) https://ec.europa.eu/food/sites/food/files/safety/docs/fs_food-improvement-agents_flavourings-guidance_modifying_properties.pdf
3.2. Organoleptic characteristics

Naringenin has been reported as an odourless off-white to light yellow powder with sweet to sour taste. Its taste, however, has not been reported to be significant as can be seen from the sensory data provided on naringenin when tested as a flavour modifier in beverages, dairy drinks, yoghurt, soft candy, cereals, flavoured snacks, soups and broths.

3.3. Existing authorisations and evaluations

The Panel is not aware of any official evaluations of FGE.410 performed by national or international authorities. In August 2015, [FL-no: 16.132] was allocated the status ‘Generally recognised as safe’ (GRAS) by the Flavour and Extract Manufactures Associations (FEMAs) expert Panel (FEMA no 4797); therefore, naringenin [FL-no: 16.132] was included in the FEMA GRAS 27 list.

3.4. Technical data

The specifications of the flavouring substance are summarised in Table 1.

3.4.1. Information on the configuration of the flavouring substance

Naringenin obtained via extraction and subsequent hydrolysis from grapefruits, is a mixture of 2R- and 2S-isomers that is variable in isomeric composition (Gaffield et al., 1975).

3.4.2. Manufacturing process

The source material for the manufacturing process of naringenin is grapefruit peel (Citrus maxima (J. Burman) Merr.).

The glycoside (naringin) of [FL-no: 16.132] is first extracted from grapefruit peel with 50% ethanol in water to obtain substances of midpolarity such as flavonoids. The fluid extract is then centrifuged to remove particles and washed through a resin column. The flavonoids are released from the resin using 65% ethanol in water. The collected ethanol fractions are then concentrated to partially remove ethanol and water. Subsequently, this concentrate is spray-dried and the glycoside is crystallised. Enzymatic or acidic hydrolysis follows during which the glycosidic bond in naringin is hydrolysed to naringenin. Finally, naringenin is crystallised and vacuum-dried.

The applicant provided data on the presence of ethanol as a residual solvent and heavy metals in the final product.

Although there are no regulatory restrictions for contaminants in flavourings, the Panel took into consideration the provisions of Commission Regulation (EU) No 231/20124 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/20085 of the European Parliament and of the Council. When ethanol is used in the manufacturing of certain food additives (e.g. food colours, gums, sweeteners, etc.), different restrictions on the levels of ethanol per food additive are reported in the Regulation (e.g. from 50 mg/kg to 2% of ethanol in the food additive). The level of 5,000 mg ethanol reported per kg of naringenin [FL-no: 16.132] is within the occurrence range of ethanol in food additives. Similarly, the concentrations of arsenic, lead, cadmium and mercury reported for naringenin, comply with the requested specifications of food additives.

3.4.3. Stability and decomposition products

The stability of naringenin [FL-no: 16.132] was evaluated over a 5-week period at room temperature and at 40°C. The substance was found to be stable at both temperatures (recovery ≥ 94%). However, no tests were conducted for the substance at temperatures higher than 40°C, even though the substance is intended to be incorporated in food categories 6.3 (breakfast cereals) and 7.2 (fine bakery wares) where higher process temperatures may apply.

Although the flavouring substance is also intended to be used in beverages, the applicant did not investigate the stability of the flavouring substance in solution. The Panel conducted a literature search

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4 Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 1-295.
5 Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16-33.
on this topic and concluded that the substance would not be expected to be unstable in solution, based on Biesaga (2011).

**Interaction with food components**

No information given.

3.4.4. **Particle size**

The particle size of the commercial material was reported to be smaller than 80 mesh (~ 0.18 mm) (Flavour Industry, 2015). No particle size distribution for the final product was provided. Based on the reported manufacturing process and the available information on solubility, consumer exposure is not anticipated to be in form of nanoparticles.

3.4.5. **Conclusion on specifications**

The Panel concluded that the specifications and manufacturing data provided for the flavouring substance are adequate. Data on stability in food are incomplete, since the heating and storage conditions for which stability in food was investigated, were not representative for all intended uses.

3.5. **Structural/metabolic similarity to substances in an existing FGE**

3.5.1. **Naringenin/naringin**

In the technical dossier, the applicant applies the read-across between naringenin [FL-no: 16.132] and naringin [FL-no: 16.058], a related flavouring substance evaluated in FGE.32 (EFSA CEF Panel, 2010b). Based on this approach, no new toxicity studies were conducted on naringenin.

To investigate the validity of the read-across and the relevance of a group-based evaluation of the flavouring substance according to the EFSA Guidance (EFSA CEF Panel, 2010a), the Panel took the structural/metabolic similarities between naringenin [FL-no: 16.132] and the substances from FGE.32 into consideration.

Naringenin (aglycone) and naringin (glycoside) (see Table 2) share as a common part the flavanone structure. However, the absence of the rhamnose-glucose moiety at the 7-position of naringenin results in major differences in absorption and bioavailability after oral exposure between the two compounds. The most important of these differences is linked to the different sites of absorption of the two substances. Briefly, naringin has to be metabolised to naringenin by hydrolases in the colon before it can be absorbed (as naringenin) (see Figure 1). However, when naringenin is administered orally as such, it will be rapidly absorbed from the small intestine. This is also supported by similar results of the glycoside/aglycone flavanone pair hesperidin/hesperetin where the same rhamnose-glucose moiety is involved (see Appendix C for more details). This difference in absorption results in major differences in pharmacokinetics between naringin and naringenin. Moreover, the difference in absorption site may affect extent and pattern of metabolism by the gut microbiota (see Section 3.8). Based on the above, the Panel concluded that despite the common flavanone moiety and the common metabolites of the two substances identified in urine, the read-across between naringenin and naringin is not applicable.

**Figure 1:** The metabolic conversion of naringin to naringenin in the colon

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6 Commission Recommendation 2011/696/EU of 18 October 2011 on the definition of nano-materials. Official Journal of the European Union L257/38-40 on 20.10.2010 (http://ec.europa.eu/environment/chemicals/nanotech/pdf/commission_recommendation.pdf).
## Table 1: Specifications

| FL-no | Chemical name | Structural formula | JECFA no | FEMA no | CoE no | CAS no | EINECS no | Odour | Phys. form | Mol. formula | Mol. weight | Impurities | Solubility\(^{(a)}\) | Solubility in ethanol\(^{(b)}\) | Others | Boiling point, °C\(^{(c)}\) | Melting point, °C | ID test | Assay minimum | Refrac. index\(^{(d)}\) | Spec. gravity\(^{(e)}\) | Specification comments |
|-------|---------------|--------------------|----------|---------|--------|--------|----------|--------|------------|--------------|-------------|------------|----------------|-----------------------------|---------|-----------------|-----------------|--------|---------------|-----------------|-----------|----------------|------------------|----------------|--------------------------|
| 16.132| 4',5,7-Trihydroxyflavanone | ![Structural formula](image) | 4797     | –       | –      | 67604-48-2 | –        | Odourless off-white to light yellow powder with sweet to sour taste | Solid | C_{15}H_{12}O_{5} | 272.257 | Ethanol ~ 1% | Poorly soluble\(^{(f)}\) | Poorly soluble | – | 251 | NMR > 95% | n.a. | n.a. | Trivial name: naringenin |

The CASrn refers to the racemate (Flavour Industry, 2017).

