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Scientific Opinion of Flavouring Group Evaluation 410 (FGE.410): 4′,5,7-trihydroxyflavanone from chemical group 25 (phenol derivatives containing ring-alkyl, ring-alkoxy, and side-chains with an oxygenated functional group)

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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.

Keywords: flavouring, 4′,5,7-trihydroxyflavanone, naringenin, FGE.410, [FL-no: 16.132]

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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 (CASrn 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.

[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 2*R*- and 2*S* 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 231/2012[4] laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008[5] 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 $\geq$ 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

[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[6]

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 microflora (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.

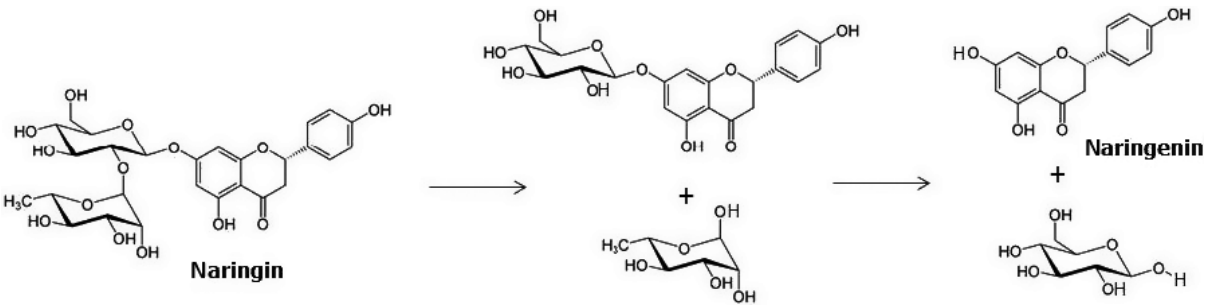
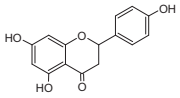


Figure 1: The metabolic conversion of naringin to naringenin in the colon

[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		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] Soluble –	– 251 NMR > 95%	n.a. n.a.	Trivial name: naringenin The CASrn refers to the racemate (Flavour Industry, 2017)

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.

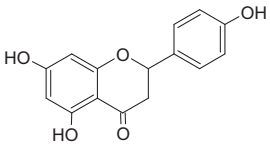
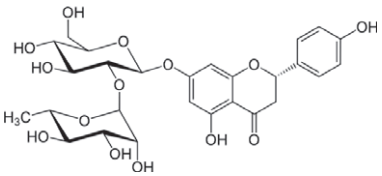
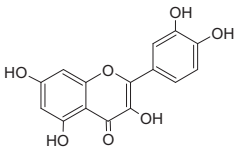
Additionally, the Panel noted that there is variability in the toxicological profiles between the two substances: naringenin was found to be non-mutagenic in bacteria when tested in *S.* Typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 (+/− S9) (see Section 3.7), whereas quercetin was found 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	
32	16.058	Naringin	
		Quercetin	

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).

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).

3.6.2. 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 4: APET – chronic dietary exposure

Chronic APET	Added as flavouring substance[(a)]		Other dietary sources[(b),(f)]		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)]	170	10,000	27	1,600	194	11,600
Children[(e)]	420	6,300	67	1,008	487	7,308

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 normal 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.
(f): Other dietary sources refer to naringenin as such.

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).

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. 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].

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.

Narenginin 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 IC_{50} values below 5 μM (Lu et al., 2011). The CYP3A-dependent metabolism of 7-benzyloxy-4-trifluoromethylcoumarin was inhibited in human (K_i = 24.6 μM), pig (K_i = 15.6 μM) and mouse (K_i = 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.

Procedure steps for long-term exposure

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[3] 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.

References

Ameer B, Weintraub RA, Jognson JV, Yost RA and Rousseff RL, 1996. Flavone absorption after naringin, hesperidin and citrus administration. Clinical Pharmacology and Therapeutics, 60, 34–40. https://doi.org/10.1016/S0009-9236(96)90164-2

Biesaga M, 2011. Influence of extraction methods on stability of flavonoids. Journal of Chromatography A, 1218, 2505–2512. https://doi.org/10.1016/j.chroma.2011.02.059

Breinholt V, Hossaini A, Brouwer C and Larsen J, 1999. *In vitro* and *in vivo* estrogenic activity of dietary flavonoids: importance of bioavailability and metabolism. Journal of Medicinal Food, 2, 227–229.

