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## Safety and efficacy of ponceau 4R for cats, dogs and ornamental fish

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### Abstract

Following a request from the European Commission, the 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 ponceau 4R for cats, dogs and ornamental fish. The following ponceau 4R concentrations in complete feed were considered safe: 31 mg/kg for cats, 37 mg/kg for dogs and 137 mg/kg for ornamental fish. Inhalation exposure of ponceau 4R is regarded as hazardous. In the absence of data, the Panel cannot conclude on the irritancy potential of ponceau 4R to skin or eyes. No conclusion could be made on the skin sensitisation of ponceau 4R. Ponceau 4R is effective in adding colour to feedingstuffs.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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. 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 7 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 Sensient Colors UK Ltd (on behalf of Feed Additives Synthetic Colours Group)<sup>2</sup> for re-evaluation of the product ponceau 4R, when used as a feed additive for cats, dogs and ornamental 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) and 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 13 September 2012.<sup>3</sup> The applicant clarified that the additive is not intended to be added to water for drinking. This latter use therefore has not been assessed in this opinion.

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 ponceau 4R, when used under the proposed conditions of use (see Section 3.1.2).

### 1.2. Additional information

Ponceau 4R is authorised without a time limit under Council Directive 70/524/EEC<sup>4</sup> as colourant for cats, dogs and ornamental fish without maximum levels. Under the same regulation, it is authorised without a time limit for all species or categories of animals, with the exception of cats and dogs, in animal feedingstuffs only in products processed from: (i) waste products of foodstuffs, (ii) other base substances, with the exception of cereals and manioc flour, denaturated by means of these agents or coloured during technical preparation to ensure the necessary identification during manufacture.

Currently, ponceau 4R is authorised as a food additive in the European Union (EU) in accordance with Annex II and Annex III to Regulation (EC) No 1333/2008 on food additives and specific purity criteria have been defined in the Commission Regulation (EU) No 231/2012.<sup>5</sup> Maximum permitted use levels for Ponceau 4R are given for 26 food categories listed in Table 4 of Regulation (EC) No 1333/2008 (Annex II) (range 1–200 mg/kg).

Ponceau 4R has been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983 (JECFA, 1983) and the EU Scientific Committee for Food (SCF) in 1984 (European Commission, 1984). In 2009, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted an opinion on the re-evaluation of ponceau 4R as a food additive (EFSA ANS Panel, 2009).

<sup>1</sup> 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.

<sup>2</sup> Sensient Colors UK Ltd. Oldmedow Road, PE30 4LA, Kings Lynn, UK.

<sup>3</sup> A new mandate was received in EFSA on 17/2/2012.

<sup>4</sup> List of the authorised additives in feedingstuffs (1) published in application of Article 9t (b) of Council Directive 70/524/EEC concerning additives in feedingstuffs. OJ C 50, 25.2.2004, p.1.

<sup>5</sup> Commission Regulation (EU) No 231/2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 of the European Parliament and of the Council. OJ L 83, 22.3.2012, p.1.

## 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<sup>6</sup> in support of the authorisation request for the use of ponceau 4R as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008<sup>7</sup> and the applicable EFSA guidance documents.

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, 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 the ponceau 4R in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>8</sup>

### 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 429/2008 and the relevant guidance documents: 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, 2011a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c) and Guidance on the assessment of additives intended to be used in pets and other non food-producing animals (EFSA FEEDAP Panel, 2011b).

## 3. Assessment

The applicant requests for the re-evaluation of the use of ponceau 4R in feed for cats, dogs and ornamental fish.

### 3.1. Characterisation

The additive under application, ponceau 4R (E 124, Cochineal Red A, CI Food Red 7, New Coccine, New Coccine Food Red 102, Cochineal red) is identical to the active substance.

Ponceau 4R is manufactured by coupling diazotised naphthionic acid to G acid (2-naphthol-6,8-disulfonic acid) and converting the coupling product to the trisodium salt.