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FL-No: FLAVIS number; JECFA: The Joint FAO/WHO Expert Committee on Food Additives; FEMA: Flavor and Extract Manufacturers Association; CoE: Council of Europe; CAS: Chemical Abstract Service; EINECS: European Inventory of Existing Commercial Chemical Substances; ID: Identity; NMR: nuclear magnetic resonance spectroscopy.

\(^{(a)}\): Solubility in water, if not otherwise stated.
\(^{(b)}\): Solubility in 95% ethanol, if not otherwise stated.
\(^{(c)}\): At 1,013.25 hPa (1 atm), if not otherwise stated.
\(^{(d)}\): At 20°C, if not otherwise stated.
\(^{(e)}\): At 25°C, if not otherwise stated.

\(^{(f)}\): Range from 9 to 475 mg/L (Shulman et al., 2011; Tomassini et al., 2004; ChemIDPlus-A Toxnet Database at https://chem.nlm.nih.gov/chemidplus/rn/480-41-1).
3.5.2. Naringenin/quercetin

Following a request for genotoxicity data on naringenin, the applicant did not proceed with the studies requested but applied a read-across between naringenin [FL-no: 16.132] and the flavonol quercetin (see Table 2) for which some genotoxicity data are available.

To investigate the validity of the read-across to quercetin and the relevance of a group-based evaluation of the flavouring substance according to the EFSA Guidance (EFSA CEF Panel, 2010a), the Panel took the structural/metabolic similarities between naringenin [FL-no: 16.132] and quercetin into consideration. Regarding the structural similarity between the two substances, the Panel noted that naringenin is a flavanone, whereas quercetin is a flavonol with a double bond in position 2 and a hydroxyl group in position 3 that is subject to tautomerism. Moreover, the two hydroxyl groups on the C-ring of quercetin are subject to oxidation and may yield reactive quinones, highly reactive electrophiles capable of reacting with thiols. This illustrates that these two substances may follow different metabolic pathways.

Additionally, the Panel noted that there is variability in the toxicological profiles between the two substances: naringenin was found to be non-mutagenic in bacteria when tested in *S. Typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 (+/- S9) (see Section 3.7), whereas quercetin was found to be mutagenic in strains TA98 and TA100 (NTP, 1992).

Based on the above, the Panel concluded that the read-across between naringenin and quercetin is not applicable.

3.5.3. Conclusion on structural/metabolic similarity

Considering the above, the Panel concluded that the read-across between naringenin and naringin and quercetin is not applicable (Table 2). Consequently, naringenin [FL-no: 16.132] will be evaluated as a stand-alone substance.

Table 2: Naringenin and substances considered for structural/metabolic similarity

| FGE | FL-no | Name       | Structural formula |
|-----|-------|------------|--------------------|
| 410 | 16.132| Naringenin | ![Naringenin](image) |
| 32  | 16.058| Naringin   | ![Naringin](image)  |
|     |       | Quercetin  | ![Quercetin](image) |

3.6. Exposure assessment (details are reported in Appendix B)

All data necessary for the calculation of normal and maximum occurrence levels for refined subcategories of foods and beverages are reported in Appendix B.

3.6.1. Natural occurrence in foods

Like its glycoside naringin, naringenin is present in grapefruit. Its concentration in grapefruit juice ranges from 0 to 12.6 mg/100 mL (Zhang, 2007). Both flavonoids also occur in tomatoes (Bugianesi et al., 2002), and in oranges (Kumpulainen et al., 1999; Erlund, 2004; e Silva et al., 2014) (Table 3).
3.6.2. Non-food sources of exposure

According to the applicant, naringenin is not used in fragrances. There is no known non-food source of exposure to humans.

3.6.3. Chronic dietary exposure

The exposure assessment to be used in the Procedure for the safety evaluation of naringenin is the chronic added portions exposure technique (APET) estimate (EFSA CEF Panel, 2010a). The chronic APET for [FL-no: 16.132] has been calculated for adults and children (see Table 4), and these values, expressed per kg body weight (bw), will be used in the Procedure (see Appendix B). The chronic APET calculation is based on the combined normal occurrence level and the standard portion size (see Appendix B). Exposure from other dietary sources is strictly related to naringenin per se.

### Table 3: Natural occurrence of naringin, naringenin in foods

| FL-no  | Name     | Food source                          | Amount                                                                 |
|--------|----------|--------------------------------------|------------------------------------------------------------------------|
| 16.132 | Naringenin| Tomato, grapefruit, oranges          | 8–42 mg/kg tomato(a), 0–12.6 mg/100 mL in grapefruit juice(b), 0.01–0.36 mg/100 mL in orange juice(c) |
| 16.058 | Naringin  | Grapefruit, oranges, tomato skin(d), tomato paste(d) | 10–86 mg/100 mL grapefruit juice(b), 0.01–0.3 mg/100 mL in orange juice(c) |

(a): Bugianesi et al. (2002).
(b): Zhang (2007).
(c): e Silva et al. (2014).
(d): No quantitative information found for naringin in the cited reference (Bugianesi et al., 2002).

3.6.4. Acute dietary exposure (Table 5)

The acute APET calculation for [FL-no: 16.132] is based on the combined maximum occurrence level and large portion size i.e. three times standard portion size (see Appendix B).
3.7. Genotoxicity (see also Appendix D)

3.7.1. Genotoxicity in vitro

Bacterial reverse mutation assay

Naringenin was tested in the Ames assay with S. Typhimurium tester strains TA98, TA100, TA1535, TA1537 and TA1538 at concentrations ranging from 33 to 10,000 µg/plate both in the absence and presence of S9 metabolic activation. The results obtained for mutagenicity indicated that the test compound did not increase the number of His+ revertants both in the absence and presence of S9 metabolic activation (see Table D.1). Data were obtained from the Chemical Carcinogenesis Research Information System (CCRIS), a database of the National Library of Medicine’s TOXNET system (Reference included as Ames Study results).

Naringenin was also shown to have no mutagenic activity when tested in S. Typhimurium tester strains TA100 and TA98 using the pre-incubation method with or without metabolic activation (Sugimura et al., 1977; Nagao et al., 1981) and in S. Typhimurium tester strains TA1535, TA100, TA1537, TA1538 and TA98 using both the pre-incubation and plate test methods with or without metabolic activation (Brown and Dietrich, 1979).

Overall, the Panel noted that there are limitations due to the absence of data on S. Typhimurium TA102 or Escherichia coli WP2 tester strains as recommended by the OECD Guideline no. 471.