Breinholt VM, Svendsen GW, Dragsted LO and Hossaini A, 2004. The citrus-derived flavonoid naringenin exerts uterotrophic effects in female mice at human relevant doses. Pharmacology and Toxicology, 94, 30–36.

Brett GM, Hollands W, Needs PW, Teucher B, Dainty JR, Davis BD, Brodbelt JS and Kroon PA, 2009. Absorption, metabolism and excretion of flavanones from single portions of orange fruit and juice and effects of anthropometric variables and contraceptive pill use on flavanone excretion. British Journal of Nutrition, 101, 664–675.

Brown JP and Dietrich PS, 1979. Mutagenicity of plant flavonols in the *Salmonella*/mammalian microsome test. Activation of flavonol glycosides by mixed glycosidases from rat cecal bacteria and other sources. Mutation Research, 66, 223–240.

Bugianesi R, Catasta G, Spigno P, D'Uva A and Maiani G, 2002. Naringenin from cooked tomato paste is bioavailable in men. Journal of Nutrition, 132, 3349–3352.
Burkina V, Zlabek V, Halsne R, Ropstad E and Zamaratskaia G, 2016. *In vitro* effects of the citrus flavonoids diosmin, naringenin and naringin on the hepatic drug-metabolizing CYP3A enzyme in human, pig, mouse and fish. Biochemical Pharmacology, 110–111, 109–116. https://doi.org/10.1016/j.bcp.2016.04.011
CCRIS (Chemical Carcinogenesis Research Information System), 2014. Ames assay results for 4′,5,7-trihydroxyflavanone. Available online: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/f?./temp/~922MAo:3
Chen J, Lin H and Hu M, 2003. Metabolism of flavonoids via enteric recycling: role of intestinal disposition. Journal of Pharmacology and Experimental Therapeutics, 304, 1228–1235. https://doi.org/10.1124/jpet.102.046409
Cramer GM, Ford RA and Hall RL, 1978. Estimation of toxic hazard-a decision tree approach. Food and Cosmetic Toxicology, 16, 255–276.
Donovan JL, Manach C, Faulks RM and Kroon PA, 2006. Absorption and metabolism of dietary plant secondary metabolites. In: Crozier A, Clifford MN and Ashihara H (eds.). *Plant Secondary Metabolites: occurrence*. Structure and Role in the Human Diet, Wiley-Blackwell Publishing, Jersey City, USA. pp. 303–351.
EFSA (European Food Safety Authority), 2012. Proposed template to be used in drafting scientific opinion on flavouring substances (explanatory notes for guidance included). EFSA Supporting Publications 2012;9(1): EN-218, 30 pp. https://doi.org/10.2903/sp.efsa.2012.en–218
EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010a. Scientific Opinion on guidance on the data required for the risk assessment of flavourings to be used in or on foods. EFSA Journal 2010;8(6):1623, 38 pp. https://doi.org/10.2093/j.efsa.2010.1623
EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2010b. Scientific Opinion on Flavouring Group Evaluation 32 (FGE.32): flavonoids (Flavanones and dihydrochalcones) from chemical groups 25 and 30. EFSA Journal 2010;8(9):1065, 61 pp. https://doi.org/10.2903/j.efsa.2010.1065
Erlund I, 2004. Review of the flavonoids quercetin, hesperetin and naringenin. Dietary sources, bioactives, bioavailability, and epidemiology. Nutrition Research, 24, 851–874. https://doi.org/10.1016/j.nutres.2004.07.005
