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Assessment of the feed additive consisting of naringin for all animal species for the renewal of its authorisation (HealthTech Bio Actives, S.L.U. (HTBA))

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Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the assessment of the application for the renewal of authorisation of naringin as a sensory additive for all animal species. The applicant provided data demonstrating that the additive currently in the market complies with the conditions of authorisation. The FEEDAP Panel confirms that the use of naringin under the current authorised conditions of use is safe for the target species, the consumers and the environment. Naringin does not cause severe irritation or corrosion to eyes, is not irritant to the skin and is not classified as a dermal sensitiser. The FEEDAP Panel cannot conclude on the possible respiratory sensitisation of the additive, due to the lack of data. There was no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

Keywords: Naringin, sensory additive, flavouring, safety, efficacy, feed, renewal

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 14(1) of that Regulation lays down that an application for renewal shall be sent to the Commission at the latest one year before the expiry date of the authorisation.

The European Commission received a request from HealthTech Bio Actives, S.L.U. (HTBA)[2] for the renewal of the authorisation of the additive naringin, when used as a feed additive for all animal species (category: sensory additive; functional group: flavouring compounds).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an under Article 14(1) (renewal of the authorisation). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 20 October 2021.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumers, users and the environment and on the efficacy of the feed additive consisting of naringin, when used under the proposed conditions of use (see Section 3.1.1).

1.2. Additional information

EFSA issued an opinion on the safety and efficacy of this product when used in feed for all animal species in 2011 (EFSA FEEDAP Panel, 2011).

EFSA also issued an opinion regarding the safety of naringenin, the aglycone of naringin, for its use in food (EFSA CEF Panel, 2017).

The additive is currently authorised as a sensory additive (functional group: flavouring compound) for use in feed for all animal species and categories without a maximum limit (2b16058).[3] The additive has been also authorised as a food flavouring (EU flavour information system (FLAVIS) No 16.058).

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[4] in support of the authorisation request for the use of naringin as a feed additive.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

The European Union Reference Laboratory (EURL) considered that the conclusions and recommendations reached in the previous assessment regarding the methods used for the control of the active substance in animal feed/marker residue in tissues are valid and applicable for the current application.[5]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Naringin is in line with the principles laid down in Regulation (EC) No 429/2008[6] and the relevant guidance

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the council of 22 September 2003 on the additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] HealthTech Bio Actives, S.L.U. (HTBA) Carretera de Zeneta 143-145 El Raiguero - La Villa, Beniel (Murcia) Spain.
[3] Commission Regulation (EU) No 870/2012 of 24 September 2012 concerning the authorisation of naringin as a feed additive for all animal species. OJ 257/10, 25.9.2012.
[4] FEED dossier reference: FAD-2021-0077.
[5] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2010-0129.pdf
[6] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP PANEL, 2012), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017) and Guidance on the renewal of the authorisation of feed additives (EFSA FEEDAP Panel, 2021a).

3. Assessment

The additive naringin is currently authorised as a sensory additive (functional group: flavouring compounds) for use in feed for all animal species. This assessment regards the renewal of the authorisation.

3.1. Characterisation

The additive consists of naringin [(2*S*)-4*H*-1-benzopyran-4-one, 7-((2-*O*-(6-deoxy-alpha-L-mannapyranosyl)-beta-D-glucopyranosyl) oxy)-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)], a flavanone glycoside, and its aglycone naringenin that occur naturally in the pericarp of citrus fruit. Naringin is extracted from grapefruit (*Citrus paradisii*) and belongs to the Chemical Group (CG) 25 (FLAVIS No is 16.058, flavour extract manufacturers association (FEMA) number is 2769 and the CAS number is 10236-47-2).[7] The molecular weight is 580.54 g/mol and the molecular formula $C_{27}H_{32}O_{14}2H_2O$.

The additive is present on the market as a beige creamy powder. The additive and the active substance are identical. It is authorised with a content of at least 90% naringin.

