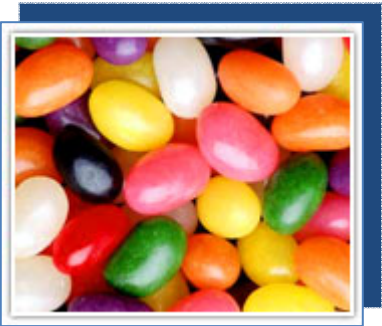


# Monitoring and **MoniQA**



## FOOD SAFETY ASPECTS OF FOOD COLOURS

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*Food colours are one of the important and essential additive class for food industry used to compensate the loss of natural colours of food destroyed during processing/storage, and to reduce batch-to-batch colour variations*



*Natural food colours* are isolated from plants, fungi and insects

These colours are:

- less stable
- less bright
- not uniform
- expensive

*The synthetic dyes* show several advantages over natural ones

These colours have:

- high stability to light, oxygen and pH
- colour uniformity
- low microbiological contamination
- low production cost

The synthetic dyes may cause a potential risk to human health.

If they are used illegally or if the amounts consumed exceed certain limits, they may be harmful to humans.

most countries

- ❑ regulate the synthetic colours that can be added to foodstuff
- ❑ establishes official tolerance levels for permitted colorants

# Permitted Food Colours in EU

E100 Curcumin

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

E102 Tartrazine

E104 Quinoline yellow

E110 Sunset Yellow FCF; Orange Yellow S

E120 Cochineal; Carminic acid; Carmines

E122 Azorubine; Carmoisine

E123 Amaranth

E124 Ponceau 4R; Cochineal Red A

E127 Erythrosine

E128 Red 2G (last year it was removed from the list)

E129 Allura Red AC

E131 Patent Blue V

E132 Indigotine; Indigo Carmine

E133 Brilliant Blue FCF

E141 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; Black PN

E153 Vegetable carbon

E154 Brown FK

E155 Brown HT

E160a Carotenes

E160b Annatto; Bixin; Norbixin

E160c Paprika extract; Capsanthin; Capsorubin

E160d Lycopene

E160e Beta-apo-8'-carotenal (C30)

E160e Ethyl ester of beta-apo-8'-carotenoic acid (C30)

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

\*Colours in pink are controversial

The Southampton Study indicated that artificial colours led to hyperactivity in children

No ban has yet been enforced, many manufacturers have taken voluntary measures to remove the additives from their product.

Many major companies, particularly those involved in producing products aimed at children, have been searching for natural alternatives to their synthetic colours



The study by McCann *et al.* (2007) has concluded that exposure to two mixtures of 4 synthetic colours plus a sodium benzoate preservative in the diet result in increased hyperactivity in 3-year old and 8- to 9-year old children

Two combinations of Tartrazine (E102), Quinoline Yellow (E104), Sunset Yellow FCF (E110), Ponceau 4R (E124), Allura Red AC (E129), Carmoisine (E122) and sodium benzoate (E211) on children's behaviour were studied.



EFSA asked to assess the results of study

The Panel concluded that study provides limited evidence

Two different mixtures of synthetic colours and sodium benzoate had a small and statistically significant effect on activity and attention in some children.

The effects were not observed for all children in all age groups

It is not possible to assess the overall prevalence of such sensitivity in the general population and reliable data on sensitivity to individual additives are not available



EFSA decided that the study gave no basis for changing acceptable daily intakes

There is the possibility for member states to initiate their own ban, the FSA had previously concluded there was insufficient evidence to support the need for individual action

But the FSA will recommend the UK push for voluntary removal of the additives through extensive reformulation while advising EFSA to implement a ban

In the European Parliament, for better labelling of additives containing azo-dyes, MPs managed to include in the compromise a new provision that foods containing some of those food colours (tartrazine, quinoline yellow, sunset yellow, carmoisine, ponceau 4R and allura red) must be labelled not only with the relevant E number but also with the words:

