Determination of Certified Color Additives in Food Products

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Outline

- Background
  - 2011 Food Advisory Committee recommendations
- Analytical method for FD&C color additives
- Sampling procedure for exposure assessment
- Contract laboratory analyses
- Method for estimating exposure

Background

- 2007 Southampton study
  - Children's consumption of color additives and possible adverse behavioral effects
    - Sunset Yellow (FD&C Yellow No. 6), Tartrazine (FD&C Yellow No. 5), Ponceau 4R, Carmoisine, Quinoline Yellow (D&C Yellow No. 10), Allura Red AC (FD&C Red No. 40)
    - Tested mixtures of color additives plus sodium benzoate
- 2008 CSPI citizen petition
  - Requests ban of eight certified color additives used in foods
  - Requests warning labels on foods containing these color additives

Background

- Certified color additives permitted for general use in food products
  - FD&C Blue No. 1
  - FD&C Blue No. 2
  - FD&C Green No. 3
  - FD&C Red No. 3
  - FD&C Red No. 40*
  - FD&C Yellow No. 5*
  - FD&C Yellow No. 6*

*These three included in Southampton study

Background

- In March 2011, FDA convened a Food Advisory Committee (FAC) to
  - consider available relevant data on the possible association between children's consumption of FD&C color additives in food and adverse behavioral effects
  - advise FDA on what action, if any, is warranted to ensure the safety of these color additives

FAC conclusions

- Causal link between children's consumption of FD&C color additives and adverse behavioral effects not established by available data
- Additional label information (i.e., warning label) unnecessary to ensure safe use of FD&C color additives

FAC recommendations

- Conduct additional research
  - Potential developmental and behavioral effects in children from exposure to FD&C color additives
- Perform comprehensive exposure assessment for FD&C color additives in foods
  - Focus on foods marketed to children

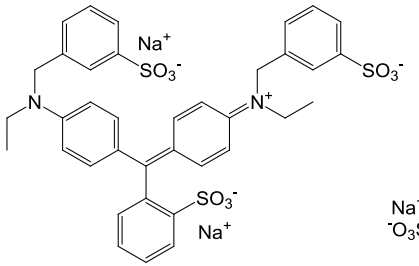
FDA's initial response to FAC recommendations

- New liquid chromatography method
  - Quantitative determination of FD&C color additives in various food matrices
  - Published in Journal of Agricultural and Food Chemistry (*J. Agric. Food Chem.* **2013**, 61, 3726−3736)

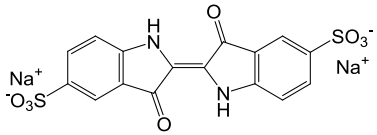
Liquid chromatography method

- Chemical structures
- Food matrices used to validate method
- Challenges
- Extraction procedure
- Analytical method
- Chromatograms

