

Safety and efficacy of a feed additive consisting of ponceau 4R for its use in baits for freshwater fish (GIFAP)

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) |
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Abstract

Following a request from the European Commission, EFSA was asked to deliver a scientific opinion on the safety and efficacy of ponceau 4R as sensory additives (functional group: (a) colourants: (i) substances that add or restore colour in feed-ingstuffs). The additive is already authorised for use with cats and dogs and ornamental fish. The additive is intended to be incorporated in freshwater fishing baits up to a maximum of 15 mg/kg in complementary feed in order to colour them. The additive is intended to be incorporated into feed directly in solid form or via an aqueous solution. The additive is not intended for use in aquaculture. The FEEDAP Panel concluded that ponceau 4R is considered safe when used in fish baits for freshwater fish at the maximum proposed dose of 15 mg/kg complementary feed. The use of the feed additive ponceau 4R fish baits for freshwater fish under the conditions of use proposed is of no concern for consumer safety. Ponceau 4R is not skin irritant but should be considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. The FEEDAP Panel could not conclude on the eye irritation potential of the additive. In absence of data no conclusion can be reached on the efficacy of ponceau 4R in freshwater fish baits.

KEYWORDS

colourant, efficacy, freshwater fish, ponceau 4R, safety, sensory additive

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1 | INTRODUCTION

1.1 | Background and Terms of Reference

Regulation (EC) No 1831/2003¹ 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 feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from GIFAP² for the authorisation of the additive consisting of ponceau 4R, when used as a feed additive for freshwater fish (category: sensory additives; functional group: (a) colourants: (i) substances that add or restore colour in feedingstuffs).

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). The dossier was received on 7th November 2022 and the general information and supporting documentation are available at <https://open.efsa.europa.eu/questions/EFSA-Q-2022-00781>. The particulars and documents in support of the application were considered valid by EFSA as of 31st March 2023.

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 feed additive consisting of ponceau 4R, when used under the proposed conditions of use (see **Section 3.1.3**).

1.2 | Additional information

Ponceau 4R is authorised as a feed additive for cats, dogs and ornamental fish (2a124).³ The EFSA FEEDAP Panel issued an opinion on the safety of this additive when used in feed for cats and dogs, ornamental fish, grain-eating ornamental birds and small rodents (EFSA FEEDAP 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 dossier⁴ in support of the authorisation request for the use of ponceau 4R as a feed additive. The dossier was received on 7 November 2022 and the general information and supporting documentation is available at <https://open.efsa.europa.eu/questions/EFSA-Q-2022-00781>.

The confidential version of the technical dossier was subject to a target consultation of the interested Member States from 31 March to 30 June 2024 for which the received comments were considered for the assessment. In accordance with Article 38 of the Regulation (EC) No 178/2002⁵ and taking into account the protection of confidential information and of personal data in accordance with Articles 39 to 39e of the same Regulation, and of the Decision of EFSA's Executive Director laying down practical arrangements concerning transparency and confidentiality,⁶ a non-confidential version of the dossier has been published on Open.EFSA.

According to Article 32c(2) of Regulation (EC) No 178/2002 and to the Decision of EFSA's Executive Director laying down the practical arrangements on pre-submission phase and public consultations, EFSA carried out a public consultation on the non-confidential version of the technical dossier from 2 February to 23 February 2024 for which no comments were received.

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' (elicitation) 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 ponceau 4R in animal feed are valid and applicable for the current application.⁷

¹Regulation (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.

²GIFAP, BP 25 Saint-Laurent Médoc - France, France.

³Commission Implementing Regulation (EU) 2020/107 of 23 January 2020. OJ L 19, 24.1.2020, p. 18–21.

⁴Dossier reference: FEED-2022-7910.

⁵Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. OJ L 31, 1.2.2002, p. 1–48.

⁶Decision available at: <https://www.efsa.europa.eu/en/corporate-pubs/transparency-regulation-practical-arrangements>.

⁷Evaluation report received on 14/3/2019 and available on the EU Science Hub https://joint-research-centre.ec.europa.eu/eurl-fa-eurl-feed-additives/eurl-fa-authorisation/eurl-fa-evaluation-reports_en.

