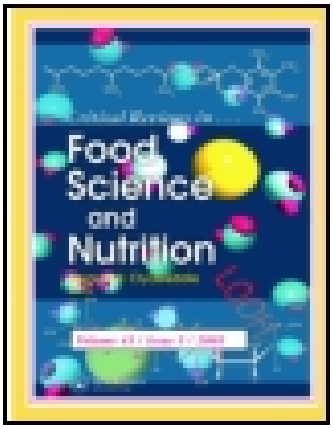

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Food Colours: Existing and Emerging Food Safety Concerns

Michalina Oplatowska-Stachowiak[a] & Christopher T. Elliott[a]
[a] Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, David Keir Building 18-30 Malone Road, BT95BN Belfast, United Kingdom., e-mail:
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Food colours: existing and emerging food safety concerns

Michalina Oplatowska-Stachowiak[a] and Christopher T. Elliott[b]

Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, David Keir Building 18-30 Malone Road, BT95BN Belfast, United Kingdom.

[a]corresponding author - e-mail: m.oplatowska@qub.ac.uk, tel: +44 (0) 2890976531

[b]e-mail: chris.elliott@qub.ac.uk

Abstract

Food colours are added to different types of commodities to increase their visual attractiveness or to compensate for natural colour variations. The use of these additives is strictly regulated in the European Union, the United States and many other countries worldwide. There is a growing concern about the safety of some commonly used legal food colourants and there is a trend to replace the synthetic forms with natural products. Additionally, a number of dyes with known or suspected genotoxic or carcinogenic properties have been shown to be added illegally to foods. Robust monitoring programs based on reliable detection methods are required to assure the food is free from harmful colours. The aim of this review is to present an up to date status of the various concerns arising from use of colour additives in food. The most important food safety concerns in the field of food colours are lack of uniform regulation concerning legal food colours worldwide, possible link of artificial colours to hyperactive behaviour, replacement of synthetic colours with natural ones and the presence of harmful illegal dyes – both known but also new, emerging ones in food. The legal status of food colour additives in the EU, US and worldwide is summarized. The reported negative health effects of both legal and illegal colours are presented. The European Rapid Alert System for Food and Feed notifications and US import alerts concerning food colours are analyzed and trends in fraudulent use of colour additives identified. The detection methods for synthetic colours are also reviewed.

Keywords: food dyes, synthetic colours, legal and illegal food colours, detection methods

Contents

1. Introduction 4

2. Regulation on the food colour additives in the European Union 6

3. Regulation on food colours in the US, India , Japan and China 9

4. Natural vs synthetic colours 10

4.1. Cochineal 11

4.2. Caramel 11

4.3. Colour additive vs colouring foodstuffs 12

5. Assessment of the safety of food colours 13

6. Illegal dyes for colouring and associated health risks 15

7. Illegal use of dyes as veterinary drugs and the associated health risks from contaminated foods 18

8. The effect of permitted food additives on the behaviour of children 19

9. The EU Rapid Alert System for Food and Feed, the US Import Alerts and monitoring of food colours 22

10. Analytical methods developed for the detection of synthetic food colours 25

11. Food colours – current issues 27

11.1. Synthetic colours 27

11.2. Dyes - illegal veterinary drugs 30

11.3. Colour migration 30

12. Conclusions and summary 31

References 33

1. Introduction

Colours are added to many foods to make them more attractive to the eye of the consumer and to compensate for natural colour variations that exist in many foods. Colour influences the perception of food quality and stimulates the appetite, since it is perceived to relate to flavour (Downham & Collins, 2000). The use of colour additives dates back to the Ancient Egyptian period (1500 BC) when colorants were added to wine and confectionaries (Watson, 2001; Downham & Collins, 2000). Since then numerous naturally-derived colours (such as indigo, alkanet (borage root), turmeric, safflower, parsley, spinach, fruits, flower petal extracts, annatto, paprika, brazilwood, and cochineal) have been used to improve the appearance of foods. The use of mineral and metal based compounds for this purpose was also reported following the industrial revolution. However, the ingestion of such toxic colouring chemicals as red lead (Pb_3O_4), vermillon (HgS) and copper arsenate in food could cause illness and death (Downham & Collins, 2000). In 1856 the British chemist Sir William Henry Perkin created the first synthetic dye ‘mauveine’ by oxidizing aniline (Watson, 2001). As a result of the “colour revolution” thousands of new colouring compounds were synthesised for industrial applications. The artificial colours were widely available and used also as food additives, due to a range of advantages over the natural counterparts such as ease of production, low cost, high stability and better colouring properties (Downham & Collins, 2000; Oreopoulou et al., 2009). Nowadays, the use of colours as food additives in the Developed World is strictly controlled by legislation, since many of the synthetic compounds were shown to be toxic and harmful to humans. However, the legislation varies in different countries. Therefore, the first important issue in the area of food

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colours is the lack of uniform regulations worldwide. It causes problems to food importers and exporters as the same food colour can be legal in one country but illegal in another.

The second current issue concerns the legally used synthetic colours and their link to hyperactive behaviour in children (Bateman et al., 2004, McCann et al., 2007). Synthetic food colours do not provide any nutritional value and there is a growing concern about their adverse health effects. Due to consumer demands there is an industry trend to remove synthetic colours from food and replace them with natural products. So far in the EU there has not been a ban placed on synthetic dyes; however in the UK voluntary action of the industry has already resulted in a marked change to the use of natural colours by major food manufacturers. The recent re-evaluations of all the synthetic food colours performed by European Food Safety Authority (EFSA) did not raise substantial safety issues when these compounds are used according to regulations (EFSA, 2009a-f). However, there is a legal requirement in the EU for foods containing six dyes (tatrazine, quinoline yellow, sunset yellow, ponceau 4R, allura red and carmoisine) that have been linked to hyperactivity to be labelled with a warning that they might have an adverse effect on the attention in children (Regulation (EC) No 1333/2008). In the USA Food and Drug Administration Food Advisory Committee did not find casual evidence to support the link between food colours and hyperactivity (F&DA, 2011a & 2011b).

The third issue is the replacement of synthetic colours with natural ones which is not that straightforward as it might appear and not all the natural colours are completely safe. The current trend of using ‘’colouring foodstuffs’’ (food ingredients such as fruits of vegetables that secondary effect is colouring) instead of colour additives can lead to more regulatory problems as these substances in the EU are not covered by the Regulation on food additives.

The fourth problem covers the illegal addition of known and emerging industrial dyes to food. There are many colouring compounds (such as sudan dyes, rhodamine B, methyl yellow or malachite green) widely used in industry for colouring fabrics, plastics or inks which are known or are suspected to have negative health effect and should not be consumed by humans. In some countries, particularly in the Developing World, harmful dyes that are not intended for human consumption can be added to foodstuffs due to a lack of legal restrictions on their use or very limited ability to monitor and control. Their presence in food entering the EU for example has been reported over a number of years on the Rapid Alert System for Food and Feed (RASFF). Such illegal practise results in economic incentives as the quality of some types of foods such as spices are assessed on the basis of colour intensity (Perva-Uzunalić et al., 2004). Globalization has resulted in the importation of food from around the world to the Developed World. The lengthening and increasing complexities of the food supply chain and substandard control over the food production in exporters' countries can lead to the exposure of humans to the hazardous chemicals in imported foods (Lineback et al., 2009). There are number of compounds such as sudan dyes for which there are monitoring programs in Europe or the United States. Due to a large number of synthetic colours available for industrial applications the well-known illegal food dyes can be replaced by other similar compounds that are not monitored for. Such unacceptable practises can be reduced in number and severity by improving legislation, developing stricter, more comprehensive monitoring programmes and by employing rapid, cost effective testing methods capable of detecting multiple hazards.

2. Regulation on the food colour additives in the European Union

Food colour additives can be synthetic, natural (derived from plant, animal or mineral sources) and also so-called 'nature identical', that are identical to those found in nature but are synthesized by human, for example synthetic carotenoids. The first regulation within the European Economic Community countries concerning colouring matters was introduced in 1962 (Council Directive 62/2645/EEC). This was replaced by European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs. Most recently Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 harmonizing the use of all food additives was introduced within the EU. This regulation defines colour as *''substances which add or restore colour in a food, and include natural constituents of foods and natural sources which are normally not consumed as foods as such and not normally used as characteristic ingredients of food. Preparations obtained from foods and other edible natural source materials obtained by physical and/or chemical extraction resulting in a selective extraction of the pigments relative to the nutritive or aromatic constituents are colours within the meaning of this Regulation.''* Only additives included in the Annex II to this Regulation can be used in food. There are currently 25 colours of natural (or nature identical) origin and 15 synthetic on the list (Figure 1.). The natural colour canthaxanthin (E161g) cannot be added to any type of food, however it is included in the list as it is used in medicinal products. Canthaxanthin can be also used in animal feed (Commission Regulation (EC) No 775/2008). The safety of this compound has been recently evaluated by EFSA (EFSA, 2010) and it was noted that the main adverse effect of this additive is the deposition of canthaxanthin crystals in the retina. It was concluded that the exposure to canthaxanthin arising from the consumption of edible products from animals which have been fed with feedstuff containing this colour is unlikely to exceed the Acceptable Daily Intake (ADI).

The legislation on food additives does not cover foods which may be used for technological function such as saffron for colouring purposes. The regulation specifies preparations obtained from foods and natural sources by selective extraction that are used as colouring agents, but does not consider foodstuffs with colouring properties (even concentrated forms or extracted in a non-selective way that retains most of the food characteristics like colour, flavour and taste) as food additives. Fruits and vegetables extracts do not fall under the regulation but chlorophylls or carotenoids selectively extracted from them do and as such they must undergo safety assessment before they can be approved. This distinction can be unclear and there is an ongoing debate about differentiating between colouring foodstuffs and food additives (FSA, 2011).

Interesting examples of colour substances that can have two functions in food are beta-carotene (E160a) and riboflavin (E101). Beta-carotene is vitamin A precursor and riboflavin is vitamin B2. These two colours provide additional nutritious value to the products they are added to. Apart from being included in the regulation on food colours they are also covered by Regulation (EC) No 1925/2006 of the European Parliament and of the Council of 20 December 2006 on the addition of vitamins and minerals and of certain other substances to foods.

The regulation on food colours specifies the approved colour additives, foods in which food colour presence is not permitted and the conditions of use of each additives in different foods. Each additive is associated with a corresponding E number. The colour number or name must be declared in the ingredients list of the product. Additionally, Annex V introduced the labelling requirement for food containing six artificial colours (E102, E104, E110, E122, E124, E129) stating that they ‘’may have an adverse effect on activity and attention in children’’**.**

A summary of the history of the legal status of each of the colour additives in the EU is presented in Figure 2. Within the first set of European regulations (Council Directive 62/2645/EEC), a number of synthetic dyes such as E103, E105, E111, E121, E125, E126 were allowed but they were later removed by an amendment as questions about their safety emerged (Council Directive 76/399/EEC). The next piece of legislation, Council Directive 94/36/EC, approved 17 synthetic dyes and 26 natural or nature identical. The use of E128 was suspended in 2007 as there was sufficient evidence relating to its carcinogenicity (Commission Regulation (EC) No 884/2007). Current legislation (Regulation (EC) No 1333/2008) specifies the colours permitted in food and is based on the state of knowledge, the technological need and their practical use. A comparison of colours permitted for use in Council Directive 94/36/EC to the Regulation 1333/2008 reveals that colours E154 and E160f were removed from the list of approved colours, the former due to the fact it was no longer used and there wasn’t sufficient data on its safety, the latter because it was no longer offered by the manufacturer (Commission Regulation (EU) No 1129/2011). Both dyes had been previously permitted for special use only (E154 for kippers and E160f for Saucisses de Strasbourg).

