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Safety and efficacy of a feed additive consisting of carmine for cats and dogs (Mars Petcare GMbH)

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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the re-evaluation of the safety and efficacy of carmine (sensory additive, functional group: colourants – substances that add or restore colour in feeds) for cats and dogs. The additive consists of aluminium lakes of carminic acid (carmines), which are complexes of aluminium and carminic acid. Carminic acid, produced from the female insect *Dactylopius coccus* Costa, is the colouring principle and is present in the final additive at 50%. The additive does not pose a risk concerning genotoxicity and is considered safe for dogs and cats at 264 and 220 mg Carmine/kg feed, respectively (which correspond to 132 and 110 mg carminic acid/kg feed, respectively). Due to the nature of the additive, the FEEDAP Panel concluded that allergic reactions may occur in the target species following the ingestion of feeds containing the additive. The FEEDAP Panel concluded that the exposure of users by inhalation is very likely, and that carmine is a respiratory and skin sensitiser; however, due to the lack of data, a conclusion cannot be reached on the potential skin and eye irritation of the additive. The additive is considered to be efficacious in feeds for dogs and cats under the proposed conditions of use.

Keywords: carmine, carminic acid, dogs, cats, colouring agent, safety, efficacy

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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 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Mars Petcare GmbH[2] for re-evaluation of the product carmine, when used as a feed additive for dogs and cats (category: sensory additives; functional group: colourants).

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 10(2) (re-evaluation of an authorised 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 24 August 2020.

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 and user and on the efficacy of the product carmine, when used under the proposed conditions of use (see Section 3.1.4).

1.2. Additional information

The product under assessment is a feed additive, hereby referred to as 'Carmine'. In the final additive, carminic acid is present as aluminium lakes (carmines), in which aluminium and carminic acid are bound in a complex.

Cochineal, carminic acid and carmine (E 120) have been evaluated by the Scientific Committee for Food (SCF) in 1983 and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2000, which set new specifications. In 2015, EFSA issued an opinion on the re-evaluation of cochineal, carminic acid, carmines (E 120) as a food additive (EFSA ANS Panel, 2015). Following this assessment, cochineal has been excluded from the authorisation of the food additive E 120.

Carminic acid and carmine (E 120) (hereby referred to as 'E 120 carminic acid, carmine') is an approved food additive in the EU.[3] According to the definition in the European Commission specifications,[4] 'carminic acid' should have not less than 90% carminic acid and 'carmine' not less than 50% carminic acid.

Carmine (Carmine Lake WSP 50%) is currently authorised in the EU for its use in cats and dogs as a colourant additive.[5]

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[6] in support of the authorisation request for the use of Carmine as a feed additive.

[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] Mars Petcare, Eitzerstrasse 215, Verden-Aller (Germany).
[3] Commission Regulation (EU) 2018/1472 of 28 September 2018 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council and the Annex to Commission Regulation (EU) No 231/2012 as regards Carminic acid, Carmine (E 120). OJ L 247, 3.10.2018, p. 4.
[4] Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p. 295.
[5] Commission List of the authorised additives in feedingstuffs published in application of Article 9 t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs (2004/C 50/01). OJ C 50, 25.2.2004, p. 144.
[6] FEED dossier reference: FAD-2010-0330.

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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of carmine[7] in animal feed. The Executive Summary of the EURL report can be found in Annex A.[8]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Carmine is in line with the principles laid down in Regulation (EC) No 429/2008[9] and the relevant guidance documents: Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017a), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017b) and the Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

3. Assessment

The current application is for re-evaluation of the use of Carmine as a feed additive (sensory additive, functional group: colourants – substances that add or restore colour in feeds) in feed for cats and dogs.

3.1. Characterisation

3.1.1. Characterisation of the active colouring principle

Carminic acid is the colouring principle of the additive Carmine and is present in the final additive at approximately 50%. The chemical formula of carminic acid is $C_{22}H_{20}O_{13}$, its molecular weight 492.4 g/mol and the chemical abstract register (CAS) number is 1260-17-9. The structural formula of carminic acid is reported in Figure 1.