The applicant stated that the manufacturing process and the composition of the additive have not been modified since the previous authorisation and data have been provided from recent batches on the composition of the additive to support this statement.

Analytical results on five batches of the additive using high-performance liquid chromatography (HPLC) confirmed compliance with the existing specifications (mean: 95.4%; range: 94.9–96%). The loss on drying on the same batches was on average 2.5% (range: 1.7–3.3%). The percentages of related substances were also reported: neoeriocitrin (mean: 0.3%; range: 0.2–0.4%), isonaringin (mean: 0.5%, range: 0.3–0.6%), neohesperidin (mean: 0.5%, range: 0.4–0.7%), rhoifolin (mean: 0.5%, range: 0.5–0.6%), naringenin (mean: 0.4%, range: 0.3–0.4%), poncirin (mean: 1.7%, range: 1.5–1.9%). The percentage of unknown compounds was on average 0.6% (range: 0.6–0.6%). Overall, the additive was characterised up to 99.8%.[8]

Three batches of the additive were analysed for impurities.[9] Cadmium, lead, mercury and arsenic concentrations/levels were below the limit of quantification (LOQ).[10] The same batches were also analysed for possible presence of several pesticides and dioxins.

Pesticides were analysed in a multiresidue test CG 250 which detected pyriproxyfen in two batches (< 0.01 mg/kg (LOQ) and 0.014 mg/kg); and in a multiresidue test LC 350 which detected thiabendazole in three batches (range 0.09–0.11 mg /kg) and acetamiprid in one batch (< LOQ 0.01 mg/kg); dithiocarbamates were not detected by a specific monoresidue test.

Polychlorinated biphenyls (PCBs) non-dioxin-like and dioxin-like measured on average 0.026 ng/g (range: 0.01–0.055 ng/g) and 0.007 pg/g (range: 0.004–0.013 pg/g), respectively. Polychlorinated dibenzodioxins (PCDDs)/polychlorinated dibenzofurans (PCDFs) measured on average 0.12 pg/g (range: 0.12–0.13 pg/g). The sum of PCDD/PCDF and dioxin-like PCBs was on average 0.13 pg/g (range: 0.12–0.14 WHO TEQ pg/g).

The analysis of mycotoxins showed that aflatoxins (B1, G1, B2, G2) were below the LOQ[11] while ochratoxin A was on average 3.5 μg/kg (range: 3.3–3.8 μg/kg).

Microbiological contamination was analysed on five batches of the additive by determination of *Escherichia coli*(not detected in 1 g), *Salmonella* spp. (not detected in 25 g), total plate count (< 1,000colony forming units (CFU)/g) and yeast and moulds (< 100 CFU/g).[12]

The detected amounts of the above-described undesirable substances do not raise safety concerns.

[7] Commission Regulation (EC) No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96 of the European Parliament and of the Council (Annex I). OL 180, 19.07.2000, p. 8.
[8] Technical dossier/Section II/Annex _II_2 and Annex_II_3.
[9] Technical dossier/Section II/Annex _II_4.
[10] LOQ (mg/kg) cadmium: 0.01, lead: 0.04, mercury: 0.005 and arsenic: 0.03I.
[11] LOQ aflatoxin B1, G1, B2, G2: 2 μg/kg.
[12] Technical dossier/Section II/Annex _II_2.

The bulk density and tapped density of five batches of the additive showed values on average 512 kg/m^3 (range: 450–550 kg/m^3) and 710 kg/m^3 (range: 630-750 kg/m^3), respectively.[13] The additive has a melting point of 83°C, a density of 1.66 g/cm^3 and the solubility in water at room temperature is around 0.1 g/L. It is soluble in alcohol, acetone and hot acetic acid but insoluble in ether, chloroform and benzene.[12]

The dusting potential of three batches of the additive was determined using the Stauber–Heubach method and showed values on average of 190 mg/m^3 (range:105–330 mg/m^3).[14]

The applicant provided analytical results on particle size distribution of the additive measured on five batches by means of laser diffraction.[15] On average, 50% of the particles have a diameter below 101.5 μm (range: 10.2–176.9 μm), and 90% of the particles have diameter below 541 μm (range: 358.4–638.3 μm). On average, 1% of the particles were below 1 μm (range: 0–4%) (only two batches reporting data different than 0).