3. Regulation on food colours in the US, India, Japan and China

The legislation concerning food colours varies widely across different countries. In the United States the use of colours in food is regulated by U.S. Food & Drug Administration (F&DA). The regulations concerning food additives are contained in the Title 21 of the Code of

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Federal Regulations (e-CFR, 2009). There are only nine synthetic food colours allowed (that are subject to certification) of which seven are for general use. Thirty five derived from vegetable, animal, mineral sources or synthetic duplicates of naturally occurring colouring compounds are exempt from certification and eight of them are for use in animal feed only. The colours subject to certification must have their identity and purity checked by F&DA. Three synthetic colorants approved for consumption in the United States are not permitted in the European Union, while nine food synthetic colours (out of 15) which are legal in Europe are not authorized in the US (Table 1.). The regulation in India permits eight synthetic colours (one of which is not legal in the EU) and 11 natural or nature identical additives, no inorganic dyes are permitted (The Prevention of Food Adulteration Rules, 1955). Japanese legislation allows use of 12 synthetic colours in food (4 of them cannot be used in the EU) (JETO, 2006). Chinese legislation permits 11 synthetic colours in food, of which one (new red) is illegal in the EU, US, Japan or India (Ministry of Health, People's Republic of China, 2011).

4. Natural vs synthetic colours

The common belief is that natural colours are better from a health perspective and consumers demand their use instead of synthetic substances (Downham & Collins, 1991). Some synthetic food dyes such as tartrazine can cause an intolerance reaction in susceptible individuals (EFSA, 2009e) and six of them (tatrazine, quinoline yellow, sunset yellow, ponceau 4R, allura red and carmoisine) have been linked to increase in the hyperactive behaviour in children (Bateman et al., 2004; McCann et al., 2007). However, natural colours have only been tested to a limited extent and their evaluations are based on a number of assumptions. With increasing use of natural colours in food, exposure to them might not be in the "natural" or "normal" ranges any longer (Nordic Council of Ministers, 2002; Watson, 2001). In the EU the natural food colours are regulated by the same legislation as synthetic substances and there are restrictions on their use. EFSA has recently decided to re-evaluate all food colours including natural products (Commission Regulation (EU) No 257/2010 setting up a programme for re-evaluation of approved food additives). This process should be completed in 2015 for all colour additives. The issues and concerns with replacing synthetic food colours with natural alternatives have been discussed elsewhere (Scotter, 2011a). The important issues pertaining to a number of natural colours are presented below.

4.1. Cochineal

Cochineal is a natural red colour obtained from crushed insects (*Dactylopius coccus)*. A substantial amount of attention recently has been directed to the use of cochineal in drinks as it makes the product to which it is added non vegan (CBSNews, 2012). However, cochineal is used widely as an alternative to artificial colours. It is used in many types of foodstuff which the consumer is unlikely to be aware of. The extract from *Dactylopius coccus* contains carminic acid that is responsible for dying properties and also proteins that can cause allergic reactions and asthma (Baldwin et al., 1997; Lizaso et al., 2000).

4.2. Caramel

Caramel is produced from sugar heated at high temperature. Some concerns have been raised recently about formation of the genotoxic or carcinogenic agents: 2- and 4-

methylimidazoles, furan and 5-hydroxymethyl-2-furfural (5-HMF) and immunotoxin 2-acetyl-4-tetrahydroxy-butylimidazole during caramel manufacturing process (Jacobson, 2012; EFSA, 2011). 5-HMF is formed during caramelisation and in Maillard reaction when carbohydrates react with proteins at high temperature. It can be found not only in caramel but also in heat-treated food such as dried fruit, coffee, honey, milk and other products (Abraham et al., 2011, EFSA, 2011). When caramelisation is performed in the presence ammonium compounds then 2- and 4-methylimidazoles and 2-acetyl-4-tetrahydroxy-butylimidazole can be formed in the reaction of ammonia with carbohydrates. Due to the safety question EFSA recommended to reduce the level of these compounds, update the purity specification for caramel colours and undertake further research into the nature of the formation of these constituents during the production process of caramel (EFSA, 2011).

4.3. Colour additive vs colouring foodstuffs

In the EU there is a distinction between colour additives (that are covered by Regulations on food additives) and what is referred to as ‘colouring foodstuffs’ that are food ingredients with colouring properties. These are foods that are used during manufacturing of the product and their secondary effect is colouring. *‘’Foods, whether dried or in concentrated form, including flavourings incorporated during the manufacture of compound foods, because of their aromatic, sapid or nutritive properties together with a secondary colouring effect”* are not considered to be colour additives by Regulation (EC) No 1333/2008. Examples of foodstuffs with colouring properties are fruits and vegetable concentrates prepared from safflower, radish, black carrot, lemon, hibiscus, red cabbage and spinach, for example. When spinach is processed by a non-selective extraction procedure to produce paste or concentrate and then added to the product it is not considered as being a food additive but a food ingredient. When spinach is selectively extracted to produce chlorophyll (E140), then this chlorophyll is classified as a food additive and is covered by the regulation on food additives. Thus the difference lies in how the extract of the natural product is prepared and if this is treated as an intrinsic part of the product or as a colour additive. This division is rather unclear and there is an ongoing debate on the distinction between colour additives and colouring foodstuffs (FSA, 2011), as shown by the example of spirulina. In 2005 Nestle decided to remove all synthetic colours from their products due to the concerns about their adverse health effects. As a result blue dyed variants of some popular sweets were removed as there was no replacement for a blue synthetic colour brilliant blue E133. Blue sweets were reintroduced in 2008 coloured with a naturally occuring blue-green dye obtained from spirulina (algae) extract, a permitted health ingredient (Scotter, 2011a). Spirulina is currently considered as a colouring foodstuff but if the European Commission decides to change its status it will require the same approval process as the other food additives do (Food Navigator, 2010 and 2011). In the US spirulina derived from the dried biomass of the cyanobacteria *Arthrospira platensis* has been recently added to a list of approved colour additives exempt from certification in response to Mars Inc. petition (F&DA, 2013).

5. Assessment of the safety of food colours

In the European Union additives can be used in food when the criteria laid down in Regulation (EC) No 1333/2008 are met: there is a technological need for its use, it does not present any hazard to health based on the scientific data available and it does not mislead the consumer. Additionally, it must provide some advantages to the consumer. The specific conditions for colours state that it should also serve one of the following functions: restore the original appearance of the food, make food more appealing and add colour to colourless foods. The additive must be subject to toxicological testing, evaluation and re-evaluation in the light of new scientific data. The Regulation specifies the foodstuffs to which the additive can be added and the level of addition, taking into accounts the Acceptable Daily Intakes (ADIs). ADI is "*an estimate of an amount of a substance in food or drinking water that can be ingested daily over the lifetime without appreciable risk, expressed in mg per kg of body weight*" (JECFA, 2009).

The approval of a colour for human consumption in the EU can only be carried after a safety assessment is prepared by the European Food Safety Authority (EFSA), specifically by The Panel on Food Additives and Nutrient Sources Added to Food (ANS). This panel is responsible for safety evaluations of new food additives (excluding flavourings and enzymes) and re-evaluation of all authorised food additives. The safety evaluation of food additives for human consumption requires a review of published scientific studies on the toxicity of the additive such as animal studies using large doses of additives for long periods to show that the substance will not cause harmful effects at the expected levels of human consumption. The additive is permitted if according to the current state of knowledge there is a reasonable certainty of no harm to the consumer from ingestion of this additive under its proposed conditions of use. The assessments of safety of food additives are based on available scientific data on the toxicity of the chemicals that are collected and evaluated by International Agency for Research on Cancer (IARC), National Toxicology Program of the US Department of Health and Human

Services (NTP) or Joint Food and Agriculture Organization of the United Nation/World Health Organization Expert Committee on Food Additives (JECFA).

In 1994 when the European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs was published the assessment of the safety of colour additives was dated back over a number of years. Therefore, when the new legislation was introduced (Regulation (EC) 1333/2008) the European Commission decided to re-evaluate all of the permitted food colours and other additives. All food additives approved before 20 January 2009 must be subjected to new risk assessments. Most of the food colours have been already evaluated. The assessment of the last group of colours should be completed by 31st of December 2015. (Commission Regulation (EU) No 257/2010).

The effect of the re-assessment can result in the change of the legal status of the food additive. An example of such a re-assessment can be demonstrated by the case of red 2G. Following the EFSA re-evaluation of the dye red 2G in 2007 (EFSA, 2007), Commission Regulation 884/2007 suspended its use. This dye was permitted previously for colouring breakfast sausages; however there was sufficient evidence that it is metabolized into the carcinogenic aniline to suspend its use. Another dye brown FK (previously permitted in kippers only) has been recently removed from the list of approved colours included in the Regulation (EC) 1333/2008 due to the fact it was no longer used and there was no sufficient data on is safety available.

6. Illegal dyes for colouring and associated health risks

Dyes used for industrial applications, under some circumstances, can be fraudulently added to a range of foods to increase their value i.e. when the colour of the product is one of the indicators of its quality or to mask low quality of a product. These dyes are cheap to produce and readily available (Tripathi et al., 2007). A number of illegal dyes have been found or have been suspected of being present in foods consumed in the EU (RASFF online database), United States (F&DA, 2013b), India (Tripathi et al., 2007) or Japan (Ministry of Health Labour and Welfare, Japan) with some examples (illegal in the EU) presented in Fig 3. Most of these dyes are considered to be genotoxic and/or carcinogenic or there is limited data available to conclude if they are safe or not. The two main groups of dyes: azo-dyes and triphenylmethanes have been identified as those most likely to be illegally added to food (EFSA, 2005). Azo dyes are a group of organic compounds widely used in industry. The toxic activity of these dyes is a result of their metabolism (Golka et al., 2004). The azoreduction (breakage of azo bond) is enzyme-mediated and results in formation of active aromatic amines that can form DNA adducts. The azoreductive enzymes present in liver, skin and gastrointestinal microflora appear to play the main role in the *in vivo* reduction (Xu et al., 2007). The metabolism of the azo-dyes is reduced by substitutions in the aromatic ring – sulphonation or carboxylation and substitution of the hydrogen of an amino group. Substitutions can reduce genotoxicity by decreasing lipid solubility of the dye and as a result its absorption (EFSA, 2005). Therefore sudan I is assumed to be carcinogenic whilst sunset yellow FCF is a legal food additive. The only difference between these two chemicals is the presence of two sulpho groups in the sunset yellow FCF structure what causes a decrease in lipid solubility.

The potential of a chemical to be metabolized into a lipid soluble aromatic amine has been considered to be an indication of genotoxic and/or carcinogenic action. Thus several dyes presented in Figure 3. have been identified to be potentially harmful (EFSA, 2005). According to the available toxicity data some of the illegal azo dyes that have been detected recently in foods in the EU are possible genotoxic and/or carcinogenic agents. A number of examples are presented below:

a) Sudan I is considered to be genotoxic both *in vitro* and *in vivo*. The subcutaneous administration of this dye in mice resulted in liver tumours. It was also shown to be carcinogenic in rats after oral administration (EFSA, 2005; NTP, 1982; IARC, 1975).

b) Sudan II was shown to be genotoxic *in vitro*; however no *in vivo* data are currently available. It is considered to be potentially genotoxic and also a possible carcinogen as the increase in the incidence of bladder cancer was reported in mice following implantation of sudan II pellets (EFSA, 2005; IARC, 1975).

c) Due to limited data, sudan III, sudan IV and para red are all considered to be potentially genotoxic and possibly carcinogenic as they are structurally related to sudan I (EFSA, 2005; IARC, 1975).

d) *In vitro* mammalian cells study and *in vivo* tests showed a potential genotoxic effect of orange II, while data on carcinogenicity are limited (EFSA, 2005).

e) Methyl yellow was shown to be carcinogenic in rats and it is classified as group 2B carcinogen – *possibly carcinogenic to humans* (IARC, 1975).

