Appendix E

Analysis of OTC drugs and vitamins
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The content of FD&C dyes in prenatal vitamins, children's vitamin tablets, children's vitamin gummies, children's pain reliever tablets, children's pain reliever syrups, children's allergy tablets, and children's cough/cold/allergy syrups was quantified using solid phase extraction (SPE) and high-performance liquid chromatography (HPLC) with a photometric diode array detector (HPLC-PDA) (Lehmkuhler et al., 2020; available at: Lehmkuhler et al., 2020, published article) . Three to five brands of each type of over-the-counter medicine (OTC) or vitamin were evaluated (Table 1). Vitamin tablets and gummy vitamins came in three colors for each brand. Therefore, the dye level in each color of vitamin was evaluated separately (Table 1).

Table 1. Sample scheme for prenatal/children's vitamins and OTC medicines

OTC/Vitamin Category	No. Brands	Lots	Colors	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

Syrups were homogenized and diluted with water prior to extraction. Gummy vitamins were homogenized with water and heated prior to extraction. Tablet vitamins (3) were crushed using a mortar and pestle and a 0.1 g sample of this composite was weighed out and diluted, prior to extraction. All samples were loaded onto a weak-anion exchange Oasis WAX SPE cartridge (60 mg, 3 mL, Waters). The cartridge was washed with 2% formic acid in water, and the dyes were eluted in 5% ammonia in methanol. Samples were dried using a SpeedVac concentrator (ThermoFisher) and brought up in 0.5 mL-1 mL of water prior to analysis. Dyes were separated and quantified using an Agilent 1200 HPLC system coupled with a 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). The mobile phase was 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. 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 authentic standards. Quantitation was achieved using external calibration of 0.25-50 µg/mL for FD&C Yellow No. 5, Yellow No. 6, and Blue No. 1 while Blue No. 2 used an external calibration of 0.15-50 µg/mL and Red No. 40 used 0.25-100 µg/mL for an external calibration curve.

Results

Dye levels varied between products with the highest levels in pain reliever and children's cough/cold/allergy syrup (Table 2).

Table 2. Ranges of FD&C Dye Amount (mg) Quantified for Each Category (per kg)

Category	FD&C Dye (mg/kg)				
	Yellow No. 5	*Yellow No. 6*	*Red No. 40*	*Blue No. 2*	*Blue No. 1*
Prenatal Vitamins		0.01-21.8	47.59-283.94	0.49-0.69	19.34-30.40
Children's Vitamin Tablets		1.16-2100.45	28.51-3561.09	7.81-461.56	
Children's Vitamin Gummies	19.62-27.71	3.90-25.75	2.76-108.83		0.41-14.73
Children's Pain Reliever Tablets				bdl	10.67-33.82
Children's Pain Reliever Syrup			8.31-67.75		0.18-12.94
Children's Allergy Tablets				bdl	10.68-33.69
Children's Cough/Cold/Allergy Syrup			4.72-102.08		0.10-13.61

Significant variability was observed within some brands. Degradation of FD&C Red No. 40, Blue No. 1 and Yellow No. 6 was observed in the children's vitamin gummies.

References – see References Section for Entire Report