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Safety and efficacy of tartrazine (E 102) for cats and dogs, ornamental fish, grain-eating ornamental birds and small rodents

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), Guido Rychen, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace, Pieter Wester, Lucio Guido Costa, Anne-Katrine Lundebye, Derek Renshaw, Orsolya Holczknecht, Maria Vittoria Vettori and Gabriele Aquilina

Abstract

Following a request from the European Commission, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of tartrazine (E 102) for cats and dogs, ornamental fish, grain-eating ornamental birds and small rodents. Tartrazine (E 102), an authorised food colourant, is intended to be used as a feed additive for cats, dogs, ornamental fish, grain-eating ornamental birds and small rodents. The following tartrazine concentrations in complete feed were considered safe for cats: 433 mg/kg; for dogs: 520 mg/kg; for ornamental birds: 63 mg/kg; for ornamental fish: 1,924 mg/kg; and for small rodents: 2,000 mg/kg. Inhalation exposure of tartrazine is regarded as hazardous. Tartrazine is considered as a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy potential of tartrazine to skin or eyes. Tartrazine is effective in adding colour to feedingstuffs.

Keywords: tartrazine, colourant, cats and dogs, ornamental fish, grain-eating ornamental birds, small rodents, safety

Requestor: European Commission

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, Giovanna Azimonti, Vasileios Bampidis, Maria de Lourdes Bastos, Georges Bories, Andrew Chesson, Pier Sandro Cocconcelli, Gerhard Flachowsky, Jürgen Gropp, Boris Kolar, Maryline Kouba, Secundino López Puente, Marta López-Alonso, Alberto Mantovani, Baltasar Mayo, Fernando Ramos, Guido Rychen, Maria Saarela, Roberto Edoardo Villa, Robert John Wallace and Pieter Wester.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. In particular, Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from Sensient Colors UK Ltd (on behalf of Feed Additives Synthetic Colours Group)[2] for re-evaluation of the product tartrazine (E 102), when used as a feed additive for cats, dogs, ornamental fish, grain-eating ornamental birds and small rodents (category: sensory additives; functional group: (a) colourants: (i) substances that add or restore colour in feedingstuff).

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 8 November 2012.[3] 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, user and the environment and on the efficacy of the product tartrazine (E 102), when used under the proposed conditions of use (see Section 3.1.2).

1.2. Additional information

Tartrazine (E 102) is authorised without a time limit under Council Directive 70/524/EEC[4] as colourant for ornamental fish without maximum levels. Under the same regulation, it is authorised without a time limit for cats and dogs and 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. Tartrazine is also authorised for grain-eating ornamental birds and for small rodents without a time limit and with maximum levels of 150 mg/kg complete feedingstuffs under Regulation (EC) No 358/2005[5].

Tartrazine (E 102) is an approved food colourant in the European Union (EU) and it is listed in Annex I of Directive 94/36/EC[6] of 30 June 1994. According to the same Directive, tartrazine is an allowed synthetic food colouring substance in the EU with a maximum permitted use level of 50–500 mg/kg food for various foodstuffs. Tartrazine is also allowed in beverages at levels up to 200 mg/L and non-alcoholic beverages at levels up to 100 mg/L.

The specific purity criteria concerning the use of tartrazine in foodstuffs are included in Commission Regulation (EU) No 231/2012[7].

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] Sensient Colors Uk Ltd. Oldmedow Road, PE30 4LA, Kings Lynn, UK.
[3] A new mandate was received in EFSA on 21/05/2012.
[4] 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.
[5] Commission Regulation (EC) No 358/2005 of 2 March 2005 concerning the authorisations without a time limit of certain additives and the authorisation of new uses of additives already authorised in feedingstuffs. OJ L 57, 3.3.2005, p. 3.
[6] European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. OJ L 237, 10.9.1994, p. 13.
[7] 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.

Tartrazine has been evaluated previously by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1966 (JECFA, 1966) and the EU Scientific Committee for Food (SCF) in 1975 and 1984 (European Commission, 1975, 1984). In 2009, the EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS) adopted an opinion on the re-evaluation of tartrazine (E 102) as food additive (EFSA ANS Panel, 2009).