Ponceau 4R is a sulfonated mono azo dye comprising primarily trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)naphthalene-6,8-disulfonate (chemical formula  $C_{20}H_{11}N_2Na_3O_{10}S_3$ , CAS number 2611-82-7, molecular weight 604.48) and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

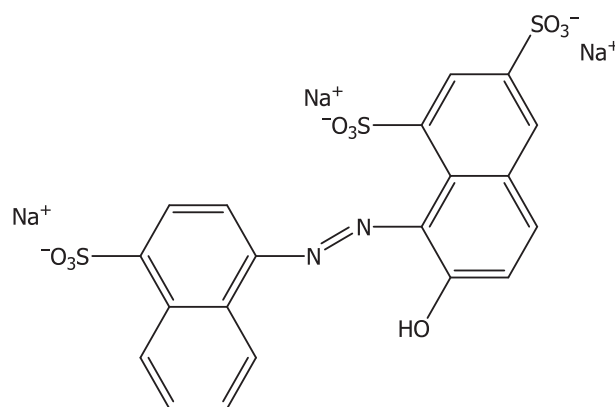
Ponceau 4R is described as the sodium salt. The calcium and the potassium salt are also permitted as food additives by Commission Regulation (EU) No 231/2012<sup>9</sup>. The structural formula of ponceau 4R is given in Figure 1. Ponceau 4R is a water-soluble reddish powder or granules.

<sup>6</sup> FEED dossier reference: FAD-2010-0349.

<sup>7</sup> 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.

<sup>8</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/FinRep\\_FAD-2010-0349\\_Ponceau4R.pdf](https://ec.europa.eu/jrc/sites/default/files/FinRep_FAD-2010-0349_Ponceau4R.pdf)

<sup>9</sup> The FEEDAP Panel notes that food legislation also permits the use of aluminium lakes (Commission Regulation (EU) No 231/2012) of this colour; however, the current application does not mention these forms of the colour. Consequently, aluminium lakes of ponceau 4R were not assessed.



**Figure 1:** Structural formula of ponceau 4R

The specifications for ponceau 4R when used as a feed additive are identical to those for ponceau 4R when used as a food additive and laid down in Commission Regulation (EU) No 231/2012<sup>10</sup>: total colouring matters calculated as the sodium salt > 80%, subsidiary colouring matter < 1%, organic compounds other than colouring matters<sup>11</sup> < 0.5%, unsulfonated primary aromatic amines (calculated as aniline) < 0.01%, arsenic < 3 mg/kg, lead < 2 mg/kg, and mercury and cadmium < 1 mg/kg each.

Five batches of ponceau 4R were analysed for the specified contents.<sup>12,13</sup> They all complied with the specifications; total colouring matter was 80.1–85.2%.<sup>12</sup>

The additive is produced in two different forms, a fine powder and a granular product. The particle size distribution was determined by laser diffraction analysis in three batches of ponceau 4R, with the following values (volume-based percentage): < 1 µm (0%, 0%, 1.0%), < 10 µm (7%, 7%, 30%), < 50 µm (39%, 46%, 74%).<sup>14</sup> Data on dusting potential were not provided.

### 3.1.1. Stability and homogeneity

No data on stability were submitted. The applicant reported a shelf-life of 4–6 years for ponceau 4R stored in a dry, cool and ventilated place based on its own experience from the use of the product in food, cosmetics, and other applications.

The applicant noted that the conditions of use for ponceau 4R in a range of foods are well established. Any substance which interacts or alters conjugated unsaturated bonds of the molecule will affect the colour. Ponceau 4R will generally be unstable in the presence of oxidising or reducing agents (e.g. sugars and acids).

Data on the capacity of the additive to homogeneously distribute in different feedingstuffs are not required for additives intended to add or restore colour to feedingstuffs. Consequently, no data have been submitted.

### 3.1.2. Conditions of use

Ponceau 4R is intended to be used in complete and complementary feed for dogs, cats and ornamental fish. No maximum content is proposed although the applicant noted that the quantity required is dependent on the properties of the feedingstuffs but, in general, is not likely to exceed 500 mg/kg complete feedingstuffs.

Upon request, the applicant provided examples from pet food manufacturers indicating that the use level recalculated to standardised complete feed with 12% moisture may reach approximately 400 mg/kg for cats, 600 mg/kg for dogs and 3,500 mg/kg for ornamental fish.<sup>15</sup>

The applicant stated that the incorporation of the additive into feed can be done directly in the solid form or via an aqueous solution.

