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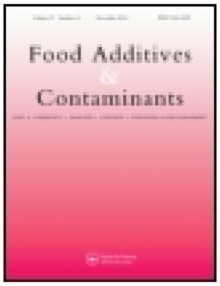

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Assessment of the exposure to Allura Red colour from the consumption of red juice-based and red soft drinks in Italy

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Assessment of the exposure to Allura Red colour from the consumption of red juice-based and red soft drinks in Italy

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This article reports the results of a survey and an exposure study, based on a probabilistic approach, concerning red juice-based and red soft drink products in Italy. It highlights the fact that the estimates of both the consumption rates and colorant intakes are related to the hypotheses of scenarios. In fact, the study estimates that, on average, consumers of red soft drinks consume 53.1 L $year^{-1}$, ranging from 39.1 to 70.7 L of soft drink products under one scenario, or 21.3 L, and from 12.7 and 35.9 L under another hypothesis; while 9.5 L of red juice-based drinks are consumed per year, ranging from 7.0 to 12.5 L, under one scenario, or on average 6.9 L, and ranging from 3.2 to 17.7 L under another scenario. The amount of colorant in a red beverage ranged from 10.9 $mg\,l^{-1}$ in a red soft drink up to 55.9 $mg\,l^{-1}$ in a red juice-based product. The risk evaluation process showed that in all cases the intake of E129 was always lower than the acceptable daily intake. The exposure assessment showed high average intakes of Allura Red in the worst-case scenario, on average, 6.5 and 13.9 $mg\,day^{-1}$, up to 25.0 and 33.0 $mg\,day^{-1}$ at the 95th percentile, for juice-based and soft drinks respectively. The most realistic scenario estimated a weighted average daily intake of Allura Red, on average from about 0.3 to 0.5 $mg\,day^{-1}$ at the 95th percentile, and from 0.4 to 0.6 $mg\,day^{-1}$ for the 95th percentile, from juice-based and soft drinks, respectively. Actually, the highest colorant intake was estimated in a 'health' juice-based drink. The intake of E129 significantly increased with a high level of colorant ($>40\,mg\,l^{-1}$).

Keywords: HPLC; probability modelling; exposure assessment; colours; beverages; fruit juices

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Introduction

Food colorants, both naturally derived and synthetic, are widely used by the food industry. Allura Red (2-hydroxy-1-(2-methoxy, 5-methyl, 4-sulphonatophenylazo)-naphthalene-6-sulphonate) disodium salt, also known as European Union additive 129 (E129) or FD&C Red No. 40 (USA), is one of the most widely used synthetic diazo colorants. Directive 94/36/EC on colours for use in foodstuffs fixes a limit of 100 $mg\,l^{-1}$ for E129 in non-alcoholic-flavoured drinks. Safety aspects have been assessed and reported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (1974, 1980, 1981) as well as by the European Union's Scientific Committee for Food (SCF) (1975, 1984, 1989). Both Committees have established an acceptable daily intake (ADI) of 0–7 $mg\,kg^{-1}$ of body weight day^{-1} ($mg\,kg\,bw^{-1}\,day^{-1}$). The studies on which the JECFA and SCF assessments were based showed a very low acute toxicity of Allura Red as measured in different species of animals (LD50: 10,000 $mg\,kg^{-1}$ bw for rats and rabbits and 5000 $mg\,kg^{-1}$ bw for dogs) (Weir 1965a, 1965b, 1967). Recently, Sasaki et al. (2002) tested 39 food additives and defined an LD50 for Allura Red of $>2000\,mg\,kg^{-1}$. Studies on sub-chronic and chronic toxicity, as well as on carcinogenity (Serota et al. 1977; Borzelleca et al. 1989, 1991), showed that the incidence of tumours or lesions in control and treated animals were very similar. Details concerning the safety aspects of Allura Red can be found in the JECFA (1980, 1981) and World Health Organization (WHO) (2009) reports, respectively.

However, after the publication of the book *Why Is Your Child Hyperactive?* (Feingold 1975), concerning the role of synthetic colorants on children's behaviour (attention deficit disorder (ADD) and/or attention deficit hyperactivity disorder (ADHD)), their use has been widely debated. Vorhees et al. (1983) showed that Allura Red in the diet significantly reduced reproductive success and organ development, and that it modified the behaviour of young rats, although it was not possible to confirm the Feingold hypothesis. Generally speaking, there is a strong feeling that a gap in the data exists regarding the assessment of exposure of infants and children to Allura Red. Often, specific toxicity tests are asked to include data on the relative sensitivity of this sub-population (Reed 1997). Studies concerning different age susceptibilities to carcinogenesis (Hattis

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et al. 2004), as well as studies on paediatric pharmacokinetics (Ginsberg et al. 2004), have been carried out. Recently, *in vivo* Comet studies (Tsuda et al. 2001; Sasaki et al. 2002) excluded a general toxicity of Allura Red, but highlighted specific DNA damage in the colon and in the stomach at the very low Allura Red levels of 10 and 100 mg kg^{-1}, respectively. Moreover, a new study reported that exposure to two mixtures of four synthetic colours (mixture A: E102, E124, E110, E122; mixture B: E110, E122, E104, E129) plus a sodium benzoate preservative in the diet resulted in increased hyperactivity in 3– and 8–9-year-old children in the general population (McCann et al. 2007). The colours, and generally the additives, may have not only the above-mentioned effects, but can increase intolerance and allergy in children (Bourrier 2006). Other studies have been carried out to assess the daily intake of some food colorants in several foodstuffs and non-alcoholic beverages. One study designed a screening method to identity food colorants used in non-alcoholic beverages, which could potentially be consumed at levels exceeding the ADIs, by using a tiered approach (Tennant 2008). Other studies concluded that children were exposed to levels of some food colorants, which exceeded the ADIs (Rao et al. 2004; Husain et al. 2006).

European Parliament and Council Directives 94/35/EC, 94/36/EC and 95/2/EC require each Member State to monitor the consumption and usage of food additives (Commission on Dietary Food Additives Intake in the European Union 2001). The European Food Safety Authority (EFSA), specifically the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials, following a request from the European Commission, was asked to assess the results of the McCann et al. study and it was concluded that this study provided limited evidence that the two different mixtures tested had a small and statistically significant effect on activity and attention in the selected children, and that the clinical significance of the observed effects remains unclear. Therefore, the panel concluded that the results of the McCann et al. study cannot be used as the basis for altering the ADIs of the respective food colorants or sodium benzoate (EFSA 2008, 2009).

