Amaranth

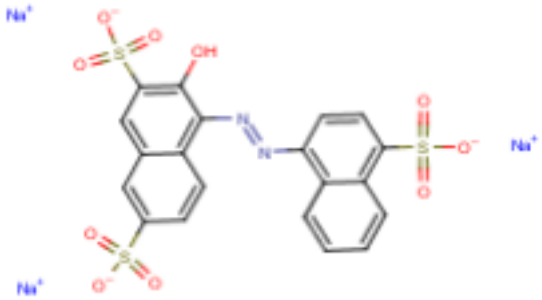
SYNONYMS

1-(4-Sulfo-1-naphthylazo)-2-naphthol-3,6-disulfonic acid
trisodium salt
1-(4-Sulpho-1-naphthylazo)-2-naphthol-3,6-disulphonic acid,
trisodium salt, 1302 Red, 1508 Red, 2,7-Naphthalenedisulfonic acid, 3-hydroxy-4-((4-sulfo-1-naphthyl)azo)-, trisodium salt,
2-Hydroxy-1,1'-azonaphthalene-3,6,4'-trisulfonic acid trisodium salt
3-Hydroxy-4-((4-sulfo-1-naphthalenyl)azo)-2,7-naphthalenedisulfonic acid trisodium salt
3-Hydroxy-4-((4-sulpho-1-naphthalenyl)azo)-2,7-naphthalenedisulphonic acid, trisodium salt
AI3-52541, Acetacid Red 2BR, Acid Amaranth, Acid Amaranth I, Acid Amaranth N, Acid Leather Red I 2BW, Acid Leather Rubine S, Acid Red 27, Acilan Red SE, Aizen Amaranth, Amacid Amaranth, Amarant, Amaranth, Amaranth (dye), Amaranth 85, Amaranth A, Amaranth B, Amaranth BPC, Amaranth Extra, Amaranth J, Amaranth Lake, Amaranth R, Amaranth S, Amaranth S Specially Pure, Amaranth USP, Amaranth WD, Amaranth red, Amaranthe, Amaranthe USP (biological stain), Azo Red R, Azo Rubine S.FQ, Azo Rubine SF, Azo Ruby S, Azorubin S, Azorubine S, Bordeaux S, Bordeaux S Extra Conc. A Export, Bordeaux S Extra Pure A, C.I. 16185, C.I. Acid Red 27, C.I. Acid Red 27, trisodium salt
C.I. Food Red 9, Calcocid Amaranth, Canacert Amaranth, Certicol Amaranth S, Cerven kysela 27, Cerven potravinarska 9, Cilefa Rubine 2B, Cranberry Red, D and C Red 2, D&C Red 2, Daishiki Amaranth, Dolkwal Amaranth, Dye FDC Red 2, Dye Red Raspberry, E 123, EEC No. 123, Edicol Amaranth, Edicol Supra Amaranth A, Eurocert Amaranth, FD & C Red No. 2, FD And C Red No. 2-Aluminium Lake, FD and C Red No. 2, FD&C Red 2, FD&C Red No. 2 - Aluminium Lake, FD&C red no.2, Fast Red, Food Red 2, Food Red 9, Fruit Red A Geigy, HD Amaranth B, HD Amaranth Supra, Hexacert Red No. 2, Hexacol Amaranth B Extra, Hidacid Amaranth, Hispacid Red AM, Java Amaranth, Kayaku Amaranth, Kayaku Food Colour Red No. 2, Kca Foodcol Amaranth A, Kiton Rubine S, L Red Z 3050, L-Red 3, L-Rot 3, Lissamine, Lissamine Amaranth AC, Maple Amaranth, NSC 215207, NSC 4300, Naphthol Red B, Naphthol Red C, Naphthol Red LZS, Naphthol Red O, Naphthol Red S, Naphthol Red S Conc. Specially Pure, Naphthol Red S Specially Pure, Naphthol Red SI, Naptholrot S, Neklacid Red A, Rakuto Amaranth, Raspberry red for jellies, Red No. 2, Red dye No. 2, S-Azo Rubine, San-ei Amaranth, Schultz Nr. 212, Shikiso Amaranth, Solar Red O, Takaoka Amaranth, Tertracid Red A, Toyo Amaranth, Trisodium salt of 1-(4-sulfo-1-naphthylazo)-2-naphthol-3,6-
disulfonic acid
Trisodium salt of 1-(4-sulpho-1-naphthylazo)-2-naphthol-3,6-disulphonic acid, UNII-37RBV3X49K, Usacert Red No. 2, Victoria Rubine O, Victoria Rubine O for food, Whortleberry Red, Wool Bordeaux 6RK, Wool Red, Wool Red 40F

CHEMICAL FORMULA

C20-H11-N2-O10-S3.3Na
C20-H14-N2-O10-S3.3Na

CHEMICAL STRUCTURE



[ToxNet, 2010]

IDENTIFIER DETAILS

CAS Number	:	915-67-3
CoE Number	:	-
FEMA	:	-
EINECS Number	:	213-022-2
E Number	:	123

SPECIFICATIONS

Melting Point: -

Boiling point: -

STATUS IN FOOD AND DRUG LAWS

CoE limits:

Beverages (mg/kg)	Food (mg/kg)	Exceptions (mg/kg)
-	-	-

Acceptable Daily Intake:

ADI (mg/kg)	ADI Set by	Date Set	Comments
0.8 mg/kg/bw	SCF	1984	-
0-0.5 mg/kg bw	JECFA	1984	-
0.15 mg/kg bw	EFSA	2010	-

FDA Status: [CFR21]

Section Number	Comments
-	-

HUMAN EXPOSURE

Natural Occurrence:

Amaranth is manufactured by the coupling of 4-amino-1-naphthalenesulphonic acid with 3-hydroxy-2,7-naphthalenedisulphonic acid [EFSA, 2010].