<sup>10</sup> OJ L 83, 22.3.2012, p.1.

<sup>11</sup> 4-aminonaphthalene-1-sulfonic acid, 7-hydroxynaphthalene-1,3- disulfonic acid, 3-hydroxynaphthalene-2,7- disulfonic acid, 6-hydroxynaphthalene-2-sulfonic acid, 7-hydroxynaphthalene-1,3,6- trisulfonic acid.

<sup>12</sup> Technical dossier/Section II/Annex 1.

<sup>13</sup> Technical dossier/Supplementary information April 2013/Annex A1 and A2.

<sup>14</sup> Technical dossier/Section II/Annex 2.

<sup>15</sup> Technical dossier/Supplementary information April 2013.

## 3.2. Safety

Following the provisions of the Regulation (EC) No 429/2008, there is no requirement for the assessment of the safety of an additive when used in pets, for the consumers and the environment. This is the case for ponceau 4R.

### 3.2.1. Toxicological studies

Ponceau 4R has been evaluated previously by JECFA in 1983 (JECFA, 1983) and the EU SCF in 1983 (European Commission, 1984). It was also evaluated by TemaNord (2002). In 2009, the ANS Panel adopted an opinion on the re-evaluation of Ponceau 4R as a food additive (EFSA ANS Panel, 2009).

#### 3.2.1.1. General toxicology

The ANS Panel (EFSA ANS Panel, 2009) noted that in 1983 JECFA had reviewed two repeat-dose short-term toxicological studies, one rats, and one in pig (JECFA, 1983), which are briefly summarised below.

In a 90-day study, groups of 16 Carworth Farm Strain-E rats of each sex were treated with 0%, 0.5%, 1% or 2% ponceau 4R in their feed (equivalent to 0, 250, 500 and 1,000 mg/kg body weight (bw) per day, respectively). Slight (31–59%) but statistically significant ( $p < 0.05$ ) increases in aspartate aminotransferase (AST) (significant only in females and alanine aminotransferase (ALT) (significant in both sexes) values in serum, and significant decreases in liver weight (significant in both sexes) and blood haemoglobin concentration (significant only in females) were demonstrated at the highest dose level. No adverse effects were seen in appearance, behaviour, growth, food consumption, red blood cell counts, most organ weights, renal function or gross pathology and histopathology (Gaunt et al., 1967). The authors concluded that the no observed adverse effect level (NOAEL) in this study was 1% ponceau 4R in the diet, equivalent to 500 mg/kg bw per day.

In a 90-day study with Large White pigs (3/sex per group), ponceau 4R was fed at doses of 0, 100, 300 and 900 mg/kg bw per day. One female from the highest dose level died on day 23; the death was attributed to an enteric infection. There was a slight reduction (statistical significance not reported) in the mean values for erythrocyte count, haemoglobin and haematocrit of the males in the highest dose group at week 6, but not at week 13. No abnormalities were observed concerning growth, composition of urine and serum, organ weights or histopathology (Gaunt et al., 1969). The ANS Panel (EFSA ANS Panel, 2009) noted that the results of this study indicate a NOAEL of 300 mg/kg bw per day; that the observed effect was transient and affected only one sex; and that the study was performed with only a limited number of animals.

The ANS Panel noted that JECFA (JECFA, 1983) had also reviewed one long-term toxicity/carcinogenicity study in mice and seven studies in rats, all performed before OECD guidelines and Good Laboratory Practices were established. The results indicated an absence of carcinogenicity. No treatment-related adverse effects were reported in any of the reports of the studies in rats. JECFA (JECFA, 1983) and the SCF (European Commission, 1984) set an acceptable daily intake (ADI) of 0–4 mg/kg bw based on the results from a long-term study in mice which revealed increased incidence of foamy reticuloendothelial cells in the liver at the 1.25% level (equivalent to 1,790 mg/kg bw per day), and glomerulonephrosis at the 0.25% and 1.25% levels (equivalent to 375 and 1,790 mg/kg bw per day) (Mason et al., 1974). The JECFA evaluation indicated that the 'no-untoward-effect level' was 0.05% in the diet. In spite of this, JECFA concluded that the level causing no toxicological effect in mice was the 0.25% dietary level, calculated by JECFA to be equivalent to 375 mg/kg bw per day. This value was used as the NOAEL in their calculation of an ADI of 0–4 mg/kg bw. In 2009, the EFSA ANS Panel noted that the results of two additional studies, that had been available at the time JECFA and the SCF set the ADI, indicate NOAEL values lower than 400 mg/kg bw per day. These were: the pig study of Gaunt et al. (1969) reporting a NOAEL of 300 mg/kg bw per day, based on a slight reduction in the number of erythrocytes at 900 mg/kg bw per day; and the mouse study of Mason et al. (1974) for which the no observed effect level (NOEL), based on the findings of glomerulonephrosis at the 0.25 and 1.25% dietary levels, was 0.05%, equivalent to 70 mg/kg bw per day.

