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# Azo dyes in the food industry: Features, classification, toxicity, alternatives, and regulation

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ABSTRACT

Azo dyes, including Tartrazine, Sunset Yellow, and Carmoisine, are added to foods to provide color, but they have no value with regard to nutrition, food preservation, or health benefits. Because of their availability, affordability, stability, and low cost, and because they provide intense coloration to the product without contributing unwanted flavors, the food industry often prefers to use synthetic azo dyes rather than natural colorants. Food dyes have been tested by regulatory agencies responsible for guaranteeing consumer safety. Nevertheless, the safety of these colorants remains controversial; they have been associated with adverse effects, particularly due to the reduction and cleavage of the azo bond. Here, we review the features, classification, regulation, toxicity, and alternatives to the use of azo dyes in food.

## 1. Introduction: overview of azo dyes

Color is one of the most valued qualities, when consumers evaluate food products, and colorants, both natural and synthetic, are often used to enhance attractiveness. Colorant use can also help to preserve the original color of a food product, which otherwise may be lost during processing (Monisha et al., 2023). Some dyes are extracted from plant or animal sources, but the most commonly used food dyes are synthetic organic compounds (Lipskikh et al., 2018) The use of natural dyes is limited by factors such as lower coloring strength, degradation during food processing, and, in some cases, undesirable flavors (Rovina et al., 2016). Azo compounds are the category of dyes produced in largest quantities (over 60%) and their importance may rise even further in the future (Cui et al., 2021).

Dyes can also be classified as cationic, anionic, or non-ionic. Most anionic and non-ionic dyes contain anthraquinone or azo chromophores. Azo dyes contain one or more $R^1$-N=N-$R^2$ bonds; these bonds may be reduced enzymatically, yielding aromatic amines (Feng et al., 2012). They may also have amphoteric properties due to the presence of carboxyl, hydroxy, amino, or sulfonyl functional groups (Ajmal et al., 2014). Azo dyes are extensively utilized in the food, pharmaceutical, cosmetics, paper, textile and leather industries (Benkhaya, M'rabet et al., 2020).

About 65% of azo dyes are used as food additives, in products such as soft drinks, jam, candy, pickles, etc. (Rovina et al., 2017b; Sun et al., 2023). The most commonly used food azo dyes are Tartrazine (E102), Sunset Yellow (E110), Carmoisine (E122), Amaranth (E123), Ponceau 4R (E124), Allura Red AC (E129), Brilliant Blue (E 133), and HT Brown (E155) (Kaya et al., 2021; Lipskikh et al., 2018) (Fig. 1). The popularity of azo dyes is based on their chemical versatility, resulting in an abundance of vibrant colors. In addition, they are low cost, readily accessible, stable, consistent, and have no off-tastes or unpleasant odors (Mahmoodi et al., 2016; Ramos-Souza et al., 2022). However, food azo dyes may have adverse health effects (Monisha et al., 2023). Below the Allowable Daily Intake (ADI) limits, intake of artificial dyes may be safe. However, larger doses may cause health effects, particularly in children (Aquino and Conte-Junior, 2020; Reza et al., 2019). Possible toxicities reported include allergy, attention deficit hyperactivity disorder (ADHD), asthma, anxiety, cytotoxicity, and genotoxicity/cancer (Kaya et al., 2021; Rovina et al., 2016; Sun et al., 2023). Azo dyes are relatively persistent, with little degradation under aerobic conditions (Benkhaya, M' rabet et al., 2020; John et al., 2022; Selvaraj et al., 2021).

Regulatory agencies, including the Food and Drug Administration (FDA; USA) and the European Food Safety Authority (EFSA) are charged with assessing and evaluating the risk of food dyes (Kaya et al., 2021; Martins et al., 2016). Azo dye food additives are restricted by Regulation (EC) 1333/2008 (Mota et al., 2021). The European Numbering System (ENS; "E numbers") facilitates identification and control of food dyes (Kaya et al., 2021). Despite legislation, regulation, and consumer notification (*e.g.*, labeling of food products), safety concerns persist. Evaluations of safety are not definitive, monitoring of imported food products

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**Abbreviations**

| | |
|---|---|
| ADI | Allowable Daily Intake |
| AM | Amaranth |
| AR | Allura red AC |
| BB | Brilliant blue |
| BN | Brilliant black |
| BR51 | Basic red 51 |
| CAR | Carmoisine |
| CAT | Catalase |
| cI | Color Index International |
| EC50 | Median effective concentration |
| EFSA | European Food Safety Authority |
| ENS | European Numbering System |
| Ery | Erythrostomin |
| FDA | Food and Drug Administration |
| GSH | Glutathione |
| GST | Glutathione transferase |
| HACCP | Hazard Analysis and Critical Control Point |
| LC50 | Median lethal concentration |
| LOAEL | Lowest adverse effects concentration |
| LOD | Limit of detection |
| MCF-7 | Human mammary gland adenocarcinoma cell line |
| mPFC | Medial prefrontal cortex |
| NaB | Sodium benzoate |
| NOAEC | No observed adverse effect concentration |
| NOAEL | No observed adverse effect level |
| P4R | Ponceau 4R |
| PCA | Principal Component Analysis |
| SOD | Superoxide dismutase |
| SY | Sunset yellow |
| TI | Teratogenicity index |
| TTZ | Tartrazine |

is complex, and, in some cases, analytical methods for assuring compliance with permitted levels of use are less than satisfactory. In this review, we discuss the characteristics of food azo dyes, their possible adverse health effects, and potential natural alternatives.

## 2. Azo dye characteristics

Azo dyes used in foods are synthetic organic compounds of low molecular weight and high water solubility (Kalia and Singh, 2020). The azo group is characterized by a double bond between N atoms (N═N) linking aromatic rings, usually with other functional groups present, such as amino (-$NH_2$) or sulphonate (-$SO_3^-$) groups (Mota et al., 2021). Azo dye synthesis entails the diazotization of a primary aromatic amine, such as phenylamine, to form a diazonium salt (Fig. 2A), which is then coupled with an aromatic compound, *e.g.,* phenol, to form an azo dye (Fig. 2B) (John et al., 2022).

An azo dye structure can be regarded as consisting of a backbone, chromophore groups, auxochrome groups, and solubilizing groups (Fig. 2C) (Benkhaya et al., 2016). The azo chromophore contributes color (optical absorbance); (Benkhaya, M'rabet et al., 2020; Gürses et al., 2016). The aromatic rings may be carbocyclic (benzene, naphthalene) or heterocyclic (Gürses et al., 2016). Azo dyes can also be categorized by the number of azo bonds in the dye molecule: monoazo, disazo, trisazo, polyazo, or azoic (Ramos-Souza et al., 2022; Zafar et al., 2022). The azo bond chromophore and the connected auxochrome groups (*e.g.*, –COOH, –$NH_2$, –$SO_3$,-OH) give azo dyes their particular colors (Cui et al., 2021). Another classification can be made according to hydrophobicity: hydrophobic azo dyes (which may be reduced intracellularly) and hydrophilic azo dyes (which may be reduced extracellularly); (Zafar et al., 2022).