Another dye rhodamine B is considered to be potentially both genotoxic and carcinogenic (EFSA, 2005). It was demonstrated to be carcinogenic in rats after subcutaneous injection

(IARC, 1978). Triphenylmethane dyes such as malachite green and its metabolite leucomalachite green are considered to be genotoxic and/or carcinogenic (EFSA, 2005).

7. Illegal use of dyes as veterinary drugs and the associated health risks from contaminated foods

Triphenylmethane dyes (such as malachite green and crystal violet) are a group of synthetic dyes that not only can be illegally added to food for colouring purposes but can also be present in fish tissues as residues due to the illegal usage as veterinary drugs. These dyes are applied in industry for colouring leather, wool, cotton, paper and fibres and in staining of tissues and bacteria (Arnold et al., 2009). They have also been used in aquaculture as veterinary drugs for the treatment of bacterial, protozoan and fungal infections in fish, as they are very cheap and easy to apply (Culp et al., 1999). European, US and Japanese surveillance systems (RASFF online database; F&DA, 2013c, Import Alert 16-124; Ministry of Health Labour and Welfare, Japan, Imported Foods Inspection Services) have reported the presence of malachite green and crystal violet in fish imported from such countries as Vietnam, China, Thailand, Taiwan and Indonesia. Triphenylmethanes have never been authorized for the use in fish for human consumption in the US or the EU due to the concerns regarding their genotoxic/carcinogenic properties. Both short-term and long-term feeding studies in animals (Culp et al., 1999; Culp et al., 2002; Culp et al., 2006) showed some toxic properties of both malachite green and its metabolite leucomalachite green. Gentian violet (comprised of 96 % crystal violet and 4% methyl violet) was demonstrated to be carcinogenic in mice and rats in a long-term exposure study (Littlefield et al., 1985).

8. The effect of permitted food additives on the behaviour of children

In the 1970s it was suggested that some food additives can cause behavioural disorders such as hyperactivity and learning disabilities in children (Feingold, 1975). Since that time a number of research studies have focused on this topic, some confirming whilst others rejecting this hypothesis (Arnold et al., 2012). In 2004 a group of researchers (Bateman et al., 2004) reported the adverse effect of the artificial food colourings (sunset yellow, tartrazine, carmoisine, ponceau 4R) and sodium benzoate on the behaviour of three year old children. In September 2007 the same group published further results of the studies on three and eight/nine year old children (McCann et al., 2007). The researchers used two mixtures of food additives: one containing sunset yellow, carmoisine, tartrazine, ponceau 4R and sodium benzoate; the second was composed of sunset yellow, carmoisine, quinoline yellow, allura red AC and sodium benzoate. The authors concluded that there was a significant reduction in hyperactive behaviour during the withdrawal phase and an increase in hyperactivity during the exposure to artificial food additives. The findings of these studies were widely discussed and debated among the scientific community. According to the Committee on Toxicity (COT) of Chemicals in Food, Consumer Products and the Environment (a scientific committee that provides an advice to the Food Standards Agency, the Department of Health and other Government Departments and Agencies on matters concerning the toxicity of chemicals in the UK) the study provided evidence that some mixtures of food additives could be linked to the increase in hyperactive behaviours in children. Additionally, it was noted that the effects were small and not clearly evident; therefore it was not possible to extrapolate the obtained results to the whole population. However, the results were consistent with previous reports concerning the hyperactivity after consuming certain foods (COT, 2007). The European Food Safety Authority (EFSA, 2008) concluded that there was limited evidence of the influence of the additives used in the research conducted on the behaviour of children. According to EFSA the effect was not statistically significant for both mixtures and age groups. It was not possible to determine the effect of individual colours since the mixtures were used in the study. According to the UK Food Standards Agency (FSA, 2008a) the Southampton study left uncertainty regarding the safety of the food additives tested in this research. It was also noted that part of the food industry was already withdrawing the artificial colours due to consumer demand. The Food Standard Agency Board agreed at its 2008 April meeting to advice UK Ministers concerning the withdrawal of these additives (FSA, 2008b). It was recommended to phase out these colours by the voluntary action of the food industry by the end of 2009 and to undertake more action to remove them from food in the whole EU. Parents of children showing hyperactive behaviour were advised to decrease the consumption of the six artificial colours.

While EFSA does not consider the evidence for dyes and hyperactivity link to be convincing enough, the legislation in the EU requires all food containing artificial colours used in Bateman et al. and McCann et al. studies to be labelled with warning: ‘’may have an adverse effect on the activity and attention in children’’(Regulation (EC) No 1333/2008). The EFSA initiated the re-assessment all of the permitted food colours and completed the evaluation of six artificial colours on 23rd of September 2009 (EFSA, 2009a-f). The Panel reduced the ADIs for quinoline yellow, sunset yellow FCF and ponceau 4R. The Panel did not change the existing ADIs for the three other colours assessed, tartrazine, carmoisine, and allura red AC. They stated that according to the evidence available quinoline yellow, sunset yellow FCF, ponceau 4R, carmoisine and allura red AC can be linked to some sensitivity reactions. Tartrazine may bring about intolerance reactions in a small part of the population. For all six colours, the Panel concluded that the evidence currently available did not substantiate a causal link between the individual colours and possible behavioural effects. There is still no conclusive answer to whether certain additives or their combination can have an adverse effect on the behaviour of children. Recently, due to new scientific data on genotoxicity of allura red, EFSA have recommended to perform more tests on all sulphonated mono azo dyes currently approved to be used in food (amaranth, ponceau 4R, sunset yellow, tartrazine, azorubine and allura red) (EFSA, 2013).

The US Food and Drug Administration Food Advisory Committee did not find the causal evidence to support the link between food colours and hyperactivity and did not recommend additional labelling requirements (F&DA, 2011a and 2011b). Nevertheless, it was advised to conduct the exposure assessment of seven US certified synthetic colours in children. The quantitative HPLC-DAD method applicable for different food matrices has been developed and validated for this purpose (Harp et al., 2013). The recent meta-analysis covering multiple studies in the area prepared by a group of US researchers found a statistically significant effect of the reduction of the ADHD symptoms when synthetic food colour additives free diet is applied (Nigg et al., 2012). Another group of US researchers have recently found a link between consumption of soft drinks and behavioural problems in 5-year old children and suggested that one of the possible explanations might be the presence of artificial food colours in these products (Suglia et al., 2013).

The exact biological mechanisms of the suspected influence of artificial food additives on hyperactive behaviour are unknown. The hyperactivity witnessed post exposure to some food additives may be caused by an altered central nervous system dopamine function that can have a genetic basis. It was demonstrated that the artificial colours can also induce IgE-independent histamine release (Bateman et al., 2004). The Southampton study provided some evidence that the hyperactive behaviour can be associated with some genetic polymorphisms thought to impair histamine clearance. In addition, it has been shown (Lau et al., 2006) that combinations of various food additives show synergistic effect to inhibit neuronal cell differentiation *in vitro*, the mechanism of this effect and its influence on the development of disorders is unknown. A study conducted by Ceyhan et al., 2013 found that artificial food colours and additives administered to female rats before and during gestation can cause an alteration in the expression of N-methyl-D-aspartate receptors (NMDARs) and nicotinic acetylcholine receptors (nACHRs) (that are linked to the learning and memory-generating processes) in their offspring.

9. The EU Rapid Alert System for Food and Feed, the US Import Alerts and monitoring of food colours

The Rapid Alert System for Food and Feed (RASFF) is an European network developed for the exchange of the information about the risks detected in relation to food and feed. It involves the Member States, the European Commission as the manager of the system and the European Food Safety Authority. Whenever a member of the network has any information relating to the risk to human health deriving from food or feed, the information is immediately notified under the RASFF. Food Inspections Authorities, Food Safety Authorities, Food Ministry and other related organizations in every European Union country are directly responsible for such exchange of information.

RASFF notifications are triggered by official controls on the internal market and controls at the border posts of the outer European Economic Area borders. Other cases can involve consumer complaints, companies notifying the outcome of an internal check or food poisoning outbreak (Kleter et al., 2009). The notifications include details of the notifying country, the risk and the origin of food, the source of notification and the action that has been taken. The notifications concern all risks connected to food such as bacterial contaminations, chemical contaminations, drug residues, illegal food additives, excessive content of food additives, foreign bodies, insects, risk of migration of package component, altered organoleptic characteristics (appearance, colour and taste) and risks of suffocation.

The notifications concerning food dyes are generally of following types (Table 2.):

1) unauthorized colour – illegal colour
2) unauthorized use of colour – colour is permitted in some foods only
3) too high content of authorized colour
4) undeclared colour

Numerous notifications for the presence of sudan dyes (mainly sudan I and IV) in food are recorded every year (Table 2.). The highest number was observed between 2003 and 2005 when a large quantity of chilli and chilli products imported to the EU was contaminated with these dyes. The contamination of fish with malachite green and crystal violet has been observed over the years with the highest number of cases recorded in 2005. Para red, orange II, methyl yellow and rhodamine B are also consistently detected in foods. The high number of notifications concerning too high use or unauthorized use of legal colours, mainly in imported foods, shows the problems faced by food importers to attempt to adhere to the EU regulations. Lack of uniform regulation worldwide results in the withdrawal from the EU market or border rejection of the products containing dyes legal in Japan (rose bengal, acid red 52 – JETO, 2006) or the US (fast green FCF – eCFR, 2009).

Legal synthetic colours such as tartrazine, sunset yellow, azorubin, amaranth, ponceau 4R, erythrosine and allura red appear on RASFF when they are used in food to which they should not be added, when there is too high content of them or food containing the colours is not properly labelled. Natural colours such as curcumin or cochineal have been also reported to be used in an illegal way (Table 2.). Annatto/bixin/norbixin (E160b) is a natural colour (extracts from seeds of annatto tree) that appears in RASFF most frequently. It is detected in spices, fish and fish products and non-alcoholic beverages, but is not authorized to be used in any of these types of food.

RASFF has also recently reported new type of the notifications concerning the lack of proper labelling of the presence of synthetic colours that have been linked to hyperactivity: "08//12/2011 undeclared colour (undeclared "Southampton" colours with absence of required health warning) in fruit flavoured sweets from Hong Kong" or "22/12/2011 insufficient labelling (absence of required health warning for "Southampton" colours) of green masala paste from the United Kingdom (RASFF online database). According to Regulation (EC) No 1333/2008 food containing colours E102, E104, E110, E122, E124, E129 should be additionally labelled with a warning stating that they ''may have an adverse effect on activity and attention in children". This labelling requirement is not strictly complied and for example in the UK it is possible to find products placed on the market with "Southampton" colours present and without the required warning information based on the observation of this report's authors.

The US Food and Drug Administration "Detention Without Physical Examination and Guidance of Foods Containing Illegal and/or Undeclared Colors" Import Alert 45-02 (F&DA, 2013b) lists the products in which illegal or undeclared colours were found and as a result they can be detained without physical examination upon entry to the US. The product is removed from the list when the importer provides the evidence of compliance with the law or provides a number of "clean shipments". The list presents the current state of the fraudulent use of colours and is updated frequently (Table 3.). Colours subject to certification (all synthetic colours) must come from F&DA certified lots, even if they are used in imported foods. As a result a high number of products containing dyes legal in the US imported from EEC or other foreign countries for which producers cannot present the required certificate are detained on entry to the US. A number of dyes illegal in the US and legal in the EU such as amaranth, brilliant black BN, brown HT, carmoisine, green S, patent blue V, ponceau 4R, quinoline yellow and illegal in both US and Europe such as rhodamine B or orange II have been also reported.

