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Safety and efficacy of a super critical carbon dioxide extract of *Humulus lupulus* L. flos when used as a feed flavouring for all animal species

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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 safety and efficacy of a super critical carbon dioxide extract of *Humulus lupulus* L. flos (hop strobiles) when used as a sensory feed additive for all animal species. The additive is specified to containing 40% beta acids and less than 0.2% alpha acids. Known substances of concern (8-prenylnaringenin, xanthohumol and 2-methyl-2-buten-2-ol) were not detected. It is intended for use as a sensory additive for all animal species at a maximum application rate of 50 mg additive/kg complete feed. Tolerance studies were provided with weaned piglets, chickens for fattening, dairy cows and fish in support of the application for all animal species. However, the FEEDAP Panel could only conclude that the additive is safe for weaned piglets, pigs for fattening and minor growing porcine species at the maximum proposed application rate. No concerns for consumer safety were identified for the use of the additive at the proposed use level in animal nutrition. In the presence of water, the additive is corrosive to skin and eyes. The additive is a potential respiratory and skin sensitiser. Use of the additive in animal production is not expected to pose a risk for the terrestrial or fresh water environment. Since harvested hop and its extracts are recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

Keywords: sensory additives, flavouring compounds, *Humulus lupulus* (L.) flos, hop, supercritical carbon dioxide, beta-acids, safety

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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 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from Simon H. Steiner Hopfen GmbH[2] for authorisation of the product extract from super critical carbon dioxide extraction of *Humulus lupulus* L. flos containing 40% beta acids with propylene glycol (Beta Rich Hop Extract - BRHE) when used as a feed additive for all animal species (category: sensory additives; functional group: flavourings).

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 application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). 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 25 October 2017.

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, consumer, user and the environment and on the efficacy of the product extract from super critical carbon dioxide extraction of *H. lupulus* L. flos containing 40% beta acids with propylene glycol (Beta Rich Hop Extract – BRHE), when used under the proposed conditions of use (see Section 3.2.2)).

1.2. Additional information

No application for re-evaluation of *H. lupulus* products as feed additives according to Article 10(2) of Regulation (EC) No 1831/2003 was submitted before the deadline of 8 November 2010. As a consequence, hop products (hop absolute, hop extract, hop extract solid and hop oil), originally on the European market as feed additives, were withdrawn from that market by Regulation (EU) No 230/2013.[3] The only exception is a tincture from *H. lupulus* L., which is currently authorised as a feed additive according to the entry in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003 (2b natural products – botanically defined).

Vegetative parts of the hop plant including hop flowers are considered to be feed materials. *H. lupulus* L., flos. have been evaluated for human medicinal traditional use by the European Medicines Agency (EMA, 2014a,b).

Propylene glycol is a feed material and an authorised food additive in the EU (E 1520) according to Annex II and III of Regulation (EC) No 1333/2008[4]. It is allowed as a carrier/carrier solvent for colours, emulsifiers, antioxidants, nutrients and enzymes, with a maximum permitted level of 1,000 mg/kg in food. In 2018, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) Panel confirmed the existing acceptable daily intake (ADI) of 25 mg/kg body weight (bw) per day for the food additive propylene glycol (E 1520) (EFSA ANS Panel, 2018).

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[5] in support of the authorisation request for the use of an extract from super critical carbon dioxide extraction of *H. lupulus* L. flos containing 40% beta acids with propylene glycol (BRHE) as feed additive.

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Simon H. Steiner Hopfen GmbH, Auhofstraβe 18, 84048, Mainburg, Germany.
[3] Commission Regulation (EC) No 230/2013 of 14 March 2013 on the withdrawal from the market of certain feed additives belonging to the group of flavouring and appetising substances. OJ L 80, 21.3.2013, p. 1.
[4] 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.
[5] FEED dossier reference: FAD-2017-0047.

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) has sought to use 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 and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of flavourings of analysis for hop beta acids from beta rich hop extract in animal feed. The Executive Summary of the EURL report can be found in Annex A.[6]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of an extract from super critical carbon dioxide extraction of *H. lupulus* L. flos containing 40% beta acids with propylene glycol (BRHE) is in line with the principles laid down in Regulation (EC) No 429/2008[7] and the relevant guidance documents: Guidance on Safety assessment of botanicals and botanical preparations intended for use as ingredients in food supplements (EFSA Scientific Committee, 2009), Compendium of botanicals that have been reported to contain toxic, addictive, psychotropic or other substances of concern (EFSA, 2012), Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011) and Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017).