In addition, the applicant provided analytical data on particle size distribution of the dust of two batches (those with particle size below 1 μm). The percentage of particles in the dust having a diameter below 100 μm is on average 100%, below 50 μm was 99.98% (range: 99.9–100%), below 10 μm 71.07% (range: 62.5–79.7%) and below 1 μm 8.76% (range: 7.9–9.5%).[16]

Stability and the capacity for homogeneous distribution of the additive were evaluated by EFSA in a previous assessment (EFSA FEEDAP Panel, 2011). No new data have been provided in the current application.

3.1.1. Conditions of use

Naringin is currently authorised for use in complete feed for all animal species without a minimum or maximum content.

The authorisation, under other provisions foresees:

1) In the directions for use of the additive and premixture, indicate the storage temperature, storage life, and stability to pelleting.
2) Recommended use up to 5 mg/kg complete feedingstuff.
3) For safety: breathing, eyes and skin protection shall be used during handling.

The applicant proposes to keep the same conditions of use as authorised.

3.2. Safety

The safety of naringin for the target species, consumers, users and the environment was evaluated in a previous opinion (EFSA FEEDAP Panel, 2011). The FEEDAP Panel considered that 'the proposed range of use levels of 1 to 5 mg naringin/kg feed as safe for all animal species with a considerable margin of safety'. The additive was also considered safe for consumers and the environment under the proposed conditions of use. No data relevant to an assessment of user safety was made available. The additive was considered to be a possible eyes and respiratory irritant based on the Safety Data Sheet provided by the applicant.

The applicant carried out a structured database search using two meta-search sites (LIVIVO and Ovid), sixteen single databases including PubMed and Web of Science, and eight publishers' search facilities including Elsevier, Ingenta, Springer, Wiley.[17] The literature search covered the period from last authorisation (2011) until February 2021. The main keywords used were: 'Naringin' OR 'Naringoside' OR 'Naringenin-7-beta-neohesperidoside' OR 'CAS No. 10236-47-2' OR 'EC No. 233-566-4'. Specific subject areas were added in order to restrict the search (such as safety for the different target animals, safety for user/workers, safety for consumers and safety for the environment). A detailed description of the iterations used, and the inclusion/exclusion criteria applied for the selection were provided.

Sixteen of the papers identified were considered relevant by the FEEDAP Panel for the assessment of the safety for the target species and the consumer. No papers were retrieved concerning the safety for the user and for the environment.

[13] Technical dossier/Section II/Annex_II_3.
[14] Technical dossier/Supplementary information (December 2021)/Annex_II_13.
[15] Technical dossier/Section II/Annex _II_3.
[16] Technical dossier/Supplementary information (December 2021)/Annex_II_13.
[17] Technical dossier/Section III/Annex_III_2_Literature_search.

The applicant states that no adverse effect notifications were received since the previous authorisation with respect to target animal safety, consumers safety, users/workers safety, and the environment.

3.2.1. Absorption, distribution, metabolism and excretion

In the previous assessment, the FEEDAP Panel (EFSA FEEDAP Panel, 2011) concluded that, based on data from mammals, no significant amounts of naringin or metabolites are to be expected in animal tissues or products, and the use of naringin in animal nutrition is considered safe for the consumer.

In the context of the literature search performed, the applicant provided ten papers describing the absorption, distribution, metabolism and excretion (ADME) profile of naringin in laboratory species (rats, mice and beagle dogs) (Liu et al., 2011; Yang et al., 2012; Zou et al., 2012; Liu et al., 2012; Lin et al., 2014; Zeng et al., 2019; Zeng et al., 2020a; Zeng et al., 2020b; Guo et al., 2020; Bai et al., 2020).