2.2 | Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of ponceau 4R is in line with the principles laid down in Regulation (EC) No 429/2008⁸ and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the consumer (EFSA FEEDAP Panel, 2017a), Guidance on the identity, characterisation and conditions of use of feed additives (EFSA FEEDAP Panel, 2017b), Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017c), Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2024), Guidance on the assessment of the safety of feed additives for the environment (EFSA FEEDAP Panel, 2019) and Guidance on the assessment of the safety of feed additives for the users (EFSA FEEDAP Panel, 2023).

3 | ASSESSMENT

Ponceau 4R is intended to be used as a sensory additive (functional group: (a) colourants: (i) substances that add or restore colour in feedingstuffs) in baits for freshwater fish. Ponceau 4R is intended to be incorporated in fishing baits in order to colour them and attract fish in freshwater (ponds, rivers), for both recreational and competitive fishing. The additive is not intended for use in aquaculture.

3.1 | Characterisation

3.1.1 | Characterisation of the additive

Ponceau 4R is a sulfonated mono azo dye and consists of trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)naphthalene-6,8-disulfonate (chemical formula $C_{20}H_{11}N_2O_{10}S_3Na_3$, CAS number 2611-82-7, molecular weight 604.48) and subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components. Ponceau 4R is described as the sodium salt.

Ponceau 4R is produced by chemical synthesis.⁹ It is prepared coupling diazotized naphthionic acid to G acid (2-naphthol-6,8-disulphonic acid) and converting the coupling product to the trisodium salt.

The applicant states that the specifications of ponceau 4R comply with those of the additive already authorised for use in dogs, cats and ornamental fish¹⁰: colouring matters calculated as the sodium salt $\geq 80\%$, subsidiary colouring matter $\leq 1\%$, organic compounds other than colouring matters $\leq 0.5\%$, $\leq 0.01\%$ unsulfonated primary aromatic amines (calculated as aniline). These specifications are also in line with those set for the use of ponceau 4R as a food additive.¹¹

Five batches of ponceau 4R were analysed for their specified components.¹² They all complied with the specifications as follow: average 87.9% colouring matter (range: 87.3–89.3), 0.4% subsidiary colouring matter (0.3–0.5), 0.30% organic compounds other than colouring matters (0.17–0.34) and $< 0.01\%$ unsulfonated primary aromatic amines.

The applicant set specifications for arsenic (< 3 mg/kg), lead (< 2 mg/kg), mercury and cadmium (< 1 mg/kg), which align with the established limits for ponceau 4R when used as a food additive. The levels analysed in the same five batches above were: 0.01 mg arsenic/kg (0.005–0.017), 0.006 mg mercury/kg (0.005–0.008), 0.32 mg lead/kg (0.126–0.767) and 0.42 mg cadmium/kg (0.371–0.564).

The additive is an odourless fine powder, freely soluble¹³ in water; a solubility test in line with OECD Test Guideline (TG)105 was submitted,¹⁴ resulting in 200–1000 g/L.

The dusting potential, as determined by the Stauber-Heubach method, was evaluated in two independent batches, yielding results of 2333 mg/m³ and 7033 mg/m³, respectively.¹⁵ For a third batch, the measurement was unfeasible due to the inability to retrieve all the dust from the filter.

⁸Commission 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.

⁹Technical dossier/Annex_11.

¹⁰Commission Implementing Regulation (EU) 2020/107 of 23 January 2020 concerning the authorisation of ponceau 4R as a feed additive for dogs, cats and ornamental fish. OJ L 19, 24.1.2020, p. 18–21.

¹¹Commission Regulation (EU) No 231/2012. OJ L 83, 22.3.2012, p. 1.

¹²Technical dossier/Annex_3, Annex_4, Annex_5, Annex_6, Annex_7.

¹³For solubility terms, see Table 2 of the Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles (EFSA Scientific Committee, 2021).

¹⁴Annex_42_dusting_potential_water_solubility CONFIDENTIAL.pdf.

¹⁵Annex_42_dusting_potential_water_solubility CONFIDENTIAL.pdf, Annex_47_dusting_potential CONFIDENTIAL.pdf, Annex_48_dusting_potential CONFIDENTIAL.pdf.

3.1.2 | Stability and homogeneity

The shelf life of the additive stored for 8 years at 15–45°C and 40%–90% relative humidity was evaluated in two batches for their colour content by measuring the extinction coefficient.¹⁶ After 8 years, average losses in pure dye content were 4.6%, moisture content increased by 4% and total dye content decreased by 1.1%.

