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Dietary exposure assessment of artificial food colours

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Abstract

Food colours are food additives that are added to foods after authorization for use by the European Commission and scientific safety assessment by the European Food Safety Authority. Each colour is added to foods to achieve a specific purpose, such as to compensate the loss of colours during food processing, to enhance natural colours, or to add colour to foods. They can be obtained via natural or synthetic pathway. Artificial (synthetic) colours are thought to increase hyperactivity in children. The current dietary exposure assessment of artificial food colours was performed using the Food Additives Chronic Exposure Assessment Tool (FAIM). The synthetic food colours used to assess the chronic exposure of children in Bulgaria are: Tartrazine, Quinoline Yellow, Sunset Yellow FCF, Azorubin, Ponceau 4R and Allura Red AC. The exposure was calculated for different food groups for which the relevant colours are authorized, according to Regulation (EC) No. 1333/2008 and the same was compared with the ADI for the respective colours. The obtained results of the exposure assessment of the considered artificial colours are below the corresponding ADI for mean and high consumers, considering that the assessment considered only those for which there is an established legislative level of use in the European legislation. However, the results cannot guarantee the actual exposure to colours of children in Bulgaria, due to the uncertainties described in the assessment.

Key words: *food colours, artificial colours, exposure assessment*

I. Introduction

Food colours are food additives that are added to foods to achieve a specific purpose, mainly to compensate the colour loss during food processing, to boost natural colours or to add colour to foods that would be colourless or coloured differently. Each food colour authorized for use in the European Union (EU) is subject to scientific safety assessment by the European Food Safety Authority (EFSA) and approval by the European Commission (EC). The Standing Working Group of the Scientific Panel on Food Additives and Nutrient Sources Added to Food (ANS) at EFSA establishes an Acceptable Daily Intake (ADI) for each azo colour which is the amount of colour that can be safely consumed, daily, during whole life of consumer. Food colours can be obtained from natural or artificial sources, which defines them as natural colours derived from plants or animals and artificial, obtained artificially. Natural colours are more and more used in food, but nevertheless azo colour (artificial colours) are widely used because they are stable in heat, light and across the whole range of food pH. They are water soluble, but not in oil or fat. Because azo colours are highly soluble in water, they do not accumulate in the body and are mainly metabolised in the liver and excreted with urine. Exposure to azo dye is thought to increase hyperactivity in children or increase ADHD (Attention Deficit Hyperactivity Disorder). The EFSA Expert Group on Food Additives and Nutrient Sources, based on scientific evidence, does not establish a causal correlation between colours and possible behavioural effects in children, but when using some of the azo colours [Sunset yellow (E110), Quinoline yellow (E104), Carmoazin (E122), Alura red (E129), Tartrazine (E102) and Ponceau 4R] have to be labelled accompanied by the text „May have an adverse effects on activity and attention in children“ (Gil, 2014).

II. Materials and methods

The purpose of this assessment is to identify, assess and characterize the risk to children's health from food colours and specifically artificial colours (azo colours), which are considered leading to adverse effects on children's health as a result of their consumption. The risk assessment was carried out using a tool to assess chronic exposure to food additives (FAIM) (EFSA, Food additive intake model (FAIM), 2012). The tool uses food consumption data from the European food consumption database (EFSA, Comprehensive European Food Consumption Database, 2022) and data on the maximum permitted levels of use in the relevant food groups according to Regulation (EC) № 1333/2008. Potential exposures for mean and high consumers, respectively for two age groups of children have been calculated for each food group for which there is an authorised level of use in food and for which there is a reported consumption (toddlers aged 12-35 months and other children aged 36 months - 9 years). All exposure measurements are expressed in mg/kg body weight/day. In order to characterize the risk, the estimated exposures to colours of children in Bulgaria was compared with the acceptable daily intake, established by EFSA for the relevant colours involved in the assessment (EFSA, Food additive intake model (FAIM): comments received from stakeholders and EFSA views., 2014).

III. Results

1. Hazard identification and characterization

Table 1 *Artificial colours in food (azo colours)*

Colours	CAS number	E number	Molecular formula
Allura Red AC	25956-17-6	E 129	$C_{18}H_{14}N_2Na_2O_8S_2$
Azorubine, carmoisine	3567-69-9	E 122	$C_{20}H_{12}N_2Na_2O_7S_2$
Ponceau 4R	2611-82-7	E 124	$C_{20}H_{11}N_2Na_3O_{10}S_3$
Quinoline yellow	8004-92-0	E 104	$C_{18}H_9NNa_2O_8S_2$
Sunset Yellow FCF	2783-94-0	E 110	$C_{16}H_{10}N_2Na_2O_7S_2$
Tartrazine	1934-21-0	E 102	$C_{16}H_9N_4Na_3O_9S_2$

Synthetic (artificial) food colours are food additives and are an important ingredient in the food industry. The natural colour of food tends to decrease during storage and processing, making synthetic food colours a technological necessity. They are mainly azo colours and are used in confectionery products, beverages, meat products and more (Aroba J., 2022).

