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Impact of Synthetic Food Colouring Agents on Aquatic Ecosystems and Human Health

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KS and KDJ have designed, conceptualized the work. Authors DU, KS and KDJ have done the literature survey and initial review writing. Authors TJ and AT have performed review proofreading and editing. All authors read and approved the final manuscript.

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ABSTRACT

Colour is an important factor to enhance the consumer acceptance and appetizing value towards food and beverages. Foods are coloured with both natural and artificial/ synthetic food dyes. Instead of natural food colours, synthetic food colours are now widely employed by the food industry because of its superior features (cheap cost, enhanced look, greater colour intensity, increased colour stability, and consistency). Life of synthetic colorants is quite long as they are

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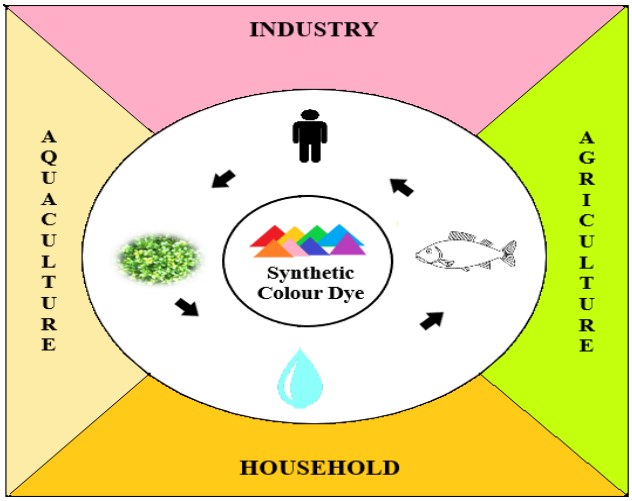
highly soluble in water and oil. Based on their solubility, synthetic colorants are classified into fat soluble synthetic colours, lake colours, water soluble synthetic colours. Colouring agents have spread across the water and soil ecosystem, this is because they are mass-produced and wider application. Samples of water, suspended particles, sediment, and wild fish have all tested positive for food dyes. As a result, they are regarded as micropollutants in aquatic systems. Cancers, mutations, neurotoxic effects, decreased haemoglobin concentrations, allergic reactions, food hypersensitivity, abdominal pain, worsened asthmatic symptoms, vomiting, and diarrhoea are just some of the potential dangers to one's health, which can result from using synthetic colours that aren't allowed or using them in excess. This review critically evaluates the effect of synthetic food colouring agents on aquatic environment and human health. We found that there is a definite unfavourable effect of synthetic organic additives on aquatic life and human health. Some synthetic colouring dyes have toxicological qualities and pharmacological action, thus it's important to keep an eye on water bodies. Synthetic organic food colour in water ecosystems may constitute a harmful effect on animals or human health's, it is urgent need to evaluate the hazard potential of these substances. For this reason, it is strongly suggested that both consumers and manufacturers of food colours should be aware about the rules and regulations regarding food colours.

GRAPHIC ABSTRACT



Keywords: Food colouring agents; toxic effects; human health; aquatic environment; health risk.

1. INTRODUCTION

Synthetic food additives or food colouring refers to any dye or chemical which generates colour after addition into food or drink or beverages. For home cooking and industrial food production purpose, we use food colouring pigments. Colorants of food are the chemical compound which added to matrixes for better food or maintain sensory characters of the food products and altered or lost during the processing of the food products storage. In modern consumerism, optical aspect plays a major character in the selection of food products. So, the usage of colours in food and beverages has become a key visual aspect to sale the food product. The usage of artificial food dyes as synthetic additives has been increased as a replacement for natural colours due to improve the look, increased colour intensity, enhanced stability and uniformity of the food product by its application [1,2]. Synthetic food dyes compared to organic dyes have good colour stability, low cost and resilience to light and pH. Various of synthetic food additives are derived from the coal tar, including an azo group in their chemical structure. In the market, both overused synthetic food additives and non-permitted synthetic food additives have been found in food and beverages. Synthetic food dyes tested on the animal model are banned because of their adverse effect. Human health concerns have been raised about several of the nine approved synthetic food additives. According to various researches, synthetic food additives key contributors to food poisoning which leads to devastating impact on human

health [3,4]. The synthetic organic dyes also contaminate the aquatic ecosystems. synthetic organic dyes now classified as Micropollutants (ng/l to ng/g) because of their low concentration in aquatic life's [5,6].

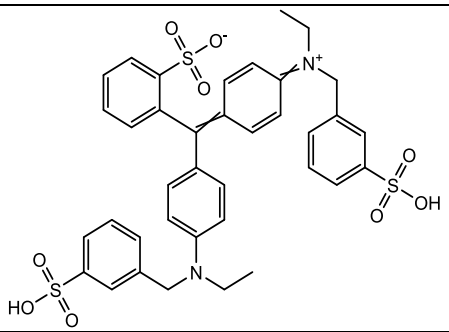
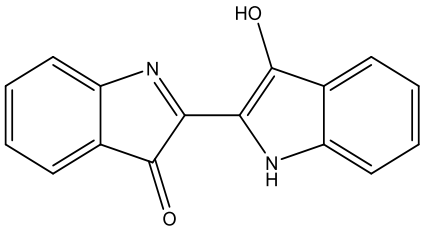
In 1865, William Perkin discovered the first synthetic organic dye, called mauveine. This breakthrough started mass manufacture of synthetic organic dyes all over the world, which revolutionize the organic dyes business [6]. European countries dominated the world market for dyestuff by the turn of the twentieth century. Now, China and India, the world's two biggest economies, are now the industry's primary manufacturers and suppliers. It becomes of big concern in between many environmentalists, because of artifical food dyes being transferred into water enviornment which causes mutagenic, carcinogenic, and genotoxic consequences in addition to aesthetic harm to aquatic bodies [7-9]. More and more people are looking into this problem, publishing studies and reviews on the environmental damage caused by textile dyes, which is a good sign that people all around the world care about this [10].