10. Analytical methods developed for the detection of synthetic food colours

Multiple chromatographic methods with different detection systems, spectrophotometric, voltammetric and capillary electrophoresis techniques have been developed for the analysis of synthetic colour compounds in foodstuffs; some examples are presented in Table 4. Reviews on the detection of natural colours (Scotter, 2011b) and sudan I-IV specifically (Rebane et al., 2010) have been presented elsewhere.

The multianalyte HPLC-DAD method developed by Yoshioka & Ichihashi (2008) can be used for the analysis of 40 synthetic legal and illegal colours in drinks and confectionary. Other HPLC-DAD methods were developed to monitor the presence of 17 legal and illegal (Bonan et al., 2013), 14 legal (Kirschbaum et al., 2003), 13 legal (Minioti et al., 2007) or 7 legal (Harp et al., 2013) synthetic food colours. Tandem mass spectrometry based detection has also been applied in the dye analysis field, mainly to determine the presence of illegal and potentially harmful dyes. The HPLC-MS/MS method developed by Feng et al. (2011) can be used for the detection of 40 synthetic dyes in soft drinks and Zhao et al., (2012) for the monitoring of the presence of 23 illegal dyes; Sun et al. (2007) of 10; Botek et al. (2007) of 8 and Fang et al. (2013a) of 7. HPLC-MS/MS was also developed for 13 triarylmethane and phenothiazine dyes (Tarbin et al., 2008). Recently Liu et al. (2011) used UPLC-MS/MS for the determination of 15 illegal dyes.

A number of antibody-based screening methods i.e. enzyme-linked immunosorbent assays (ELISA) were developed for the analysis of limited number of illegal synthetic colours: sudan dyes, para red, orange II, triphenylmethanes (malachite green, crystal violet, brilliant green), rhodamine B, methyl yellow and chrysoidine. ELISA tests were also established for the detection of legal synthetic dyes – sunset yellow and tartrazine.

A number of commercial companies in Europe provide analytical services for sudan and related dyes. *Eurofins* can screen for 28 (Eurofins, 2013), *Premier Analytical Services* for 21 (Premier Analytical Services, 2013*)* or *QTS Analytical* for 14 illegal dyes (QTS Analytical, 2012).

Generally no safe level of the presence of illegal dyes in foods has been established; however the FSA in the UK has recommended a limit 0.5 ppm for products containing illegal dyes to be withdrawn from the market (FSA, 2007). With regards malachite green in fish, the minimum required performance limit (MRPL) for the method used for the detection of the sum of malachite green and its leuco metabolite is set at 2 ppb in the EU according to Commission Decision 2004/25/EC.

11. Food colours – current issues

11.1. Synthetic colours

As a result of large number of cases contamination of food with sudan dyes in the EU in 2003 the emergency measures were taken and all chilli, chilli products, curcuma and palm oil imported into the EU had to be screened for the presence of sudan I-IV dyes (Commission Decisions 2003/460/EC, 2004/92/EC and 2005/402/EC). In 2005 and 2006 the Food Standards Agency (FSA) in the UK tested 893 samples of spices, sauces and oils for sudan dyes, para red, rhodamine B, orange II, red 2G, methyl yellow and metanil yellow and found six to be contaminated (FSA, 2006). Additionally, 18 samples contained annato/bixin/norbixin (E160b) a natural food colour that should not be used in spices. Our own study on the application of the ELISA method for the detection of sudan I has shown presence of this contaminant in 3 samples (out of 85 tested) that were purchased within the EU (1 sample) and outside (2 samples) (Oplatowska et al., 2011a). The data from RASFF also support the necessity for monitoring for the presence of sudan dyes in food, as contamination events can occur, but not on the same scale as between 2003 and 2005. There are also a number of dyes with similar properties that might be illegally used in food as a replacement for monitored sudan dyes. The compounds such as metanil yellow, methyl yellow, rhodamine B, toluidine red and other sudan-similar azo dyes should be also included in the screening programmes to ensure safe food for the consumer.

A number of commercial companies offer screening services for several synthetic dyes. However, there is still a need for the development of multianalyte methods that could target a large range of suspect compounds. Such trends that are currently observed in mycotoxin analysis (Varga et al., 2013) and veterinary drug residues (Smith et al., 2009). Further expansion of the existing tools for fast, low cost screening methods such as ELISA (limited to sudan dyes, para red, methyl yellow, rhodamine B, orange II, chrysoidine and triphenylmethane dyes thus far) would serve to provide further data on the occurrence of these contaminants in foods. The list of possible targets is close to impossible to determine as there is such a large number of synthetic dyes used in industry and widely available nowadays. New dyes are being detected in food, for example in 2012 Ruf et al. reported the presence of common industrial azo-dye basic red 46 in sumac spice sample during the routine analysis for sudan dyes. Studies performed in countries from which the dye-contaminated food is often imported can provide some background on the targets that the analytical methods should focus on. One such study in India reported the presence of a number of illegal dyes in coloured food commodities purchased from urban and rural areas (Tripathi et al., 2007). Thirty one percent of samples were found to contain illegal dyes: metanil yellow, rhodamine B, orange II, sudan dyes, auramine, malachite green, amaranth and quinoline yellow (the last two are not permitted in India), with more contaminants found in rural area (38% of the total samples, while 25% in urban area) probably due to the less frequent sampling and analysis of samples. This high level occurrence of illegal synthetic colours shows the potential scale of the problem in Asian countries and indeed across the Developing World. Another Indian study (Rao & Sudershan, 2008) reported the increase in usage of some permitted synthetic colours in food that may lead to exceeding the ADIs. The illegal dyes (namely fast red, amaranth, rhodamine B, orange II and auramine) were also identified in some foods analyzed. In the study performed by Dixit et al., 2013 the illegal synthetic colours such as rhodamine B, metanil yellow, orange II and malachite green were found in 16.4 % food samples collected in different regions of India in addition to excessive amount of legal colorants identified in 58 % of samples. Due to the common occurrence of illegal dyes in food the *Times of India* advices how to detect food adulteration with illegal dyes in ordinary kitchen (*Times of India*, 2012). The author suggests that illegal metanil yellow in turmeric can be exposed by dissolving spices in water and adding any commonly available acid as metanil yellow should turn purple, violet or pink under acidic conditions. The presence of malachite green on green vegetables can be detected by simply placing food on moistened white paper. The adulteration with malachite green is indicated by formation of the coloured impressions.

Dyes can be also added illegally to food to compensate for their low quality. In a recent study the synthetic dye E154 was found in 9 out of 51 fishery products that should not contain this dye according to the EU legislation (Bonan et al., 2013). Carcinogenic chrysoidine has been demonstrated to be used illegally as dyes in soybean milk or to dye low-cost fish to imitate expensive and superior in quality yellow-fin tuna (Gui et al., 2010; Reyns et al., 2009; Wang et al., 2008). Chrysoidine has also antimicrobial and antispectic activities and as such it has been applied to disinfect fish skin in some Asian countries (Reyns et al., 2009).

11.2. Dyes - illegal veterinary drugs

Monitoring programs for carcinogenic triphenylmethane dyes: malachite green and crystal violet are already in place in Europe and the US. However, there are a number of dyes belonging to this group with similar properties that can be used as alternatives and may not be detected in these monitoring programmes. Tarbin et al., 2008 has suggested eleven other compounds belonging to triarylmethanes, xanthenes and phenothiazine groups that should be under suspicion i.e. brilliant green, ethyl violet, pararosaniline, victoria blue B, victoria blue R, victoria pure blue BO, rhodamine 6G, methylene blue, azure B, new methylene blue and nile blue. One of these compounds – victoria pure blue BO appeared on RASFF in 2010 as it was found in fish imported from Vietnam.

11.3. Colour migration

The migration of a colour from the package material or other food contact materials has been recorded on RASFF since 2005 (RASFF online database). RASFF does not generally give details about the identity of the dye that was detected in food as a result of the migration. These are most probably dyes used in industrial applications e.g. dying cutlery, napkins or wrappings and should not be consumed by humans. A recent study demonstrated the migration of synthetic colour brilliant green from green paper towels (Oplatowska et al., 2011c). Brilliant green is a triphenylmethane dye that is likely to have similarly toxic properties as the known illegal veterinary drug malachite green. A measurable amount of the paper towel dye was shown to migrate from the towel trough the skin when the towel was used for hand drying. Additionally, it was also demonstrated that large amounts of colour can accumulate in food (fish was used as the model) when towels containing dye were used for wrapping. This study raised a concern about the safety of use of dyed paper towels for hygiene purposes and in food preparation area.

A previously unidentified mean of exposure to legal synthetic colours was demonstrated in a study performed by Lucova et al., 2013. Patent blue V (E131) and brilliant blue (E133) were shown to directly enter the epithelium and then possibly the bloodstream through the tongue from the saliva when used for example in lollipops. These dyes could also penetrate the shaven skin when used in aftershave products. This type of exposure does not include gastrointestinal tract degradation and result in direct access of the dyes to the systemic circulation. The authors concluded that due to the systemic availability these compounds should not be used in topical products intended for use on slightly damaged skin and for confectionary and lollipops for licking. Currently E131, E133 and other synthetic dyes are commonly used in cosmetics, oral mouthwashes, pharmaceutical lozenges and confectionary.

12. Conclusions and summary

The use of synthetic colours in food has resulted in a substantial amount of negative press in recent years due to the possible harmful side effects. One of the most important issues concerning the legal synthetic food colours is that there is still no definitive answer if these dyes play any role in hyperactive behaviour in children. However, due to the current suspicions there is a trend to remove them from the food supply chain. The voluntary withdrawal action in the UK supported by the Food and Standards Agency has already resulted in major food suppliers changing from synthetic colours to natural products, especially in confectionery. However, the replacement is not always straightforward and not all natural colours are completely safe.

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Food globalization has resulted in humans consuming foods from many different parts of the world. Another important issue is the lack of uniform regulations on food colours what causes disruption to the international trade. Additionally, due to the lack of uniform control policies worldwide, the distinct possibility of illegal and harmful chemicals being present in foods locally produced or indeed imported cannot be ruled out and indeed is quite likely, particularly in the Developing World. The sudan scare in 2003 and contamination of hundreds of products with illegal sudan dyes demonstrates the need for rigorous monitoring programs and identifying and targeting of possible hazards. Currently, a number of analytical methods have been developed for the analysis of food colours and a number of companies across Europe and the US provide testing services for detecting illegal dyes present in foods. Nevertheless, there is still a clear need for additional research and monitoring activities to better protect the consumer. Therefore, another challenge in the area of food colours is identification of known and emerging dye adulterants/contaminants in food, not only in the Developed World but as a matter of priority in the Developing World is needed. To support this need the availability of rapid and low cost multicolourant screening and confirmatory methods is identified as a priority.

Further exposure assessments of legal synthetic colours, especially in children are required as a matter of priority to better understand the link between consumption and adverse health effects. Without this data the ability to protect the consumer, most especially the most vulnerable (children) is highly compromised.

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Table list

Table 1. Comparison of the synthetic dyes authorized for use in food in different global regions.

EU	US	Japan	India	China
E102 tartrazine	FD&C yellow No. 5.	food yellow No. 4	tartrazine	tartrazine
E104 quinoline yellow				quinoline yellow
E110 sunset yellow FCF	FD&C yellow No. 6.	food yellow No. 5	sunset yellow FCF	sunset yellow
E122 azorubine			carmoisine	carmoisine
E123 amaranth		food red No. 2		amaranth
E124 ponceau 4R		food red No. 102	ponceau 4R	ponceau 4R
E127 erythrosine	FD&C red No. 3.	food red No. 3	erythrosine	erythrosine
E129 allura red AC	FD&C red No. 40.	food red No. 40		allura red
E131 patent blue V				
E132 indigotine	FD&C blue No. 2.	food blue No. 2	indigo carmine	indigotine
E133 brilliant blue FCF	FD&C blue No. 1.	food blue No. 1	brilliant blue FCF	brilliant blue
E142 green S				
E151 brilliant black BN				
E155 brown HT				
E180 litholrubine BK				
	FD&C green No. 3	food green No. 3	fast green FCF	
	orange B			
	citrus red No. 2.			
		food red No.104 (phloxine B)		
		food red No.105 (rose bengal)		
		food red No.106 (acid red 52)		
				new red

Table 2. Number of notifications concerning colouring compounds in food recorded by RASFF (RASFF online database).

