

Interactions of Azo Dyes Commonly Used in Oral Drug Products with the Organic Anion Transporting Polypeptide 2B1 (OATP2B1) and Human Gut Bacteria

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Azo dyes are Found in Foods and Drug Products: FD&C Red No. 40



FD&C Red No. 40

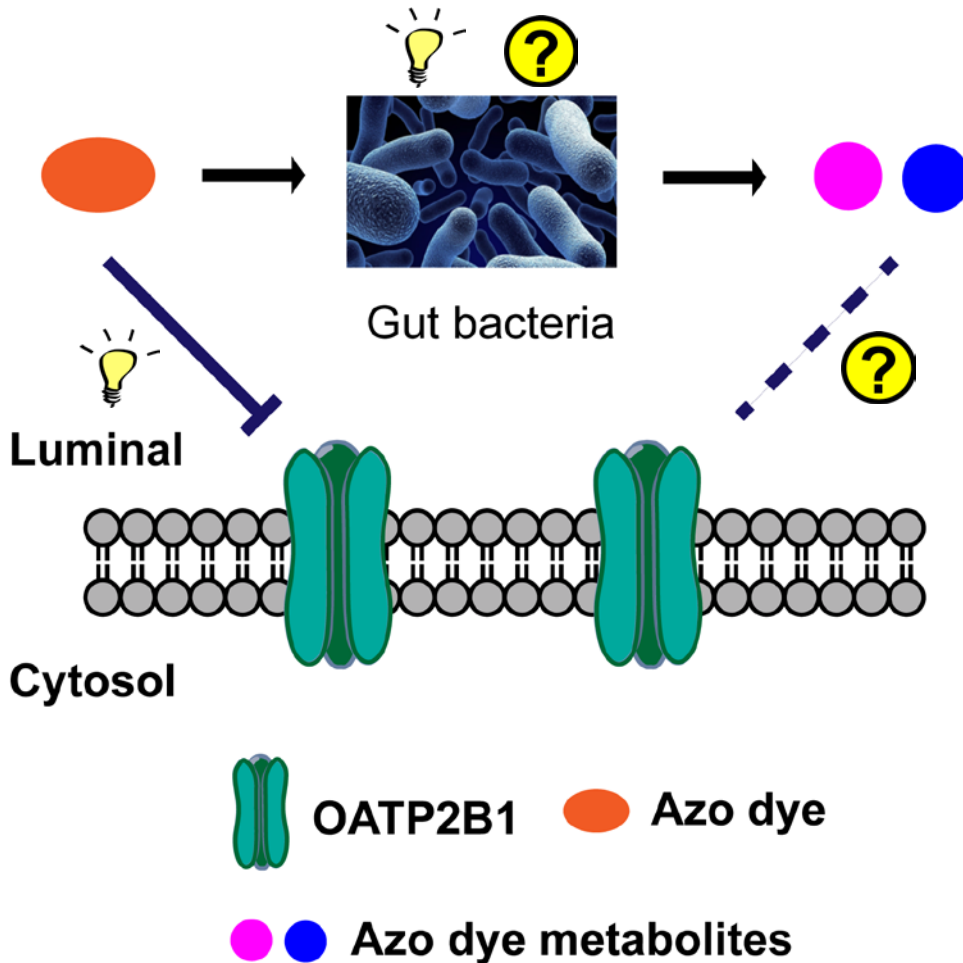


- **Used in 35 drugs with active ingredients such as phenytoin sodium and valproic acid (CERSI Excipient Browser¹)**
- **Commonly used in food industry.**
- **Estimated daily intake per person is 25 mg², which equals to 220 μM in intestine.**