Erlund I, Meririne E, Alfthan G and Aro A, 2001. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. The Journal of Nutrition, 131, 235–241.
FAO/WHO, 2008. Evaluation of certain food additives. Sixty-ninth report of the joint FAO/WHO expert committee on food additives. Rome, 17–26 June 2008. WHO technical report series, No 952.
Felgines C, Texier O, Morand C, Manach C, Scalbert A, Regerat F and Remesy C, 2000. Bioavailability of the flavanone naringenin and its glycosides in rats. American Journal of Physiology. Gastrointestinal and Liver Physiology, 279, G1148–G1154.
Gaffield W, Lundin RE, Gentili B and Horowitz RM, 1975. C-2 stereochemistry of naringin and its relation to taste and biosynthesis in maturing grapefruit. Bioorganic Chemistry, 4, 259–269. https://doi.org/10.1016/0045-2068(75)90036-X
Hsiu S-L, Huang T-Y, Hou Y-C, Chin D-H and Chap P-D, 2002. Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. Life Sciences, 70, 1481–1489.
Jagetia GC, Venkatesha VA and Reddy TK, 2003. Naringin, a citrus flavonone, protects against radiation-induced chromosome damage in mouse bone marrow. Mutagenesis, 18, 337–343. https://doi.org/10.1093/mutage/geg001
Kanaze FI, Bounartzi MI, Georgarakis M and Niopas I, 2007. Pharmacokinetics of the citrus flavanone aglycones hesperetin and naringenin after single oral administration in human subjects. European Journal of Clinical Nutrition, 61, 472–477.
Ke J-Y, Schwartz SJ, Riedl KM, Yee LD, Kliewer KL and Belury MA, 2013. Accumulation of dietary naringenin and metabolites in mice. The FASEB Journal, 27 (1 Suppl.), abstract 636.2.
Kumpulainen J, Lehtonen M and Mattila P, 1999. Trolox equivalent antioxidant capacity of average flavonoid intake in Finland. In: Kumpulainen JT and Salonen JT (eds.). Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry, Cambridge, UK. 240, 141–150.
Lu WJ, Ferlito V, Xu C, Flockhart DA and Caccamese S, 2011. Enantiomers of naringenin as pleiotropic, stereoselective inhibitors of cytochrome P450 isoforms. Chirality, 23, 891–896. https://doi.org/10.1002/chir.21005
Ma Y, Li P, Chen D, Fang T, Li H and Su W, 2006. LC/MS/MS quantitation assay for pharmacokinetics of naringenin and double peaks phenomenon in rats plasma. International Journal of Pharmaceutics, 307, 292–299.
Nagao M, Morita N, Yahagi T, Shimizu M, Kuroyanagi M, Fukuoka M, Yoshihira K, Natori S, Fujino T and Sugimura T, 1981. Mutagenicities of 61 flavonoids and 11 related compounds. Environmental and Molecular Mutagenesis, 3, 401–419. https://doi.org/10.1002/em.2860030402
Nielsen IL, Chee WS, Poulsen L, Offord-Cavin E, Rasmussen SE, Frederiksen H, Enslen M, Barron D, Horcajada MN and Williamson G, 2006. Bioavailability is improved by enzymatic modification of the citrus flavonoid hesperidin in humans: a randomized double-blind, crossover trial. Journal of Nutrition, 136, 404–408.
NTP (National Toxicology Program), 1992. Technical Report series No.409. Toxicology and Carcinogenesis studies of quercetin (CAS no.117-39-5) in F344/N rats (Feed studies) https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr409.pdf
OECD (Organisation for Economic Co-operation and Development), 1997. Test No. 471: Bacterial reverse mutation test. OECD Guideline for testing of chemicals. Section 4.