Azo dyes are organic compounds that can be used to give colour to food. They are classified according to colour, origin, chemical structure and type of material to which they are applied. The most accurate and scientific classification of food colours is based on their chemical structure. Azo colours contain azo group -N = N-, but some of them contain two (diazo), three (triazo) or more. Aromatic azo compounds (R = R '= aromatic) are usually stable and have bright colours, such as red, orange and yellow. That fact can be explained by the presence of side groups around the azo bond that help stabilize the N = N group making it part of a decentralized system that often absorb the visible frequencies of light. Some azo colours are prohibited for adding them in food because of toxic side effects. They are prohibited not due to the colours itself, but to the degradation products of the azo colours, such as aniline, toluene, benzidine, naphthalene. The azo bond can be reduced by enzyme called azo reductase. It is non-specific enzyme found in various microorganisms (such as intestinal bacteria) and present in various organs, such as liver, kidney, lung, etc.

Food additives have also been associated with adverse effects such as asthma and allergic reactions. There are clinical trials performed to evaluate the effects and to report adverse effects after intake of Tartrazine. The exact mechanism by why Tartrazine increased allergic reactions or asthma is still not fully understood. It has also been suggested that exposure to azo colours is associated with an increased risk of hyperactive behaviour in children or exacerbate ADHD (attention deficit hyperactivity disorder). The Southampton study (McCann D., 2007) tested the effects of azo colours in mixture of sodium benzoate by 3-year-old and 8/9-year-old children. It is reported that a combination of additives commonly found in infant formula increasing levels of hyperactivity in children on this age (Gil, 2014).

Miller and colleagues make a scientific review based on a meta-analysis and a detailed review of the scientific literature of a potential impact of artificial colours on activity and attention in children. The team of scientists concludes that the current evidence from the studies supports the interaction between exposure to synthetic dyes and adverse behavioural effects in children, both those with behavioural disorders and those without. There appears to be considerable variability in each individual's sensitivity to synthetic food colours (Miller M., 2022). The ANS Panel concludes that the currently available scientific evidence does not demonstrate a causal interaction between individual colours and the possible behavioural effects. According to the Southampton study, the European Parliament and the Council of the European Union decided that foodstuffs containing any of the 6 azo colours listed should be accompanied by the following text „May have adverse effect on activity and attention in children" (Gil, 2014).

Allura Red AC (E 129) is azo colour authorized as a food additive in the EU. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1980 and the Scientific Committee on Food (SCF) in 1984 and 1989 established an Acceptable Daily Intake (ADI) of 0-7 mg/kg body weight/day. Based on *in vivo* and *in vitro* studies SCF, JECFA assessments concluded, that Alura Red AC does not exhibit any genotoxic activity. The EFSA ANS Panel noted that Alura Red AC had no adverse effect in vitro genotoxicity studies as well as in long-term carcinogenicity studies. As regards the Southampton study, EFSA concluded that the study provides limited evidence that the two different mixtures of the tested synthetic colours and sodium benzoate have a small but significant effect on activity and attention in selected children from the general population, except children taking medication for Attention deficit hyperactivity disorder (ADHD). Since the study tested mixtures rather than individual additives, it is not possible to say which of the individual substances is responsible for the observed adverse effects. Overall, the ANS Panel concludes that the data on genotoxicity, semi-chronic, reproductive, developmental and long-term toxicity, and carcinogenicity do not justify a review of the ADI of 7 mg/kg bw/day. The Panel also notes for some sensitivity reactions following the intake of Allura Red AC, such as urticaria, rhinitis and asthma, especially when Alura Red AC is taken in combination with other synthetic dyes, and that sensitive individuals may react at doses within ADI (EFSA 2. , Scientific Opinion on the reevaluation of Allura Red AC (E 129) as a food additive on request from the European Commission, 2009).

Azorubine, carmoisine (E 122) is an azo dye authorised as a food additive in the EU. It was evaluated by JECFA and SCF in 1983 and 1984 with an established ADI of 0-4 mg/kg bw/day. The EFSA ANS Panel notes that Azorubine/Carmoizine (E 122) had no adverse effect in in vitro genotoxicity studies as well as in long-term carcinogenicity studies and concludes that the current database does not provide a basis for revising the ADI of 0-4 mg/kg bw/day. Hypersensitivity reactions have been reported after the application of the dye, but mainly when mixed with other dyes, but no conclusions can be drawn due to insufficient scientific evidence. The Panel also notes that sensitive individuals may react to doses within the ADI range (EFSA 2. , Scientific Opinion on the re-evaluation of Azorubine/Carmoisine (E 122) as a food additive on request the European Commission., 2009).