The ANS Panel concluded that overall these findings give reason for re-definition of the ADI of 4 mg/kg bw per day. Applying a safety factor of 100 to the lowest NOAEL of 70 mg/kg bw per day (based on increased prevalence of glomerulonephrosis at 375 mg/kg bw per day or greater) from the long-term mouse study, the ANS Panel derived an ADI of 0.7 mg/kg bw (EFSA ANS Panel, 2009).

For reproduction/developmental toxicity, the ANS Panel made reference to the studies already reviewed by JECFA (1983): (i) a three-generation reproduction study in rats showing no adverse reproductive or developmental effects at any dose tested (up to a dose of 1,250 mg/kg bw per day), (ii) three teratogenicity studies with NOAELs of 100, 500 and 4,000 mg/kg bw per day corresponding to the highest dose tested in each study) and (iii) one long-term study which considers also some reproductive and developmental parameters with a NOAEL of 500 mg/kg bw per day.

In a more recent extended one-generation reproduction study (Tanaka, 2006, as described in EFSA ANS Panel, 2009), the authors concluded that the dose levels of ponceau 4R tested (dietary concentrations up to 0.48%, equal to 819 mg/kg bw per day) produced no adverse effects on reproduction, and a few adverse effects on neurobehavioural parameters in mice. The authors concluded that the NOAEL was presumed to be 0.12% in the diet (approximately 205 mg/kg bw per day) for maze learning by males in the F1 generation. The ANS Panel noted that 'these neurobehavioural findings were not consistent among the sexes and were especially observed because of reduced values in the control group'.

The ANS Panel made reference to the conclusions of the EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) regarding the finding of behavioural effects in children exposed to mixtures of food additives that included ponceau 4R (McCann et al., 2007). The AFC Panel noted the weakness of the findings and considered that they could not be used as the basis for setting an ADI for any of the individual food additives in the mixtures. The ANS Panel considered other reports of adverse effects in exposed humans and studies of hypersensitivity and intolerance in laboratory animals, and concluded that while some sensitivity reactions after ponceau 4R intake have been reported, mostly when ponceau 4R is taken within mixtures of other synthetic colours, no conclusion on the induction of sensitivity by ponceau 4R could be drawn from the limited scientific evidence available. The ANS Panel noted that sensitive individuals may react at dose levels within the ADI.

### 3.2.1.2. Genotoxicity of ponceau 4R

The genotoxicity of ponceau 4R was first assessed in 2009 by EFSA ANS Panel (2009) and more recently in EFSA ANS Panel statement on allura red and other sulfonated mono azo dyes, authorised as food and feed additives, including ponceau 4R (EFSA ANS Panel, 2013). In this latter statement, it was concluded that further investigation on the *in vivo* genotoxicity of the sulfonated mono azo dyes, including ponceau 4R, using an internationally validated experimental protocol for comet assay is recommended.

The applicant has subsequently submitted a new *in vivo* genotoxicity study that is assessed below.

#### ***In vivo* mammalian comet assay**

Ponceau 4R was evaluated in the comet assay for its genotoxic potential to induce DNA damage in liver, kidney, colon, urinary bladder and stomach cells of male mice, in compliance with OECD guideline 489.<sup>16</sup> Deionised water was selected as the vehicle. Test and control article formulations were administered at a dose volume of 10 mL/kg by oral gavage. Six animals per dose level were administered orally with test article doses of 25, 500, and 2,000 mg/kg body weight per day, for two consecutive days. On the second day, the positive control group for urinary bladder (three animals) was dosed by intraperitoneal route with 20 mg methyl methanesulfonate (MMS)/kg bw, while the positive control group for the other target organs (three animals) received orally 40 mg MMS/kg bw, approximately 3–4 h before euthanasia.

