

Contents lists available at ScienceDirect



Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Certified food dyes in over the counter medicines and supplements marketed for children and pregnant women



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ARTICLE INFO

Keywords: FD&C dyes Over-the-counter medicine Vitamins Gummies High performance liquid chromatography

ABSTRACT

Food, Drug, & Cosmetic (FD&C) dyes are synthetic color additives used in food, prescription drugs and over-thecounter medicines (OTCs). Consumption of FD&C dyes has been associated with neurobehavioral behavior in some children. The amount of dye used in commercial products is proprietary, making it difficult to assess dietary intake and determine exposure in children. To date, no studies have examined FD&C dyes in OTCs or vitamins in the United States. To address this, FD&C Red No. 40, Yellow No. 5, Yellow No. 6, Blue No. 1, and Blue No. 2 levels were measured in prenatal vitamin tablets, children's chewable and gummy vitamins, pain reliever tablets and syrups, and cough/cold/allergy tablets and syrups. Dyes were isolated using solid phase extraction (SPE) and quantified by high performance liquid chromatography (HPLC). Dye levels varied between products with highest levels in pain reliever and cough/cold/allergy syrups. Significant variability was observed within some brands. Degradation of Red No. 40, Blue No. 1, and Yellow No. 6 was observed in the vitamin gummies. Intake of FD&C Red No. 40 is two times the US FDA ADI (accepted daily intake) for some children's pain reliever syrups and almost three times the US FDA ADI for some cough/cold/allergy syrups.

1. Introduction

Food, Drug, & Cosmetic (FD&C) dyes are synthetic color additives approved for use in food, drugs, and cosmetics to correct natural color, enhance appearance, and provide color identity (U.S. Food and Drug Administration, 2017). FD&C straights are dyes that are water-soluble powders, granules, or liquids not mixed with or chemically reacted with any other substance. FD&C lakes are the products of FD&C straights coated onto an insoluble alumina material. FD&C aluminum lakes are oil-dispersible and tint through dispersion. The Food & Drug Administration (FDA) certifies all synthetic batches of straights and lakes used as FD&C color additives. The amount of food dyes (i.e. straights and aluminum lakes) being certified has risen from 12 mg/capita/day in 1950 to 68 mg/capita/day in 2018 (Stevens et al., 2014). In 2019, the FDA certified 10.4 million kilograms of FD&C food dyes. (U.S. Food & Drug Administration) Only 3–5% of the certified dyes are exported and the remaining 95–97% are used in US products. Certified dyes can be used in food products for humans and animals, for cosmetic products, and in prescription drugs and over-the-counter medications (OTCs). The amounts of dye used in commercial products is proprietary and not disclosed on the product label.

Currently, there are seven FD&C dyes approved for use in foods which include FD&C Yellow No. 5 (Tartrazine), Yellow No. 6 (Sunset Yellow), Red No. 40 (Allura Red), Blue No. 2 (Indigo Carmine), Blue No. 1 (Brilliant Blue), Green No. 3 (Fast Green), and Red No. 3 (Erythrosine). Amaranth (formerly FD&C Red No. 2) is no longer approved for use in the U.S. (Fig. 1).

FD&C dyes are frequently added to vitamins and OTCs to enhance their appeal and provide brand identity. Many of the active components of OTCs are extremely bitter and must be masked using flavoring agents. Frequent flavors used in OTCs marketed to children include grape, cherry, and bubblegum. FD&C dyes are used to create color in OTCs in order to associate the visual appearance with the appropriate flavor (Pérez-Ibarbia et al., 2016). A survey of food items marketed

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https://doi.org/10.1016/j.fct.2020.111499

Received 25 February 2020; Received in revised form 1 June 2020; Accepted 3 June 2020 Available online 27 June 2020

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Abbreviations: ADI, Accepted Daily Intake; AFCs, Artificial Food Colors; EU, European Union; EFSA, European Food Safety Authority; FAO, Food and Agriculture Organization; FD&C, Food Drug & Cosmetics; HPLC, High performance liquid chromatography; JECFA, Joint FAO/WHO Executive Committee on Food Additives; OTCs, Over-The-Counter Medicines; PDA, photo diode array; SPE, Solid Phase Extraction; FDA or US FDA, United States Food and Drug Administration; WHO, World Health Organization

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Fig. 1. Chemical structures of 7 FD&C approved dyes for use in foods and amaranth (internal standard).

toward children established that the most frequently used dyes in these products include FD&C Red No. 40, Blue No. 1, Yellow No. 5 and Yellow No. 6 (Batada and Jacobson, 2016). There is no comparable survey on OTCs marketed to children.

The concept of an acceptable daily intake (ADI) was established in 1961 by a Joint World Health Organization (WHO)/Food and Agriculture Organization of the United Nations (FAO) Expert Committee on Food Additives (JECFA) to protect consumer's health and aid international food trade (Lu, 1988). An ADI is the amount of a specific substance (e.g. food additive) that can be consumed on a daily basis over a lifetime without an increased health risk. The ADI for the FD&C dyes are established by the US FDA. A similar ADI is established for the European Union (EU) by EFSA (European Food Safety Authority) and JECFA informs ADIs at an international level. A summary of the current ADI values set be JECFA, EFSA, and the US FDA is given in Table 1 (Lehto et al., 2017). ADIs are periodically evaluated and adjusted.

Attention deficit hyperactivity disorder (ADHD) is one of the most common behavioral disorders in children, affecting 6.1% of school-aged children between 1997 and 1998 and 10.2% of school-aged children

Table 1

Accepted daily intake (ADI) of selected FD&C dyes comparing US FDA, JECFA, and EFSA recommendations.^a.

