Safety of Some Synthetic Food Colours: Review

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ABSTRACT

Food additives are used to protect food, increase quality and extend shelf life in many stages, from production to consumption of food. Colorants added by food producers to color food or to adjust the color to desired level are among the commonly used food additives. Considering today's developing production technologies, foods fade or discolor at various stages of processing, storage, and sale due to physical and chemical conditions such as heat, light, pH and oxygen. Colorants are used to regain these color losses, to enhance weak colors, to give color to the food that is actually colorless, and to win back the favour of customers by hiding low quality. Therefore, the most used food colorants in Canada, China, European Union, Mexico and United States of America were presented. The chemical name, chemical structure, common uses, acceptable daily intake (ADI), and toxicity literature of six most used artificial food colors were reviewed.

Introduction

Food colour considered as an additive and according to the regulation definition, “it is any dye, pigment, or other substance that can impart colour to a food, drug, or cosmetic or to the human body.” (FDA, 2018). Addition of colorants to foods is thought to have occurred in Egyptian cities, where candy makers around 1500 BC added natural extracts and wine to improve the products appearance (Meggos, 1995). In 1856 the first synthetic colour (mauvine), was developed by Sir William Henry Perkin and the first chemically synthesized colorants were created from aniline in 1900s (Maronpot et al., 2020). Colour is an important characteristic and selection criterion for food choice. Recent studies have highlighted this importance and have shown how selection may change among certain populations, and over time (Clydesdale, 1993). Colorants are used in the production of soft drinks, candies, chewing gums, chocolates, jelly, bakery products, canned and vegetable products, dairy products, and meat and fish products (Asif Ahmed et al., 2021). There was no control over this use of colour and so inevitably legislation came into force. In particular that was as a result of health concerns over some of the toxic compounds used. An established list of permitted synthetic colours eventually came into force in most countries early in this century. In the last twenty years however, consumers have become increasingly aware of the ingredients in their foods and as such they require foods to be as ‘natural’ as possible. This combined with technological developments has fuelled the increase in the usage of naturally derived colours. Naturally derived colours are usually less stable to heat, light, pH and oxygen. Stability of natural pigments is considered the main challenge to overcome for their utilization as food colorants (Jadhav and Bhujbal, 2020). They may interact with other ingredients, resulting in the development of unwanted colours and flavours and all hues might not be available. Therefore, this article reviews the scope of some synthetic colours in food processing and increasing the awareness of safety limits of food colorants.
Synthetic Food Colours Impacts

Some of food colours and additives were declassified as human carcinogens following the comprehensive evaluation of results of at least three tests: cytotoxicity, genotoxicity and mutagenicity in vitro and some in vivo to guarantee safety of these colours. (Thomas and Adegoke, 2015).

The objectives of genotoxicity tests are to detect mutagens and carcinogens (Sasaki et al., 2000). The tests are designed and aimed at detecting compounds that induce genetic damage by various mechanisms.

The Most Used Synthetic Food Colours

The most used food colorants in Canada, China, European Union, Mexico and United States of America are presented in Table 1.

Synthetic Colours Safety Literature

The following tables (Tables 2-7) represent the chemical name, chemical structure, common uses, ADI, and toxicity literature commentary of six most used artificial food colours.

Table 1. The most popular synthetic food colours.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Amaranth</th>
<th>Erythrosine</th>
<th>Sunset Yellow</th>
<th>Allura Red</th>
<th>Tartrazine</th>
<th>Brilliant Blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD&amp;C Name</td>
<td>Red</td>
<td>Red</td>
<td>Yellow</td>
<td>Red</td>
<td>Yellow</td>
<td>Blue</td>
</tr>
<tr>
<td>E-Number</td>
<td>No.2</td>
<td>No.3</td>
<td>No.6</td>
<td>No.40</td>
<td>No.5</td>
<td>No.1</td>
</tr>
<tr>
<td>Canada, Australia, EU</td>
<td>E-123</td>
<td>E-127</td>
<td>E-110</td>
<td>E-129</td>
<td>E-102</td>
<td>E-133</td>
</tr>
<tr>
<td>Japan, Brazil</td>
<td>E-129</td>
<td>E-102</td>
<td>E-133</td>
<td>E-129</td>
<td>E-102</td>
<td>E-133</td>
</tr>
<tr>
<td>USA</td>
<td>E-129</td>
<td>E-102</td>
<td>E-133</td>
<td>E-129</td>
<td>E-102</td>
<td>E-133</td>
</tr>
</tbody>
</table>