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[8] in support of the authorisation request for the use of tartrazine 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[9] 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.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the tartrazine in animal feed. The Executive Summary of the EURL report can be found in Annex A.[10]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of tartrazine (E 102) 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), Technical Guidance for assessing the safety of feed additives for the environment (EFSA, 2008a), Guidance for the preparation of dossiers for the re-evaluation of certain additives already authorised under Directive 70/524/EEC (EFSA, 2008b), 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, 2012d), 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 tartrazine (E 102) in feed for cats, dogs, ornamental fish, grain-eating ornamental birds and small rodents.

3.1. Characterisation

The additive under application, tartrazine (E 102, FD&C Yellow No. 5, Cl Food Yellow 4), is identical to the active substance.

Tartrazine is prepared from 4-aminobenzenesulfonic acid, which is diazotised using hydrochloric acid and sodium nitrite. The resultant azo compound is then coupled to 4,5-dihydro-5-oxo-1-(4'-sulfophenyl)-1*H*-pyrazole-3-carboxylic acid, its methyl ester, ethyl ester or salt. The resulting dye is purified and isolated as the sodium salt.

Tartrazine is a disulfonated mono azo dye comprising essentially of trisodium-5-hydroxy-1-(4-sulfonatophenyl)-4-(4-sulfonatophenylazo)-*H*-pyrazole-3-carboxylate (chemical formula $C_{16}H_9N_4Na_3O_9S_2$, CAS number 1934-21-0, molecular weight 534.37) and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components. Tartrazine is described as the sodium salt. The use of the calcium and the potassium salts are also permitted as food

[8] FEED dossier reference: FAD-2010-0342.

[9] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[10] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2010-0342-tartrazine.pdf

additives by Commission Regulation (EU) No 231/2012[11]. The structural formula of tartrazine is given in Figure 1. Tartrazine is a water-soluble light orange powder or granules.

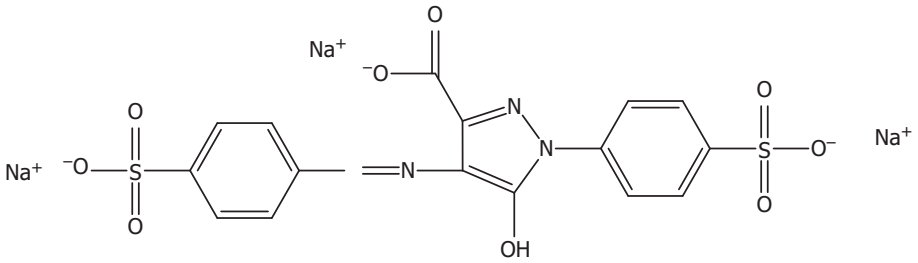

Figure 1: Structural formula of tartrazine

The specifications for tartrazine when used as a feed additive are identical to those for tartrazine when used as a food additive and laid down in Commission Regulation (EU) No 231/2012[12]: total colouring matters calculated as the sodium salt > 85%, subsidiary colouring matter < 1%, organic compounds other than colouring matters (4-hydrazinobenzene sulfonic acid, 4-aminobenzene-1-sulfonic acid, 5-oxo-1-(4-sulfophenyl)-2-pyrazoline-3-carboxylic acid, 4,4′-diazoaminodi(benzene sulfonic acid) and tetrahydroxysuccinic acid) < 0.5%, unsulfonated primary aromatic amines < 0.01%, arsenic < 3 mg/kg, lead < 2 mg/kg, and mercury and cadmium < 1 mg/kg each.