FD&C Dye	US FDA	JECFA	EFSA
	ADI (mg/kg bw/	ADI (mg/kg bw/	ADI (mg/kg bw/
	d)	d)	d)
FD&C Yellow No. 5	0–5	07.5	0-7.5
FD&C Yellow No. 6	0–3.75	0–4	0-4
FD&C Red No. 40	0–7	0–7	0-7
FD&C Blue No. 2	0–2.5	0–5	0-5
FD&C Blue No. 1	0–12	0–6	0-12.5

^a Leto, S. et al., 2017 [7].

between 2015 and 2016 (Xu et al., 2018). In the early 1970s, the pediatric allergist Dr. Benjamin Feingold suggested a link between ADHD and the intake of synthetic food dyes (Feingold, 1975). Since this time, several studies have demonstrated a link between food dye intake and hyperactive behavior in children whereas others have not (Schab and Trinh, 2004; McCann et al., 2007; Lok et al., 2013). For example, the Southampton Study (2007) demonstrated a significant increase in hyperactivity for all children (with no missing data) who consumed 85% of a drink mixture containing Allura Red [E129], Sunset Yellow [E110], Tartrazine [E102], and Quinoline Yellow [E104] in various mixtures alongside several artificial food colors (AFCs) not approved for use by the US FDA (Carmoisine [E122] and Ponceau 4R [E124]) (McCann et al., 2007). Quinoline Yellow is the same structure as D&C Yellow No. 10 and identified as such when batch certified by the US FDA. D&C Yellow No. 10 is not permitted for use in foods in the US, but is allowed in prescription drugs and OTCs. In July of 2008 the European Parliament released a document announcing the required labelling of AFCs on food products (European Parliament, 2008). Conversely, in March of 2011, the US FDA Food Advisory Committee voted 8-6 to not recommend requiring any warning label on products containing FD&C color additives (Arnold et al., 2012). To date, there is no information on the bioavailability of FD&C dyes when consumed as aluminum lakes as compared with straights however; this has the potential to influence bioavailability. Additionally, no studies to date address how the food matrix (e.g. given with or without food; given with high protein food vs given with liquid beverage) may influence the bioavailability of food dyes. However, it is well established that the bioavailability of natural pigments (e.g. anthocyanidins, carotenoids) and most pharmaceuticals vary depending upon the food matrix and gut contents (Yang et al., 2011a; Failla et al., 2019; Khan and Singh, 2016).

To understand any potential health risk from the consumption of food dyes, accurate knowledge of dietary intake is needed to ensure thorough exposure assessments. Exposure assessments for color additives in the US have relied on quantifying FD&C dyes in a range of foods, including foods commonly consumed by children, and linking that information to food consumption data from the National Health and Nutrition Examination Survey (NHANES) (Center for Disease Control and National Center for Health Statistics, 2020). For example, Bastaki et al. (2017) used the 2-day food consumption recall from What We Eat In America (WWEIA) from the National Health and Nutrition Examination Survey (NHANES) data from 2009 to 2012, whereas Doell et al. (2016) utilized the NHANES data from 2007 to 2010 for a 2-day consumption survey and 10-14 day consumption survey using NPD Group, Inc. National Eating Trends- Nutrient Intake Database (NPD NET-NID) (Doell et al., 2016; Bastaki et al., 2017). Both studies had comparable results highlighting drinks as a primary food source of dye intake for all age groups, while breakfast cereals, decoration/chips for baking, and ice cream cones were highest in food dye content, though consumption was lower overall. The dyes contributing the highest exposure in all age groups examined include FD&C Red No. 40, Yellow No. 6, and Yellow No. 5 (Doell et al., 2016). These studies estimated

that exposure levels were below the ADI for each dye based upon consumption (Bastaki et al., 2017). A recent study of Korean foods demonstrated similar results (Lim et al., 2019).

To date, only one study from Slovakia assessed the amount of FD&C dye from OTCs and vitamins (not stated if vitamins were for children or adults) (Šuleková et al., 2016). The levels of FD&C dyes found in this study did not exceed maximal permissible levels for EFSA. With the exception of the study in Solvakia, all previous studies of FD&C dye exposure focus only on food intake and do not include OTCs or children's vitamins. However, assessing the amount of dye in OTCs and vitamins is critical towards understanding their contribution to total FD &C dye intake, especially given that these products can be consumed acutely (e.g. pain relief), subchronically (e.g. allergy relief, pain relief) and chronically (e.g. vitamins).

2. Materials and methods

2.1. Chemical reagents and standards

The analytical standards were purchased from Millipore Sigma (Missouri, USA) for each of the following food dyes: FD&C Yellow No. 5 (Tartrazine), Yellow No. 6 (Sunset Yellow FCF), Red No. 40 (Allura Red AC), Blue No. 2 (Indigo Carmine), Blue No. 1 (Brilliant Blue FCF), Green No. 3 (Fast Green), Red No. 3 (Erythrosine) and Amaranth (formerly FD &C Red No. 2). Waters Oasis WAX 3 cc Vac Cartridges (60 mg sorbent per cartridge, 60 µm Particle Size) were purchased from Neta Scientific (New Jersey, USA). Ammonium acetate, HPLC Grade was purchased from Spectrum Chemicals (California, USA). VWR provided the formic acid (High Purity Grade), methanol (HPLC Grade) while acetonitrile, HPLC Grade, > 99.9% was purchased from Millipore Sigma (Missouri, USA). Ammonium hydroxide solution, ACS Reagent 28.0–30.0% was purchased from Millipore Sigma (Missouri, USA).