Recently, in order to harmonise the use of food additives in foods in the community, the European Parliament and the Council of the European Union adopted Regulation (EC) No. 128/2008 of 22 December 2008 on food additives. This regulation defines 'colours' as substances that 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 the Regulation.

Despite this scientific advice, since July 2010, Annex V of Regulation (EC) No. 1333/2008 (European Commission 2008b) provides a list of food colours (E110, E104, E122, E129, E102, E124) which must be included on food labels with the following information: 'may have an adverse effect on activity and attention in children.'

Over the last few years, after a period where 100% juices seemed to be very popular and widespread, the market showed a slowdown in sales of these products and a significant increase in sales of fruit juice-based drinks (Rossi 2006; Muraca 2007). These beverages allowed both a product innovation and, overall, a significant reduction of fruit and, as a consequence, a reduction in costs (Rossi 2006). The beverages sector is one of the most important food areas where Allura Red is widely used, mainly in fruit-based drinks and soft drinks based on red fruit juices The success of these beverages comes from the increasing consumer demand for foods containing anthocyanins due to their health properties (Bitsch et al. 2004; Galvano et al. 2004; Bonina et al. 2005; Mazza 2007). Many foods are important sources of anthocyanins (Wu et al. 2006), but in Italy these are immediately associated with red (blood) oranges (Maccarone et al. 1983, 1985). The chemical composition (Fallico et al. 1996; Arena et al. 1998, 2006; Maccarone et al. 1998; Rapisarda et al. 1998), the influence of processing (Arena et al. 2000, 2001a, 2001b), the recovery of valuable products (Di Mauro et al. 2000, 2002) and the safety aspects (Fallico et al. 2009) of red oranges have been thoroughly investigated.

Risk assessment is based on a deterministic approach, when the calculated risk is based on a point estimate, or on a probabilistic approach, where both the variability and uncertainty of input variables are taken into consideration by using probability distributions to represent the input variables instead of point estimates (Vose 2000; Spanjersberg et al. 2007). The probabilistic approach was used by Slob and Pieters (1998) to estimate acceptable human exposure limits (RfD, ADI, TDI) by using distributions of extrapolation factors instead of single uncertainty factors, which are more conservative. Besides this, Slob (2006) published a study on probabilistic dietary exposure assessments, taking into account the variability in both amount and frequency of consumption. The probabilistic model was also used (Spanjersberg et al. 2007) for allergen risk assessment, resulting in a more exhaustive risk assessment than traditional deterministic risk assessments.

This article reports the results of a research project that investigated the characteristics of red beverages, both juice-based and soft drinks, in Italy. In particular,

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the intake and exposure of the Italian population to Allura Red from these beverages was estimated. This assessment was carried out using different modelling intakes.

Materials and methods

Sampling and chemical analysis

HPLC analyses of colorants

A sample (10 ml) was appropriately diluted with water and filtered through a 0.45 μm (Albet). HPLC analyses were conducted on a Shimadzu Class VP LC-10ADvp equipped with a SPD-M10Avp diode array detector. The column was a C18 Omnispher (Varian) (150 × 4.6 mm, 5 μm) and the chromatographic conditions were in agreement with those of Minioti et al. (2007). Each colorant was identified by comparing the retention time, splitting the peak of colours detected in the sample with the colour standard, and comparing the UV-vis spectra of the colour standards with those of the samples. The amount of each colour was determined using an external calibration curve, measuring the signal at 504 nm for E129, 485 nm for E110 and 516 nm for E122. The purity of the colours was evaluated according to Minioti et al. (2007) and European Commission (2008a): they were 88.1% for E129, 88.7% for E110 and 86.9% for E122. Details (uncertainty, limits of detection and precision) of the HPLC method are reported in Fallico et al. (2010).

Estimation of the consumption of red beverages

For the first two scenarios the consumers' characteristics (age, weight), the food categories and the daily consumption rates of juice-based and soft drinks were those reported in the INRAN-SCAI 2005–06 survey (Leclercq et al. 2009). For all others the estimates of yearly and daily consumption, as well as the number of consumers, of total red beverages, red fruit juice-based and red soft drinks, were made using yearly sales data of the 27 selected shops. Personal communication with the managers of the shopping chain was essential in order to obtain both sets of data.

Risk assessment

The estimated daily intake (EDI) of Allura Red was made by following the methods described in Petersen and Barraj (1996) and Douglass and Tennant (1997). A tired approach was used to estimate the exposure with different scenarios. Details of each scenario are reported in Table 1. First, a maximum permitted level (MPL) (EFSA 2009) than a single point method (SPM) (Petersen and Barraj 1996), were hypothesised. After, as to our knowledge no specific data, both for consumption and for colorant levels, in Italian red fruit based beverages were available, more refined scenarios were attempt. This part of the study was carried out with the collaboration of the Roberto Abate spa company, which owns a chain of more than 40 shops, each having 200–6000 m^2 of selling area. It represents about the 10% of food retailers in the metropolitan area of Catania (Italy) (about 1.0–1.5 million people). One-year selling data from 27 shops, including all of the largest ones, were used for this study. Moreover, from these shops, all of the red fruit juice-based and red soft drinks were sampled in triplicate, excluding 100% juices. A total of 17 different brands were collected. For these scenarios (3A, 3B and 4) a probabilistic approach was used. The distribution of the colour concentration (mg kg^{-1}) in non-alcoholic beverages was multiplied by the distribution of beverages. In order to carry out a safety evaluation of colorant E129 exposure, a risk assessment model with a probabilistic approach from @Risk software ver. 5.5 was used (Palisade Inc., Newfield, NY, USA). In this kind of analysis the single values are replaced from distributions of probability; a value selected at random from the food consumption distribution curve was multiplied by one drawn at random from the colour concentration distribution curve; this process was repeated thousands of times until the pre-planned number of repetitions was reached.