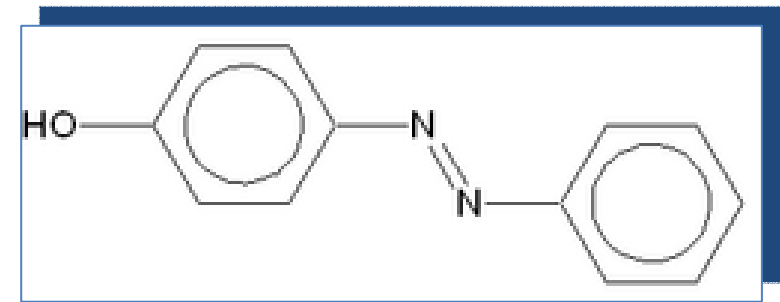
*"may have an adverse effect on activity and attention in children"*

The most common synthetic food colorants are azo dyes

Tartrazine, Sunset yellow, Carmoisine, Amaranth, Black PN, Ponceau 4R and Chocolate brown HT are permitted as azo food colours

Sudan (I-IV) dyes are not the food colours

Auremine and Rhodamine which are hazardous to human health and not permitted in food.



General structure of azo dyes

The azo dyes are used in solvents, oils, fats, waxes, plastics, printing inks, household commodities, textile and leather