2.2. HPLC conditions

HPLC analysis was performed on an Agilent 1200 HPLC system coupled with a photodiode array (PDA) detector (Agilent Technologies, Memphis, TN) using an Agilent InfinityLab Poroshell 120 EC-C₁₈ column (250 mm × 4.6 mm, i. d. with 4 µm particle diameter, Agilent Technologies, Memphis, TN). Resolution was achieved using a mobile phase composed of A (10 mmol L⁻¹ ammonium acetate in water) and B (acetonitrile) with a gradient elution of 0 min (3% B), 0–2 min (3% B), 2–5 min (10% B), 5–10 min (30% B), 10–12 min (33% B), 12–15 min (3% B), 15–17 min (3% B) at a flow rate of 1 mL min⁻¹. The sample injection volume was 50 µL. The column temperature was maintained at 25 °C. The full scan PDA was run over a range of 200–650 nm. Peaks were detected at 420 nm, 480 nm, 520 nm, 609 nm, and 620 nm. The identity of each peak was confirmed by the retention time and spectral characteristics of the corresponding standards.

2.3. Standards and linearity for quantification

The standard solution mixture was used to establish an eight point calibration curve at concentrations of 0.25, 0.5, 1, 2.5, 5, 10, 25, 50 mg L⁻¹. The mixture included analytical standards of FD&C Yellow No. 5, FD&C Yellow No. 6, FD&C Red No. 40, FD&C Blue No. 2, and FD &C Blue No. 1. Internal calibration was established to correct for instrument error and normalize integration. Amaranth was used as the internal standard (Fig. 1).

2.4. Limit of detection and limit of quantification

The limits of detection and quantification were determined using EURACHEM: The Fitness for Purpose of Analytical Methods, Second Edition 2014 (Magnusson and Örnemark, 2014). Ten injections of a low concentration of each standard solution was evaluated for their

standard deviation. The limit of detection (LOD) was accepted at 3 times the standard deviation and the limit of quantification (LOQ) at 10 times the standard deviation.

2.5. Syrups/gummy vitamins/vitamins extraction

Three grams of syrup or gummy vitamin were placed in a 50 mL conical tube and MilliQ water was added for a final volume of 30 mL. For vitamin tablets, 0.5-1 g samples were placed in a 50 mL conical tube and MilliQ water was added for a final volume of 10 mL. Each sample was ultrasonically extracted at 55 \pm 5 °C for 30 min with intermittent vortexing every 10 min. The homogenous samples were centrifuged for 10 min at 4000 RPM for the syrup and gummy vitamin samples, while all other samples were centrifuged at 5000 RPM. The supernatant (3 mL) was loaded on an Oasis WAX SPE cartridge (3 cc, $60 \text{ mg} \times 60 \text{ um}$) conditioned with 1 mL of methanol followed by 1 mL of water. After loading, the cartridge was washed with 1 mL of 2% formic acid in water and 1 mL of methanol. The samples were eluted with 2 mL of 5% ammonia hydroxide in methanol and dried (Savant SpeedVac, ThermoFisher, Massachusetts, USA). The dried samples were spiked with 10 μ L of internal standard (amaranth at 250 μ g/mL) and reconstituted in 500 µL of MilliQ water for HPLC analysis.

2.6. Vitamins/pain reliever/allergy tablets extraction

A composite was made from each sample by grinding 2-4 tablets together with a mortar and pestle. A 0.1 g sample of the composite was transferred to a 2 mL centrifuge tube containing 0.01 g of EDTA. An aliquot of 0.25 M NaOH was added for a final volume of 2 mL. The samples were vortexed for 30 s and ultrasonically extracted at 40 ± 5 °C for 30 min with intermittent vortexing every 10 min. The samples were centrifuged for 10 min at 12,000 RPM. The supernatant was transferred to a 2 mL centrifuge tube and the pH was adjusted with a 5% formic acid solution to pH 6-7. The sample (1 mL) was loaded on an Oasis WAX SPE cartridge (3 cc, 60 mg \times 60 μ m) conditioned with 1 mL of methanol followed by 1 mL of water. After loading, the cartridge was washed with 1 mL of 2% formic acid in water and 1 mL of methanol. The samples were eluted with 2 mL of 5% ammonia hydroxide in methanol and dried (Savant, Massachusetts, USA). The dried samples were spiked with 10 μ L of a 250 μ g/mL solution of amaranth and reconstituted in 500 µL of MilliQ water for HPLC analysis.

2.7. Spike recovery

The extraction methods were evaluated by spike recovery of a blank matrix, in this case a clear allergy syrup and clear gummy candy. Matrix blanks were spiked at three concentrations 0.75 mg L^{-1} , 7.5 mg L^{-1} ,

Table 2

Sampling scheme for prenatal/children's vitamins and children's OTCs.

and 15mgL^{-1} of standard mixture and repeated in triplicate.

2.8. Over the counter medicine and vitamin samples

A range of commercial OTCs and vitamins were purchased from Target, CVS Pharmacy, WalMart, and RiteAid Pharmacy across California, Indiana, and Georgia. Samples included prenatal vitamins tablets, children's chewable and gummy vitamins, children's pain reliever tablets and syrups, and children's cough/cold/allergy tablets and syrups. Three different lots of each brand were purchased. Each lot was extracted and analyzed in duplicate to give analytical replication. Lots were identified by expiration date and/or manufacturing codes. Two composite samples from each lot were made (e.g. using 2–4 tablets or gummies from each lot). The children's vitamin tablets and gummies came in 3 colors per bottle. Each color was analyzed in duplicate from each lot. The tablets were ground with a mortar and pestle prior to weighing out samples. To ensure homogeneity, syrups were vortexed 30 s before sampling.

2.9. Food dye exposure calculation

The amount of dye quantified was used to calculate how much would be consumed by an average weight child at 6 to < 11 years old (31.8 kg), as determined from EPA Exposure Factors Handbook from September 2011 (U. S. EPA, 2011).