Five batches of tartrazine were analysed for the specified contents. They all complied with the specifications as follow: total colouring matter 88.2–89.2%; subsidiary colouring matter < 0.05%; sum of organic compounds other than colouring matters < 0.5% and unsulfonated primary aromatic amines < 0.01%.[13] The levels of heavy metals (Pb, Cd, Hg) and arsenic also met the specifications.[14]

The particle size distribution of two sources of tartrazine was determined by laser diffraction analysis.[15] For the first source (five batches), the volume-based percentage of particles of < 10 μm diameter was between 2.1% and 39.3%, that of particles of < 50 μm diameter between 47.3% and 67.9%, and that of particles of < 100 μm diameter between 78.9% and 86.4%. For the second source (three batches), the volume-based percentage of particles of < 10 μm diameter was between 0.5% and 0.9%, that of particles of < 50 μm diameter between 62.0% and 66.7%, and that of particles of < 100 μm diameter between 96.8% and 97.5%. 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 tartrazine 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 tartrazine in a range of foods are well established. Any substance which interacts or alters conjugated unsaturated bonds of the molecule will affect the colour. Tartrazine 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 homogenously distribute in different feedingstuffs were not required for additives which are used to add or restore colour to feedingstuffs. Consequently, no data have been submitted.

3.1.2. Conditions of use

Tartrazine is intended to be used in complete and complementary feed for dogs, cats, ornamental fish, grain-eating ornamental birds and small rodents. No maximum content is proposed for dogs, cats and ornamental fish although the applicant noted that the quantity required is dependent on the properties of the feedingstuff but, in general, is not likely to exceed 500 mg/kg complete feedingstuff

[11] 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 tartrazine were not assessed.
[12] OJ L 83, 22.3.2012, p. 1.
[13] Technical dossier/Section II.
[14] Technical dossier/Supplementary information/April 2013/Appendix A-1.
[15] Technical dossier/Supplementary information/April 2013/Appendix A-2.

for cats, dogs and ornamental fish. For grain-eating ornamental birds and small rodents, the applicant further proposed a maximum content of 150 mg/kg complete feedingstuff.

Upon request, the applicant provided data from pet food manufacturers indicating that the use dose may vary from up to 250 mg/kg for cats, 257 mg/kg for dogs, 1,000 mg/kg for rodents and up to 5,000 mg/kg for ornamental fish based on dry matter content. Values up to 200 mg/kg were indicated by pet food manufacturers for grain-eating birds.[16]

The applicant stated that the incorporation of the additive into feed can be done directly in the solid form or via an aqueous solution. The additive is not intended to be added to water for drinking. This latter use therefore has not been assessed in this opinion.

3.2. Safety

3.2.1. Toxicological studies

Tartrazine has been evaluated previously by JECFA in 1966 and 2016 (JECFA, 1966, 2016) and by the EU SCF in 1975 and 1984 (European Commission, 1975, 1984). In 2008, the EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids, and Materials in Contact with Food (AFC) assessed several food additives, including tartrazine, against claims of them causing hyperactivity in children (EFSA, 2008c). In 2009, the EFSA ANS Panel adopted an opinion on the re-evaluation of tartrazine (E 102) as food additive (EFSA ANS Panel, 2009).

3.2.1.1. General toxicology

The ANS Panel noted that, in 1964, JECFA had reviewed several repeat-dose short-term toxicological studies in rats, cats and dogs (JECFA, 1966). The ANS Panel made reference to a more recent rat study (Aboel-Zahab et al., 1997, as described in a review by TemaNord, 2002), which showed effects on haematology, blood biochemistry and the histopathology of the liver and kidneys when certain mixtures of food colours were fed at a level of 800 mg mixture/kg body weight (bw) per day for 30 or 60 days. However, no no-observed-adverse-effect-level (NOAEL) was identified due to the use of only one dose level and lack of information about the levels of each colour in the mixtures tested.