3. Assessment

The additive under assessment is a super critical carbon dioxide extract of *H. lupulus* L. flos dissolved in propylene glycol (an authorised food additive in the EU, E 1520) and is intended for use as a flavour in feeds for all animal species.

3.1. Origin and extraction

Hop plants (*H. lupulus* L.) are dioecious climbing plants belonging to the Cannabaceae family and are native to Europe and North America. They are now grown throughout the world finding principal use in the brewing of beers and production of other beverages. Only the flowers of the hop plant (often referred to as seed cones or strobiles) are used for all industrial applications.[8]

The freshly dried flowers (strobiles) contain on average 30% secondary plant metabolites, mainly hop bitter resins (24%), polyphenols (5%) and essential oils (1%).[9] The content of secondary plant metabolites differs depending on the hop variety, the crop year, the growing region, the timing of harvest and the handling just before drying.

Hop bitter resins belong to two subcategories depending on their solubility in organic solvents: (i) 'soft resins', soluble in *n*-hexane and (ii) 'hard resins', insoluble in *n*-hexane but soluble in ether and methanol. The main components of the soft resin fraction are alpha acids (or humulones) and beta acids (or lupulones), which are both prenylated phloroglucinol derivatives. The major representatives of the hard resin fraction are prenylated flavonoids.[10] The essential oil fraction in fresh hop strobiles can range from 0.6 to 4.4 mL/100 g.[11] The composition of this fraction is complex and variable, with nearly 500 compounds identified (reviewed by (Eyres et al., 2007)),[12] the majority of them present only at trace concentrations. In addition to the prenylated flavonoids from the hard resin fraction, hop flowers contain a number of other phenylpropanoid derivatives.

[6] The full report is available on the EURL website https://ec.europa.eu/jrc/sites/default/files/FinRep-FAD-2017-0047.pdf
[7] 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.
[8] Technical dossier/Section II.
[9] Technical dossier/Section II, p. 18.
[10] Technical dossier/Section II, pp. 21–22.
[11] Technical dossier/Section II, p. 23.
[12] Technical dossier/Section II/Annex_II_47.

The extent to which individual compounds or groups of secondary metabolites are represented in hop products is highly dependent on the method of preparation and extraction.

3.2. Characterisation of the additive

The applicant provided a detailed description of the manufacturing process.[13] The starting material is the dried flowers of *H. lupulus* (strobiles), which are pelleted and then extracted with supercritical carbon dioxide (CO_2). [redacted]

The resulting additive is a dark brown liquid viscous liquid at room temperature. It has a density of 1.07 ± 0.01 g/mL (20°C), a typical viscosity of 300 cP and a pH in water of 11.0 ± 0.5 (1 part additive, 3 parts water).[14]

The Chemical Abstracts Service (CAS) number 8060-28-4, the European Inventory of Existing Chemical Substances (EINECS) number 232-504-3 and Flavor Extract Manufacturers Association (FEMA) number 2578 are applied indiscriminately to different kinds of extracts and derivatives from *H. lupulus,* none of which accurately describes the additive under application.

The product specification is based on the content of beta acids which are set as 40 ± 1.5%. Specifications are also set for the other major components, including alpha acids 0.4 ± 0.3%, hop oils 1.5 ± 0.3%, propylene glycol 20 ± 15%, moisture from 0 to < 8%, crude ash 10 ± 2% and other resins 25 ± 8%.