Dye	Number of notifications recorder by RASFF										Type of notification	Product category[2]	Conc. [mg/kg – ppm]
	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**			
acid red 52 (sulforhodamine B)					3						Unauthorized colour	**I, J**	
basic red 46									1		Unauthorized colour	**J**	500 – 1500
crystal violet and leucocrystal violet			2	5	2					2	Unauthorized substance	**G**	0.0009 – 0.654
fast garnet					1		1				Unauthorized colour	**J**	0.77-1.3
fast green FCF						2		3	2	1	Unauthorized colour	**A, C, M**	
gardenia yellow							5	1	1		Unauthorized colour	**A, C, K, S**	
malachite green and leucomalachite green	11	18	50	17	9	2	5	4	7	2	Unauthorized substance	**D, G**	0.0003 – 4.875
methyl yellow (butter yellow)			2	1			3	1			Unauthorized colour	**J**	0.24–44
oil orange ss							1				Unauthorized colour	**J**	0.15

Dye	Number of notifications recorder by RASFF										Type of notification	Product category[2]	Conc. [mg/kg – ppm]
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012			
orange II		1	2	2		1	3	2			Unauthorized colour	**H, J, N, S**	0.56–12000
orange RN						1					Unauthorized colour	**J**	
para red			42	12		1	1				Unauthorized colour	**I, J, S**	0.02–416
red 2G					2				1		Unauthorized colour	**L**	1.4 – 6.3
rhodamine B			5	3	5	2	1	3	5	3	Unauthorized colour	**A, C, H, I, J, N, S, U**	0.01–89.2
rose bengal			1				3				Unauthorized colour	**C, I**	
sudan I	118	190	135	40	22	17	17	14	15	2	Unauthorized colour	**A, F, I, J, O, R, S**	0.0013 – 4938
sudan II	1										Unauthorized colour	**R**	
sudan III	1	1	4	1				1			Unauthorized colour	**F, J, S**	0.1 – 181
sudan IV	5	170	94	25	13	12	15	12	9	6	Unauthorized colour	**A, F, I, J, L, S**	0.01 – 2105
sudan 7B				1							Unauthorized colour	**A, C, I, J, N, S**	0.05

Dye	Number of notifications recorder by RASFF										Type of notification	Product category[2]	Conc. [mg/kg – ppm]
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012			
sudan red G				1		1			1		Unauthorized colour	**F, J, S**	0.2 – 25.2
toluidine red			1	1					2		Unauthorized colour	**J, S**	2.5 – 179
victoria pure blue BO								1			Unauthorized substance	**G**	0.0089
E 100 curcumin				1	1	1			1		Too high content, unauthorized use	**A, J, S**	450 – 3200
E 102 tartrazine			3	36	10	7	12	5	7	3	Too high content, unauthorized use, undeclared colour	**A, B, C, G, I, J, M, N, O, R**	1.2 – 6000
E 104 quinoline yellow			2	2	1	1					Too high content, unauthorized use	**A, C, G**	70.6– 128
E 110 sunset yellow FCF	1	2	11	7	15	16	11	11	7	2	Too high content, unauthorized use, undeclared colour	**A, C, D, G, I, J, K, L, M, N, O, R, S, T**	5–2265

Dye	Number of notifications recorder by RASFF										Type of notification	Product category[2]	Conc. [mg/kg – ppm]
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012			
E 120 cochineal	1						1		4	4	Too high content, unauthorized use	**G, I, L, M, O**	115–356
E 122 azorubine			7	8	2	2	5	2	6	7	Too high content, unauthorized use, undeclared colour	**A, B, C, G, I, J, K, L, M, N, T**	15.6–314
E 123 amaranth	7	2	7	5		3	4	4	4	1	Unauthorized use, undeclared colour	**B, C, E, H, J, K, M, N, S**	1.2–3300
E 124 ponceau 4R		4	6	11	8	8	17	8	4	7	Too high content, unauthorized use, undeclared colour	**A, C, D, G, I, J, K, L, M, N, P, S**	0.0027–1800
E 127 erythrosine	2	5	15	6	11	17	12	4	11	7	Unauthorized use	**A, B, C, D, E, G, H, I, J, K, M,**	1.6–145

Dye	**Number of notifications recorder by RASFF**										**Type of notification**	**Product category**[2]	**Conc. [mg/kg – ppm]**
	2003	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**			
E 129 allura red AC	2		2	7	8	4	5	2	3	1	Too high content, unauthorized use, undeclared colour	**N, O, R, S,W A, C, D, E, G, I, J, L, M, N**	1.5 – 850
E 131 patent blue V							1		1		Unauthorized use undeclared colour	**C, I**	
E 132 indigotine/indigo carmine				1	1	1		1	1		Too high content, unauthorized use, undeclared colour	**C, E, G, I**	14–309
E 133 brilliant blue FCF			3	2	2	4		2	2		Too high content, unauthorized use, undeclared colour	**A, B, C, E**	91–2500
E 141i chlorophylls			1								Unauthorized use	**A**	
E 142 green S			1								Undeclared colour	**C**	
E 150a plain caramel							1		1		Unauthorized use	**S, M**	
E 151 brilliant black BN				2		1	1	2			Too high content, undeclared colour	**C, G**	410 – 630
E 153 vegetable carbon									1		Unauthorized use	**A**	
E 155 brown HT				1					1		Too high content, undeclared colour	**C, G**	

Dye	Number of notifications recorder by RASFF										Type of notification	Product category[2]	Conc. [mg/kg – ppm]
	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012			
E 160b a73nnatto/bixin/norbixin	2	9	22	11	5	6	3	6	1	9	Too high content, unauthorized use	**A, C, F, G, J, M, S**	0.6 – 3486
E 170 calcium carbonate										2	Unauthorized use	**H, S**	
E 171 titanium dioxide		19	7	17	5						Unauthorized use	**I**	0.44–217
E 173 aluminium										1	Unauthorized use	**C**	

[2]**Product categories:**

A – cereals and bakery products

B – cocoa and cocoa preparations, coffee and tea

C – confectionery

D – crustaceans and products thereof

E – dietetic foods, food supplements, fortified foods

F – fats and oils

G – fish and products thereof

H – food additives

I – fruit and vegetables

J – herbs and spices

K – ices and desserts

L – meat and meat products (other than poultry)

M – non-alcoholic beverages

N – nuts, nut products and seeds

O – other food product / mixed

P – poultry meat and poultry meat products

R – prepared dishes and snacks

S – soups, broths, sauces and condiments

T – wine

U – food contact materials

W – milk and milk products

Table 3. Number of import alerts for each colour additive on "Detention Without Physical Examination and Guidance of Foods Containing Illegal and/or Undeclared Colors" F&DA list as on 06/09/2013.

	Food colour	Nr of products on Import Alert 45-02 as on 06/09/2013
Synthetic colours legal in the US	FD&C yellow No. 5. tartrazine	2500
	FD&C yellow No. 6 sunset yellow	1905
	FD&C red No. 3. erythrosine	416
	FD&C red No. 40. allura red	1300
	FD&C blue No. 1.brilliant blue	1359
	FD&C blue No. 2 indigotine	210
	FD&C green No. 3 fast green FCF	24
	citrus red No. 2	1
Natural colours legal in the US	turmeric	33
	annatto	9
	astaxanthin	3
	betanin/beetroot	1
	carmine/cochineal	34
	carotene	45
	chlorophylls/copper complexes of chlorophylls	20
	iron oxides	13
	paprika colour	9
	riboflavin	2
	titanium dioxide	50
Synthetic colours illegal in the US	acid red 52	16
	acid yellow G	1
	amaranth	291
	brilliant black BN	22
	brown HT	5
	carmoisine	1072
	chrysoin S	6
	green S	33
	lake red CBA	6
	light green SF	3
	malachite green	12(rice and tapioca)+35(seafood)*
	orange II	24
	patent blue V	82
	phloxine B	11
	polar yellow 5G	5
	ponceau 4R	1162

	ponceau 6R	1
	ponceau SX	14
	quinoline yellow	187
	quinoline yellow spirit soluble	10
	red 2 G	6
	rhodamine B	99
Natural colours illegal in the US	anthocyanin	21
	calcium carbonate	6
	carbon black, charcoal, vegetable carbon	134
	carthamin	13
	gardenia blue pigment	7
	gardenia yellow pigment	17

*Import Alert 16-124 (F&DA, 2013c)

Table 4. Detection methods for synthetic food colours.

Colour	CI number	Detection methods
acid blue 1	42045	**Liquid chromatography –** Yoshioka & Ichihashi, 2008
acid green 9	42100	**Liquid chromatography –** Yoshioka & Ichihashi, 2008
acid green 25	61570	**Liquid chromatography –** Feng et al., 2011
acid red 13 (fast red E)	16045	**Liquid chromatography –** Feng et al., 2011; Yoshioka & Ichihashi, 2008
acid red 52 (sulforhodamine B)	45100	**Liquid chromatography –** Feng et al., 2011; Yoshioka & Ichihashi, 2008
acid violet 7	18055	**Liquid chromatography –** Yoshioka & Ichihashi, 2008
acid violet 17	42650	**Liquid chromatography –** Feng et al., 2011
acid yellow 9 (fast yellow AB)	13015	**Liquid chromatography –** Feng et al., 2011, Yoshioka & Ichihashi, 2008
alizarin yellow GG	14025	**Liquid chromatography –** Feng et al., 2011
amido black 10B (acid black 1)	20470	**Liquid chromatography –** Feng et al., 2011; Yoshioka & Ichihashi, 2008
astrazon orange G (basic orange 21)	48035	**Liquid chromatography –** Fang et al., 2013a
astrazon orange R (basic orange 22)	48040	**Liquid chromatography –** Fang et al., 2013a
auramine O (basic flavine O)	41000	**Liquid chromatography –** Feng et al., 2011
azocarmine B	50090	**Liquid chromatography –** Bonan et al., 2013
azocarmine G	50085	**Liquid chromatography –** Bonan et al., 2013
azure B	52010	**Liquid chromatography –** Tarbin et al., 2008
benzyl violet 4B	42640	**Liquid chromatography –** Yoshioka & Ichihashi, 2008
brilliant black PN	28440	**Liquid chromatography –** Yoshioka & Ichihashi, 2008
brilliant green	42040	**Liquid chromatography –** Andersen et al., 2007; Andersen et al., 2009; Hurtaud– Pessel et al., 2011; Tarbin et al., 2008 **ELISA –** Oplatowska et al., 2011b; Shen et al., 2011