The ANS Panel noted that JECFA (1966) had reviewed one long-term toxicity/carcinogenicity study in mice and six in rats. JECFA (1966) and the SCF (European Commission, 1975, 1984) set an acceptable daily intake (ADI) of 7.5 mg/kg bw on the basis of an NOAEL of 1.5% in the diet (750 mg/kg bw per day), which was the highest dose tested in a long-term rat study (Mannell et al., 1958). Although these studies were not performed according to modern standards, they gave no indication of any carcinogenicity of tartrazine. The results of a 2-year dog study are reported in the ANS opinion (Davis et al., 1964 as referenced in EFSA ANS Panel, 2009) (see also Section 3.2.1). The ANS Panel noted that in the TemaNord report (2002) more recent reports (Maekawa et al., 1987; Borzelleca and Hallagan, 1988a,b; Moutinho et al., 2007) of long-term toxicity/carcinogenicity studies (one in mice; two in rats) were reviewed and reported also as negative for carcinogenicity. The highest dose of tartrazine given in any of the long-term toxicity/carcinogenicity studies was 5% in the diet (equal to 9,735 mg/kg bw per day weeks for mice and 3,348 mg/kg bw per day for female rats). In addition, the ANS Panel noted that a more recent study (2007) in male rats showed that 7.5 mg tartrazine/kg bw per day, administered in water for drinking for 46 weeks, caused no increased incidence of tumours in the stomach or duodenum.

For reproduction/developmental toxicity, the ANS Panel made reference to three rat studies reviewed by TemaNord (2002). These showed no adverse effects at up to the highest dose tested, approximately 1,000 mg/kg bw per day. Two more recent studies (Tanaka, 2006; Tanaka et al., 2008; as described in EFSA ANS Panel, 2009) showed no effects on reproduction or neurobehaviour in mice given up to 773 mg/kg bw per day. The ANS Panel considered studies of hypersensitivity and intolerance in laboratory animals and observations in humans, and concluded that the use of tartrazine as a food additive appears to trigger intolerance reactions in a small fraction of the exposed population, in some cases at doses lower than the ADI. Consequently, the ANS Panel stated that 'the presence of this dye must be clearly mentioned on the labelling of products destined for human food and pharmaceuticals, in order to provide clear and essential information to intolerant people'. The ANS Panel concurred with the conclusions of the AFC Panel (EFSA, 2008c) about the paucity of evidence to

[16] Technical dossier/Supplementary information/April 2013.

associate exposure to certain food additives including tartrazine with hyperactivity in children, and considered there to be insufficient evidence to justify a restriction on use of tartrazine as a food additive. The ANS Panel concluded that there was no reason to change the existing ADI of 7.5 mg/kg bw (EFSA ANS Panel, 2009).

In 2016, JECFA withdrew the current ADI of 0–7.5 mg/kg bw and replaced it with an ADI of 0–10 mg/kg bw on the basis of a NOAEL of 984 mg/kg bw per day for reduction in body weight in a chronic rat study and applying an uncertainty factor (UF) of 100 (JECFA, 2016).

3.2.1.2. Genotoxicity of tartrazine

The genotoxicity of tartrazine was firstly assessed in 2009 by the EFSA ANS Panel (EFSA ANS Panel, 2009), and more recently, in the EFSA ANS Panel statement on allura red and other sulfonated mono azo dyes, authorised as food and feed additives, including tartrazine (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 tartrazine, using an internationally validated experimental protocol for comet assay is recommended.

The applicant has subsequently submitted two new *in vivo* genotoxicity studies that are assessed below.

In vivo *mammalian comet assay*

Tartrazine was evaluated for DNA damage (single-strand breaks, double-strand breaks and strand breaks induced by alkali labile sites) in cells of selected organs of mice using the comet assay in compliance with the OECD guideline 489.[17] Concomitantly, the test item was also assayed in a bone marrow mice micronucleus assay (see below). Deionised water was used as the vehicle. Test and/or control article formulations were administered at a dose volume of 10 mL/kg via oral gavage once per day on 3 consecutive days.

Six male mice were administered with test article doses of 25, 500 and 2,000 mg/kg bw per day. On study day 3, 3–4 h after dosing, the animals were euthanised, and the stomach, liver and colon were processed for the comet assay. Methyl methanesulfonate (MMS) was administered to three animals as a positive control on study day 3 at a dose of 40 mg/kg bw.

No statistically significant increase in the % tail DNA (DNA damage) in the test article-treated groups (stomach, liver or colon) was observed relative to the concurrent vehicle control group (ANOVA, $p > 0.05$, Dunnett's post hoc analysis).