Amaranth

A dark reddish powder is soluble in water and slightly soluble in ethanol. The color intensity of the solution cannot be changed by hydrochloric acid while, sodium hydroxide increases it. Table (2) shows the chemical name, chemical structure, common uses and ADI of Amaranth.

Gene mutation

There are many reports documenting the lack of genotoxicity of Amaranth on bacteria (Brown et al., 1978; Haveland-Smith and Combes, 1980; Chung et al., 1981; Das and Mukherjee, 2004).

In vivo assays

There is still a large amount of uncertainty and discrepancies on the reported in vivo follow up tests. Sasaki et al. (2002) reported the genotoxicity of Amaranth using comet assay on eight mouse organs. Following a 24 h single dose treatment, amaranth at doses lower than the admissible daily intake (2000 mg/kg-body weight (b.w.)) showed genotoxicity damage in three different organs. There is insufficient evidence to confirm carcinogenicity of Amaranth in humans and it is presently unclassified as a human carcinogen.
Tartrazine
A bright-orange yellow powder freely soluble in water. Hydrochloric acid does not change the aqueous solution while, becomes redder in the presence of sodium hydroxide. The chemical name, chemical structure, common use and ADI of Tartrazine are tabulated in Table 3.

Cytogenetic evaluation
The interaction of Tartrazine and endogenous material like bovine hemoglobin was described as spontaneously involving Van der Waal’s forces and hydrogen bonds between the oxygen atoms at position 31 and 15 in the dye (Li et al., 2014). In a similar study, Tartrazine showed extensive DNA binding, cytostatic potential and reduced mitotic index (Mpointoukas et al., 2010).

Gene mutation assays
Several reports have indicated the non-mutagenicity of Tartrazine using Salmonella typhimurium and Escherichia coli (Chung et al., 1981; Das and Mukherjee, 2004; Elkhim et al., 2007; EFSA, 2009a).

In vivo assays
Sasaki et al. (2002) reported the results of comet assay on eight mouse organs following oral administration of Tartrazine up to doses of 2000 mg/kg-b.w. After only 3 h post-administration, DNA damage was reported at dose levels of 10 mg/kg-b.w in the colon and the glandular stomach at doses higher than 10 mg/kg-b.w. (Sasaki et al., 2002). Poul et al., (2009) demonstrated the non-mutagenicity of Tartrazine when administered as oral gavage up to doses of 2000 mg/kg-b.w.

Allura red
A red azo dye used as a color in both food and beverages. It is usually supplied as its red Na salt and can also be used as Ca or K salts. These salts are soluble in H2O. The chemical name, chemical structure, common uses and ADI of Allura red are presented in Table 4.

Cytogenetic evaluation
Allura red is non-genotoxic in many gene mutation tests involving prokaryotic and eukaryotic cells with or without activation (Chung et al., 1981; Combes and Haveland-Smith, 1982; (Kobylewski and Jacobson, 2012). However, Allura red was reported to show direct genotoxic effect when different concentrations of the dye ranging from 9.76 to 5000 μg/ml was incubated with a culture of Saccharomyces cerevisiae at 37°C. Comet assay revealed dose-related DNA damage starting at concentration of 1250 μg/ml, though no positive correlation could be established with exposure time (Jabeen et al., 2013).