No data were submitted on the stability of the additive when incorporated in feed. The applicant states that ponceau 4R is generally unstable in the presence of oxidising or reducing agents (e.g. sugars and acids).

3.1.3 | Conditions of use

The additive ponceau 4R is intended to be incorporated in fishing baits for freshwater fish up to a maximum content of 15 mg/kg in complementary feed. The applicant stated that it can be used either as small fish baits of approximately 4 g attached to the hooks or fishing lines or as small pellets (ground bait) which are cast in water to attract fish to the fishing area. It is not intended for use in aquaculture feed.¹⁷

3.2 | Safety

Ponceau 4R is intended to be used in the preparation of baits for freshwater fish only. According to the proposed conditions of use, it can be used either as small fish baits of ~4 g attached to the hooks or fishing lines or as small pellets (ground bait) which are cast in water to attract fish to the fishing area (Section 3.1.3). Therefore, the FEEDAP Panel considers that the safety assessment of this additive should consider this peculiar use, which would result in an occasional exposure of the target animals to ponceau 4R and not to a continuous exposure during their whole life, as it is normally the case with other feed additives.

The safety of ponceau 4R was previously evaluated by Joint FAO/WHO Expert Committee on Food Additives in 1983 and 2011 (JECFA, 1983, 2011), by the Scientific Committee on Food (SCF) (European Commission, 1984), by EFSA in 2009 when re-evaluating ponceau 4R as a food additive (EFSA, 2015; EFSA ANS Panel, 2009) and in 2018 as a feed additive for cats, dogs and ornamental fish (EFSA FEEDAP Panel, 2018).

No new studies were submitted in support of the safety of the additive, but the applicant performed a literature search on the safety of ponceau 4R for the target species, consumers and users, covering the period up to October 2023 and for the safety for the environment up to November 2022, to identify any relevant information that might be available since the previous evaluations. The literature search was conducted using Google Scholar, PubMed and EFSA publications databases. A total of 29 publications were retrieved (9 on the safety for the target species, 17 for the consumer, 1 for the users and 2 for the environment).

3.2.1 | Absorption, distribution, metabolism and excretion and residues

No data on absorption, distribution, metabolism, excretion (ADME) and residues of ponceau 4R in fish were made available to the FEEDAP Panel.

The ADME of ponceau 4R in laboratory animals have been previously reviewed by EFSA (EFSA, 2015; EFSA ANS Panel, 2009; EFSA FEEDAP Panel, 2018) and JECFA (JECFA, 2011) which considered that the absorption of ponceau 4R in humans and laboratory animals is typically low. Once absorbed, ponceau 4R is primarily excreted unchanged in the urine, while the remaining portion undergoes reductive metabolism by the intestinal microbiota. Small amounts of some metabolites (e.g. sulfanilic acid and 4-amino-3-carboxy-5-hydroxy-1-(4-sulfophenyl)pyrazole (SCAP)) and aniline derivatives are then absorbed by the intestine and excreted via urine (JECFA, 2011).

The applicant provided a literature search (see Section 3.2) to support that the anatomical organisation of the intestine, the gene expression regulated by gut microbes and the core gut microbiota assembly are similar in fish and mammals (Tyagi & Shukla, 2002; Rawls et al., 2004; Roeselers et al., 2011; Wu et al., 2012; Turnbaugh & Gordon, 2009; Turnbaugh et al., 2009, cited in Li et al., 2014). Although these findings might suggest a commonality of the enteric metabolism in fish and mammals, the FEEDAP Panel considered that the information provided by the applicant is of limited relevance for the characterisation of the ADME profile of ponceau 4R in fish.

No data on possible residues of ponceau 4R in fish was made available to the FEEDAP Panel. However, taking into account the conditions of use of ponceau 4R in freshwater fish (as described in Section 3.1.3), the Panel considers that the use of the additive for recreational or sportive fishing would result in a limited consumption of baits before the animals are caught. Therefore, considering the limited amount ingested by the animals over a short period of time, the deposition of ponceau 4R in fish flesh is considered unlikely.

¹⁶Technical dossier/Annex_13 and Annex_41.

¹⁷Technical dossier/Conditions_of_use.pdf.