Micronucleus assay

Chinese hamster V79 cells were used to evaluate DNA intercalation, metal reactivity, reactive oxygen species (ROS) generation, functional DNA topo II interactions and clastogenicity using modifications to the Chinese hamster V79 in vitro micronucleus assay of different bioflavonoids including naringenin. The details of the micronucleus test are given below.

Chinese hamster V79 cells were treated with naringenin for three hours in the absence of S9 metabolic activation. The authors concluded that naringenin was non-clastogenic in this assay (Snyder and Gillies, 2002). However, the Panel noted that the study was designed for research purposes and shows significant shortcomings that include the use of a short treatment (only 3 h), treatment in the presence of S9 metabolic activation was not performed and the number of binucleated cells scored for induction of micronuclei was very low (300–500 binucleated cells). The outcome of the study cannot be considered reliable on this basis, and conclusions drawn by the authors do not appear to be supported by convincing experimental evidence.

In response to a request for additional genotoxicity studies for naringenin, the applicant submitted studies on the flavonol quercetin which, for reasons detailed in Section 3.5 were not considered relevant and were not taken into account in this evaluation.

Similarly, an in vivo chromosomal aberration study on naringin (Jagetia et al., 2003) was not considered for the evaluation of naringenin [FL-no: 16.132].

Table 5: APET – acute Dietary Exposure

| Acute APET | Added as flavouring substance (a) | Other dietary sources (b) | Combined (c) |
|------------|----------------------------------|--------------------------|--------------|
|            | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day | µg/kg bw per day | µg/person per day |
| Adults (d) | 1,875 | 112,500 | 0 | 0 | 1,875 | 112,500 |
| Children (e) | 4,725 | 70,875 | 0 | 0 | 4,725 | 70,875 |

APET: added portions exposure technique; bw: body weight; n.a. not applicable: the acute APET calculation is based on the combined maximum occurrence level.

(a): APET Added is calculated on the basis of the maximum amount of flavouring added to a specific food category.

(b): APET Other dietary sources is calculated based on the natural occurrence of the flavouring in a specified food category.

(c): APET Combined is calculated based on the combined amount of added flavouring and naturally occurring flavouring in a specified food category.

(d): For the adult APET, calculation a 60-kg person is considered representative.

(e): For the child APET, calculation a 3-year-old child with a 15 kg bw is considered representative.
3.7.2. Conclusion on genotoxicity assessment

Overall, the Panel concluded that the available data set is insufficient to conclude on the genotoxic potential of naringenin. A bacterial gene mutation assay and an *in vitro* micronucleus assay (according to OECD guidelines 471, 487 and GLP) (OECD, 1997 and 2014) are required for [FL-no: 16.132].

3.8. Absorption, distribution, metabolism and elimination (Appendix C)

Naringenin is rapidly absorbed following oral administration and is subject to phase II metabolic reactions (primarily conjugation with glucuronic acid and/or sulfate). This is followed by excretion of the conjugates via bile and urine. Naringenin that is not absorbed within the upper part of the gastrointestinal (GI) tract passes into the colon, where it is subject to microbial metabolism to phenolic acids such as *p*-hydroxyphenylpropionic acid, *p*-coumaric acid and *p*-hydroxybenzoic acid. The metabolic fate of a major part of the dose in humans is unknown.

Studies pertaining to the metabolic fate of naringenin are summarised in detail in Appendix C. Naringenin cannot be assumed to be metabolised into innocuous products since in humans most of the dose is unaccounted for in terms of metabolites.

3.9. Toxicity data

No toxicity studies were submitted for naringenin [FL-no: 16.132].

3.10. Naringenin and estrogenic activity

Studies on the oestrogenic effects of naringenin were already considered by the Panel in FGE.32 (EFSA CEF Panel, 2010b), where the Panel concluded that: ‘naringenin is a weak estrogen compared with e.g. the phytoestrogens genistein and 8-prenylnaringenin, although its potential estrogenic effects are controversial’.

In uterotrophic assays (Breinholt et al., 1999, 2004), indications for oestrogenic activity were obtained at dose levels that approach the APET (11.6 mg/adult per day).

The Panel is aware that more recent studies on this topic are available. Considering all the data gaps in the available information and the fact that consequently this evaluation cannot be finalised, the Panel decided not to go into this aspect in depth in this opinion.

3.11. Naringenin and drug interaction

Naringenin is an inhibitor of several CYP 450 species and may interact with the metabolic elimination of medicines. Such interactions have already been demonstrated in several *in vitro* and *in vivo* studies in animals. Potential interaction of naringenin with medicines have been listed in the DRUGBANK database (https://www.drugbank.ca/).

Naringenin dose-dependently increased the bioavailability of felodipine 10 mg/kg bw approx. twofold when administered orally to Wistar rats in doses from 25 to 100 mg/kg bw for 15 consecutive days (Sandeep et al., 2014). When rats were treated orally with naringenin at doses of 12.5 and 25 mg/kg bw for 15 consecutive days (Pingili et al., 2016), the bioavailability of rasagiline mesylate (2 mg/kg) was increased: its area-under-the-curve (AUC) increased from 60 to 138 ng.h/mL (with 12.5 mg naringenin/kg) and to 232 ng.h/mL (with 25 mg naringenin/kg). At 25 mg/kg naringenin, also the rasagiline concentration in the brain of the rats was significantly increased. The Panel noted that there is no adequate margin of safety between the chronic combined APET for naringenin (11.6 mg/adult per day) and the dose levels of 12.5–100 mg/kg bw that were administered in the above *in vivo* studies.

Naringenin inhibited the metabolism of simvastatin in rat and human hepatocytes and microsomes (Ki 5–30 μM; Ubeaud et al., 1999), and various cytochrome P450 isoforms with IC₅₀ values below 5 μM (Lu et al., 2011). The CYP3A-dependent metabolism of 7-benzyloxy-4-trifluoromethylcoumarin was inhibited in human (Ki = 24.6 μM), pig (Ki = 15.6 μM) and mouse (Ki = 19.6 μM) microsomes (Burkina et al., 2016); the glycoside naringin had no inhibitory effect in this system. Moreover, the substance suppressed CYP1B1 transactivation *in vitro* at 5 μM (Poon et al., 2013).

Considering the above, the applicant was asked to convincingly demonstrate that the substance does not present any risk of drug interactions in its estimated levels of intake based on the APET approach.

In their reply, the applicant referred to the Panel conclusion in FGE.32 according to which ‘the potential for drug interactions from the intakes of the candidate flavanones based on the MSDI
approach does not give rise to concern’ (EFSA CEF Panel, 2010b). However, the Maximised Survey-derived Daily Intake (MSDI) does no longer apply for the estimation of the exposure to flavouring substances falling within the remit of Regulation (EC) No 1331/2008 of the European Parliament and of the Council establishing a common authorisation procedure for food additives, food enzymes and food flavourings.

The Panel concluded that in the light of currently applicable exposure (APET) estimates for naringenin, the opinion for naringin in FGE.32 cannot be used to support the evaluation of naringenin. This is also supported by the differences between the toxicokinetics of naringenin and naringin (see section 3.5.1 and Appendix C).