Zou et al. (2012) described that in rats after oral administration of 42 mg naringin/kg body weight (bw), the highest values of the compound were found in liver (C_{max}: 231 ng/g at 0.08 h), followed by lower levels in trachea, lung and kidney. After 12 h naringin was not anymore detected in the analysed tissues (LOQ: 10 ng/g, liquid chromatography with tandem mass spectrometry (LC–MS/MS)). In a similar experiment, Zeng et al. (2019) observed that absorption and excretion of orally administered naringin were delayed in aged rats compared to adult rats. Analyses of urine and faeces showed that the excretion of naringin and its metabolites has been completed 48 h post-dose.

The FEEDAP Panel noted that the ADME profile of naringin has been evaluated also in the context of the safety assessment of bitter orange extract (EFSA FEEDAP Panel, 2021b).

Overall, all 10 studies demonstrated that the limited amount of absorbed naringin is distributed to several organs, but it seems not to accumulate. Naringin and its metabolites are excreted in faeces and urine.

3.2.2. Toxicological studies

3.2.2.1. Genotoxicity studies

In the previous assessment (EFSA FEEDAP Panel, 2011), genotoxicity endpoints were not evaluated. Three papers retrieved from the literature search (Section 3.2), have been considered relevant by the FEEDAP Panel and are described below.

Naringin effects on genome stability were investigated in the frame of a study conducted on diabetic Wistar albino rats (Bakheet and Attia, 2011). Non-diabetic control animals were treated by gavage with 25 or 50 mg/kg bw naringin for 4 weeks and killed 24 h after the last administration. The tested doses were selected based on the antihyperglycemic and antioxidant effects induced by naringin in a previous study on Wistar rats with streptozotocin-induced diabetes. No increase in the levels of DNA strand breaks and chromosome damage was observed in the bone marrow, as measured by the Comet assay and micronucleus test, respectively. No toxicity was induced in the target tissue by naringin treatment since the ratio polychromatic/normochromatic erythrocytes was not modified in comparison with the control group. The frequency of chromosomal aberrations analysed in spermatocytes was not affected by naringin treatment.

The genotoxic potential of naringin was evaluated *in vitro* in human lymphocytes and V79 cells applying the cytokinesis-blocked micronucleus (CBMN) test and the alkaline Comet assay (Bacanli et al., 2015). The micronucleus frequency was analysed after a continuous treatment with 50, 100, 500, 1,000 and 2,000 μM naringin and no increase was observed in any experimental condition compared to the negative controls. No significant cytotoxicity was induced by naringin up to the highest concentration tested. In human lymphocytes, no changes in the level of DNA strand breaks were detected after treatments with naringin at 50, 100, 500 and 1,000 μM, while the highest concentration caused a statistically significant increase in DNA damage compared to negative control. The Fpg-modified comet assay detected a significant increase of oxidative DNA damage at 50 μM naringin and above. No DNA damage was observed in V79 cells in any experimental condition. The FEEDAP Panel noted that anti-oxidant compounds might exert a pro-oxidant activity in experimental conditions associated to high concentrations of oxygen, as those experienced by mammalian cells in culture. Hence, a bias in the estimation of the pro-oxidant effect could be envisaged.

A study was performed to assess the interactions between naringin and nucleic acid using an electrochemical DNA biosensor, a tool for the assessment of the interaction of nucleic acid with xenobiotics (Szczepanek et al., 2020). Calf thymus DNA was used as a model of dsDNA. Naringin

showed an antioxidant protective effect at concentration below 10 μM. Above this level, structural deformations in the tested nucleic acids were detected, indicating that an excessive amount of naringin might induce DNA oxidation. However, the biological absorption of naringin is limited, thus only an antioxidant effect could be expected *in vivo*.