Colour	CI number	Detection methods
chrysoidine (basic orange2)	11270	**Liquid chromatography** – Fang et al., 2013a; Fang et al., 2013b; Feng et al., 2011; Gui et al., 2010; Reyns et al., 2009; Zhao et al., 2012 **Gas chromatography** – Wang et al., 2008 **Chemiluminescence sensor with magnetic molecularly imprinted polymer** – Lu et al., 2012 **ELISA** – Lei et al., 2011
chrysoine (acid orange 6, tropaeolin O, resorcinol yellow)	14270	**Liquid chromatography** – Yoshioka & Ichihashi, 2008; Fang et al., 2013a
crystal violet and/or leuco crystal violet	42555	**Liquid chromatography** – Andersen et al., 2007; Andersen et al., 2009; Ascari et al., 2012; Dowling et al., 2007; Hurtaud-Pessel et al., 2011; Long et al., 2009; Rushing et al., 1995; Rushing & Hansen, 1997; Rushing & Thompson, 1997; Tao et al., 2011; Tarbin et al., 1998; Tarbin et al., 2008; Wu et al., 2007 **Spectrophotometry** – Safarik & Safarikova, 2002 **ELISA** – Oplatowska et al., 2011b; Shen et al., 2011; Yang et al., 2007
disperse blue 106	111935	**Liquid chromatography** – Zhao et al., 2012
disperse blue 124	111938	**Liquid chromatography** – Zhao et al., 2012
disperse orange 3	11005	**Liquid chromatography** – Zhao et al., 2012
disperse orange 11	60700	**Liquid chromatography** – Zhao et al., 2012
disperse orange 37	11132	**Liquid chromatography** – Zhao et al., 2012
disperse red 1	1110	**Liquid chromatography** – Zhao et al., 2012
disperse yellow 3	11855	**Liquid chromatography** – Zhao et al., 2012
eosine Y	45380	**Liquid chromatography** – Feng et al., 2011; Yoshioka & Ichihashi, 2008
ethyl violet	42600	**Liquid chromatography** – Tarbin et al., 2008
fast green FCF	42053	**Liquid chromatography** – Feng et al., 2011; Harp et al., 2013; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008
fast red E	16045	**Liquid chromatography** – Yoshioka & Ichihashi, 2008
guinea green B	42085	**Liquid chromatography** – Feng et al., 2011

Colour	CI number	Detection methods
light green SF	42095	**Liquid chromatography** – Feng et al., 2011
malachite green and /or leuco malachite green	42000	**Liquid chromatography** – Allen et al., 1994; Andersen et al., 2006; Andersen et al., 2009; Ascari et al., 2012; Bergwerff & Scherpenisse, 2003; Chen et al., 2013; Doerge at al., 1998; Dowling et al., 2007; Hajee & Haagsma, 1995; Halme et al., 2007; Hashimoto et al., 2012; Hurtaud-Pessel et al., 2011; Lee et al., 2006; Liu et al., 2011; Long et al., 2009; Martínez Bueno et al., 2010; Mitrowska & Posyniak, 2004; Mitrowska et al., 2008; Nebot et al., 2013; Roybal et al., 1995; Rushing & Hansen, 1997; Rushing & Thompson, 1997; Scherpenisse & Bergwerff, 2005; Smith et al., 2009; Tao et al., 2011; Tarbin et al., 1998; Tarbin et al., 2008; Turnipseed et al., 1995; Valle et al., 2005; Wu et al., 2007 **Spectrophotometry** – Safarik & Safarikova, 2002 **ELISA** – Oplatowska et al., 2011b ; Shen et al., 2011; Singh et al., 2011; Xing et al., 2009; Yang et al., 2007;
martius yellow	10315	**Liquid chromatography** – Yoshioka & Ichihashi, 2008
metanil yellow	13065	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Fuh & Chia, 2002; Zhao et al., 2012
methylene blue	52015	**Liquid chromatography** – Tarbin et al., 2008; Zhao et al., 2012
methyl orange (acid orange 52)	13025	**Liquid chromatography** – Fang et al., 2013a
methyl yellow (butter yellow, sudan yellow)	11020	**Liquid chromatography** – Feng et al., 2011; Sun et al., 2007; Zhao et al., 2012 **ELISA** – Oplatowska & Elliott, 2011
napthol yellow S	10316	**Liquid chromatography** – Yoshioka & Ichihashi, 2008
new methylene blue	52030	**Liquid chromatography** – Tarbin et al., 2008
new red		**Liquid chromatography** – Feng et al., 2011
nile blue A	51180	**Liquid chromatography** – Tarbin et al., 2008
orange I (acid orange 20)	14600	**Liquid chromatography** – Fang et al., 2013a; Fuh & Chia, 2002; Yoshioka & Ichihashi, 2008;
orange II (acid orange 7)	15510	**Liquid chromatography** – Bonan et al., 2013; Fang et al., 2013a; Feng et al., 2011; Fuh & Chia, 2002; Yoshioka & Ichihashi, 2008; Zhao et al., 2012; Zou et al., 2013; **ELISA** – Xue et al., 2012
orange G	16230	**Liquid chromatography** – Yoshioka & Ichihashi, 2008
orange RN	15970	**Liquid chromatography** – Yoshioka & Ichihashi, 2008

Colour	CI number	Detection methods
para red	12070	**Liquid chromatography** – Botek et al., 2007; Ertaş et al., 2007; Fan et al., 2009; Liu et al., 2011; Mazzotti et al., 2008; Pardo et al., 2009; Stuart, 2006; Sun et al., 2007; Uematsu et al., 2007; Zhao et al., 2012 **ELISA** – Chang et al., 2011; Ju et al., 2008; Liu et al., 2012; Qi et al., 2012; Shan et al., 2012; Wang et al., 2012; Xu et al., 2010a
pararosaniline	42500	**Liquid chromatography** – Tarbin et al., 2008
phloxine B	45410	**Liquid chromatography** – Feng et al., 2011; Yoshioka & Ichihashi, 2008
ponceau 2R	16150	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Fuh & Chia, 2002; Yoshioka & Ichihashi, 2008
ponceau 3R	16155	**Liquid chromatography** – Feng et al., 2011; Fuh & Chia, 2002; Yoshioka & Ichihashi, 2008
ponceau 6R	16290	**Liquid chromatography** – Bonan et al., 2013; Yoshioka & Ichihashi, 2008
ponceau SX	14700	**Liquid chromatography** – Feng et al., 2011; Yoshioka & Ichihashi, 2008
ponceau xylidine	16150	**Liquid chromatography** – Zou et al., 2013
quinoline yellow spirit soluble	47000	**Spectrophotmetry** – Capitan et al., 1996
red 2G (acid red 1, azophloxine)	18050	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008; Zou et al., 2013 **Capillary electrophoresis** – Ryvolova et al., 2007
red 10B	17200	**Liquid chromatography** – Yoshioka & Ichihashi, 2008
rhodamine B	45170	**Liquid chromatography** – Botek et al., 2007; Feng et al., 2011; Liu et al., 2011; Stuart, 2006; Zhao et al., 2012 **ELISA** – Oplatowska & Elliott, 2011
rhodamine 6G	45160	**Liquid chromatography** – Liu et al., 2011; Tarbin et al., 2008
rose bengal	45440	**Liquid chromatography** – Feng et al., 2011; Yoshioka & Ichihashi, 2008
sudan I	12055	**Liquid chromatography** – Botek et al., 2007; Calbiani et al., 2004a; Calbiani et al., 2004b; Chailapakul et al., 2008, Cornet et al., 2006; Di Donna et al., 2004; Ertaş et al., 2007; Fan et al., 2009; He et al., 2007; Liu et al., 2007a; Liu et al., 2011; Ma et al., 2006; Mazzetti et al, 2004; Mazzotti et al., 2008;

Colour	CI number	Detection methods
		Murty et al., 2009; Pardo et al., 2009; Puoci et al., 2005; Schummer et al., 2013; Stuart, 2006; Sun et al., 2007, Tateo & Bononi, 2004; Uematsu et al., 2007; Yu et al., 2008; Zhang et al., 2006; Zhao et al., 2012 **Gas chromatography –** He et al., 2007 **Capillary electrophoresis –** Mejia et al., 2006 **Voltammetry –** Du et al., 2007; Gan et al., 2008; Lin et al., 2008; Mo et al., 2010 **Amperometry –** Liu et al., 2007b **Spectrophotometry –** Capitan et al., 1996 **Chemiluminescence –** Liu et al., 2007c **Plasmon resonance light scattering –** Wu et al., 2006 **ELISA –** Anfossi et al., 2009; Chang et al., 2011; Han et al., 2007; Ju et al., 2008; Liu et al., 2012; Oplatowska et al., 2011a; Qi et al., 2012; Shan et al., 2012; Wang et al., 2009; Wang et al., 2011; Xiao et al., 2011; Xu et al., 2010a; Xu et al., 2010b **Flow injection chemiluminescence –** Chen et al., 2010
sudan II	12140	**Liquid chromatography –** Botek et al., 2007; Calbiani et al., 2004a; Calbiani et al., 2004b; Chailapakul et al., 2008, Cornet et al., 2006; Ertaş et al., 2007; Fan et al., 2009; He et al., 2007; Liu et al., 2007a; Liu et al., 2011; Ma et al., 2006; Mazzotti et al., 2008; Murty et al., 2009; Pardo et al., 2009; Schummer et al., 2013; Stuart, 2006; Sun et al., 2007; Tateo & Bononi, 2004; Uematsu et al., 2007; Yu et al., 2008; Zhang et al., 2006; Zhao et al., 2012 **Gas chromatography –** He et al., 2007 **Capillary electrophoresis –** Mejia et al., 2006 **Amperometry –** Liu et al., 2007b **Plasmon resonance light scattering –** Wu et al., 2006 **ELISA –** Anfossi et al., 2009; Chang et al., 2011; Liu et al., 2012; Qi et al., 2012; Shan et al., 2012
sudan III	26100	**Liquid chromatography –** Botek et al., 2007; Calbiani et al., 2004a; Calbiani et al., 2004b; Chailapakul et al., 2008, Cornet et al., 2006; Ertaş et al., 2007; Fan et al., 2009; He et al., 2007; Liu et al., 2007a; Liu et al., 2011, Ma et al., 2006; Mazzotti et al., 2008; Murty et al., 2009; Pardo et al., 2009; Schummer et al., 2013; Stuart, 2006; Sun et al., 2007; Uematsu et al., 2007; Yu et al., 2008; Zhang et al., 2006; Zhao et al., 2012 **Gas chromatography –** He et al., 2007 **Capillary electrophoresis –** Mejia et al., 2006

Colour	CI number	Detection methods
		Amperometry – Liu et al., 2007b **Plasmon resonance light scattering** – We et al., 2006 **ELISA** – Chang et al., 2011; Ju et al., 2008; Liu et al., 2012; 2008; Qi et al., 2012; Shan et al., 2012
sudan IV	26105	**Liquid chromatography** – Botek et al., 2007; Calbiani et al., 2004a; Calbiani et al., 2004b; Chailapakul et al., 2008, Cornet et al., 2006; Ertaş et al., 2007; Fan et al., 2009; He et al., 2007; Liu et al., 2007a; Liu et al., 2011; Ma et al., 2006; Mazzotti et al., 2008; Murty et al., 2009; Pardo et al., 2009; Schummer et al., 2013; Stuart, 2006; Sun et al., 2007; Uematsu et al., 2007; Xinyi et al., 2010; Yu et al., 2008; Zhang et al., 2006; Zhao et al., 2012 **Gas chromatography** – He et al., 2007 **Capillary electrophoresis** – Mejia et al., 2006 **Amperometry** – Liu et al., 2007b **Plasmon resonance light scattering** – Wu et al., 2006 **ELISA** – Chang et al., 2011; Liu et al., 2012; Qi et al., 2012; Shan et al., 2012
sudan blue 2	61554	**Liquid chromatography** – Zhao et al., 2012
sudan orange G	11920	**Liquid chromatography** – Botek et al., 2007; Liu et al., 2011; Pardo et al., 2009; Sun et al., 2007; Zhao et al., 2012
sudan red B	26110	**Liquid chromatography** – Sun et al., 2007
sudan red 7B	26050	**Liquid chromatography** – Botek et al., 2007; Liu et al., 2011; Pardo et al., 2009; Sun et al., 2007; Zhao et al., 2012
sudan red G	12150	**Liquid chromatography** – Liu et al., 2011; Sun et al., 2007; Zhao et al., 2012 **ELISA** – Chang et al., 2011; Liu et al., 2012; 2011; Qi et al., 2012; Shan et al., 2012
toluidine red	12120	**Liquid chromatography** – Liu et al., 2011; Zhao et al., 2012
uranine	45350	**Liquid chromatography** – Feng et al., 2011; Yoshioka & Ichihashi, 2008
victoria blue B	44045	**Liquid chromatography** – Tarbin et al., 2008
victoria pure blue BO	42595	**Liquid chromatography** – Tarbin et al., 2008
victoria blue R	44040	**Liquid chromatography** – Tarbin et al., 2008
yellow 2G (acid yellow 17)	18965	**Liquid chromatography** – Feng et al., 2011, Yoshioka & Ichihashi, 2008

