

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of indigo carmine (E 132) for cats and dogs and ornamental fish¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{2,3}

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ABSTRACT

Indigo carmine (E 132), an authorised food colourant, is intended to be used as a feed additive for dogs, cats and ornamental fish without a maximum content. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) Panel concludes that indigo carmine (purity of at least 93 % colouring matter) is safe for cats and dogs at levels up to 250 mg/kg complete feed and for ornamental fish up to 1 000 mg/kg complete feed. Users may be at risk of inhalation exposure to dust from indigo carmine. In the absence of information on the inhalation toxicity, such exposure is regarded as hazardous. Indigo carmine is not an irritant to skin and eyes. Indigo carmine should be considered as a potential skin and respiratory sensitiser in humans. Indigo carmine is effective in adding colour to feedingstuffs for dogs, cats and ornamental fish.

KEY WORDS

indigo carmine, sensory additive, colourant, cats, dogs, ornamental fish, safety

¹ On request from the European Commission, Question No EFSA-Q-2010-01533, adopted on 28 April 2015.

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⁴ This scientific opinion has been amended following the adoption of the decision of the European Commission on confidentiality claims submitted by the applicant, in accordance with Article 8(6) and Article 18 of Regulation (EC) No 1831/2003. The modified sections are indicated in the text.

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver an opinion on the safety and efficacy of indigo carmine in feed for cats and dogs and ornamental fish.

Indigo carmine (E 132), an authorised food colourant, is intended to be used as a feed additive for dogs, cats and ornamental fish without a maximum content.

The FEEDAP Panel concludes that indigo carmine (purity of at least 93 % colouring matter) is safe for cats and dogs at levels up to 250 mg/kg complete feed and for ornamental fish up to 1 000 mg/kg complete feed.

Users may be at risk of inhalation exposure to dust from indigo carmine. In the absence of information on the inhalation toxicity, such exposure is regarded as hazardous. Indigo carmine is not an irritant to skin and eyes. Indigo carmine should be considered as a potential skin and respiratory sensitiser in humans.

Indigo carmine is effective in adding colour to feedingstuffs for dogs, cats and ornamental fish.

It is recommended to increase the specified minimum purity of indigo carmine from 85 % to 93 % total colouring matter to ensure that the safety assessment is applicable.

It is noted that routine control should be possible if maximum contents of an additive in feed are proposed for safety reasons. However, no such analytical method is available for indigo carmine.

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BACKGROUND

Regulation (EC) No 1831/2003⁵ 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/EEC⁶ 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 time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Sensient Colors UK Ltd (on behalf of Feed Additives Synthetic Colours Group)⁷ for re-evaluation of the product indigo carmine (E 132), when used as a feed additive for cats, dogs and ornamental fish (category: 2. sensory additive; functional group: (a) colourants/substances that add or restore colour in feedingstuff) under the conditions mentioned in Table 1.

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).⁸ EFSA received directly from the applicant the technical dossier in support of this application.⁹ According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 1 July 2011.

Indigo carmine (Indigotine) (E 132) is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. It is currently authorised for its use in cats and dogs as colourant additive (colouring agents authorised for colouring foodstuffs by Community rules). The additive is also authorised for all species or categories of animals with the exception of cats and dogs for 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. It is also authorised for its use in ornamental fish as colourant additive. No maximum levels of indigo carmine (E 132) in feeds are established in the EU.

Indigo carmine (E 132) is an approved food colourant in the EU and it is listed in Annex I of Directive 94/36/EC¹⁰ of 30 June 1994 with maximum permitted levels ranging from 50 to 500 mg/kg in food.¹¹

⁵ 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.

⁶ Council Directive of 23 November 1970 concerning additives in feedingstuffs. OJ L 270, 14.12.1970, p. 1.

⁷ Sensient Colors UK Ltd. Old Meadow Road, Kings Lynn, PE30 4LA, UK.