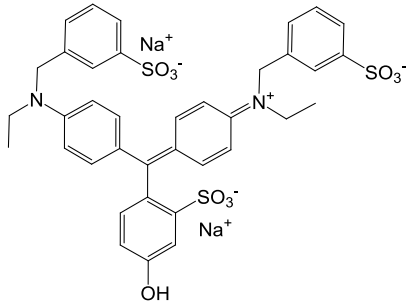
Chemical structures



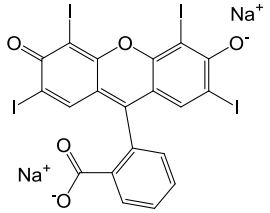
FD&C Blue No. 1



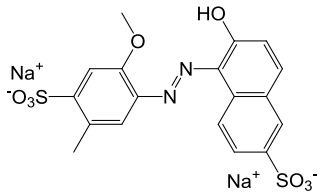
FD&C Blue No. 2



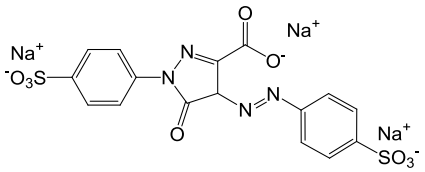
FD&C Green No. 3



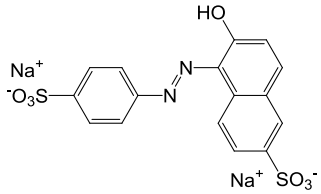
FD&C Red No. 3



FD&C Red No. 40



FD&C Yellow No. 5



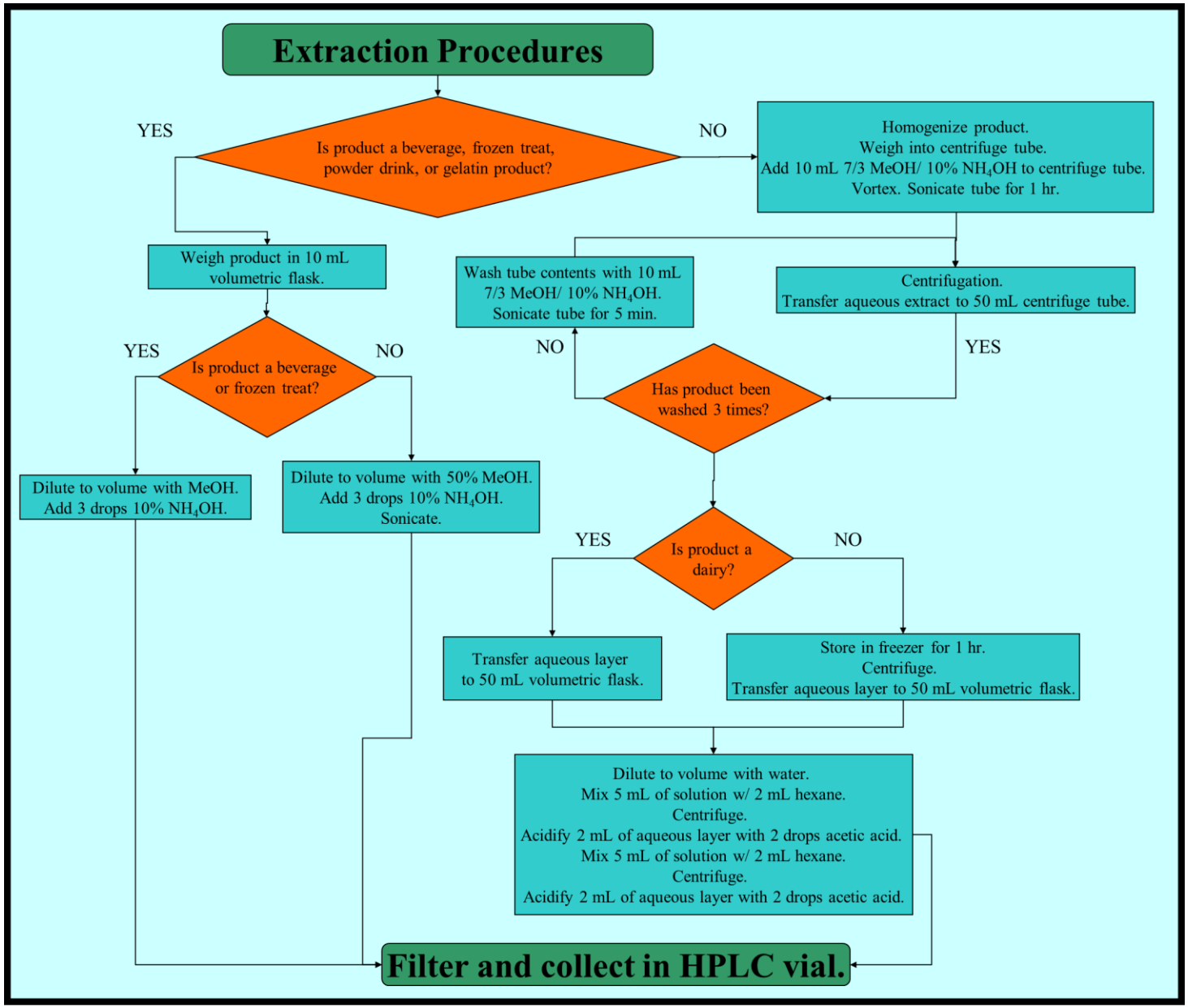
FD&C Yellow No. 6

Food matrix categories

- Beverages
  - Soda
  - Powdered drinks
  - Popsicles
- Dairy
  - Milk
  - Ice cream
  - Yogurt
- Sauces
  - Salad dressing
  - Jelly
- Sweets and spices
  - Chocolate
  - Gummies
  - Decorations
  - Seasonings
- Baked goods
  - Cookies
  - Cereal
  - Chips