Analysis of five batches of the additive made from starting materials from different suppliers and from different harvest years showed compliance with specifications (Table 1). The analysis of the five batches was by high-performance liquid chromatography with spectrophotometric detection (alpha acids and beta acids) and gas chromatography and accounted for 98.6% (98.0–99.3%) of the product.[15]

Table 1: Analysis of five batches of the additive

Constituent	FLAVIS No	CAS No	Percentage in the extract	
			Mean	Range
Resins				
1. Total beta acids			40.3	39.6–41.0
• Colupolone		468-27-9	• 52.5(b)	• 47.7–56.0(b)
• Adlupolone + lupulone		28374-71-2 + 468-28-0	• 47.5(b)	• 44.0–52.3(b)
2. Total alpha acids			0.2	0.1–0.2
3. Other resins(a)			24.0	20.3–32.8
Hop oils		8007-04-3	1.5	1.3–1.7
• beta-Myrcene	01.008	123-35-3	• 22.8(c)	• 22.3–23.5(c)
• beta-Caryophyllene	01.007	87-44-5	• 5.6(c)	• 5.4–5.8(c)
• alpha-Humulene		6753-98-6	• 20.5(c)	• 19.9–21.0(c)
Propylene glycol		57-55-6	14.9	7.7–19
Moisture			7.1	5.6–7.7
Ash			10.5	9.6–11.5
Total			98.6	98.0–99.3

FLAVIS: The EU Flavour Information System; CAS: Chemical Abstracts Service.
(a): Results calculated: Other resins = total resins – (beta acids + alpha acids).
(b): Relative percentage of total beta acids.
(c): Relative percentage of hop oils.

Although uncharacterised at the molecular level, the fraction 'other resins' is mostly constituted of many compounds structurally related to alpha and beta acids and their transformation products. As these compounds are present in very small concentrations and are difficult to be fully characterised, they are grouped together as uncharacterised resins.

[13] Technical dossier/Section II/Annex_II_89.
[14] Technical dossier/Section II/Annex_II_52.
[15] Technical dossier/Section II/Annex_II_53 and 95.

Evidence was provided to show that prenylflavonoids are not extracted by carbon dioxide and remain in the spent medium. Particularly, 8-prenylnaringenin, a flavanone which exhibits phytoestrogen activity and therefore considered a substance of toxicological concern, was below the limit of detection (LOD) in five batches of the additive (LOD 500 mg/kg).[16] In addition, analysis of a further three batches confirmed the absence of 8-prenylnaringenin, xanthohumol (another prenylflavonoid, LOD 500 mg/kg) and 2-methyl-2-buten-2-ol (a product of oxidative degradation of hop resins, LOD 10 mg/kg).[17]

These three batches were examined for the presence of possible impurities deriving from the raw material used for extraction.[18] Heavy metals (mercury, cadmium and lead) and arsenic were all below their respective limits of detection (LODs < 0.02 for mercury and < 0.06 mg/kg for the others). In the same batches, copper was < 10 mg/kg and nitrate < 250 mg/kg. The sum of dioxins was 0.07 ng WHO PCDD/F-TEQ (World Health Organization polychlorinated dibenzo-*p*-dioxin (PCDD) and polychlorinated dibenzofuran (PCDF) toxic equivalents)/kg extract and dioxin-like polychlorinated biphenyls (PCBs) was 0.11 ng WHO-PCDD/F-PCB-TEQ (World Health Organization PCDD, PCDF and polychlorinated biphenyl (PCB) toxic equivalents)/kg extract. Aflatoxin B1 could not be detected (LOD 0.8 μg/kg). Screening for 156 pesticide residues showed compliance with legislation on pesticide residues.[19] None of the data on chemical impurities raised concerns.

The applicant also provided additional data on impurities in the dried hops before extraction (heavy metals and arsenic, aflatoxin B1, B2, G1, G2 and ochratoxin A, radionuclide contamination, dioxins and dioxin-like PCBs, microbial contamination, pyrrolizidine alkaloids and polycyclic aromatic hydrocarbons) which, again, raised no concerns.[20]

3.2.1. Stability

The shelf-life of a single batch of the additive under application and a more concentrated form of beta acids was tested at 10°C, 20°C and 30°C for 4 years by monitoring the beta acids content. Even at the higher temperature losses of beta acids over this period were less than 4%.[21]

Although data on stability in premixtures and feeds are generally not required for flavours, the applicant provided a stability study [redacted]

3.2.2. Conditions of use

The additive is intended to be added directly to compound feed or via premixtures and preparations for all animal species without withdrawal. The recommended maximum use level is 50 mg additive/kg complete feed, providing 20 mg beta acids/kg feed.

3.3. Safety

The assessment of safety is based on the highest use level proposed by the applicant (50 mg additive/kg complete feed).