Positive and vehicle control values were within the expected ranges and all criteria for a valid assay were met.

In vivo *micronucleus assay*

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.

3.1.1.3. Conclusions on toxicity

Toxicological studies in laboratory animals showed no alerts for particular adverse effects that need to be taken into consideration when assessing target species safety. Tartrazine was not carcinogenic and did not cause reproduction/developmental toxicity.

The administration of tartrazine via oral gavage up to 2,000 mg/kg bw per day did not induce a significant increase in DNA damage in liver, colon or stomach cells relative to the concurrent vehicle control. It is concluded that tartrazine has no genotoxic potential *in vivo*.

3.2.2. Safety for the target species

A number of subchronic and chronic feeding studies with tartrazine in mice and rats for durations of over 1 year without any reported significant adverse effects have been previously described and assessed (EFSA ANS Panel, 2009). Minor effects of discoloured fur, urine and faeces had been

[17] Technical dossier/Supplementary information/May 2016.

reported for doses from 10,000 mg/kg feed onwards (Borzelleca and Hallagan, 1988a). The identified NOAEL of these studies was 50,000 mg/kg feed, however, a slight but significant decrease in final body weight of rats was found at this dose level; the next lower dose 20,000 mg/kg feed (corresponding to 984 and 1,225 mg/kg bw per day for males and females, respectively) was considered for further assessment of target animals safety, and the lowest value used being 984 mg tartrazine/kg bw per day.

Considering a dose of 20,000 mg/kg feed and an UF of 10 for intraspecies variability, the FEEDAP Panel concludes that 2,000 mg tartrazine/kg complete feed is considered safe for small rodents.

Three groups of dogs (two of each sex/group) were given 0, 10,000 or 20,000 mg tartrazine/kg in their diets for 2 years (Davis et al., 1964). Blood was taken for haematology[18] at unspecified intervals before and during treatment. All dogs were sacrificed at the end of the treatment period, and necropsies were performed and organs were weighed. The following organs from each dog were examined microscopically: bone marrow (myeloid:erythroid ratio), heart, lung, liver, gall bladder, kidney, urinary bladder, spleen, pancreas, mesenteric lymph node, adrenal, thyroid, skeletal muscle, bone, testis (or ovary) and prostate (or uterus). The stomach and thymus were also examined for the controls and top-dose group; and the following organs were examined only in the top-dose group (not in the controls): intestines, salivary glands, submaxillary lymph node, brain and pituitary. No clinical signs, haematological changes, altered organ weights or gross pathology attributable to treatment were seen. One top-dose female had mild chronic gastritis at the pyloric end of the stomach; it was not clear whether this was a treatment-related effect and as the stomach was not examined for the low- and mid-dose groups it was not possible to identify a NOAEL for the effect. An area of interstitial nephritis was seen in the kidney of one top-dose male, but due to the small group sizes it was not clear whether or not the effect was treatment-related and a NOAEL could not be reliably identified. Chronic lymphocytic thyroiditis was found in one low-dose female, but was not considered to be a treatment-related effect. In conclusion, although microscopic lesions that might be treatment-related were only seen at the top-dose level, a NOAEL could not be identified.

The applicant submitted a study performed in canary birds (10 per group).[19] The diet containing tartrazine (purity $\geq$ 85%) at 0 or 1,500 mg/kg diet (10-fold the authorised maximum content) was given *ad libitum* for 14 days. No signs of intolerance were observed. However, the short study period prevents a full assessment of the results for target animal safety.