In vivo assays
The non-teratogenicity of Allura red has been reported after groups of 11 days old pregnant rats were fed up to 2000 mg/kg-b.w. of single oral doses of Allura red (Tsuda et al., 2001). Comet assay was used to assess DNA damage in the embryo at 3, 6 and 24 h sampling times. When assessed by comet assay, colon-specific DNA damage was reported in mice at doses of 10 mg/kg-b.w. three hours post-administration but no damage was observed in rats at any of the tested dye doses or exposure times (Shimada et al., 2010).

Table 3. Tartrazine

<table>
<thead>
<tr>
<th>Colour</th>
<th>Tartrazine FD&amp;C Yellow No. 5, Food Yellow No.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>3-carboxy-5-hydroxy-1-(4’sulphophenyl)-4-(4'-sulphophenylazo) pyrazoletrisodium salt.</td>
</tr>
<tr>
<td>Chemical structure</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Tartrazine Structure" /></td>
<td></td>
</tr>
<tr>
<td>Physical description</td>
<td>Tartrazine is a lemon yellow dye that provides a yellow to orange shade in applications. FD&amp;C Yellow 5 is principally the trisodium salt of 4,5-dihydro-5-oxo-1-(4-sulfophenyl) 4-[(4-sulfophenylazo) -1H-pyrazole -3-carboxylic acid. It is soluble in water and sparingly soluble in ethanol. The chemical name is 5-oxo-1-(p-sulfophenyl)-4-[(p-sulfophenyl) azo]-2-pyrazoline-3-carboxylic acid, trisodium salt.</td>
</tr>
<tr>
<td>Common uses</td>
<td>FD&amp;C Yellow 5, also known as Tartrazine, has been used in foods since 1916 and provides a pleasing lemon yellow colour when used in foods, drugs, and cosmetics. It is used to colour beverages, dessert powders, candy and confections, ice cream, custards, puddings, preserves, bakery, dairy fats and oil, meat, seafood, snacks, dry mixes and seasonings, fruit preparations, convenient foods and flavours.</td>
</tr>
<tr>
<td>Consumption levels</td>
<td>The FDA established a maximum ADI for FD&amp;C Yellow No. 5 of 5.0 mg/kg of body weight per day (Kobylewski and Jacobson, 2012). This level is equivalent to 300 mg (about 0.01 oz) per day for a 60 kg (132 lb) person. EU established a maximum ADI of 7.5 mg/kg of body weight per day, based on the result of animal studies. This level is equivalent to 450 mg (0.016 oz) per day for a 60 kg person. USA: GMP (FDA, 21 CFR 74.705), FDA also maintains an ADI of 5 mg/kg bw/day. EU: ADI of 0-7.5 mg/kg body weight; EFSA has also established MPLs for use of Tartrazine in foods and beverages in Europe.</td>
</tr>
<tr>
<td></td>
<td>JECFA: ADI of 0-10 mg/kg body weight (JECFA 82nd Meeting Summary &amp; Conclusions, 2016).</td>
</tr>
</tbody>
</table>
Table 4. Allura red

<table>
<thead>
<tr>
<th>Colour</th>
<th>Allura Red FD &amp; C Red 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>disodium 2-hydroxy-1-(2-methoxy-5methyl-4-sulphonatophenylazo) naphthalene-6sulphonate.</td>
</tr>
<tr>
<td>Chemical structure</td>
<td><img src="image1" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Physical description</td>
<td>Allura Red is an orange red dye that has a red to brownish shade in applications.</td>
</tr>
<tr>
<td>Common uses</td>
<td>Allura Red is a highly versatile food colour used in dairy fats and oil, snacks, convenient food, confectionery, dry mixes and seasonings, flavours, gelatin, puddings, custards, alcoholic and non-alcoholic beverages, fruit preparations, canned and frozen fruit juices, dairy products, bakery products, sausage casings, jams, jellies, condiments, candy, frostings, meat, poultry and seafood. FD&amp;C Red No. 40 is also used in pharmaceuticals and cosmetics (Kobylewski and Jacobson, 2012). USA: GMP (FDA, 21 CFR 74.340); US FDA also maintains ADI of 7.0 mg/kg-bw day (Kobylewski and Jacobson, 2012).</td>
</tr>
<tr>
<td>Consumption levels</td>
<td>EU: ADI of 0-7 mg/kg body weight (EFSA, 2009b); EFSA has also established MPLs for use of Allura Red in foods and beverages in Europe. JECFA: ADI of 0-7 mg/kg body weight (JECFA, 25th Report, 1981). An acceptable daily intake (ADI) of 7 mg/kg body weight per day is equivalent to 420 mg (.015 oz) per day for a 60 kg (132 lb) person.</td>
</tr>
</tbody>
</table>