Simulations

Simulations were run in order to obtain estimations of the following parameters: the number of customers (total, red beverages, red soft drinks and red juice-based drinks (Table 4); the distribution of red beverage consumption for consumers only (Table 5); and yearly and daily intakes (mg) of E129 for each beverage (Tables 6–8). The conditions of each simulation were as follows: number of iterations: 10,000; sampling type: Latin hypercube. For each simulation output the probability distribution between the 5th and 95th percentiles, the mean, the standard deviation and the uncertainty parameters were recorded. Moreover the best fitting distribution, according to the K-S value, was extrapolated.

Results and discussion

The estimation of daily intake of a substance from a food source needs data concerning the amount of food eaten and the level at which the compound is present in that food. Many methods can be used to obtain these data; the most common refer to the literature and specific surveys of food intake (Douglass and Tennant 1997). Data concerning the consumption of juices, nectars and soft drinks in Italy in 2009 indicates a consumption of 66 L $year^{-1}$ per capita for carbonated, still and sports/energy drinks and of 15 L $year^{-1}$ per

Table 1. Description of the input used for the models.

	Method	Input	References
1	Maximum permitted level (MPL)	• Consumption of fruit juices, ml day^{-1}: 130.9; 330 (mean; 95th) • Consumption of soft drinks, ml day^{-1}: 64.4; 250 (mean; 95th) • Maximum concentration allowed of E129 in beverages: 100 mg l^{-1}	• The Italian National Food Consumption Survey 2005–06 (Leclercq et al. 2009). • Annex II, REG. EC 1333/08 (L 354/16)
2	Single-point method (SPM)	• Consumption of fruit juices, ml day^{-1}: 130.9; 330 (mean; 95th) • Mean concentration of E129 in juice-based drinks: Table 3 • Consumption of soft drinks ml day^{-1}: 64.4; 250 (mean; 95th) • Mean concentration of E129 in soft drinks: Table 3	• The Italian National Food Consumption Survey 2005–06 (Leclercq et al. 2009)
3A	Probabilistic	*Red juice-based drinks* • Consumption of beverages in the selected shops (L year^{-1}): Table 2 • Distribution of the tendency to drink: uniform (0.35; 0.56) • Distribution of the number of buyers: $\sum$(binomial$_i$ (number of receipts; P_i)/purchases per year$_i$) • Distribution of market share in the selected shops: Normal: Table 2 • Number of consumers/buyers: 1 *Soft drinks* • Consumption of red soft drinks in the selected shops (L year^{-1}): Table 2 • Distribution of the tendency to drink: uniform (0.218; 0.35) • Distribution of the number of buyers: $\sum$(binomial$_i$ (number of receipts; P_i)/purchases per year$_i$) • Distribution of market share in the selected shops: Normal: Table 2 • Number of consumers/buyers: 1	• Audipress (2008). • Bevitalia (2010a. 2010b). • Giovenali (2008). • The Italian National Food Consumption Survey 2005–06 (Leclercq et al. 2009). • Arcella and Leclercq (2005)
3B	Probabilistic	*Red juice-based drinks* • Consumption of beverages in the selected shops (L year^{-1}): Table 2 • Distribution of the tendency to drink: uniform (0.35; 0.56) • Distribution of the number of buyers: $\sum$(binomial$_i$ (number of receipts; P_i)/purchases per year$_i$) • Distribution of market share in the selected shops: Table 2 • Distribution of the number of consumers: Triang (1, 1.21, 2) *Soft drinks* • Consumption of soft drinks in the selected shops (L year^{-1}): Table 2 • Distribution of the tendency to drink: uniform (0.218; 0.35) • Distribution of the number of buyers: $\sum$(binomial$_i$ (number of receipts; P_i)/purchases per year$_i$) • Distribution of market share in the selected shops: Table 2 • Distribution of the number of consumers: Triang (1, 3, 4)	• Audipress (2008). • Bevitalia (2010a, 2010b). • Giovenali (2008). • ISTAT (2010), The Italian National Food Consumption Survey 2005–06 (Leclercq et al. 2009). • Arcella and Leclercq (2005)
4	Probabilistic	*Red juice-based drinks* • Consumption of each red fruit based beverages (L year^{-1}) • Distribution of the number of consumers: Scenario 3B *Soft drinks* • Consumption of each soft drink in the selected shops (L year^{-1}) • Distribution of the number of consumers: Scenario 3B	• Leclercq et al. (2003)

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Table 2. Data from the shopping centres.

Number of shopping centres	27
Number of receipts	5,100,630
Number of items year^{-1} (red beverages)	49,856
Litres year^{-1} (red beverages)	62,682.8
Litres year^{-1} (red juice based)	32,640.8
Litres year^{-1} (red soft drinks)	30,042
Incidence of red soft drink	1.5%
Incidence of red juice-based drinks	5.7%

capita for pure juices, nectars and juice-based drinks (Bevitalia 2010a, 2010b). However, to the best of our knowledge, specific data concerning the red beverages sector do not exist.

The sales data of the selected 27 shops over a one-year period, covering a total area with 1.0–1.5 million people, are reported in Table 2. In one year 5,100,630 receipts were given out and 49,856 red beverages were sold with a total of 62,682.8 L. In particular, the red juice-based drinks accounted for 32,640.8 L and the red soft drinks for 30,042 L. Furthermore, from the collected data it was possible to establish that red juice-based drinks represented about 5.7% of all juice-based drinks, while red soft drinks represented 1.5% of all soft drinks sold during this period.