⁸ Although in the forwarding sheet for this application the European Commission included a new use (Article 4) for use in water, the original request of the applicant was restricted to use in feed. Therefore, the current assessment is restricted to the use of indigo carmine in feed.

⁹ EFSA Dossier reference: FAD-2010-0346.

¹⁰ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.09.1994, p. 13.

¹¹ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

The specific purity criteria concerning the use of indigo carmine (E 132) in foodstuffs are included in Commission Regulation (EU) No 231/2012.¹²

Indigo carmine (E 132) has previously been evaluated by the Scientific Committee on Food (SCF) (EC, 1984) and the Joint Food and Agricultural Organisation (FAO) and World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (JECFA, 1975). In 2000, the Nordic Working Group on Food Toxicology and Risk Assessment reviewed the current status and safety data on all food additives permitted in the EU (TemaNord, 2002), including indigo carmine. In 2014 EFSA Panel on food additives and nutrient sources added to food (ANS) adopted an opinion on the re-evaluation of indigo carmine (E 132) as food additive (ANS EFSA Panel, 2014).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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, user, environment and the efficacy of the product indigo carmine (E 132), when used under the conditions described in Table 1.

¹² 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.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Indigo Carmine
Registration number/EC No/No (if appropriate)	E132
Category(-ies) of additive	2 (Sensory)
Functional group(s) of additive	A (Colourants)

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulfonate; Dark-blue powder or granules	$C_{16}H_8N_2Na_2O_8S_2$	Colour (Blue); Spectrometry (610 nm); Assay (Minimum 85 % total colouring matters calculated as sodium salt)	Annex II Methods of Analysis Relating to the Criteria of Purity of Food Additives Commission Directive 81/712/EEC

Trade name (if appropriate)	Indigo Carmine
Name of the holder of authorisation (if appropriate)	Not relevant

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
Cats and dogs; Ornamental fish	Not relevant	Not relevant	Not relevant	Not relevant

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Not relevant
Specific conditions or restrictions for handling (if appropriate)	Not relevant
Post-market monitoring (if appropriate)	Not relevant
Specific conditions for use in complementary feedingstuffs (if appropriate)	Not relevant

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
Not relevant	Not relevant	Not relevant	Not relevant

ASSESSMENT

1 Introduction

The current application is for re-evaluation of the use of indigo carmine (E 132) as a feed additive to add or restore colour in feedingstuffs for cats, dogs and ornamental fish.

Indigo carmine is included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. It is currently authorised for its use in cats, dogs and ornamental fish as a colourant additive. The additive is also authorised for animal feedingstuffs for all species or categories of animals, with the exception of cats and dogs, for products processed from (i) waste products of foodstuffs and (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. No maximum levels of indigo carmine in feeds are established in the European Union (EU).

Indigo carmine is an approved food colourant with maximum permitted levels ranging from 50 to 500 mg/kg.^{13,14} The specific purity criteria concerning the use of indigo carmine in foodstuffs are included in Commission Regulation (EU) No 231/2012.¹⁵

Indigo carmine has previously been evaluated by the Scientific Committee on Food (SCF) (EC, 1984) and the Joint Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) Expert Committee on Food Additives (JECFA) (JECFA, 1969, 1975). In 2000, the Nordic Working Group on Food Toxicology and Risk Assessment (NTT) reviewed the current status and safety data on all food additives permitted in the EU, including indigo carmine (TemaNord, 2002). In 2014, the EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) adopted an opinion on the re-evaluation of indigo carmine (E 132) as food additive (EFSA ANS Panel, 2014).

2 Characterisation

2.1 Characterisation of additive

The additive under application, indigo carmine (indigotine, CI Food Blue, FD&C Blue No 2), is identical to the active substance.