3.3.1. Safety for the target species

A total of five studies in four different species (weaned piglets, chickens for fattening, rainbow trout and dairy cows) were provided to support the safety for the target species. One of the studies in chickens for fattening was not considered because the test item used was not the additive under

[16] Technical dossier/Section II and Supplementary information February 2018/Annex_SIn_7.
[17] Technical dossier/Supplementary information February 2018/Annex_SIn_8.
[18] Technical dossier/Section II/Annex_II_73.
[19] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJL 70, 16.3.2005, p. 1.
[20] Technical dossier/Section II/Annex_II_74.
[21] Technical dossier/Section II/Annex_II_92.
[22] Technical dossier/Section II/Annex_II_93.

assessment and no relationship with it could be established.[23] Two more studies (one in chickens for fattening[24] and one in rainbow trout[25]) were not considered further due to limitations on the study design. In the study with chickens birds were kept in pens [redacted], the trouts were kept in tanks [redacted]. The experimental unit is the smallest entity to which the feed is administered, this means the pen for the chicken study or the tank for the fish study. The experimental units per group in these two studies were three and two, respectively, which the Panel considers too small to reach a reasonable power in the statistical analysis. Consequently the studies were not considered further. In the study in dairy cows all the animals [redacted][26] Therefore in the absence of data on the feed intake the study cannot be considered.

3.3.1.1. Weaned piglets

[redacted] There were no significant differences in any of these parameters. No significant differences were seen in the measured blood chemistry and haematological parameters other than a significant increase in the highest dose in platelet count in the treated groups compared to the control. However, these changes were small, remained within reference values and were not accompanied by any other changes in haematology and are thus considered incidental.

3.3.1.2. Conclusions on safety for the target species

The tolerance study made with weaned piglets showed that the additive was safe at the proposed application rate of 50 mg additive/kg complete feed with a margin of safety of at least 10. This conclusion is extended to pigs for fattening and extrapolated to minor growing porcine species. No conclusions could be drawn for any other animal species/category.

3.3.2. Safety for the consumer

No specific studies on absorption, distribution, metabolism and excretion (ADME) and toxicology were provided with the product under assessment.

[23] Technical dossier/Supplementary information February 2018/Annex_SIn 14 and Annex SIn 15.
[24] Technical dossier/Section III/ Annex_III_12_[redacted] and Supplementary information February 2018/Annex SIn 13.
[25] Technical dossier/Section III/ Annex_III_14_[redacted] and Supplementary information February 2018/Annex SIn 21.
[26] Technical dossier/Section III/ Annex_III_15_[redacted] and Supplementary information February 2018/Annex SIn 17.
[27] Technical dossier/ Section III/ Annex III_9.2_[redacted]

The applicant performed an extensive literature search to retrieve data for the main components of the additive, focussing on the alpha and beta acids and other bitter resins, hop oils and their constituents and the solvent propylene glycol. The databases searched were: Pubmed, Web of Science, FSTA, Agris, TOXNET; Websites (EFSA, EMA, GRAS Inventory, Clinical trials.gov) and Books and Monographs (Library OPAC of the Technical University of Munich). The search included the terms: *humulus lupulus or beta acids, lupulone, colupulone, adlupulone, alpha acids, humulone, cohumulone, adhumulone, hop oil, myrcene, beta-caryophyllene, alpha-humulene, and propylene glycol* – in combination with the search term *toxicology*. The search covered a period until 1940. The search identified 411 publications.[30] The outcome of the search is presented below.

3.3.2.1. Absorption, distribution, metabolism and excretion

The transport of hop alpha and beta acids *in vitro* across a Caco-2 monolayer was studied by (Cattoor et al., 2010).[31] After tests for monolayer integrity, acids were added to the apical or basolateral chambers. The apparent permeability coefficient for apical to basolateral transport was substantially higher for the alpha acids (humulone, adhumulone and cohumulone) compared to the beta acids (lupulone, colupulone and adlupulone). From these results, the authors concluded that alpha acids are likely to be efficiently absorbed while the permeability to beta acids would be comparatively low. However, no *in vivo* studies made with hop alpha and beta acids confirming these *in vitro* results could be identified.