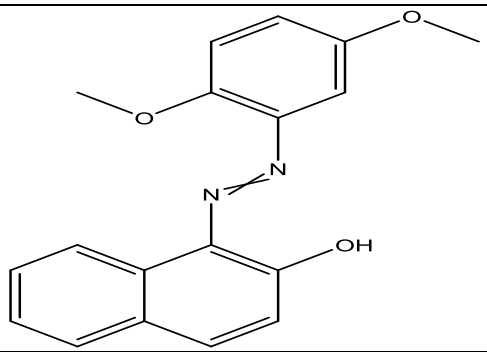
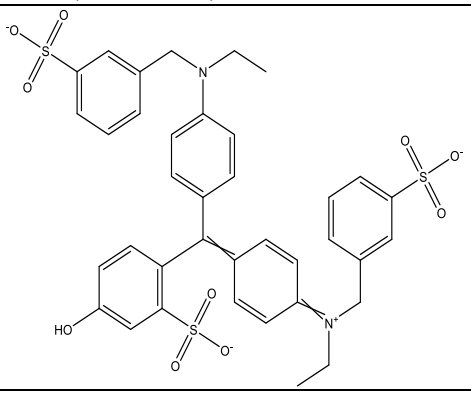
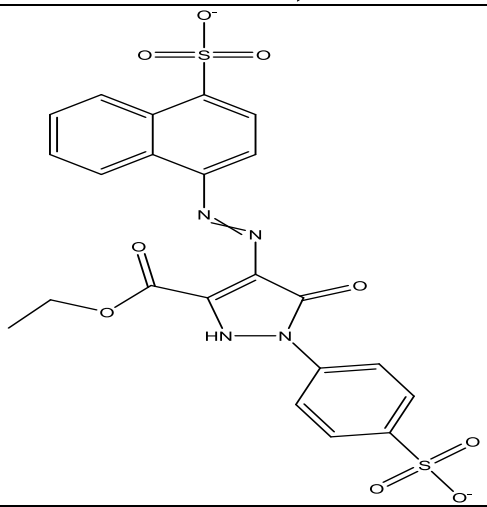
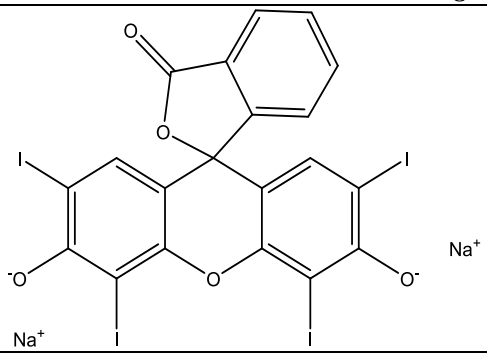
In aquatic environment, the important source of dyes is textile industry. There is at least a 5% dye wastage rate and as high as a 50% dye wastage rate across the different dyeing processes, leading to almost 200 billion litters of coloured effluents every year, making the textile sector a significant contributor to dyes in aquatic habitats [11,12]. The delayed or nonbiodegradability of several synthetic organic dyes adds another layer of difficulty to the removal of these substances from wastewater using currently available technology at sewage treatment plants. (STPs) [12]. Multiple reports have come out in recent research as well as past years, the predominance of artificial colouring agents in water and water bodies [13,14], in sediment [15], and in exotic fish [16,17]. There has to be a thorough evaluation of the effects of organic food colours on aquatic ecosystems before efforts can be made to reduce the amount of these colours in the water.

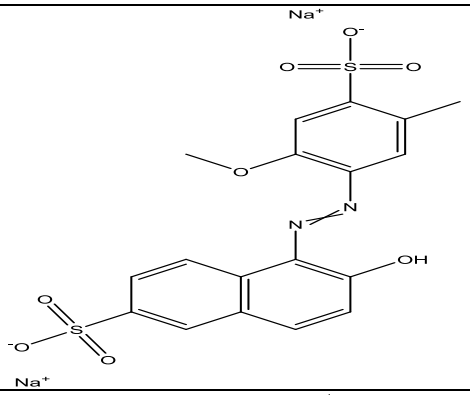
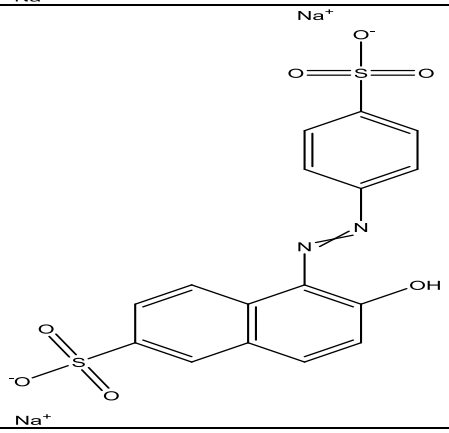
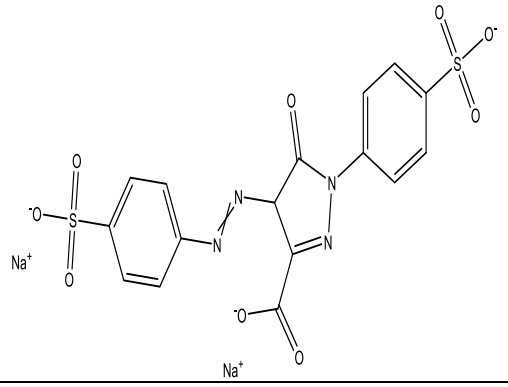
2. CLASSIFICATION OF SYNTHETIC FOOD COLOURING AGENTS

The usage of artificial food colouring is increased dramatically since 1955, when the U.S. Food and Drug Administration (FDA) first began tracking its use. The FDA verifies each batch of dyes/colours which satisfy the legal standards further used in food. The certification method helps by providing data on annual shipments of food colour used in food, medicine, and cosmetics [18]. Ninety percent of all artificial food dyes are made up of only three different colours: Allura red, sunset yellow, and tartrazine. Major food colours (according to CSPI, Summary of research on Food Dyes report) and their structures are mentioned in Table 1.

Table 1. Major Synthetic Colours and Their Molecular structures

Sr. no.	Food colour	Structures	Colour
1.	**Brillant** Blue		Blue
2.	**Indigo Blue**		Indigo

Sr. no.	Food colour	Structures	Colour
3.	**Citrus Red 2**		Dark Red
4.	**Fast Green**		Turquoise
5.	**Orange B**		Red Orange
6.	**Erythrosine**		Watermelon red

Sr. no.	Food colour	Structures	Colour
7.	Allura Red		Red
8.	Sunset Yellow		Orange
9.	Tartrazine		Yellow

Mostly research looked at combinations and individual use of synthetic food colours, whereas many products and diets contain other chemicals that are common in many goods and diets. They may contribute to additive or synergistic effects of the food additives. Some children have been shown to exhibit hyperactivity, in addition to other behavioural issues, when exposed to some synthetic colour mixes (and Yellow 5 tested solo) were used, which have been linked to organ damage, cancer, birth abnormalities, allergic responses, and more [19,20]. By the end of 2009, the British government recommended that businesses phase out the majority use of synthetic food colours. While the European Union warned many members, no more consumption of food dye containing products after July 20, 2010. Food colourings cannot be deemed healthy because of their considerations of toxicological, including carcinogenicity, hypersensitivity reactions and behavioural consequences [21].