Indigo carmine is prepared by the sulphonation of indigo ((1,3-dihydro-3-oxo-5-sulpho-2H-indol-2-ylidene)-2,3-dihydro-3-oxo-1H-indole) with sulphuric acid or oleum (fuming sulphuric acid). The indigo (or indigo paste) used is manufactured by the fusion of *N*-phenylglycine (prepared from aniline and formaldehyde) in a molten mixture of sodamide and sodium and potassium hydroxides under ammonia pressure. The indigo is isolated and subjected to purification procedures prior to sulphonation. Material safety data sheets of the raw materials used in the synthesis are provided in the dossier.

Indigo carmine consists essentially of a mixture of disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulphonate and disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,7'-disulphonate (chemical formula $C_{16}H_8N_2Na_2O_8S_2$; Chemical Abstracts Service (CAS) number 860-22-0; European Inventory of Existing Commercial chemical Substances (EINECS): 212-728-8; molecular weight 466.36) and

¹³ European Parliament and Council Directive 94/36/EC of 30 June 1994 on colour for use in foodstuffs. OJ L 237, 10.09.1994, p. 13.

¹⁴ Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives. OJ L 354, 31.12.2008, p. 16.

¹⁵ Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annex 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.

subsidiary colouring matters together with sodium chloride and/or sodium sulphate as the principal uncoloured components.

Indigo carmine is described as the sodium salt; however, the calcium and potassium salts are also permitted as food additives Commission Regulation (EU) No 231/2012.¹⁶ 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 indigo carmine were not assessed.

The structural formula of indigo carmine is given in Figure 1. Indigo carmine is water soluble (1.6 % at 20 °C). It is produced as a fine powder of dark-blue colour or as a granular product (mainly for liquid application).

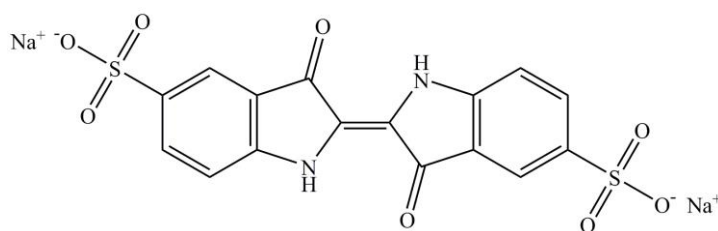


Figure 1: Structural formula of disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,5'-disulphonate

The specifications for indigo carmine when used as a feed additive are identical to those for indigo carmine when used as a food additive and laid down in Commission Regulation (EU) No 231/2012¹⁷ (total colouring matter > 85 %, disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,7'-disulphonate < 18 %, subsidiary colouring matter < 1 %, organic compounds other than colouring matters (isatin-5-sulphonic acid, 5-sulphoanthranilic acid and anthranilic acid) < 0.5 %, unsulphonated primary aromatic amines < 0.01 %) except those for lead (10 mg/kg instead of 2 mg/kg), which follows the previous food legislation (Regulation (EC) 128/2008).¹⁸

Five batches of indigo carmine were analysed for the specified contents. They all complied with the specifications (total colouring matter 88.4–90.1 %;¹⁹ disodium 3,3'-dioxo-2,2'-bi-indolylidene-5,7'-disulphonate 12.4–12.8 %;²⁰ subsidiary colouring matter 0.06–0.21 %; sum of organic compounds other than colouring matters < 0.5 %, and unsulphonated primary aromatic amines < 0.01 %). Heavy metals (lead, cadmium, mercury) and arsenic also met the specifications.²¹

The particle size distribution of samples from three producers was determined by laser diffraction/scattering analysis.²² For the first manufacturer (five batches) the volume-based percentage of particles < 10 µm diameter was between 0 and 14.7 %, that of particles < 50 µm diameter between 10.0 and 66.4 % and that of particles < 100 µm diameter between 38.5 and 83.7 %. For the second manufacturer (one batch) the volume-based percentage of particles < 10 µm diameter was 11.4 %, that of particles < 50 µm diameter 54.5 % and that of particles < 100 µm diameter 75 %. For the third manufacturer (one batch) the volume-based percentage of particles < 10 µm diameter was 100 % and that of particles < 1 µm diameter 22.3 %. Data on dusting potential were not provided.