3.2.2 | Toxicological studies

Based on the results from long-term carcinogenicity studies, reproductive toxicity studies and in vivo genotoxicity studies, described in the above-mentioned assessments (EFSA, 2009; EFSA FEEDAP Panel, 2018; JECFA, 2011), ponceau 4R was considered not carcinogenic nor genotoxic and not a reproductive/developmental toxicant. In 2009, the ANS Panel confirmed the existing acceptable daily intake (ADI) of 0.7 mg/kg bw, derived from a no observed adverse effect level (NOAEL) of 70 mg/kg bw per day based on the reduction in body weight observed in a chronic study in rats and applying an uncertainty factor (UF) of 100.

The literature search retrieved four papers which were not considered in the previous assessments. Three papers (Grigorenko et al., 2021; Mustafina et al., 2022; Yeroshenko et al., 2021), describe toxicological studies in rats reporting adverse effects on various organs after oral administration of a mixture of dyes, including ponceau 4R. The FEEDAP Panel considered these papers not relevant for the current assessment, since it was not possible to establish whether these effects were due to the single substance or to their potential additive or synergistic effects.

Another paper (De Souza et al., 2022) reported positive genotoxicity results obtained testing a ponceau 4R of unknown purity. However, the representativeness of the test item used in this study for the additive under assessment is not established and therefore, this paper was not further considered for the current assessment.

The paper by Sasaki et al. (2002), already considered in the previous ANS Panel opinion (EFSA ANS Panel, 2009), evaluated the acute oral toxicity of ponceau 4R in male mice. The median lethal dose (LD50) of ponceau 4R was found to be greater than 2000 mg/kg bw, with no observed mortality following single-dose administration across all dosage groups. The FEEDAP Panel considered the results of this study relevant for the assessment on the safety of the additive for the target species.

3.2.3 | Safety for the target species

Ponceau 4R is intended to be used only in the preparation of fishing baits for freshwater fish. The Panel considers that this use will likely result in an occasional exposure of the animals to the additive and not to a continuous exposure during the whole life. In addition, for those fish that are caught and killed after capture, the assessment of the safety of the additive is irrelevant. Therefore, the Panel considers that the assessment of the safety for the target species is relevant only for those fish that are either caught and released in the water, or those that eat the ground baits and are not caught. In both cases, it is not possible to make an accurate estimate of the actual consumption of the additive, which, as mentioned before, is going to be occasional, in a very short time and not continuous during long periods of the life of the animals. In addition, the number of baits ingested represents a small fraction of the total feed intake of fish during a given day.

The FEEDAP Panel considered appropriate to make an estimation of the potential exposure to ponceau 4R from its use in baits. In that regard, the applicant proposed as a worst-case scenario for the consumer safety assessment that a 300 g fish might eat 20 baits (total of 80 g). The FEEDAP Panel considers that this scenario can be appropriate for the assessment of the safety for the target animals. Under these conditions, considering the maximum use level of 15 mg ponceau 4R/kg complementary feed, a single fish would ingest approximately 1.2 mg ponceau 4R. Considering an average weight of the fish of 300 g, this would result in an exposure of 4 mg/kg bw.

The FEEDAP Panel also notes that ponceau 4R is already authorised in feed for ornamental fish at 137 mg/kg complete feed.

Considering that ponceau 4R is of low acute toxicity in mammalian species (LD50, mice), and the peculiarity of the conditions of use which would likely result in an occasional exposure, the FEEDAP Panel concludes that the use of ponceau 4R in the preparation of baits for freshwater fish under the proposed conditions of use (maximum use level of 15 mg/kg complementary feed) is of no concern for the target animals.

3.2.4 | Safety for the consumer

Considering that ponceau 4R is already authorised for use as food additive in several food categories and the conditions of use of the feed additive as proposed by the applicant, the FEEDAP Panel considers that the exposure to residues of ponceau 4R from fish for recreational or sportive fishing would not significantly contribute to the overall exposure to ponceau 4R via food for the consumers (EFSA, 2015; EFSA ANS Panel, 2009). Therefore, the FEEDAP Panel concludes that the use of ponceau 4R under the proposed conditions of use is safe for the consumers.