They are genetically toxic for humans and determined as carcinogenic

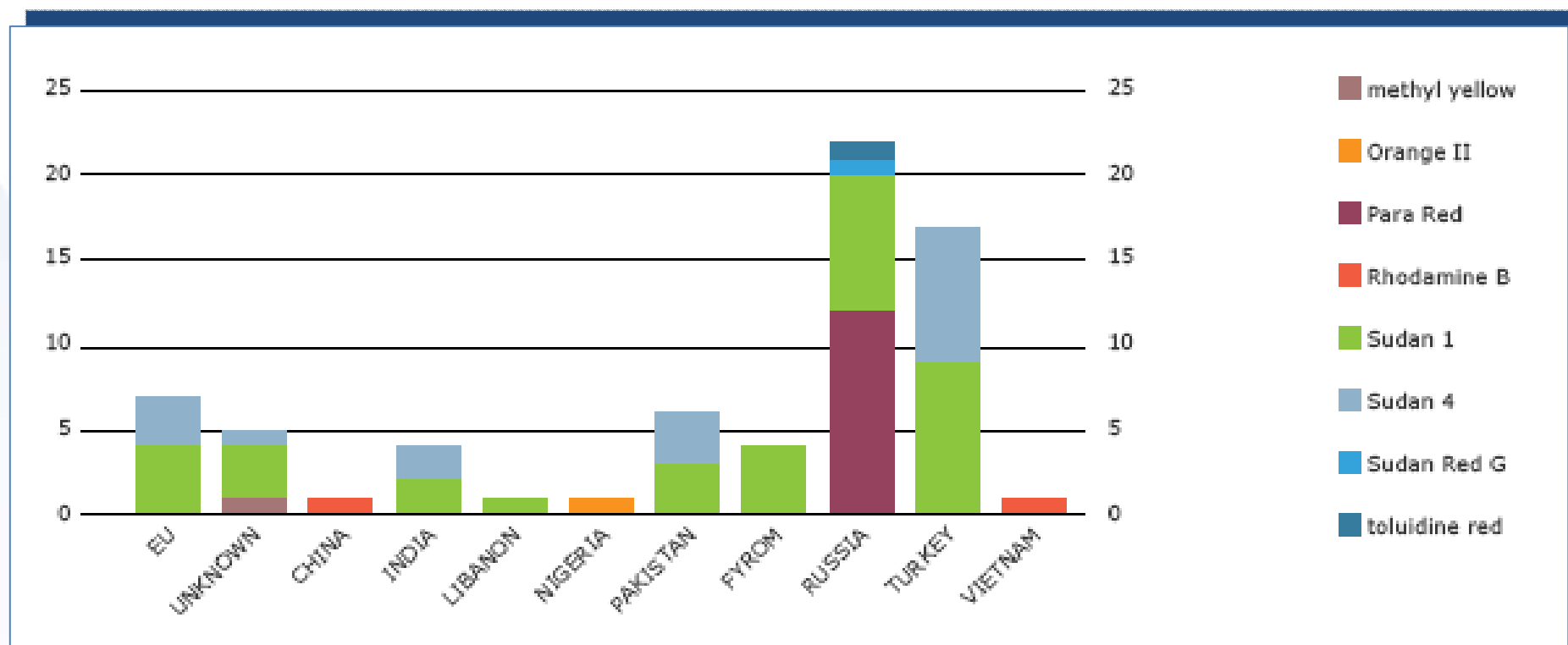


Although recognized as carcinogens, they recently have been found in food products in some European countries.

- ❑ They are added to foods such as chili powders to mimic, intensify, and prolong the appearance of natural red hues.
- ❑ More than six hundred products containing Sudan dyes have been recalled in the UK in 2005.
- ❑ Commission requires products to have documentation confirming the absence of Sudan dyes.
- ❑ Since 2003, European nations have required random product testing and testing of suspected adulterated products.
- ❑ Items found to contain Sudan dyes must be disposed of as hazardous waste

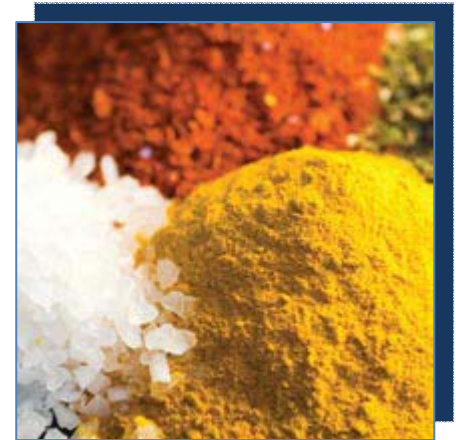


It was indicated that a sharp decrease in numbers of notifications about the illegal use of dyes in food has been observed since 2003 according to the data collected through the Rapid Alert System



Sudan I may be formed as an impurity during the production of sunset yellow.

Its presence in sunset yellow should therefore be restricted to an amount below the limit of detection, i.e. 0,5 mg/kg.



## Analytical Methods for Food Colours

Since the illegal use of dyes have an impact on public health, sensitive, selective and accurate analytical methods should be developed in order to identify and quantify synthetic food colorants in foods

Methods should be simple, rapid, and inexpensive

Chromatography (such as HPLC, HPLC-MS, HPIC), capillary electrophoresis, spectrometry and electrochemical methods are more frequently used

## Chromatographic techniques

They are still widely tested and improved

Raw samples cannot be introduced in the column prior to chemical treatment

The chromatographic methods involve solid–liquid extraction followed by HPLC separation with UV/Vis and/or MS detection.

HPLC with chemiluminescence detection and electrophoretic separation with UV detection have also been utilized.

## UV visible spectrometry

It is also a useful tool

The overlapping of the peaks leads to difficulties in analysis.

It is impossible to distinguish easily between synthetic and natural dyes.

## Capillary electrophoresis (CE)

It offers good performance and fast analysis times and minimal amounts of sample and solvent

Compared with HPLC, CE can usually be completed in less time than by HPLC

For example, 13 sweeteners, preservatives and food colours were separated by a CE based method within less than 15 minutes

Lower sample capacity and a shorter optical pathlength for on-capillary UV detection are some drawbacks of CE

## Large-volume sample stacking (LVSS) for CE

The technique has been used to concentrate and investigate charged analytes

The method is effective in detecting low concentrations of food colorants in some popular foods when on-capillary UV detection is used.

The detection limits for colorant standards were reduced up to 80-fold by LVSS when compared to conventional CE without sample stacking limits of detection (LODs) at 0.18–1.76 mg/ ml.