¹⁶ OJ L 83, 22.3.2012, p. 1.

¹⁷ OJ L 83, 22.3.2012, p. 1.

¹⁸ Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs. OJ L 6, 10.1.2009, p. 20.

¹⁹ Technical dossier/Section II/Annex 1.

²⁰ Technical dossier/Supplementary information February 2015.

²¹ Technical dossier/Section II/Annex 1.

²² Technical dossier/Supplementary information September 2011/Annex 1.

2.2 Stability and homogeneity

The applicant reported a shelf-life of four to six years for indigo carmine 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. However, no supporting evidence was provided. No data on the stability or on the potential of the additive to homogeneously distribute in different feedingstuffs of the additive when incorporated to feed were submitted.

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

2.3 Conditions of use

The additive is to be used in feedingstuffs for cats, dogs and ornamental fish, to add or restore colour. No maximum or minimum contents are specified; however, the applicant provided typical inclusion levels of 10 to 200 mg/kg feedingstuffs for cats and dogs, 1 000 to 5 000 mg/kg in tropical fish flakes and 500 to 2 500 mg/kg in extruded tropical fish food and pond fish food.²³

The applicant stated that the additive is intended for addition to feed only. The additive can be incorporated directly to feed as solid substance or dissolved in water.

2.4 Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of the active substance in the feed additive. The Executive Summary of the EURL report can be found in the Annex.

The FEEDAP Panel notes that no method for the determination of indigo carmine in premixtures or feedingstuffs was provided by the applicant and could therefore not be recommended for official control by the EURL.

3 Safety

In 1975, JECFA established an Acceptable Daily Intake (ADI) of 5 mg/kg body weight (bw) for indigo carmine, based on a no observed adverse effect level (NOAEL) of 1 % (inhibition of growth of male rats) in the diet for two years in rats (approximately 500 mg/kg bw per day) and an uncertainty factor of 100. The same value was then retained in 1983 by SCF and in 2002 by NTT.

In 2014, the ANS Panel evaluated the safety of indigo carmine (EFSA ANS Panel, 2014). Based on the available genotoxicity data, including a newly performed *in vivo* study on micronuclei induction (also available for the current assessment),²⁴ the ANS Panel concluded that indigo carmine does not give rise to concerns for genotoxicity (EFSA ANS Panel, 2014). No adverse effects in sub-acute, chronic, reproduction and developmental toxicity studies, and no modifications of haematological and biological parameters in chronic toxicity studies were identified at doses less than or equal to 500 mg/kg bw per day.

In this assessment, the ANS Panel took account of the results of a study of indigo carmine of unknown purity, which showed adverse effects on the testes (including thickening of the tubular basement membrane and arrest spermatogenesis) of mice at oral doses of 17 mg/kg bw per day or greater but did not identify a NOAEL (Dixit and Goyal, 2013). It was not clear to the ANS Panel whether the adverse effects observed were due to the food additive itself or to impurities and/or contaminants present in the

²³ Technical dossier/Supplementary information September 2011.

²⁴ Technical dossier/Supplementary information January 2014.

material tested and/or to the conduct of the study. It was noted that other studies showed no histopathological effects on the testes of mice when doses of up to 8 259 mg/kg bw per day of indigo carmine (purity of 93 % colouring matter and 7 % volatile matter) were given orally for up to 23 months (Hooson et al., 1975; Borzelleca and Hogan, 1985). Further assurance was given by the absence of adverse effects on reproduction of in rats given oral doses of up to 250 mg/kg bw per day over three generations (Borzelleca et al., 1987). Thus, the adverse effects on the testes reported by Dixit and Goyal (2013) were not evident when indigo carmine of 93 % colouring matter was tested at much higher doses. Consequently, the ANS Panel reaffirmed the existing ADI of 5 mg/kg bw with the condition that the indigo carmine should have a purity of 93 % colouring matter or more.

The FEEDAP Panel endorses the above conclusions of the ANS Panel.