3.11.1. Conclusion on drug interaction

The Panel noted that the applicant did not provide appropriate information to convincingly demonstrate that there is no risk of drug interactions with [FL-no: 16.132] in humans at its estimated levels of exposure based on the APET.

Therefore, it remains to be established whether exposure to naringenin at the level of the APET is devoid of such interactions with drug metabolism.

3.12. Procedure for the safety assessment

The Procedure and its principles are reported in Appendix A.

Does naringenin give rise to concern with respect to genotoxicity?

The Panel requested additional data on the candidate substance. Since this data did not become available and the available data is insufficient to conclude on the genotoxic potential of naringenin, the substance cannot be evaluated through the Procedure (Appendix A).

4. Conclusions

4',5,7-Trihydroxyflavanone or naringenin [FL-no: 16.132] evaluated in this FGE is a flavanone. Several other flavanones or related substances have been evaluated in FGE.32. The Panel investigated the possibility to use a group-based evaluation for naringenin, applying read-across. However, major differences have been identified in bioavailability and elimination between naringenin and naringin in FGE.32. In addition, there are also differences in the presence or absence of functional groups that will result in differences in their toxicological profiles. Therefore, the Panel concluded that the application of a group approach, including read-across is not justified. Consequently, the Panel decided to conduct an individual evaluation for naringenin.

Specifications and manufacturing process

Specifications including complete purity criteria and identity for the material of commerce for [FL-no: 16.132] have been provided.

The information provided on the manufacturing process was considered adequate by the Panel. Data on stability in food are incomplete, since the heating and storage conditions for which stability in food was investigated, were not representative for all intended uses.

Use and exposure

Naringenin [FL-no: 16.132] is intended to be used as a flavouring substance with flavouring modifying properties in a variety of foods. In addition, naringenin [FL-no: 16.132] occurs in grapefruit, mainly in the form of its glycoside (naringin) but also as such. It can be also naturally found in tomatoes and oranges.

The chronic combined dietary exposure to naringenin has been estimated at 11,600 μg/person per day (194 μg/kg bw per day) for a 60-kg adult and 7,308 μg/person per day (487 μg/kg bw per day) for a 15-kg 3-year-old child. The Panel notes that ~ 84% of the chronic combined exposure estimate would originate from the use of naringenin as flavouring substance.

The highest acute intake of naringenin (as added flavouring substance) results from the consumption of dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt) containing 300 mg/kg of naringenin consumed by a 60-kg adult or a 15-kg 3-year-old child. This results in an intake of 112.5 mg/person per
day (1,875 μg/kg bw per day) for a 60-kg adult and an intake of 70.9 mg/person per day (4,725 μg/kg bw per day) for a 15-kg 3-year-old child.

Absorption, distribution, metabolism and elimination

Absorption, distribution, metabolism and elimination (ADME) studies with [FL-no: 16.132] in rats and humans indicate that naringenin is rapidly absorbed after oral administration; it is converted to conjugates and also metabolised in the GI tract. In humans, most of the dose is unaccounted for in terms of metabolites.

Genotoxicity

The Panel noted that the available genotoxicity studies have significant shortcomings. A bacterial gene mutation assay and an in vitro micronucleus assay (according to OECD guidelines 471, 487 and GLP) are required for [FL-no: 16.132]. Consequently, the substance cannot be evaluated through the Procedure.

Toxicity

The applicant did not provide subchronic or chronic toxicity studies on naringenin.

Oestrogenic activity

Naringenin has been reported to exhibit weak oestrogenic activity. The Panel, considered that the current evaluation of naringenin cannot be finalised and decided not to go into this aspect in depth.

Interaction with drugs

The Panel noted that inhibition of CYP 450 species by [FL-no: 16.132] has been clearly demonstrated in animal species in vivo which implies that the substance may interact with the metabolism and elimination of medicines. For this, it needs to be convincingly demonstrated that there is no risk of drug interactions resulting from exposure to [FL-no: 16.132] in humans at its estimated levels of exposure.

Conclusion

Overall, the Panel cannot reach a conclusion as to the safety of naringenin [FL-no: 16.132] since the available data on genotoxicity are not adequate. Depending on the outcome of the assessment on genotoxicity more toxicological data are needed to finalise the evaluation.

Documentation provided to EFSA

1) Flavour Industry, 2015. Technical dossier of one new flavouring substance, 4’,5,7-trihydroxyflavanone.
2) Flavour Industry, 2017. Technical dossier of one new flavouring substance, 4’,5,7-trihydroxyflavanone, updated in February 2017.

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**Abbreviations**

ADME absorption, distribution, metabolism and elimination  
APET added portions exposure technique  
BW body weight  
CAS Chemical Abstract Service  
CCRIS Chemical Carcinogenesis Research Information System  
CEF EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids  
CoE Council of Europe  
EFFA European Flavour Association  
EINECS European Inventory of Existing Commercial Chemical Substances  
FAO Food and Agriculture Organization of the United Nations  
FEMA Flavor and Extract Manufacturers Association  
FGE Flavouring Group Evaluation  
FLAVIS Flavour Information System database  
GI gastrointestinal  
GLP Good Laboratory Practice  
GRAS Generally recognised as safe  
HPLC HIGH-performance liquid chromatography  
IC$_{50}$ half maximal inhibitory concentration
| ID | Identity |
|----|----------|
| JECFA | The Joint FAO/WHO Expert Committee on Food Additives |
| LC | liquid chromatography |
| MS | mass spectrometry |
| MRT | mean residence time |
| MSDI | Maximised Survey-derived Daily Intake |
| NMR | nuclear magnetic resonance spectroscopy |
| NOAEL | no observed adverse effect level |
| OECD | Organisation for Economic Co-operation and Development |
| ROS | reactive oxygen species |
| SCF | Scientific Committee on Food |
| SPET | single portion exposure technique |
| WHO | World Health Organisation |
Appendix A – Procedure for evaluation of a new flavouring substance

**Figure A.1:** The stepwise procedure for evaluation of new flavouring substances according to Commission Regulation (EC) No 1334/2008
Appendix B – Use levels and exposure calculations

