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Safety of Allura Red AC in feed for cats and dogs

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

Abstract

No evidence of genotoxicity was found in an *in vivo* micronucleus test with Allura Red AC; however, no evidence of target cell exposure was provided. In an *in vivo* comet assay, Allura Red AC was clearly negative for the induction of DNA damage in all the analysed tissues (stomach, colon, and liver). Consequently, the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concludes that Allura Red AC is not genotoxic. Since no data on the safety of Allura Red AC for cats and dogs were provided, the highest safe dietary concentration of Allura Red AC was derived from the no observed adverse effect level obtained in toxicity studies with rats, applying an uncertainty factor of 100. The calculated values were 370 mg/kg complete feed for dogs and 308 mg/kg complete feed for cats.

Keywords: colourant, Allura Red AC, cats, dogs, safety, genotoxicity

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Correspondence: feedap@efsa.europa.eu

Panel members: Gabriele Aquilina, 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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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 a scientific opinion on the safety of Allura Red in feed for cats and dogs.

No evidence of genotoxicity was found in an *in vivo* micronucleus test with Allura Red AC, however, no evidence of target cell exposure was provided. In an *in vivo* comet assay, Allura Red AC was clearly negative for the induction of DNA damage in all the analysed tissues (stomach, colon, and liver). Consequently, the FEEDAP Panel concludes that Allura Red AC is not genotoxic.

Since no data on the safety of Allura Red AC for cats and dogs were provided, the highest safe dietary concentration of Allura Red AC was derived from a no observed adverse effect level obtained in toxicity studies with rats and applying an uncertainty factor of 100. The calculated values are 370 mg/kg complete feed for dogs and 308 mg/kg complete feed for cats.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Regulation (EC) No 1831/2003 establishes rules governing the Community authorisation of additive for animal nutrition and, in particular, Article 9 defines the terms of the authorisation by the Commission.

The applicant, Feed Additives Synthetic Colour Group, c/o Sensient Colors UK Ltd., is seeking a Community authorisation of Allura Red AC to be used as a sensory additive (colourant) in feed for cats and dogs (Table 1).

Table 1: Description of the substances

Category of additive	Sensory additive
Functional group of additive	Colourants/substances that add or restore colour in feedingstuffs
Description	Disodium-2-hydroxy-1-(2-methoxy-5-methyl-4-sulfonatophenylazo)naphthalene-6-sulfonate
Target animal category	Cats and dogs
Applicant	Feed Additives Synthetic Colour Group, c/o Sensient Colors UK Ltd.
Type of request	New opinion

EFSA ANS Panel adopted three opinions on Allura Red used as food additive on 23 September 2009, on 15 May 2013 and 27 January 2015. On 24 April 2012 the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ('Authority') adopted an opinion on the use of Allura Red as feed additive. In this opinion on the safety and efficacy of the product it is considered that:

'Genotoxicity of Allura Red AC cannot be excluded; (ii) the mouse carcinogenicity study may not be appropriate for investigating the risk of colon cancer that might result from local DNA damage; and (iii) Allura Red AC is proposed for lifetime use in cats and dogs, leading to a much higher exposure in target animals than in humans. So the FEEDAP Panel concluded that the available data are insufficient to demonstrate the safety of Allura Red AC for cats and dogs.

Furthermore, in the absence of any information, the substance should be considered to be potentially harmful as a result of skin, eye, or inhalation exposure by users of the additive.'

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment on the safety and to allow a revision of the Authority's opinion.

The Commission has now received supplementary information concerning the genotoxicity of Allura Red AC, in particular:

- In vivo Micronucleus and Comet Assay in mice

In view of the above, the Commission asks the Authority to deliver a new opinion on the safety of Allura Red AC as sensory additive (colourant) in feed for cats and dogs based on the additional data submitted by the applicant.