The applicant submitted a report on a trial conducted to determine the toxicity of 10 colourants, including tartrazine, on three species of ornamental fish, namely firemouth cichlid (*Thorichthys meeki*), ornate tetra (*Hyphessobrycon bentosi*) and red barb (*Puntius conchonius*).[20] This study included groups (two aquaria each with 30–40 fish of each species) that were provided feed containing 0 (control) or 15,000 mg tartrazine/kg feed for a period of 84 days. Mortality, weight increase 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 tartrazine-containing diet was within normally accepted limits: 1.7%, 8% and 1 % for firemouth cichlid, ornate tetra and red barb, respectively (mortality rates in the control group were 0%, 11% and 0%, respectively). Body weight gain of the firemouth cichlid, ornate tetra and red barb fish fed the diet containing tartrazine were increased by 11%, 8% and 18%, respectively, in comparison to the control group. Slight differences were observed in the feed to gain ratio for the different species fed the tartrazine 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 were available, and considering the limitations of the studies in dogs, canary birds and fish, the FEEDAP Panel applied the procedure described in the FEEDAP Panel guidance for additives already authorised for use in food (EFSA FEEDAP Panel, 2012c) to derive safe feed concentrations for these species/categories (Table 1). The NOAEL used in the calculation was 984 mg/kg bw per day and an UF of 100 was applied.

[18] Haemoglobin and haematocrit determinations, total white cell counts, white cell differential counts, packed cell volume (PCV), white blood counts (WBC) and differential leucocyte count.
[19] Technical dossier/Section III/Annex 40.
[20] Technical dossier/Supplementary information/January 2013/Appendix III.

Table 1: Calculated maximum safe dietary levels of tartrazine in complete feeds for cats, dogs and ornamental fish and birds

Species	Body weight (kg)	Feed intake (g dry matter/day)	Safe intake (mg/day)	Maximum safe dietary level (mg/kg complete feed)(1)
Cat	3	60	29	433
Dog	15	250	148	520
Ornamental fish	0.012	0.055	0.12	1,924
Ornamental birds	0.025	3.4	0.24	63

(1): Complete feed containing 88% DM.

Tartrazine is considered safe for the target species at the following concentrations in complete feed: 433 mg/kg for cats; 520 mg/kg for dogs; 63 mg/kg for ornamental birds; 1,924 mg/kg for ornamental fish; and 2,000 mg/kg for small rodents.

3.2.3. Safety for the user

3.2.3.1. Effects on the respiratory system

Particle size distribution analysis of two sources of tartrazine showed it to contain a large proportion of particles of respirable size. In the absence of information on dusting potential, users are considered as being at risk of inhalation exposure to dust from the additive. In the absence of information on the inhalation toxicity of tartrazine, such exposure is regarded as hazardous.

3.2.3.2. Effects on the eyes and skin

No information was available on the irritancy of tartrazine to skin or eyes.

Bär and Griepentrog (1960) tested a large number of substances for skin sensitisation in guinea pigs. Tartrazine did not produce sensitisation in this test.

Safford and Goodwin (1985) tested tartrazine and two of its metabolites, sulfanilic acid and 1-sulfofenyl-3-carboxyl-4-amino-5-oxy-pyrazole (SCAOP), in guinea pigs using a Magnusson and Kligman maximisation test for delayed contact sensitisation that broadly conformed to the OECD Guideline 406. Tartrazine was not a skin sensitiser in this test but sulfanilic acid and SCAOP developed strong skin reactions upon challenge. Animals given induction doses of tartrazine gave skin reactions when challenged with SCAOP (but not sulfanilic acid), indicating a cross-reactivity. Safford and Goodwin (1985) also performed an immediate skin reactivity test in guinea pigs using preconjugated (to bovine serum albumin) forms of tartrazine, sulfanilic acid and SCAOP. Challenges with the unconjugated form of each test material all produced immediate skin test reactivity (confirmed by the presence of specific antibodies in the serum), and tartrazine cross-reacted to SCAOP but not to sulfanilic acid.

Allergy to tartrazine has been demonstrated in some eczema patients by skin patch tests (Grater, 1976; as reported in Safford and Goodwin, 1985).

Kalender (2000) studied the effects of tartrazine on dermal mast cell degranulation in the mouse 1, 6, 12, and 24 h after a single intradermal injection. After 1 and 12 h, the dye caused partial degranulation; at the end of the 6-h period, an internal degranulation was predominant. The effects were no longer visible after 24 h.