The metabolism of beta acids and alpha acids was evaluated *in vitro*, in liver microsomal fractions of rabbit and human. The extension of biotransformation was very similar between rabbit and human liver microsomes, with beta acids almost completely transformed (> 90%) and alpha acids transformed by about 70%. Metabolism of beta acids was mainly characterised by conversion into hupulones and the formation of a series of tricyclic oxygenated products (Cattoor et al., 2013).[32] These data suggests that any absorbed fraction of hop alpha and beta acids will be extensively metabolised *in vivo*.

Hop oils represent only 1.5% of the additive and are constituted by monoterpenes (e.g. beta-myrcene), sesquiterpenes (e.g. alpha-humulene) and oxygenated compounds (e.g. beta-caryophyllene). These compounds are known to be rapidly absorbed, oxidised and excreted as conjugated metabolites.[8]

Propylene glycol is metabolised to lactic and pyruvic acid by non-ruminant mammals (EFSA ANS Panel, 2018) and to glucose by ruminants.

3.3.2.2. Toxicological studies

No data on the genotoxicity of hop alpha and beta acids or their possible effects on reproduction and development could be found in the literature search. This is in agreement with the statement of EMA that no data on genotoxicity studies and no preclinical data on reproductive and developmental toxicity on hop strobiles or its preparations are available (EMA, 2014a).

The studies identified deal with some individual compounds. Acute oral toxicity studies were made with individual alpha and beta acids in mice, rats, rabbits or guinea pigs given single doses between 100 and 1,800 mg/kg bw of lupulone, a prominent beta acid (Chin and Anderson, 1950; Hansel and Wagener, 1967).[33] No acute effects were seen when humulone, an alpha acid, was given orally to mice at a dose of 1,500 mg/kg bw.[34] However, repeated dose studies in experimental animals with lupulone or other beta acids, other than a 13-day study in monkeys, appear not to be available. In this short-term study, monkeys were given a daily oral dose of 500 mg/kg bw lupulone (Chin and Anderson, 1950).[35] No effects were seen on body weight, blood parameters, electrocardiogram or liver function.

A published trial in humans investigating the effects of lupulone in the treatment of tuberculosis reported that intake of 5,000 mg lupulone/day by 10 patients for up to 3 months reported that the only side effects seen were irritation of the gastrointestinal (GI) tract with no toxic effects on liver, kidney, bone marrow or myocardium (Farber et al., 1950).[36]

[30] Technical dossier/Section III and Supplementary information February 2018.
[31] Technical dossier/Section III/Annex_III_64.
[32] Technical dossier/Section III/Annex_III_59.
[33] Technical dossier/Section III/Supplementary information February 2018/Annex SIn_31-32.
[34] Technical dossier/Section III/Supplementary information February 2018/Annex SIn_33.
[35] Technical dossier/Section III/Annex_III_74.
[36] Technical dossier/Section III/Annex_III_84.

Toxicological data exists on other components of the additive (e.g. the components of the hop oil, beta-myrcene and beta-caryophyllene). Under the proposed conditions of use, their concentrations in the extract would result in feed concentrations well below the threshold of toxicological concern (TTC) for the relevant Cramer classes, and are not further considered.

When considering the toxicology of hops and hop extracts used as traditional herbal medicine in Europe, the Committee on Herbal Medicinal Products of the European Medicines Agency (HMPC) reported that the 'experimental toxicological data on hop preparations are rather limited and incomplete but as a whole show low toxicity'. The HMPC further stated that 'In view of its long term use and present use in humans, hops is considered to be non-toxic and safe with no significant adverse effects'. The EMA concluded that 'the safety assessment of hops and hop preparations is mainly based on many years of experience from the extensive medicinal use in humans, which indicate hop preparations to be safe pharmaceutical agents' (EMA, 2014a).[37]

The ADI of 25 mg/kg bw per day for the food additive propylene glycol (E 1520) was recently confirmed by the ANS Panel (EFSA ANS Panel, 2018).