The administration of ponceau 4R did not cause a significant increase in DNA damage in liver, kidney, colon, stomach and bladder relative to the corresponding vehicle control. In bladder, variations between animals were observed in all dose groups, however, the summary data appears to be negative. The positive control performed as expected in all the analysed organs but in bladder, where no statistically significant DNA damage was observed, therefore the criteria for a valid assay were not met for this organ.

The FEEDAP Panel considered that the *in vivo* genotoxicity of ponceau 4R for bladder can be excluded on the basis of the overall available data set, taking into account the following: (i) in the long-term toxicity studies in rats and in mice mentioned in the ANS Panel re-evaluation of ponceau 4R (see Section 3.2.1.1), no carcinogenicity nor induction of preneoplastic lesions was reported in any tissue, including bladder; any genotoxic effect on bladder would be expected to produce observable

<sup>16</sup> Technical dossier/Supplementary information November 2016.



effects in the long-term toxicity studies, and (ii) the *in vivo* genotoxicity in other tissues can be excluded by the result of the comet assay, that are reliably negative in all the other tissues.

Bone marrow samples from the comet assay described above were analysed for the induction of micronuclei. No statistically significant increase in the incidence of micronucleated polychromatic erythrocytes in the test article-treated groups was observed relative to the vehicle control group. However, no evidence of target cell exposure (alteration of polychromatic erythrocytes/total erythrocyte ratio) was provided; therefore, this observation adds no information relevant to risk assessment.

### 3.2.1.3. Conclusions on toxicity

Ponceau 4R was negative for the induction of DNA damage in liver, kidney, colon and stomach in an *in vivo* comet assay. Although in this test no evaluation could be made in the urinary bladder, since the positive control substance did not produce the expected effect in this organ, on the basis of the overall available data set, the *in vivo* genotoxicity of ponceau 4R in bladder can be excluded. The FEEDAP Panel concludes that there is no concern for the *in vivo* genotoxicity of ponceau 4R.

Toxicological studies in laboratory animals showed no alerts for particular adverse effects that need to be taken into consideration when assessing target species safety. Ponceau 4R was not carcinogenic and did not cause reproduction toxicity. The FEEDAP Panel notes the ADI of 0.7 mg/kg bw that was set by the ANS Panel, and notes the comment of the ANS Panel that some sensitive individuals may have adverse reactions to oral doses of ponceau 4R within the range of this ADI.

### 3.2.2. Safety for the target species

The applicant submitted a report on a trial conducted to determine the toxicity of 10 colourants, including ponceau 4R, for three species of ornamental fish, namely firemouth cichlid (*Thorichthys meeki*), ornate tetra (*Hyphessobrycon bentosi*) and red barb (*Puntius conchonius*).<sup>17</sup> This study included groups (two aquaria each with 30–40 fish of each species) that were provided feed containing 0 (control) or 4,000 mg ponceau 4R/kg feed for a period of 84 days. Mortality, weight gain and feed intake were recorded every 3 weeks, feed to gain ratio was calculated correspondingly. The rate of mortality for each of the three species fed the ponceau 4R-containing diet was within normally accepted limits: 2%, 3% and 1% for firemouth cichlid, ornate tetra and red barb, respectively (mortality rates in control group were 0%, 11% and 0%, respectively). Body weight gain of the firemouth cichlid fed the diet containing ponceau 4R was decreased by 4% in comparison to the control group, that of ornate tetra and red barb fish fed the diet containing ponceau 4R were increased by 10% and 3 %, respectively, in comparison to the control group. Slight differences were observed in the feed to gain ratio for the different species fed the ponceau 4R supplemented diets but all were considered to fall within normal experimental variation. In the absence of haematology and clinical chemistry in ornamental fish, this study is of limited value.