3.2.3.3. Conclusions on safety for the user

Inhalation exposure of tartrazine is regarded as hazardous. Tartrazine is considered as a skin sensitiser. In the absence of data the Panel cannot conclude on the irritancy potential of tartrazine to skin or eyes.

3.2.4. Safety for the environment

Following the provision of the Regulation (EC) No 429/2008, 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 tartrazine.

3.3. Efficacy

Tartrazine is intended to be used to add or restore colour in the feedingstuffs for cats, dogs, ornamental fish, grain-eating ornamental birds and small rodents. 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).[21] However, considering the wide variety of feedingstuffs used in complete and complementary feed for cats and dogs, ornamental fish, grain-eating ornamental birds as well as small rodents and the uncertainty which concentration of tartrazine would result in a visible effect, the FEEDAP Panel requested an effect demonstration. The applicant provided one study in which graded amounts of tartrazine were supplemented to a complementary feed (biscuits) for pets.[22]

Samples of standard biscuits were prepared containing wholemeal flour, milk powder and vegetable oil. Tartrazine was added at 0, 50 and 500 mg/kg biscuit. Colour of the samples was measured by reflectance spectrophotometry. By the addition of tartrazine, the 'a' value increased from 7.35 (blank sample) to 10.43 (50 mg tartrazine) and 11.51 (500 mg tartrazine). The 'L' value decreased accordingly (from 61.00 to 51.19 and 53.99, respectively) and the 'b' value increased from 22.41 to 29.71 and 45.93, respectively.[23]

The data demonstrated that tartrazine is effective in colouring a typical complementary feed for pets at a minimum dose of 50 mg/kg.

4. Conclusions

Tartrazine is considered safe for the target species at the maximum concentrations in complete feed: 433 mg/kg for cats; 520 mg/kg for dogs; 63 mg/kg for ornamental birds; 1,924 mg/kg for ornamental fish; and 2,000 mg/kg for small rodents.

Inhalation exposure of tartrazine is regarded as hazardous. Tartrazine is considered as a skin sensitiser. In the absence of data, the FEEDAP Panel cannot conclude on the irritancy potential of tartrazine to skin or eyes.

Tartrazine is effective in adding colour to feedingstuffs.

Documentation provided to EFSA

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

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[21] OJ L 133, 22.5.2008, p. 1.

[22] Technical dossier/Section IV/Annex 1.

[23] 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).

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Abbreviations

ADI	acceptable daily intake
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids, and Materials in Contact with Food

ANOVA	analysis of variance
ANS	EFSA Panel on Food Additives and Nutrient Sources added to Food
bw	body weight
CAS	Chemical Abstracts Service
EURL	European Union Reference Laboratory
FAO	Food and Agriculture Organization of the United Nations
FEEDAP	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
OECD	Organisation for Economic Co-operation and Development
SCAOP	1-sulfofenyl-3-carboxyl-4-amino-5-oxy-pyrazole
SCF	Scientific Committee for Food
UF	uncertainty factor
WHO	World Health Organization

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for tartrazine

In the current application authorisation is sought under articles 4(1) and 10(2) for *Tartrazine* under the category/functional group 2(a) "sensory additives"/"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, grain-eating ornamental birds and small rodents.

Tartrazine is a synthetic orange powder or granules soluble in water, consisting of a minimum of 85% "*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 are also applicable for the *feed additive*. *Tartrazine* is intended to be incorporated directly in *feedingstuffs* or as a solution in *water*, with a proposed maximum content of 150 mg/kg complete *feedingstuffs*.

For the quantification of the *Tartrazine* "total colouring matters content" in the *feed additive*, the Applicant submitted the internationally recognised FAO JECFA monograph for food additives. Determination of total colouring matters content of *Tartrazine* is based on spectrophotometry at 426 nm in aqueous solution, as described in the monograph and specified in 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 this spectrophotometric method - described in the JECFA monograph and specified in Commission Directive 2008/128/EC - for the quantification of the *Tartrazine* "total colouring matters content" in the *feed additive*.

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