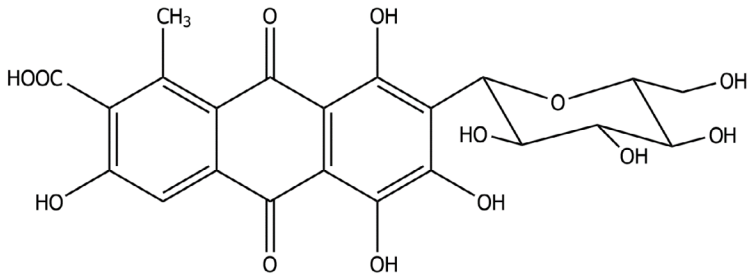

Figure 1: Structural formula for carminic acid

In the final additive, carminic acid is present as aluminium lakes (carmines), in which aluminium and carminic acid are bound in a complex.

Carminic acid is obtained from the female insect of the species *Dactylopius coccus* Costa (commonly known as cochineal). [redacted]

[7] Referred as 'Carmine lake WSP 50%' in the EURL report.
[8] The full report is available on the EURL website: https://joint-research-centre.ec.europa.eu/publications/fad-2010-0330_en
[9] 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.

[redacted][10]

The applicant sent data on the content of total ash measured on three batches of the primary extract carmine lake/paste, demonstrating compliance with the existing specifications for 'E 120 carminic acid, carmine' (not more than 12%) for use in food (mean: [redacted], min-max: [redacted]).[11]

In addition, the applicant has provided analysis on possible presence of residual solvents, including methanol and ethanol, measured on a single batch of carmine aluminium lake. All the solvents were below the respective LOQ,[12] except for ethanol which was 19.5 mg/kg.[13] According to the applicant, ethanol is likely carried over from the source materials or released by hydrolysis during the extraction of the carminic acid at high temperature.

The applicant has provided analysis on a single batch of carmine lake tested for possible presence of lead (0.11 mg/kg), cadmium (0.008 mg/kg), mercury (0.006 mg/kg) and arsenic (< 0.05 mg/kg).[14]

In the final additive, carminic acid is present as aluminium lakes (carmines), in which aluminium and carminic acid are bound in a complex (Figure 2).

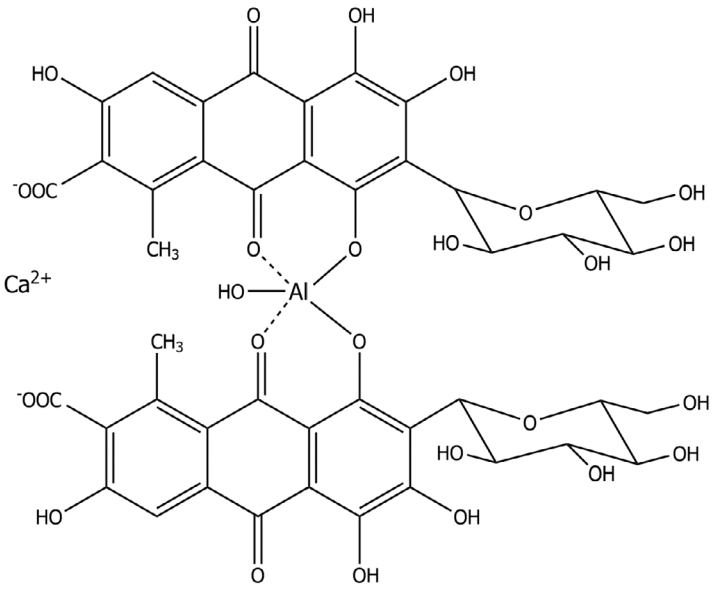

Figure 2: Structural formula for aluminium lakes of carminic acid (carmines)

3.1.2. Characterisation of the additive

To form the final additive (Carmine), maltodextrin is added to ensure a final content of carminic acid of 48.3–51.9%.

The applicant stated that the aluminium lakes of carminic acid, used in the preparation of the feed additive Carmine, complies with the specifications set for carmine used as a food additive ('E 120 carminic acid, carmine'): not less than 50% carminic acid in the chelates, not more than 3% relative to carminic acid for 4-aminocarminic acid and not more than 1% matter insoluble in dilute ammonia.