1. <http://excipients.ucsf.bkslab.org/>

2. Nutr Rev. 2013 May;71(5):268-81.

Complex Interplay Among Azo Dyes, Transporters and Gut Bacteria



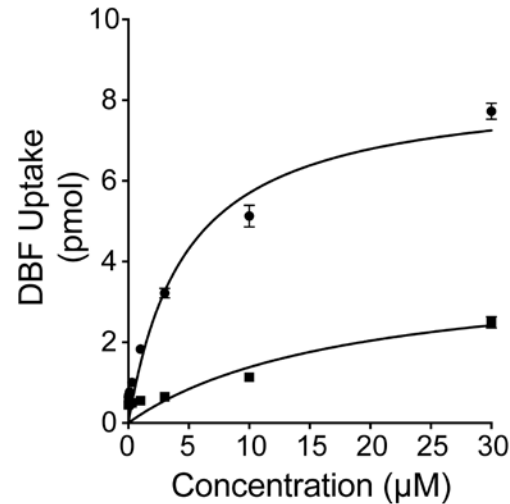
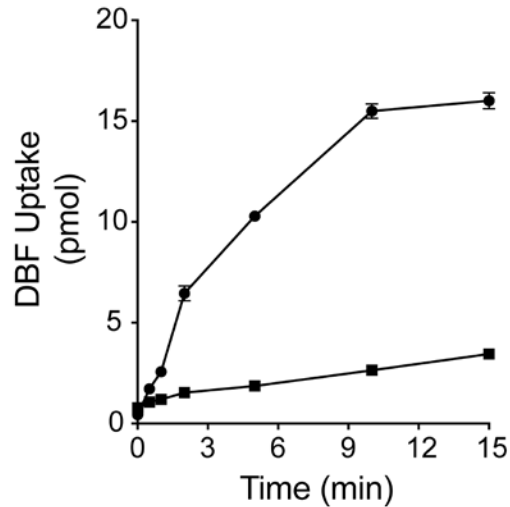
Some azo dyes commonly added in food and drug products appear to be inhibitors of intestinal influx transporter, OATP2B1, in vitro.

Azo dyes can be metabolized by human gut bacteria, ex vivo.

Do azo dye metabolites inhibit OATP2B1?

What is the rate and extent of metabolism of azo dyes by human gut bacteria?

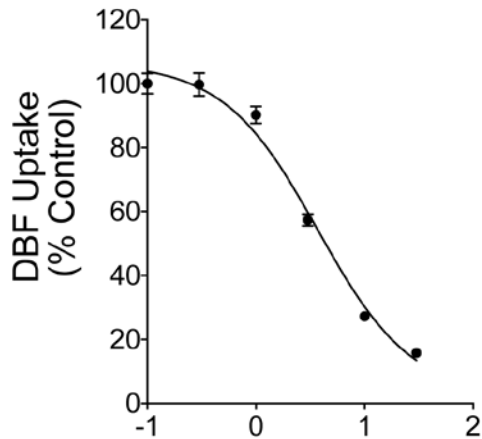
Azo Dyes are Potent Inhibitors of OATP2B1



◆ Human OATP2B1
 ■ Empty Vector

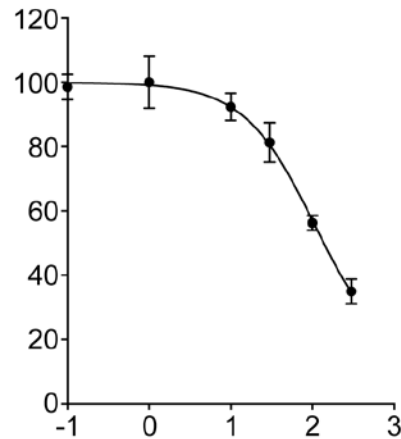
DBF: 4',5'-Dibromofluorescein
 HEK FlpIn cells