## 3. Azo dye toxicity

Azo food dyes may present health risks (Dey and Nagababu, 2022; Khataee et al., 2022). Most toxic effects following oral ingestion are probably due to reduction of the dyes by intestinal microflora, *i.e.*, cleavage of azo bonds leading to formation of aromatic amines, which may subsequently be N-hydroxylated or N-acetylated (Kaya et al., 2021; Ngo and Tischler, 2022). In mammals, reduction of ingested azo dyes is principally due to metabolism catalyzed by bacterial azoreductase enzymes in the anaerobic environment of the lower gastrointestinal tract (Ameur et al., 2020; Elbanna et al., 2017). Azo dye reduction catalyzed by mammalian enzymes (in organs such as the liver and kidneys) has also been suggested, but it is not clear that it contributes significantly.

After azo dye reduction in the intestinal tract, the released aromatic amines may be absorbed by the intestine and subsequently excreted by the kidneys (Elbanna et al., 2017). Bacterial reduction in the anaerobic environment of the colon converts an azo dye into two metabolites, such as sulfanilic acid (a sensitizing aromatic amine) and aminopyrazolone. These metabolites are absorbed more easily than the parent azo dye and can be excreted in the feces (Atlı Şekeroğlu et al., 2017; Kiziltan et al., 2022); urinary excretion of intact azo dyes is very low or negligible (*e.g.*, <5% for tartrazine) (Villaño et al., 2016). If the oral absorption rate is 2–5%, the peak concentration in the blood of a 70 kg adult (5 L blood) could be 2–5 mg/mL (Jiang et al., 2020).

Sulfanilic acid may affect cell division and cause regenerative hyperplasia, potentially contributing to carcinogenesis (Bezerra et al., 2016). However, sulfonate groups may be cleaved from aromatic amines by metabolism (JECFA, 2019). Multiple mechanisms of azo dye toxicity have been proposed. Metabolites of azo dyes may induce liver injury due to oxidative stress caused by reactive oxygen species (ROS), among other adverse hematological/biochemical effects (Elbanna et al., 2017). Azo dye toxicity may require reduction of the azo bond, giving rise to aromatic amines. However, some azo dyes contain free aromatic amine groups that may be metabolically oxidized (independent of azo reduction) and cause toxicity (Feng et al., 2012). Toxic effects of dyes may depend on the absorption, metabolism, and excretion of the food product containing the dyes, and there may be interactions between colorants and other food constituents (Kuswandi et al., 2017).

Previous research has indicated that azo dyes such as Tartrazine, Ponceau 4R, Allura Red AC, and HT Brown could affect neurodevelopment or be carcinogenic. As discussed below, EFSA does not anticipate adverse effects of the food dyes on neurobehavioral development or cell proliferation in humans (Villaño et al., 2016). However, toxicity depends on dose, and some dyes generally considered "non-toxic" may become toxic at high doses (Pérez-Ibarbia et al., 2016).

## 4. Food azo dyes: properties and health effects

Tartrazine (TTZ) and sunset yellow (SY) are widely used in food processing; they are often used in combination, to impart yellow color (Kaya et al., 2021; Okeke et al., 2022). Both dyes are approved by EFSA and FDA. ADI values for SY and TTZ have been established as 4.0 and 7.5 mg/kg bw/day, respectively (Dey and Nagababu, 2022; Lipskikh et al., 2018).

### 4.1. Yellow azo dyes in food products

#### 4.1.1. Sunset yellow

SY (E110) is a synthetic azo dye commonly used as a food colorant; it

| AZO DYE | ENS | COLOR | FOOD PRODUCT | ADI | MOLECULAR STRUCTURE |
|---|---|---|---|---|---|
| Tartrazine<br>(Yellow 5[A]; cl 19140[B])<br>$C_{16}H_9N_4Na_3O_9S_2$ | E102 | | | 7.5 mg/kg | |
| Sunset Yellow<br>(Yellow 6[A]; cl 15985[B])<br>$C_{16}H_{10}N_2Na_2O_7S_2$ | E110 | | | 4 mg/kg | |
| Carmoisine<br>(Acid Red 14[A]; cl 14720[B])<br>$C_{20}H_{12}N_2Na_2O_7S_2$ | E122 | | | 4 mg/kg | |
| Amaranth<br>(Acid Red 27[A]; cl 16185[B])<br>$C_{20}H_{11}N_2Na_3O_{10}S_3$ | E123 | | | 0-0.8 mg/kg | |
| Ponceau 4R<br>(Acid Red 18[A]; cl 16255[B])<br>$C_{20}H_{11}N_2Na_3O_{10}S_3$ | E124 | | | 0.7 mg/kg | |
| Allura Red<br>(FD&C Red 40[A]; cl 16035[B])<br>$C_{18}H_{14}N_2Na_2O_8S_2$ | E124 | | | 7 mg/kg | |
| Brilliant blue<br>(FD&C Red 40[A]; cl 16035[B])<br>$C_{37}H_{34}N_2Na_2O_9S_3$ | E133 | | | 6 mg/kg | |
| Brown HT<br>(Food Brown 3[A]; cl 20285[B])<br>$C_{27}H_{18}N_4Na_2O_9S_2$ | E155 | | | 1.5 mg/kg | |

**Fig. 1.** Features of the azo dyes most used in the food industry (ENS: European Numbering System; [A] Additional nomenclatures; [B] cI: Color Index International; ADI: Acceptable Daily Intake). Created with BioRender.com. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