Table 5. Sunset yellow

<table>
<thead>
<tr>
<th>Colour</th>
<th>Sunset yellow FCF Food Yellow No.6, CAS No. 2783-94-0.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>Disodium 6-hydroxy-5-[(4sulfophenyl) azo]-2-naphthalenesulfonate.</td>
</tr>
<tr>
<td>Chemical structure</td>
<td><img src="image2" alt="Chemical structure" /></td>
</tr>
<tr>
<td>Physical description</td>
<td>Sunset Yellow is a yellow dye that provides a reddish-orange shade in applications. FD&amp;C Yellow No. 6 is principally the disodium salt of 6-hydroxy-5-[(4-sulfophenyl)azo]-2- naphthalenesulfonic acid. The trisodium salt of 3-hydroxy-4[(4-sulfophenyl)azo]-2,7- naphthalenesulfonic acid may be added in smaller amounts.</td>
</tr>
<tr>
<td>Common uses</td>
<td>FD&amp;C Yellow No. 6, also known as Sunset Yellow, has been used in foods since 1929 and provides a pleasing orange color when used in foods, drugs and cosmetics. FD&amp;C Yellow No. 6 is used to color dessert powders, cereals, bakery goods, snack foods, confectioneries, cherries, sausage, ice cream, sherbet, dairy products, dairy fats and oil, meat, seafood, dry mixes and seasonings, fruit preparations, convenient food, flavours and beverages (Kobylewski and Jacobson, 2012).</td>
</tr>
<tr>
<td>Consumption levels</td>
<td>USA: maximum ADI of 3.75 mg/kg of body weight per day (Kobylewski and Jacobson, 2012). This level is equivalent to 225 mg per day for a 60 kg person. EU: ADI of 0-4 mg/kg body weight JECFA: ADI of 0-4 mg/kg body weight (JECFA, 74th Report, 2011)</td>
</tr>
</tbody>
</table>

**Sunset yellow (FCF)**

Sunset yellow in the form of orange-red crystals is soluble in water. It forms reddish-orange solution in concentrated sulfuric acid, changing to yellow on dilution. Table 5 shows the chemical name, chemical structure, common uses and ADI of Sunset yellow.

**Cytogenetic evaluation**

Early reports demonstrated the non-mutagenicity of Sunset yellow on *E. coli*, four tester strains of *S. typhimurium* TA 1538, 1535, 100 and 98 strains with or without metabolic activation (Chung et al., 1981; JECFA, 1982; Wever et al., 1989). Haveland-Smith and Combes (1980), also tested the ability of twenty five dyes to induce mutations in a tryptophan-requiring *E. coli* strain (sensitive to base substitutions) and a histidine auxotroph of *S. typhimurium* strain TA1538 (specific for frame shifts). Sunset yellow was demonstrated to be non-mutagenic with or without metabolic activation.

**In vivo assays**

Sunset Yellow FCF did not induce DNA damage in any of the eight mouse organs-glandular stomach, colon, liver, kidney, urinary bladder, lung, brain, bone marrow-assessed by comet assay following a single oral dosing of the dye up to 2000 mg/kg (Sasaki et al., 2002; Kobylewski and Jacobson, 2012).

