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# Yellow and Red Synthetic Food Dyes and Potential Health Hazards: A Mini Review

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REVIEW

## Abstract

Synthetic dyes are widely used in the food industry to maintain or restore the colour of food during processing or storage, the azo ones occupying the first place in terms of the extent of use. Compared to dyes obtained from natural sources, synthetic ones present relevant advantages, such as higher stability and lower cost. Despite these advantages, their use has been linked to side effects, such as allergic reactions, carcinogenic effects, behavioural and neurocognitive effects, but also medium and long-term toxicity. In this context, this review describes the most used yellow and red synthetic dyes, namely Tartrazine, Quinoline Yellow, Sunset Yellow FCF, Carmoisine/Azorubine, Amaranth, Ponceau 4R, Allura Red AC, as well as their applications in food products. Moreover, it aims to provide current data on the toxicity issues and the possible negative effects on children's behaviour of these dyes, based on the evaluations previously carried out by European Food Safety Authority (EFSA), but also on subsequent studies available in the literature.

**Keywords:** artificial food colours; azo dyes; food safety; toxicity; children's behaviour

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## INTRODUCTION

Food dyes or colour additives are substances that are added to food or drinks to give them colour or to restore their colour affected during the manufacturing, packaging, transport, or storage process. Since the visual appearance of the food product is important for the consumer, adding food colourings to food not only increase their visual appeal, but also stimulate the appetite. Thus, the colour used in processed foods influences the perception of flavour, which they visually reinforce (Oplatowska-Stachowiak & Elliott, 2017; Ramos-Souza et al., 2023; "Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives," 2008; Spence, 2023). Because the selection of foods and their acceptance by consumers are still influenced by colour, and food colouring pigments are generally unstable and do not maintain their colour during processing, artificial food dyes are widely used to colour beverages, foods, and sweets around the world (Silva et al., 2022; Stevens et al., 2015). Compared to natural dyes, synthetic food colours (SFCs) present a series of economically relevant characteristics for producers in the food industry, namely: accessibility - being easier to obtain, and with lower costs; stability to different agents – being more resistant to light, oxygen, and pH changes; better colouring properties and high colour stability (Oplatowska-Stachowiak & Elliott, 2017; Silva et al., 2022). In terms of the extent of use of synthetic dyes, in the USA,

for example, beverages rank first, due to the large volume of liquid consumed in a serving, but these dyes are also used in many other products, such as beverage syrups, cakes, cookies, ready-to-eat cereals, ice cream and sherbets, popsicles, gelatin, puddings, boxed dinners, snacks, and candy (Stevens et al., 2015).

Despite the advantages they present, the still extensive use of synthetic organic dyes in processed foods raises many concerns regarding the potential risks, due to the toxic potential and the numerous side effects reported in the medium or long term (Oplatowska-Stachowiak & Elliott, 2017; Silva et al., 2022). Thus, allergic reactions, as well as behavioural and neurocognitive effects, have been reported when using them. In addition, the link between the consumption of foods containing these dyes by children and the effects they have on their behaviour has made their use subject to increased attention (Doell et al., 2016; Silva et al., 2022). In the European Union (EU), the complete name and/or E number of the colour additives used in foods must be disclosed in the ingredients list. Colourants range from E100 (Curcumin) to E180 (Litholrubine BK) (Durazzo et al., 2022). Also, for six synthetic food dyes (Table 1), the food labels containing them must include additional information, namely that they can negatively affect children's activity and attention ("Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on Food Additives," 2008).

**Table 1.** Selection of studies on the effects of "Southampton Six" food dyes on the behaviour of children and youth

| Dye | Study type | Country | Population | Outcomes | Reference |
|---|---|---|---|---|---|
| **Tartrazine**<br>**Sunset Yellow FCF**<br>**Carmoisine/Azorubine**<br>**Ponceau 4R** | Randomised, placebo controlled, double blind, crossover challenge study | UK | 1873 children aged 3 years | Significantly greater increases in hyperactive behaviour during the SFCs food challenge period than during the placebo period, based on parental reports | (Bateman et al., 2004) |
| **Tartrazine**<br>**Quinoline Yellow**<br>**Sunset Yellow FCF**<br>**Carmoisine/Azorubine**<br>**Ponceau 4R**<br>**Allura Red AC** | Randomised, double-blinded, placebo-controlled, crossover trial | UK | 297 children aged 3 and 8/9 years from the general population | Significantly increased hyperactivity in children who consumed SFCs, versus placebo | (McCann et al., 2007) |
| **Tartrazine**<br>**Quinoline Yellow**<br>**Sunset Yellow FCF**<br>**Carmoisine/Azorubine**<br>**Ponceau 4R**<br>**Allura Red AC** | Meta-analyse of randomized controlled trials | Variable | Participants aged 3 to 18 years with a diagnosis of attention deficit hyperactivity disorder (ADHD) of any subtype | Beneficial effects on ADHD symptoms following the exclusion of artificial food dyes from the diet | (Sonuga-Barke et al., 2013) |
| **Tartrazine**<br>**Sunset Yellow FCF**<br>**Carmoisine/Azorubine**<br>**Ponceau 4R** | Randomised, double-blind, placebo-controlled trial | Hong Kong | 70 boys and 60 girls with a mean age of 8.64 years from the general population | No significant associations between SFCs consumption and children's behaviour | (Lok et al., 2013) |
| **Tartrazine**<br>**Sunset Yellow FCF**<br>**Allura Red AC** | Double-blind placebo-controlled crossover trial | USA | 18 participants with ADHD and 41 controls, 18–24 years old | AFC exposure: impairment of brain wave activity and ADHD symptoms in the ADHD group; no effects in the control group | (Kirkland et al., 2022) |

In addition, since 2008, the UK has a voluntary ban on the same six food colours because research supported by the UK Food Standards Agency (FSA) suggested that consumption of mixtures of food colours and the preservative sodium benzoate could increase hyperactivity in some children (Bakthavachalu et al., 2020). Table 1 summarizes

some of the studies that investigated the effects of these six so-called "Southampton colours" on the behaviour of children and young people, including two studies (Bateman et al., 2004; McCann et al., 2007) that were the subject of EFSA's 2008 assessment (EFSA, 2008).

Furthermore, many other adverse health effects such as DNA damage, carcinogenesis and high genotoxicity have been reported following excessive consumption of synthetic dyes (Han et al., 2021). Although the risk assessment of food dyes is carried out by the regulatory agencies, the European Food Safety Authority (EFSA), and, the Food and Drug Administration (FDA; USA), respectively (Barciela et al., 2023), safety evaluations are not definitive, periodic re-evaluations being required so that the level of exposure to synthetic dyes in foods for which there is an established legislative use level does not represent a concern for health. Considering the importance of understanding the risks associated with the consumption of artificial colours in food, this review aims to discuss the characteristics of yellow and red SFCs, their applications in food and possible adverse health effects, emphasizing the potential hazards of oral exposure in children, as the main consumers of brightly coloured processed foods.