The analysis presented in the current study will offer an update on the latest studies that have arisen in the accessible in-depth literatures about the usage and safety of food colours. We have tried to remain focused on these aspects of safety: cytotoxicity, genotoxicity, carcinogenicity, and mutagenicity induction or potential.

3. ROLE OF SYNTHETIC FOOD COLOURING AGENTS ON ENVIRONMENT

The food laws have been constantly updated by the Central Committee for Food Standards of India, as food colours have harmful effects on humans. Because of the portion of these CCFS regulations, synthetic colours used in certain foods such as Fast red E and Amaranth have been prohibited and advised to restrict the use of artificial food additives cap from 200 to 100 ppm in all goods for human consumption except in canned food items, jams and jellies [22,115]. Several synthetic food dyes are now in use on a large scale, with some nations using far more than others. Only seven synthetic food additives are permitted by USA; In contrast to India, where only eight coal tar food colours are allowed, the European Union permits the use of sixteen synthetic food additives are required to be used in such food items under the Prevention of Food Adulteration regulations (PFA). These include Carmosine, Ponceau 4R, Erythrosine, Tartrazine, Sunset yellow, Indigo Carmine, Bright Blue and Strong Green FCF. Metanil black, Rhodamine B, Orange G, Blue VRS, Auramine are unregulated food colours and some dye and lake synthetic food colours [23].

In the sector of industrial area, the quality of synthetic additives is subject to checks, but in the non-industrial sector, the quality of synthetic colours is not, despite statutory controls, leading to an expansion in the production of substandard food goods, that are harmful to the health of human and, consequently, a financial burden on the national economy. Some synthetic additives are approved, the indiscriminate intake is unsafe, which leads to greater frequency of gastrointestinal disorders including cholera, enteric fever, TB etc. Mutagenic and possibly carcinogenic, the other unapproved food additives provide significant health risks [24]. In no case may a food include more than 0.1 milligrams per kilogram of colour additives. To determine, what amount of food colouring is acceptable to consume on a daily basis, The Level of Food Additives with Maximum Allowable was established by the Joint FAO/WHO Expert Committee on Food Additives which is called as Acceptable Daily Intake limit (ADI) (Table 3) [25].

Many environmental and health problems are caused by chemicals which are used in the industries for making synthetic food colour. While they impact the immediate discharge of untreated effluent and leakage of toxic chemicals from water factories. Due to the presence of hydrosulphides, the concentration of oxygen in the water body is decreased and prevents the flow of light through the water body. Approximately 40 per cent of colorants used worldwide contain organically bound chlorine that is a carcinogen. Synthetic food colour effluent untreated or incompletely processed can be detrimental to both marine and terrestrial organism's creatures by disrupting the natural ecosystem and having long-term negative impacts.

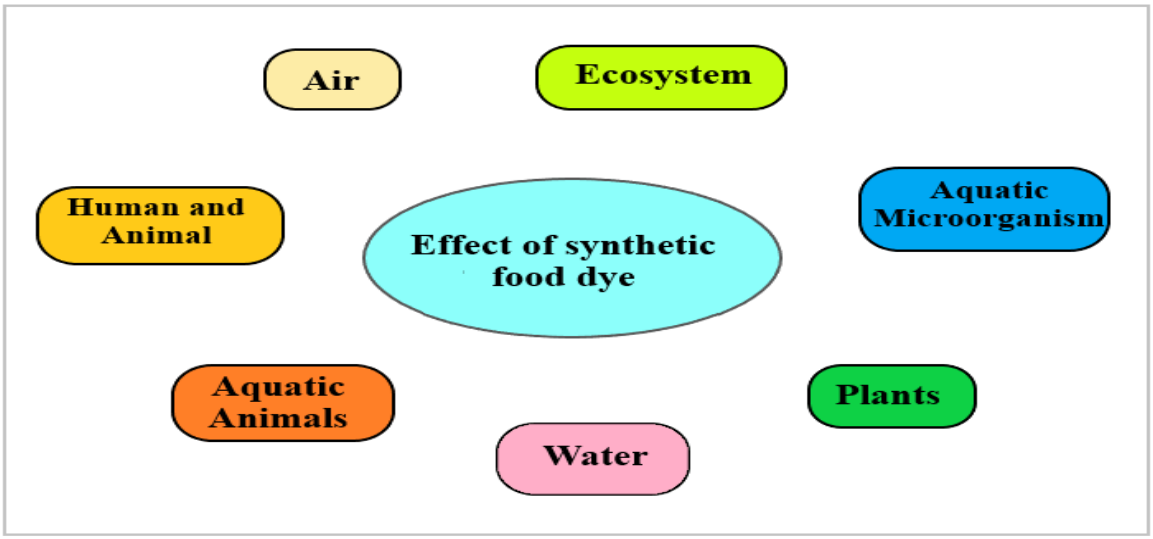


Fig. 1. Impact of Synthetic Food Dye on Environment

The International Conference on Harmonization in 2003, 2011and 2018, the Organization for Economic Co-operation and Development and the European Food Safety Authority have recommended numerous measures to achieve targeted assessments. It has been noted that there is no single test that can provide definitive evidence of the health problems of these synthetic food dyes and artificial additives. So, it is advised that minimum set of tests be carried out to ensure the safety of these items or goods. The survey of published literature disclosed that, once any licensed chemicals or synthetic dyes become a focus of safety concerns, researchers carry out thorough analysis resulting into the removal of genotoxic or carcinogenic labels from substances that have previously been reported. Certain food dyes and chemicals are routinely removed from the list of known human carcinogens after careful review of genotoxicity and mutagenicity data from in vivo and in vitro investigations in tissues of mammalian and entire animals. Therefore, the safety criteria for repeated reactions to the substances must be stressed to avoid the development of noticeable and terrible side effects arising from the ingestion over time of subtoxic doses of the additives.