**Chronic toxicity**

A number of studies describing the administration of Sunset yellow in doses of 0 to 2% in mice for periods of 52
to 80 weeks have reported no significant difference in the incidence of tumours when compared with appropriate control groups (Bonser et al., 1956; Gaunt et al., 1974; JECFA, 1982; EFSA, 2009c). Similarly long-term studies in rats, hamster and dogs have not detected any carcinogenic effects associated with the dye when administered up to 5% doses (JECFA, 1982; EFSA, 2009c).

**Erythrosine**
A brown powder. It is soluble in H₂O and ethanol. It reacts with hydrochloric acid and produces a yellowish brown precipitate. It also reacts with sodium hydroxide and produces a precipitate soluble in access of the reagent. Table 6 presents the chemical name, chemical structure, common uses and ADI of Erythrosine.

**Cytogenetic evaluation**
A high degree of cytotoxicity and cytostaticity has been reported at Erythrosine doses of 2.4 and 8 mM when tested on human peripheral blood cells in vitro (Mpountoukas et al., 2010). In the same study, extensive direct binding of the dye to calf thymus DNA was reported.

**Long-term carcinogenicity**
Long-term carcinogenicity studies have demonstrated that there was no significant difference in the incidence of non-neoplastic lesions or malignant tumours observed in the control and treated groups of Charles River CD weanling rats of both genders that have been exposed in utero to the dye and subsequently fed Erythrosine doses of 0.1, 0.5, or 1.0% for 30 months (Borzelleca et al., 1987; EFSA, 2011).

In another study, no tumour was observed in groups of Osborne Mendel rats that received oral gavages (0, 0.5, 1.0, 2.0 or 5.0%) or sub-cutaneous injections (12 mg/rat) for 24 months (Hansen et al., 1973). No mortality or dose-related adverse effects were reported in a similar study where groups of three female and three male beagle dogs were fed 0, 0.5, 1.0, 2.0 or 5.0% for 2 years (Hansen et al., 1973).

**Gene mutation assays**
The inability of Erythrosine to induce gene mutation in *S. typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 at concentrations of up to 10,000 µg/plate have been demonstrated. The dye or its metabolites are reportedly non-mutagenic (Auletta et al., 1977; Bonin and Baker, 1980; Ishidate et al., 1984; JECFA, 1986; EFSA, 2011). In another report, Erythrosine was not only nonmutagenic in *S. typhimurium* strains TA 97a, TA 98, TA 100, TA 102 and TA 104 but also reduced the antimutagenic potential of benzopyrene, sodium azide and ethidium bromide (Lakdawalla and Netrawali, 1988).

Erythrosine (at doses of 50, 100 and 200 mg/kg-b.w. repeated after 24 h) did not increase the frequency of sister chromatid exchanges in peripheral blood lymphocytes in male B6C3F1 mice. No increase in the frequency of micronuclei in the bone marrow polychromatic erythrocytes or peripheral blood reticulocytes was also observed. The authors suggested that the lack of a clastogenic potential provides support for the non-genotoxic mechanism of the carcinogenicity of Erythrosine (Zuno et al., 1994).

**Brilliant Blue (FCF)**
A reddish violet powder or granules with a metallic luster. It is soluble in H₂O and alcohol. It has pale amber solution in concentrated sulfuric acid, changing to yellow then greenish blue on dilution. The chemical name, chemical structure, common uses and ADI of Brilliant blue are presented in Table 7.