Ponceau 4R (E 124) was evaluated by JECFA and SCF in 1983 and 1984 with an established ADI of 0-4 mg/kg bw/day. The EFSA ANS Panel noted that Ponceau 4R (E 122) had no adverse effect in in vitro genotoxicity studies as well as in long-term carcinogenicity studies. The Panel also reviewed a long-term study with mice reporting glomerulonephrosis, from which study they derived a no observed adverse effect level (NOAEL) of 70 mg/kg body weight/day, from which they calculated an ADI of 0,7 mg/kg bw/day (EFSA 2. , Scientific Opinion on the reevaluation of Ponceau 4R (E 124) as a food additive on request from the European Commission, 2009).

Quinoline yellow (E 104) was evaluated by JECFA and SCF in 1975, 1978 and 1984 with an established ADI of 0-10 mg/kg bw. The studies that aren't evaluated by JECFA and SCF were chronic toxicity and carcinogenicity studies with a reproductive toxicity phase in rats. The EFSA ANS Panel noted that Quinoline yellow had no adverse effect in in vitro genotoxicity studies as well as in long-term carcinogenicity studies. It concludes that the currently available database of semi-chronic, reproductive developmental and long-term toxicity of Quinoline yellow, including a study in rats, which was not taken into account in the JECFA or SCF, provides a justification for the re-determination of the ADI. Extrapolating a NOAEL of 50 mg/kg bw/day from the reproductive phase chronic toxicity and carcinogenicity study in rats and applying factor of uncertainty 100 to this NOAEL, the Panel established an ADI of 0.5 mg/kg bw /day.

Sunset Yellow FCF (E 110) was evaluated by the ANS Panel to EFSA with an established ADI of 1 mg/kg bw/day in 2009. Following a request from the European Commission, the ANS Panel was asked to evaluate the newly submitted data from a study carried out as a result of the recommendations contained in the 2009 opinion and to make a precise exposure assessment of Sunset Yellow FCF. The new information to be assessed includes an assessment of the 28-day study report, the data reviewed by JECFA in its last evaluation in 2011, as well as any additional toxicological information that became available after the completion of the previous evaluation by the ANS Panel. It is considered that the newly submitted data from the 28-day study and the commonly available toxicological database for Sunset Yellow FCF provide a basis for revising the established provisional ADI and the ANS Panel concluded that a new ADI for Sunset Yellow FCF of 4 mg/kg bw/day can be established, based on a NOAEL of 375 mg/kg bw/day from the long-term rat feeding study and factor of uncertainty 100 in line with the latest JECFA assessment. Estimated exposure for Sunset Yellow FCF, based on both current authorized legislative use levels and reported use levels, are well below the new ADI level of 4 mg/kg bw/day for all population groups (EFSA, Scientific opinion on the reconsideration of the temporary ADI and refined exposure assessment for Sunset Yellow FCF (E 110), 2014).

Tartrazine (E 102) was evaluated by JECFA and SCF in 1975 and 1984 with an established ADI of 0-7.5 mg/kg bw/day. The EFSA ANS Panel notes that Tartrazine (E 102) had no adverse effect in genotoxicity studies as well as in long-term carcinogenicity studies in mice and no effect on nuclear DNA migration observed in mice and concludes that the current database does not provide a basis for revising ADI of 0-7,5 mg/kg bw/day. The Panel noted that Tartrazine may cause reactions of intolerance in a small number of individuals and that sensitive individuals may react to Tartrazine at doses within the ADI (EFSA, Scientific Opinion on the reevaluation Tartrazine (E 102) on request from the European Commission, 2009).

2. Assessment

2.1. Level of use of food colours

This exposure assessment uses data on levels of use of food colours according to Regulation (EC) № 1333/2008 (maximum permitted levels of use defined in current EU legislation).

In table 2 below in yellow are shown the food groups for which maximum levels of use of food colours have been established but no consumption data have been reported for the relevant child groups.

Table 2 Level of use of food colours

Food categories	Food groups	Maximum level (mg/l or mg/kg, as appropriate)					
		Tartrazine E 102	Quinoline Yellow E 104	Sunset Yellow FCF E 110	Azorubine, Carmoisine E 122	Ponceau 4R E 124	Allura Red AC E 129
1. Dairy products and analogues	1.1. Flavoured fermented milk products including heat-treated products		10	5		5	
	1.2. Cream and cream powder		10	5		5	
	1.3. Processed cheese	100			100		
2. Fruit and vegetables	2.1. Processed fruit and vegetables	100	30	35	200	20	200
3. Confectionery	3.1. Other confectionery including breath refreshing micro sweets		30	35		20	
	3.2. Chewing gum		30	10		10	
4. Meat	4.1. Non-heat-treated meat products			15		50	
	4.2. Heat-treated meat products						25
5. Fish and fisheries products	5.1. Processed fish and fishery products including molluscs and crustaceans	100		200	100	200	250
	5.2. Fish roe		200	200		200	
6. Salts, spices, soups, sauces, salads and protein products	6.1. Seasonings and condiments		10				
	6.2. Mustard		10	50		35	
	6.3. Sauces		20	30			
	6.4. Protein products, excluding products covered in category 1.8		10	20		10	
7. Foods intended for particular nutritional uses as defined by Directive 2009/39/EC	7.1. Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)		10	10		10	
	7.2. Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)		10	10		10	
8. Beverages	8.1. Flavoured drinks		10	20		10	
	8.2. Alcoholic beverages, including alcohol-free and low-alcohol counterparts	100	50	50	100	50	100
9. Food supplements as defined in Directive 2002/46/EC	9.1. Food supplements excluding food supplements for infants and young children		35	10		35	