OECD (Organisation for Economic Co-operation and Development), 2014. Test No. 487: *in vitro* mammalian cell micronucleus test. OECD Guidelines for the Testing of Chemicals, Section 4.
Orrego-Lagaron N, Vallverdu-Queralt A, Martinez-Huelamo M, Lamuela-Raventos RM and Escribano-Ferrer E, 2016. Metabolic profile of naringenin in the stomach and colon using liquid chromatography/electrospray ionization linear ion trap quadrupole-Orbitrap-mass spectrometry (LC-ESI-LTQ-Orbitrap-MS) and LC-ESI-MS/MS. Journal of Pharmaceutical and Biomedical Analysis, 120, 38–45.
Pingili R, Vemulapalli S, Mullapudi SS, Nuthakki S, Pendyala S and Kilaru N, 2016. Pharmacokinetic interaction study between flavanones (hesperetin, naringenin) and rasagiline mesylate in wistar rats. Drug Development and Industrial Pharmacy, 42, 1110–1117.
Poon CH, Wong TY, Wang Y, Tsuchiya Y, Nakajima M, Yokoi T and Leung LK, 2013. The citrus flavonone naringenin suppresses *CYP1B1* transactivation through antagonising xenobiotic-responsive element binding. British Journal of Nutrition, 109, 1598–1605. https://doi.org/10.1017/S0007114512003595
Sandeep MS, Sridhar V, Puneeth Y, Babu PR and Babu KN, 2014. Enhanced oral bioavailability of felodipine by naringenin in Wistar rats and inhibition of P-glycoptrotein in everted rat gut sacs *in vitro*. Drug Development and Industrial Pharmacy, 40, 1371–1377. https://doi.org/10.3109/03639045.2013.819885
Shulman M, Cohen M, Soto-Gutierrez A, Yagi H, Wang H, Goldwasser J, Lee-Parsons CW, Benny-Ratsaby O, Yarmush ML and Nahmias Y, 2011. Enhancement of Naringenin Bioavailability by Complexation with Hydroxypropoyl-β-Cyclodextrin. PLoS ONE, 6, e18033. https://doi.org/10.1371/journal.pone.0018033
e Silva CRC, David JM, Borges RdSQ, Ferreira SLC, David JP, dos Reis PS and Bruns RE, 2014. Determination of flavanones in orange juices obtained from different sources by HPLC/DAD. Journal of Analytical Methods in Chemistry, Volume 2014, Article ID 296838, 5 pages. https://doi.org/10.1155/2014/296838
Snyder RD and Gillies PJ, 2002. Evaluation of the clastogenic, DNA Intercalative, and topoisomerase II-interactive properties of bioflavonoids in Chinese hamster V79 cells. Environmental and Molecular Mutagenesis, 40, 266–276. https://doi.org/10.1002/em.10121
Sugimura T, Nagao M, Matsushma T, Yahagi T, Seino Y, Shirai A, Sawamura M, Natori S, Yoshihira K, Fukuoka M and Kuroyangai M, 1977. Mutagenicity of flavone derivatives. Proceedings of the Japan Academy, Series B, 53, 194–197. https://doi.org/10.2183/pjab.53.194
Tommasini S, Raneri D, Ficarra R, Calabrò ML, Stancanelli R and Ficarra P, 2004. Improvement in solubility and dissolution rate of flavonoids by complexation with beta-cyclodextrin. Journal of Pharmaceutical and Biomedical Analysis, 35, 379–387. https://doi.org/10.1016/S0731-7085(03)00647-2
Ubeaud G, Hagenbach J, Vandenschrieck S, Jung L and Koffel JC, 1999. *In vitro* inhibition of simvastatin metabolism in rat and human liver by naringenin. Life Sciences, 65, 1403–1412.
Wan L, Sun X, Wang X, Li Y, Yu Q and Guo C, 2011. A stereospecific HPLC method and its application in determination of pharmacokinetics profile of two enantiomers of naringenin in rats. Journal of Chromatographic Science, 49, 316–320.
Yáñez JA, Remsberg CM, Miranda ND, Vega-Villa KR, Andrews P and Davies NM, 2008. Pharmacokinetics of selected chiral flavonoids: hesperetin, naringenin and eriodictyol in rats and their content in fruit juices. Biopharmaceutics and Drug Disposition, 29, 63–82.
Zhang J, 2007. Flavonoids in grapefruit and commercial grapefruit juices: concentration, distribution and potential health benefits. Proceedings of the Florida State Horticultural Society, 120, 288–294.