Based on the new evidence provided by the applicant, showing no induction of structural and numerical chromosome aberrations as well as no biologically relevant increase in DNA damage possibly related to gene mutations, the FEEDAP Panel concluded that the use of naringin as feed additive is not of concern in relation to genotoxicity.

3.2.2.2. Repeated dose toxicity studies

Three papers were retrieved by the applicant following the literature search described above (Section 3.2). These are toxicological studies conducted in rats or dogs and have been considered relevant for the assessment.

Studies in rats

A suspension of naringin (98.3% purity) in sterile water was administered 6 days per week by gavage to groups of 22 Sprague–Dawley rats of each sex at doses of 0, 50, 250 or 1,250 mg/kg bw per day for 13 weeks (6/sex) (Li et al., 2013), or 26 weeks (8/sex) (Li et al., 2014). Groups of 4 rats/sex were allowed to recover from each phase of treatment for 4 weeks before necropsy. Clinical observations, BW and feed intake (FI) were recorded throughout the study. At the end of the study blood samples were collected for haematology and clinical chemistry measurements. All animals were subject to necropsy with gross examination and all major organs were weighed. Histological examination was initially limited to high-dose and control animals, but with potential to be extended to other groups if treatment-related changes were seen.

Results of the 90-day study showed a dose-related reduction in BW gain in all the animals in the highest dose group. Lymphocyte percentage was higher ($p < 0.05$) in high-dose animals (pooled data for both sexes). In the same group there was a reduction in urea. The total bilirubin (TBIL) concentration was significantly ($p < 0.05$) lower than that of controls in all treated groups but little evidence for a dose relationship. Some absolute organ weights of treated groups showed significant differences from the control, but these were not present when weights were expressed relative to BW. Necropsy examination and histology revealed no differences between treated and control groups. The no observed adverse effect level (NOAEL) from the 90-day study is 1,250 mg/kg bw per day, the highest dose tested.

Results of the 6-month study showed a statistically significant reduction of the BW gain of high-dose rats of both sexes compared to the controls. From week 26 onwards, the growth curves showed a body weight reduction also for the control and mid-dose females. There were a number of statistically significant differences between the treated and control groups in results of haematological (higher haemoglobin (Hb) level in 50 mg/kg bw per day female group, lower neutrophiles percentage in 1,250 mg/kg bw per day male group, higher red blood cell count (RBC), Hb and haematocrit (HCT) levels in 250 and 1,250 mg/kg bw per day male groups at the end of 6-month treatment period) and serum chemistry parameters (levels of creatinine, total proteins, albumin and TBIL of female rats and the levels of urea, glucose and chlorine of male rats from 1,250 mg/kg bw per day group were significantly decreased compared with corresponding control values). In addition, the levels of high-density lipoprotein (HDL-C) in 250 mg/kg bw per day female group and of HDL-C and cholesterol in 250 mg/kg bw per day male group were significantly increased compared with corresponding control values. Relative liver and spleen weights were higher ($p < 0.05$) than those of controls in high-dose females. A similar difference was not seen in males, but slightly higher relative weights were seen for brain, lungs, kidneys, prostate and testis in high dose males. These differences were not reproduced in the recovery group. Necropsy examination and histology revealed no differences between treated and control groups. The NOAEL from the 6-month study is 1,250 mg/kg bw per day.

The NOAEL of 1,250 mg/kg bw per day is supported by the reported data from both 3- and 6-month studies. The effect on BW is insufficient to be considered an adverse effect. In principle, NOAEL should be reduced to compensate for the 6-days per week dosing which would be 1,071 mg/kg bw per day; however as this is the highest dose tested, the 1,250 mg/kg bw per day is retained.