## AZO FOOD COLOURS: PROPERTIES, USES, SAFETY, AND ADVERSE HEALTH EFFECTS

Azo compounds are the most frequently used artificial dyes, representing over 60% of production worldwide (Ramos-Souza et al., 2023; Silva et al., 2022). They are characterized by the presence in their molecule of one or more chromophoric groups –N=N– that unit two symmetrical and/or asymmetrical azo alkyl or aryl radicals (Ramos-Souza et al., 2023). Azo food colours used in food products are shown in Figure 1.

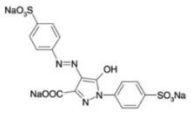
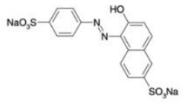
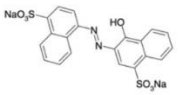
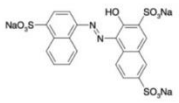
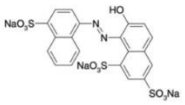
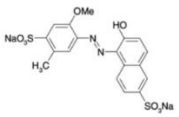
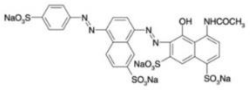
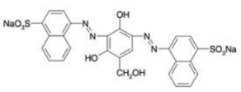
| Common name | INS | ENS | FD&C | Colour | Molecular structure |
|---|---|---|---|---|---|
| Tartrazine | 102 | E 102 | Yellow #5 |  |  |
| Sunset Yellow FCF | 110 | E 110 | Yellow #6 |  |  |
| Carmoisine/Azorubine | 122 | E 122 | |  |  |
| Amaranth | 123 | E 123 | Red #2 |  |  |
| Ponceau 4R | 124 | E 124 | |  |  |
| Allura Red AC | 129 | E 129 | Red #40 |  |  |
| Brilliant Black BN | 151 | E 151 | |  |  |
| Brown HT | 155 | E 155 | |  |  |

**Figure 1.** The common name, numbering, colour, and molecular structure of eight azo dyes used in the food industry. ENS–European Numbering System; FD&C–Food, Drug and Cosmetic; INS–International Numbering System

Among the azo dyes permitted in food, the yellow and red ones are widely used within the food industry compared to the brown ones (EFSA, 2010a). Table 2 shows the applications of yellow and red azo dyes in the food industry.

**Table 2.** Food uses of yellow and red azo dyes

| Azo dye | Food products | References |
|---|---|---|
| **Tartrazine** | soft drinks, juices, candies, jellies, jams, flavoured chips, popcorn, cakes, ice cream, soups, sauces, condiments, and cereals | (Dey & Nagababu, 2022; Rovina, Siddiquee, et al., 2017a) |
| **Sunset Yellow FCF** | aromatized and fermented beverages, candies, chewing gum, jams, jellies, preserved fruits, ice cream, baked goods, confectionery, desserts, soups, sauces, condiments, fish roe, fish paste, and crustaceans | (Barciela et al., 2023; Dey & Nagababu, 2022; Rovina, Acung, et al., 2017) |
| **Carmoisine/ Azorubine** | beverages, ice cream, candies, chewing gum, chocolate, fruit syrup, canned red fruits, jellies, jams, yogurts, Swiss rolls, cheesecakes mixes, chips, breadcrumbs, ketchup, sauces, seasoning, seafood, fish roe, fish paste, and crustacean paste | (Khataee et al., 2022; Monisha et al., 2023; Silva et al., 2022) |
| **Amaranth** | soft drinks, syrups, some alcoholic drinks (including spirits, aperitif wines), ice cream, cake mixes, tinned fruit pie fillings, soups, prawns, cereals, salad dressings, chewing gums, jams, chocolate, coffee, and fish roe | (Rovina, Siddiquee, et al., 2017b; Silva et al., 2022) |
| **Ponceau 4R** | alcoholic beverages, flavoured drinks, powder juices, chewing gum, milk products, edible ices, desserts, fruit syrups, red fruit preserves, jellies, jams, fine baked goods, pastries, confectionery, soups, edible cheese rind, sausages, appetizers, seasoning, sauces, seafood, fish roe, and fish paste | (Mohamed et al., 2023; Silva et al., 2022) |
| **Allura Red AC** | flavoured drinks, alcoholic beverages, syrups, powder mixes, chewing gum, sweets, candies, ice cream, bakery products, pastry products, confectionery, jellies, gelatins, dairy foods, flavoured fermented milk products, edible cheese rinds, desserts, preserves of fruits, baked crustaceans, seafood, sausages, appetizers, sauces, spices, and soups | (Barciela et al., 2023; Mohamed et al., 2023; Silva et al., 2022) |

Azo dyes can be easily reduced by intestinal flora under specific circumstances, resulting in the formation of twenty different carcinogenic aromatic amines. These amines have the potential to cause DNA mutations, harm the human body, trigger the development of malignant tumours, and induce neurotoxic effects (Zhang et al., 2022).

An investigation was conducted to examine the ability of bacterial strains from the human gut microbiome to reduce four azo dyes (Allura Red, Amaranth, Sunset Yellow, and Tartrazine). The results revealed that Amaranth was reduced by the highest number of bacterial species, while Tartrazine was reduced by the lowest number. Additionally, certain correlations were observed between the rates of reduction and the structure of the dyes. Among the products resulting from the azo reduction were sulfanilic acid (from the reduction of Sunset Yellow and Tartrazine), 1-amino-2-naphthol-6-sulfonic acid (from the reduction of Allura Red and Sunset Yellow), 4-aminonaphthalene-1-sulphonic acid (from Amaranth reduction), potential disruptors of the intestinal microbial ecosystem (Elder et al., 2023). Since the toxicity of azo dyes may be mediated by the parent compound, or the metabolites generated during their reduction, it is critical to investigate the potential adverse health effects of these substances.

## Yellow azo food colours

*Tartrazine*

Tartrazine, a lemon-yellow, water-soluble mono azo dye, was first discovered in 1884 and is derived from coal tar (Ameur et al., 2019; Rovina, Siddiquee, et al., 2017a). The synthesis of Tartrazine consists in the condensation of phenylhydrazine-*p*-sulfonic acid with oxalacetic diethyl ester, the combination of the product with diazotized sulfanilic acid and then the hydrolysis of the ester with sodium hydroxide. Another method is the condensation of two moles of phenylhydrazine-*p*-sulfonic acid with one mole of dihydroxytartaric acid (Barciela et al., 2023; Rovina, Siddiquee, et al., 2017a).

It is the second most used food dye to colour many beverages and foods (Table 2) in a bright yellow, being considered a cheaper substitute for natural food colourants like curcumin and saffron in developing countries (Ameur et al., 2019; Sambu et al., 2022; Stevens et al., 2015). Tartrazine is the most investigated dye, for which the Joint FAO/WHO Expert Committee on Food Additives (JECFA) carried out the first risk assessment in 1964 and established an acceptable daily intake (ADI) of 0–7.5 mg/kg body weight per day (mg/kg bw/day) in 1966 (Ramos-Souza et al., 2023; Rovina, Siddiquee, et al., 2017a). In 2009, following the safety re-evaluation of Tartrazine by EFSA, the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel) concluded that there was no reason to revise the ADI of 7.5 mg/kg bw/day, given that at the maximum reported use levels, the refined intake estimates were below the ADI (EFSA, 2009d).