Colour	CI number	Detection methods
E 102 tartrazine	19140	**Liquid chromatography** – Alves et al., 2008; Bonan et al., 2013; Chen et al., 1998; Culzoni et al., 2009; Feng et al., 2011; Fuh & Chia, 2002; Garcia-Falcon & Simal-Gandara, 2005; Gianotti et al., 2005; Gonzalez et al., 2003a; Gonzalez et al., 2003b; Hajimahmoodi et al., 2013; Harp et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Ma et al., 2006; Minioti et al., 2007; Vidotti et al., 2006; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008; Perez-Urquiza & Beltran, 2000 **Voltammetry** – Ghoreishi et al., 2011; Ghoreishi et al., 2012; Medeiros et al., 2012a; Medeiros et al., 2012b; Ni et al., 1996; Ni et al., 1997; Silva et al., 2007; Song et al., 2010 **Spectrophotometry** – Aktas & Ertokus, 2011; Capitan-Vallvey et al., 1998; Llamas et al., 2009; Ni & Gong, 1997; Ni et al., 2009; Vidotti et al., 2005 **Photoacoustic spectroscopy** – Coelho et al., 2010 **ELISA** – Lei et al., 2013
E 104 quinoline yellow	47005	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Garcia-Falcon & Simal-Gandara, 2005; Gianotti et al., 2005; Hajimahmoodi et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Spectrophotometry** – Capitan et al., 1996
E 110 sunset yellow FCF	15985	**Liquid chromatography** – Alves et al., 2008; Bonan et al., 2013; Chen et al., 1998; Culzoni et al., 2009; Feng et al., 2011; Fuh & Chia, 2002; Garcia-Falcon & Simal-Gandara, 2005; Gianotti et al., 2005; Hajimahmoodi et al., 2013; Harp et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Ma et al., 2006; Minioti et al., 2007; Vidotti et al., 2006; Yoshioka & Ichihashi, 2008; Zou et al., 2013 **Capillary electrophoresis** – Del Giovine & Bocca, 2003; Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008; Perez-Urquiza & Beltran, 2000 **Voltammetry** – Ghoreishi et al., 2012; Medeiros et al., 2012a; Medeiros et al., 2012b; Nevado et al., 1997; Ni et al., 1996; Ni et al., 1997; Ni & Bai, 1997; Silva et al., 2007; Song, 2010 **Spectrophotometry** – Aktas & Ertokus, 2011; Capitan et al.,

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Colour	CI number	Detection methods
		1996; Capitan-Vallvey et al., 1998; Llamas et al., 2009; Ni & Gong, 1997; Ni et al., 2009; Vidotti et al., 2005 **Photoacoustic spectroscopy** – Coelho et al., 2010 **ELISA** – Xing et al., 2012
E 122 azorubine	14720	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Garcia-Falcon & Simal-Gandara, 2005; Gennaro et al., 1997; Gianotti et al., 2005; Hajimahmoodi et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Del Giovine & Bocca, 2003; Jager et al., 2005; Ryvolova et al., 2007 **Differential pulse polarography** – Chanlon et al., 2005
E 123 amaranth	16185	**Liquid chromatography** – Alves et al., 2008; Bonan et al., 2013; Chen et al., 1998; Culzoni et al., 2009; Feng et al., 2011; Fuh & Chia, 2002; Gennaro et al., 1997; Gianotti et al., 2005; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Ma et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Jager et al., 2005; Lee et al., 2008; Perez-Urquiza & Beltran, 2000; Ryvolova et al., 2007 **Voltammetry** – Ni et al., 1996; Ni et al., 1997; Ni & Bai, 1997 **Spectrophotometry** – Llamas et al., 2009; Nevado et al., 1995; Ni & Gong, 1997; Ni et al., 2009; Vidotti et al., 2005
E 124 ponceau 4R	16255	**Liquid chromatography** – Bonan et al., 2013; Chen et al., 1998; Feng et al., 2011; Fuh & Chia, 2002; Garcia-Falcon & Simal-Gandara, 2005; Gennaro et al., 1997; Gianotti et al., 2005; Hajimahmoodi et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Ma et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008; Zou et al., 2013 **Capillary electrophoresis** – Del Giovine & Bocca, 2003; Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008; Perez-Urquiza & Beltran, 2000; Ryvolova et al., 2007 **Voltammetry** – Ni et al., 1996; Ni et al., 1997 **Spectrophotometry** – Capitan-Vallvey et al., 1998; Nevado et al., 1995; Ni & Gong, 1997; Ni et al., 2009 **Differential pulse polarography** – Chanlon et al., 2005
E 127 erythrosine	45430	**Liquid chromatography** – Feng et al., 2011; Gonzalez et al., 2003a; Harp et al., 2013; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka &

Colour	CI number	Detection methods
		Ichihashi, 2008; Zou et al., 2013 **Capillary electrophoresis** – Kuo et al., 1998; Lee et al., 2008; Jager et al., 2005; Ryvolova et al., 2007
E 129 allura red AC	16035	**Liquid chromatography** – Alves et al., 2008; Bonan et al., 2013; Chen et al., 1998; Feng et al., 2011; Fuh & Chia, 2002; Hajimahmoodi et al., 2013; Harp et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008; Zou et al., 2013 **Capillary electrophoresis –** Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008; Perez-Urquiza & Beltran, 2000 **Voltammetry –** Silva et al., 2007 **Spectrophotometry –** Aktas & Ertokus, 2011 **Differential pulse polarography** – Chanlon et al., 2005
E 131 patent blue V	42051	**Liquid chromatography** – Bonan et al., 2013; Feng et al., 2011; Gianotti et al., 2005; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Dossi et al., 2007; Jager et al., 2005; Perez-Urquiza & Beltran, 2000
E 132 indigotine/indigo carmine	73015	**Liquid chromatography** – Bonan et al., 2013; Chen et al., 1998; Feng et al., 2011; ; Gonzalez et al., 2003a; Gonzalez et al., 2003b; Hajimahmoodi et al., 2013; Harp et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis –** Huang et al., 2002; Huang et al., 2003; Huang et al., 2005; Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008
E 133 brilliant blue FCF	42090	**Liquid chromatography** – Alves et al., 2008; Bonan et al., 2013; Chen et al., 1998; Feng et al., 2011; Gianotti et al., 2005; Gonzalez et al., 2003a; Gonzalez et al., 2003b; Harp et al., 2013; Hajimahmoodi et al., 2013; Khanavi et al., 2012; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Vidotti et al., 2006; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis –** Huang et al., 2002; Huang et al., 2003; Huang et al., Jager et al., 2005; Kuo et al., 1998; Lee et al., 2008 **Voltammetry –** Ghoreishi et al., 2011; Medeiros et al., 2012a; Medeiros et al., 2012b **Spectrophotometry –** Ni & Gong, 1997; Ni et al., 2009;

Colour	CI number	Detection methods
		Vidotti et al., 2005 **Photoacoustic spectroscopy** – Coelho et al., 2010
E 142 green S	44090	**Liquid chromatography** – Feng et al., 2011; ; Gonzalez et al., 2003a; Gonzalez et al., 2003b; Kirschbaum et al., 2003; Kirschbaum et al., 2006; Minioti et al., 2007; Yoshioka & Ichihashi, 2008 **Capillary electrophoresis** – Dossi et al., 2007
E 151 brilliant Black BN	28440	**Liquid chromatography** – Gonzalez et al., 2003a; Gonzalez et al., 2003b; Kirschbaum et al., 2003; Kirschbaum et al., 2006

Figure captions

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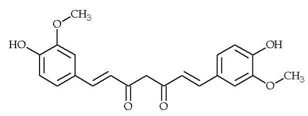
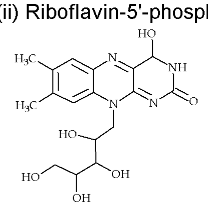
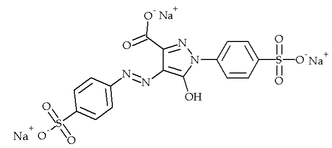
E100 Curcumin



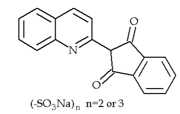
E101 (i) Riboflavin,
(ii) Riboflavin-5'-phosphate



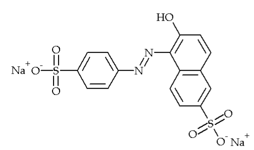
E102 Tartrazine



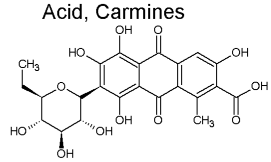
E104 Quinoline Yellow



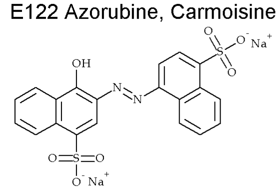
E110 Sunset Yellow FCF



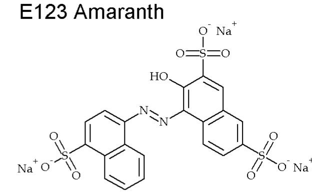
E120 Cochneal, Carminic Acid, Carmines



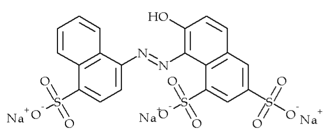
E122 Azorubine, Carmoisine



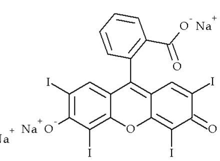
E123 Amaranth



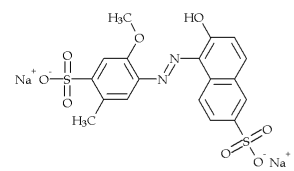
E124 Ponceau 4R, Cochineal Red A



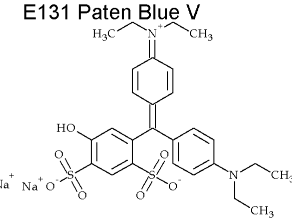
E127 Erythrosine



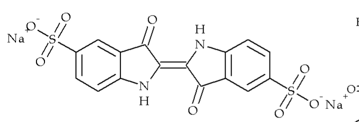
E129 Allura Red AC



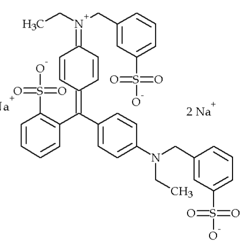
E131 Paten Blue V



E132 Indigotine, Indigo Carmine



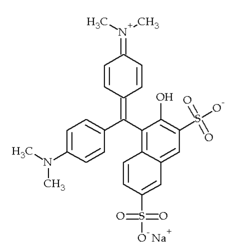
E133 Brilliant Blue FCF



E140 (i) Chlorophylls,
(ii) chlorophyllins

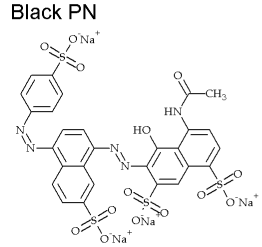
E141 (i) Copper complexes of chlorophylls,
(ii) copper complexes of chlorophyllins

E142 Green S



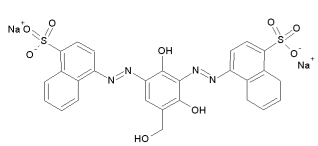
E150a Plain caramel
E150b Caustic sulphite caramel
E150c Ammonia caramel
E150d Sulphite ammonia caramel

E151 Brilliant Black BN, Black PN



E153 Vegetable carbon

E155 Brown HT



E160a (i) Mixed carotenes, (ii) beta-carotene
E160b Annatto, bixin, norbixin
E160c Paprika extract, capsanthin, capsorubin
E160d Lycopene
E160e Beta-apo-8'- carotenal
E161b Lutein
E161g Canthaxanthin
E162 Beetroot Red, betanin
E163 Anthocyanins

E170 Calcium carbonate
E171 Titanium dioxide
E172 Iron oxides and hydroxides
E173 Aluminium
E174 Silver
E175 Gold

E180 Litholrubine BK

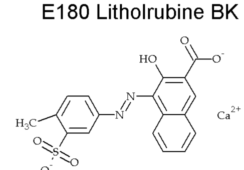


Figure 1. Food colour additives currently permitted in the EU.