Table B.1: Normal and maximum occurrence levels for refined categories of foods and beverages

| Food categories (a) | Standard portions (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources (mg/kg) | Combined occurrence level from all sources (mg/kg) |
|---------------------|-----------------------|-------------------------------------------------------|---------------------------------------------|---------------------------------------------------|
|                     |                       | Normal | Maximum | Average (d) | Maximum | Normal | Maximum |
| 01.1                | Milk- and dairy-based drinks | 200    |         |             |         |        |         |
| 01.2                | Fermented and renneted milk products (plain), excluding food category 01.1.2 (dairy-based drinks) | 200    |         |             |         |        |         |
| 01.3                | Condensed milk and analogues (plain) | 70     |         |             |         |        |         |
| 01.4                | Cream (plain) and the like | 15     |         |             |         |        |         |
| 01.5                | Milk powder and cream powder and powder analogues (plain) | 30     |         |             |         |        |         |
| 01.6                | Cheese and analogues | 40     |         |             |         |        |         |
| 01.7                | Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt) | 125    | 30      | 300         | 30      | 300    |
| 01.8                | Whey and whey products, excluding whey cheeses | 200    |         |             |         |        |         |
| 02.1                | Fats and oils essentially free from water | 15     |         |             |         |        |         |
| 02.2                | Fat emulsions mainly of type water-in-oil | 15     |         |             |         |        |         |
| 02.3                | Fat emulsions mainly of type water-in-oil, including mixed and/or flavoured products based on fat emulsions | 15     |         |             |         |        |         |
| 02.4                | Fat-based desserts excluding dairy-based dessert products of category 1.7 | 50     |         |             |         |        |         |
| 03.0                | Edible ices, including sherbet and sorbet | 50     | 20      | 100         | 20      | 100    |
| 04.1.1              | Fresh fruit | 140    |         |             | 8       | 42     |
| 04.1.2              | Processed fruit | 125    |         |             | 8       | 42     |
| 04.1.2.5            | Jams, jellies, marmalades | 30     |         |             |         |        |         |
| 04.2.1              | Fresh vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed | 200    |         |             |         |        |         |
| Food categories<sup>(a)</sup> | Standard portions<sup>(b)</sup> (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources<sup>(c)</sup> (mg/kg) | Combined occurrence level from all sources<sup>(e)</sup> (mg/kg) |
|-------------------------------|----------------------------------|---------------------------------|-------------------------------|-------------------------------|
|                              |                                  | Normal                          | Maximum                       | Average<sup>(d)</sup>          | Maximum          |
| 04.2.2 Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter) and nuts and seeds | 200 | | | |
| 04.2.2.5 Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g. peanut butter) | 30 | | | |
| 05.1 Cocoa products and chocolate products, including imitations and chocolate substitutes | 40 | 30 | 100 | 30 | 100 |
| 05.1.3 Cocoa-based spreads, including fillings | 30 | | | |
| 05.2 Confectionery, including hard and soft candy, nougats, etc., other than 05.1, 05.3 and 05.4 | 30 | | | |
| 05.3 Chewing gum | 3 | | | |
| 05.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces | 35 | | | |
| 06.1 Whole, broken or flaked grain, including rice | 200 | | | |
| 06.2 Flours and starches (including soya bean powder) | 30 | | | |
| 06.3 Breakfast cereals, including rolled oats | 30 | 30 | 100 | 30 | 100 |
| 06.4 Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles) | 200 | | | |
| 06.5 Cereal and starch based desserts (e.g. rice pudding, tapioca pudding) | 200 | | | |
| 06.6 Batters (e.g. for breading or batters for fish or poultry) | 30 | | | |
| 06.7 Pre-cooked or processed rice products, including rice cakes (Oriental type only) | 200 | | | |
| 06.8 Soya bean products (excluding soya bean products of food category 12.9 and fermented soya bean products of food category 12.10) | 100 | | | |
| Food categories<sup>(a)</sup> | Standard portions<sup>(b)</sup> (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources<sup>(c)</sup> (mg/kg) | Combined occurrence level from all sources<sup>(e)</sup> (mg/kg) |
|--------------------------------|---------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|                                |                                 | Normal | Maximum | Average<sup>(d)</sup> | Maximum | Normal | Maximum |
| 07.1 Bread and ordinary bakery wares | 50                              |        |         |                    |         |        |         |
| 07.2 Fine bakery wares (sweet, salty, savoury) and mixes | 80                              | 30     | 200     |                    |         |        |         |
| 08.1 Fresh meat, poultry and game | 200                             |        |         |                    |         |        |         |
| 08.2 Processed meat, poultry and game products in whole pieces or cuts | 100                             | 20     | 50      |                    |         |        |         |
| 08.3 Processed comminute meat, poultry and game products | 100                             |        |         |                    |         |        |         |
| 08.4 Edible casings (e.g. sausage casings) | 1                               |        |         |                    |         |        |         |
| 09.1.1 Fresh fish | 200                             |        |         |                    |         |        |         |
| 09.1.2 Fresh molluscs, crustaceans and echinoderms | 200                             |        |         |                    |         |        |         |
| 09.2 Processed fish and fish products, including molluscs, crustaceans and echinoderms | 100                             |        |         |                    |         |        |         |
| 09.3 Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms | 100                             |        |         |                    |         |        |         |
| 09.4 Fully preserved, including canned or fermented, fish and fish products, including molluscs, crustaceans and echinoderms | 100                             |        |         |                    |         |        |         |
| 10.1 Fresh eggs | 100                             |        |         |                    |         |        |         |
| 10.2 Egg products | 100                             |        |         |                    |         |        |         |
| 10.3 Preserved eggs, including alkaline, salted and canned eggs | 100                             |        |         |                    |         |        |         |
| 10.4 Egg-based desserts (e.g. custard) | 125                             |        |         |                    |         |        |         |
| 11.1 Refined and raw sugar | 10                              |        |         |                    |         |        |         |
| 11.2 Brown sugar excluding products of food category 11.1 | 10                              |        |         |                    |         |        |         |
| 11.3 Sugar solutions and syrups, and (partially) inverted sugars, including molasses and treacle, excluding products of food category 11.1.3 (soft white sugar, soft brown sugar, glucose syrup, dried glucose syrup, raw cane sugar) | 30                              |        |         |                    |         |        |         |
| Food categories\(^{(a)}\) | Standard portions\(^{(b)}\) (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources\(^{(c)}\) (mg/kg) | Combined occurrence level from all sources\(^{(e)}\) (mg/kg) |
|--------------------------|-------------------------------|---------------------------------------------|---------------------------------------------|-----------------------------|
|                          |                               | Standard portions\(^{(b)}\) (g) | Normal | Maximum | Average\(^{(d)}\) | Average\(^{(d)}\) | Maximum | Normal | Maximum |
| 11.4                     | Other sugars and syrups (e.g. xylose, maple syrup, sugar toppings) | 30 |  |  |  |  |  |  |  |
| 11.5                     | Honey                         | 15 |  |  |  |  |  |  |  |
| 11.6                     | Table-top sweeteners, including those containing high-intensity sweeteners | 1 |  |  |  |  |  |  |  |
| 12.1                     | Salt and salt substitutes     | 1 |  |  |  |  |  |  |  |
| 12.10                    | Protein products other than from soybeans | 15 |  |  |  |  |  |  |  |
| 12.2                     | Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles) | 1 |  |  |  |  |  |  |  |
| 12.3                     | Vinegars                      | 15 |  |  |  |  |  |  |  |
| 12.4                     | Mustards                      | 15 |  |  |  |  |  |  |  |
| 12.5                     | Soups and broths              | 200 | 20 | 50 | 8 | 42 | 28 | 92 |
| 12.6                     | Sauces and like products      | 30 |  |  |  |  |  |  |  |
| 12.7.a                   | Salads 120 g (e.g. macaroni salad, potato salad) excluding cocoa- and nut-based spreads of food categories | 120 |  |  |  |  |  |  |  |
| 12.7.b                   | Sandwich spreads (20 g), excluding cocoa- and nut-based spreads of food categories | 20 |  |  |  |  |  |  |  |
| 12.8                     | Yeast and like products       | 1 |  |  |  |  |  |  |  |
| 12.9                     | Soybean-based seasonings and condiments | 15 |  |  |  |  |  |  |  |
| 12.9.1                   | Fermented soya bean products (e.g. miso) | 40 |  |  |  |  |  |  |  |
| 12.9.2                   | Soybean sauce                 | 15 |  |  |  |  |  |  |  |
| 12.9.3                   | Fermented soybean sauce       | 15 |  |  |  |  |  |  |  |
| 13.2.a                   | Complementary foods for infants and young children: Dry instant cereals (with or without milk), including pasta | 110 |  |  |  |  |  |  |  |
| 13.2.b                   | Complementary foods for infants and young children: Meat based or fish based dinner | 170 |  |  |  |  |  |  |  |
| 13.2.c                   | Complementary foods for infants and young children: Dairy based dessert | 110 |  |  |  |  |  |  |  |
| Food categories<sup>(a)</sup> | Standard portions<sup>(b)</sup> (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources<sup>(c)</sup> (mg/kg) | Combined occurrence level from all sources<sup>(e)</sup> (mg/kg) |
|-----------------------------|----------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| 13.2.d Complementary foods for infants and young children: Vegetables, potatoes, broth, soups, pulses | 170 | | | |
| 13.2.e Complementary foods for infants and young children: Biscuits and cookies | 20 | | | |
| 13.2.f Complementary foods for infants and young children: Fruit purée | 110 | | | |
| 13.2.g Complementary foods for infants and young children: Fruit juice | 120 | | | |
| 13.2.h Milk for young children | 200 | | | |
| 13.3 Dietetic foods intended for special medical purposes (excluding food products of category 13.1 'Infant formulae, follow-up formulae and other formulae for special medical purposes for infants') | 200 | | | |
| 13.4 Dietetic formulae for slimming purposes and weight reduction | 200 | | | |
| 13.5 Dietetic foods (e.g. supplementary foods for dietary use), excluding products of food categories 13.1 (Infant formulae, follow-up formulae and other formulae for special medical purposes for infants), 13.2–13.4 and 13.6 | 200 | | | |
| 13.6 Food supplements | 5 | | | |
| 14.1 Other non-alcoholic ('soft') beverages (expressed as liquid) | 300 | 20 | 100 | 20 | 100 |
| 14.2.1 Beer and malt beverages | 300 | 20 | 100 | 20 | 100 |
| 14.2.2 Cider and perry | 300 | | | |
| 14.2.3 Grape wines | 150 | | | |
| 14.2.4 Wines (other than grape) | 150 | | | |
| 14.2.5 Mead | 150 | | | |
| 14.2.6 Distilled spirituous beverages containing more than 15% alcohol | 30 | | | |
| Food categories<sup>(a)</sup> | Standard portions<sup>(b)</sup> (g) | Occurrence level as added flavouring substance (mg/kg) | Occurrence level from other sources<sup>(c)</sup> (mg/kg) | Combined occurrence level from all sources<sup>(e)</sup> (mg/kg) |
|-------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                               |                                 | Normal | Maximum | Average<sup>(d)</sup> | Maximum | Normal | Maximum |
| 14.2.7 Aromatised alcoholic beverages (e.g. beer, wine and spirituous cooler-type beverages, low alcoholic refreshers) | 300 | | | |
| 15.1 Snacks, potato-, cereal-, flour- or starch-based (from roots and tubers, pulses and legumes) | 30 | 30 | 500 | 30 | 500 |
| 15.2 Processed nuts, including coated nuts and nut mixtures (with e.g. dried fruit) | 30 | | | |
| 15.3 Snacks – fish based | 30 | | | |
| 16.0 Composite foods (e.g. casseroles, meat pies, mincemeat) – foods that could not be placed in categories 01–15 | 300 | | | |