3.1 Safety for the target species²⁵

In a study in beagle dogs, Hansen et al. (1966)²⁶ administered indigo carmine in the diet at concentrations of 0, 10 and 20 mg/kg feed (reported to be approximately equivalent to 0, 250 and 500 mg/kg body weight per day, respectively) for a period of two years. The control group consisted of one male and one female, whereas the test groups each had two males and two females. There were unplanned deaths from viral infectious canine hepatitis in all treatment groups and the dead animals were replaced. Bone marrow smears showed terminal depletion of nearly all the mature myeloid forms of cells in dogs from groups receiving 10 or 20 mg/kg feed. No clinical signs attributable to the test material were observed, and no compound-related effects on body weight, haematology, gross lesions or microscopic pathology were reported. A NOAEL could not be established for this study as a result of the high mortality due to infection and as adverse effects were seen at all doses tested.

A study conducted to determine the toxicity of 10 colourants, including indigo carmine, on three species of ornamental fish, specifically firemouth cichlid (*Thorichthys meeki*), ornate tetra (*Hyphessobrycon bentosi*) and red barb (*Puntius conchonius*), was provided.²⁷ This study included groups (two aquaria, each containing 30 to 40 fish of each species) that were provided with feed containing 0 (control) or 350 mg indigo carmine²⁸/kg feed for a period of 84 days. Mortality, weight increase and feed intake were recorded every three weeks and feed-to-gain ratio was calculated.

The rate of mortality for each of the three species fed the indigo carmine-containing diet was within normally accepted limits: 0, 8 and 1 % for firemouth cichlid, ornate tetra and red barb, respectively (mortality rates in control groups were 0, 11 and 0 %, respectively). Body weight gains of the firemouth cichlid, ornate tetra and red barb fish fed the diet containing indigo carmine were, respectively, 8, 2 and 10 % higher than in the control group. Slight differences were observed in the feed-to-gain ratio for the different species fed the indigo carmine supplemented diets but were all 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 were available, and considering the limitations of the available dog and fish studies, the FEEDAP Panel set a maximum dietary safe level for cats, dogs and ornamental fish (Table 2) using the lowest NOAEL (500 mg/kg bw per day) and applying an uncertainty factor of 100, as described in the FEEDAP Panel guidance for additives already authorised for use in food (EFSA FEEDAP Panel, 2012).

²⁵ This section has been amended following the provisions of Article 8(6) and Article 18 of Regulation (EC) No 1831/2003.

²⁶ Technical dossier/Section III/Annex 16.

²⁷ Technical dossier/Supplementary information January 2103/Annex 3.

²⁸ From 10 000 mg of a commercial indigo carmine preparation.

Table 2: Calculated maximum safe dietary levels of indigo carmine in complete feeds for cats, dogs and ornamental fish

Species	Body weight (kg)	Safe intake (mg/day)	Feed intake (g dry matter/day)	Maximum safe dietary level	
				(mg/kg dry matter)	(mg/kg complete feed)
Cat	3	15	60	250	220
Dog	15	75	250	300	264
Ornamental fish	0.012	0.06	0.054	1 050	924

The maximum safe dietary concentration in complete feed for dogs is 264 mg/kg and for cats is 220 mg/kg. These levels are above the maximum typical inclusion rate reported by the applicant (200 mg/kg). For ornamental fish, the maximum safe concentration is 924 mg indigo carmine/kg feed. For practical reasons, the FEEDAP Panel rounds these values to 250 mg/kg complete feed for cats and dogs and 1 000 mg/kg complete feed for ornamental fish.

The above safe concentrations were derived from the lowest NOAEL observed in a two-year rat study. It should be mentioned that the ADI derived from the NOAEL of 500 mg/kg bw per day was confirmed by the ANS Panel under the condition of a 93 % colouring matter of indigo carmine. Consequently, any extrapolation from the NOAEL to target animals refers to a feed additive with at least the same purity. The additive under assessment shows a purity of only 88 to 90 %.