The applicant set the following specifications for the additive under assessment: carminic acid 47.5–52.5% with a moisture content of ≤ 10.0%. These specifications were confirmed by the analysis of seven batches of Carmine, dissolved in hydrochloric acid and analysed by spectrophotometry at 494 nm,[15] showing the following values: carminic acid average 50.83% (range 48.3–51.9%). The moisture content was 7.4% (range 7.0–7.5%).[16]

[10] Technical dossier/Section II/Annex_II_Production Flow (NEW).pdf.
[11] Technical dossier/Supplementary Information (July 2022)/Section II/Annex_01.
[12] LOQ for methanol: 10 mg/kg.
[13] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_05.
[14] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_06.
[15] Technical dossier/Section II/Annex_II_Assay.
[16] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_01.

The applicant has provided also analytical results on the content in the final additive of 4-aminocarminic acid (average: 475.2 mg/kg; range: 330–651 mg/kg), protein (average: 19.7 g/100 g; range: 18.1–21.8 g/100 g) and ash (average: 32.38 g/100 g, range: 25.41–49.95 g/100 g), analysed on five batches of the product.[17]

According to the applicant, the high content of ash is due to the high amount of alkali cations used for the dissolution of the insoluble carmine paste/lake.[18]

Impurities

Five batches of the additive were analysed for possible presence of cadmium (average: 0.01 mg/kg, range: 0.005–0.014) lead (average: 0.23 mg/kg, range 0.15–0.27 mg/kg), mercury (average: 0.0148 mg/kg, range 0.011–0.0.02 mg/kg) and arsenic (below limit of quantification, LOQ).[19]

Microbiological contamination was analysed in seven batches of the additive, by determination of *Escherichia coli* (absent in 1 g), staphylococci (absent in 1 g), Enterobacteriaceae (< 10 colony forming unit (CFU)/g), *Salmonella* spp. (absent in 25 g) and *Listeria monocytogenes* (absent in 25 g).[20]

Analysis on the possible presence of pesticides was not provided but the applicant submitted a statement declaring that the additive is produced from *D. coccus* Costa which grow on cladodes pesticides free, since pesticides are lethal to cochineal growth.[21]

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

3.1.3. Physical properties of the additive

The additive appears as a dark red powder. The solubility is up to [redacted] in water.[22]

Considering the results on solubility, there is no need to further characterise the fraction of small particles potentially present in the additive (EFSA Scientific Committee, 2021).

The loss on drying is max. 9% and pH value of 10% aqueous solution is 8 to 11.

The dusting potential measured on three batches of the additive under assessment, by using the Stauber–Heubach method was on average 6,545 mg/m^3 (range: 5,515–7,285 mg/m^3).[23]

3.1.4. Stability and homogeneity

The shelf life of the additive (2 batches) was studied when stored at ambient conditions for 12 months. The initial concentrations of carminic acid were 49 and 49.5%. Losses, in terms of carminic acid content, at the end of the storage period were 1.2 and 2.4%.

No specific data on the stability of the additive in feed was provided. However, the data on the efficacy provided some indications on the effect of the additive over time which may sustain the stability of the active substance in feed (see Section 4).

3.1.5. Conditions of use

Carmine is intended to be used in complementary or complete feed for dogs and cats. Although the applicant reports typical use levels of 200 mg Carmine/kg complementary or complete feed which would correspond to 100 mg carminic acid/kg, higher use levels might be used (e.g. 300 mg Carmine/kg feed).

3.2. Safety

No new studies were submitted by the applicant in support of the safety of the additive.

Cochineal, carminic acid and carmine (E 120) have been evaluated by the SCF in 1983 and by JECFA in 2000. Both committees established an acceptable daily intake (ADI) of 5 mg carmine/kg body weight (bw) per day.