Studies in dogs

A suspension of naringin (98.8% purity) was administered 6 days per week by gavage to groups of 6 beagle dogs of each sex at doses of 0, 20, 100 or 500 mg/kg bw per day for 13 weeks (2/sex), or

26 weeks (2/sex) (Li et al., 2020). Groups of 1/sex were allowed to recover from each phase of treatment for 4 weeks before necropsy. The FEEDAP Panel considered that the small numbers of animals used in this study reduce the value of the data obtained and consequently, the study was not further considered for the assessment.

3.2.3. Safety for the target species

In the previous assessment (EFSA FEEDAP Panel, 2011), the safety of the target species was assessed based on the NOAEL found in Patterson (1960, 1962), available only as reviewed in FASEB (1982). In that study, rats were fed diets containing 2.5% naringin; half of the rats were necropsied after 75 days and the remainder after 400 days. After 400 days, treated rats had a significantly lower body weight than controls, which was associated with a reduced feed intake and the results were attributed to an effect on palatability. Otherwise, no treatment-related histopathological effects were noted. From this study, the FEEDAP Panel identified a NOAEL of 1,250 mg/kg bw per day and applying a safety factor of 100, derived the maximum safe intake of naringin (12.5 mg/kg bw per day).

The FEEDAP Panel considered 1,250 mg/kg bw per day as the NOAEL for naringin derived from the studies conducted in rats (Section 3.2.2.2). The maximum safe concentration in feed has been calculated following the procedure described in the Guidance on safety for the target animals (EFSA FEEDAP Panel, 2017) using an uncertainty factor of 100 and is reported in Table 1.

Table 1: Maximum safe concentration in feed in all animal species, based on the NOAEL of 1,250 mg/kg bw per day

Animal species/categories	Body weight (kg)	Feed intake (g/DM per day)	Maximum safe concentration in feed (mg/kg complete feed)*
Chicken for fattening	2	158	139
Laying hen	2	106	207
Turkey for fattening	3	176	187
Piglet	20	880	250
Pig for fattening	60	2,200	300
Sow lactating	175	5,280	364
Veal calf (milk replacer)	100	1,890	582
Cattle for fattening	400	8,000	550
Dairy cow	650	20,000	357
Sheep/goat	60	1,200	550
Horse	400	8,000	550
Rabbit	2	100	220
Salmon	0.12	2.1	628
Dog	15	250	660
Cat	3	60	550
Ornamental fish	0.12	0.054	2,444

*: Complete feed dry matter (DM) = 88%, milk replacer DM = 94.5%.

3.2.3.1. Conclusions on safety for the target species

Based on the new evidence provided by the applicant, the Panel concludes that the additive remains safe for all animal species at the current authorised conditions of use (recommended use level 5 mg/kg complete feed).

3.2.4. Safety for the consumer

In the previous assessment the FEEDAP Panel (EFSA FEEDAP Panel, 2011) concluded that, based on data from mammals, no significant amounts of naringin or metabolites are to be expected in animal tissues or products, and the use of naringin in animal nutrition is considered safe for the consumer.

The review of the new scientific papers on ADME confirmed the conclusions reached in the former EFSA evaluation. Based on the new evidence provided by the applicant, the FEEDAP Panel concludes that the additive remains safe for the consumers under the conditions of the authorisation.

3.2.5. Safety for the user

No data relevant to the assessment of user safety was made available in the context of the former evaluation. In the current application, new studies were submitted and are described below.

3.2.5.1. Effects on respiratory system

No inhalation toxicological studies have been provided. Based on the dusting potential (up to 330 mg/m^3) and particle size data of the dust (up to 9.5% of the particles were below 1 μm), the FEEDAP Panel considered that the exposure of the user to the additive through inhalation is possible.

3.2.5.2. Effects on the skin and eyes

The skin irritation potential of naringin was tested in a study performed according to OECD test guideline (TG) 439, which showed that it is not a skin irritant.[18]

The dermal toxicity of naringin was tested in a study performed according to OECD TG 402. It was concluded that the LD_{50} of the test material is higher than the tested dose (2,000 mg/kg bw) when applied by the dermal route to rats.[19] With this test result the substance does not need to be classified in accordance with the CLP regulation (1272/2008).

