



# Photodegradation of FD&C Red No. 40 Dye in Synthetic Hummingbird Nectar

W. Muscara, L. Carrington; E. Eberts, Dr. P. Auger, Dr. E. Strauss  
Center For Urban Resilience | Loyola Marymount University | Spring 2017

LMU LA  
Frank R. Seaver College  
of Science and Engineering

## Abstract

It is common among many homeowners and bird enthusiasts alike to provide supplemental nutrition for Hummingbirds through nectar feeder set-ups. Often commercially available nectar powders and concentrates contain a variety of red dyes solely to make the nectar look more appealing for the customer. Based on the lack of information regarding the safety of the dyes for the birds, investigation into how FD&C red #40 (the most common red dye) degrades in a sucrose solution exposed to sunlight is warranted. Ultra High Pressure Liquid Chromatography and mass spectrometry were used to identify the chemical structure of FD&C Red #40 and determine ideal ppm concentrations for best analysis of potential metabolites. Ultimately, degradation of dye in the presence of controlled artificial sunlight in a sucrose solution (~25% sucrose) and subsequent analysis will help to determine the degraded metabolites that arise and gain insight into their potential harm to hummingbirds.

## Introduction

- Hummingbirds play key roles in local ecosystems acting as a form of pollination, insect control, and indicator of climate shifts.
- Hummingbirds are particularly susceptible to sustain harm from low levels of toxins due to their small size.
- Initial goals were to evaluate microbial communities in hummingbird feeder nectar.
- With the advice of Lisa Tell at UC Davis, the goal was refocused to investigate the photodegradation of FD&C red #40 phase
- Metabolites of this dye may potentially be harmful to hummingbirds as Red #40 has only been FDA approved for use after studies on mammals, no studies on toxicity in birds.

### Question:

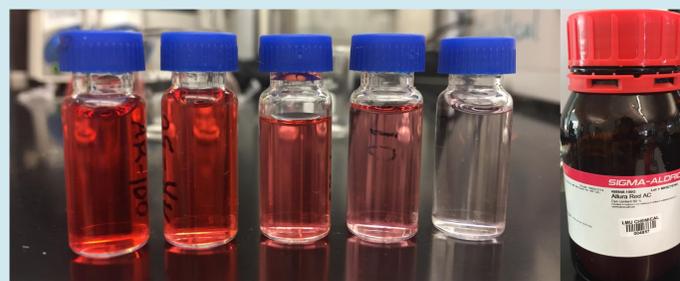
- How does FD&C red #40 degrade in a sucrose solution exposed to sunlight?

### Hypotheses

- **H1:** The photodegradation products of FD&C red #40 present in the nectar after prolonged sun exposure will cause harm to the hummingbirds
- **H2:** The photodegradation products of FD&C red #40 present in the nectar are unlikely to cause adverse health effects when ingested by hummingbirds

## Methods

- **Dilution:** In preparation for initial analysis, the dye was diluted to 100ppm, 50ppm, 20ppm, 10ppm, and 1ppm.
- **Ultra high pressure liquid chromatography (uHPLC):**
  - **Absorbance:** 510nm
  - **Column:** Luna Reverse phase C18 5um 120A 150x 4.6mm
  - **Mode:** Negative ion mode for Mass Spec. PDA wavelength 254 & 510
  - **Run:** 12 min with 1 Min. equilibration
    - 0-10 min 90/10 to 10/90 and 10-12 min 5/95
  - **Flow:** 0.5ml/min
  - 90%A /10% B (A=0.1% formic acid in water B= pure methanol)
- **Mass Spec:** following preparation of ppm dilutions and run through uHPLC products are analyzed by mass spectrometry for identification



Left: Ppm dilutions of azo red dye preparation for run through ultra high pressure liquid chromatography assay.  
Right: FD&C Red #40