3.3.2.3. Assessment of safety for the consumer

The FEEDAP Panel recognises that a full data set establishing the safety of the additive is not available but considers the following:

- harvested hops and hop extracts (including supercritical carbon dioxide extracts) are widely used in beer brewing and to flavour food. Consequently, consumers are directly exposed to their constituents, including defined and undefined components of the resin fractions, by food consumption;
- consumer exposure to these constituents may also result from the intake of preparations of hop strobiles (*H. lupulus* L., flos) as traditional herbal medicinal products, which are considered safe for human use (EMA, 2014a,b);
- *in vitro* data of hop beta acids, the major component of the additive, indicates that they are poorly absorbed by target animals. Any absorbed fraction is extensively metabolised (as demonstrated *in vitro* and residues in edible tissues are not expected;
- the other identified components of the additive derived from hops are present only in low concentrations and are known to be metabolised and eliminated by target animals;
- substances of known concern due to associated pharmacological activity (8-prenylnaringenin, xanthohumol and 2-methyl-2-buten-2-ol) are below the limit of detection and expected to be of no concern in the hop extract under application;
- the solvent propylene glycol will be fully metabolised by the target species and is without concern in the hop extract under application;
- the unidentified part of the mixture is composed of hard and soft resins that are not considered to be substances of concern.

Based on the above, the FEEDAP Panel concludes that the use of the additive in animal feed would not appreciably increase the existing human exposure to the individual defined and undefined constituents of the extract and can therefore be considered as safe for the consumer.

3.3.3. Safety for the user

No data on safety for users was provided other than reference to reports of allergic responses found most commonly amongst workers in hop plantations (reviewed by Van Cleemput et al., 2009).[38]

The additive in water solution has a pH of 11.0, which would be corrosive to skin and eyes.

The additive should be considered to have a potential for respiratory and skin sensitisation of susceptible individuals.

3.3.4. Safety for the environment

H. lupulus (L.) is a native species to Europe, where harvested hops and its downstream products are used by the brewery industry. The maximum concentration of the additive in feed is 50 mg/kg. This amount is not likely to change the concentration of compounds from hops in the environment.

[37] Technical dossier/Section III/Annex_III_72.
[38] Technical dossier/ Section III/ Van Cleemput et al., 2009 Annex_III_[58],

The use of the extract from the plant in animal production is not expected to pose a risk for the terrestrial or fresh water compartments.

3.4. Efficacy

Under the terms Regulation (EC) No 1334/2008[39] flavouring preparations produced from food, may be used without an evaluation and approval as long as 'they do not, on the basis of the scientific evidence available, pose a safety risk to the health of the consumer, and their use does not mislead the consumer'. Consequently, there is no specific EU authorisation for any *H. lupulus* (L.) extract when used to provide flavour in food. However, hop extract, hop extract solid and hop oil are listed in Fenaroli's Handbook of Flavour Ingredients (Burdock, 2009) and by FEMA with the reference numbers 2587, 2579 and 2580, respectively.[40]

Since harvested hop and its extracts are universally recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

4. Conclusions

The conclusions on safety in this opinion apply specifically to the extract under application obtained by supercritical carbon dioxide extraction and cannot be safely extended to encompass all extracts derived from *H. lupulus* (L.).

The additive is safe for weaned piglets, pigs for fattening and minor growing porcine species when used at the recommended dose of 50 mg additive/kg complete feed. There is insufficient evidence to conclude on the safety of the additive for all animal species.

No concerns for consumer safety were identified for the application of the additive at the proposed use level in animal nutrition.

In the presence of water, the additive is corrosive to skin and eyes. The additive is a potential respiratory and skin sensitiser.

The use in animal production of the extract from *H. lupulus* (L.) is not expected to pose a risk for the environment.

Since harvested hop and its extracts are recognised to flavour food and its function in feed would be essentially the same as that in food, no further demonstration of efficacy is considered necessary.

5. Recommendations

It is recommended that the method of extraction should be specified in any potential authorisation of the additive.

Considering the three substance of toxicological concern, 8-prenylnaringenin, xanthohumol and 2-methyl-2-buten-2-ol, the Panel recommends a maximum concentration of 500, 500 and 10 mg/kg additive, respectively.

Documentation provided to EFSA

1) Extract from super critical carbon dioxide extraction of *Humulus lupulus* L. flos containing 40% beta acids with propylene glycol (Beta Rich Hop Extract - BRHE) for all animal species. September 2017. Submitted by Simon H. Steiner Hopfen GmbH.
2) Extract from super critical carbon dioxide extraction of *Humulus lupulus* L. flos containing 40% beta acids with propylene glycol (Beta Rich Hop Extract – BRHE) for all animal species Supplementary information. February 2018. Submitted by Simon H. Steiner Hopfen GmbH.
3) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for hop beta acids from beta rich hop extract.
4) Comments from Member States.