Some heavy metals are the part of food colouring agents, majority of which have a negative impact on health of living organisms which may be very severe [26]. The human body needs about 70 pleasant heavy metals, but certain metals such as Cadmium, Arsenic, Chromium, Iron, Lead, Copper, Mercury and Zinc can cause considerable health implications because they interfere with the enzyme system and metabolism as they are readily available in consumption water making it very problematic round the world [27,28]. Many metals are essential for organisms at low concentrations, but when in high concentrations, toxic impacts are observed. Several heavy metals, such as cadmium, mercury and arsenic are among the omnipresent harmful metals which endow in drinking water and for the body, have no useful functions [29-31].

4. IMPACT OF FOOD COLOURING AGENT USE IN INDIAN SUBCONTINENT

It is well-documented that animals and humans subjected to experiments suffer when exposed to unapproved synthetic food colours, according to the results of several research published from India on the topic of permitted and non-permitted food colour. The educated populace in and around Chittoor district of Andhra Pradesh, Tirupati city, was polled to determine their knowledge of the risks associated with consuming products containing synthetic food colours because of the reports that have been made about the public's increased awareness of their use. The synthetic food additives such as sunset yellow, Tartrazine, and erythrosine were collected from imperial chemical industry, Uttarakhand (UK food guide *et al*;2007). Mixtures of Tartrazine / Carotid, Sunset Yellow / Curcumin and Erythrosine / Anthocyanin were make ready by adding 1:1 (w / w) ratio of each other. Sample of colourants were used to add colouring to yellow and red food items. The study indicated that as comparing to natural colourants, synthetic colourants are adversely affecting hepatic and renal parameters [32]. Synthetic dyes have been found to increase overall lipids, total cholesterol, LDL-C, lipid peroxide, alkaline phosphatase activities; acid phosphatase; lactate phosphatase and urea plasmas. While each of the HDL-C, GSH, SoD, catalase, IgA, IgM, and IgA material decreased. Therefore, it's crucial that you be conscious of the dangerous consequences using of these synthetic colorants in the food [33].

In recent times, emphasis is being given to children's sensitivity trials for higher susceptibility for eating. A nationwide study was conducted for the data on usage pattern of colours in 16 major states in India defining food commodities through which colour can surpass ADI limits [32]. The data showed that 87.8% included approved colours, out of which only 48% met with the specified 100mg/kg limit. Majority of the samples of candies, sugar dolls, beverages, ice cream, mouth fresheners and products of bakery reached the legal restraints. Restricted colours were prevalent in tests of sweets and sugar toys [32]. The most common colours were sunset yellow and tartrazine. Other samples used a combination of the two or more colours. One study found that a composition of sunset yellow and tartrazine exceeded the allowable threshold by 37% factor, with median and 95% values of 4.5 and 25.7, respectively [32].

Table 2. Quality assurance data on standard permitted and non-permitted colours

Analyte	LOD ($mgml^{-1}$)	LOQ ($mgml^{-1}$)	Recovery range (%)	RSD % (n¼3)	Linearity range ($mgml^{-1}$)	Measurement uncertainty (SE)
BBFCF	0.04	0.48	70.4–77.7	2.61–4.65	0.3–3.75	0.002–0.004
Carmoisine	0.75	1.41	85.8–99.0	3.70–6.84	1.25–7.5	0.002–0.003
Erythrosine	0.12	2.60	40.2–48.5	1.69–6.19	1.5–9.0	0.001–0.003
Ponceau 4R	0.14	1.93	84–90.2	4.41–8.01	1.5–9.0	0.002–0.003
SSYFCF	0.05	3.49	72.6–88.0	3.09–7.75	1.25–7.5	0.001–0.004
Tartrazine	0.08	1.61	75.6–78.7	2.12–8.38	0.6–5.0	0.001–0.004
Metanil Yellow	0.24	0.37	79.6–84.4	2.48–4.19	1.25–5.0	0.002–0.005
Rhodamine B	0.01	0.11	67.2–68.8	2.32–7.41	0.6–5.0	0.002–0 .003

Source: Dixit et.al. (2010), Food Additives and Contaminants Vol. 27, No. 2, Feb 2010, 181–189
Note: LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation, SE, standard error

Dose measurements showed that ingestion of erythrosine exceeded the limit of Acceptable Daily Intake by 2-6 times at median levels of observed colours, and by 3-12 times at the 95th percentile level for all five age groups [32]. The Indian quality assurance data of standard permitted colours and non-permitted colours are mentioned in Table 2. Thus, the Indian government's current recommendation of a maximum intake of 100 milligrams per kilogram of synthetic colours should be revised and replaced with regulations based on consumption patterns of individual food items. Those who are more likely to be affected by the health risks associated with the unnecessary use of excessive colours should be the focus of any such tests [32]. According to global market trend in usage of food colours, India is at the third place whereas USA is at the first.

5. EFFECT OF FOOD COLOURING AGENTS AS A DIETARY ADDITIVE

Food colour is an essential component since it gives each food item its own identity. Because of the inevitable loss of colouration that occurs during processing and storage, artificial colouring has become a technical requirement. Conventional toxicity research on food colours often revealed harmful effects only at very high doses. Most cases of food poisoning may be traced back to the ingestion of dyes for textiles that are banned in the United States [34,35].

Hyperactivity in Children: In a tiny trial, researchers discovered that removing artificial food colours and preservatives helped Seventy-three percent of children of ADHD (with hyperactivity disorder) have trouble paying attention [36,37]. Yet another research disclosed that both age of 3 and an age group of 8 to 9 had elevated hyperactivity after consuming food colours combined with sodium benzoate. When used with vitamin C, it may produce benzene, a known carcinogen. Although many companies have reformulated their goods to avoid this mix, it is still present in some [38]. A possible link between sodium benzoate consumption and agitation is also discussed. However, it is not clear what caused the hyperactivity in this study's subjects since they were given a variety of chemicals. Changes in behavior, such as irritation, restlessness, despair, and inability to sleep, have been linked to tartrazine, often known as Yellow 5 [39,40]. Young children are more prone to hyperactivity after consuming artificial food colours. On the other hand, it seems that few kids have the similar reaction to the food-coloured goods. Scientists from Southampton University identified a genetic factor that predicts a child's sensitivity to food dyes. Scientists have found a weak but statistically significant correlation between kids who eat foods with artificial colours and

behavioural problems. It seems that some kids are more vulnerable to the synthetic colours than others [41,42].