1.2. Additional information

Allura Red AC, a synthetic food colouring substance, is an approved food colourant in the EU, and it is listed in Annex II of Regulation (EC) No 1333/2008[1] for a limited number of foodstuffs with a maximum allowed usage level of 25–500 mg/kg food for various foodstuffs. Allura Red AC is also permitted in alcoholic beverages at levels up to 200 mg/L and in non-alcoholic beverages at levels up to 100 mg/L.

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

Allura Red AC has previously been evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1974 (JECFA, 1974), 1980 (JECFA, 1981) and 1981 (JECFA, 1981) and by the Scientific Committee for Food (SCF) in 1975 (EC, 1975), 1984 (EC, 1984) and 1989 (EC, 1989). Allura Red AC was evaluated in 2000 by the National Toxicological Program (NTP, 2000). In 2002, the Nordic Working Group on Food Toxicology and Risk Assessment (NNT, 2002) reviewed the current status and safety data on all food additives permitted in the EU, including Allura Red AC.

The EFSA Panel on Food Additives and Nutrient Sources Added to Food (ANS) issued a scientific opinion on the re-evaluation of Allura Red AC as a food additive (EFSA ANS Panel, 2009). The ANS Panel noted that Allura Red AC was negative in *in vitro* genotoxicity studies as well as in long-term carcinogenicity studies and that the effects on nuclear DNA migration observed in the mouse *in vivo* comet assay were not expected to result in carcinogenicity. The ANS Panel also concurred with the conclusion from a previous EFSA opinion adopted by the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC) (EFSA, 2008) on the McCann et al. study (1975) that the findings of the study cannot be used as a basis for altering the Acceptable Daily Intake (ADI). The ANS Panel concluded in 2009 that '*the present database does not give reason to revise the ADI of 7 mg/kg bw per day*.'

The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) concluded in 2012 (EFSA FEEDAP Panel, 2012a) that the available data are insufficient to demonstrate the safety of Allura Red AC for cats and dogs. In particular, on the basis of the experimental results, it was not possible to exclude a genotoxic potential of Allura Red AC. Although Allura Red AC was negative in bacterial reverse mutation assays, no other validated *in vitro* tests were provided. Allura Red AC was positive in two *in vivo* comet assays in mice (stomach and colon), but these findings were not seen in rats. The FEEDAP Panel noted that the mouse carcinogenicity study may not be appropriate for investigating the risk of colon cancer that might result from local DNA damage.

Based on the above, in 2013 the ANS Panel was asked to deliver a scientific opinion addressing the new scientific information that became available since the adoption of the opinion on the re-evaluation of Allura Red AC (E 129) when used as a food colouring agent in 2009 and to assess whether this would have altered its conclusions.

In the more recent ANS opinion (EFSA ANS Panel, 2013), the new findings were interpreted in conjunction with all the available relevant data from genotoxicity testing, metabolism and carcinogenicity, and in consideration of possible species differences between mouse and rat. These new data were considered in the context of the overall relevant data available not only for Allura Red AC, but also for a number of other structurally related sulphonated mono azo dyes authorised as food additives. The ANS Panel concluded that the new data, by themselves, were insufficient at that time to change the conclusions of the 2009 opinion on the safety of Allura Red AC and that there was at that time no reason to revise the ADI. The read-across exercise had highlighted a shared pattern of effects for this class of substances that would warrant further investigation. The Panel therefore recommended a repetition of the *in vivo* comet assay in mice, to be performed in compliance with the most recent and internationally validated experimental protocol, using whole cells and examining a wide range of tissues. These recommendations apply to all the sulphonated mono azo dyes included in the review (EFSA ANS Panel, 2013).

In 2015, EFSA ANS Panel issued an opinion on the refined assessment of Allura Red AC (EFSA ANS Panel, 2015).

The present opinion addresses the request from the Commission to deliver a new opinion on the safety of Allura Red AC as sensory additive (colourant) in feed for cats and dogs based on the additional data submitted by the applicant (an *in vivo* micronucleus and comet assay in mice).