Adverse effects reported for Tartrazine include allergic skin reactions such as urticaria, angioedema and eczema, and, also, disorders of the respiratory tract, gastrointestinal tract, and central nervous system. Many studies have shown that Tartrazine causes hives and asthma symptoms, and people who are allergic to aspirin are more likely to be allergic to Tartrazine as well. Tartrazine, but also Allura Red AC and Sunset Yellow FCF, are the azo dyes for which there is the highest probability of causing allergic reactions, being also the most consumed azo dyes (Dey & Nagababu, 2022; John et al., 2022). In a 5-year-old girl who suffered from recurrent reactions accompanied by urticaria, angioedema, headaches, dyspnea, loss of consciousness and abdominal pain that were not eradicated, her diet diary showed that the symptoms appeared after ingestion of colourful sweets, such as candies and jellybeans. Open challenge tests with Tartrazine (300 μg), aspirin (50 mg), and acetaminophen (10 mg) were positive, thus intolerance to azo dyes and nonsteroidal anti-inflammatory drugs, such as aspirin, was diagnosed (Inomata et al., 2006).

Results of oral challenge studies used to determine the prevalence of food or food additive sensitivity in a subject population have indicated a prevalence between 1% and 68%. Although many previous studies have reported positive reactions in the case of Tartrazine, it is not clear yet whether these are the result of breakthrough urticaria from withholding the antihistamines or a false-positive reaction due to the order effect of administering the placebo first (Rajan et al., 2014). According to the FDA, Yellow #5 could cause an allergic reaction in 1 in 10000 people, and the ADI established for this dye is 5 mg/kg bw/day. In a scientific opinion, EFSA estimates that the frequency of intolerance to Tartrazine is <1% of cases of food-induced urticaria and angioedema (EFSA, 2010a).

Potential deleterious consequences of Tartrazine on digestion were reported by Ameur et al. (2019), who evaluated the effect of this dye on the enzymatic activities of amylase, lipase, and protease after subchronic ingestion in mice. Although at a dose close to the ADI the activities of digestive enzymes were not affected, the consumption of Tartrazine in high doses could induce a decrease of proteases activities (trypsin and chymotrypsin) *in vivo*. Among small children, a strict control of the dose of Tartrazine in high-consumption foods is required, even more so as their oral exposure to this colour could also be done through the toys and accessories for children that contain it (Ameur et al., 2019).

In an *in vivo* study in the animal model *Caenorhabditis elegans*, exposure to Tartrazine produced aging-related damage such as oxidative stress and lipofuscin accumulation, with Tartrazine also shortening lifespan and down-regulating longevity genes (Guerrero-Rubio et al., 2023). Also, adverse effects such as cardiomegaly, hepatorenal damage and splenic pigmentation have been reported for Tartrazine, and its intake could be strongly associated with mutagenicity in the form of genetic mutations and chromosomal abnormalities (Sambu et al., 2022).
Regarding the carcinogenic potential, for Tartrazine there is no conclusive evidence that this dye causes cancer, but it may contain carcinogenic contaminants, such as benzidine, 4-aminobiphenyl and 4-aminoazobenzene (Dey & Nagababu, 2022; John et al., 2022). The presence of carcinogenic aromatic amine residues in azo dyes is allowed if they are present in low levels, which are supposed to be safe (Dey & Nagababu, 2022).

*Sunset Yellow FCF*

Sunset Yellow, a monoazo dye, is presented in the form of powder, or in the form of granules, orange-red in colour, soluble in water and sparingly soluble in ethanol. It has the chemical name disodium;2-hydroxy-1-(4-sulphonatophenylazo) naphthalene-6-sulphonate and is obtained by diazotizing 4-aminobenzenesulfonic acid using hydrochloric acid and sodium nitrite, and subsequent coupling with 6-hydroxy-2-naphthalene-sulfonic acid (Rovina, Acung, et al., 2017).

In 2014, the new ADI for Sunset Yellow is established by EFSA at a dose of 4 mg/kg bw/day, replacing the temporary ADI of 1 mg/kg bw/day, previously established by the ANS Panel in the 2009 scientific opinion (EFSA, 2014).

Among the health problems reported for Sunset Yellow are allergic reactions, intolerances, behavioural disorders in children, or sleep disturbances (Barciela et al., 2023; Ramos-Souza et al., 2023; Silva et al., 2022). In addition, this dye may be teratogenic, genotoxic, and carcinogenic (Barciela et al., 2023; Rovina, Acung, et al., 2017). Intolerance reactions have been reported to a small extent for this dye, in sensitive individuals, and have included urticaria, angioedema, wheezing, and leukocytoclastic vasculitis. Following a request from the European Commission (EC), in 2010, the Panel on Dietetic Products, Nutrition and Allergies has considered all food azo-colours (Tartrazine, Sunset Yellow, Carmoisine, Amaranth, Ponceau 4R, Allura Red AC, Brilliant Black BN, Brown FK, Brown HT, and Litholrubine BK), and concluded in its scientific opinion that "oral consumption of the food colours under consideration, either individually or in combination, is unlikely to trigger severe adverse reactions in human subjects at the current levels of use" (EFSA, 2010a).

In terms of purity specifications, Sunset Yellow FCF may contain residues of potentially carcinogenic substances such as benzidine, 4-aminobiphenyl and 4-aminoazobenzene at low levels, that should be considered safe (Dey & Nagababu, 2022). Both EFSA, and FDA, noted that no carcinogenic potential of this dye was observed in mice and rats in long-term feeding studies (Barciela et al., 2023; EFSA, 2014). The International Agency for Research on Cancer (IARC) has classified this substance as not carcinogenic to humans (Class 3) (Ramos-Souza et al., 2023).

Although, based on previous *in vivo* and *in vitro* studies, there are no data on the genotoxic activity of Sunset Yellow, it can be considered that there could be a risk of exposure to aromatic amines that are associated with genotoxicity or carcinogenicity, the azo-reduction products of this dye, such as 1-amino-2-naphthol-6-sulfonic acid, sulfanilic acid, and N-acetylated forms, being found mainly in urine (EFSA, 2009c; Rovina, Acung, et al., 2017).

Experimental data have shown that Sunset Yellow in high doses has xenoestrogenic activity and can contribute to the development of cholestasis, similar to Tartrazine, being able to predispose to the development of primary biliary cirrhosis, in combination with other factors (Axon et al., 2012). Since oestrogenic activity has been demonstrated in an *in vitro* model system, and in long-term studies, including an *in utero* phase in mice and rats, no effects on endocrine and reproductive organs were observed, EFSA did not consider the results of this study in the risk assessment (EFSA, 2014).