2.2. Food consumption

The FAIM tool in this assessment uses food consumption data from the EFSA European Food Consumption Database. Based on these consumption data, the estimated exposure for the relevant persons for whom consumption was reported in the Bulgarian study is calculated. As this assessment covers the chronic exposure of children in Bulgaria to food colours, the consumption data used by the tool are for children in Bulgaria who reported chronic consumption of foods for which there are established maximum levels of use of the azo dyes involved in the assessment. The Bulgarian population nutrition survey was performed in 2007. The used consumption data are the average consumption value for the whole population (medium consumers) and the high consumption value of the 95th percentile (high consumers) only for consumers of two age groups children (small children aged 12-35 months and other children aged 36 months-9 years). Food consumption is expressed in grams per kilogram body weight per day (g/kg bw/day).

Table 2 *Food consumption for children in Bulgaria aged 12 months to 9 years*

Food categories	Food groups	Toddlers 12 - 35 months		Other children 36 months - 9 years	
		Mean (Total population)	High level (consumers only)	Mean (Total population)	High level (consumers only)
		g/kg bw/day		g/kg bw/day	
1. Dairy products and analogues	1.1. Flavoured fermented milk products including heat-treated products	12.6	38.5	6.3	21.5
	1.2. Cream and cream powder	0.1	1.2	0.0	0.9
	1.3. Processed cheese	0.0	0.9	0.0	0.8
2. Fruit and vegetables	2.1. Processed fruit and vegetables	1.9	9.4	1.7	8.2
3. Confectionery	3.1. Other confectionery including breath refreshing micro sweets	0.1	1.2	0.1	0.8
	3.2. Chewing gum				
4. Meat	4.1. Non-heat-treated meat products	4.9	11.0	4.6	10.2
	4.2. Heat-treated meat products	0.8	4.2	1.2	5.2
5. Fish and fisheries products	5.1. Processed fish and fishery products including molluscs and crustaceans				
	5.2. Fish roe				
6. Salts, spices, soups, sauces, salads and protein products	6.1. Herbs, spices, seasonings	0.2	0.4	0.2	0.3
	6.2. Mustard				
	6.3. Sauces	0.0	0.4	0.0	0.5
	6.4. Protein products, excluding products covered in category 1.8				
7. Foods intended for particular nutritional uses as defined by Directive 2009/39/EC	7.1. Dietary foods for special medical purposes defined in Directive 1999/21/EC (excluding products from food category 13.1.5)				
	7.2. Dietary foods for weight control diets intended to replace total daily food intake or an individual meal (the whole or part of the total daily diet)				
8. Beverages	8.1. Flavoured drinks	2.2	20.5	4.6	23.3
	8.2. Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.2	0.0	0.3
9. Food supplements as defined in Directive 2002/46/EC	9.1. Food supplements excluding food supplements for infants and young children				

The food groups, for which maximum levels of use for food colours have been established but no consumption data for the relevant child groups have been reported, are shown in yellow. Therefore, no exposure assessment has been performed for these food groups.

2.3. Exposure assessment

The exposure assessment was calculated using the FAIM tool. Intake statistics were calculated based on individual consumption data. Surveys with only one day per subject were excluded. Surveys with less than 60 subjects were considered as non-reliable for estimating high levels of exposure. The mean consumption is calculated based on the whole population, whereas the high level consumption is calculated based on consumers only. When the number of consumers per age class for a food category was above 60, the highest calculated level was the 95th percentile. When the number of consumers per age class for a food category was below 60, the high level was estimated as the mean consumption of consumers only. This is due to the minimum number of observations necessary to estimate the 95th percentile. The calculations were performed using individual body weights of the consumers. All the exposure assessment is expressed in mg/kg bw/day. The total exposure for mean consumers is calculated by sum of all mean exposure values for each food group. If the same approach is applied to estimate the total exposure for high consumers, this will lead to an overestimation of exposure, as people are unlikely to be high level consumers of more than one food category when a limited number of food categories are used in the assessment. Therefore, the tool applies a method for calculating exposure to high level consumers, adding the highest level of exposure from one food category (calculated for consumers only) to the mean exposure values for the other categories (calculated for the total population). Thus, it is assumed that a person can be a high level consumer of only one food category and mean consumer of all other food groups involved in the assessment (EFSA, Food additive intake model (FAIM), 2012), (EFSA, Food additive intake model (FAIM): comments received from stakeholders and EFSA views., 2014). The potential exposures for mean and high level consumer children in two age groups (toddlers aged 12-35 months and other children aged 36 months - 9 years) are calculated for all food groups for which there is an authorised level of use with the theoretical maximum food colour. The exposure assessment was carried out for those food groups for which there is a legally permitted maximum level of use and reported consumption in the consumption survey for children in Bulgaria.