<table>
<thead>
<tr>
<th>Table 6. Erythrosine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
</tr>
<tr>
<td>-</td>
</tr>
<tr>
<td>Chemical structure</td>
</tr>
<tr>
<td>Physical description</td>
</tr>
<tr>
<td>Common uses</td>
</tr>
<tr>
<td>Consumption levels</td>
</tr>
</tbody>
</table>

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Table 7. Brilliant Blue

<table>
<thead>
<tr>
<th>Colour</th>
<th>Brilliant Blue FCF Food Blue No. 1, FD &amp; C Blue No.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical description</td>
<td>FD&amp;C Blue No. 1 is a greenish blue dye that adds a blue shade in applications. Brilliant Blue FCF is soluble in water and slightly soluble in ethanol.</td>
</tr>
<tr>
<td>Common uses</td>
<td>It has been used in foods in the US since 1929. This color adds a distinctive, bright blue hue to beverages, beverage powders, dairy products, baked goods, dessert powders, confections, condiments, icings, syrups, jams, jellies, marmalades, liqueurs, extracts, dairy fats and oil, meat, seafood, snacks, dry mixes and seasonings, fruit preparations, convenient food, and flavors (Kobylewski and Jacobson, 2012).</td>
</tr>
<tr>
<td>Consumption levels</td>
<td>Brilliant Blue is approved for use in many countries throughout the world, including the US, Canada, India, Japan and the European Union. USA: GMP (FDA, 21 CFR 74.101); US FDA also maintains ADI of 12.0 mg/kg-bw day (Kobylewski and Jacobson, 2012). EU: ADI of 0.6 mg/kg body weight (EFSA, 2010); EFSA has also established MPLs for use of Brilliant Blue in foods and beverages in Europe JECFA: ADI of 0-12.5 mg/kg-body weight (JECFA, 13th Report; 1969) JECFA’s maximum ADI is equivalent to 750 mg (about .02 oz) per day for a 60 kg (132 lb) person.</td>
</tr>
</tbody>
</table>

In vivo assays

Brilliant Blue did not increase the frequency of micronuclei in the bone marrow of groups of mice that received intra-peritoneal doses of 0, 500, 1000 or 2000 mg/kg-bw (EFSA, 2010). In another study that was not considered by the last EFSA re-evaluation of brilliant blue because of the inconsistent osmolality of cell culture medium used, the dye was reportedly genotoxic in the chromosome aberration assay of over 190 additives using Chinese hamster fibroblast cell line (Ishidate et al., 1984; EFSA, 2010). The colourant at doses up to 2000 mg/kg-bw gave negative results in a genotoxicity assessment of 39 food additives by comet assay on eight mouse organs (Sasaki et al., 2002).

Long-term carcinogenicity

In a lifetime/carcinogenicity study that involve F0 and F1 generation, dye doses of 0.1, 1.0 or 2.0% were fed to groups of Charles River CD rats for a period of 116 weeks for the males and 111 weeks for the females. A non-observed-adverse-effect-level (NOAEL) of 2 and 1% was established for the male and female groups respectively. In the same study with CD mice fed up to 5% dye, no adverse effects was observed and the NOAEL was established at 5% for both genders (Borzelleca et al., 1990).

In a study conducted before the OECD guidelines were formulated and publicized, dietary concentrations of brilliant Blue, 0.03, 0.3 or 3% when fed to groups of 30 rats for 75 weeks did not produce any dose-related adverse effects on the growth or mortality (Mannell et al., 1962). Similar results were seen with the sub-cutaneous injection (Mannell and Grice, 1964).

Gene mutation assays

The non-mutagenicity of brilliant blue in various Salmonella strains with or without metabolic activation has been demonstrated in many studies (Brown et al., 1978; Bonin and Baker, 1980; Haveland-Smith and Combes, 1980; Ishidate et al., 1984).

Conclusion

It could be concluded that the present review indicated that synthetic colorants are adversely affecting hepatic and renal parameters comparing to natural colorants. Therefore, it is necessary be aware about the hazardous effects of consuming such synthetic food colorants. Furthermore, the implementation of laws, regulations, proper labelling and awareness programs of artificial food dyes for customers and food processors are highly recommended looking for food safety. Also, further research is needed to evaluate toxicity of synthetic food colors.

Conflict of Interest

The authors have declared no conflict of interest.

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