The equation to evaluate syrups is as follows:

$$\frac{Dye (mg)}{Body Weight (kg)} per day = \frac{mg of dye}{mL of syrup} * mL of dose per day * \frac{1}{bodyweight (kg)}$$

The equation to evaluate tablets and gummies if as follows:

$$\frac{Dye (mg)}{Body Weight (kg)} per \ day = \frac{mg \ of \ dye}{kg \ of \ tablet/gummy} * \ kg \ of \ dose \ per \ day$$

$$* \ \frac{1}{bodyweight (kg)}$$

These results can be directly compared to the ADI provided by the US FDA.

3. Results/discussion

The OTC and vitamin products sampled (Table 2) were chosen by first identifying products that had a minimum of 3 national brands within each category (i.e. vitamins, pain relievers and cold/allergy) and that could be purchased with 3 replicate lots of each brand. Identifying three replicate lots, was not always possible for some brands which

OTC/Vitamin Category	No. Brands	Lots	Colored Units in Product	No. of Replicates from Each Lot	Total Samples
Vitamins					
Prenatal Vitamin Tablets	5	3		2	30
Children's Chewable Vitamin Tablets	5	3	3	2	90
Children's Vitamin Gummies	3	3	3	2	54
Pain Relievers					
Children's Pain Reliever Tablets	3	3		2	18
Children's Pain Reliever Syrup	5	3		2	30
Cold/Allergy					
Children's Cough/Cold/Allergy Tablets	4	3		2	24
Children's Cough/Cold/Allergy Syrup	5	3		2	30

Table 3

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FD&C and D8	C dves listed	on each sample	label ^a .
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Sample Identification	Flavor	FD&C Dyes of Interest Listed
Vitamins		
Prenatal Vitamir	15	
1 2		FD&C Blue No. 1, FD&C Red No. 40 FD&C Red #40 Dye, FD&C Red #40 Lake, FD
3		FD&C Blue No. 2 Lake, FD&C Red No. 40 Lake, FD&C Yellow No. 6 Lake
4 5		FD&C Red #40 Lake, FD&C Yellow #6 Lake FD&C Blue No. 2 Lake, FD&C Red No. 40 Lake, FD&C Yellow No. 6 Lake
Children's Table	t Vitamins	
1		FD&C Blue No. 2 Lake, FD&C Red No. 40 Lake, FD&C Yellow No. 6 Lake ED&C Blue #2 Aluminum Lake, ED&C Red
-		#40 Aluminum Lake, FD&C Yellow #6 Aluminum Lake FD&C Blue No. 2 Lake, FD&C Red No. 40
4		Lake, FD&C Yellow No. 6 Lake FD&C Red #40 Aluminum Lake, FD&C Yellow #6 Aluminum Lake, FD&C Blue #2
5		Aluminum Lake Blue 2 Lake, Red 40 Lake, Yellow 6 Lake
Children's Gumn	ny Vitamin	
1		FD&C Blue #1, FD&C Red #40, FD&C Yellow #5
2 3		FD&C Blue #1, FD&C Red #40 FD&C Blue #1, FD&C Red #40, FD&C Yellow #6
Pain Relievers		
Children's Pain	Reliever Tablets	
1 2	Grape Grape	FD&C Blue #1, D&C red #30 Lake FD&C Blue No. 2 Aluminum Lake, D&C Red
3	Grape	No.30 Aluminum Lake FD&C Blue No. 1 Aluminum Lake, D&C Red No.27 Aluminum Lake
Children's Pain F	Reliever Syrup	
1 2 3 4 5	Berry Grape Bubblegum Grape Bubblegum	FD&C Red No.40, D&C Yellow No. 10 FD&C Blue No. 1, FD&C Red No.40 FD&C Red No. 40, D&C Red No. 33 FD&C Blue No. 1, D&C Red No. 33 FD&C Red #4, D&C Red #33
Cold/Allergy		
Children's Allerg	y Tablets	
1	Grape	FD&C Blue No. 2 Aluminum Lake, D&C Red No. 27 Aluminum Lake
2	Grape	FD&C Blue No. 1 Aluminum Lake, D&C Red No. 30 Aluminum Lake, D&C Red No.7 Calcium Lake

No. 27 Aluminum Lake

No. 27 Aluminum Lake

FD&C Blue No. 2 Aluminum Lake, D&C red

FD&C Blue No. 1, FD&C Red No. 40

FD&C Blue No. 1, FD&C Red No. 40

FD&C Red No. 40, D&C Red no.33

Grape

Grape

Cherry

Grape

Children's Allergy/Cough/Cold Syrup

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Sample Identification	Flavor	FD&C Dyes of Interest Listed
4	Very Berry	FD&C Blue #1, FD&C Red #40, D&C Red #30
5	Grape	FD&C Blue No. 1, FD&C Red No. 40

as listed verbatim on ingredient label.

influenced sampling. The FD&C dyes listed on the labels of these products is given in Table 3. Out of the allowable FD&C dyes for these products, all were identified in samples with the exception of FD&C Red No. 3 and Green No. 3. The five identified FD&C dyes were quantified using high performance liquid chromatography (HPLC) and UV-vis detection at their respective absorption maximum (Table 4). HPLC conditions were optimized to give complete resolution between dyes. A representative HPLC chromatogram obtained at 420 nm is given in Fig. 2.