Table 3 *Exposure assessment from food colours*

Food categories	Food groups	Toddlers 12 - 35 months			Other children 36 months - 9 years		
		Mean mg/kg bw/day	High level mg/kg bw/day	Contribution to the total exposure %	Mean mg/kg bw/day	High level mg/kg bw/day	Contribution to the total exposure %
Tartrazine E 102							
Dairy products and analogues	Processed cheese	0.0	0.1	1.2%	0.0	0.1	1.3%
Fruit and vegetables	Processed fruit and vegetables	0.2	0.9	98.5%	0.2	0.8	98.3%
Beverages	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.3%	0.0	0.0	0.4%
Total estimated exposure		0.2	0.9		0.2	0.8	
Quinoline Yellow E 104							
Dairy products and analogues	Flavoured fermented milk products including heat-treated products	0.0	0.1	5.7%	0.0	0.0	1.2%
	Cream and cream powder	0.0	0.0	0.7%	0.0	0.0	0.3%
Fruit and vegetables	Processed fruit and vegetables	0.1	0.3	63.3%	0.1	0.2	49.0%
Confectionery	Other confectionery including breath	0.0	0.0	3.1%	0.0	0.0	3.1%

	refreshing micro sweets						
Salts, spices, soups, sauces, salads and protein products	Seasonings and condiments	0.0	0.0	1.9%	0.0	0.0	1.5%
	Sauces	0.0	0.0	0.2%	0.0	0.0	0.4%
Beverages	Flavoured drinks	0.0	0.2	24.8%	0.0	0.2	44.1%
	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.4%	0.0	0.0	0.3%
Total estimated exposure		0.1	0.3		0.1	0.3	
Sunset Yellow FCF E 110							
Dairy products and analogues	Flavoured fermented milk products including heat-treated products	0.0	0.0	1.3%	0.0	0.0	0.3%
	Cream and cream powder	0.0	0.0	0.2%	0.0	0.0	0.1%
Fruit and vegetables	Processed fruit and vegetables	0.1	0.3	34.5%	0.1	0.3	26.5%
Confectionery	Other confectionery including breath refreshing micro sweets	0.0	0.0	1.7%	0.0	0.0	1.7%
Meat	Non-heat-treated meat products	0.1	0.2	39.0%	0.1	0.2	30.2%
Salts, spices, soups, sauces, salads and protein products	Sauces	0.0	0.0	0.1%	0.0	0.0	0.3%
Beverages	Flavoured drinks	0.0	0.4	23.2%	0.1	0.5	41.0%
	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.0%	0.0	0.0	0.0%
Total estimated exposure		0.2	0.6		0.2	0.6	
Azorubine, Carmoisine E 122							
Dairy products and analogues	Processed cheese	0.0	0.1	0.6%	0.0	0.1	0.7%
Fruit and vegetables	Processed fruit and vegetables	0.4	1.9	99.3%	0.3	1.6	99.1%
Beverages	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.2%	0.0	0.0	0.2%
Total estimated exposure		0.4	1.9		0.3	1.6	
Ponceau 4R E 124							
Dairy products and analogues	Flavoured fermented milk products including heat-treated products	0.0	0.0	0.8%	0.0	0.0	0.2%
	Cream and cream powder	0.0	0.0	0.1%	0.0	0.0	0.1%
Fruit and vegetables	Processed fruit and vegetables	0.0	0.2	12.0%	0.0	0.2	11.0%
Confectionery	Other confectionery including breath	0.0	0.0	0.6%	0.0	0.0	0.7%

	refreshing micro sweets						
Meat	Non-heat-treated meat products	0.2	0.6	79.3%	0.2	0.5	73.0%
Beverages	Flavoured drinks	0.0	0.2	7.1%	0.0	0.2	14.9%
	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.1%	0.0	0.0	0.1%
Total estimated exposure		0.3	0.6		0.3	0.6	
Allura Red AC E 129							
Fruit and vegetables	Processed fruit and vegetables	0.4	1.9	95.0%	0.3	1.6	92.0%
Meat	Heat-treated meat products	0.0	0.1	4.9%	0.0	0.1	7.8%
Beverages	Alcoholic beverages, including alcohol-free and low-alcohol counterparts	0.0	0.0	0.2%	0.0	0.0	0.2%
Total estimated exposure		0.4	1.9		0.4	1.7	