(a): Most of the categories reported are the subcategories of Codex GSFA (General Standard for Food Additives) used by the JECFA in the SPET technique (FAO/WHO, 2008). In the case of category 13.2 (complementary foods for infants and young children), further refined categories have been created so that a specific assessment of dietary exposure can be performed in young children.

(b): For Adults. In case of foods marketed as powder or as concentrates, occurrence levels must be reported for the reconstituted product, considering the instructions reported on the product label or one of the standard dilution factors established by the JECFA (FAO/WHO, 2008):
- 1/25 for powder used to prepare water-based drinks such as coffee, containing no additional ingredients,
- 1/10 for powder used to prepare water-based drinks containing additional ingredients such as sugars (ice tea, squashes, etc.),
- 1/7 for powder used to prepare milk, soups and puddings,
- 1/3 for condensed milk.

(c): As natural constituent and/or developed during the processing and/or as carry over resulting from their use in animal feed.

(d): In order to estimate normal values in each category, only foods and beverages in which the substance is present in significant amount will be considered (e.g. for the category ‘Fresh fruit’ 04.1.1., the normal concentration will be the median concentration observed in all kinds of fruit where the flavouring substance is known to occur).

(e): As added flavouring or from other sources. The normal and maximum combined occurrence levels of the substance will be assessed by the applicant either by adding up occurrence levels from added use to that from other sources or by expert judgment based on the likelihood of their concomitant presence. This will be done both for normal use levels and for maximum use levels.
B.1. **Calculation of the Dietary Exposure – ‘Added Portions Exposure Technique’ (APET)**

**Chronic Dietary Exposure**\(^7\)

The chronic APET calculations are based on the normal combined occurrence level by adding the highest contributing portion of food and highest contributing portion of beverages (either among soft drinks or alcoholic beverages). APET for children is calculated by adding the highest contributing portion of food and highest contributing portion of beverages (among soft drinks). Furthermore, in the APET calculation for children the portion sizes listed in Table B.1 are adjusted by a factor 0.63 to take into account the smaller portion sizes consumed by children.

**Adults**

*On the basis of normal occurrence level from the added flavouring only*

- **Solid food**: The maximum intake will be from category 12.5 (Soups and broths) with the normal combined occurrence level of 4,000 \(\mu\)g/adult per day.
- **Beverage**: The two categories (14.1 and 14.2.1) to which naringenin is added have the same normal combined occurrence level of 6,000 \(\mu\)g/adult per day.

The total APET will be 10,000 \(\mu\)g/adult per day corresponding to 170 \(\mu\)g/kg bw per day for a 60-kg person.