The t_R, linear dynamic range (LDR), and limit of detection (LOD) for each dye is given in Table 4. Standard curves for all FD&C Dyes were linear over the range of 0.25–50 mg $\rm L^{-1}$ with exception of FD&C Blue No. 2 which was linear over 0.15–50 mg L^{-1} and FD&C Red No. 40 which was linear over 0.25–100 mg L^{-1} . The correlation coefficient (R^2) was greater than 0.999 for all dyes, with exception to FD&C Blue No. 1, which was > 0.994. There is limited information available on the extraction of FD&C dye from OTCs and vitamins. The one study available, examined dye levels in gelatin and tablet vitamins using HPLC without SPE extraction for sample clean-up and concentration (Šuleková et al., 2016). Yang et al. (2011) established an extraction method using SPE for concentration of FD&C aluminum lake dyes in chewing gum using SPE and HPLC (Yang et al., 2014). This method was applied to the analysis of dyes in jelly and gummy candy (Yang et al., 2011b). The extraction method from Yang et al. (2014) suggested using ammonium sulfate for protein precipitation from gelatin or another food additive. Herein, this step did not have any significant benefits and was omitted. The spike and recoveries of dyes ranged from 71.0% to 127.2% and are given in Table 5.

Prenatal and children's vitamin tablets and gummies came in multiple colors, therefore the FD&C dyes in each different color tablet or gummy were assessed independently (Table 6). Prenatal vitamins evaluated were either softgel (brand 1) or tablet form (brands 2-5). Prenatal vitamins contained four of the five FD&C dyes examined across samples (Table 3) with levels ranging between 0.59 \pm 0.10 mg kg⁻¹ (Blue No. 2) to 197.83 \pm 57.75 mg kg⁻¹ (Red No. 40) (Table 6). The levels of FD&C Red No. 40 were highest per tablet in the softgel vitamins $(0.35 \pm 1.01 \text{ mg tablet}^{-1})$, as seen in Table 7. Prenatal vitamin consumption is considered a highly frequent event since women are instructed to take one or two a day, depending on the product. US FDA ADI's are not specifically established for sensitive populations, such as children and pregnant women. Our data suggest that food dye exposure from the recommended dosage of prenatal vitamins would not exceed the US FDA ADI for any of the measured FD&C dyes. The potential effects of prenatal food dye exposure to the fetus are unknown.

Similar to prenatal vitamins, children's chewable vitamin tablets are consumed daily if taken as instructed. All brands of children's chewable vitamin tablets came in three distinct colors within the bottle (red, purple, and orange). Levels of FD&C Yellow No. 6 ranged between 15.40 \pm 13.70 and 1801.35 \pm 197.47 mg kg⁻¹ across brands and colors (Table 6). Levels of FD&C Red No.40 ranged between 58.21 ± 15.70 and 2847.75 ± 543.76 mg kg⁻¹ across brands and colors whereas levels of FD&C Blue No. 2 were between 10.49 \pm 2.70 and 419.60 \pm 22.97 mg kg⁻¹ (Table 6). If considering the amount of dye consumed per dose (i.e. tablet), the amount of FD&C Red No. 40 ranged between 0.27 and 3.77 mg tablet⁻¹ indicating that no brands would result in doses of FD&C Red No. 40 (or any of the other dyes)

Table 4			
Summary of FD&C	dyes used	for analytical	standards.

Peak	RT (min)	FD&C Dye	Common Name	λ_{max} (nm)	LDR (mg L^{-1})	R (Stevens et al., 2014)	LOD	LOQ
1	7.576	FD&C Yellow No. 5	Tartrazine	420	0.25–50	0.9999	0.0015	0.0049
3	9.059	FD&C Blue No. 2	Indigo Carmine	609	0.15–50	0.9996	0.0005	0.0018
4	10.645	FD&C Yellow No. 6	Sunset Yellow	480	0.25–50	0.9999	0.0010	0.0033
5	11.313	FD&C Red No. 40	Allura Red	520	0.25–100	0.9995	0.0018	0.0061
6	13.408	FD&C Blue No. 1	Indigo Carmine	620	0.25–50	0.9946	0.0039	0.0130



Fig. 2. Chromatogram at 420 nm for FD&C dye Analytical Standards; 1, FD&C Yellow No. 5; 2, FD&C Red No. 2; 3, FD&C Blue No. 2; 4, FD&C Yellow No. 6; 5, FD&C Red No. 40; 6, FD&C Blue No. 1.

above the US FDA ADI for an average 45 kg child of 11 years (Table 1).

Significant variability was observed "between lots" of the same brand, and for one case (red tablet vitamins in brand 1), there was "within lot" variability. Variability within lots of the same brand reflect inconsistent manufacturing, whereas variability between lots of the same brand can reflect many things including inconsistent manufacturing, degradation due to shipping and storage difference, and variable exposure to heat and light during shelf-life. For example, the levels of FD&C Yellow No. 6 measured in the red vitamin tablets of brand 3 were as follows: lot 1, 46 mg kg^{-1} and 51 mg kg- (U.S. Food and Drug Administration, 2017); lot 2, 25 mg kg⁻¹ and 26 mg kg⁻¹; lot 3, 1.4 mg kg⁻¹ and 1.2 mg kg⁻¹ (data not shown). The in-lot variability was low with an RSD < 8% for these samples, whereas the between-lot variability was high with an RSD 77%. In-lot variability was also observed in brand 1 in the red vitamin tablets. For example, the levels of FD&C Red No. 40 in brand 1 ranged between 660.75 and 1083.91 mg kg⁻¹ for five samples yet was 71.75 mg kg⁻¹ in one sample. In-lot variability and between-lot variability for manufactured products needs to be understood and considered when using values to make exposure assessments.

Children's gummy vitamins came in combinations of red, yellow, and green or orange, purple, and red within a bottle. Gummy vitamins were analyzed separately by color described as above (Table 6). FD&C Yellow No.5 and 6, Red No. 40, and Blue No. 1 were present in these vitamins. On average the levels of measured dyes were low in the gummy vitamins, and suggest that daily consumption would not significantly contribute to the ADI of any of the FD&C dyes evaluated. Significant in-lot and between-lot variability was observed in these samples. For example, the different lots of gummy vitamins from brand 1 had similar expiration dates (May 2020, September 2020, and October 2020), yet a single outlier influenced their variability overall for the brand. Within brand variability was low (< 22%) for the Red No.