Table 3 shows the results concerning the presence of colorants, the percentages of colorants and the amounts in litres of the 17 studied beverages. Almost 48% of the red beverages was made up by the red soft drinks, with one alone representing the 35.2% of the market. Concerning the 15 red juice-based drinks, they ranged from a minimum of 0.17% to a maximum of 11.96% of the market. Most of these were referred to as red orange juice (Fallico et al. 2010), and, concerning their colour, in seven this was due to the addition of anthocyanin extracts, but in eight it was due to the presence of Allura Red. The colour of the two red soft drinks was due to colorants: one contained a mixture of E122 and E110 and the other was coloured with E129. The amount of colorants ranged from 10.86 mg l^{-1} in the first soft drink (the sum of E122 and E110) to 55.91 mg l^{-1} in a juice-based drink (number 14). It is interesting to note that all but the soft drinks, which are usually perceived negatively, were very similar to fruit juices. The contribution of actual fruit juices in these drinks to their colour is very small or even sometimes negligible (Fallico et al. 2010). Instead, they have significant amounts of colorants added, which are often quite higher than the amounts in soft drinks. The levels of added colorants were very high in beverage numbers 5, 11 and 14, with 42.83, 48.51 and 55.91 mg l^{-1} of E129, respectively. The first one claimed to have red orange juice properties whilst the other two claimed to have strong antioxidant activity and berries. It is important to note that consumption of the colorant depended on both its concentration in the beverages and on beverage consumption rates. For instance, sample number 17 represented the 35.2% of the market, but it contributed 50.6% of Allura Red consumption. Beverage numbers 11 and 14 each represented each less than 3% of the market, but contributed to the 5.8% and 7.5% of Allura Red consumption, respectively.

The average level of colorant in the red juice-based drinks was 29.6 mg l^{-1} and the levels were log-normally distributed, while in the two soft drinks the average value was 20.5 mg l^{-1} and the levels were normally distributed (Table 3).

In order to build a more realistic scenario to assess the daily intake of Red Allura from red beverages, estimation of the number of consumers and a survey of these beverages was a primary goal of this research. Literature data and the number of receipts were used to estimate the total number of customers of the shopping centres. In turn, estimation for red drink products consumers in one year was attempt.

Studies aimed to understand buyers' habits in Italy (Audipress 2008; TNS Italia 2010) highlighted that the majority of consumers, about the 75%, prefer shopping chain. Considering these as our population, their percentages as well as the frequency of purchases are reported in Table 4. First, the total number of customers was divided in four categories, considering the frequency of purchases. Considering that the number of receipts for each category, it is given by the number of customers multiplied by the number of purchases, the inverse formula (number of receipts/number of purchases) was used to estimate the number of customers for each category. The number of receipts for each category was the result of a binomial distribution of the total number of receipts and the related percentage. The total number of customers was given by adding the customers of all categories. The resulting number was 137,370 as average, with a minimum value of 123,648 and maximum of 153,921 total customers. The best-fit distribution is a β-general one (Table 4).

From this, taking into account the tendency of customers to buy red beverages, the incidence of red products on the category and the number of consumers per buyer, were estimated the number of consumers for red beverages for the two Scenarios 3A and 3B, respectively (Table 4). The two scenarios differ from each other for the number of consumers per buyer (or item). In fact, the Scenario 3A hypotheses that each buyer is the consumer of the item, while the Scenario 3B differs between the buyer and the number of consumers. In order to build Scenario 3B during 2 consecutive days in the largest of the 27 shopping centres, 30 buyers for each category of red beverages, juice-based

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Table 3. Market incidence and colorant data of each beverage.

		Litres per year			Colorants				
Beverage number	Drinks	Litres	Percentage of total	Percentage of category	Colorant	$mg\,l^{-1}$ (SD)	$mg\,year^{-1}$ (SD)	Percentage of total	Percentage of category
1	Juice based	4774	7.62	14.63	E 129	29.30 (0.52)	139,878.2 (2482.4)	10.6	25.00
2		1043	1.66	3.20	E 129	23.76 (1.06)	24,781.7 (1105.5)	1.9	4.00
3		2536	4.05	7.77	No colorants				
4		7497	11.96	22.97	E 129	16.71 (0.36)	125,274.9 (2699.0)	9.5	22.00
5		862	1.38	2.64	E 129	42.83 (1.79)	36,919.5 (1543.1)	2.8	7.00
6		4966	7.92	15.21	No colorants				
7		729.75	1.16	2.24	No colorants				
8		594	0.95	1.82	No colorants				
9		786.75	1.26	2.41	No colorants				
10		1647.75	2.63	5.05	E 129	16.03 (0.22)	26,413.4 (362.5)	2.0	5.00
11		1586.25	2.53	4.86	E 129	48.51 (2.44)	76,949.0 (3870.4)	5.8	14.00
12		109.5	0.17	0.34	No colorants				
13		1803	2.88	5.52	No colorants				
14		1759.8	2.81	5.39	E 129	55.91 (0.44)	98,390.4 (774.4)	7.5	17.00
15		1946	3.10	5.96	E 129	17.93 (1.58)	34,891.8 (3074.8)	2.6	6.00
						Log-normal (29.6; 18.1)			
16	Soft	7977	12.73	27.00	E122 + E110	10.86 (1.80)	86,630.2 (4360)	6.6	11.00
17		22,065	35.20	73.00	E 129	30.22 (1.49)	666,804.3 (32876)	50.6	89.00
						Normal (20.5; 10.9)			

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Table 4. Estimated number of red beverages per year.

		Frequency of purchases	***%***	***Purchases per year***	***Number of customers***
Total customers of the shopping centres	***Input***	Never	25	–	
		1 per month	13.1	12	Binomial (5100630; 0.131)/12
		2–3 per month	23.6	Uniform (24; 36)	Binomial (5100630; 0.236)/Uniform (24; 36)
		4–8 per month	52.8	Uniform (48; 96)	Binomial (5100630; 0.528)/Uniform (48; 96)
		> 21 per month (daily)	10.5	252	Binomial (5100630; 0.105)/252
	Output	***Total customers***		***Mean: 137370; st.dev.:9212; min (5%): 123648; Max (95%): 153921 β–General (1.99; 3.2; 119333; 166273); K–S: 0.01***	
Scenario 3A	***Input***	Total Customers		β–General (1.99; 3.2; 119333; 166273)	
				Red Soft	***Red Juice based***
		Tendency to buy		Uniform (0.218; 0.35)	Uniform (0.35; 0.562)
		Incidence on category		Normal (0.015; 0.0015)	Normal (0.057; 0.0057)
		Number of consumers per buyer		1	1
	Output	**Number of consumers** *(Uncertainty (rank and regr.))*		***Mean: 585; st. dev.: 106; min (5%): 426; Max (95%): 768 LogNormal (585; 106); K–S: 0.0173*** ***(1. tendency to buy: 0.74; 2. Incidence %: 0.55).***	***Mean: 3571; st. dev.: 654; min (5%): 2586; Max (95%): 4705 LogNormal (3571; 654); K–S: 0.013*** ***(1. tendency to buy: 0.74; 2. Incidence %: 0.55).***
Scenario 3B	***Input***	Total Customers		β–General (1.99; 3.2; 119333; 166273)	
				Red Soft	***Red Juice based***
		Tendency to buy		Uniform (0.218; 0.35)	Uniform (0.35; 0.562)
		Incidence on category		Normal (0.015; 0.0015)	Normal (0.057; 0.0057)
		Number of consumers per buyer		Triang (1; 3; 4)	Triang (1; 1.21; 2)
	Output	***Number of consumers*** *(Uncertainty (rank and regr.))*		***Mean: 1560; st. dev.: 465; min (5%): 836; Max (95%): 2360 β–General (4.9; 9.4; 252; 4080); K–S: 0.0074*** **(1. N. of consumers: 0.78; 2. tendency to buy: 0.45; 3. Incidence %: 0.34).**	***Mean: 5009; st. dev.: 1194; min (5%): 3290; Max (95%): 7210 LogNormal (5009; 1194); K–S: 0.0075*** ***(1. N. of consumers: 0.64; 2. tendency to buy: 0.57; 3. Incidence %: 0.42).***