## Data

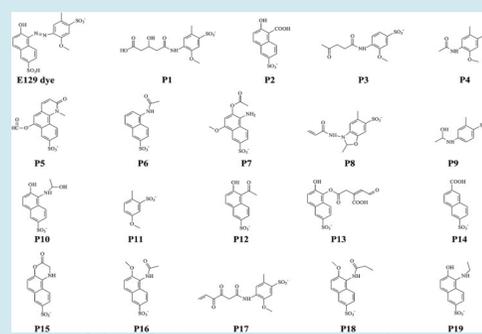
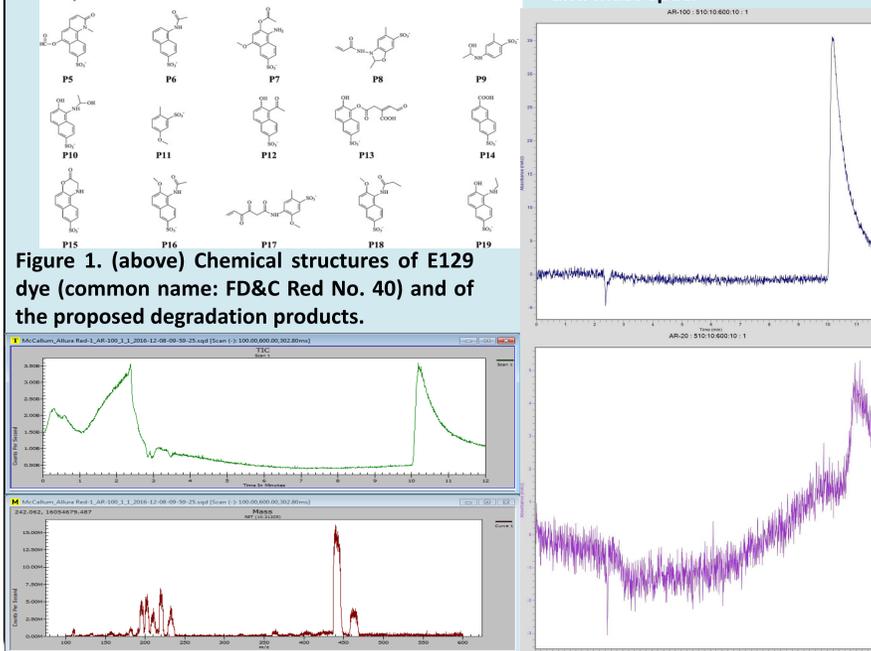


Figure 1. (above) Chemical structures of E129 dye (common name: FD&C Red No. 40) and of the proposed degradation products.

Figure 2. (3 graphs below) Raw data from 1<sup>st</sup> run through uHPLC and mass spec.



## Preliminary Findings

- The preliminary phase of the experiment involved research into possible metabolites of the dye (Figure 1) as well as determining the calibration curve for FD&C #40 (Figure 2).
- The goal is to use this calibration curve and known metabolites to compare with the mass spectrometry data of the dye after it degrades in artificial sunlight.

## Discussion

### Implications:

- Assess possibility of unexpected side reactions to form degradation products in the presence of sunlight and sucrose.
- Gain insight into potentially harmful health effects of red dye degradation on Hummingbirds.
- Evaluate potential for small levels of ingested metabolites to have an adverse effect on the health of these environmentally valuable birds.

### Future Work:

- A repeated conformation uHPLC run of the dye itself, similar to the one performed already.
- A uHPLC analysis with mass spectrometry for the degradation of dye in the presence of controlled artificial sunlight in water only.
- A uHPLC analysis with mass spectrometry for the degradation of dye in the presence of controlled artificial sunlight in a sucrose solution (~25% sucrose).
- Identification of the resultant metabolites and investigation into their toxic effects on hummingbirds.

## Literature Cited

- Borzelleca, J.F., J.W. Olson, and F.E. Reno. 1989. Lifetime toxicity/carcinogenicity study of FD & C Red No. 40 (Allura Red) in Sprague-Dawley rats. *Food and Chemical Toxicology*. 27:701-705.
- Gosetti, F., U. Chiominatto, E. Mazzucco, G. Calabrese, M.C. Gennaro, and E. Marengo. 2012. Identification of photodegradation products of Allura Red AC (E129) in a beverage by ultra high performance liquid chromatography-quadrupole-time-of-flight mass spectrometry. *Analytica Chimica Acta*. 746:84-89.
- McKone, H.T., and K. Ivie. 1980. An introduction to high performance liquid chromatography: Separation of some FD&C dyes. *Journal of Chemical Education*. 57:321.

## Acknowledgements

- LMU's Center for Urban Resilience
- Dr. McCallum
- Dr. Lisa Tell of UC Davis