Regarding teratogenic potential, the morphological and skeletal malformations induced due to *in ovo* administration of Sunset Yellow and Tartrazine at dose 14 times the ADI of both (1.575 mg/egg, and 0.375 mg/egg, respectively), was studied by El-Borm et al. (2020). Malformations in the feathers, head and limbs were revealed, as well as an evident reduction in the weight and length of the embryos. In addition, there were skeletal malformations in embryos injected individually with the two dyes, at the level of the skull cap, beak, vertebral column, sternum, and ribs (El-Borm et al., 2020).

**Red azo food colours**

*Carmoisine/Azorubine*

Carmoisine is a red monoazo dye, which gives a red to brown shade in applications, soluble in water, slightly soluble in ethanolic solutions, and insoluble in vegetable oil, having high stability against pH, heat, light, and oxygen. It has the chemical name disodium;4-hydroxy-3-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-1-sulfonathenate (Barciela et al., 2023; Khataee et al., 2022). It is used in food products which, after fermentation, are subjected to thermal treatments (Khataee et al., 2022).

The use of Carmoisine is subject to strict rules, in Japan, USA and Canada, not being permitted in food products, while in EU, products containing it must carry the warning label (Mohamed et al., 2023). The established ADI for Carmoisine/Azorubine is 4 mg/kg bw/day. In 2015, EFSA performed the refined exposure assessment for this dye, and concluded that the ADI was not exceeded for any of the population groups, neither at the mean, nor at the higher exposure level (EFSA, 2015a).

Due to the unavoidable presence of the carcinogen β-naphthylamine, Carmoisine has been associated with health problems such as hyperactivity in children and increased risk of cancer in animals. In addition, excessive consumption could lead to other side effects, such as skin rashes, cholinesterase inhibition and breathing difficulties (Mohamed et al., 2023; Peksa et al., 2015). It can also modify biochemical markers of renal and hepatic functions and induce oxidative stress. In high doses, it can have toxic, carcinogenic and mutagenic potential (Micheletti et al., 2020).

The toxic effects and carcinogenicity of Carmoisine were evaluated in a mouse animal model, being administered orally, in four different doses (control, low, medium and, respectively, high, equivalent to 0, 4, 200 and, respectively, 400 mg/kg bw), for 120 days. Medium and high doses led to a significantly decrease in body weight, increased levels of biochemical parameters (serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total protein, globulin, urea and creatinine), altered levels of haematological parameters (increased number of platelets, white blood cells and monocytes, and drastic reduction of haemoglobin (Hb) and red blood cells), and changes in the tumour-related gene expressions (increased Bcl-x and PARP, decreased p53), in the mice of the two groups, compared to the control group. The results of the study confirmed that the safe level of this food dye was a dose equivalent to the ADI (4 mg/kg bw/day), while high doses have been associated with renal failure, hepatotoxicity, and liver tumours (Reza et al., 2019).

Available reports showed that excessive use of Carmoisine triggers anxiogenic behaviour in mice by inducing oxidative stress. Thus, in the study by Subramaniyan et al. (2022), brain tissue damage induced by Carmoisine was confirmed by the imbalance in neurochemicals (significant increase in glutamate and decrease in gamma-aminobutyric acid ($p < 0.001$)) and depletion of antioxidant enzymes levels (catalase and superoxide dismutase) ($p < 0.05$)) (Subramaniyan et al., 2023).

A recent study investigated the effects of exposure to Carmoisine at a wide range of doses, which included recommended doses, as well as overexposure (from 4 to 2000 ppm), on the embryonic development of zebrafish. Carmoisine caused severe malformations, reduced the eye height and diameter, increased the free oxygen radicals, apoptotic cells, and lipid accumulation, reduced the locomotor activity in a dose-dependent manner, and, at the highest dose, it decreased blood flow rate. Therefore, exposure to high doses of Carmoisine can lead to increased oxidative stress and an increased level of some metabolites that cause neurodegenerative effects and behavioural changes (Kiziltan et al., 2022).

Regarding reproductive effects, Carmoisine showed hazardous effects on fertility, namely a decrease in sperm count, when it was administered orally to male Sprague-Dawley albino rats in doses of 50 mg/kg bw/day or higher.

Histopathological studies indicated a deleterious effect with medium and high doses of Carmoisine (250, and 500 mg/kg bw/day, respectively) (Montaser et al., 2018).

In terms of sensitivity to this food dye, there are no data and no well-documented cases of intolerance reactions after oral exposure have been reported (EFSA, 2010a).

*Amaranth*

Amaranth is presented as reddish-brown powder or granules, soluble in water and sparingly soluble in ethanol, having the chemical name trisodium;3-hydroxy-4-[(4-sulfonatonaphthalen-1-yl)diazenyl]naphthalene-2,7-disulfonate (Silva et al., 2022).

The use of Amaranth is very restricted, in only a few foods and drinks, both in the EU and in Japan, while in the USA this dye has been banned since 1976. The World Health Organization (WHO) has established the ADI for Amaranth at 0.5 mg/kg bw/day (Elder et al., 2023; Mohamed et al., 2023; Mohammadi et al., 2022).

Amaranth was previously evaluated by JECFA in 1972, 1975, 1978 and 1984, and by the Scientific Committee for Food (SCF) in 1976, 1979 and 1983. In 1984, SCF established an ADI for Amaranth of 0-0.8 mg/kg bw/day based on the results of a 90-day study in rats, while JECFA allocated an ADI of 0-0.5 mg/kg bw/day amaranth, based on a long-term carcinogenicity study in rats (EFSA, 2010b). In 2010, following the re-evaluation of Amaranth safety, the ANS Panel established an ADI for this dye of 0.15 mg/kg bw/day, concluding that the anticipated dietary exposure of the adult population at a high level (97.5 percentile) may exceed the ADI. Considering this conclusion, in 2013 EFSA carried out a refined exposure assessment of Amaranth, concluding that the refined exposure estimates for children, as well as adults and the elderly, remain below the ADI (EFSA, 2013).

Among the adverse effects reported in the case of excessive Amaranth consumption are hyperactivity in children, restlessness, anxiety, dizziness, allergies, and cancer (Mohamed et al., 2023; Mohammadi et al., 2022). However, IARC has classified Amaranth as non-carcinogenic to humans (Class 3) (Ramos-Souza et al., 2023). Intolerance reactions have been documented in sensitive persons to a lesser level for Amaranth, similar to the reactions that have been described for Sunset Yellow (EFSA, 2010a). Furthermore, it has been reported that this food colour exhibits significant genotoxicity, cytostaticity, and cytotoxicity (Alizadeh et al., 2022).