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and soft drinks, were asked to say for who and for how many people they were going to buy the red beverage. Regarding the red juice-based drinks they readily answered 'for children' and the most common number of consumers were one or two, up to three people per item. For this reason a Triangular distribution was chosen (1; 1.37; 3) centred on 1.37 (the average number of children per female in Italy (ISTAT 2010) ranging from one to three). For soft drinks the most common number of consumers declared per item was three, ranging from one to four. So, a triangular distribution (1; 3; 4) was chosen.

For Scenario 3A the average number of customers buying red soft drinks was estimated to be 585, log-normal distributed, ranging from 426 to 768 consumers (Table 4). While the average number of customers of red juice-based drinks was 3571, log-normal distributed, ranging from 2586 to 4705. For Scenario 3B it was estimated as 1560 customers for soft drinks, β-general distributed, ranging from 836 to 2360 customers. The estimated number of juice-based drinks consumers was 5009, log-normal distributed, ranging from 3290 to 7210 customers. The sensitivity analysis highlighted that, in Scenario 3A, the uncertainty on the estimation of the number of customers was related to the tendency to buy and to the incidence of the red products; while in Scenario 3B it was first related to the number of consumers per buyer, followed by the two input described above (Table 4).

The yearly (litres) and daily (ml) consumptions of both red soft drinks and red juice-based drinks were estimated (10,000 iterations each) by dividing the litres sold for each category by the appropriate customer distribution. These results are reported in Table 5. Both consumption distributions were positively skewed, as found for other foods (Zhang et al. 2009). In fact, for both red soft drinks and red juice-based drinks the best-fitting distribution was a log-normal one. Data suggest that in Scenario 3A, on average, a consumer drinks 53.1 L of a red soft beverage per year. However, none of the values between 39.1 and 70.7 L year^{-1} can be excluded (Table 5). This would mean a daily consumption of 145.4 ml of red soft drinks, ranging from 107.1 to 193.8 ml. These data fully agree with the literature, both with the industrial and food consumption surveys. In fact, of the 65 L of yearly consumption per capita, 79% (51.4 L) must be ascribed to carbonated drinks (Bevitalia 2010a), while the Italian food consumption survey reports a mean of 130.9 ml up to 330 ml (95th percentile) as daily consumption (Leclercq et al. 2009). Thus, the approach used here to estimate the number of consumers of red beverages seemed to be appropriate. Moreover, the consumption of red soft drinks only concerned a small fraction of the population; however, this was at the same level as all of the other soft drinks. It seems that the consumption of red soft drinks was not added to that of the other soft drinks, but rather it was treated as an alternative to them. The yearly estimated consumption of red juice-based drinks, for the same scenario, was 9.5 L (25.6 ml day^{-1}), ranging from a minimum of 7.0 L (19.0 ml day^{-1}) to a maximum of 12.5 L (34.3 ml day^{-1}). The values reported here are similar to those in Bevitalia (2010b) (12.5 L year^{-1} (34.6 ml day^{-1})) for the category of nectars and juice drinks; they are lower than those, reported as fruit and vegetable juices, found in the Italian food consumption survey (64.4 ml up to 250 ml at the 95th percentile, Leclercq et al. 2009). Lower values were estimated for Scenario 3B (Table 5). In fact, the yearly estimated consumption of red soft drinks ranged from 12.7 to 35.9 L, with a most probable value of 21.3 L. It means a daily consumption of 58.3 ml ranging from 34.8 to 98.4 ml day^{-1}. As concerns the estimation of consumption of red juice-based products in Scenario 3B the results are as follows: 6.9 L year^{-1} (18.9 ml day^{-1}) ranging from 12.4 to 27.2 L year^{-1}. The sensitivity analysis, analogous to the number of consumers estimation, has shown that in Scenario 3A the main uncertainty factors are the tendency to buy and the incidence of the red beverages on the category, while in Scenario 3B these factors are overcome by the number of consumers/buyer.

Combining the daily beverages consumption value with the concentration of the colorant (Scenarios 1 and 2) or the distributions of E129 concentrations with those of beverage consumption (Scenarios 3 and 4), the per capita estimation of yearly and daily intakes of E129 were obtained (Tables 6–8). In the worst case (Scenario 1), considering a consumption per capita of 64.4 and 130.9 ml day^{-1} on average, 250 and 330 ml day^{-1} at the 95th percentile, for juice-based and soft drinks (Leclercq et al. 2009), the average colorant intake was 6.5 and 13.0 mg day^{-1} for juice-based and soft drinks, respectively. The daily intakes for consumers at the 95th percentile were 25.0 and 33 mg day^{-1} for juice-based drinks and soft drinks, respectively (Table 6). The second scenario took into account the same consumption rates of Scenario 1, but instead of the MPL of the colorant (100 mg l^{-1}), the averages found in these beverages were used (Table 3). Under these hypotheses the intake of colorant was 1.9 and 2.7 mg day^{-1} from juice-based and soft drinks, respectively. At the 95th percentile the intake was 7.4 and 6.8 mg day^{-1}, respectively (Table 6).