The EFSA ANS Panel (EFSA ANS Panel, 2015) evaluated the safety of cochineal, carminic acid and carmine (E 120) and confirmed the ADI of 5 mg carmine (containing approximately 50% carminic acid)/kg bw corresponding to 2.5 mg carminic acid/kg bw per day. The ADI was derived by applying an uncertainty factor of 100 to the no observed adverse effects level (NOAEL) of 500 mg carmine/kg

[17] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_03.
[18] Technical dossier/Supplementary Information (July 2022).
[19] LOQ for arsenic: 0.05 mg/kg.
[20] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_07.
[21] Technical dossier/Section II/Annex_II_Pesticides statement NEW.pdf.
[22] Technical dossier/Supplementary Information (May 2022)/Section II/Annex_09.
[23] Technical dossier/Supplementary Information (November 2021)/Section II/Annex_09.

bw per day, corresponding to 250 mg carminic acid/kg bw per day (highest dose tested) identified in a combined chronic and carcinogenicity study conducted in rats (Ford et al., 1987).

The applicant made reference to the evaluations mentioned above and submitted in the dossier all the relevant papers,[24] including some of the unpublished reports.[25]

3.2.1. Toxicological studies

The FEEDAP Panel reviewed the relevant studies, and a summary of the main results is given below.

3.2.1.1. Genotoxicity

Negative results from Ames tests performed mostly with carminic acid and using as test model *Salmonella* Typhimurium strains and/or other microorganisms were reported in a series of publications (Kada et al., 1972; Brown and Brown, 1976; Barale et al., 1978; Haveland-Smith and Combes, 1980; Ishidate et al., 1984; Loprieno et al., 1992; Sarıkaya et al., 2012; Andres, 2014). Negative results for the induction of structural chromosome damage were observed in two *in vitro* studies performed in CHO or CHL cells (Ishidate et al., 1984; Loprieno et al., 1992). A significant increase of polyploidy was reported by Ishidate et al. (1984); however, it was considered not biologically relevant because of the possible presence of impurity in the test item used (e.g. indigo carmine). Loprieno et al. (1992) conducted an *in vivo* micronucleus test in mice exposed to carminic acid, while Geissel (2014) [redacted]. All these studies gave negative results. Two unscheduled DNA synthesis (UDS) studies (Kornbrust and Barfknecht, 1985; Mori et al., 1988) performed in rat hepatocytes reported negative results.

Overall, the genotoxicity studies available show that carminic acid does not induce gene mutations or chromosomal damage.

3.2.1.2. Short-term and subchronic toxicity studies

Helal et al. (2000) evaluated the effects on liver and kidneys in male albino rats when given carmine at 1.25 mg/kg bw per day in the diet for 30 days. No effects were observed, the only finding was an increase of serum urea, AST and total bilirubin. The last two parameters were normal after a recovery of 15 days. According to the authors, the increase of serum urea may suggest an impairment in renal function due to the exposure to carmine. However, the FEEDAP Panel noted that only one low dose was tested.

A 90-day study investigated the effects in rats exposed to cochineal (content of carminic acid unknown) in combination with potassium aluminium sulfate (Kawasaki et al., 1994). The cochineal was given to animals up to 1,500 mg/kg bw per day. Decrease of serum phospholipid and triglyceride levels were recorded in males at the highest dose tested; while in females, serum triglyceride and total cholesterol levels were lower, but gamma-glutamyl transferase levels were significantly higher. Kidney, adrenal and spleen weights were significantly lower in females receiving the highest dose tested; however, no histopathological changes were recorded.

[redacted] (Batelle Memorial Institute, 1962). [redacted] The FEEDAP Panel considered this a very old study with limited relevance.

3.2.1.3. Chronic toxicity and carcinogenicity studies

A combined chronic toxicity/carcinogenicity study was conducted in Wistar rats fed with carmine up to 500 mg/kg bw per day (containing a mean of 48.5% of carminic acid) for 109 weeks (Ford et al., 1987). No adverse effects were reported. Mammary gland hyperplasia was observed in all

[24] Technical dossier/Supplementary Information (May 2022)/Section III/Annex_06.
[25] Technical dossier/Supplementary Information (May 2022)/Section III/Annex_07.

treated groups but without a dose–response relationship. Therefore, the Panel concluded that the effect was not treatment related. The NOAEL identified from this study was 500 mg carmine/kg bw per day (corresponding to 250 mg carminic acid/kg bw per day).