Table	5	
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40 dye in red vitamins of all brands examined. However, variability in Red No. 40 increased in the purple vitamins (RSD 45% in brand 2 and 39% in brand 3).

The gummy vitamins from brands 2 and 3 showed significant between-lot variability, which was related to the expiration date. In these brands, the color of the vitamins were visibly faded in the samples with early expiration dates as compared with those of later expiration dates. For example, in brand 2 the lot expiring September 2019 had an average of 28.82 mg kg⁻¹ FD&C Red No. 40 in the purple vitamin whereas the lots expiring in June 2020 and September 2020 contained 94.11 and 94.75 mg kg⁻¹ respectively. For brand 3, FD&C Yellow No. 6 in the orange vitamin decreased from average values of 24.74 mg kg⁻¹ and 21.71 mg kg⁻¹ in lots expiring in March and January 2020 to an average value of 4.06 mg kg $^{-1}$ in the lot expiring in November 2019. FD&C Red No. 40 in the purple gummy vitamins of brand 3 also decreased from average values of 85.46 mg kg^{-1} and 70.58 mg kg^{-1} for the March and January 2020 lots as compared to 28.99 mg kg⁻¹ in the November 2019 lot. FD&C Red No. 40 and Yellow No. 6 are azo dyes. The azo bond can be reduced to form amine cleavage products. Reduction of the azo bond results in color fade and is accelerated in the presence of ascorbic acid (Parkinson and Brown, 1981). The reductive action of ascorbic acid is enhanced by UV light. Brands 1 and 2 contained 15 mg of Vitamin C per gummy, while Brand 3 contained 62.5 mg of vitamin C per gummy based upon the manufactures label. Although the dye degradation does not correlate with the level of ascorbic acid listed on the label, the label gives no information on the vitaminer (i.e. form of ascorbic acid used in the product) however this can influence the activity of ascorbic acid.

Understanding how manufacturing and product formulation influence color stability and variability in products is critical for making accurate intake assessments. Many things can influence levels of dyes in manufactured products of the same brand including manufacturer

Concentration	FD&C Yellow No. 5	FD&C Yellow No. 6	FD&C Red No. 40	FD&C Blue No. 2	FD&C Blue No. 1
0.75 mg L ⁻¹ 7.5 mg L ⁻¹ 15 mg L ⁻¹	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$

Table 6

Summary of dye content in (mg of dye per kg of sample).

Brand	Color	FD&C Yellow No. 5 Avg. $(mg kg^{-1})$	%RSD	FD&C Yellow No. 6 Avg.(mg kg	%RSD	FD&C Red No. 40 Avg.(mg kg ⁻¹)	%RSD	FD&C Blue No. 2 Avg.(mg kg ⁻¹)	%RSD	FD&C Blue No. 1 Avg.(mg kg ⁻¹)	%RSD
Vitamii	ns										
Prenat	al Vitami	ns									
1 2 3 4 5	Purple Pink Pink Pink Pink			$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	116% 25% 35% 8%	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	29% 10% 19% 43% 7%	bdl 0.59 ± 0.10	bdl 17%	24.55 ± 4.51	18%
Childre	en's Vitan	nin Tablets									
1	Red Purple					697.21 ± 314.45 1060.34 ± 495.70	45% 47%	419.60 ± 22.97	5%		
2	Orange Red Purple			134.95 ± 37.06 15.40 ± 13.70	27% 89%	719.91 ± 354.08 58.21 ± 15.70	49% 27%	193.84 ± 19.95	10%		
3	Red Purple			147.56 ± 10.70 25.07 ± 19.29	7% 77%	2847.75 ± 543.76 271.09 ± 44.49	19% 16%	329.56 ± 26.18	8%		
4	Red Purple Orange			112.36 + 11.97	11%	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	6% 23%	364.22 ± 50.61	14%		
5	Red Purple Orange			83.12 ± 25.01	30%	257.51 ± 104.82 148.69 \pm 56.93	41% 38%	$10.49 ~\pm~ 2.70$	26%		
Childre	en's Vitan	in Gummies									
1	Red Yellow Green	22.84 ± 2.49	11%			104.33 ± 10.30	10%			3.11 ± 0.57 0.93 ± 0.44 11.55 ± 2.80	18% 47% 24%
2	Purple Red Orange			16.84 ± 9.15	54%	$\begin{array}{rrrrr} 72.56 \ \pm \ 32.50 \\ 27.06 \ \pm \ 5.01 \end{array}$	45% 19%			3.23 ± 0.37	12%
	Purple Red					61.67 ± 24.22 58.14 ± 13.03	39% 22%			2.90 ± 0.45	16%
Pain R	elievers										
Childro	en's Pain	Reliever Tablets									
1 2 3	Purple Purple Purple							bdl	bdl	30.13 ± 3.00 14.74 ± 2.02	10% 14%
Childre	en's Pain	Reliever Syrups									
1 2 3	Red Purple Pink					8.66 ± 0.29 65.59 ± 2.40 19.77 ± 1.20	3% 4% 6%			11.87 ± 0.53	5%
4 5	Purple Pink					23.54 ± 1.33	6%			0.31 ± 0.14	44%
Cold/A	llergy										
Childre	en's Allerş	gy Tablets									
1 2	Purple Purple							bdl	bdl	19.62 ± 7.43	38%
3 4	Purple Purple							bdl bdl	bdl bdl		
Childre	en's Allers	gy/Cough/Cold Syrup									
1 2	Purple Red					32.59 ± 1.72 5.20 ± 0.32	5% 6%			5.43 ± 0.51	9%
3 4 5	Purple Red Purple					31.43 ± 0.90 69.88 ± 8.98 95.21 ± 4.42	3% 13% 5%			$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	5% 53% 6%