Cancer: Toxicity tests on various food colours showed mixed results, with most showing no harmful effects. However, some people worry that the colours may contain harmful chemicals. It's possible that the additives used to create Yellow 5, Yellow 6 and Red 40, contain carcinogens. Potential carcinogens including 4-aminobiphenyl, 4-aminoazobenzene and benzedrine, have been discovered in food dyes [113]. Because their presence in the dyes is assumed to be harmless at low concentrations, these pollutants are permitted [43]. There is no solid proof that artificial food colours, with the exception of Red 3, cause cancer at this time. The rising use of food dyes suggests the need for more study. This investigation taught us that synthetic food colours, while their potential use in the aesthetic enhancement of food, drink, pharmaceutical, and other products, can offer health risks to humans. Products containing synthetic food colours, even in the little amounts permitted by Federal Food, 1958, are preferable to those that are organically derived. As a result, the study indicated that artificial food colours are bad for human health [44].

Allergies: So many studies have shown that Yellow 5, sometimes known as tartrazine, may trigger asthma attacks and hives. Curiously, those who are allergic to aspirin also seem to be sensitive to Yellow 5 [45]. One of the research found that 52 percent of persons with persistent hives or swelling were allergic to artificial food colours. Even severe allergic responses seldom end in fatality. But if you're experiencing allergic problems, cutting out artificial food colours might help to have a good health. Common food colours that might trigger allergy reactions include sunset yellow, Allura red, and tartrazine [1,46-48]. Blue 1, sunset yellow, Allura red, and tartrazine are only some of the artificial food colours that may trigger an allergic reaction in individuals form [49-51].

The health of humans is affected by various factors:

I. The assertion that synthetic food additives might cause cancer is the most disturbing one to yet.
II. There is less evidence to back up this assertion. Consumption of food colours is unlikely to cause cancer, according to the existing evidence.
III. Some individuals have allergic responses to food dyes, but unless you're experiencing symptoms, there's no need to avoid them.
IV. The argument that children who consume food colours are more likely to have attention deficit hyperactivity disorder is the major key with the most supporting scientific evidence.
V. Some children seem to be more susceptible to the impact of food colouring agents than others, but several studies have indicated that these additives enhance hyperactivity in children with and without ADHD.
VI. You may want to limit or eliminate your child's use of artificial food colours if he or she exhibits hyperactive or aggressive behaviour.
VII. Colorants in food are employed to improve the visual appeal of the product, which brings us to point number seven. Adding food colouring to your meal does not improve its nutritional value in any way.
VIII. The majority of food colouring comes from manufactured meals that are bad for your health in other ways.
IX. Reducing or eliminating your consumption of processed meals and replacing them with whole, nutritious foods is a great way to boost your health and significantly cut down on your exposure to artificial food dyes.
X. Most people probably won't become sick from eating food with added colours, but it's still best to steer clear of processed goods that include them.

A chemical added for a reason, while it is being processed or storage of food and it has the potential to alter the food's characteristics or become a part of the food itself. To keep foods from spoiling, manufacturers often add preservatives to them. To prevent or delay deterioration, degraded quality, nutritional value or eat ability content, and increase the duration that food can be stored for, managing food goods followed by food preservation [52,53]. Some storage techniques include adding beneficial bacteria or fungus to the food, while others focus on halting the development of harmful microorganisms like bacteria and yeasts. In the widest sense, antioxidants in food include everything that has even a little influence on keeping foods from deteriorating due to oxidation [54].

Table 3. Synthetic Colours and their Adverse Effect on Human Health

Sr. No.	Colour name	Adverse effect on human health	Accepted Intake	References
1	Tartrazine	allergic and intolerance reactions, childhood hyperactivity	7.5 mg/kg bw	[55-58]
2	Sunset Yellow FCF	Allergic reactions, Carcinogenic effect	4 mg/kg bw	[59,60]
3	Amaranth	Allergic reactions, Asthma, Carcinogenic effect	0.15 mg/kg bw	[58,31]
4	Ponceau 4R	It may cause adverse responses much as azo food dyes, suspected to hyperkinesia	0.7 mg/kg bw	[57,114]
5	Erythrosine	Allergic reactions and cancer	0.1 mg/kg bw	[25,57,58]
6	Litholrubine BK	Hyperkinesia leading to cancer	-	[57]
7	Brown HT	skin allergies, hyperkinesia	1.5 mg/kg bw	[57,61]
8	Allura Red AC	Carcinogenic, induces asthma, rhinitis, or hives	7 mg/kg bw	[28,57,58]
9	Patent Blue V	Carcinogenic	5 mg/kg bw	[26,57]
10	Indigotine	Allergy and carcinogenic	5 mg/kg bw	[57,62]
11	Brilliant blue FCF (E133)	Could be deposit in kidney and lymphatic vessel, carcinogenic; Gastrointestinal cancers; ADHD in children.	6 mg/kg bw	[36,37,58,63-67]
12	Green S	Harmful side effects, Carcinogenic	5 mg/kg bw	[57,68,69]
13	Brilliant Black PN	induce allergic reactions, suspected to hyperkinesia	1 mg/kg bw	[57,60]
14	Indigo carmine	Worse, brain gliomas and breast cancer both increased significantly in the rat model. for humans because rats develop more cancers, notably brain gliomas. Mitochondria were not shielded from the dye's potentiating effects, damaging hepatocyte membranes.	5mg/kg bw	[60,70-72]
15	Citrus red	carcinogenic	4 mg/kg bw	[73]

Table 4. CSPI Studies Carried Out Positive and Negative Effect of Food Dyes

Synthetic food colours	Total Positive Studies	Positive Studies (In-vivo)	Total Negative Studies
Indigo Carmine	1	0	10
Brilliant Blue	2	0	7
Erythrosine	4	1	8
Fast Green	3	0	6
Tartrazine	6	2	5
Allura Red AC	3	3	7
Sunset Yellow	2	1	6

Source: Sarah Kobylewski and Michael F. Jacobson (2010). Food Dyes A Rainbow of Risks