Mori et al. (1991) reported a 2-year study conducted in mice exposed to cochineal extract (containing carminic acid at 29.8%) up to 9,000 mg/kg bw per day. No adverse effects were reported. Tumour incidence in animals exposed to cochineal extracts was not significantly different compared to control.

3.2.1.4. Reproductive and developmental toxicity studies

Rats were administered by gavage with carmine up to 1,000 mg/kg bw per day (containing carminic acid > 50%) for 19 days during pregnancy (Grant et al., 1987). The study design involved an additional control group (named 'salt group') in which animals received a solution of sodium, potassium and ammonium chlorides. Overall, no treatment-related effects were observed with the exception of an increased degree of ossification in the animals exposed to carmine and those included in the 'salt group'. However, the relevance of these findings remains unclear.

In a three-generation reproduction study, Wistar-derived rats were fed with a diet containing carmine at 0, 50, 150 or 500 mg/kg bw per day (content of carminic acid around 50%) for 60 days before mating and during all phases of the study (Grant and Gaunt, 1987). F3 generation showed significantly increased degree of ossification compared to control, without a dose–response relationship. These findings were consistent with other findings reported in literature (Grant et al., 1987), but were not considered sufficient to identify a treatment-related effect due to exposure to carmine.

Tanaka (1995) reported a reproductive and neurobehavioural study performed in mice exposed to a cochineal extract, containing carminic acid at 10% approximately, up to 2% in the diet. The starting animals (F0 generation) received the treatment from 5 weeks of age to mating until weaning, and to F1 generation from 4 to 9 weeks of age. Due to the limitations in the study design and in the characterisation of the test item, this study was not further considered.

The FEEDAP Panel considers that the NOAEL previously identified for carmine (500 mg/kg bw per day corresponding approximately to 250 mg carminic acid/kg bw per day) from the publication by Ford et al. (1987) can be used to derive the safe level of Carmine in feeds for cats and dogs. Based on the evidence provided, the FEEDAP Panel also confirmed the conclusions previously reached by the ANS Panel on the lack of genotoxicity potential of the additive under assessment.

3.2.1.5. Conclusion on toxicology

The FEEDAP Panel concluded that carmine has no genotoxic potential, is not carcinogenic and did not cause reproduction/developmental toxicity. The toxicological studies in laboratory animals showed no adverse effects that need to be taken into consideration when assessing the safety for the target species. The lowest NOAEL is 500 mg carmine/kg bw per day (corresponding approximately to 250 mg carminic acid/kg bw per day) which is identified from the publication by Ford et al. (1987).

3.2.2. Safety for the target species

The applicant did not provide any studies to support the safety for the target animals but submitted all the relevant papers,[24] including some of the unpublished reports,[25] that were evaluated in the previous EFSA assessment (EFSA ANS Panel, 2015) (see Section 3.2.1).

In addition, the applicant submitted the results of a literature search covering the time period from 2015 to 2022 and including all the different aspects of the safety for the additive. The databases used were LIVIVO and Google Scholar. The search strategy was described and reported in detail;[26] twenty-eight papers were retrieved.[27] Out of these twenty-eight publications, the FEEDAP Panel noted that nineteen papers were related to the allergenicity potential of the additive and were considered relevant for the assessment of the safety for the users. Due the limited relevance, the remaining papers were not further considered in the safety assessment of Carmine.

The NOAEL identified in the study by Ford et al. (1987) (500 mg carmine/kg bw and day), was used to calculate the maximum safe level of the additive Carmine. The NOAEL identified corresponds to 250 mg carminic acid/kg bw per day. In accordance with the procedure described in the Guidance

[26] Technical dossier/Supplementary Information (May 2022)/Section III/Annex_01.
[27] Technical dossier/Supplementary Information (May 2022)/Section III/Annex_05.

on the safety for the target species (EFSA FEEDAP Panel, 2017b) the maximum safe concentration would be 132 and 110 mg carminic acid/kg complete feed for dogs and cats, respectively. These levels would correspond to maximum safe concentration of the additive Carmine of 264 and 220 mg/kg complete feed for dogs and cats, respectively.