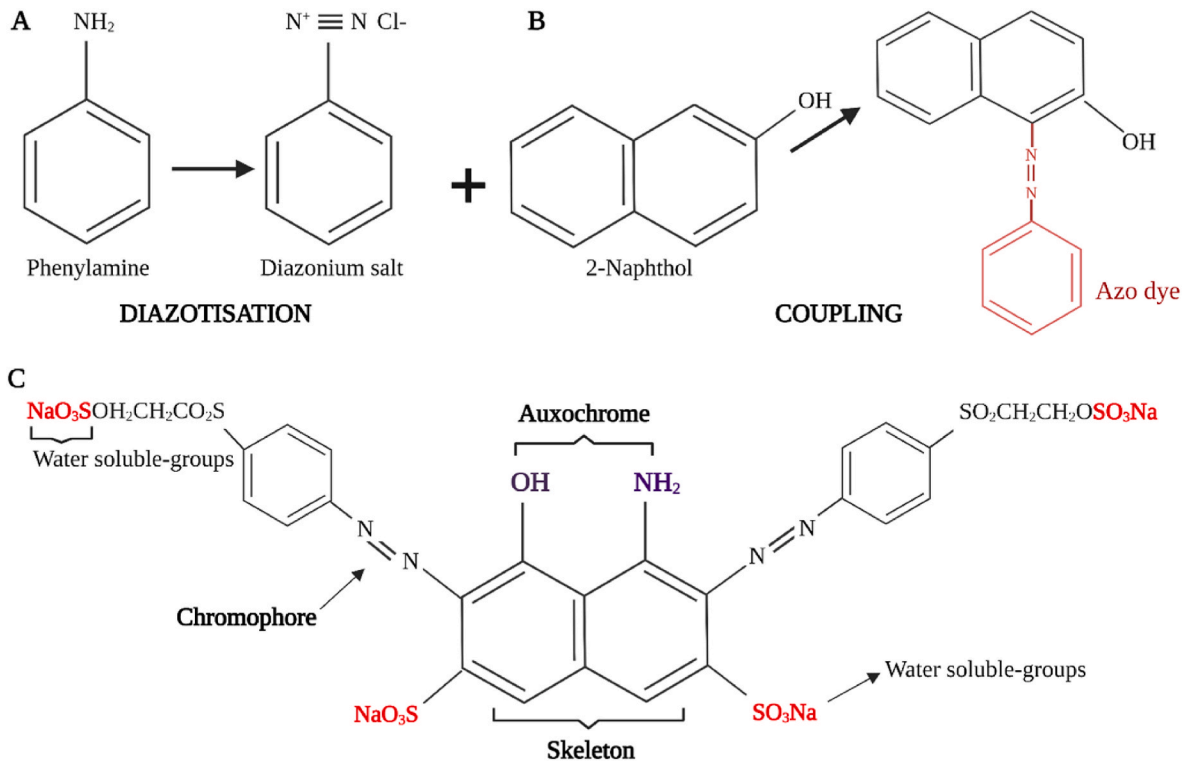


**Fig. 2.** Azo dye formation and structure. Created with BioRender.com.

is also known as Cl Food Yellow Cl 3, Orange Yellow S, and Yellow 6 (Ali et al., 2020; Villaño et al., 2016); (El-Borm et al., 2020). SY is polar, water soluble, and poorly soluble in ethanol. Aqueous solutions are orange-yellow, becoming red-brown in alkaline and neutral solutions (Rovina et al., 2017). SY can be found in a wide variety of food products, such as aromatized and fermented beverages, ice cream, condiments, confectionery, chewing gum, jams, jellies, desserts, soups, fish roe, fish paste, and crustaceans (Silva et al., 2022).

SY may be teratogenic and may cause gastric upset, diarrhea, and vomiting; allergic reaction, intolerances, and behavioral disorders in children; or sleep disturbances (McCann et al., 2007; Van Bever et al., 1989; Ward, 1997). Dwivedi and Kumar, 2015, reported the effects of SY on chromosomal aberrations (genotoxic and cytotoxic effects) in the plant species *Brassica campestris* L. (Dwivedi & Kumar, 2015). Ali et al. (2020), assessed the genotoxicity of combinations of SY and sodium benzoate (NaB) *in vivo* in rats and observed structural abnormalities at SY doses of 50 mg/kg bw/day, much higher than the ADI (Ali et al., 2020). El-Borm et al. (2020), studied morphological and skeletal malformations induced in chicken embryos caused by doses of SY and TTZ, 1.575 and 0.375 mg/egg, respectively (14 × ADI). Severe adverse effects were seen, such as malformations of the feathers, head, and neck, reduction of embryo weight and length, and a marked increase in congenital malformations, such as short beak, exencephaly, pointed tail and pygostyle, curved scapula, and retarded ossification (El-Borm et al., 2020).

However, no consensus has been reached with regard to possible adverse effects of SY in humans. EFSA and FDA have noted that SY has never shown carcinogenic or genotoxic effects in long-term studies in mice and rats, either *in vitro* or *in vivo* (Rovina et al., 2017). Bastaki et al. (2019), fed diets containing SY to rats for 28 days to evaluate toxicity. The no observed adverse effect level (NOAEL) was 1.475 mg/kg and no clinical signs of toxicity, differences in feed intake, body weight, hematology, clinical chemistry, or coagulation parameters considered adverse were seen (Bastaki et al., 2019). Table 1 summarizes published studies, with some results finding adverse effects associated with SY and others not. SY (0.1–5.0 mM) causes aggregation of β-lactoglobulin protein; potential interactions of SY with food components need to be considered (Khan et al., 2022; Ramos-Souza et al., 2022).

#### 4.1.2. Tartrazine

Tartrazine (TTZ; E102), also known as Cl Food Yellow 4 and FD & C Yellow 5, provides a lemon-yellow color; it is soluble in water and poorly soluble in ethanol (Ameur et al., 2020; Silva et al., 2022). TTZ synthesis can be accomplished by condensation of phenylhydrazine-*p*-sulfonic acid with oxaloacetic ester; the product is combined with diazotized sulfanilic acid, giving rise to an ester which is then hydrolyzed with NaOH. An alternative method is the condensation of 2 mol of phenylhydrazine-*p*-sulfonic acid with 1 mol of dihydroxytartaric acid (Ziegler and Locher, 1887).

TTZ is added to ices, candies, jellies, jams, potato chips, cakes, ice cream, sauces, cereals, etc. (Rovina et al., 2017a). TTZ is occasionally used as a substitute for saffron (Zoughi et al., 2021). Among all azo food dyes, TTZ is suspected to trigger the most serious allergic and intolerance reactions, as well as hyperactivity, and it has been reported that TTZ preparations may contain residues of aromatic amine carcinogens (John et al., 2022; Silva et al., 2022). Children are the most vulnerable sector of the population, particularly since they are the main consumers of brightly colored processed foods (Atlı Şekeroğlu et al., 2017). Asif Ahmed et al. evaluated the intake of fruit juice and drinks, ice cream, and cakes by children aged 6–17 years; TTZ was present in 42.3% of the products (Asif Ahmed et al., 2021).

Vani et al., 2018, determined the no-observed-effect concentration (NOAEC), median lethal concentration ($LC_{50}$), median effective concentration ($EC_{50}$), and teratogenic potential (TI) of TTZ to zebrafish embryos *in vivo*. TTZ had no effect up to 5 mM; concentrations 20–30 mM caused anomalies such as cardiac deformations; bradycardia was observed in embryos exposed to 30 mM TTZ, followed by cardiac deformations (Joshi and Katti, 2018).