Since no specific data on tolerance of cats and dogs were available, and considering the limitations of the studies in fish, the FEEDAP Panel applied the procedure described in the guidance for additives already authorised for use in food (EFSA FEEDAP Panel, 2012b) to derive safe feed concentrations these species/categories (Table 1). The NOAEL used in the calculation was 70 mg/kg bw per day (based on increased prevalence of glomerulonephrosis at 375 mg/kg bw per day or greater from a long-term mouse study) and an uncertainty factor of 100 was applied.

**Table 1:** Calculated maximum safe dietary levels of ponceau 4R in complete feeds for cats, dogs and ornamental fish

	Body weight (kg)	Feed intake (g dry matter/day)	Safe intake (mg/day)	Maximum safe dietary level (mg/kg complete feed) <sup>(a)</sup>
Cat	3	60	2.1	31
Dog	15	250	10.5	37
Ornamental fish	0.012	0.055	0.0084	137

(a): Complete feed containing 88% DM.

<sup>17</sup> Technical dossier/Supplementary information January 2013/Annex 3.

Ponceau 4R is considered safe for the target species at the following concentrations in complete feed: 31 mg/kg for cats, 37 mg/kg for dogs and 137 mg/kg for ornamental fish.

### 3.2.3. Safety for the user

#### 3.2.3.1. Effects on the respiratory system

Particle size distribution analysis of three batches of ponceau 4R showed a large proportion of particles of respirable size (up to 30% (v/v)  $\leq 10 \mu\text{m}$ ). In the absence of information on dusting potential, users are considered at risk of inhalation exposure to dust from the additive. In the absence of information on the inhalation toxicity of ponceau 4R, such exposure is regarded as hazardous.

#### 3.2.3.2. Effects on the eyes and skin

No information was provided on the irritancy of ponceau 4R to skin or eyes.

In a guinea-pig skin sensitisation test using the protocol of Landsteiner and Jacobs (1935), ponceau 4R was found not to be a skin sensitiser (Bär and Griepentrog, 1960). The study was conducted prior to the development of current protocols.

The ANS Panel (EFSA ANS Panel, 2009) and Mancuso et al. (1990) assessed the hypersensitivity of ponceau 4R, including skin sensitisation following oral/dermal challenge (Mikkelsen et al., 1978; Lindemayer and Schmidt, 1979; Weber et al., 1979; Rapaport, 1980; Ibero et al., 1982; Veien and Krogdhal, 1991; Fuglsang et al., 1993, 1994). There is no convincing evidence that exposure to ponceau 4R by oral or cutaneous routes causes sensitisation in humans.

A review article concluded that well-controlled studies failed to confirm additives in food as a cause of chronic idiopathic urticaria/angioedema, but tartrazine and other dyes including ponceau 4R may occasionally aggravate the pre-existing condition (Simon, 2003).

#### 3.2.3.3. Conclusions on safety for the user

Inhalation exposure of ponceau 4R is regarded as hazardous. In the absence of data, the Panel cannot conclude on the irritancy potential of ponceau 4R to skin or eyes.

There is no convincing evidence that exposure to ponceau 4R by oral or cutaneous routes causes sensitisation in humans, although it can aggravate pre-existing allergic conditions in some individuals.

### 3.3. Efficacy

Ponceau 4R is intended to be used to colour the food for cats, dogs and ornamental fish. It is an authorised colourant for use in food. Where the function requested for feed is the same as that used in food, no further demonstration of efficacy might be necessary (Regulation (EC) No 429/2008).<sup>18</sup> However, considering the wide variety of feedingstuffs used in complete and complementary feed for cats, dogs and ornamental fish and the uncertainty of which concentration of Ponceau 4R would result in a visible effect, the FEEDAP Panel normally requires an effect demonstration. The applicant provided one study in which graded amounts of Ponceau 4R were supplemented to a feed (biscuits) for pets.<sup>19</sup>

Samples of standard biscuits were prepared containing wholemeal flour, milk powder and vegetable oil. Ponceau 4R was added at 0, 50 and 500 mg/kg biscuit. Colour of the samples was measured by reflectance spectrophotometry.<sup>20</sup> By the addition of ponceau 4R the 'a' value increased from 7.4 (blank sample) to 19.7 (50 mg ponceau 4R) and 37.6 (500 mg ponceau 4R). The 'L' value decreased accordingly from 61.0 to 52.3 and 40.9, respectively, and the 'b' value decreased from 22.4 to 22.3 and 19.0, respectively.