In its opinion, the ANS Panel pointed out that allergic reactions are associated with exposure to cochineal extract and carmines and that these substances are able to trigger both acute and chronic hypersensitivity reactions (EFSA ANS Panel, 2015). The FEEDAP Panel considered that the same considerations on the possible occurrence of allergic reactions observed in humans may apply also to the target species (dogs and cats). However, the FEEDAP Panel considered that these reactions are unpredictable and linked to the individual sensitivity.

3.2.2.1. Conclusions on the safety for the target species

The additive does not pose any risk as concerns genotoxicity. The additive is considered safe for dogs and cats at 264 and 220 mg Carmine/kg complete feed which correspond to 132 and 110 mg carminic acid/kg complete feed, respectively.

Due to the nature of the additive, the FEEDAP Panel concluded that allergic reaction may occur in the target species following the ingestion of feeds containing the additive. However, the FEEDAP Panel considered that these reactions are unpredictable and linked to the individual sensitivity.

3.2.3. Safety for the user

The applicant did not provide any studies to support the safety for the users.

Due to the high dusting potential of the additive (highest measured value: 7,285 mg/m^3), the FEEDAP Panel considered that the exposure by inhalation is very likely.

The applicant has submitted results of two literature searches: one conducted covering the period from 2015 to 2021. The database used were LIVIVO and Google Scholar and the basic keywords were: "carmine", "carminic", "cochineal", "allerg*", "occupation*", "derma*.[28] In total, 27 papers were retrieved and 22 were considered relevant by the applicant.[29] The other one was conducted covering the period from 2015 to 2022 and it is described under Section 3.2.1. Overall, 19 papers were related to allergenicity of carmine, carminic acid or cochineal dye. However, 9 of these 19 articles were already submitted by the applicant following the first literature search, described above (Hirase et al., 2020; Lemoine et al., 2020; Rundle et al., 2018; Takeo et al., 2018; Gulseren et al., 2017; Miyakawa et al., 2017; Liippo and Lammintausta, 2015; De Pasquale et al., 2015; Sanchez-Machin et al., 2016).

A total of 32 papers were screened by the FEEDAP Panel for their relevance in the assessment of the user safety for Carmine/carminic acid. Nineteen papers (Catli et al., 2015; De Pasquale et al., 2015; Gosetti et al., 2015; Liippo and Lammintausta, 2015; Miyakawa et al., 2016; Sanchez-Machin et al., 2016; Ferris et al., 2017; Gulseren et al., 2017; Ramesh and Lieberman, 2017; Machler and Jacob, 2018; Takeo et al., 2018; Fok, 2020; Hirase et al., 2020; Lemoine et al., 2020; Morin and Sasseville, 2020; Ozceker et al., 2020; Suzuki et al., 2021; Warshaw et al., 2021; Sadowska et al., 2022) were considered by the FEEDAP Panel as relevant and confirmed that the additive should be considered as an allergen through dermal exposure. Only one single paper (Yamashita et al., 2018) reported negative effects of carmine tested for its skin sensitisation potential using a modified lymph node assay with an elicitation phase. However, the FEEDAP Panel considered that the experimental design of this study had some limitations (e.g. one dose level only).

No information on skin and eye irritation potential of the additive have been provided, consequently, the FEEDAP Panel could not reach a conclusion for these endpoints.

3.2.3.1. Conclusions on the safety for the user

The FEEDAP Panel concluded that carmine is a dermal and a respiratory sensitiser. Due to the lack of data, the FEEDAP Panel could not conclude on potential skin and eye irritation of the additive.

3.3. Efficacy

Carmine (Carmine Lake WSP 50%) is currently authorised in the EU for its use in cats and dogs as a colourant additive.

[28] Technical dossier/Supplementary Information (November 2021)/Section III/Annex_12.
[29] Technical dossier/Supplementary Information (November 2021)/Section III/Annex_15.

Carmine is also authorised as a colouring agent in a broad range of foods (including processed cereals, raw and processed meat and fish). Considering that the foods in which it is authorised and the matrices of the feeds for cats and dogs are essentially the same (cereals, meat and fish), it is reasonable to expect that the effect seen in food will be observed in feed for cats and dogs when this additive is used at comparable concentrations and under similar conditions.