3.1.1 Conclusion on the safety for the target species

The FEEDAP Panel concludes that indigo carmine (purity of at least 93 %) is safe for cats and dogs at levels up to 250 mg/kg complete feed and for ornamental fish up to 1 000 mg/kg complete feed.

3.2 Safety for the user

3.2.1 Effects on the respiratory system

Particle size distribution analysis of a fine powder formulation of indigo carmine showed it to contain a large proportion of particles of respirable size. In the absence of information on dusting potential and of any information on the granulated form, it would be prudent to regard users as being at risk of inhalation exposure to dust from the additive. In the absence of information on the inhalation toxicity of indigo carmine, such exposure is regarded as hazardous.

Asthma was documented in a case report on one man working in an animal feed mill following occupational exposure to indigo carmine and other dyes (Miller et al., 1995).²⁹ After withdrawing the patient's normal medication for asthma, bronchial challenge with lactose powder containing 40 g/kg of indigo carmine produced dyspnoea and wheezing after 5 minutes of exposure, followed by a reduction in FEV₁ (forced expiratory volume in one second) 20 minutes post exposure, confirming an asthmatic response to the challenge. Lower concentrations did not produce an asthmatic response. Skin-prick testing with 20 mg/mL indigo carmine produced no skin reaction. Serum contained no specific IgE, IgA, IgM or IgG to indigo carmine–HSA (human serum albumin) conjugates. Bronchial challenge with lactose powder containing 6 g/kg of sodium sulphate (another constituent of the material being produced) caused no asthma in the patient. The mechanism by which indigo carmine caused asthma was not clear.

3.2.2 Effects on eyes and skin

Production workers exposed to indigo carmine were reported to have developed conjunctivitis (Arutyunov, 1935). No further details were available.

Burnett and Opdyke (1971) tested a series of 38 colouring agents (including indigo carmine) for eye irritancy in albino rabbits using the Draize test. Indigo carmine produced slight transient irritation

²⁹ Technical dossier/Section III/Annex 25.

when 0.2 mL of 10 % aqueous solution was applied twice daily, on five days per week for four weeks, to the eyes of six rabbits. Three days after the last administration, there were no signs of eye irritation in any of the treated animals. This indicates that indigo carmine dye is not an eye irritant.

Although no experimental studies of skin irritation were available, it is reasonable to assume that indigo carmine is also non-irritant to skin, as eyes tend to be more sensitive to irritation than skin.

Indigo carmine was reported to have no skin-sensitising activity in a test in guinea pigs that was performed before the currently recommended protocols were developed (Bär and Griepentrog, 1960).³⁰

In a study on 204 workers from 15 northern Italian feed mills (Mancuso et al., 1990), subjects were interviewed and patch-tested for allergy to 34 commonly used additives, including indigo carmine. The prevalence of occupational contact dermatitis was 13.7 %. Skin reactions occurred in 16.7 % (2 out of 12) of subjects with allergic contact dermatitis and 6.8 % of asymptomatic subjects who were patch-tested with indigo carmine. There was no information on the prevalence of allergy to indigo carmine in an unexposed population.

3.2.3 Conclusions on user safety

In the absence of information on dusting potential, and given that a powdered form of the additive contained up to 100 % fine respirable particles, it would be prudent to regard users as being at risk of inhalation exposure to dust from the additive. In the absence of information on the inhalation toxicity of indigo carmine, such exposure is regarded as hazardous.

Indigo carmine is not an eye or skin irritant. Indigo carmine should be considered as a potential skin and respiratory sensitiser.

3.3 Safety for the environment

Following the provision of the guidance on environmental risk assessment (EFSA, 2008), there is no requirement for the assessment of the environmental impact of the use of a feed additive when used in pets. This is the case for indigo carmine.