The Food and Drug Administration divided food colourings into 2 broad categories: those that must be certified and those that do not. Food colouring may be either natural or artificial. The FDA classifies synthetic food colours as "those colours which need certification"[74]. One of the study conducted by Sahar et.al. [75] for the studying influence of food colour use on the rats for thirteen weeks showed a momentous rise in Total Cholesterol and Triacyl-Glycerol levels in

tomato ketchup and potato chips (30 percent). Both ALT and AST values were dramatically elevated in the rat group, which was administered with colour fruit juice for 12 hours, with an increase in TKPC for 30%, serum creatinine and albumen. Consumption of high and low food colours demonstrated significant reduction in liver GSH [75]. As a consequence of the immune system's response to the inflammation, the number of WBCs was observed to rise in the study's subjects when they consumed foods with high concentrations of food colouring. Their outcome has shown that coloured juice containing sunset yellow, tartrazine, and carnosine can increase in Rat serum ATL [76]. As a result of their investigation, the researchers determined that the synthetic food colours employed in the study negatively impacted various aspects of metabolic, liver, and kidney function, Mekkawy et.al [77] accepted those findings well. Amin et.al, 2010 [54] advised that, the high amount artificial food colours (Tartrazine, Carmoisin, Sunset Yellow and Quick Green) in rats had a momentous rise in ALT and AST serum relative to stock. There may be a correlation between the elevated levels of intracellular enzymes in the blood as hepatocellular impairments and the improvement in liver function showed ALT and AST as clearly evident in the trials of histopathology. The liver has shown a damage to the liver's cells and a disintegration of tissue around the principal vein at low dose/amount organic food colours as Sharma et al. [78] have indicated those findings. The release into the bloodstream of a high amount of different enzymes tissue rely on both the degree and form of impairment caused by the administration of the toxic agent. A decline in renal function is linked to a large increase in serum creatinine. In accordance to the Centre for Science in the Public Interest (CSPI) research and report, a number of the most popular artificial food colourings may have links to cancer.

According to the report of CSPI "The three most widely used synthetic food colour; Red 40, Yellow 5, and Yellow 6; are contaminated with known Carcinogens. Another food colour, Red 3, has been acknowledged for years by the Food and Drug Administration to be a Carcinogen, yet is still in the food supply". Despite the fact that the chemical industry's own research has shown connected nine of the food colours now used in the United States to health problems such as cancer, hyperactivity, and allergy-like responses, as reported by the Centre for Science in the Public Interest (CSPI) [79]. The human cell lines derived central nervous system (CNS) of was negatively affected by the colour combinations of red 40, tartrazine, red 2, yellow 4, yellow 6, and Blue 1. In processed foods the consolidation of synthetic food colouring are commonly used [80]. Red 40, most commonly used colour, may induce hyperactivity in children as well as stimulate the growth of immune system tumors in animals. Blue 2, a common food dye used in candy, drinks, pet food, and other products, has been related to tumors in the brain. While, tartrazine used in baked items, cookies, popcorn and many more, may can be contaminated with a wide range of chemical substances known to cause cancer in children due to hyperactivity and other behavioural consequences [24,81,82]. The chemical industry and research advisors commissioned, performed and reviewed almost all the toxicological studies on food colour. Ideally, independent researchers would be studying the organic colours (and other sensitive chemicals). In comparison, nearly all of the experiments examined individual synthetic colours, while many edible products include blended synthetic food colours with other ingredients, this may lead the impact of additives or synergies [24,81,82]. The Washington Post (June 3, 2008) reported “Beyond the behavioural problems and risk of cancer, the greatest hazard that synthetic food colour pose for children may also be the most obvious: They draw children away from nutritious diet and toward brightly coloured processed products that are high in calories but low in nutrients, such as fruit-flavoured drinks and snack foods. Those types of foods are a major force in America’s obesity epidemic.” In placebo-controlled study [83]. The study analysed objectively the effects of popular synthetic food colours used in drinks like soda, juices of fruits and dressings of salads. This study shows that few children were more hyperactive and distractible because of the food colours. The "Annals of Allergy" [84] study work in the year 1994, (volume 73) reported that around 73 percent of kids with attention deficit hyperactivity disorder benefited from artificial food colours free diet [36,37]. According to research published in Lancet, children whose diets included organic food dyes with E numbers saw a substantial drop in IQ, similar to that seen in children exposed to lead in gasoline [83]. The British Food Standards Agency urged the food industry to take aggressive measures to eliminate the six food colours identified in the 2009 study and substitute them with Organic Materials, and it cautioned parents to limit their children's exposure to food colouring agents. The

Food Standards Agency in Britain has voluntarily prohibited several food colours, including tartrazine, quinine pink, sunset purple, carmoisine, ponceau 4R, and allura red [83].

Before refined petroleum products were available, the basic material for producing synthetic food colors came from waste oil (coal tar). Thousands of items, including breakfast cereals, candies, pills, and beverages, make use of these colours. Auramine, a yellow dye, has been reported to damage organs and rhodamine produced as a byproduct, damage kidney, liver and retards growth in rats. Rhodamine is excellent in red blood cell degradation [2]. Food Colours of Edibles Chromate leads to anemia, stomach discomfort, neurological issues, asthma, and even lead poisoning effect, when used abusively [54]. Reproductive organs (the ovaries and the testes) are impacted by yellow metanil.; Also detected in the abdominal organs degeneration after ingestion of rice coloured by it may result in oxygen deficient skin cells leading to skin discoloration because of inadequate blood oxygenation causing methemoglobinemia in adults within 2-4 hours [2]. Food colours such as Allura purple, Amaranth, Erythrosine, Tartrazine, Phloxine and Rose Bengal can affect liver, colon, and urinary bladder, and weakened the DNA. Induced immunosuppressive activity by Amaranth [31] and Tartrazine, Allura could react allergically. Tartrazine and Brown dye are liberator of histamine, which heightens asthma. Yellow 5, Yellow 6, and Red 40 contain a carcinogen called benzidine. Benzidine exists in a free or restricted form (detected in higher food colour concentrations).

Table 5. The Eight Artificial Food Dyes Approved by Both the FDA and the EFSA

S. No.	Artificial food dyes	Banned (in Country)
1.	Erythrosine	United States only in topical cosmetics and medications
2.	Tartrazine	Norway, Austria.
3.	Quinolone yellow	Australia, Japan, Canada, U.S.
4.	Sunset light	Norway, Sweden, Finland
5.	Indigo Carmine	America, Japan, Canada, Norway
6.	Brilliant Blue	France, Belgium, Austria, Denmark, Germany, Switzerland, Norway
7.	Allura red	France, Belgium, Denmark, Germany, Switzerland, Norway, Austria, and Sweden
8.	Carmosine	Japan, Canada, Norway, Austria, Sweden and U.S.