2. Data and Methodologies

2.1. Data

The present assessment is based on the data submitted by the applicant in the form of additional information[2] to a previous application on the same product.[3]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety of Allura Red AC is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance for the preparation of dossiers for sensory additives (EFSA FEEDAP Panel, 2012b), Guidance for the preparation of dossiers for additives already authorised for use in food (EFSA FEEDAP Panel, 2012c), Guidance on the assessment of additives intended to be used in pets and other non-food-producing animals (EFSA FEEDAP Panel, 2011, revised in 2012).

3. Assessment

3.1. Safety

3.1.1. Genotoxicity studies including mutagenicity

Allura Red AC was evaluated in the comet assay for its potential to induce DNA damage in liver, stomach and colon cells of male Hsd:ICR (CD-1) mice, in compliance with OECD Guideline 489.

Groups of six animals (five in the positive controls) were dosed by gavage once per day on three consecutive days (study days 1, 2 and 3) with the test article at doses of 25, 500 or 2000 mg/kg body weight (bw) in deionised water (10 mL/kg bw). Cyclophosphamide (CP) and methyl methanesulphonate (MMS) were administered on study days 2 and 3, respectively, to the positive control groups.

All animals were sacrificed three to four hours after the last administration and cell suspensions from the organs listed above were prepared to be analysed for DNA damage. The following parameters were measured in at least 150 cells per animal: tail length, percentage tail DNA, Olive tail moment. None of the animals treated with the test article showed significant increases in the measured parameters over the vehicle controls in any of the analysed tissues, while the positive controls performed as expected.

Concomitantly to the comet assay described above, groups of six animals treated by gavage on three consecutive days with 25, 500 or 2000 mg/kg bw were analysed for the induction of micronucleated polychromatic erythrocytes (PCEs) in bone marrow, in compliance with OECD Guideline 474 (rev. 1997). The animals were sacrificed three to four hours after the last administration and 2 000 PCEs per animal were scored for the presence of micronuclei.

No treatment-related effect was reported in the test article-treated groups relative to the negative control group, while the positive control induced a statistically significant increase in the incidence of micronucleated PCEs. However, it should be noted that no local cytotoxicity at bone marrow (detected as alteration of the ratio between PCEs and normochromatic erythrocytes) was observed in the test article groups compared with the vehicle control group; therefore, there was no evidence of target cells exposure.

[2] FAD-2015-0019.
[3] FAD-2010-0347.
[4] 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.

Conclusions on genotoxicity

In an *in vivo* comet assay, Allura Red AC was clearly negative for the induction of DNA damage in all the analysed tissues (stomach, colon and liver). No evidence of genotoxicity was found in an *in vivo* micronucleus test with Allura Red; however, no evidence of target cell exposure was provided.

Based on the new data provided, the FEEDAP Panel concludes that Allura Red AC is not genotoxic.

3.1.2. Safety for target animals

No data on the safety of Allura Red AC for cats and dogs were provided by the applicant. Instead, the FEEDAP Panel derived safe dietary levels for target animals using a no observed adverse effect level (NOAEL) obtained in toxicity studies with laboratory animals and a default value for body weight to estimate for feed intake (FEEDAP Panel guidance for additives already authorised for use in food (EFSA FEEDAP Panel, 2012c).

This NOAEL was derived from studies in rats resulting in a NOAEL of 695 mg/kg body weight per day for growth suppression in pups in a reproduction study and a NOAEL of 701 mg/kg body weight per day for males in a developmental toxicity study (EFSA ANS Panel, 2009, 2013).

Applying a safety factor of 100, the highest safe dietary concentration of Allura Red AC was calculated for dogs (default value 15 kg bw and 250 g dry matter intake/day) with 370 mg/kg complete feed and for cats (default value 3 kg bw and 60 g dry matter intake/day) with 308 mg/kg complete feed.

4. Conclusions

No evidence of genotoxicity was found in an *in vivo* micronucleus test with Allura Red; however, no evidence of target cell exposure was provided. In an *in vivo* comet assay in male mice, Allura Red AC was clearly negative for the induction of DNA damage in all the analysed tissues (stomach, colon, and liver). Consequently, the FEEDAP Panel concludes that Allura Red AC is not genotoxic.