The genotoxic, cytotoxic, and cytostatic potential of Amaranth was tested at 0.02–8 mM in human peripheral blood cells *in vitro*. At the highest concentration, the level of sister chromatid exchanges/cell was increased 1.7 times over the control level, but Amaranth showed a genotoxic effect at all tested concentrations. These results suggested that the two symmetrical rings in the chemical structure of Amaranth could act as intercalating agents to the double strand of DNA, with the consequence of chromosomal breaks and high genotoxicity. At the highest concentrations, 4 and 8 mM, Amaranth was found to have both cytostatic and high cytotoxic effects, the strong positive correlation between the proliferation rate index and the mitotic index supporting these results (Mpountoukas et al., 2010).

In the study by Sarikaya et al. (2012), the somatic mutation and recombination test (SMART) was used in *Drosophila melanogaster* to assess the genotoxic potential of Amaranth. Various concentrations of Amaranth produced positive results in this test. Inconsistent findings were obtained for small single spots, total multiple wing hair spots and total spots, at the lowest concentration (1 mg/mL). Nevertheless, the frequency of all mutation types was noticeably increased by a high concentration of Amaranth (50 mg/mL), suggesting that this dye might induce genotoxic effects (Sarikaya et al., 2012).

The developmental toxicity of Amaranth on zebrafish embryos was evaluated by Jiang et al. (2020). Amaranth caused embryonic lethality in zebrafish in a dose-dependent manner. Higher doses induced morphological alterations such as pericardial oedema, yolk sac oedema, body malformation, and spine curvature; in addition, incubation difficulties were reported, which ultimately led to developmental abnormalities and teratogenic potential. The development of oedema can be attributed to the azo dyes' high-water solubility, which induces an excessive uptake of water in the embryo and disturbs osmotic equilibrium (Jiang et al., 2020).

Research on the interaction of Amaranth and human serum albumin (HSA) revealed that the dye's binding to HSA caused a conformational change in HSA, resulting in the disruption of hydrogen bond networks (Rovina, Siddiquee, et al., 2017b). According to the findings of a biophysical investigation examining the interaction between Amaranth and Hb, the presence of Amaranth altered the polarity surrounding tryptophan residues but had little effect on the polarity surrounding tyrosine residues. Results from three-dimensional fluorescence, Fourier transform infrared spectroscopy, and circular dichroism revealed that the binding reaction induced structural changes in Hb (Basu & Kumar, 2015).

*Ponceau 4R*

Ponceau 4R is a monoazo dye, in the form of reddish powder or granules, soluble in water and sparingly soluble in ethanol, having the chemical name trisodium;2-hydroxy-1-(4-sulfonato-1-naphthylazo)-naphthalene-6,8-disulfonate. It is obtained by coupling diazotized naphthionic acid to G acid (2-naphthol-6,8-disulfonic acid), followed by the conversion of the coupling product to the trisodium salt (Silva et al., 2022).

According to JECFA, the ADI for this dye is 4 mg/kg bw/day. EFSA established for Ponceau 4R an ADI of 0.7

mg/kg bw/day (Ramos-Souza et al., 2023). In 2009, the EFSA Panel ANS re-evaluated Ponceau 4R and concluded that, at maximum levels of use, intake estimates for adults at the high percentile (97.5th) and for children aged 1 to 10 years at the mean and high percentiles (95th/97.5th) were generally above the ADI, which required the modification of the maximum permitted levels of Ponceau 4R, depending on the food category, in Annex II to Regulation (EC) No. 1333/2008. As a result of changes, in the 2015 refined exposure assessment for Ponceau 4R, none of the exposure estimates exceeded the ADI of 0.7 mg/kg bw per day in any population (EFSA, 2015b).

Previous research has indicated that Ponceau 4R is associated with adverse health effects, such as allergies, increased rates of hyperactivity in children, and cancers, when consumed in excess, due to the poisonous and carcinogenic aromatic rings and azo functional groups in its structure (de Moraes et al., 2018; Qin et al., 2020). Similar to Tartrazine, only a few cases of intolerance reactions have been reported for Ponceau 4R, including urticaria, angioedema, wheezing and leukocytoclastic vasculitis, so that, in 2010, the Panel on Dietetic Products, Nutrition and Allergies concluded that oral consumption of this food dye, individually or in combination, is unlikely to trigger severe adverse reactions in human subjects at the levels of use (EFSA, 2010a).

As allergic reactions to food additives are often suspected by families, the aim of a recent study was to investigate the results of oral food challenge (OFC) in a paediatric cohort, including twenty-three patients who underwent an open OFC to Carmine Red (E 120), Cochineal Red (E 124), Erythrosine (E 127), Patent Blue V (E 131), Tartrazine (E 102), Sunset Yellow (E 110), and/or sodium benzoate (E 211). Although the diagnosis of IgE-mediated allergy was formally confirmed in only one OFC out of forty-five (2.2%), for a patient developing localized urticaria 60 minutes after ingestion of Carmine and Cochineal Red (E 120, E 124), which was resolved with oral antihistamine treatment, the families remained suspicious regarding ready-made products, despite the fact that the results of this study, and literature, support the rarity of hypersensitivity to food additives (Lemoine et al., 2020).

Ponceau 4R purity requirements permit the presence of unknown unsulfonated aromatic amines at amounts of up to 50 μg/kg of food. In spite of the fact that certain aromatic amines might be linked to genotoxicity or even carcinogenicity, the ANS Panel observed that Ponceau 4R did not show any evidence of carcinogenicity in long-term studies. Based on a neurotoxicity and reproductive toxicity study of this dye administered to mice in the diet, the ANS Panel concluded that there were no consistent neurobehavioural findings between sexes, and Ponceau 4R has no adverse effects on reproduction or development up to a dose of 1250 mg /kg bw/day (EFSA, 2009a).

Although there is no indication of carcinogenicity, genotoxicity, neurotoxicity, or reproductive and developmental toxicity for Ponceau 4R at the permissible dietary exposure, toxicity is dose-dependent, and some food colours that are typically considered to be "non-toxic", may become harmful when exposure is in large amounts (Barciela et al., 2023; EFSA, 2009a).

*Allura Red AC*

Allura Red AC is a monoazo dye, in the form of dark red powder or granules, soluble in water and poorly soluble in 50% ethanol, with the chemical name disodium;6-hydroxy-5-((2-methoxy-5-methyl-4-sulfophenyl)azo)-2-naphthalenesulfonate (Barciela et al., 2023; Mohamed et al., 2023; Silva et al., 2022). It is synthesized by coupling diazotized 5-amino-4-methoxy-2-toluenesulphonic acid with 6- hydroxy-2-naphthalene sulphonic acid (Rovina et al., 2016).