These intakes, for both Scenarios 1 and 2, according to categories of consumers reported in Leclercq et al. (2009) (children (3.0–9.9 years), teenagers (10.0–17.9 years) and adults (18.0–64.9 years)), were converted into μg kg bw^{-1} day^{-1} (Table 6). These data, which are analogous with those in the literature (Tennant 2008; EFSA 2009; EXPOCHI 2010), highlighted that the most exposed were children because of their high consummation/body weight ratio. In fact,

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Table 5. Estimation of yearly and daily consumption of red beverages.

		Red soft drinks	***Uncertainty (rank and regr.)***	***Red juice based***	***Uncertainty (rank and regr.)***
Scenario 3A		***Yearly (litres)***	1) tendency to buy: –0.73 2) Incidence %: –0.55	***Yearly (litres)***	1) tendency to buy: –0.73 2) Incidence %: –0.55
	Mean (St. dev.):	***53.1 (9.8)***		***9.5 (1.8)***	
	Min (5%):	39.1		7.0	
	Max (95%):	70.7		12.5	
	Best fit:	LogNormal (53.1; 9.8); K–S: 0.0154		LogNormal (9.5; 1.8); K–S: 0.0185	
		Daily (milliliters)		***Daily (milliliters)***	
	Mean (St. dev.):	***145.4 (27)***		***25.6 (4.8)***	
	Min (5%):	107.1		19.0	
	Max (95%):	193.8		34.3	
	Best fit:	LogNormal (145.4; 27); K–S: 0.0154		LogNormal (25.6; 4.8); K–S: 0.0185	
Scenario 3B		***Yearly (litres)***	1) n. of consumers: –0.79 2) tendency to buy: –0.38 3) Incidence %: – 0.30	***Yearly (litres)***	1) n. of consumers: –0.61 2) tendency to buy: –0.58 3) Incidence %: –0.43
	Mean (St. dev.):	***21.3 (7.4)***		***6.9 (1.6)***	
	Min (5%):	12.7		3.2	
	Max (95%):	35.9		17.7	
	Best fit:	LogNormal (21.3; 7.4); K–S: 0.0082		LogNormal (6.9; 1.6); K–S: 0.0071	
		Daily (milliliters)		***Daily (milliliters)***	
	Mean (St. dev.):	***58.3 (20.4)***		***18.9 (4.5)***	
	Min (5%):	34.8		12.4	
	Max (95%):	98.4		27.2	
	Best fit:	LogNormal (58.3; 20.4); K–S: 0.0082		LogNormal (18.9; 4.5); K–S: 0.0071	

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Table 6. Estimation of E129 daily intake from red beverages.

	Intake (mg/d)	**Intake (μg/d/kg bw)**			**Uncertainty (rank and regr.)**
Scenario 1		***3–9.9 years***	***10–17.9 years***	***18–64.9 years***	
Red juice based	Intake $_{av.}$ = 0.0644 * 100 = 6.4	247.5	122.8	92.7	
	Intake $_{95th}$ = 0.250 * 100 = 25.0	957.9	475.3	358.7	
Red soft	Intake $_{av.}$ = 0.131 * 100 = 13.1	498.1	247.1	186.5	
	Intake $_{95th}$ = 0.330 * 100 = 33.0	1264.4	627.4	473.5	
Scenario 2					
Red juice based	Intake $_{av.}$ = 0.0644 * 29.6 = 1.9	73.2	36.3	27.4	
	Intake $_{95th}$ = 0.250 * 29.6 = 7.4	283.5	140.7	106.2	
Red soft	Intake $_{av.}$ = 0.131 * 20.5 = 2.7	102.3	50.8	38.3	
	Intake $_{95th}$ = 0.330 * 20.5 = 6.8	260.5	129.3	97.6	
Scenario 3A					
Red juice based	*Intake (mg/d) = (LogNormal (29.6; 18.1)*LogNormal (9.5, 1.8)/(LogNormal (3571; 654))/365*				
	Intake $_{av.}$ = 0.8 (0.5)	35.5 (64.7)	16.3 (12.3)	12.0 (8.0)	1) mg/l of E129: 0.94 2) tendency to buy: –0.213 3) incidence %: –0.157
	Intake $_{5th}$ = 0.2	9.3	4.8	3.7	
	Intake $_{95th}$ = 1.7	87.4	37.5	26.9	
Red soft	*Intake (mg/d) = (LogNormal (20.5; 10.8)*LogNormal (53.1, 9.8)/LogNormal (585; 106))/365*				
	Intake $_{av.}$ = 3.0 (1.7)	130.5 (304.4)	61.2 (42.4)	44.9 (28.2)	1) mg/l of E129: 0.935 2) tendency to buy: –0.24 3) incidence %: –0.181
	Intake $_{5th}$ = 0.4	38.3	19.7	15.0	
	Intake $_{95th}$ = 5.9	310.7	135.1	97.7	
Scenario 3B					
Red juice based	*Intake (mg/d) = (LogNormal (29.6; 18.1)*LogNormal (6.9, 1.6)/LogNormal (5009; 1194))/365*				
	Intake $_{av.}$ = 0.6 (0.4)	29.1 (262.3)	12.2 (9.5)	9.0 (6.5)	1) mg/l of E129: 0.91 2) n. of consumers: –0.215 3) tendency to buy: –0.202 4) Incidence %: –0.149
	Intake $_{5th}$ = 0.2	6.6	3.4	2.5	
	Intake $_{95th}$ = 1.3	67.7	29.0	21.1	
Red soft	*Intake (mg/d) = (LogNormal (20.5; 10.8)*LogNormal (21.3, 7.4)/LogNormal (1560; 465))/365*				
	Intake $_{av.}$ = 1.2 (0.8)	54.0 (100.8)	24.5 (20.6)	17.9 (12.7)	1) mg/l of E129: 0.81 2) n. of consumers: –0.417 3) tendency to buy: –0.202 4) Incidence %: –0.161
	Intake $_{5th}$ = 0.1	12.9	6.7	5.1	
	Intake $_{95th}$ = 2.6	137.8	58.1	41.8	

Table 7. Estimation of E129 daily intake from single red beverage.