Reported Uses:

A sulfonic acid-based naphthylazo dye used as a coloring agent for foodstuffs and medicines and as a dye and chemical indicator. It was banned by the FDA in 1976 for use in foods, drugs, and cosmetics (From Merck Index, 11th ed) [ToxNet, 2010].

TOXICITY DATA

Organism	Test type	Route	Reported dose	Effect
Mouse	LD_{50}	Intraperitoneal	1gm/kg (1000mg/kg)	BEHAVIORAL: Somnolence (general depressed activity)
Rat	LD_{50}	Intraperitoneal	1gm/kg (1000mg/kg)	
Rat	LD_{50}	Intravenous	1gm/kg (1000mg/kg)	

[ToxNet, 2010]

Rat	LD_{50}	Oral	6gm/kg bw	
Mouse	LD_{50}	Oral	>10gm/kg	

[EFSA, 2010]

JECFA reviewed the use of Amaranth on several occasions, the latest being 1984 whereby an ADI of 0-0.5mg/kg bw was set. This was a reduction from 0.75mg/kg bw due to the availability of new information that suggested 50mg/kg bw in the diet caused no toxicological effects in rats over 2yrs including *in utero* exposure. This level was deemed appropriate to avoid renal calcification and hyperplasia that was observed at higher doses [JECFA, 1984]. However in 2010 EFSA reviewed the available literature on Amaranth. EFSA disagreed with JECFA with regards to a 2yr rat study, stating that the NOAEL was actually a LOAEL. Furthermore that review of the reproductive /developmental literature suggests that the most sensitive NOAEL is 15 mg/kg bw/day as identified in rabbits and rats. EFSA basing their assessment on the NOAEL of 15 mg/kg bw/day suggested an ADI of 0.15 mg/kg bw [EFSA, 2010].

A study by Poul *et al.,* (2009) assessed the faecal excretion and genotoxic effect of Amaranth (90% purity) in male Swiss mice (7/treatment group with 12 in the control group). Amaranth was administered via oral gavage at doses of 200 or 1000 mg/kg bw. Treatment of mice with Amaranth was not found to increase the frequency micronucleated colonic cells above the background incidence at either dose. Amaranth was also found to significantly increase the frequency of mitotic figures compared to water treated controls. Analysis of

concentrations of Amaranth and its main metabolite naphthionic acid in faeces showed that the parent compound reached at maximum concentration at 4h, and was no longer detectable at 24h, and naphthionic acid was eliminated 'in about 24h'. Furthermore, the metabolic rate was found to be greater in the lower dose group compared to the higher dose group [Poul *et al.,* 2009].

In Vivo Toxicity Status

A CD 1 (ICR) mouse study in pregnant and male mice fed Amaranth by gavage 'at the limit dose of 2000 mg/kg (10ml/kg) was performed to assess DNA damage. In pregnant mice increased DNA damage was observed at 3hrs in the colon, and less strongly at 6hrs in the lu ng (stomach, liver, kidney, bladder, brain and embryo showed no change). In male mice Increased DNA damage was observed in the colon at 3hrs . Effects were observed as low as 10 mg/kg. Other organs showing increased DNA damage by Amaranth were the stomach, liver, bladder, brain and bone marrow, however doses were at least 1000 mg/kg [Tsuda *et al.,* 2001].

A 21-day study in Sprague-Dawley rats assessed the effects of high-dose Amaranth inclusion into a range of diets (n=6/sex/group). Amaranth at 5% in stock r ations was found to have "little if any adverse effect on weight increment or gross appearance" but when included at this level in purified diet growth was significantly inhibited, fur appeared "unthrifty" and death resulted within 2 weeks. The authors ass essed the addition of supplements (including vitamins, salt mixture etc) and plant fibre-contianing materials (10% cellulose, 10% alfalfa meal, alfalfa residue, watercress powder or parsley powder . The toxic effects were only found to be abrogated by the plant fibre-containing diet. The authors highlighted that the 5% dose (50,000ppm) was far higher than was an expected exposure (although this was based on data from around the time of the study in 1974) [Ershoff & Thurston, 1974].

Carcinogenicity / Mutagenicity

IARC has classified Amaranth as an IARC Group 3 – the agent is not classifiable as to its carcinogenicity to humans [IARC, 1975].