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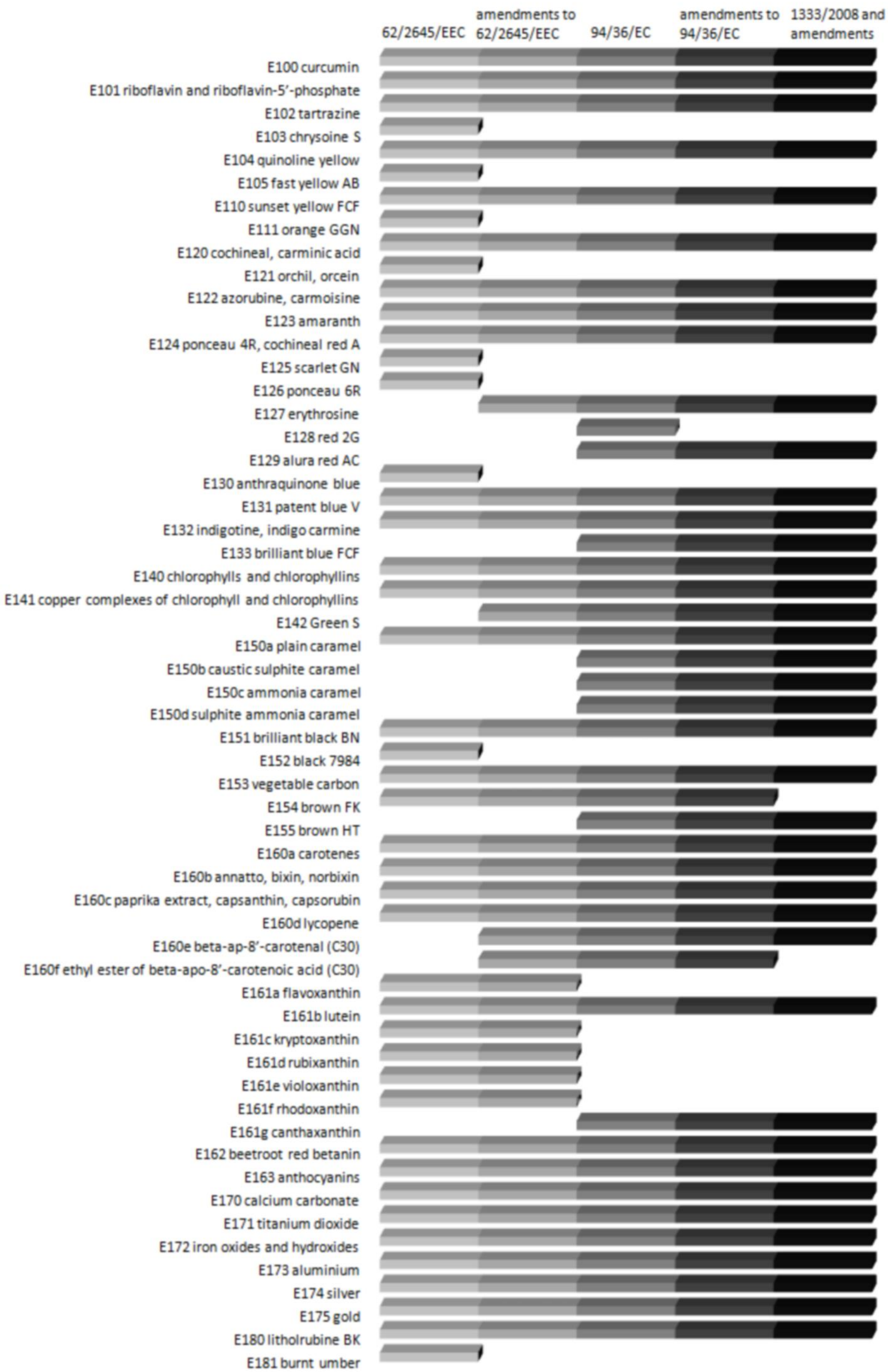
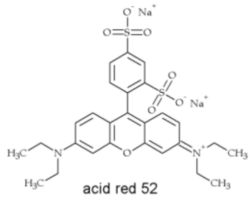
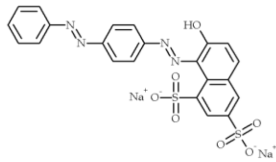
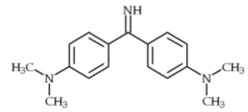
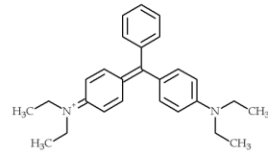

62/2645/EEC
amendments to 62/2645/EEC
94/36/EC
amendments to 94/36/EC
1333/2008 and amendments
E100 curcumin
E101 riboflavin and riboflavin-5'-phosphate
E102 tartrazine
E103 chrysoine S
E104 quinoline yellow
E105 fast yellow AB
E110 sunset yellow FCF
E111 orange GGN
E120 cochineal, carminic acid
E121 orchil, orcein
E122 azorubine, carmoisine
E123 amaranth
E124 ponceau 4R, cochineal red A
E125 scarlet GN
E126 ponceau 6R
E127 erythrosine
E128 red 2G
E129 alura red AC
E130 anthraquinone blue
E131 patent blue V
E132 indigotine, indigo carmine
E133 brilliant blue FCF
E140 chlorophylls and chlorophyllins
E141 copper complexes of chlorophyll and chlorophyllins
E142 Green S
E150a plain caramel
E150b caustic sulphite caramel
E150c ammonia caramel
E150d sulphite ammonia caramel
E151 brilliant black BN
E152 black 7984
E153 vegetable carbon
E154 brown FK
E155 brown HT
E160a carotenes
E160b annatto, bixin, norbixin
E160c paprika extract, capsanthin, capsorubin
E160d lycopene
E160e beta-ap-8'-carotenal (C30)
E160f ethyl ester of beta-apo-8'-carotenoic acid (C30)
E161a flavoxanthin
E161b lutein
E161c kryptoxanthin
E161d rubixanthin
E161e violoxanthin
E161f rhodoxanthin
E161g canthaxanthin
E162 beetroot red betanin
E163 anthocyanins
E170 calcium carbonate
E171 titanium dioxide
E172 iron oxides and hydroxides
E173 aluminium
E174 silver
E175 gold
E180 litholrubine BK
E181 burnt umber

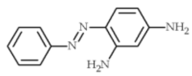
Figure 2. The history of the legal status of colour additives in the EU. The graph illustrates changes to the status of each colour additive after the introduction of the new regulation or amendment. For example E100 has always been legal, while E103 had been allowed by Council Directive 62/2645/EEC, then it was removed from the list by the amendments and it has been illegal since then.

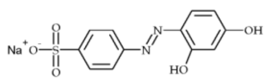

acid red 52

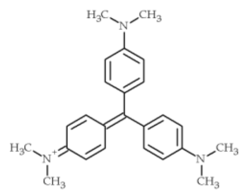

acid red 73

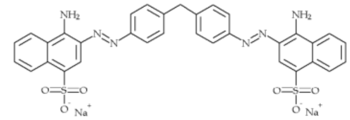

auramine

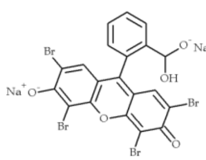

brilliant green

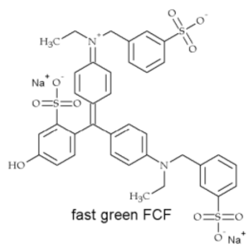

chrysoidine

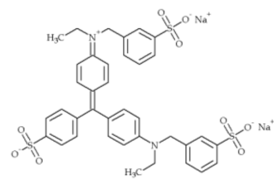

chrysoine

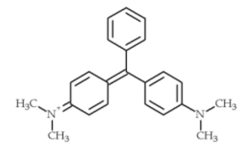

crystal violet

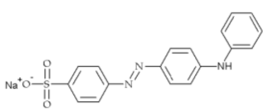

congo red

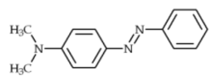

eosin Y

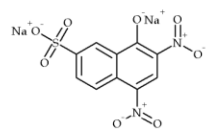

fast green FCF

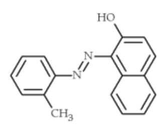

light green SF

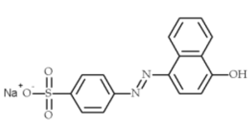

malachite green

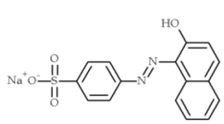

metanil yellow

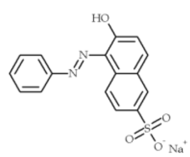

methyl yellow

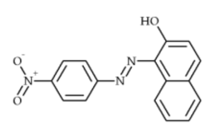

naphthol yellow S

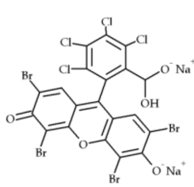

oil orange SS

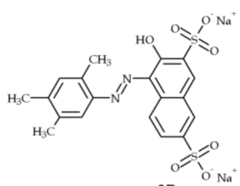

orange I

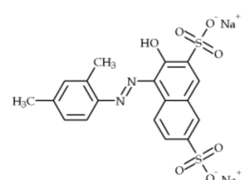

orange II

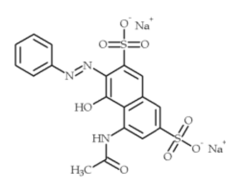

orange RN

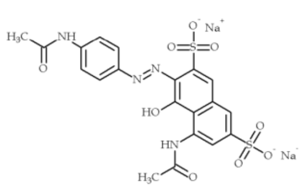

para red

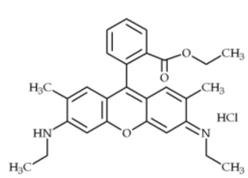

phloxine B

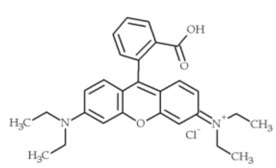

ponceau 3R

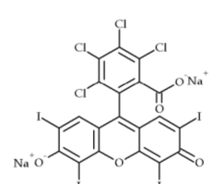

ponceau MX

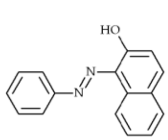

red 2G

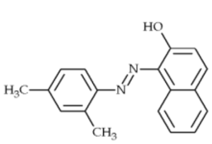

red 6B


rhodamine 6B


rhodamine B


rose bengal


sudan I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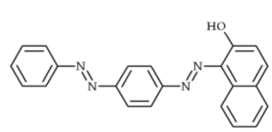
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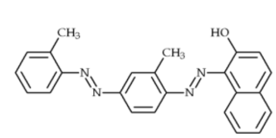
sudan IV

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Figure 3. Dyes not permitted for use in foods in the EU but possibly used/abused in the EU and other countries (EFSA, 2005; RASFF online database; Tripathi et al., 2007).