	Percentage litres	Percentage colorants	Consumers	Daily intake (mg)
1				
Minimum (5%)			484	0.36
Maximum (95%)			1050	0.79
Mean (SD)	14.6	24.8	733 (175)	0.55 (0.13)
Best fit			Log-normal (484; 175)	Log-normal (0.55; 0.13)
2				
Minimum (5%)			106	0.29
Maximum (95%)			229	0.65
Mean (SD)	3.2	4.4	160 (38)	0.45 (0.11)
Best fit			Log-normal (160; 38)	Log-normal (0.45; 0.11)
4				
Minimum (5%)			759	0.21
Maximum (95%)			1649	0.45
Mean (SD)	23.0	22.1	1150 (275)	0.32 (0.08)
Best fit			Log-normal (1150; 275)	Log-normal (0.32; 0.08)
5				
Minimum (5%)			87	0.53
Maximum (95%)			190	1.03
Mean (SD)	2.6	6.6	132 (32)	0.74 (0.20)
Best fit			Log-normal (132; 32)	Log-normal (0.74; 0.20)
10				
Minimum (5%)			167	0.20
Maximum (95%)			362	0.43
Mean (SD)	5.1	4.7	253 (60)	0.30 (0.07)
Best fit			Log-normal (253; 60)	Log-normal (0.30; 0.07)
11				
Minimum (5%)			161	0.60
Maximum (95%)			349	1.32
Mean (SD)	4.9	13.7	243 (58)	0.92 (0.22)
Best fit			Log-normal (243; 58)	Log-normal (0.92; 0.22)
14				
Minimum (5%)			178	1.85
Maximum (95%)			387	3.59
Mean (SD)	5.4	17.5	270 (65)	2.58 (0.55)
Best fit			Log-normal (270; 65)	Log-normal (1.55, 0.55; shift 1.02)
15				
Minimum (5%)			83	0.70
Maximum (95%)			161	1.52
Mean (SD)	6.0	6.2	121 (24)	1.06 (0.25)
Best fit			Log-normal (121; 24)	Log-normal (1.06; 0.25)
16				
Minimum (5%)			878	0.11
Maximum (95%)			1906	0.29
Mean (SD)	26.6	11.5	1330 (318)	0.19 (0.06)
Best fit			Log-normal (1330; 318)	Log-normal (0.19; 0.06)
17				
Minimum (5%)			2428	0.34
Maximum (95%)			5272	0.76
Mean (SD)	73.4	88.5	3679 (879)	0.52 (0.13)
Best fit			Log-normal (3679; 879)	Log-normal (0.52; 0.13)

Note: Uncertainty = number of consumers (regression > 0.90).

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for Scenario 1, the mean exposure values for red juice-based and red soft drinks were 247.5 and 498.1 μg kg bw^{-1} day^{-1}, respectively. At the 95th percentile the children's exposures were 957.9 and 1264.4 μg kg bw^{-1} day^{-1}, respectively. The above data represent 3.5% and 7.1% of the mean, up to 13.7% and 18.1% at the 95th percentile of the ADI value, for juice-based and soft drinks, respectively. Moreover, comparing the above data with those reported in EXPOCHI (2010) (MPL scenario) concerning the total

Table 8. Weighted estimation of E129 daily intake from red beverages (Scenario 4).

Intake ($mg\,day^{-1}$)		Intake ($\mu g\,kg\,bw^{-1}\,day^{-1}$)		
		3.0–9.9 Years	10.0–17.9 Years	18.0–64.9 Years
Red juices based drinks				
Mean (SD)	0.34 (0.09)	14.7 (34.9)	6.7 (2.8)	4.9 (1.7)
Minimum (5%)	0.22	6.9	3.7	2.8
Maximum (95%)	0.50	28.2	11.7	8.0
Best fit	Log-normal (0.34; 0.09); K-S: 0.007			
Red soft drinks				
Mean (SD)	0.44 (0.11)	19.8 (53.3)	9.0 (5.2)	6.6 (2.3)
Minimum (5%)	0.28	9.1	4.8	3.8
Maximum (95%)	0.63	37.6	15.3	10.7
Best fit	Log-normal (0.44; 0.11); K-S: 0.003			

Note: Uncertainty = number of consumers (regression > −0.9).

exposure to E129 of Italian children, 773.7 and 6635.4 $\mu g\,kg\,bw^{-1}\,day^{-1}$ as the mean and 95th percentile, respectively. They represent, on average, 32% to 64.4% of total intake for juice-based and soft drinks, respectively; and lower values of 14.4% and 19.1% at the 95th percentile.

Using the experimental average levels of the colorant (Scenario 2) took to a much lower intake (Table 6). In fact, under this scenario the daily intake per kilogram of body weight for Italian children was 73.2 and 102.3 µg for juice-based and soft drinks, respectively. It could achieve 283.5 and 260.5 µg at the 95th percentile. This represents 1.0% and 4.1% of the ADI at the mean and 95th percentile for juice-based drinks and 1.5% and 3.7% for soft drinks, respectively. A comparison with results of the 'analytical scenario' reported in the EXPOCHI (2010) study (524.3 and 1257.5 $\mu g\,kg\,bw^{-1}\,day^{-1}$ as the mean and 95th percentile, respectively) highlights that the consumption of these beverages provides the 14.0% of colorant total daily intake (up to the 22.5%) from juice-based drinks or the 19.5% (up to 20.7%) for soft drinks.

The above scenarios took into account the results of surveys not specific for red beverages. Moreover, these did not consider properly the amount of knowledge contained in the input parameters in terms of uncertainty and the associated probability. In order to obtain a more realistic E129 daily intake and to manage the uncertainty and the variability about the number of consumers, as well as the colorant level in the beverages, a probabilistic approach was used (Scenarios 3A, 3B and 4).

Under the hypotheses of Scenarios 3A and 3B, using the distributions of colorant levels, the year of beverage intake and the appropriate number of consumers, and dividing by 365, it was possible to obtain an estimation of Red Allura daily intake (Table 6). Under Scenario 3A it was 0.8 $mg\,day^{-1}$, ranging from 0.2 to 1.7 $mg\,day^{-1}$ for juice-based drinks. The intake of E129 from red soft drinks was estimated to be 3.0 $mg\,day^{-1}$, ranging from 0.4 to 5.9 $mg\,day^{-1}$. The best-fitting distribution for the daily intake was, in both cases, a log-normal one. Under Scenario 3B the daily intake from juice-based drinks was 0.6 mg (range = 0.2–1.3 $mg\,day^{-1}$); it was 1.2 $mg\,day^{-1}$ from red soft drinks, ranging from 0.1 to 2.6 $mg\,day^{-1}$.