**Children (3-year-old child of 15 kg body weight)**

- **Solid food**: The maximum intake will be from category 12.5 (Soups and broths) with the normal combined occurrence level of 4,000 \(\times\) 0.63 = 2,520 \(\mu\)g/child per day.
- **Beverage**: The maximum intake will be from category 14.1 (Non-alcoholic (‘soft’) beverages) with the normal combined occurrence level of 6,000 \(\times\) 0.63 = 3,780 \(\mu\)g/child per day.

The total APET will be 6,300 \(\mu\)g/child per day corresponding to 420 \(\mu\)g/kg bw per day for a 15-kg child.

**Conclusion**

The higher of the two values among adults and children, expressed per kg/bw per day, should be used as the basis for the safety evaluation of naringenin, i.e. the value of 487 \(\mu\)g/kg bw per day for a 15 kg child should be compared to the appropriate no observed adverse effect level (NOAEL) for naringenin.

**Combined Dietary Exposure**

This is an estimate of total dietary exposure derived from both the addition of the flavouring substance to foods and beverages and other dietary sources. To estimate the APET for combined dietary exposure, the occurrence of the substance in grapefruit and tomatoes was also taken into account in the estimation.

**Adults**

- **Solid food**: The maximum intake will be from category 12.5 (Soups and broths) with the normal combined occurrence level of 5,600 \(\mu\)g/adult per day.
- **Beverage**: The two categories (14.1 and 14.2.1) to which naringenin is added have the same normal combined occurrence level of 6,000 \(\mu\)g/adult per day.

The total APET will be 11,600 \(\mu\)g/adult per day corresponding to 194 \(\mu\)g/kg bw per day for a 60-kg person.

**Children (3-year-old child of 15 kg body weight)**

- **Solid food**: The maximum intake will be from category 12.5 (Soups and broths) with the normal combined occurrence level of 5,600 \(\times\) 0.63 = 3,528 \(\mu\)g/child per day.
- **Beverage**: The maximum intake will be from category 14.1 (Non-alcoholic (‘soft’) beverages) with the normal combined occurrence level of 6,000 \(\times\) 0.63 = 3,780 \(\mu\)g/child per day.

\(^7\) The APET has been calculated based on the occurrence levels in the food subcategories reported in the above table, with the exclusion of categories 13.2 (complementary foods for infants and young children).
The total APET will be 7,308 \( \mu \text{g/child per day} \) corresponding to 487 \( \mu \text{g/kg bw per day} \) for a 15-kg child.

**Acute Dietary Exposure**

The calculation is based on the maximum use levels and large portion size, i.e. three times standard portion size (see Table B.1). The APET calculation for children applies the portion sizes listed in Table B.1 adjusted by a factor 0.63 to take into account the smaller portion sizes consumed in this case.

**Adults**

The highest contribution comes from three portions of category 01.7 (Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)) and is \( 3 \times 125 \text{ g} \times 300 \text{ mg/kg} = 112.5 \text{ mg/adult} \).

**Children**

The highest contribution comes from three portions of category 01.7 (Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)) and is \( 3 \times 125 \text{ g} \times 300 \text{ mg/kg} = 112.5 \times 0.63 = 70.875 \text{ mg/child} \).

**Infants and young children (0–1 year)**

Acute dietary exposure is not calculated for infants and young children.

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*Based on the same considerations as for adults but using the special factors used for chronic exposure to infants.*
Appendix C – Absorption, distribution, metabolism and elimination

4',5,7-Trihydroxyflavanone (naringenin) [FL-no: 16.132]

Naringenin is the aglycone of naringin, i.e. it does not have the glucose and rhamnose sugars that are part of naringin at position 7 (see Figure C.1).

Figure C.1: Structures of naringin and naringenin

Human pharmacokinetics data: difference in pharmacokinetics between naringin and naringenin

Some reliable human data are available on naringenin [FL-no: 16.132]. Kanaze et al. (2007) studied the pharmacokinetics and metabolism of 95% pure racemic naringenin (and hesperitin) in six human volunteers after a single oral dose of 135 mg in a solid dispersion capsule. Naringenin is rapidly absorbed: already after 20 min it appeared in plasma, and reached a peak concentration of 2,010 ± 770 ng/mL plasma (7.4 µM, determined as total naringenin, including the glucuronide and sulfate conjugate; mean ± SD in six volunteers) after 4 h. Half-life of elimination was 2.31 ± 0.40 h. Only 5.81 ± 0.81% of the dose was recovered in urine during 24 h after dosing. This suggests a major contribution of metabolism other than conjugation, such as cleavage of the C-ring in the intestine, as discussed by the authors. They also discuss extensively the difference in pharmacokinetics between naringenin and its precursor naringin: the latter is more slowly absorbed after hydrolysis in the colon in humans. This was confirmed by studies in the rat (Felgines et al., 2000) who fed rats with naringin and naringenin. The appearance, plasma concentrations and urinary excretion of naringenin conjugates after administration of naringin, were later, or lower (respectively) than after administration of naringenin.

The rate-limiting step is the hydrolysis of the rhamnose group, which occurs only in the colon. This has very elegantly been shown for the hesperidin/hesperitine pair by Nielsen et al. (2006) in a cross-over trial in human volunteers. Hesperidine has exactly the same diglycoside moiety (rhamnose-glucose) as naringin. Absorption of hesperitine is (much) more rapid when it is a glucoside (i.e. it is a glycoside with only glucose, rather than a glycoside with rhamnose-glucose) than when it is the naturally occurring hesperidine, containing the rhamnose-glucose (‘rutoside’) group. The reason is that the glucoside is already rapidly hydrolysed in the small intestine, while the hydrolysis of the rutoside (starting with the rhamnose) only takes place in the colon. When hesperitine (and by analogy naringenin) itself will be administered, obviously no hydrolysis will be required and uptake in the small intestine will be rapid.

Animal absorption and pharmacokinetics data

Similar rapid absorption of naringenin after oral administration was also observed in several other species, such as rat, mice and rabbit (Hsiu et al., 2002; Ma et al., 2006; Ke et al., 2013).

Ma et al. (2006) studied pharmacokinetics of naringenin (purity over 95%) in detail in the rat. Naringenin (purity > 95%) was administered to male and female Wistar rats (5/sex per group) at doses of 30, 90 or 270 mg/kg bw by oral gavage following an overnight fast. They also investigated the role of enterohepatic circulation of naringenin in bile duct-cannulated rats. The maximum plasma concentration of free naringenin was reached within 15 min of naringenin administration (2.9, 3.7 and 4.4 ng/mL for the 30, 90 or 270 mg/kg bw groups, respectively), whereas the Cmax for total naringenin (including the conjugates) was reached at 0.5, 2 and 2 h following administration of 30, 90 or 270 mg naringenin/kg bw, respectively (16.9, 28.0 and 43.8 ng/mL, respectively). A dose-dependent increase in the area-under-the-curve (AUC) of free and total naringenin was observed. The elimination half-time of total naringenin was determined to be 7.6 and 10.5 h for the 90 and 270 mg/kg bw groups,
respectively, and the mean respective mean residence time (MRT) was 7.9 and 8.6 h. The authors noted that the slow rate of elimination of naringenin may be attributed to glucuronidation and enterohepatic circulation: naringenin was excreted in bile as glucuronide conjugate. Twice as much naringenin was excreted in bile (12%) than in urine (6.25%). In comparison to control rats, the total plasma concentration of naringenin in bile duct-cannulated rats was lower and the concentration versus time profile demonstrated no double peaks, thereby confirming that naringenin undergoes enterohepatic circulation.