Table Averag	7 e and range of dye quantified in milligr:	ams per tablet	or milliliter.							
Bran	1 Color FD&C Yellow No. 5 Avg. (mg tablet ⁻¹)	Range	FD&C Yellow No. 6 Avg. (mg tablet ⁻¹)	Range	FD&C Red No. 40 Avg. (mg tablet ⁻¹)	Range	FD&C Blue No. 2 Avg. (mg tablet ⁻¹)	Range	FD&C Blue No. 1 Avg. (mg tablet ⁻¹)	Range
Vitan	uins									
Prení	ttal Vitamins									
,	Dumle				0.35 + 0.101	0 24-0 50			0.04 + 0.007	0.03_0.04
- 7	Pink		$1.2e-4 \pm 1.4e-4$	9.0e-6-3.8e-4	0.2 ± 0.019	0.17-0.23				000
З	Pink		0.02 ± 0.004	0.011 - 0.021	0.1 ± 0.018	0.007-0.12	bdl	bdl		
4 D	Pink Pink		0.02 ± 0.007 0.03 ± 0.006	0.009-0.026 0.020-0.036	0.16 ± 0.07 0.14 ± 0.01	0.06-0.24 0.13-0.15	0.0007 ± 0.0001	0.0006-0.0008		
Child	ren's Vitamin Tablets									
-	Red				0.87 ± 0.391	0.09-1.35				
	Purple				1.32 ± 0.616	0.79–2.35	0.52 ± 0.029	0.48 - 0.57		
2	Orange Red		0.17 ± 0.046 0.02 ± 0.017	0.119-0.247 0.0001-0.054	0.92 ± 0.45	0.13 - 1.35				
	Purple				0.07 ± 0.02	0.04 - 0.10	0.25 ± 0.025	0.22-0.28		
c	Orange		0.03 ± 0.014	0.161-0.201	0120 + 22 0	12 1 22 0				
o	Purple		070'0 I CT'0	100.00200.00	0.36 ± 0.059	2.03-4./1 0.25-0.43	0.44 ± 0.027	0.39-0.48		
	Orange		2.38 ± 0.261	2.098–2.777						
4	Red Durnle				1.52 ± 0.096 1.12 ± 0.254	1.45-1.66 0.65-1.30	0.45 + 0.063	0 37_0 53		
	r urpre Orange		0.14 ± 0.015	0.118-0.160	L07-0 - 7T-T	6C.1-CU.U	con.o - ct.o	00.0-10.0		
ъ	Red Č				0.46 ± 0.189	0.25-0.76				
	Purpie Orange		0.15 ± 0.045	0.092-0.216	0.2/ ± 0.103	0.12-0.40	c00.0 ± 20.0	0.01-0.03		
Child	ren's Vitamin Gummies									
1	Red				0.256 ± 0.027	0.207-0.290			0.008 ± 0.001	0.005-0.01
	Yellow 0.056 ± 0.007	0.049-0.0	72						0.002 ± 0.001	0.001-0.004
2	Green Orange								0.028 ± 0.007	0.017-0.037
	Purple				0.179 ± 0.081	0.038 - 0.265			0.008 ± 0.001	0.006-0.01
c:	Red Orange				0.066 ± 0.013 0.071 + 0.026	0.048-0.091 0.034-0.104				
	Purple Red		0.033 ± 0.018	0.008-0.051	0.122 ± 0.048	0.048-0.172			0.006 ± 0.001	0.004-0.006
Pain	Relievers									
Child	ren's Pain Reliever Tablets									
	Purple						-		0.04 ± 0.004	0.03-0.04
nσ	Purple						IDG		0.01 ± 0.001	0.008-0.012
Child	ren's Pain Reliever Syrup									
0	Red				1.15 ± 0.04^{a}	$1.11 - 1.22^{a}$				
00 M	Purpie Pink				$8.01 \pm 0.29^{\circ}$ 2.45 ± 0.15 ^a	7.66-8.50 2.21-2.66 ^a			1.45 ± 0.0/	-086.1-665.1
									(contin	ued on next page)

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Brand Color FD&C Yellow No. 5 Avg. (mg tablet ⁻¹)	Range	FD&C Yellow No. 6 Range Avg. (mg tablet ⁻¹)	FD&C Red No. 40 Avg. (mg tablet ⁻¹)	Range	FD&C Blue No. 2 Range Avg. (mg tablet ⁻¹)	FD&C Blue No. 1 Avg. (mg tablet ⁻¹)	Range
4 Purple 5 Pink			3.44 ± 0.19^{a}	3.21–3.72 ^a		0.039 ± 0.02^{a}	0.023-0.063 ^a
Cold/Allergy							
Children's Allergy Tablets							
1 Purple					bdl		
2 Purple 3 Purple					bdl	0.01 ± 0.004	70.0-10.0
4 Purple					Dal		
Children's Cough/Cold/Allergy Syrup							
1 Purple			4.20 ± 0.22^{a}	3.83-4.44 ^a		0.70 ± 0.07^{a}	0.594–0.777 ^a
2 Red			0.64 ± 0.04^{a}	$0.58 - 0.67^{a}$			
3 Purple			3.31 ± 0.09^{a}	$3.10 - 3.38^{a}$		0.59 ± 0.03^{a}	$0.536-0.619^{a}$
4 Red			9.29 ± 1.19^{a}	8.29–11.7 ^a		0.026 ± 0.01^{a}	$0.013 - 0.051^{a}$
5 Purple			10.47 ± 0.49^{a}	9.93–11.23 ^a		1.39 ± 0.08^{a}	$1.257 - 1.497^{a}$
^a Values in mg mJ. ⁻¹							

differences, shipping and storage conditions, and placement on the retailer shelf as this can affect product exposure to light and heat.