Eye irritation was assessed in an *in vitro* study performed in compliance with GLP and following the OECD TG 437, which showed that naringin does not induce severe eye irritation or damage.[20]

The potential of naringin to cause eye irritation or severe eye damage was tested in an *in vitro* study performed according to OECD TG 438. Naringin scored higher than control for corneal opacity and fluorescein retention. Thus, although the results indicate that naringin does not cause severe irritation or corrosion of the eyes, the results do not allow a final classification.[20]

In a skin sensitisation study following OECD TG 442-B, naringin did not show any skin sensitisation potential.[21]

3.2.5.3. Conclusions on safety for the user

Naringin does not cause severe irritation or corrosion to eyes, is not irritant to the skin and is not classified as a dermal sensitiser. The FEEDAP Panel considered that the exposure of the user to the additive through inhalation is possible. However, due to the lack of data, the FEEDAP Panel cannot conclude on the potential of the additive to be a respiratory sensitiser.

3.2.6. Conclusions on safety

Based on the above and the fact that the manufacturing process, the composition of the additive and the conditions of use for the species/categories for which the additive is authorised have not been modified, the Panel considers that there is no evidence to reconsider the conclusions reached in the previous assessment.

The FEEDAP Panel concludes that naringin remains safe for the target species, the consumers and the environment under the conditions of use currently authorised.

Naringin is not irritating to the skin or eyes and is not classified as a dermal sensitiser. The FEEDAP Panel could not conclude on the possible respiratory sensitisation of the additive, due to the lack of data.

3.3. Efficacy

The present application for renewal of the authorisation does not include a proposal for amending or supplementing the original conditions that would have an impact on the efficacy of the additive. Therefore, there is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

4. Conclusions

The applicant has provided data demonstrating that the additive currently in the market complies with the conditions of authorisation.

[18] Technical dossier/Supplementary information (December 2021)/Annex_III_44.
[19] Technical dossier/Supplementary information (December 2021)/Annex_III_43.
[20] Technical dossier/Supplementary information (December 2021)/Annex_III_46.
[21] Technical dossier/Supplementary information (December 2021)/Annex_III_47.

The FEEDAP Panel concludes that naringin remains safe for the target species, the consumer, and the environment under the conditions of use currently authorised.

Naringin does not cause severe irritation or corrosion to eyes, is not irritant to the skin and is not classified as a dermal sensitiser. The FEEDAP Panel cannot conclude on the possible respiratory sensitisation of the additive, due to the lack of data.

There is no need for assessing the efficacy of the additive in the context of the renewal of the authorisation.

5. Documentation provided to EFSA/Chronology

Date	Event
26/03/2021	Dossier received by EFSA. Naringin for all animal species. Submitted by Saqual GmbH on behalf of HealthTech Bio Actives, S.L.U. (HTBA).
26/05/2021	Reception mandate from the European Commission
20/10/2021	Application validated by EFSA – Start of the scientific assessment
01/12/2021	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: characterisation/safety for the user*
13/12/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
21/01/2022	Comments received from Member States
24/03/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
bw/BW	body weight
CBMN	cytokinesis-blocked micronucleus
CFU	colony forming unit
CLP	Classification, Labelling and Packaging
CG	Chemical group
ECG	electrocardiogram
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FI	feed intake
GLP	good laboratory practices
Hb	haemoglobin
HCT	haematocrit
HDL-C	high-density lipoprotein concentration
HPLC	high-performance liquid chromatography
LC–MS/MS	liquid chromatography with tandem mass spectrometry
LD_{50}	median lethal dose
LOQ	limit of quantification
NOAEL	no observed adverse effect level

OECD	Organisation for Economic Co-operation and Development
PCB	polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxin
PCDF	polychlorinated dibenzofuran
RBC	red blood cell count
TBIL	total bilirubin
TG	test guidelines