[39] 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 Regulation (EC) No 1601/91 of the Council, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34.

[40] Technical dossier/ Section IV/ Page 5.

Chronology

Date	Event
17/08/2017	Dossier received by EFSA
13/09/2017	Reception mandate from the European Commission
25/10/2017	Application validated by EFSA – Start of the scientific assessment
13/12/2017	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 target species*
19/12/2017	Clarification teleconference during risk assessment with the applicant according to the "EFSA's Catalogue of support initiatives during the life-cycle of applications for regulated products"
26/01/2018	Comments received from Member States
13/02/2018	Reception of supplementary information from the applicant - Scientific assessment re-started
19/02/2018	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
03/10/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ADME	absorption, distribution, metabolism and excretion
ADI	acceptable daily intake
ALAT	alanine aminotransferase
ANOVA	analysis of variance
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
AP	alkaline phosphatase
ASAT	aspartate aminotransferase
BRHE	Beta Rich Hop Extract
Bw	body weight
CAS	Chemical Abstracts Service
CK	creatine kinase
EBC	European Brewery Convention
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FEMA	Flavor Extract Manufacturers Association
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
GGT	gamma glutamyl transferase
GI	gastrointestinal
GLDH	glutamate dehydrogenase
HPLC	high-performance liquid chromatography
HMPC	Herbal Medicinal Products of the European Medicines Agency
LOD	limit of detection
MCHC	mean corpuscular haemoglobin concentration
MCV	mean corpuscular volume
MIC	minimum inhibitory concentration
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofuran
TEQ	toxic equivalents
TTC	threshold of toxicological concern
UV	ultraviolet
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for hop beta acids from beta rich hop extract – BRHE

In the current application, authorisation is sought under article 4(1) for the extract from supercritical carbon dioxide extraction of *Humulus lupulus* L. flos containing 40% beta acids with propylene glycol (beta rich hop extract – BRHE) under the category/ functional group (2 b) 'sensory additives'/'flavouring compounds', according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the feed additive for all animal species and categories.

According to the Applicant, the *feed additive* is a dark brown viscous liquid consisting of 38.5–41.5% *hops beta-acids* as active substance(s) and containing minor amounts of hops alpha-acids, hop oils, and propylene glycol. The maximum moisture content is 8%.

The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures* with no proposed minimum or maximum limits. However, the Applicant suggested a maximum inclusion level of 20 mg of *hops beta-acids*/kg *feedingstuffs*.

For the quantification of *hops beta-acids* in the *feed additive* and *premixtures*, the Applicant submitted a European Brewery Convention (EBC) ring-trial validated method. This method was originally designed for the determination of individual hops alpha- and beta-acids in all hops, in hop powder products and in all conventional hop extracts. This method is based on reversed phase high-performance liquid chromatography coupled to UV detection (HPLC-UV) at 313 nm wavelength.

The Applicant applied this HPLC-UV method for the analysis of five batches of the *feed additive* containing 40% of *hops beta-acids* and obtained a relative standard deviation for *repeatability* (RSDr) of 1.6%, which is in good agreement with the RSDr reported in the frame of the ring-trial validation study published by EBC for hop extracts (2.6–4.3%). Based on the acceptable performance characteristics available, the EURL recommends this method for official control to quantify *hops beta-acids* in the *feed additive*.

Furthermore, the Applicant applied the above mentioned HPLC-UV method for the analysis of *premixtures* sample consisting of sprayed *BRHE* on silica, and reported satisfactory performance characteristics derived from the validation and verification studies. While the sprayed silica is not representative of the various *premixtures* that may be used, this HPLC-UV method is considered fit-for-purpose for the quantification of *hops beta-acids* in silica based *premixtures*.

Since the accurate quantification of *hops beta-acids* from *BRHE* added in *feedingstuffs* is not achievable experimentally, the EURL cannot evaluate nor recommend any method for official control to quantify *hops beta-acids* from *BRHE* added content in *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761) is not considered necessary.