Allura Red AC was evaluated by JECFA in 1980 and by SCF in 1984 and 1989, both committees establishing an ADI of 0-7 mg/kg bw/day. The safety of Allura Red AC was re-evaluated in 2009 by EFSA, considering the results of two studies, one of which reported *in vivo* genotoxicity in mice, and the second (McCann et al., 2007) reported increased hyperactivity in children aged 8 to 9 years, exposed to a mixture that included Allura Red AC. Noting that Allura Red AC was negative in *in vitro* genotoxicity as well as in long-term carcinogenicity studies, and the findings of the study by McCann et al. (2007) cannot be used as a basis for altering the ADI, the ANS Panel concluded that these results does not give reason for revising the ADI of 7 mg/kg bw/day (EFSA, 2009e). Given the presence of aromatic rings and azo structure in Allura Red, precise dosage regulation is crucial for the human body (Mohamed et al., 2023).

Although most food dyes have not been linked to any negative effects in toxicity studies, the presence of contaminants in the dyes is a source of concern. Potentially hazardous impurities found in Allura Red include benzidine, 4-aminobiphenyl, and 4-aminoazobenzene. These contaminants are permitted in dyes due to their low concentrations, which are considered to be secure (Dey & Nagababu, 2022).

Consumption of Allura Red has been linked to sporadic behavioural changes in both people and animals, including an increase in hyperactivity in children's behaviour (Barciela et al., 2023). Other reported adverse effects include allergies, asthma, brain damage, and cancer. Furthermore, it has been claimed that this dye has detrimental effects on both the liver and the kidneys (Mohamed et al., 2023). As with Carmoisine, EFSA considered that there are no available data on sensitivity to Allura Red AC and no well-documented cases of intolerance reactions following oral exposure have been reported (EFSA, 2010a).

The neurotoxicity of Allura Red was evaluated in the study by Noorafshan et al. (2018). For six weeks, rats were administered by gavage low (ADI) and high doses (10 x ADI) of Allura Red, with or without taurine. As indicated by

the results, administration of this dye in low dose equivalent to the ADI may impair memory and spatial learning, in addition to decreasing the number of glial cells. Learning and memory were adversely affected by a high dose of Allura Red, which also caused structural damage to the medial prefrontal cortex, including the loss of cortex volume, cells, and the dendritic tree (Noorafshan et al., 2018).

Recent research examined the effect of Allura Red on antioxidant, haematological and biochemical parameters in male Swiss albino mice, after 30, 45 and 60 days of treatment, respectively, with a dose of 172.2 mg/kg bw/day. Following a 60-day treatment period, evident reductions were observed in platelets, haematocrit percentage, white blood cell count, red blood cell count, and Hb. Additionally, catalase, superoxide dismutase, and glutathione levels decreased significantly in the treatment groups. Conversely, serum alkaline phosphatase, serum total protein, albumin, mean corpuscular Hb concentration, aspartate aminotransferase, alanine aminotransferase, glutathione peroxidase, and lipid peroxidase values all increased significantly. The variations of different parameters were very significant after 60 days of treatment, the toxicity of Allura Red being evident at the level of the given dose (Sharma et al., 2022).

Oxidative stress-mediated renal and hepatic toxicity and the genotoxic effect of Allura Red and Sunset Yellow in male rats were investigated in the study by Khayyat et al. (2018). The doses administered orally over a period of four weeks did not exceed the ADI of the two dyes. The findings revealed that azo dye-treated animals had elevated levels of biochemical markers of hepatic and renal function (aspartate aminotransferase, alanine aminotransferase, urea, uric acid, and creatinine), a discernible increase in malondialdehyde, and a substantial reduction in total antioxidant levels, when compared to the control group. Both dyes had detrimental effects on the liver and kidney of albino rats, resulting in changes to their fine structure and histology. Specifically, expression of Bcl2 was downregulated, while COX2 was upregulated. In summary, the results of this study indicated that male Wistar albino rats exposed to Allura Red (7 mg/kg bw/day) and Sunset Yellow (2.5 mg/kg bw/day) developed pathological and physiological hepatic and renal toxicity. While Allura Red does showed no genotoxicity, Sunset Yellow exhibited a slight genotoxic effect (Khayyat et al., 2018).

## QUINOLINE DERIVATIVE FOOD COLOURS: PROPERTIES, USES, SAFETY, AND ADVERSE HEALTH EFFECTS

### Yellow food colours of the quinophthalone class

The chromophoric system of quinoline dyes consists of the quinophthalone or 2-(2-quinolyl)-1,3-indandione heterocyclic ring system. Quinoline Yellow (Figure 2, Table 3) is the sole dye within this class that holds significance in food colouring applications (Damant, 2011).

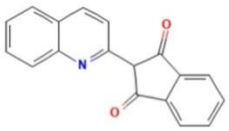
| Common name | INS | ENS | FD&C | Colour | Molecular structure |
|---|---|---|---|---|---|
| Quinoline Yellow | 104 | E 104 | Yellow #10 |  |  |

**Figure 2.** The common name, numbering, colour, and molecular structure of Quinoline Yellow. ENS–European Numbering System; FD&C–Food, Drug and Cosmetic; INS–International Numbering System

**Table 3.** Food uses of Quinoline Yellow

| Quinoline dye | Food products | Reference |
|---|---|---|
| **Quinoline Yellow** | flavoured drinks, alcoholic beverages, chewing gum, marmalades, jams, jellies, desserts, ice cream, pastry and fine bakery products, confectionery, decorations and coatings, flavoured fermented milk products, cheeses, edible cheese rind, seasoning, smoked fish, shells, fish paste, crustacean paste, fish roe | (Silva et al., 2022) |

*Quinoline Yellow*

Quinoline yellow is a bright yellow dye with green shade, that is available as granules or powder. It is poorly soluble in ethanol and soluble in water (Dey & Nagababu, 2022; Silva et al., 2022). It has the chemical name disodium

salts of the disulphonates of 2-(2-quinolyl)indan-1,3-dione and is synthesized by condensing quinaldine(2-methylquinoline) with phthalic anhydride at a temperature of approximately 220 °C in the presence of zinc chloride. The resulting product is then sulfonated at the quinoline ring using oleum at a temperature of around 90 °C. The disulfonated product is the predominant component of the obtained mixture, although monosulfonates and trisulfonates are also present (Damant, 2011).

Previous evaluations of Quinoline Yellow were carried out by the JECFA in 1975, 1978, and 1984, as well as by the EU SCF in 1984. Both committees established an ADI range of 0-10 mg/kg bw/day (EFSA, 2009b). The regulations governing the use of Quinoline Yellow in Europe and Yellow #10 in the USA differ. Although this dye is authorised for use in food and medicine inside the EU, its usage is restricted to medicine and cosmetics in the US, and it is not permitted in food. In contrast, its usage in food or medication is completely prohibited in Japan (Pérez-Ibarbia et al., 2016).

Mild genotoxic effects of Quinoline Yellow were observed in two *in vitro* cell models, namely human lymphocytes and Vicia faba root tip meristems, after exposure to extremely high concentrations of this dye, as determined by the micronucleus and comet assays. Furthermore, Quinoline Yellow demonstrated the ability to inhibit plasma pseudocholinesterase and erythrocyte cholinesterase; however, the enzyme's binding was dialyzable, suggesting that the process was reversible (Amchova et al., 2015). These data were included in EFSA's reassessment of the toxicity and ADI of this dye. Thus, in 2009, the ANS Panel decided that the available information on the semi-chronic, reproductive, developmental, and long-term toxicity of Quinoline Yellow, including a study in rats that was not considered by JECFA or SCF, provides a justification for redefining the ADI, and consequently established a lower ADI of 0.5 mg/kg bw/day (EFSA, 2009b).