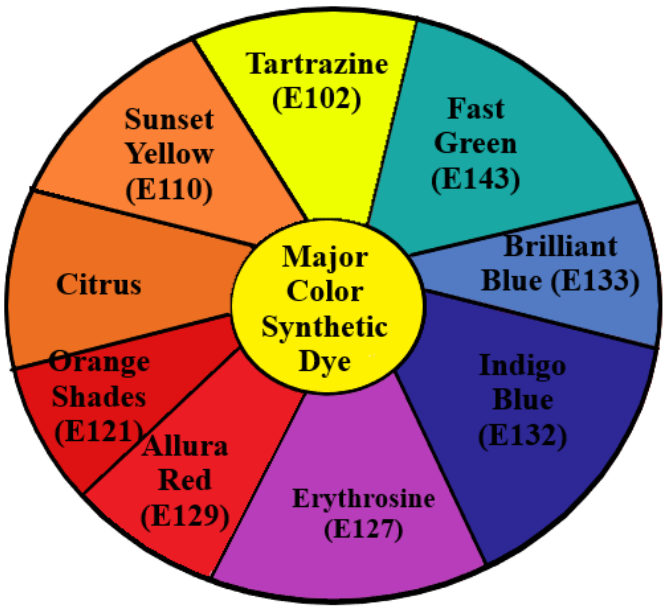


Fig. 2. Artificial Food Dyes Approved by Both the FDA and the EFSA

6. FOOD COLOURING AGENTS - AQUATIC ENVIRONMENT: CURRENT STATUS AND FUTURE PERSPECTIVE

Food colours containing azo dyes have carcinogenic effects, when aromatic amines are produced by microbial anaerobic metabolism in the intestines, that induce intestinal cancer as its azo dyes Benzidine bond [85]. Azo colours such as methyl purple, methyl orange, methyl violet, Ponceau S, tartrazine, sunset yellow, amaranth and acid black are commonly taken into consideration of food colouring also clothing colouring, resulting in an elevated cancer rate among the urban community. For U.S. manufacturing of packaged macaroni and cheese, these shades are commonly used. Through FDA, erythrosine was approved as a thyroid cancer drug in 1990 [86]. P-dimethylaminoazonbenzene, an azo dye found carcinogen, and non-azo dyes are found mutagenic. In animals after body metabolization, bladder cancer is caused by Allura red which produces a product. Few other synthetic food colours are mutagenic, such as Metanil Black, Auramine, Lead Chromate, Rhodamine, Malachite green and sudan-3. Sudanese colours are bright, organic, fat-soluble azo colours that cannot be used as food colours due to harmful effects on health. Oestrogen enhancement has been identified with extensive use of Sunset Yellow and Tartrazine, increased oestrogen levels that linked to breast cancer and diminish male sex drive with excessive amounts of sunset yellow and tartrazine [19,45]. Histamine was previously unknown to be a significant neurotransmitter. Neurons produce it, store it in vesicles, and then secrete it either from the cell body or axonal varicosities, where it can act on post-synaptic membrane receptors [87]. Some neurons produce several neurotransmitters, whereas others are specifically histaminergic (serotonin, dopamine, GABA). Histamine is broken down in the brain primarily by HNMT and then by MAO [88]. Red 3 has been identified with oestrogen such as growth-stimulating capacity, potentially genotoxic and promoting breast cancer forks.

7. SYNTHETIC FOOD COLOUR TOXICITY IN AQUATIC ENVIRONMENT

When evaluating the environmental implications of synthetic food colour, it is important to consider the potential damage that the dyes provide to aquatic organisms across all trophic levels. The OECD and the Globally Harmonized System for the Classification and Labelling of Chemicals both recommend testing the aquatic toxicity of coloured chemicals on plants, algae, vertebrates and invertebrates to determine the potential dangers to the aquatic environment [89,90]. The OECD and GHS hazards on aquatic environments are included here.

Even within the same chemical class, synthetic food colours are highly diverse in terms of aquatic toxicity. For example, an aquatic toxicity research conducted by *Daphnia pulex* for two xanthenic dyes on crustaceans found that EC50 was 0.423 mg/l for phloxin B compared with 337 mg/l for EC50 [91]. In contrast, a rhodamine B and fluorescein effect research has shown that all these synthetic dyes cannot be classified as poisonous to these species (LC50 N100 mg/l) on fish such as *Ictalurus punctatus*, Lepomis macrochirus and Oncorhynchus mykiss [92]. However, another research of other synthetic food colour, the malachite green, reveals that *Heteropneustes fossilis* is significant, and that malachite green is category I toxicant. Malachite green poisoning caused difficulty with the respiration and the swimming [93].

Table 6. Substances in water that pose a risk to aquatic environment are classified according to OECD and GHS guidelines

Aquatic organism		Acute/chronic toxicity		
Aquatic plants or algae	Acute toxicity chronic toxicity	72 or 96 h ErC_{50} ≤1 mg/l	72 or 96 h ErC_{50} >1–≤10 mg/l	72 or 96 h ErC_{50} 10–≤100 mg/
Crustacea	Acute toxicity chronic toxicity	48 h EC_{50} ≤1 mg/l	48 h EC_{50} >1–≤10 mg/l	48 h EC_{50} >10–≤100 mg/l
Fish	Acute toxicity chronic toxicity	96 h LC_{50} ≤1 mg/l	96 h LC_{50} >1–≤10 mg/l	96 h LC_{50} >10–≤100 mg/l

Unless the experimentally determined BCF <500, the log Kow is not less than 4, and the chronic toxicity NOECs are less than 1 mg/l, thus the substance is not readily biodegradable. [89,90]

Other writers have found harmful effects of green malachite on fish, including lethargy, uneven movement and restlessness [94,95]. It is worth noting that, among diverse animal species, dye toxicity is varied. For example, *Oreochromis niloticus* LC50 was poisonous to malachite green at 0.437 mg/l, whereas *Heteropneustes fossilis* LC50 at 1 mg/l [93,94].

8. CURRENT STATUS

Food industry colourants are used to make foods more appealing or to offset differences in colour following food preparation. Azo, quinone, indigoid, xanthene and triphenyl methane are often employed in dyes. When synthetic food colouring agents are used in the European Union, they are identified by their respective E numbers, which are assigned by the European Food Safety Authority (EFSA). The number of synthetic food colours is 15 out of 40 which got foods licensed in Europe till now [96]. The Food and Drug Administration regulates the use of synthetic food colourants in the United States, which authorizes just 9 synthetic food colours for colouring food products/items [97]. Japanese law allows the utilize as food additives of 12 organic artificial colouring agents [23]. There are, of course, variances in the license for synthetic food colours usage between nations; for example, 4 colours acceptable for Japanese use but prohibited in the EU (phloxine B, fast green FCF, Acid Red 52 and Rose Bengals) [85].