The highest safe dietary concentration of Allura Red AC was calculated to be 370 mg/kg complete feed for dogs and 308 mg/kg complete feed for cats.

Documentation provided to EFSA

1. Supplementary dossier on Allura Red AC for cats and dogs. May 2015. Submitted by Feed Additives Synthetic Colour Group, c/o Sensient Colors UK Ltd.

References

EC (European Commission), 1975, online. Reports of the Scientific Committee for Food (1st Series), opinion expressed on 31 December 1975. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_01.pdf

EC (European Commission), 1984, online. Reports of the Scientific Committee for Food (14th Series), opinion expressed on 7 July 1983. Available online: http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_14.pdf

EC (European Commission), 1989. Reports of the Scientific Committee for Food (21st Series), opinion expressed on 10 December 1987.

EFSA (European Food Safety Authority), 2008. Assessment of the results of the study by McCann et al. (2007) on the effect of some colours and sodium benzoate on children's behaviour. Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC). The EFSA Journal, 660:1-54.

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2009. Scientific Opinion on the re-evaluation of Allura Red AC (E 129) as a food additive. EFSA Journal, 7(11):1327, 39 pp. doi:10.2903/j.efsa.2009.1327

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2013. Statement on Allura Red AC and other sulphonated mono azo dyes authorised as food and feed additives. EFSA Journal, 11(6):3234. doi:10.2903/j.efsa.2013.3234

EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources Added to Food), 2015. Refined exposure assessment for Allura Red AC (E 129). EFSA Journal, 13(2):4007. doi:10.2903/j.efsa.2015.4007

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2011, revised in 2012. Guidance on the assessment of additives intended to be used in pets and other non food-producing animals. EFSA Journal 2011;9(2):2012, 3 pp. doi:10.2903/j.efsa.2011.2012

EFSA Panel on Additives and Products or Substances added to Feed (FEEDAP), 2012a. Scientific Opinion on the safety and efficacy of Allura Red AC (E 129) in feed for cats and dogs. EFSA Journal,10(5):2675, 14 pp. doi:10.2903/j.efsa.2012.2675

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012b. Guidance for the preparation of dossiers for sensory additives. EFSA Journal 2012;10(1):2534, 26 pp. doi:10.2903/j.efsa.2012.2534

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP), 2012c. Guidance for the preparation of dossiers for additives already authorised for use in food. EFSA Journal 2012;10(1):2538, 4 pp. doi:10.2903/j.efsa.2012.2538

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1974. Report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series, No. 557.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1980. 24th Report. Toxicological evaluation of certain food additives. WHO Food Additive Series, No. 15.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 1981. 25th Report. Evaluation of certain food additives. WHO Technical Report Series, No. 669.

McCann J, Choi E, Yamasaki E and Ames BN, 1975. Detection of carcinogens as mutagens in the Salmonella/microsome test: assay of 300 chemicals. Proceedings of the Natural Academy of Sciences of the USA, 72, 5135–5139.

NNT (Nordic Working Group on Food Toxicology and Risk Assessment), 2002. Food additives in Europe 2000—Status of safety assessments of food additives presently permitted in the EU.

NTP (National Toxicology Program), 2000. Allura red. Available from NTP website: http://ntp.niehs.nih.gov/index.cfm?objectid=E87EF85F-BDB5–82F8-FDED9AB34EFB2FAD

Shimada C, Kano K, Sasaki YF, Sato I and Tsuda S, 2010. Differential colon DNA damage induced by azo food additives between rats and mice. Journal of Toxicological Sciences, 35, 547–554.

Tsuda S, Murakami M, Matsusaka N, Kano K, Taniguchi K and Sasaki YF, 2001. DNA damage induced by red food dyes orally administered to pregnant and male mice. Toxicological Sciences, 61, 92–99.