The EC amended Annex II to Regulation (EC) No. 1333/2008, regarding the conditions of use, in response to the ADI's decrease. Depending on the food category, this resulted in either a withdrawal of the maximum permitted levels for this dye, or a reduction of them by a factor of 1.1 to 50. As a result, for all population groups, the estimates of medium and high-level exposure to Quinoline Yellow in the EFSA review conducted in 2015 were below the ADI (EFSA, 2015c).

The Quinoline Yellow's specifications permit the presence of relatively high concentrations of unsulphonated aromatic amines (such as aniline) and 2-methylquinoline and/or 2,6-dimethylquinoline in food, up to 50 μg/kg for non-sulfonated aromatic amines and 2.5 mg/kg for methylquinolines. Although some aromatic amines may be associated with genotoxicity or even carcinogenicity, Quinoline Yellow was found to be negative in both long-term carcinogenicity studies and *in vitro* genotoxicity, according to the Panel (EFSA, 2009b). However, subsequent research showed that this colour was genotoxic at low concentrations (0.5–20 μg/mL) in HepG2 cells, with a dose-dependent effect on two assays (the comet assay and the cytokinesis-block micronucleus cytome assay). Furthermore, the dye disrupted the stability of DNA, leading to chromosomal losses (aneugenic) and breakage (clastogenic) (Chequer et al., 2015).

A recent study used spectroscopic, molecular dynamics, and computational methods to examine the molecular interactions and binding affinity between Quinoline Yellow and pepsin. The outcomes demonstrated that this artificial dye could attach to pepsin with a high degree of affinity, altering the structure and conformation of the enzyme (Hashemi-Shahraki et al., 2021).

Although there have been reports of sensitivity reactions, such as urticaria, rhinitis, and asthma, following the consumption of Quinoline Yellow, the ANS Panel determined that these reactions mostly occurred when Quinoline Yellow was consumed in combination with other synthetic colours. Therefore, the scientific data are insufficient to draw firm conclusions about the ability of Quinoline Yellow to induce sensitivity. In addition, the Panel noted that doses within the ADI could induce adverse effects in susceptible individuals (EFSA, 2009b).

## THE EFFECT OF PERMITTED SYNTHETIC FOOD COLOURS ON CHILDREN'S BEHAVIOUR

Since the 1970s, SFCs have been suspected of inducing behavioural disorders and learning difficulties in children. Thus, a research that considered a regime for eliminating food additives, known as the Feingold diet, suggested that ADHD could be controlled in 30–50% of children through this diet (Ramos-Souza et al., 2023; Turner & Kemp, 2012).

A study whose results have generated much controversy, the "Southampton Study", published in 2007, was a randomised, double-blinded, placebo-controlled, crossover trial that tested whether the intake of SFCs and additives affected childhood behaviour. In the study, 153 3-year-old children and 144 8/9-year-old children were included, who had an additive-free diet and received one of two mixtures of food dyes (E 102, E 110, E 122, and E 124; and, respectively, E 104, E 110, E 122, and E 129) and sodium benzoate, or fruit juice as placebo, over a period of 6 weeks. The daily doses of the two mixtures were equivalent to the amount of food colouring in two 56 g bags of sweets, in the case of 3-year-old children, and respectively, two or four bags of sweets, in the case of 8/9-year-old children. The study concluded that SFCs or sodium benzoate (or both) may lead to increased levels of hyperactivity, compared to placebo, in 3-year-old and 8/9-year-old children in the general population, although the overall effect did not reach a statistical significance, not being consistent in both age groups (McCann et al., 2007). After evaluating the results of this study, in the 2008 scientific opinion, EFSA concluded that "the findings of the study cannot be

used as a basis for altering the ADI of the respective food colours or sodium benzoate due to considerable uncertainties, such as the lack of consistency and relative weakness of the effect and the absence of information on the clinical significance of the behavioural changes observed" (EFSA, 2008).

In a meta-analysis that included randomized controlled trials of nonpharmacological intervention for ADHD, it was shown that the exclusion of SFCs from the diet produced beneficial effects on ADHD symptoms, but these effects were often limited to ADHD patients with food sensitivities (Sonuga-Barke et al., 2013).

Even though the hypothesis that allergies or hypersensitivity to certain foods or ingredients cause learning and behavioural problems has appeared in the literature since the 1920s, later, this hypothesis focused on the influence of food additives on hyperactivity in children, via allergenic or pharmacological mechanisms, suggesting that aspirin-allergic children are especially susceptible to SFCs (Nigg et al., 2012).

Although, according to Annex V of Regulation EC No. 1333/2008, only for yellow and red dyes the labels of food products must include additional mentions, studies have shown that blue dyes are also suspected to be associated with ADHD symptoms, but the literature is limited in this regard. A recent research conducted by Kirkland et al. (2022) in the USA, examined the effects of SFCs on ADHD symptoms and electroencephalography in college students with and without ADHD, who avoided SFCs in their diet for 2 weeks and then received either 225 mg of mixed SFC powder (i.e., Red #40, Red #3 (Erythrosine), Yellow #5, Yellow #6, Blue #1 (Brilliant Blue FCF), Blue #2 (Indigo Carmine)) disguised in chocolate cookies, or placebo chocolate cookies without SFCs. The results of the study indicated the negative impact of SFCs in students with ADHD, versus the extended control group (Kirkland et al., 2022). Unlike previous studies that examined the effects of a combination of SFCs on ADHD symptoms in children, a recent review that included four studies aimed to evaluate these effects only for Blue #1 or Blue #2 dyes. Blue #1 has been observed to impact neurodevelopment and cause hyperactive behaviour in mice and rats, however Blue #2 has not demonstrated any conclusive toxicity (Rambler et al., 2022).

## DIETARY EXPOSURE OF CHILDREN TO ARTIFICIAL FOOD COLOURS

Dietary exposures to the seven FD&C colour additives approved for general use in food in the USA were estimated in the study by Doell et al., (2016) for the US population aged 2 years and older, children 2–5 years, and teenage boys aged 13–18 years, based on analytical levels of the FD&C colour additives in approximately 600 foods. The major food categories contributing to exposure to multiple FD&C colour additives for all three populations were breakfast cereal, juice drinks, soft drinks, and frozen dairy desserts/sherbet (also referred to as ice cream, frozen yogurt, sherbet – including bars, sticks, sandwiches). Using three different exposure scenarios: low exposure, medium exposure, and high exposure, it was found that for all populations and all exposure levels, the highest cumulative consumer-only exposures were determined for FD&C Red #40, FD&C Yellow #5 and FD&C Yellow #6 (Doell et al., 2016).