Ameur et al. (2020), evaluated the effects of TTZ on the enzymatic activities of amylase, lipase, and proteases - digestive enzymes - after subchronic ingestion in mice. TTZ at the established ADI does not detrimentally affect activity. Nevertheless, excessive dye intake appears

**Table 1**
Experimental studies with azo dyes.

| AD | ENS | Trial | Dose* | ET** | Analysis/Assay | Results* | Impact in health | Ref. |
|---|---|---|---|---|---|---|---|---|
| TTZ | 102 | Zebrafish | 0–100[C] | 7 | Graded dilutions during growth | NOAEC (5[C]), LOAEC (10[C]), $LC_{50}$ (29.4[C]); $EC_{50}$ (59.60[C]), TI (0.49) | Tail distortion, yolk sac and cardiac edema (20–30 mM) | Joshi and Katti (2018) |
| TTZ | 102 | Mice | 7.5–75[A] | 91 | Pancreatic enzymes activity | Slight decrease in protease activity only at highest concentration | No changes in body weight (non-harmful use based on ADI) | Ameur et al. (2020) |
| TTZ | 102 | Rats | 100 mg/ 200mL[E] | 49 | Hematological, histopathology and biochemical assays | Hepatic congestion, hyperplasia of the bile ducts, and so on | Histopathological alterations, which caused tissue damage to the liver, kidney, and spleen | Balta et al. (2019) |
| TTZ | 102 | Eukaryotic cells | 100, 200, 400[D] | 3 | Cell viability assay | Capacity to block cell division | Toxic, cytotoxic, and mutagenic effects | Dos Santos et al. (2022) |
| TTZ | 102 | Zebrafish | 0–100[C] | 3 | Randomized | $LC_{50}$ (47.1[C]); $EC_{50}$ (42.66[C]), TI (1.1) | Toxicity/teratogenic potential | Jiang et al. (2020) |
| TTZ | 102 | Rats | 10[A] | 56 | Randomized | Decrease of total proteins, antioxidants, among others | Kidney and liver dysfunction | Al-Seeni et al. (2018) |
| TTZ | 102 | Chicken embryos | 0.2[F] | 20 | Morphological, morphometric and mortality rate | Short beak, exencephaly, pointed tail, curved scapula, and so forth (10–60%) | Malformations in the endoskeleton | Subramaniyan et al. (2022) |
| TTZ | 102 | Rats | 10[A] | 56 | Randomized | Increase in triglycerides, cholesterol, LDL, and decrease in HDL | Drastic tissue injury to the stomach, liver, kidney, and testicles. | El Rabey et al. (2019) |
| TTZ | 102 | Rats | 7.5[A] | 40 | Oxidative stress biomarkers | Increased MDA levels and decreased CAT, GST, and GR activity | Alteration of cerebral biochemical markers at prescribed IDA levels | Bhatt et al. (2018) |
| TTZ | 102 | Rats | 500[A] | 21 | Histopathology and biochemical assays | Nephrotoxicity on histopathological and biochemical parameters | Oxidative stress | Erdemli et al. (2017) |
| TTZ | 102 | Rats | 700[A] | 14 | Measurement of Oxidative Stress | Decreased level of ascorbic acid in the brain | Genotoxicity and tumorigenic potential | Alsalman et al. (2019) |
| TTZ | 102 | Rats | 500[A] | 21 | Histopathology and biochemical assays | Inhibition of antioxidant enzyme activities in ileum and colon | Oxidative stress | Altinoz et al. (2021) |
| TTZ | 102 | Mice | 2.5–5[A] | 35 | Behavior, histological and biochemical assays | Pyknosis, chromatolysis and neuron degeneration | Tissue damage in several brain regions | Albasher et al. (2020) |
| SY | 110 | Chicken embryos | 0.2[F] | 20 | Morphological, morphometric and mortality rate | Severe endoskeletal abnormalities (10–50%) | Malformations in the endoskeleton | El-Borm et al. (2020) |
| SY | 110 | Zebrafish | 0–100[C] | 7 | Graded dilutions during growth | NOAEC (0.1[C]), LOAEC (10[C]), $LC_{50}$ (42.57[C]); $EC_{50}$ (19.41[C]), TI (2.1) | Toxicity/teratogenic potential | Joshi and Pancharatna (2019) |
| SY | 110 | Zebrafish | 0–100[C] | 3 | Randomized | $LC_{50}$ (38.93[C]); $EC_{50}$ (29.81[C]), TI (1.31) | Toxicity/teratogenic potential | Jiang et al. (2020) |
| SY | 110 | Rats | 2.5[A] | 28 | Randomized | Increased of NO, AST, ALT, and MDA levels (stress biomarkers) | Histopathological and physiological aberrations in the liver and kidney and slight genotoxicity | Khayyat et al. (2018) |
| SY | 110 | Rats | 500–1500[A] | 28 | Sperm quality | NOAEL (375[A]) | Absence any adverse effects | Bastaki et al. (2019) |
| SY | 110 | Rats | 5–200[A] | 84 | CA and comet | CA (0.5–1.5%), comet (86,9% with 50[A]) | Genotoxicity to liver and bone marrow cells (high doses) | Ali et al. (2020) |
| SY | 110 | *Zea mays* | 15, 17.5, 20, 22.5, 25, and 30[B] | 3 | Shoot inhibition test, chlorophyll content, α-amylase activity and | $EC_{50}$ (22.5[B]) | Decrease in chlorophyll content and α-amylase activity and 53% polymorphism | Dikilitaş and Aksoy (2018) |
| CAR | 122 | | | | RAPD PCR technique | $EC_{50}$ (20[B]) | Decrease in chlorophyll content and α-amylase activity and 71% polymorphism | |
| CAR | 122 | Mice | 0.4–400[A] | 120 | Randomized | NOAEC (0.4[A]) | Liver cell damage and cancer | Reza et al. (2019) |
| CAR | 122 | *P. vicina* | 0.05–14[B] | 4 | Randomized | $LC_{50}$ (5.491[B]) | Mortality and gene expression | Li et al. (2022) |
| CAR | 122 | Zebrafish | 4–2000[A] | 4 | Randomized | NOAEC (5[A]), $LC_{50}$ (1230.53[A]) | Low oxygen levels, increased oxidative stress and behavioral alterations | Kiziltan et al. (2022) |
| CAR | 122 | Mice | 20[A] | 30 | Behavioral and biochemical assays | Increase in neurochemicals and decrease in antioxidant enzymes | Brain injury | Subramaniyan et al. (2022) |
| AM | 123 | Zebrafish | 0–100[C] | 3 | Randomized | $LC_{50}$ (39.86[C]); $EC_{50}$ (31.94[C]), TI (1.25) | Toxicity/teratogenic potential | Jiang et al. (2020) |
| AR | 129 | | | | | $LC_{50}$ (47.42[C]); $EC_{50}$ (40[C]), TI (1.18) | | |
| AR | 129 | Rats | 7–70[A] | 42 | NOR and RAM | Loss of neurons, glial cells, and dendritic trees | Impaired learning, memory and mPFC structure. | Noorafshan et al. (2018) |
| AR | 129 | Mice | 25,500 and 2000[A] | 3 | Bone marrow micronucleus and comet assays | No genotoxicity of the compound or its metabolites after oral intake up to the limit dose | Absence of genotoxic effects | Bastaki et al. (2017) |