Exposure to Tartrazine has been calculated for three food groups and is in the range of 0.2 – 0.9 mg/kg bw/day. The highest contribution to Tartrazine exposure was in the food group „Processed fruits and vegetables", respectively 98.5% for toddlers and 98.3% for other children, and the lowest - for beverages. For Quinoline Yellow, the exposure was in the range of 0.1 – 0.3 mg/kg bw/day for both groups of children. The highest contribution to Quinoline Yellow exposure was in the food group „Processed fruits and vegetables", respectively 63.3% for toddlers and 49.0% for other children, and the lowest for the sauces. Exposure to Sunset Yellow FCF was in the range of 0.2 – 0.6 mg/kg bw/day for both groups of children. The highest contribution to Sunset Yellow FCF exposure was in food group „Non-heat-treated meat products", 39.0%, and for other children in flavoured drinks was 41.0%. Exposure to Azorubin was in the range of 0.3 – 1.9 mg/kg bw/day. The highest contribution to Azorubin exposure was in food group „Processed fruits and vegetables", respectively 99.3% for toddlers and 99.1% for other children, and the lowest - the beverages. Exposure to Ponceau 4R was in the range of 0.3 – 0.6 mg/kg bw/day for both groups of children. The highest contribution to Ponceau 4R exposure was in the food group „Non-heat-treated meat products", respectively 79.3% for toddlers and 73.0% for other children, and the lowest - the cream and cream powder. Exposure to Alura Red AC was in the range of 0.4 – 1.9 mg/kg bw/day. The highest contribution to Alura Red AC exposure was in food group „Processed fruits and vegetables", respectively 95.0% for toddlers and 92.0% for other children, and the lowest - the beverages.

Table 4 shows that overall the largest contribution to colour exposure was observed in the food group "Processed fruits and vegetables". The contribution of food to the overall exposure is either due to the higher amount of food consumed by consumers or to the high content of a food colouring agent. In general, confectionery is the food in which food colouring is often used. However, some of these foods are not included in the present assessment due to a lack of consumption data such as chewing gum, decorations, glazes and therefore no exposure calculations.

Figures 1 and 2 show the total exposures of the relevant colour, theoretically incorporated in all food groups for which it is authorised for use at the maximum level and consumed with the relevant food for the two age groups involved in the assessment.

Figure 1 *Chronic exposure assessment from food colours for toddlers*

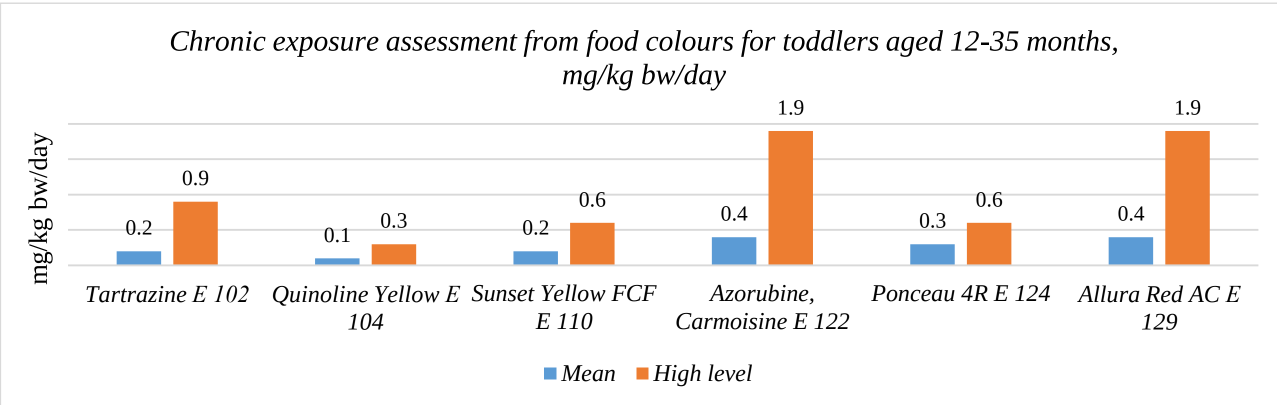
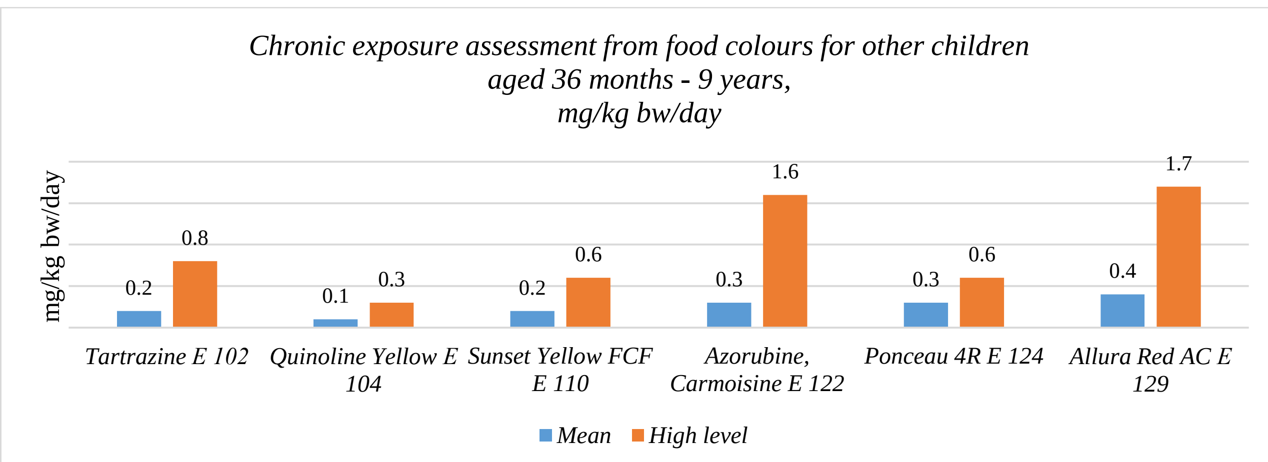