Indeed, these three dyes account for 90% of all food dyes used in the USA, and of these, Red #40 is the most commonly used in food (Zhang et al., 2023). Worrying is the consumption of Red #40 dye by 94% of people over 2 years old in the USA, as well as the presence of artificial dyes in over 40% of the foods marketed to children (Batada & Jacobson, 2016; Zhang et al., 2023).

A study conducted in Saudi Arabia followed the consumption pattern of food products containing SFCs by 6-17-year-old school-going children. The highest consumption was recorded for juices and drinks, ice cream and cakes, and the most frequently used colours were Brilliant Blue (54.1%) and Tartrazine (42.3%) (Asif Ahmed et al., 2021). In India, following the exposure assessment to synthetic dyes through two major groups, sweets and savouries, an exceeding of the uniform maximum permissible limit of 100 mg/kg was found in the case of Tartrazine, which was used in doses between 12.5 and 1091 mg/kg. Another cause of concern was related to the intake of Sunset Yellow FCF which saturated the ADI limit to a maximum of 47.8% in children (Dixit et al., 2013).

Assessment of the exposure of Polish children (3 and 8/9 years, n = 149) to "Southampton colours" (Tartrazine, Quinoline Yellow, Sunset Yellow, Azorubine, Ponceau 4 R, and Allura Red) targeted 49 products containing these dyes. 21.7% of the children in the 3-year-old group (n = 18) consumed three of these dyes (Tartrazine, Sunset Yellow, Allura Red), mostly in desserts and candies, while 28.8% of the children in the 8/9-year-old group (n = 19) consumed one of the dyes (Azorubine) mostly in soft drinks. In both age groups of children, the intake of these dyes did not exceed the established ADIs in any case (on any day of the study). These results suggested that the changes in the EU legislation regarding the reduction of the permitted levels of these target food colours in certain food categories and the mention of warning messages on the labels of products containing these dyes led to the reduction of the consumption of these colours used in the Southampton study to a level where the ADIs are not exceeded, even for children (Gajda-Wyrębek et al., 2017).

## FUTURE PERSPECTIVES ON THE USE OF SYNTHETIC FOOD DYES

Significant factors to consider when determining the dyes allowed in foods and their permissible levels are the ADI, toxicological data, and variations among international regulations (Barciela et al., 2023). In this regard, while regulations may vary between nations, it would be important for ADI values and toxicological parameters to be

harmonized internationally (Pérez-Ibarbia et al., 2016).

The evaluation of food dye consumption in different populations must consider sociodemographic and physiological variables (Ramos-Souza et al., 2023). The development of international databases, which would provide information on the doses of synthetic dyes in food, the use of these dyes by various categories of the population, their authorization, and potential risks, would be of great necessity (Pérez-Ibarbia et al., 2016; Ramos-Souza et al., 2023). By means of such databases, there could be an adequate control of dietary exposure to synthetic dyes in various populations, avoiding exceeding the recommended daily intake levels established by regulatory agencies (Ramos-Souza et al., 2023).

Strict monitoring of the use of synthetic dyes in the food industry by government agencies could represent another important future perspective. Industries should evaluate and perform "Hazard Analysis Critical Control Point (HACCP)" for any azo dye used for food processing, thus ensuring that each dye falls within the dietary limit, showing at most minimal toxicity to humans and the environment (Barciela et al., 2023; Okeke et al., 2022).

Since food safety is of major importance, the development of nano-detection technologies for azo dyes is required to improve the sensitivity of physical-chemical detection technologies, even in the case of small amounts of food dyes. The development and applicability of the nano-ELISA system in the analysis of azo dyes could promote improvements in their sensitivity and selectivity (Okeke et al., 2022).

The adoption of artificial intelligence technologies could contribute to the successful detection and quantification of azo dyes in food. The results of a recent study revealed the accurate detection and quantification of illegally added azo dye Sudan 1 in ketchup samples using colour histograms (obtained from digital images) and multivariate analysis (Reile et al., 2020). Using artificial intelligence, another study developed an electrochemical detection platform by applying a binary classification evaluation. The results showed the improvement of the accuracy of tartrazine determination in food samples (Wu et al., 2022).

These studies have demonstrated the possibility of future realization of software applications on mobile devices and computers, for the effective identification and quantification of synthetic dyes in food. Thus, consumers could correctly and strictly evaluate their intake of processed foods containing such food additives and limit their consumption according to the recommended daily intake limits (Okeke et al., 2022).

In addition, educating consumers about the harmful effects of SFCs on health can play an essential role in reducing the population's exposure to these substances. Public awareness campaigns could contribute to ensuring the safety of the population by orienting consumers towards food choices that promote health, at the expense of unhealthy foods (Durazzo et al., 2022; Okeke et al., 2022).

## CONCLUSIONS

Food dyes represent an essential tool of the food sector, and among synthetic dyes, azo ones play a key role. The use of dyes as food additives is strictly regulated by legislation, as many of the synthetic compounds have been shown to be harmful to humans. Artificial food colours have been associated with a multitude of detrimental and toxicological consequences, encompassing allergic reactions, behavioural and neurocognitive effects, and toxicity on both medium and long term.

The primary issue in the domain of food dyes remains the lack of uniform regulations worldwide. Since 2009, EFSA has reassessed the safety of SFCs and revised the ADI values. According to current EFSA recommendations, the consumption ADI levels of the yellow and red dyes included in this review are reported to be 0.15–7.0 mg/kg, with a wider range (0.5–10.0 mg/ kg) in case of JECFA recommendations. Consequently, EFSA concluded that these substances are unlikely to have a significant negative impact on human health, even in children, at the recommended intake level, based on the literature that is currently available and clinical investigations.

The toxicity of a certain dye is always dependent on the dosage, and numerous investigations conducted both *in vitro* and *in vivo* have consistently shown that most artificial food colours exhibit harmful effects only at concentrations far higher than the ADI. Sensitive ADI values, however, have prompted several nations to ban the use of several SFCs. Moreover, the concerns regarding the safety of these substances arise from the fact that most studies have documented the negative consequences linked to the ingestion of a specific dye, rather than in combination with other dyes or food additives, as is most commonly encountered in the general population. Among patients, those with allergies and intolerances are the risk categories that need to be carefully considered. According to reports, adverse reactions linked to the consumption of synthetic dyes are uncommon. Furthermore, when positive reactions do occur, they are frequently the result of substantial individual variation and complex diagnoses.

Despite the extensive literature available, the data on the potential side effects and toxicity of the synthetic dyes that are the subject of the present review remain controversial, and the long-term substitution of these substances with natural compounds remains the solution of choice in order to ensure food safety. Furthermore, parental involvement in restricting or avoiding the consumption of foods containing "Southampton colours" by their own children is crucial for mitigating the potential hazards associated with the ingestion of these additives.

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**Conflicts of Interest**

The authors declare that they do not have any conflict of interest.

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