(*continued on next page*)

**Table 1** (*continued*)

| AD | ENS | Trial | Dose* | ET** | Analysis/Assay | Results* | Impact in health | Ref. |
|---|---|---|---|---|---|---|---|---|
| AR | 129 | Mice | 172.2[A] | 60 | Hematological and biochemical assays | Decrease in white and red blood cells, hemoglobin, hematocrit, platelets, CAT, SOD, and GSH | High toxicity | (P. Sharma et al., 2023) |
| P4R | 124 | Rats | 7.5[A] | 28 | Randomized | Increased of NO, AST, ALT, and MDA levels (stress biomarkers) | Histopathological and physiological aberrations in the liver and kidney | Khayyat et al. (2018) |
| BB | 133 | *Allium cepa* | 100, 200, 400 and 500[D] | 3 | FRAP, TEAC, RAPD-PCR, and comet assay | Disappearing bands of DNA | May cause genotoxic effects | Koç and Pandir (2018) |
| BB | 133 | Rats | 1.2[A] | 90 | Randomized | Neutrophilia and lymphopenia | Hematological alterations | Motwadie et al. (2021) |

***Abbreviations:*** AD: azo dye; ET: exposure time NOAEC: no observed adverse effect concentration; LOAEC: lowest adverse effects concentration; NOAEL: no observed adverse effect level *Polyrhachis vicina: P. vicina;* $LC_{50}$**:** median lethal concentration; $ED_{50}$**:** median effective concentration; SY: sunset yellow; CA: chromosomal aberration assay; MDA: malondialdehyde; NO: nitric oxide; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TTZ: tartrazine; GST: glutathione-S-transferase; SOD: superoxide dismutase; CAT: catalase; GSH: glutathione; GR: glutathione reductase; CAR: carmoisine; MN: micronucleus; AM: amaranth; AR: allura red AC; FRAP: ferric reducing antioxidant power; TEAC: trolox equivalent antioxidant capacity; HDL: high-density lipoproteins; LDL: low-density lipoproteins; mPFC: medial Prefrontal Cortex; NOR: novel object recognition test; RAM: eight-arm radial maze test; ADI: acceptable daily intake; P4R: ponceau 4R; BB: brilliant blue. **Results are expressed in*: [A]mg/kg; [B]g/L;[C]mM; [D]μg/mL, mg/mL[E]; mL[F]. ***Days.*

to disrupt the enzymatic activity of proteases *in vivo*, which could have deleterious consequences on digestion (Ameur et al., 2020). These results are summarized in Table 1.

The toxicogenic studies of TTZ are not conclusive since, in *in vivo* studies in which animals received TTZ, no cytotoxic changes in tissues or organs were noticed and absence of genotoxic activity (Bastaki et al., 2017). The effects of dyes will not be the same for each individual; they may vary according to dose, age, sex, nutritional status, genetic factors, and lengths of exposure (Dwivedi & Kumar, 2015). Al-Seeni et al. (2018), found that TTZ given to rats (10 mg/kg bw/day) lowered total proteins, antioxidants, and high-density lipoproteins. Furthermore, it led to pathological changes in the liver, kidney, testis, and feces tissues (Al-Seeni et al., 2018). In a further investigation by El Rabey et al., 2019, in rats, TTZ (10 mg/kg bw/day) caused elevation of serum liver enzymes (hepatotoxicity), abnormal renal indexes, lipid peroxidation, elevated triglycerides and total cholesterol, and alterations in low- and very low-density lipoproteins. Additionally, there was toxicity to the stomach, liver, kidney, and testicular tissues (El Rabey et al., 2019).

Altinoz et al. (2021), reported that oral intake of TTZ inhibited the activity of antioxidant enzymes in the ileum and colon. TTZ and its metabolites might have triggered free radical formation, such as $H_2O_2$, and superoxide dismutase (SOD) reduction. MDA and total oxidant status were increased by TTZ (Altinoz et al., 2021). Albasher et al. (2020), reported that TTZ at the established ADI induced neurobehavioral, hematological, and locomotor behavioral alterations in neonatal mice (Albasher et al., 2020). Nasri and Pohjanvirta, 2021, confirmed that TTZ exhibited a proliferative effect on the human mammary gland adenocarcinoma cell line (MCF-7) with an $EC_{50}$ of 7.46 nM, suggesting that it might induce estrogenic impact in humans (Nasri and Pohjanvirta, 2021).

### 4.2. Red azo dyes in food products

#### 4.2.1. Carmoisine

Carmoisine (CAR) or azorubine (E122) is a disodium salt consisting of two naphthalene subunits and an azo functional group (-N═N-), with ADI = 4 mg/kg bw/day (Khataee et al., 2022; Monisha et al., 2023). It is soluble in water, slightly in ethanolic solutions, but insoluble in vegetable oil. CAR is stable against pH, heat, light and oxygen (Silva et al., 2022). CAR has naphthionic acid and 1-naphthalene sulfonic-3-amino-4-hydroxyl acid within its structure (Dikilitaş and Aksoy, 2018). It is used as an artificial red colorant in beverages and food such as Swiss rolls, jellies, jams, yogurts, cheesecake mixes, breadcrumbs, etc. It is commonly used when food is subjected to heat treatments after fermentation (Khataee et al., 2022).

Subramaniyan et al. (2022), studied the ameliorative capacity of *Vernonia cinerea* extract against CAR-induced brain damage and anxiogenic effects in mice. They concluded that CAR, at dose = 20 mg/kg bw/day, injured brain tissue by increasing glutamate levels or decreasing γ-aminobutyric acid and antioxidant enzymes such as catalase (CAT) and SOD (Subramaniyan et al., 2022). Reza et al. (2019), assessed the toxic effects of CAR and its relation to carcinogenicity. They orally administered CAR to mice at four doses for 120 days. Results highlighted the abnormal function of vital organs, with the liver as the primary target organ of CAR exposure. The total body weight, platelet, hemoglobin, white and red blood cell, and monocyte cell counts of the medium- and high-dose treated mice were decreased compared to the control group. Biochemical parameters (aminotransferase, alkaline phosphatase, and globulin) were raised, while serum cholesterol levels decreased post-treatment. CAR was found to increase the overall risk of hepatocellular damage at elevated doses and might lead to cancer (Reza et al., 2019). According to available reports, overuse of this dye triggers anxiogenic behavior in mice by inducing oxidative stress (Subramaniyan et al., 2022). Li et al. (2022), reported dose-dependent mortality of CAR to *Polyrhachis vicina* (black ant). CAR altered gene expression, decreased survival, and affected the growth and development of the ants (Li et al., 2022). Kiziltan et al. (2022), assessed the effects of CAR exposure on zebrafish embryonic development at recommended and overexposure doses. They concluded that CAR caused severe malformations, reduced ocular size and diameter, increased oxygen free radicals, apoptotic cells, and lipid accumulation, caused a dose-dependent decrease in locomotor activity, and at the highest dose, decreased blood flow rate (Kiziltan et al., 2022). Dikilitas and Aksoy, 2018, evaluated the toxicity of CAR and SY to corn (*Zea mays*). Root inhibition test revealed $EC_{50}$ values of 20 g/L for CAR and 22.5 g/L for SY. The levels of a, b, and total chlorophyll were decreased by both azo dyes but more sharply by CAR. The concentration of α-amylase decreased particularly with SY. Random Amplification of Polymorphic DNA (RAPD) analysis showed 71% polymorphism for the CAR-treated groups and 53% for the SY-treated ones, confirming the genotoxicity of the dyes (Dikilitaş and Aksoy, 2018).