Challenges

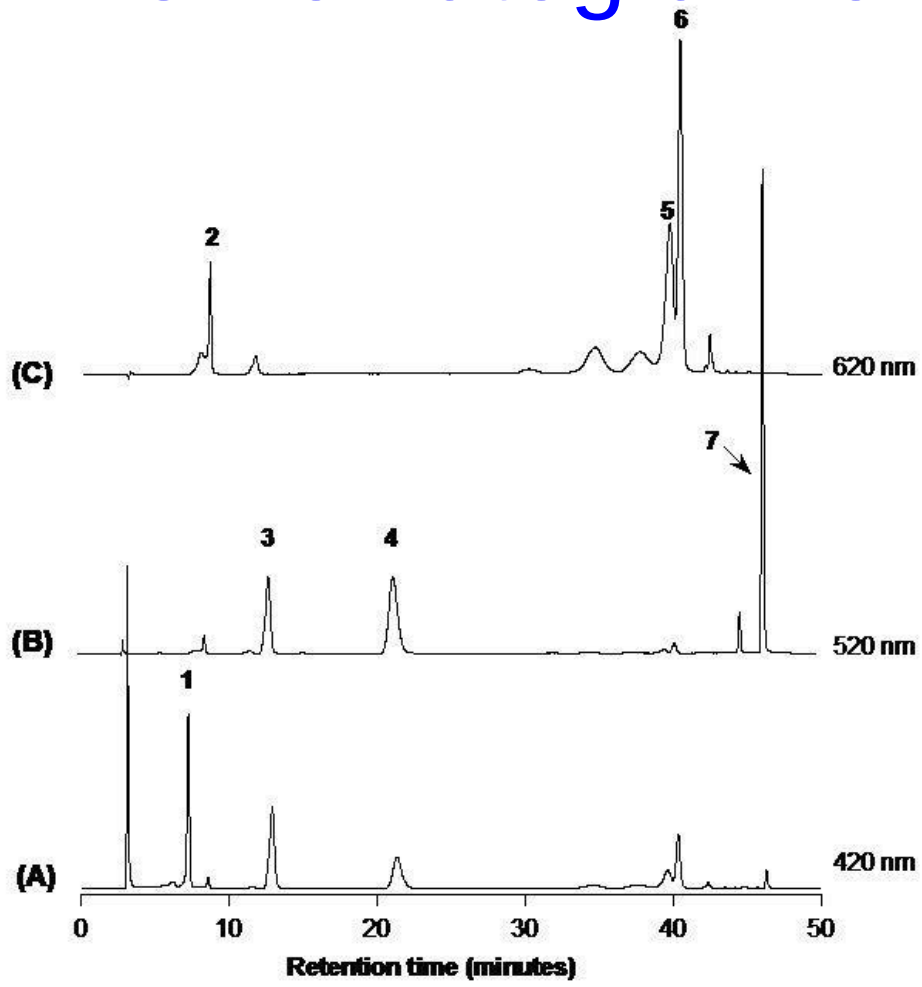
- Different extraction methods needed to be developed for different food matrices
  - Candies and beverages
  - More complex foods (dairy products and baked goods)
- Stability of FD&C Blue No. 2
  - Poor stability in light and under basic conditions
- Recovery of FD&C Red No.3
  - Adheres to food matrices and filter under neutral or acidic conditions