Figure 2 *Chronic exposure assessment from food colours for other children*



Exposure to Azorubin and Alura Red AC was highest for both age groups in children, due to higher authorised levels in food use for these colours. The lowest level of use is Quinoline Yellow.

2.4. Risk characterization

In this step, the likelihood of adverse toxic effects in humans shall be assessed as a result of dietary exposure to the additive. The risk characterisation is usually done by comparing the ADI values of the additive with the exposure levels calculated in the assessment. The risk shall be characterised using the scenario used in the assessment, where the content of the colour in the authorised foodstuffs is within the maximum levels laid down by European legislation and all consumed foodstuffs in which the addition of a colour is permitted are assumed to contain a colour. The calculated percentage of ADI expresses the hazard index. If the result is less than 100%, there is no risk of adverse effects.

__Figure 3__ Characterization of the risk of exposure to colours for toddlers

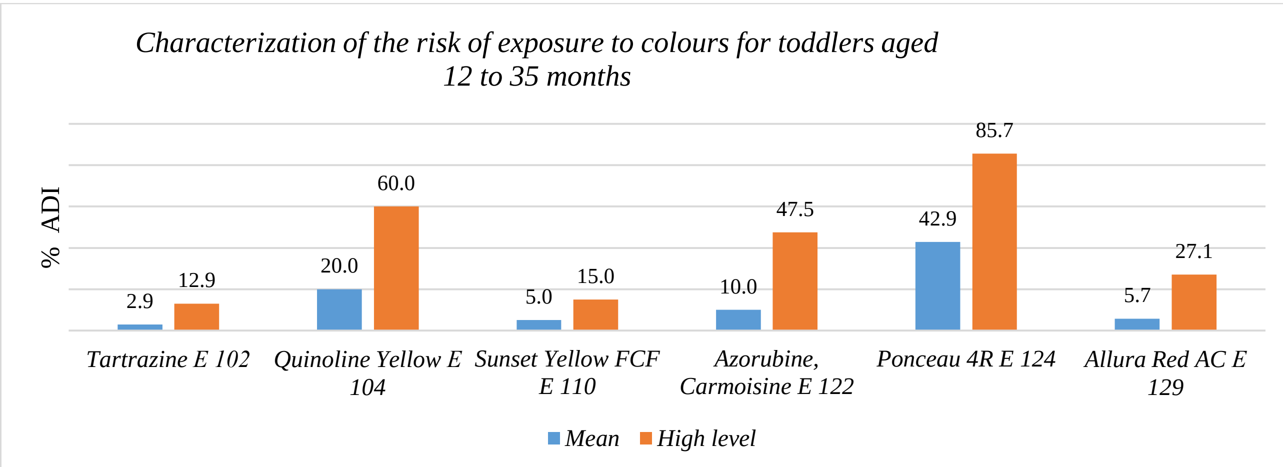
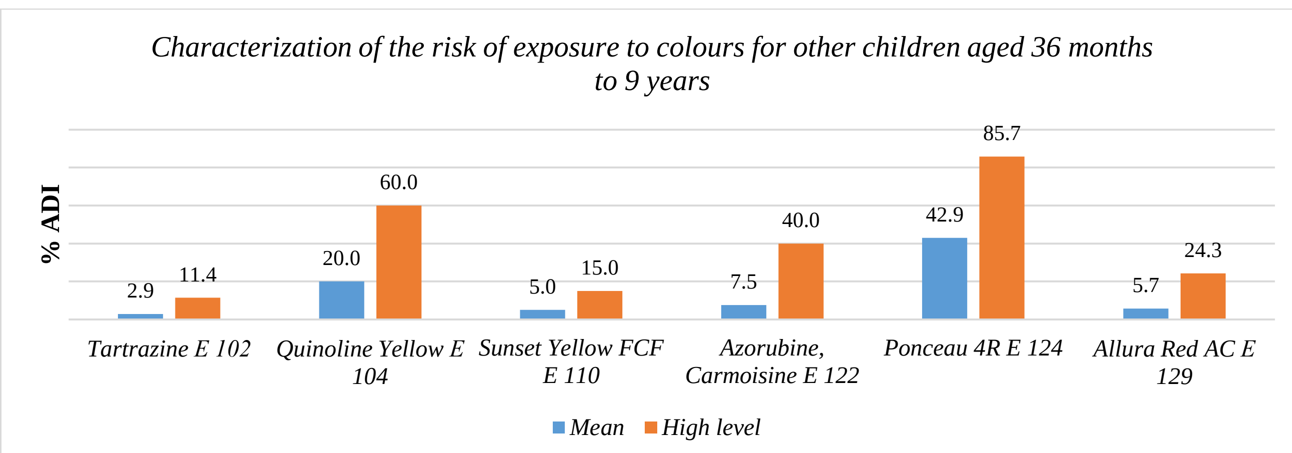