#### 4.2.2. Allura red AC

Allura Red AC (AR; E129) is a mono-azo red dye used in powder mixes, candy, ice cream, spices, drink coloring, sauces, jellies, gelatins, and dairy foods (P. Sharma et al., 2023). This dye is soluble in water and poorly soluble in 50% ethanol (Rovina et al., 2016). It can be delivered as a sodium, calcium, or potassium salt (Silva et al., 2022). AR consumption has been related to occasional behavioral alterations in humans and animals, including increased hyperactivity in children, although further studies are needed (McCann et al., 2007; Noorafshan et al., 2018). The EFSA has advised an ADI of 7.0 mg/kg bw/day (Al-Shabib et al., 2019).

Noorafshan et al. (2018), assessed the effects of AR, administered for six weeks by gavage with or without taurine, on the medial prefrontal cortex (mPFC) of rats. AR (ADI dose) affected the number of glial cells and spatial learning and memory. High doses of AR could damage CPFm structure, including loss of volume, cells, and the dendritic tree of the cortex (Noorafshan et al., 2018). Khayyat et al. administered AR orally to rats, 7 mg/kg bw/day, for 30 days. The dye caused hepatic and renal toxicities. No genotoxicity was observed in white blood cells by the comet assay (Khayyat et al., 2018).

### 4.3. Blue azo dyes in food products

#### 4.3.1. Brilliant blue

Brilliant Blue (BB; E133), is a synthetic dye with moderate stability when exposed to light, heat, and acidic conditions, but with low oxidative stability (Ferreira et al., 2016). BB is a triphenylmethane dye, not an azo dye, but it will be discussed briefly here because it is widely used in foods, including drinks, dairy products, tinned foods, peas, soup envelopes, glazes, candies, and ice cream (Motwadie et al., 2021). The extensive π-conjugated structure of BB provides the color strength of the dye (Sivasankaran et al., 2017). Asif Ahmed et al. (2021), concluded that BB is a common dye in food products such as juices and soft drinks, ice cream, and cakes, with children having the highest intake (Asif Ahmed et al., 2021).

Motwadie et al. (2021), assessed the effects of daily oral exposure of rats for 90 days to 1.2 mg/kg bw/day of BB. Results indicated that BB intake causes neutrophilia and lymphopenia (Motwadie et al., 2021). Koç and Pandir, 2018, evaluated the toxicity of BB (100, 200, 400, and 500 ppm) and SY (25, 50, 100, and 500 ppm) on rootstock, meristematic cells for periods of 24, 48, and 72 h. The results suggest possible genotoxic effects of both dyes because of decreased antioxidant capacity. It was concluded that BB and SY have intake dose-dependent cytotoxic and mutagenic effects (Koç and Pandir, 2018). Table 1 summarizes several studies that evaluated the possible toxicity of BB.

## 5. Natural compound-based alternatives to azo dyes in foodstuffs

The use of non-synthetic additives is an ongoing trend in the food industry, driven by consumer choice and by the perceived benefits of naturally occurring nutrients (Landim Neves et al., 2021). Natural food dyes can be obtained from fungi, bacteria, plants, fruits, vegetables, and structural components of animals (Nabi et al., 2023). Natural food dyes have been categorized into four main chemical groups: flavonoid derivatives, such as anthocyanins; isoprenoid derivatives, such as carotenoids; pyrrole derivatives, such as chlorophyll; and nitrogenous heterocyclic derivatives, such as betalains (Echegaray et al., 2023). Natural food dyes can achieve a coloring effect comparable to synthetic dyes but they can offer some technological advantages, preservative properties, and better environmental sustainability, and they can be extracted from by-products and waste (Castro et al., 2021). However, even if their toxic risks are less, they are not necessarily non-toxic (Manzoor et al., 2021). Abe et al., 2019, evaluated the toxicity of the azo dye Basic Red 51 (BR51) and the natural dye erythrostomin (Ery) in *Daphnia magna*. Both dyes caused effects after short- or long-term exposures. Nevertheless, BR51 was up to 100 times more toxic than Ery. The toxic effects of Ery may be reduced by photodegradation (Abe et al., 2019).

Teixeira et al. (2022), investigated the replacement of artificial food dyes, such as TTZ, by natural dyes. The study was conducted on natural yogurt, and the highest color equivalence was obtained with curcumin. The antioxidant capacity of the natural dyes was also evaluated, with satisfactory results. This approach may give information about the interactions between natural dyes and food pH, as well as similarities or differences in color vs. synthetic dyes (Teixeira et al., 2022). De Faria Amaral De Faria Silva et al., 2022, produced a natural yellow dye based on cochlioquinol II and riboflavin isolated from the fungus *Arcopilus aureus*. The yellow dye was resistant to temperature and pH variations and was proposed as a practical substitute for synthetic yellow dyes (Amaral De Faria Silva et al., 2022).

Anthocyanins, betalains, and carotenoids are in wide use for providing red, orange, and yellow shades. Use of natural green and blue food dyes remains very limited, but research into new alternative food dyes from natural sources is ongoing (Neri-Numa et al., 2017). For example, the fruit of *Genipa americana* L. may provide an alternative to synthetic blue dyes. Few studies have evaluated its possible toxicity (Neri-Numa et al., 2020). Use of natural food dyes could be increased by improving the stability, intensifying the color, or reducing the cost of known dyes or by the identification of new ones (Landim Neves et al., 2021). Red beet betalains are an attractive substitute for CAR and AR. They are water-soluble nitrogenous pigments with high dyeing properties and antioxidant activities. They are approved as food additives by EFSA and have not raised safety concerns. ADI has not been set, based on known toxicology data (Calva-Estrada et al., 2022; EFSA, 2015a; Fu et al., 2020). Lycopene from tomatoes provides an orange to brilliant red hue, but it is often discarded due to its lack of stability and its degradation in food products. Paprika from peppers is less expensive and more resistant to oxidative degradation, giving a very similar shade (Nabi et al., 2023). Research on natural substitutes for azo dyes is broad and growing, and is essential to offer alternatives with good pigmenting capacity, high stability, scalability, cost-effectiveness, and safety. Table 2 shows some natural alternatives that have already been approved as food colorants, and their properties and characteristics.