The data demonstrated that ponceau 4R is effective in colouring a typical feed for pets at a dose of 50 mg/kg.

## 4. Conclusions

Ponceau 4R is considered safe at a concentration in complete feed of 31 mg/kg for cats, 37 mg/kg for dogs and 137 mg/kg for ornamental fish.

<sup>18</sup> OJ L 133, 22.5.2008, p.1.

<sup>19</sup> Technical dossier/Supplementary information April 2013/Annex A-2.

<sup>20</sup> Colour quantification standardised by the Commission International de l'Eclairage. L (lightness, black to white reflectance, 1-100), a (red=positive, green=negative), b (yellow = positive, blue = negative).

Inhalation exposure of ponceau 4R is regarded as hazardous. In the absence of data, the Panel cannot conclude on the irritancy potential of ponceau 4R to skin or eyes. No conclusion could be made on the skin sensitisation of ponceau 4R.

Ponceau 4R is effective in adding colour to feedingstuffs.

## Documentation provided to EFSA

- 1) Ponceau 4R. November 2010. Sensient Colors UK Ltd.
- 2) Ponceau 4R. Supplementary information (spontaneous). January 2013. Submitted by Sensient Colors UK Ltd.
- 3) Ponceau 4R. Supplementary information. April 2013. Submitted by Sensient Colors UK Ltd.
- 4) Ponceau 4R. Supplementary information. February 2015. Submitted by Sensient Colors UK Ltd.
- 5) Ponceau 4R. Supplementary information. November 2016. Submitted by Sensient Colors UK Ltd.
- 6) Ponceau 4R. Supplementary information. May 2017. Submitted by Sensient Colors UK Ltd.
- 7) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for ponceau 4R.
- 8) Comments from Member States.

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## Abbreviations

ADI	acceptable daily intake
AFC	EFSA Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ALT	alanine aminotransferase
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
AST	aspartate aminotransferase
bw	body weight
CAS	Chemical Abstracts Service
DM	dry matter
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MMS	methyl methanesulfonate
NOAEL	no observed adverse effect level
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
Rf	retention factor
SCF	Scientific Committee for Food
TLC	thin layer chromatography
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 ponceau 4R**

In the current application authorisation is sought under articles 4(1) and 10(2) for Ponceau 4R under the category/functional group 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. Authorisation is sought for the use of the feed additive for ornamental fish, cats and dogs.

Ponceau 4R is a synthetic reddish powder or granules, consisting of a minimum of 80 % of total colouring matters content calculated as sodium salt. The Applicant states that the purity criteria set in the Commission Directive 2008/128/EC for the food additive apply to the requirement for the feed additive.

Ponceau 4R is intended to be incorporated directly in feedingstuffs as a solution in water (either added directly as a solid to the feedingstuffs in the presence of water or by addition of an aqueous solution), with no recommended minimum or maximum levels. However, a typical maximum concentration of 500 mg/kg feedingstuffs is suggested by the Applicant.

For the determination of total colouring matters content of Ponceau 4R in the feed additive, the Applicant proposed the internationally recognised FAO JECFA monographs for food additives (recommended by Commission Directive 2008/128/EC) where identification of Ponceau 4R is based on (i) spectrophotometry at 505 nm in aqueous solution and (ii) Thin Layer Chromatography (TLC) with Retention factors (R<sub>f</sub>) determined using several chromatographic conditions for confirmation, while quantification of total colouring matters content of Ponceau 4R is based on i) spectrophotometry at 505 nm in aqueous solution and ii) titration with Titanous chloride. Even though no performance characteristics are provided, the EURL recommends for official control the above mentioned methods recommended by Commission Directive 2008/128/EC and described in the FAO JECFA monographs for the determination of Ponceau 4R in the feed additive.

The Applicant did not provide any experimental method or data for the determination of Ponceau 4R in premixtures, feedingstuffs and water. Therefore the EURL cannot evaluate nor recommend any method for official control to determine Ponceau 4R in premixtures, feedingstuffs and water.

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) is not considered necessary.