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

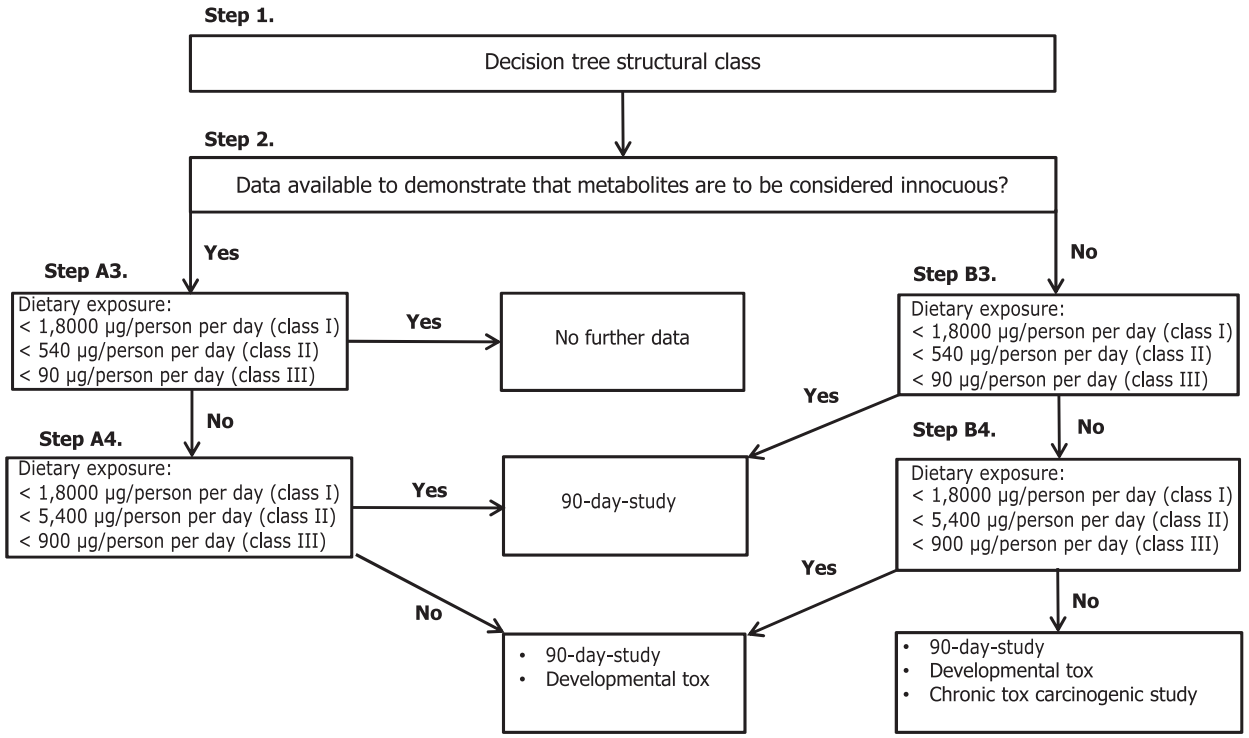


Figure A.1: The stepwise procedure for evaluation of new flavouring substances according to Commission Regulation (EC) No 1334/2008

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[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)	
			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[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)	
			Normal	Maximum	Average[d]	Maximum	Normal	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[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)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
07.1	Bread and ordinary bakery wares	50						
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	80	30	200			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			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)	
			Normal	Maximum	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[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)	
			Normal	Maximum	Average[d]	Maximum	Normal	Maximum
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[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)	
			Normal	Maximum	Average[d]	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 μg/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 μg/adult per day.

The total APET will be 10,000 μg/adult per day corresponding to 170 μg/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 × 0.63 = 2,520 μg/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 × 0.63 = 3,780 μg/child per day.

The total APET will be 6,300 μg/child per day corresponding to 420 μg/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 μg/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 μg/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 μg/adult per day.

The total APET will be 11,600 μg/adult per day corresponding to 194 μg/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 × 0.63 = 3,528 μg/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 × 0.63 = 3,780 μg/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 μg/child per day corresponding to 487 μg/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 × 125 g × 300 mg/kg = 112.5 mg/adult.

Children[8]

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

Infants and young children (0–1 year)

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

[8] 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).

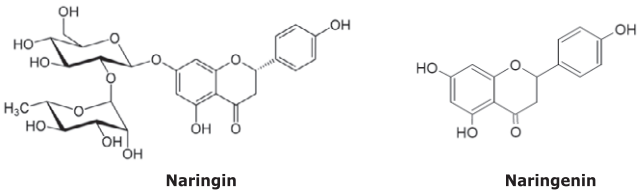


Figure C.1: Structures of naringin and naringenin

Human pharmacokinetics data: difference in pharmacokinetics between naringin and naringenin

Some reliable human data are available on [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 C_{max} 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-Lagaron et al., 2016). Ring cleavage by the intestinal microflora has been reported, resulting in metabolites such as *p*-hydroxyphenylpropionic 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 ~ 230 μmol/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 ± 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 μmol/L (41 mg/L) and 1,283 μmol/L (349 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 ± 0.4 μmol/L from orange juice and 6.0 ± 5.4 μmol/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 ± 25% and 1.1 ± 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_{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 narengenin 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.