## 6. Food colors: applicable legislation

Regulation (EC) Nº 178/2002 lays down the general requirements for use of food dyes (Regulation (EC) No 178/2002 Laying down the General Principles and Requirements of Food Law, Establishing the European Food SafetyAuthority and Laying down Procedures in Matters of Food Safety, 2002). Regulation (EC) Nº 1333/2008 lists authorized food additives (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008). This regulation establishes rules covering definitions, terms of use, labeling, and authorization procedures. It includes five annexes; the third annex concerns authorized food additives and their requirements. Regulation (EC) Nº 1129/2011 modified Annex III and included specific rules for food colorants (Part B), maximum quantities, conditions of use, restrictions, and individual assignment, with E-numbers, to facilitate identifying, controlling, and reducing risk (Kaya et al., 2021). For instance, SY (E110) is authorized at levels up to 100 mg/kg in processed cheeses (Regulation (EU) Nº 1129/2011 of November 11, 2011 Amending Annex II to Regulation (EC) Nº 1333/2008 of the European Parliament and of the Council by Establishing a Union List of Food Additives, 2011). Annex V of Regulation (EC) Nº 1333/2008 establishes additional mandatory information on food labeling; specifically, if azo dyes are used, the E-number must be included following the warning: "*may have adverse effects on activity and attention in children*" (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008).

Regulation (EC) Nº 1169/2011 standardizes food labeling for protection of consumer health (Regulation (EU) No 1169/2011 of the European Parliament and of the Council of October 25, 2011 on the Provision of Food Information to Consumers, 2011). Regulation (EC) Nº 1331/2008 establishes an ordinary authorization procedure for food additives, enzymes, and flavorings (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008). Regulation (EU) Nº 231/2012 laying down specifications for food additives are listed in Annexes II and IIIto Regulation (EC) Nº 1333/2008 (Regulation (EU) Nº 231/2012 of March 9, 2012 Laying down Specifications for Food Additives Listed in Annexes II and IIIto Regulation (EC) Nº 1333/2008 of the European Parliament

**Table 2**
Natural food dyes allowed as alternatives to azo dyes; specifications and salient properties.

| AZ | Natural alternative | Molecular formula | Chemical group | EN | Source of origin | Color | Solubility | Stability as a food coloring | **Maximum dose** (mg/L or mg/kg) | **ADI** (mg/kg bw/day) | Toxicity level | Food application | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TTZ | Curcumin | $C_{21}H_{20}O_6$ | Tetraterpenoids (carotenoid) | E100 | *Curcuma longa* L. (rizome) | Orange and yellow | Oil and polar solvents soluble | Optimal stability at pH < 7, becomes orange and unstable at pH > 7 | Food group dependent (*quantum satis* up to 250 mg/kg) | 0–3 | Low | Baked goods and egg-colored appetizers | (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Landim Neves et al., 2021; Pasias et al., 2015; Tvrdá et al., 2016) |
| SY/TTZ | Riboflavin (vitamin B2) | $C_{17}H_{20}N_4O_6$ | Flavin (nitrogenous base) | E101 | *Ashbya gossypi* (fungi) | Yellow | Soluble in alkaline solution | Heat resistant in protected lighting and moisture conditions | Food group dependent (*quantum satis* up to 100 mg/kg) | 0–0.5 | Low | Foodstuff and drinks | (Amaral De Faria Silva et al., 2022; Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Toma et al., 2023) |
| CAR | Cochineal or carmines | $C_{22}H_{20}O_{13}$ | Anthraquinone | E120 | *Dactylopius coccus* (insect) | Red | Varies with the nature of cations. | Stable in darkness and light sensitive | Combined maximum limit (*quantum satis* up to 250 mg/kg) | 2.5 | High | Extensive range of foodstuffs | (Aguilar et al., 2015; Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Pasias et al., 2015; Villaño et al., 2016) |
| CAR TTZ | Annatto Extracts | $C_{24}H_{28}O_4$ | Bixin and norbixin (carotenoid) | 160b | *Bixa orel-Lana* (seeds) | Red and orange | Water insoluble and soluble in ethanol | Good stability to heat and exposure to light when they are linked to proteins | Food group dependent (8–80 mg/kg) | 0.3 | Low | Flavored fermented milk products, cheese, ices, oils and so on | (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Pasias et al., 2015; Younes et al., 2019) |
| CAR AR | Paprika extract | $C_{40}H_{56}O_3$ | Capsanthin (xanthophyll carotenoid) | E160c | *Capsicum annum* (pepper) | Reddish orange | Oil soluble | Influenced by the lipid composition of the food | Food group dependent (10 mg/kg *up to quantum satis)* | 1.7 | Low | Dairy products, cheese, edible ice, cereals, and so on | (EFSA, 2015b; Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Pasias et al., 2015) |
| CAR AR | Lycopen extracts | $C_{40}H_{56}$ | Tetraterpene (carotenoid) | E160d | *Lycopersicon esculentum* L. (tomatoe) | Red | Not water-soluble and soluble in chloroform | Oxygen- and light-sensitive (antioxidants required) | Food group dependent (10–500 mg/kg) | 0.5 | Low | Salmon, jam and jellies, surimi, cheese, soups, drinks and snacks | (Aguilar et al., 2008; Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Pasias et al., 2015) |
| CAR AR | Beetroot red | $C_{24}H_{26}N_2O_{13}$ | Betacyanins | E162 | *Beta vulgaris* L. | Pink and red | Water soluble | Stable in the pH range of most foodstuff | *quantum satis* | N/A | Low | Creams, cheese, wine, and so on | (Calva-Estrada et al., 2022; EFSA, 2015a; Fu et al., 2020; Pasias et al., 2015) |
| BN | Vegetable or black carbon | C | N/A | E153 | Carbonization of vegetable materials | Black | Insoluble in water and any solvent | Highly stable to heat, light, and pH variations | *quantum satis* | N/A | Medium | Beverages, kippers, soups and supplements | (EFSA, 2012; Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008; Pasias et al., 2015) |

***Abbreviations:*** AZ: azo dye; TTZ: tartrazine; SY: sunset yellow; ADI: acceptable daily intake: QS: quantum satis; CAR: carmosine; AR: allura red AC; BN: brilliant black; N/A: not applicable.

and of the Council, 2012). Regulation (EU) Nº 257/2010 establishes a program to re-evaluate authorized food additives (Regulation (EU) Nº 257/2010 of March 25, 2010 Setting up a Programme for the Re-Evaluation of Approved Food Additives in Accordance with Regulation (EC) Nº 1333/2008 of the European Parliament and of the Council on Food Additives, 2010). Lastly, if the dye is intended for animal feed, it must follow Regulation (EC) Nº 1831/2003 (Regulation (EC) Nº1831/2003 of the European Parliament and of the Council of September 22, 2003 on Additives for Use in Animal Nutrition, 2014). Table 3 summarizes these statutes.