In India, only eight thymes were suggested as edible under the parameters of the 1954 Prevention of Food Adulteration Act, which was enacted to ensure the safety of different foods and food products (now the Food safety law, 2006).The history and paths of these fascinating materials that have shone across our planet for over 3500 years, their physical and chemical characteristics as well as the use and the toxicity of the eight synthetic food additives allowed in India [98].

It should be noted that some synthetic food dyes that are authorized in the industries sector may be utilizes as food colours unlawfully; methanil yellow, malachite green, rhodamine B and orange II, these colours have been identified in various foodstuffs such as sweets [3,99,100]. Fish products also include colours removed (red 2) from the food processing sector or never permitted in the fish business (cochineal red A) [101]. The food meant for human consumption contains no permitted synthetic organic colours. Whether the meal was deliberately or inadvertently tainted with dyes is not known. However, inadequate inspection or disregard of the rules on food might encourage food forging. Unlawful colorants are detected in foodstuffs in the Fast Food and Feed Alert System (RASFF). Most alerts to date relate to unlawful utilize of artificial organic teeth, like para red, sudan I-IV, orange II and rhodamine B, in foodstuffs [47,85].

9. FUTURE PERSPECTIVE

The studies referring to health implications of synthetic food additives provided information about the effects and it has been revealed that these are prime culprits for causing cancer, hypersensitivity reaction, behavioural effects and other toxicological considerations. Dangerous to both people and the planet, they must be avoided at all costs as they exhibit certain groups of organic compounds that interfere with the metabolism. Synthetic food colours are added to food items which is intended for popularizing and marketing by making it more palatable. The Indian food colorants market is dominated by synthetic food colour. Since the colour of the food plays a vital part in boosting the aesthetics and the hunger, it is no surprise that the processed food sector in the nation uses more synthetic colours as a result of the preference of the local population for inexpensive food products/items. The Food Safety and Standards Authority of India (FSSAI) approves synthetic food colours, which are used in various foods. Tartrazine, sunset yellow, quinoline yellow, indigo carmine, and amaranth are only some of the synthetic colours often used in the researched industry [23].

It has also been observed that many of the synthetic food colours are toxic and causing damages in mammalian cells [73]. Currently, the studies have produced national-level data on food colour usage patterns and defined commodities through which they can reach the limits of Acceptable Daily Intake (ADI). All SSYFCF and erythrosine reached the respective ADI thresholds at the 95th percentile stage [32]. The use synthetic food colour at high concentrations contributes to an improvement in the histopathological composition of the liver and kidney along with a rise in WBC which has shown as hyperactive, sticky, and reddish skin. The marked increase in ALT in rats treated with Tartrazine indicates serious cytotoxicity. This clearly showed the genotoxic, cytotoxic and mutagenic studies for the impact of synthetic

food colouring agents before they reach the consumers [75]. Rats consumed high dose/amount synthetic food colours to momentous increase in serum ALT and aspartate aminotransferase test (AST) [54,77]. High quantity usages of sunset yellow, tartrazine and Red3 revealed several estrogenic potentials [102]. Food additives have harmful impact on vital organs [78]. Nine of the licensed food colours in the United States are currently associated with pathological state of health ranging from obesity to agitated to allergy-like reactions. (Public interest science centre, 2013) CSPI.

The complexity and difficulty of treating textile wastes have increased, prompting a renewed quest for effective and practical curing techniques. Thus, the need for dye substitutes is urgently needed to reduce environmental issues. A suitable choice for wastewater breakdown is microbial and enzymatic, as these processes don't produce a lot of sludge, don't harm the environment, and are also reasonably priced [103]. The next step should be to scale up and implement bacterial decolorization techniques in actual industrial effluents based on the positive laboratory results. Furthermore, given the most recent developments in genomes and proteomics, it may be possible to improve the efficiency of enzymatic or bacterial treatments of textile effluent. Given all the encouraging research results and continuous advancements, it is anticipated that microbiological treatment will predominate in the removal of harmful compounds and pigments from textile wastewater. Developing environmentally safe dyes, pigments, and auxiliaries should be a top priority for action worldwide due to the emotive nature of the argument and increased consumer concerns.

10. CONCLUSION

The studies reveal that, synthetic food additive, such as Allura red shows genotoxic effect in mice with colon epithelium got severely affected [104]. In in-vivo and in-vitro assays, Red 3 is genotoxic and in animals it shows carcinogenic effects [21]. Sunset yellow shows genotoxic and cytotoxic effect after treating on actively proliferating root tip cells of *Brassica compestris L* [105]. As a result of toxicity, synthetic food colours cannot be considered safe. For the sake of protecting and bettering people's health, it is important that food should be of high quality and safe. The Food Safety and Standards Authority of India (FSSAI), Food and Drug Administration (FDA) and European Food Safety Authority (EFSA) are the most important approved regulatory authorities. The FSSAI is responsible for establishing and enforcing ADI limits for additions of food colouring agents to food and their safety. The industries of food manufacturers first get approval from the FSSAI for use of new synthetic or natural food additives in food production. In other advanced nations the usage of synthetic colours in the edible products is passed through a battery of toxicity tests. Currently, only eight synthetic food colors are approved for usage in India (Table 2). About the dangers to one's health posed by artificial food dyes, it is necessary to create public awareness and encourage them, to consuming synthetic food colours within ADI limits and to fulfil a certain function according to FSSAI, EFSA and FDA legal framework [23,46,111,112]. Nowadays, all the countries worldwide are focusing on use of food additive and colorant-related regulations [106]. The food and medicine administrations of many nations are urged to act and outlaw the use of artificial colours in foods [107]. In addition, we currently know very little about what happens to the wastes produced by the factories that make food colouring agents in the environment. Many clinical investigations have been conducted to determine the effects of various colourant compositions as they would be consumed in daily life [83,108]. Due to the serious limitations of these investigations, it is regrettably impossible to reach a firm conclusion.

Consequently, despite the seeming wealth of knowledge, future research on unidentified dyes pharmacological mechanisms should be encouraged and toxicity studies should be conducted with new methodological approaches. However, despite the discovery of new and potentially helpful pharmacological pathways for the commonly used colourants, the problem of the pharmacological characteristics and toxicity of food colourants has not yet been fully explored., e.g., Anti-inflammatory and anti-depressive properties of Brilliant Blue [109] and Inhibition of hematopoietic prostaglandin D2 synthase by erythrosine [110]. In order to understand the potential impact that different colourants or their combinations may have on neurodevelopment, pharmacology, and other factors, systematic studies should be carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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