Extraction Procedures
Is product a beverage, frozen treat, powder drink, or gelatin product?
YES
NO
Homogenize product.
Weigh into centrifuge tube.
Add 10 mL 7/3 MeOH/ 10% NH4OH to centrifuge tube.
Vortex. Sonicate tube for 1 hr.
Weigh product in 10 mL volumetric flask.
Wash tube contents with 10 mL 7/3 MeOH/ 10% NH4OH. Sonicate tube for 5 min.
Centrifugation.
Transfer aqueous extract to 50 mL centrifuge tube.
Is product a beverage or frozen treat?
YES
NO
NO
YES
Has product been washed 3 times?
Dilute to volume with MeOH. Add 3 drops 10% NH4OH.
Dilute to volume with 50% MeOH. Add 3 drops 10% NH4OH. Sonicate.
Is product a dairy?
YES
NO
Transfer aqueous layer to 50 mL volumetric flask.
Store in freezer for 1 hr.
Centrifuge.
Transfer aqueous layer to 50 mL volumetric flask.
Dilute to volume with water.
Mix 5 mL of solution w/ 2 mL hexane.
Centrifuge.
Acidify 2 mL of aqueous layer with 2 drops acetic acid.
Mix 5 mL of solution w/ 2 mL hexane.
Centrifuge.
Acidify 2 mL of aqueous layer with 2 drops acetic acid.
Filter and collect in HPLC vial.

Analytical method

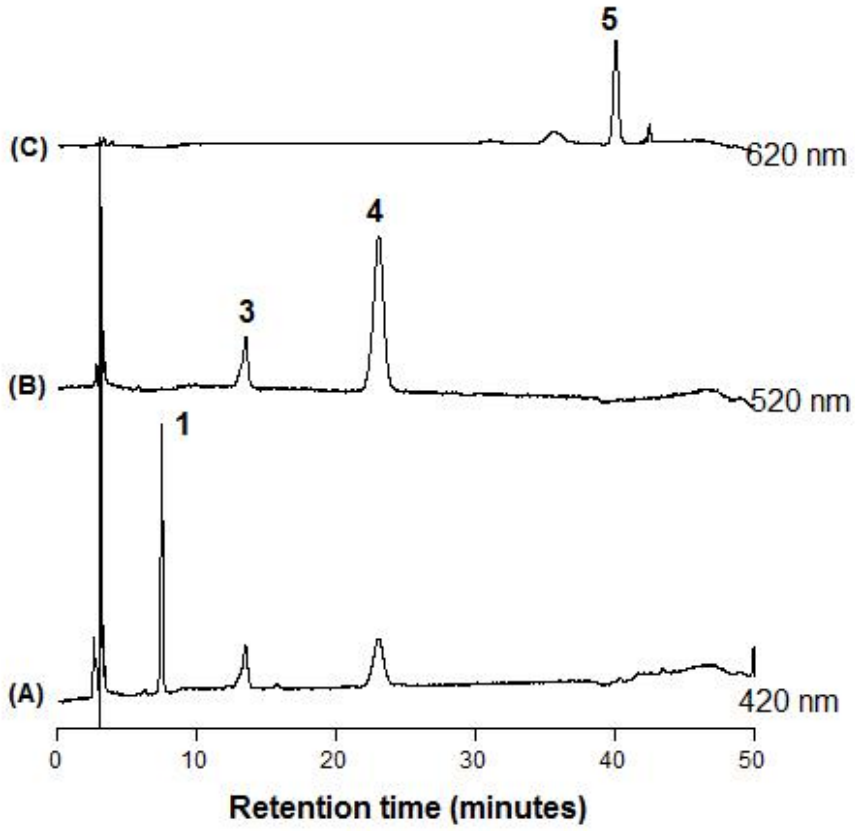
- Color additives extracted and analyzed using liquid chromatography (LC) with photodiode array (PDA) detection
  - Gradient elution using C18 column
  - Ammonium acetate in water and methanol
- Calibration curves used for quantitation