For the determination of Sudan dyes, beside the chromatographic and capillary electrophoresis methods, there are a few electrochemical methods in the literature.

The electrochemically activated glassy carbon electrode (AGCE) was utilized to determine Sudan I based on its reduction

Another electrochemical method for determination of Sudan dyes has been developed based on its oxidation and using montmorillonite calcium- modified carbon paste electrode

The limit of detection is  $0.02 \text{ mg L}^{-1}$

Food colours in several different food matrices were studied with the methods summarized

Sudan dyes were studied in red chilli pepper, chilli powder, chilli and curry containing foodstuffs, and egg.

Methods were reported for the water-soluble food colorants in soft and alcoholic drinks, solid juice and jelly powders, jams, sweets, ice-cream, syrups, candies, marshmallows, salted fish and salted vegetable, milk beverages.



Nordic Committee on Food Analysis (NMKL) Method is one of the validated and standard analytical methods used throughout Europe

(FSA) Method 145A and Method 145B are other two validated and standard methods for Sudan dyes.

# Works done by HCTU in MoniQA project

HCTU is the coordinator of the food colors in the additives working group. Based on this, HCTU prepared a database in excel format about the methods used for the detection of food colours.

	A	B	C	D	E
	Food Matrix	Analytical Method	% Recovery	Limit of Detection (LoD)	Is detection limit adequate for legislation limit?
1					
2	Chilli products	HPLC-UV			Yes
3	Chilli powder and products containing chilli powder.	HPLC using rapid scanning or diode-array UV/VIS detection			Yes
4		Liquid chromatography (LC)			Yes
5				0.04 mg/L (E-102)	
6				0.05 mg/L (E-110)	
7	solid juice powders, solid jelly powders and soft drinks.	HPLC-UV-Photodiode Array Detection (DAD)		0.10 mg/L (E-123)	Yes
8				0.01 mg/L (E-129)	
9				0.15 mg/L (E-133)	
10			Range: 89-98%	Range: 1.2-5.4 µg/kg	
11			96-98% (Sudan I)	1.2 µg/kg (Sudan I)	
12	Red Chilli Pepper	HPLC-UV-VIS	95-98% (Sudan II)	3.9 µg/kg (Sudan II)	Yes
13			97-98% (Sudan III)	1.2 µg/kg (Sudan III)	
14			89-95% (Sudan IV)	5.4 µg/kg (Sudan IV)	
15			93-96% (Para Red)	3.6 µg/kg (Para Red)	
16			Range: 79.8 and 95.7%	Range: 4.0-4.8 µg/kg	
17			80.8-95.7% (Sudan I)	4.6 µg/kg (Sudan I)	
18	various types of eggs	HPLC-UV-VIS	79.8-95.2% (Sudan II)	4.0 µg/kg (Sudan II)	Yes
19			81.0-95.7% (Sudan III)	4.8 µg/kg (Sudan III)	
20			81.6-94.2% (Sudan IV)	4.2 µg/kg (Sudan IV)	

The database considers the following headings:

- Food Matrix
- Analytical Method
- % Recovery
- Limit of Detection (LoD)
- Is detection limit adequate for legislation limit?
- Limit of Quantification (LoQ)
- Reproducibility (%)
- Repeatability (%)
- Is the method official?
- Status of validation
- Interlaboratory tests
- Number of samples analysed
- Reference
- Comments

Some standard methods and several non-official methods were examined in the database.

Most frequently used methods were

- The High-performance liquid chromatography (HPLC)
- High-performance liquid chromatography-mass spectrometry (HPLC-MS)
- Capillary electrophoresis (CE)
- Ultra performance liquid chromatography-tandem quadrupole mass spectrometry (UPLC-MS/MS)
- High-performance ion chromatography (HPIC)
- Pulse polarography
- Cathodic stripping voltammetry
- UV-VIS spectrometry
- Electrochemical determination

Thanks for your attention!

**MoniQA**

[www.moniqa.org](http://www.moniqa.org)



SIXTH FRAMEWORK PROGRAMME