In a series of research articles describing the contents of the Carcinogenicity Potency Database (CPDB) a study of A maranth was shown to have a TD_{50} of 632 mg/kg/day in rats, positive test result however was only observed for male and female rats combined [Gold *et al.,* 1993].

Groups of four ddY mice were used to test for DNA damage by Amaranth. Mice were orally dosed at 10 and 100 mg/kg and assessed at 3 and 24h post treatment. Amaranth was shown to induce dose-dependant DNA damage in the glandular stomach, colon, and urinary bladder using the Comet assay [Sasaki *et al.,*2002].

Dermal Toxicity

No data identified

Reproductive / Developmental toxicity

A number of reproductive animal studies were reviewed by EFSA in 2010. A NOAEL of 15 mg/kg bw/day in rats and rabbits was found to be the most sensitive and an ADI of 0.15 mg/kg bw was based on this [EFSA, 2010].

Inhalation Toxicity

No data identified

Other Relevant Studies

No data identified

Behavioural Data

No data identified

In Vitro Toxicity Status

Carcinogenicity / Mutagenicity

Amaranth failed to induce mutations in the Ames test [TA92, TA1535, TA100, TA1537, TA94 and TA98] , at concentrations up to 5 mg/plate, but was found to induce chromosome aberrations in a Chinese hamster fibroblast cell line [1 mg/ml] [Ishidate *et al.*, 1984].

Amaranth up to 1x10-3 M was found to be negative in the rat (male Spr ague-Dawley caesarean derived rats) *in vitro* hepatocyte primary culture/ DNA repair (HPC/DR) assay and also negative at levels up to 500 mg/kg bw in the *in vivo/in vitro* HPC/DR assay [Kornbrust & Barfknecht, 1985].

Amaranth (E123) and Allura red (E129), v ery important food azo dyes used in food, drug, paper, cosmetic and textile industries, were assessed for their genotoxic potential through comet assay in yeast cells. Comet assay was standardized by with different concentration of H_2O_2. Concentrations of Amaranth and Allura red were maintained in sorbitol buffer starting from 9.76 to 5,000 µg/mL and 1 × 104 cells were incubated at two different incubation temps. 28 and 37°C. Amaranth (E123) and Allura red (E129) were found to exhibit their genotoxic effect directly in Saccharomyces cerevisiae. No significant genotoxic activity was observed for Amaranth and Allura red at 28°C but at 37°C direct relation of Amaranth concentration with comet tail was significant and no pos. relation was seen with time exposure factor. At 37°C the minimum concentration of Amaranth and Allura red at which significant DNA damage observed through comet assay was 1,250 µg/mL in 2nd h post

exposure time. The results indicated that food colors should be carefully used in baking products as heavy concentration of food colors could affect the fermentation process of baking [Jabeen, *et al*., 2013].

REFERENCES

Gold *et al.,* (1993). The Fifth Plot of the Carcinogenic Potency Database : Results of Animal Bioassays Published in the General Literature through 1988 and by the National Toxicology Program through 1989. *Environmental Health Perspectives* **100:** 65-135 (Found in Legacy, 2010. Legacy National Tobacco Documents Library Collections. http://legacy.library.ucsf.edu Bates Number: 2063639106/9605).

EFSA (2010). Scientific Opinion on the Re-evaluation of Amaranth (E123) as a Food Additive. http://www.efsa.europa.eu/en/scdocs/doc/1649.pdf

Ershoff & Thurston (1974). Effects of Amaranth (FD & C No.2) Toxicity I the Rat. *The Journal of Nutrition* **104(7):** 937-42.

IARC (1975). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 8: Some Aromatic Azo Compounds -Summary of Data Reported and Evaluation.
http://monographs.iarc.fr/ENG/Monographs/vol8/volume8.pdf

Ishidate *et al.,* (1984). Primary mutagenicity screening of food additives currently used in Japan. *Fd. Chem. Toxic.*, **22**, 623.

Jabeen, *et al*. (2013). "Genotoxicity assessment of amaranth and allura red using Saccharomyces cerevisiae." Bull. Environ. Contam. Toxicol. **90**: 22-26.

JECFA (1984). WHO Food Additives Series 19.
http://www.inchem.org/documents/jecfa/jecmono/v19je02.htm

Kornbrust & Barfknecht , (1985). Testing of 24 Food, Drug, Cosmetic, and Fabric Dyes in the In Vitro and the In vivo/In Vitro Rat Hepatocyte Primary Culture/DNA Repair Assays. *Environmental Mutagenesis* **7:**101-120 (Found in Legacy, 2010. Legacy National Tobacco Documents Library Collections. http://legacy.library.ucsf.edu Bates Number: 2057934708/4727).

Legacy, 2002. Legacy National Tobacco Documents Library Collections. http://legacy.library.ucsf.edu Bates Number: 87689371/9387.

Poul *et al*., (2009). Lack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice. *Food Chem Toxicol.* **47(2):**443-8.

Sasaki *et al.,* (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutat Res.* **519(1-2):**103-19.

ToxNet, searched 2010. http://toxnet.nlm.nih.gov/

Tsuda *et al.,* (2001). DNA damage induced by red food dyes orally administered to pregnant and male mice. *Toxicological Sciences* **61:**92-99.