In addition, the applicant provided supporting visual evidence on the effect of the use of the additive in marketed compound feeds supplemented with Carmine at various levels (from 26 to 200 mg/kg) over time. However, the Panel noted that there is limited objective measurement of the changes in colour. The addition of Carmine resulted in a darker and redder colour in comparison to an untreated feed, as in the visual evidence provided.[30,31] The additive is considered to be efficacious in feeds for dogs and cats.

4. Conclusions

The additive is considered safe for dogs and cats at 264 and 220 mg Carmine/kg complete feed. These levels would correspond to 132 and 110 mg carminic acid/kg complete feed, for dogs and cats, respectively.

Due to the nature of the additive, the FEEDAP Panel concluded that allergic reactions may occur in the target species following the ingestion of feeds containing the additive. However, the FEEDAP Panel considered that these reactions are unpredictable and linked to the individual sensitivity.

The FEEDAP Panel concluded that Carmine is a dermal and a respiratory sensitiser. Due to the lack of data, the FEEDAP Panel could not conclude on potential skin and eye irritation of the additive.

The additive is considered to be efficacious in feeds for dogs and cats.

5. Documentation provided to EFSA/Chronology

Date	Event
05/11/2010	Reception mandate from the European Commission
16/10/2019	Dossier received by EFSA. Carmine for dogs and cats. Submitted by Mars Petcare GmbH
24/08/2020	Application validated by EFSA – Start of the scientific assessment
06/11/2020	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 target species/safety for the user/efficacy*
23/11/2020	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
25/11/2020	Comments received from Member States
05/11/2021	Reception of supplementary information from the applicant - Scientific assessment re-started
08/12/2021	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 target species/efficacy*
16/05/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
21/06/2022	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*
12/07/2022	Reception of supplementary information from the applicant - Scientific assessment re-started
27/09/2022	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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[30] Technical dossier/Supplementary Information (November 2021)/Section IV/Annex_10.
[31] Technical dossier/Supplementary Information (November 2021)/Section IV/Annex_11.

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Abbreviations

ADI	acceptable daily intake
ANS Panel	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
CFU	colony forming unit
CHL	Chinese hamster lung
CHO	Chinese hamster ovary
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
IR	infrared

JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOQ	limit of quantification
MW	molecular weight
NOAEL	no observed adverse effect level
SCF	Scientific Committee on Food
TG	Testing guidelines
UF	uncertainty factor
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 the Analysis for Carmine

In the current application an authorisation is sought under Article 10(2) for a carmine preparation, so-called Carmine lake WSP 50%, under the category/functional group/subgroup 2(a)(i) 'Sensory additives'/'colourants'/'substances that add or restore colour in feedingstuffs' according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the feed additive for dogs and cats.

The Applicant described Carmine lake WSP 50% as a preparation containing carmine (aluminium complex of carminic acid) as active substance. The feed additive is a dark red powder obtained from the aqueous extract of coccus cacti. Carmine lake WSP 50% is composed of a minimum of 50% (w/w) carmine and maltodextrin.

Carmine lake WSP 50% is intended to be incorporated directly in complementary feedingstuffs, with no recommended minimum or maximum levels. However, the Applicant suggested a typical inclusion level of 750 mg carmine/kg complementary feedingstuffs.

For the quantification of carmine in Carmine lake WSP 50% the Applicant submitted a spectrophotometric method based on the one for carmine products described in the "Carmine monograph" of the Food Chemical Codex (FCC).

Additionally, the Applicant provided experimental data for the analysis of carmine in several batches of Carmine lake WSP 50%.

Based on the information available the EURL recommends for official control the spectrophotometric method described in the "Carmine monograph" of the Food Chemical Codex (FCC) for the quantification of carmine in Carmine lake WSP 50%.

The Applicant did not provide experimental data or analytical methods for the determination of carmine in feedingstuffs. Therefore, the EURL cannot evaluate nor recommend any method for official control for the determination of carmine 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.