Children's pain relievers are available in tablets or as syrups in flavors such as grape and bubblegum. Pain relievers are typically consumed episodically and for short time periods however, in some situations sub-chronic and chronic use may occur. All pain reliever tablets examined herein contained only FD&C Blue No. 1 at levels less than $30.13 \pm 3.00 \text{ mg kg}^{-1}$ (Table 6) which is less than $0.04 \pm 0.004 \text{ mg}$ tablet ⁻¹. Although D&C (i.e. dyes permissible for use in drug and cosmetics only but not foods) were used in many of these products to aid in coloration, the levels of D&C were not measured in this study. However, future studies may want to consider the amount of these dyes in OTCs if a negative association is found between their consumption and behavior in children.

Children's pain reliever syrups contained FD&C Red No. 40 and Blue No. 1. The amount of FD&C Red No. 40 in these syrups ranged between $8.66 \pm 0.29-65.59 \pm 2.40 \text{ mg kg}^{-1}$ correlating to 1.15 ± 0.004 to $8.01 \pm 0.29 \text{ mg mL}^{-1}$. The dose of a pain reliever syrup is dependent upon body weight (age) and varies from 5 to 15 mL for children 2–11 years, 4–5 times a day depending upon brand. This means a 6 to < 11 year old child consuming brand 2 ($8.01 \pm 0.29 \text{ mg mL}^{-1}$) at the maximum recommend daily dose of 40–60 mL would consume 10.1–15.1 mg kg⁻¹ bw/d of Red No. 40. This is well above the 7 mg kg⁻¹ bw/d ADI (Table 1). Even the brand with the lowest amount of Red No. 40 (brand 1) would contribute 1.4–2.2 mg kg⁻¹ bw/d if the child receives the full 60 mL dose, which is 21–31% of the ADI for this dye. This data indicates that pain reliever syrups can be a significant source of Red No. 40 in children who use these products for acute, sub-chronic, and chronic pain relief.

Children's cough/cold/allergy medicine is available in tablets or as syrups. Syrups come in a variety of flavors and colors. All cough/cold/ allergy tablets examined contained only FD&C Blue No. 1 at levels less than 19.62 \pm 7.43 mg kg⁻¹ and FD&C Blue No. 2 but below the limit of detection (Table 6). The rest of the coloring came from the use of D&C dyes.

The dose of cough/cold/allergy syrups are dependent upon body weight, similar to the pain reliever syrups, and have a maximum dose of 10–20 mL for children 4–12 years in the brands examined. The amount of FD&C Red No. 40 in the pain cough/cold/allergy OTCs ranged from 5.20 \pm 0.32 to 95.21 \pm 4.42 mg kg⁻¹ (Table 6). Brands 4 and 5 contained significant amounts of Red No, 40 when considering total dose. For example, an average of 9.29 \pm 0.49 mg mL ⁻¹ (range 8.29–11.7 mg mL ⁻¹) was found in brand 4, and 10.47 \pm 0.49 mg mL⁻¹ (range 9.93–11.23 mg mL ⁻¹) was found in brand 5 (Tables 6 and 7). The recommended dose for brand 5 is 10 mL up to 6 times a day for a 6 to < 11 year old child. This means a 6 to < 11 year child consuming the maximum dose of brand 5 (60 mL of the syrup) would consume 19.8 mg kg⁻¹ of Red No. 40 in one day. This is almost three times the US FDA ADI of 7 mg kg⁻¹ day⁻¹.

These data indicate that in general vitamins are not a significant source of FD&C dyes when taken as directed. Pain reliever tablets and cough/cold/allergy tablets are also not significant sources of FD&C dyes. However, pain reliever syrups and cough/cold/allergy syrups can be a significant contributor of Red No. 40 when taken episodically, subchronically, or chronically and levels can be significantly above the US FDA ADI in children. It is still unclear the extent that FD&C dyes influence behavior in children. However, understanding the amount of FD &C dyes present in OTCs given to children when they are ill or suffering from chronic conditions such as allergies is important as these conditions can exacerbate behavioral changes in children. This information will be useful in helping to better understand variability in commercial products and assessment of FD&C dye exposure in children.

Disclaimer

The analyses reported in this paper were performed by the Mitchell

Laboratory at UC Davis under contract # 18-E0035 from the Office of Environmental Health Hazard Assessment, California EPA. The views expressed by the authors are their own and do not necessarily represent those of the Office of Environmental Health Hazard Assessment, the California Environmental Protection Agency, or the State of California.

CRediT authorship contribution statement

Arlie L. Lehmkuhler: Methodology, Formal analysis, Data curation, Writing - original draft, Writing - review & editing. Mark D. Miller: Conceptualization, Funding acquisition, Project administration, Writing - review & editing. Asa Bradman: Conceptualization, Writing - review & editing. Rosemary Castroina: Conceptualization, Writing - review & editing. Alyson E. Mitchell: Conceptualization, Data curation, Funding acquisition, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was funded by the Office of Environmental Health Hazard Assessment, California EPA (contract # 18-E0035). Authors would like to thank Ruwan Thilakaratne, and Mayela Gillian for assistance in sampling selection and market research, and Dr. Melanie Marty, Ph.D., Deputy Director for Scientific Affairs and Adjunct Assistant Professor at the University of California, Davis, Department of Environmental Toxicology for editorial comments and support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2020.111499.

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