3.2.5 | Safety for the user

The highest dusting potential measured was 7033 mg/m³, indicating that exposure of users by inhalation is likely.¹⁸

¹⁸Technical dossier/Annex_42, Annex_47, Annex_48.

The skin irritation potential of the additive was tested in a study conducted according to OECD TG 439.¹⁹ The results indicated that the additive is non-irritant to the skin in accordance with UN GHS 'No Category'.

An eye irritation test according to OECD TG 405 was submitted by the applicant.²⁰ However, it is not considered relevant by the FEEDAP Panel as the test item used is considered not representative of the additive under assessment.

The applicant submitted some published papers (Safford & Goodwin, 1985; Kalender, 2000; Leung et al., 2019) reporting dermal sensitisation effects for azo dyes other than ponceau 4R (e.g. Patent Blue V, tartrazine). The FEEDAP Panel considered that these results can be extrapolated to the additive under assessment.

3.2.5.1 | *Conclusions on the safety for the users*

The additive is not skin irritant but should be considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. No conclusions can be reached on the eye irritation potential of the additive.

3.2.6 | Safety for the environment

Ponceau 4R is intended to be used only in fishing baits for freshwater fish, and the additive will not be used in aquaculture operation involving the use of cages. Therefore, surface water is considered the environmental compartment potentially at risk.

In line with the requirements of the FEEDAP Panel guidance on the safety of the additive for the environment (EFSA FEEDAP Panel, 2019) the applicant calculated the predicted environmental concentration for surface water (PEC_{sw}) using the FERA calculation tool,²¹ assuming that 100% of the ingested dose is excreted as the parent compound.

At the maximum concentration of ponceau 4R used in fishing baits (15 mg/kg complementary feed), the PEC_{sw} does not exceed the threshold value of 0.1 µg/L for aquaculture from land-based fish farms. Thus, the assessment stops in Phase I of the FEEDAP guidance on the safety of the additive for the environment (EFSA FEEDAP Panel, 2019). The FEEDAP Panel considers that the use of ponceau 4R, according to the proposed conditions of use, is not expected to pose a risk to the environment.

3.3 | Efficacy

Ponceau 4R is intended to be used to colour the fishing baits of freshwater fish. Ponceau 4R is authorised as a food additive. In principle, where the function requested for feed is the same as that used in food, no further demonstration of efficacy is necessary, provided that the effect seen when used in food could reasonably be expected to be seen when used in feed at the recommended concentration and that food and feed matrices are of comparable nature. However, considering the uncertainty in the equivalence of the food and feed matrices and the use levels, efficacy demonstration for this use as feed additive was considered necessary.

No data on efficacy were submitted by the applicant. Therefore, no conclusion on the efficacy of ponceau 4R when used in fish baits for freshwater fish can be reached.

4 | CONCLUSIONS

The FEEDAP Panel concludes that the use of ponceau 4R in the preparation of baits for freshwater fish under the proposed conditions of use (maximum use level of 15 mg/kg complementary feed) is of no concern for the target animals.

The use of ponceau 4R as a feed additive under the proposed conditions of use is considered safe for the consumer and the environment.

Regarding the user safety, the additive is not skin irritant but should be considered a skin and respiratory sensitiser. Inhalation and dermal exposure are considered a risk. The FEEDAP Panel could not conclude on the eye irritation potential of the additive.

In absence of data no conclusion can be reached on the efficacy of ponceau 4R in freshwater fish baits.

ABBREVIATIONS

ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BW	body weight
CAS	Chemical Abstracts Service
CD	Commission Decision
CV	coefficient of variation

¹⁹Technical dossier/Annex_62.

²⁰Technical dossier/Annex_63.

²¹FERA calculation tool <https://www.efsa.europa.eu/en/applications/feedadditives/tools>.

DM	dry matter
ECHA	European Chemicals Agency
EINECS	European Inventory of Existing Chemical Substances
EURL	European Union Reference Laboratory
FAO	Food Agricultural Organization
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FLAVIS	The EU Flavour Information System
FL-no	FLAVIS number
HACCP	hazard analysis and critical control points
IUPAC	International Union of Pure and Applied Chemistry
JECFA	The Joint FAO/WHO Expert Committee on Food Additives
LOD	limit of detection
LOQ	limit of quantification
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
RH	relative humidity
UF	uncertainty factor
WHO	World Health Organization

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European Commission

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