Chromatogram of standards



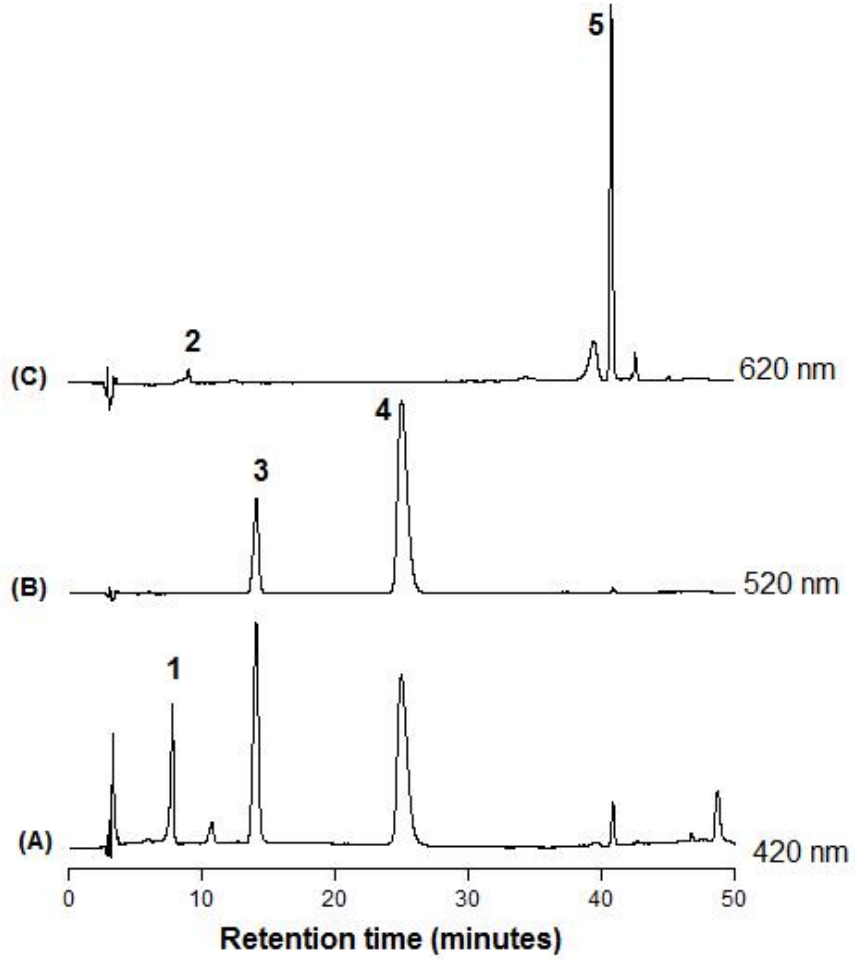
1. Yellow 5
2. Blue 2
3. Yellow 6
4. Red 40
5. Blue 1
6. Green 3
7. Red 3

Iced tea sample



1. Yellow 5
3. Yellow 6
4. Red 40
5. Blue 1

Cereal sample



1. Yellow 5
2. Blue 2
3. Yellow 6
4. Red 40
5. Blue 1

Product	Amount of color additive in food products (mg/kg)							Total color additives	
	Blue 1	Blue 2	Green 3	Red 3	Red 40	Yellow 5	Yellow 6	Concentration (mg/kg)	Per Serving (mg)
Orange soda					2.1		33.4	35.5	8.5
Chocolate candy	34.0				60.8	32.1	152.8	279.7	13.4
Barbecue sauce	2.7				89.7		97.8	190.2	6.7
Cereal	37.5	6.7			310.4	33.9	126.1	514.6	7.6
Strawberry milk				3.9				3.9	0.39

Results highlights

- FDA laboratory survey of 44 foods
  - "Sprinkles" contained highest levels of color additives
- Most common color additives found
  - FD&C Red No. 40, FD&C Blue No. 1, FD&C Yellow 5, and FD&C Yellow No. 6
- Less common color additives found
  - FD&C Blue No. 2, FD&C Green No. 3, and FD&C Red No. 3

Steps for obtaining analytical data for exposure assessment

- Development and validation of LC method
- Selection of contract laboratory
- Identification of foods containing color additives
- Contract laboratory analyses
- Evaluation of contract laboratory results

Steps for identifying foods containing FD&C color additives and determining their levels

- LabelBase by FoodEssential with product label data provided by Gladson and Mintel
- Product label survey
  - Surveyed local grocery stores in the greater Washington DC area, as well as label information from online sources
  - June 2012 to December 2013
- Analytical data to determine levels
- Market share information