__Figure 4__ Characterization of the risk of exposure to colours for other children



The risk has been assessed for a scenario where a child of the appropriate age group consumes a certain amount of food from all food groups for which there is an authorised level of use with the maximum authorised amount of food colouring. In the absence of sufficient studies on mixtures of colours and the derivation of a reference point for comparison and risk monitoring, it is not possible to compare the results of the total exposure of a mixture of colours with the ADI of a single colour.

The highest percentage of ADI is observed for the colours Ponso 4R and Quinoline Yellow, for which the maximum level of intake, compared to the other colours, is much lower. The percentage of the ADI for Ponso 4R is 85.7% and for Quinoline Yellow is 60%. The lowest percentage of the ADI was for Tartrazine, 2.9%.

IV. Uncertainties in the assessment

Table 4 *Uncertainties in the assessment of exposure to colours*

Consumption data
The consumption data is from a survey done in Bulgaria in 2007, which could be an uncertainty in the quantity of food consumed today.
Food consumption survey are short-term.
Consumption data are often available only for the largest food groups, not for specific foods.
Data on the content of food colour
Use maximum permitted levels of use as set in the current EU legislation as no analytical results of food colours are available
Exposure assessment
No reporting of food categories for which there is no consumption data
Linking availability data or maximum levels to food categories in the EFSA food consumption database

V. Conclusion

The results obtained from the exposure assessment of the artificial colours considered in this assessment, based on the mean and maximum exposure values, indicate that the food exposure values of the two groups of children (toddlers aged 12-35 months and other children aged 36 months - 9 years) are below the respective ADI, for both mean consumers and high consumers. Although the current exposure levels of artificial colours added to food (only for those with established a legislative level of use) do not represent a health concern for Bulgarian children, the obtained results cannot guarantee the actual exposure to colours due to some uncertainties described in Table 5 and the fact that the exposures are calculated for only one colour in different food groups and compared to the ADI of the respective colorant. In the absence of sufficient studies on mixtures of colours and the derivation of a reference point for comparison and risk monitoring, it is not possible to compare the results of the total exposure of a mixture of colours with the ADI of a single colour. The absence of data on the content of colours in food may not provide a representative estimate of the exposure of children to colours in Bulgaria at the present time.

References

Aroba J., H.-H. Y. (2022). Cross Talk between Synthetic Food Colors (Azo Dyes), Oral Flora, and Cardiovascular Disorders. *Applied sciences*, *12*(14), 7084.

EFSA. (2009). Scientific Opinion on the reevaluation Tartrazine (E 102) on request from the European Commission. *EFSA Jornal, 7*(11), 1331.

EFSA. (2012). Food additive intake model (FAIM). Italy.

EFSA. (2014). Food additive intake model (FAIM): comments received from stakeholders and EFSA views. Italy.

EFSA. (2014). Scientific opinion on the reconsideration of the temporary ADI and refined exposure assessment for Sunset Yellow FCF (E 110). *EFSA Journal*, *12*(7), 3765.

EFSA. (2022). Comprehensive European Food Consumption Database. Italy.

EFSA, 2. (2009). Scientific Opinion on the reevaluation of Allura Red AC (E 129) as a food additive on request from the European Commission. *EFSA Journal*, *7*(11), 1327.

EFSA, 2. (2009). Scientific Opinion on the re-evaluation of Azorubine/Carmoisine (E 122) as a food additive on request the European Commission. *EFSA Journal, 7*(11), 1332.

EFSA, 2. (2009). Scientific Opinion on the reevaluation of Ponceau 4R (E 124) as a food additive on request from the European Commission. *EFSA Journal, 11*(7), 1328.

Gil, C. (2014). *Toxicological effects of food additives.* Sweden: Uppsala.

McCann D., B. A. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial. *The Lancet*(9598), 1560-1567.

Miller M., S. C. (2022). Potential impacts of synthetic food dyes on activity and attention in children: a review of the human and animal evidence. *Environmental Health, 21*(45).