Two studies were conducted to assess potential differences in the pharmacokinetics related to the different enantiomers \((R/S)\) of naringenin (Yáñez et al., 2008; Wan et al., 2011). Following oral administration, the pharmacokinetic parameters for the two enantiomers were largely similar.

**Metabolism of naringenin**

Metabolism of naringenin takes place primarily in the cells of the intestine walls, the liver and the microflora in the colon.

Conjugation of naringenin to form sulfate and glucuronide conjugates is a major pathway in humans. However, in the available studies most of the compound administered is unaccounted for: recovery in urine as (un)conjugated [FL-no: 16.132] is relatively low (5–30% in 24 h). Presumably, most of an oral dose is either excreted with the faeces and/or converted (by the colon microflora) to other metabolites which remain unknown. Results of intestinal perfusions and isolated cells of rats and mice demonstrated that it was also metabolised to apigenin (Chen et al., 2003; Orrego-Lagarón et al., 2016). Ring cleavage by the intestinal microflora has been reported, resulting in metabolites such as \(p\)-hydroxyphenylpropanionic acid, \(p\)-coumaric acid and \(p\)-hydroxybenzoic acid (Felgines et al., 2000; Donovan et al., 2006), which in fact may be major metabolites if they are also excreted with the faeces. Felgines et al. (2000) fed a diet containing 9.2 mmol/kg feed of naringenin to rats of 170 g for one meal, resulting in an intake of \(\sim 230 \mu\text{mol/rat}\). Approx 30% was excreted as conjugated naringenin and 10% as ring-cleaved metabolites in the next 24 h.

**Further toxicokinetic data with naringenin in humans**

Six healthy volunteers received orally 135 mg of each compound (hesperetin and naringenin), under fasting conditions (Kanaze et al., 2007). Blood samples were collected at 14 different points over a 12 h period. Urine was collected over 24 h, in five sequential time intervals. Plasma and urine concentrations were measured using high-performance liquid chromatography (HPLC), and pharmacokinetic parameters were calculated from these.

Naringenin was absorbed from the gastrointestinal (GI) tract and was measureable in almost all subjects 20 mins following oral administration and reached a peak in 3.5 h. The mean peak plasma concentration for naringenin was 2,009 ng/mL. The elimination half-life was 2.31 h. The urinary recovery was 5.8 \(\pm\) 0.8% of the administered dose.

All six subjects successfully completed the study and were discharged in good health with no reported undesirable or adverse effects after oral administration of the aglycone naringenin. Oral administration of the flavanone aglycones, hesperetin and naringenin, lead to their rapid absorption as their conjugated forms. The cumulative urinary recovery data indicated low bioavailability for both flavanone aglycones, owing to extensive first-pass metabolism partly by cleavage of the C-ring by the enzymes of intestinal bacteria leading to degradation products such as phenolic acids (Kanaze et al., 2007).

In a second study (Erlund et al., 2001), eight healthy human volunteers were given a one-time drink of orange or grapefruit juice at 8 mL/kg to evaluate bioavailability of naringenin. The concentration of naringenin in the orange juice was 151 \(\mu\text{mol/L} (41 \text{mg/L})\) and 1,283 \(\mu\text{mol/L} (349 \text{mg/L})\) in the grapefruit juice as determined after hydrolysis of the naringin. The orange juice therefore represents a low dose compared with the grape fruit juice. Blood samples and urine were collected between 0 and 24 h. The resulting peak plasma concentrations of naringenin were 0.6 \(\pm\) 0.4 \(\mu\text{mol/L}\) from orange juice and 6.0 \(\pm 5.4 \mu\text{mol/L}\) from grapefruit juice. The elimination half-life was 1.3–2.2 h, and therefore, plasma concentrations reflect short-term intake. The relative urinary excretion varied depending on the dose and was 30 \(\pm\) 25% and 1.1 \(\pm\) 0.8% for naringenin from grapefruit and orange juice, respectively.

There was a high interindividual variation in the bioavailability of the compounds as indicated by variation in their \(C_{\text{max}}\) and AUC values and this is attributed by the author to differences in GI microflora.
In a third study (Brett et al., 2009), 20 volunteers ingested orange juice and orange fruit segments and the appearance of naringenin in plasma and urine was measured. The oranges contained 11.8 mg naringenin (as naringin) and the orange juice contained 9.4 mg naringenin (as naringin). Plasma and urine samples were analysed for naringenin following hydrolysis. The half-life for plasma naringenin was 4–5 h. The percentage of naringenin excreted in the urine ranged from 6.8% to 14.5%. Low concentrations of naringenin were detected in the plasma within 15 min of ingestion of the fruit or juice. This study showed that there were no significant differences in the bioavailability of naringenin consumed in fresh fruit or in commercially processed juice. This study also confirmed that interindividual variation with respect to absorption and excretion of naringenin was indeed very high and not related to age, sex, or BMI of the subjects.

Ameer et al. (1996) showed that naringenin is methylated at various sites after intake of naringin by a human volunteer. Bugianesi et al. (2002) confirmed that conjugated naringenin is found in plasma after consumption of cooked tomato paste.

**Conclusion on metabolism**

Naringenin cannot be assumed to be metabolised into innocuous products since in humans most of the dose is unaccounted for in terms of metabolites.
Appendix D – Genotoxicity

Table D.1: Summary of *in vitro* genotoxicity studies considered by the Panel

| Chemical name [FL-no] | Test system | Test object | Concentration | Result | Reference | Comments |
|-----------------------|-------------|-------------|---------------|--------|-----------|----------|
| Naringenin [FL-no: 16.132] | Reverse mutation | S. Typhimurium TA1535, TA1537, TA1538, TA98, TA100 | 33–10,000 µg/plate | Negative\(^{(a)}\) | CCRIS (2014) | See Section 3.7.1 |
|                        | Reverse mutation | S. Typhimurium TA98, TA100 | –            | Negative\(^{(a),(b)}\) | Sugimura et al. (1977), Nagao et al. (1981) | See Section 3.7.1 |
|                        | Reverse mutation | S. Typhimurium TA1535, TA1537, TA1538, TA98, TA100 | –            | Negative\(^{(a),(c)}\) | Brown and Dietrich (1979) | See Section 3.7.1 |
|                        | Micronucleus Assay | Chinese hamster V79 cells | –            | Negative\(^{(d)}\) | Snyder and Gillies (2002) | See Section 3.7.1 |

FL-no: FLAVIS number.
(a): With and without metabolic activation.
(b): Including pre-incubation.
(c): Including pre-incubation or plate tests.
(d): Without metabolic activation.