2012-2013 product label survey

- >7300 products surveyed covering 60+ food categories
  - Comprehensive survey of product categories previously or currently known to contain FD&C color additives
- Survey is a snapshot in time
- Exposure reflects the products that were in the marketplace at the time of the survey

Examples of food categories

- Baby Food
- Baking Chips
- Baking Mixes
- Beverages (e.g., energy drinks, soda, juice drinks)
- Bread
- Brownies
- Cakes and Cupcakes
- Candy (e.g., hard, soft, chocolate)
- Cereals
- Cereal Bars
- Cheese Spreads and Cream Cheese
- Chewing Gum
- Condiments
- Cookies
- Covered Nuts
- Crackers
- Decorating Items (e.g., jimmies, sugar crystals)
- Dessert Toppings
- Dips
- Drink Mixers
- Flavored Milk
- Fillings
- Frosting and Icing
- Fruit in a Cup
- Frozen Desserts
- Frozen Yogurt
- Fruit Snacks
- Gelatin Dessert
- Glazes
- Granola Bars
- Ice Cream Bars, Cones, and Sandwiches
- Ice Cream
- Jams and Jellies
- Marshmallows and Marshmallow Topping
- Meal Replacements
- Pie Crust
- Popsicles, Fruit Bars, Italian Ices
- Prepared Meals
- Pudding
- Refrigerated Cookie Dough
- Savory Snacks
- Sherbet
- Soup
- Toaster Pastries
- Trail Mix
- Yogurt

Label survey observations

- Products are continually being reformulated to remove FD&C color additives
  - One brand of macaroni and cheese removed FD&C Yellow No. 5 and FD&C Yellow No. 6 in 2013 in products marketed to children
  - A brand of potato bread has removed FD&C Yellow No. 5 and FD&C Yellow No. 6 since December 2013

- Based on the label survey, over 580 representative products were chosen for analysis of FD&C color additives
  - Emphasis was on products marketed to children

Contract laboratory analyses of food products

- Contract laboratory used FDA's method to determine levels of FD&C color additives in food products
- Analytical data obtained for over 580 representative products
  - Prior to analyses, all products were prepared as they would be consumed
  - Results were provided in mg/kg
  - Limit of detection (LOD) for the method was 1 mg/kg
  - Photos of product labels, including the ingredient lists were provided

Method for estimating exposure for FD&C color additives

- Three different population groups were chosen
  - U.S. population, aged 2+ years
  - Children, 2-5 years
  - Teenage boys, aged 13-18 years
- Children and teenage boys were chosen because they are typically high consumers of foods that would be expected to contain FD&C color additives

Method for estimating exposure for FD&C color additives

- Exposure is estimated on an “eaters-only” basis at the mean and 90th percentile
  - “Eaters-only” means that individuals included in the estimate consumed one or more of the foods identified as containing an FD&C color additive over the survey period
  - The 90th percentile represents the high intake consumers of a given food

Method for estimating exposure for FD&C color additives

- 2-day food consumption data from the 2007-2010 National Health and Nutrition Examination Survey (NHANES) were used
- Over 60 food categories where products contained at least one of the FD&C color additives were identified
- >300 food codes from the NHANES survey were assigned across these food categories for each FD&C color additive
  - NHANES food codes represent either a specific product (e.g., Peanut M&Ms) or a general product type (e.g., soft drink, fruit flavored, caffeine containing)
- Levels of each FD&C color additive found in the analyzed food were assigned to the appropriate food code

Method for estimating exposure for FD&C color additives

- Market share data were used for certain NHANES food codes where there was a broad range of FD&C levels among different brands for a FD&C color additive for a given food code

- Three different exposure scenarios were performed based on:
  - The lowest FD&C level for a given color for each food code
  - The highest FD&C level for a given color for each food code
  - A typical FD&C level for a given color for each food code

FAC Follow-Up

- FDA has collected data on amounts of FD&C color additives used in food products
  - These data are being used to estimate dietary exposure for the U.S. population and various population subgroups, including children
- FDA is reassessing safety studies conducted on FD&C color additives that are available in its files
- FDA will determine whether additional safety studies are needed
- Results from the exposure estimate will be presented at the American Chemical Society (ACS) annual meeting in August