The above data suggest that the intake (µg) per children's kg of body weight (Table 6) is 35.5 µg, ranging from 9.3 to 87.4 $\mu g\,kg\,bw^{-1}\,day^{-1}$ for juice-based drinks under Scenario 3A; and 29 µg, ranging from 6.6 to 67.7 $\mu g\,kg\,bw^{-1}\,day^{-1}$ under Scenario 3B. Representing, under the two scenarios, 0.5% (0.1–1.3%) and 0.4% (0.1–1.0%) of the ADI, respectively.

The daily intake of E129 from red soft drinks was 3.0 mg, ranging from 0.4 to 5.9 $mg\,day^{-1}$, under Scenario 3A; and 1.2 mg, ranging from 0.1 to 2.6 $mg\,day^{-1}$, under Scenario 3B (Table 6). These mean 130.5 $\mu g\,kg\,bw^{-1}\,day^{-1}$ (38.3–310.7 $\mu g\,kg\,bw^{-1}\,day^{-1}$) under Scenario 3A; and 54.0 $\mu g\,kg\,bw^{-1}\,day^{-1}$ (12.9–137.8 $\mu g\,kg\,bw^{-1}\,day^{-1}$) under Scenario 3B. These represent 1.9% (0.6–4.4%) and 0.8% (0.2–2.0%) of the ADI, respectively.

Sensitivity analysis highlighted that most of the uncertainty in the estimation of E129 daily intake in both scenarios was due to the variability of E129 level in red beverages, followed by the tendency to buy and the market incidence for Scenario 3A; and the number of consumers per buyer, the tendency to buy and the market incidence in Scenario 3B, respectively. The number of consumers per buyer becomes meaningful in the estimation of daily intake from soft drinks under Scenario 3B (Table 6).

Although the intakes shown above can be considered as realistic since they were based on both the true E129 concentration distribution and on red beverages consumption, they do not provide information about

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brand loyalty behaviour. Moreover, this approach did not take into account the contribution of each beverage to E129 intake. For these reasons, an estimation of E129 intake was made for each beverage and a weighted average taken (Scenario 4). Similar to other foods (Leclercq et al. 2003; Zhang et al. 2009), the market incidence, calculated using the percentage of litres (Table 3) and the number of consumers found in Scenario 3B, was used to estimate the per capita consumption of each beverage. The E129 intake values were obtained for each beverage by dividing the mg year^{-1} of colorant (Table 3) by the consumer distribution (Table 4), from which, in turn, the estimated daily intake was obtained (Table 7).

A new average intake (Table 8), both for red juice-based and red soft drinks, was estimated using the following equation:

$$\mathrm{EDI} = \sum (EDI_i * L_i/L_T)$$

where EDI_i is the estimated daily intake for a single beverage; L_i is litres of the beverage; and L_T is total litres for each category of juice-based and soft drinks. For the juice-based drinks (Table 7), three beverages (11, 14 and 15) showed a daily intake higher or equal to 1 mg day^{-1}. For beverage 14, under the brand loyalty scenario, the intake of E129 achieved very high levels: 2.6 mg day^{-1} (3.6 mg day^{-1} at the 95th percentile). All other juice-based drinks containing E129 (1, 2, 4, 5 and 10) showed a daily intake equal or lower than 0.7 mg day^{-1}. As mentioned above, beverage 5 claims to have red orange juice properties, whereas the other two, 11 and 14, claim to have very high antioxidant levels and healthy properties of berries. The EDI values for the two soft drinks (Table 7) were 0.2 and 0.5 mg day^{-1} with the highest values of 0.3 and 0.8 mg day^{-1}, respectively. The weighted ADI for Allura Red showed (Table 8) a log-normal distribution centred on 0.3 mg day^{-1} for red juice-based drinks and 0.4 mg day^{-1} for red soft drinks. The highest values (95% percentile) were 0.5 and 0.6 mg day^{-1}, respectively. These data suggest child intakes of 14.7 μg kg bw^{-1} day^{-1} (6.9–28.2 μg kg bw^{-1} day^{-1}) from juice-based drinks and 19.8 μg kg bw^{-1} day^{-1} (9.1–37.6 μg kg bw^{-1} day^{-1}) from soft drinks, respectively. Representing 0.2% (0.1–0.4%) of the ADI for juice-based drinks and 0.3% (0.1–0.5%) for soft drinks, respectively. Under this scenario the uncertainty was due exclusively to the number of consumers.

The above data show that the estimation of the E129 daily intake changes significantly with the hypotheses under each scenario. There is a deep difference between the first scenario and all the others. In fact, under Scenario 1, the daily intake at the 95th percentile of the colorant from red soft drinks can achieve 64% of the ADI for children. The more the scenario becomes realistic, the lower is the anticipated intake of E129, at least from red beverages. In fact, the anticipated daily intake from these beverages is always far from the ADI.

The more refined the scenario becomes, the lower is the difference of colorant intake from juice-based and soft drinks, respectively: the colorant level in the beverages is the most important factor affecting its daily intake. Loyalty to a single red beverage, if it has high E129 levels, can significantly increase the daily intake of the colorant.

All of the beverages with colorant concentrations below 30 mg l^{-1} did not show any potential risk. Considering the findings reported in risk evaluation processes (EFSA 2009), these data clearly suggest that the main risk from the colorants in beverages may be from the overall levels used. Although the risk assessment process did not call for a modification of the allowed amounts of E129, the obligation of Art. 24 of REG 1333/2008 (European Commission 2008b) to include the additional information 'may have an adverse effect on activity and attention of children' can have, in practice, the same effect as a ban on these products. A significant reduction in the amount of E129 allowed in non-alcoholic beverages would help to protect young consumers. Moreover, the data above highlight that if a risk does exist, it often comes from beverages claiming to have healthy properties on their labels. Major restrictions from this point of view would be very useful.

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