4 Efficacy

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).³¹ 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 indigo carmine would result in a visible effect, a demonstration of dose–effect relationship in a typical complementary feedingstuff appeared necessary and was provided.³²

Samples of standard biscuits containing wholemeal flour, milk powder and vegetable oil were prepared. Indigo carmine was added at 0, 50 and 500 mg/kg feed. The colour of the samples was measured by reflectance spectrophotometry. By the addition of indigo carmine the a^* value decreased from 7 (blank sample) to -2 (50 mg indigo carmine/kg) and -13 (500 mg indigo carmine/kg). The L^* value decreased accordingly (from 61 to 52 and 39, respectively) and the b^* value decreased from 22 to 14 and -2 , respectively.³³ The effect was also confirmed visually.

³⁰ Technical dossier/Section III/Annex 4.

³¹ 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.

³² Technical dossier/Supplementary information January 2012.

³³ 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).

The data demonstrated that indigo carmine is effective in colouring a typical complementary feed for dogs at the minimum dose tested (50 mg/kg feed).

It is concluded that indigo carmine is effective in adding colour to feedingstuffs for dogs, cats and ornamental fish.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

The FEEDAP Panel concludes that indigo carmine (purity of at least 93 % colouring matter) is safe for cats and dogs at levels up to 250 mg/kg complete feed and for ornamental fish up to 1 000 mg/kg complete feed.

It would be prudent to regard users as being at risk of inhalation exposure to dust from the additive. In the absence of information on the inhalation toxicity of indigo carmine, such exposure is regarded as hazardous. Indigo carmine is not an irritant to skin and eyes. Indigo carmine should be considered as a potential skin and respiratory sensitiser in humans.

Indigo carmine is effective in adding colour to feedingstuffs for dogs, cats and ornamental fish.

RECOMMENDATIONS

It is recommended to increase the specified minimum purity of indigo carmine from 85 % to 93 % total colouring matter to ensure that the safety assessment is applicable.

REMARK

It is noted that routine control should be possible if maximum contents of an additive in feed are proposed for safety reasons. However, no such analytical method is available for indigo carmine.

DOCUMENTATION PROVIDED TO EFSA

1. Indigo Carmine. November 2010. Sensient Colors UK Ltd.
2. Indigo Carmine. Supplementary information. September 2011. Sensient Colors UK Ltd.
3. Indigo Carmine. Supplementary information. January 2012. Sensient Colors UK Ltd.
4. Indigo Carmine. Supplementary information. January 2013. Sensient Colors UK Ltd.
5. Indigo Carmine. Supplementary information. January 2014. Sensient Colors UK Ltd.
6. Indigo Carmine. Supplementary information. February 2015. Sensient Colors UK Ltd.
7. Evaluation report of the European Union Community Reference Laboratory for Feed Additives on the methods(s) of analysis for Indigo Carmine.
8. Comments from Member States received through the ScienceNet.

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Annex – Executive Summary of the evaluation report of the European Union reference laboratory for feed additives on the method(s) of analysis for indigo carmine¹

In the current application authorisation is sought under articles 4(1) and 10(2) for *Indigo carmine* under the “sensory additives”, functional group 2(a) “colourants”, 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 cats and dogs, ornamental fish.

Indigo carmine is a synthesized dark-blue powder or granules, soluble in water, consisting of a minimum of 85 % total colouring matters calculated as sodium salt. *Indigo carmine* 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.

For the determination of *Indigo carmine* in the *feed additive*, the Applicant proposed the internationally recognised FAO JECFA monograph in food additives. Identification is based on spectrophotometry with a maximum in water at 610 nm and Thin Layer Chromatography (TLC), while quantification of *Indigo carmine* in the *feed additive* is based on spectrophotometry at 610 nm in aqueous solution, as recommended by Commission Directive 2008/128/EC laying down specific purity criteria concerning colours for use in foodstuffs. Even though no performance characteristics are provided, the EURL recommends for official control the JECFA monograph based on spectrophotometry for the quantification of the *Indigo carmine* in the *feed additive*.

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

¹ The full report is available on the EURL website: http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep_FAD-2010-0346.pdf.