**Table 3**
Principle European regulations applied to additives and dyes in the food industry.

| Regulation | Application | Specification | Ref. |
|---|---|---|---|
| ***Food additives*** | | | |
| Reg (EC) Nº178/2002 | Generic | General requirements and principles of food legislation | (Regulation (EC) No 178/2002 Laying down the General Principles and Requirements of Food Law, Establishing the European Food Safety Authority and Laying down Procedures in Matters of Food Safet, 2002) |
| Reg (EC) Nº1333/2008 | Generic | Harmonize, ensure safety, quality, and ease of storage and use | (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of December 16, 2008 on Food Additives, 2008) |
| Reg (EC) Nº 1169/2011 | Generic | Specifies how additives must be labeled on foodstuffs | (Regulation (EU) No 1169/2011 of the European Parliament and of the Council of October 25, 2011 on the Provision of Food Information to Consumers, 2011) |
| Reg (EU) Nº 231/2012 | Generic | Specifications on origin, purity criteria and pertinent information | (Regulation (EU) Nº 231/2012 of March 9, 2012 Laying down Specifications for Food Additives Listed in Annexes II and IIIto Regulation (EC) Nº 1333/2008 of the European Parliament and of the Council, 2012) |
| Reg (EC) Nº 1831/2003 | Animal feed | Sets out the classification of feed additives by categories | (Regulation (EC) Nº1831/2003 of the European Parliament and of the Council of September 22, 2003 on Additives for Use in Animal Nutrition, 2014) |
| ***Food dyes*** | | | |
| Reg (EC) Nº1129/2011 | Part B and E | Regulations for food dyes (Annexes) | (Regulation (EU) Nº 1129/2011 of November 11, 2011 Amending Annex II to Regulation (EC) Nº 1333/2008 of the European Parliament and of the Council by Establishing a Union List of Food Additives, 2011) |
| Reg (EU) Nº 257/2010 | Re-evaluation | Carmoisine (2015) and Amaranth and Brilliant Blue FCF (2010) | (Regulation (EU) Nº 257/2010 of March 25, 2010 Setting up a Programme for the Re-Evaluation of Approved Food Additives in Accordance with Regulation (EC) Nº 1333/2008 of the European Parliament and of the Council on Food Additives, 2010) |

***Abbreviations:*** Reg: regulation.

The FDA and EFSA are responsible for safety regulations, assessing potential toxicity, and estimating human dietary exposure to colors (and other food additives) (Durazzo et al., 2022). In Europe, EFSA re-evaluates additives, whether to maintain or definitively change the ADI or, if scientific evidence is still inconclusive, to recommend a temporary ADI (Mota et al., 2021). For instance, SY had a temporary ADI of 1 mg/kg bw/day after its evaluation in 2009 (EFSA, 2009). In 2014, after re-evaluation, it was increased to 4 mg/kg bw/day. It was concluded that consumer exposure would be below this new ADI for all age groups (EFSA, 2014). In 2022, EFSA issued a communication evaluating SY as a component of animal feed and provided data on the safe concentrations for cats, dogs, fish, and poultry (Bampidis et al., 2022).

Food additive regulation is extensive in industrialized economies, where foods are routinely monitored for possible fraud or producer misconduct. However, there are discrepancies with regard to regulatory guidelines. Comparing the USA and the EU. Certain food dyes may be prohibited in one jurisdiction while allowed in the other, although they are produced in the same form and used for identical purposes. CAR (E122) or AM (E123) can be used at specified levels in the EU but are prohibited in some other countries. These discrepancies have led some countries to distrust EFSA and FDA guidance and to set their legislation on food dyes independently (Durazzo et al., 2022).

## 7. Future perspectives; alternatives

The use of artificial food dyes raises questions. Is the ADI an easily understood parameter for guiding consumer choice (Feitosa et al., 2017)? Are the amounts used by the industry safe? Dietary exposures vary widely, due to differences in food consumption patterns and in the quantities of dyes used (Villaño et al., 2016). Some improvements and alternatives that may help to resolve concerns about the use of food azo dyes are noted:

i Food color affects the ability of consumers to identify and perceive flavor, shapes distinctive flavor preferences and patterns, and may dominate the effects of labeling and even taste. Coloration is, in many cases, a food industry marketing strategy. Consequently, the effective use of food color for point-of-sale promotional purposes and understanding of the hedonic and sensory expectations triggered by food colors could bring greater objectivity to the use of these food additives (Dey and Nagababu, 2022).

ii. Regulations might differ across countries, but ADI values and toxicological specifications should be the same, worldwide. An international database would provide clearer knowledge of dye use, authorization, and potential risks (Pérez-Ibarbia et al., 2016).

iii. The use of natural food colorants can be optimized, yields can be enhanced, and consumer demand for health-promoting choices can be encouraged (Durazzo et al., 2022).

iv. Government agencies ought to strengthen their monitoring of the use of these dyes, ensuring that the food industry (with regard both to domestically produced and imported foods) follows the applicable regulations. Industries should carry out a “Hazard Analysis and Critical Control Point (HACCP)" for every azo dye used for food processing, to ensure that each is within appropriate limits and is no more than minimally toxic to humans and the environment (Okeke et al., 2022).

v. Using molecular dynamics simulations, it may be possible to compare the binding affinities of azo dyes with other dyes and food components and to track likely interactions, aiding risk assessment of the dyes (Ramos-Souza et al., 2022).

vi. Artificial intelligence technologies can be applied to detection and quantitation of azo dyes in food. Wu et al. (2022), developed

an electrochemical detection platform by applying a binary classification evaluation, using artificial intelligence, that improves the accuracy of TTZ determination in food samples (Wu et al., 2022).

## 8. Conclusions

Food dyes are important tools for the food industry, and azo dyes play a fundamental role. Tartrazine, Sunset Yellow, or Carmoisine are among the most used, mainly due to the coloring properties associated with their chemical structures. The Acceptable Daily Intake (ADI), toxicological data, and differences in international regulations are crucial in determining the dyes allowed in foods, and their quantities. Despite the considerable literature available regarding dietary exposures and potential adverse effects, the data are controversial. Several *in vivo* and *in vitro* studies have demonstrated dye toxicity after exposure to concentrations above the ADI. Substituting synthetic dyes with natural colorants is feasible in many cases. However, unfortunately, the technological and economic advantages of azo dyes may outweigh the advantages of natural dyes, when they are commercialized as colorants for the food industries.

Consumers are demanding improved information about the use of dyes in the food industry. Research on food dye toxicology, performed using assays accepted by regulatory agencies and applying laboratory best practices, can improve the consistency and reliability of data used for regulatory purposes. Development of natural alternatives to azo dyes can promote long-term substitutions and help to ensure food safety.

## CRediT authorship contribution statement

**P. Barciela:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **A. Perez-Vazquez:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **M.A. Prieto:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

No data was used for the research described in the article.

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