FD&C Red No. 40



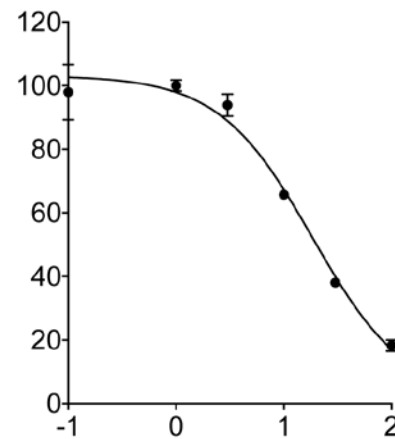
$K_i = 2.59 \mu\text{M}$

FD&C Yellow No. 6



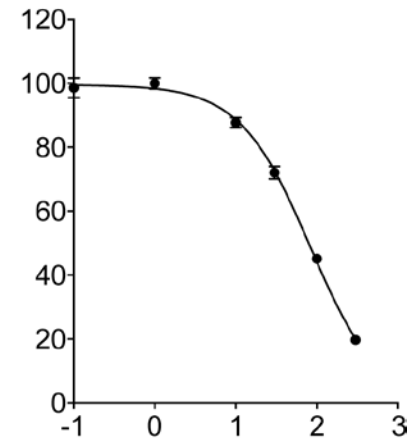
$K_i = 68.4 \mu\text{M}$

D&C Red No. 6



$K_i = 11.3 \mu\text{M}$

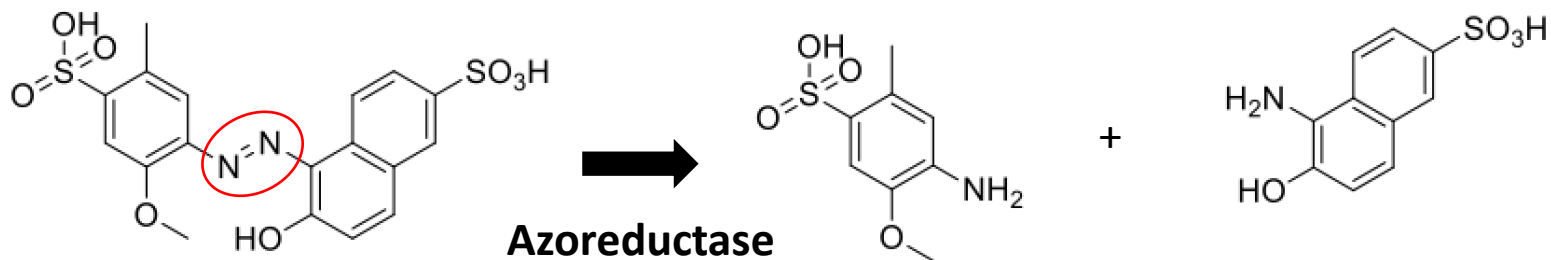
D&C Red No. 33



$K_i = 58.1 \mu\text{M}$

Screening concentration: 2 µM; Uptake time: 3 min

Metabolites of Azo Dyes are Weak Inhibitors of OATP2B1



FD&C Red No.40
 $K_i = 2.59 \mu\text{M}$

Metabolite 1
 $K_i > 50 \mu\text{M}$

Metabolite 2
 $K_i > 200 \mu\text{M}$

Excipients with Azo Group	K_i (μM)	Reduced Metabolites	K_i (μM)
FD&C Red No. 40	2.59	1-amino-2-methoxy-5-methylbenzene-4-sulfonic acid	> 50
		5-amino-6-hydroxy-2-naphthalenesulphonic acid	> 200
FD&C Yellow No. 6	68.4	Sulfanilic acid	> 200
		5-amino-6-hydroxy-2-naphthalenesulphonic acid	> 200
D&C Red No. 6	11.3	4-amino-3-hydroxy-[2]naphthoic acid	> 200
		4-Aminotoluene-3-sulfonic acid	> 200
D&C Red No. 33	58.1	3,5-diamino-4-hydroxy-naphthalene-2,7-disulfonic acid	> 50
		Aniline	> 200

Azo dyes are metabolized by bacteria from human stool samples

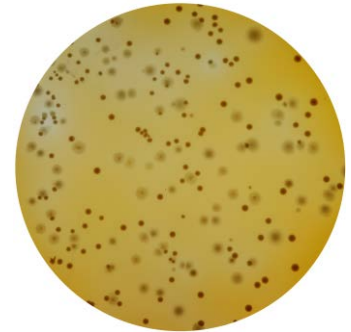
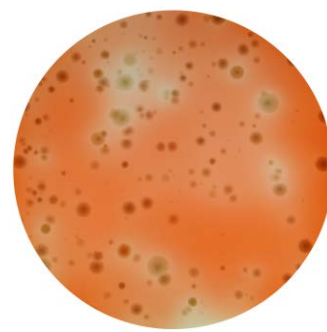
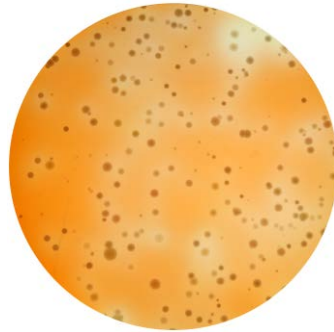
D&C Red No. 33

D&C Red No. 6

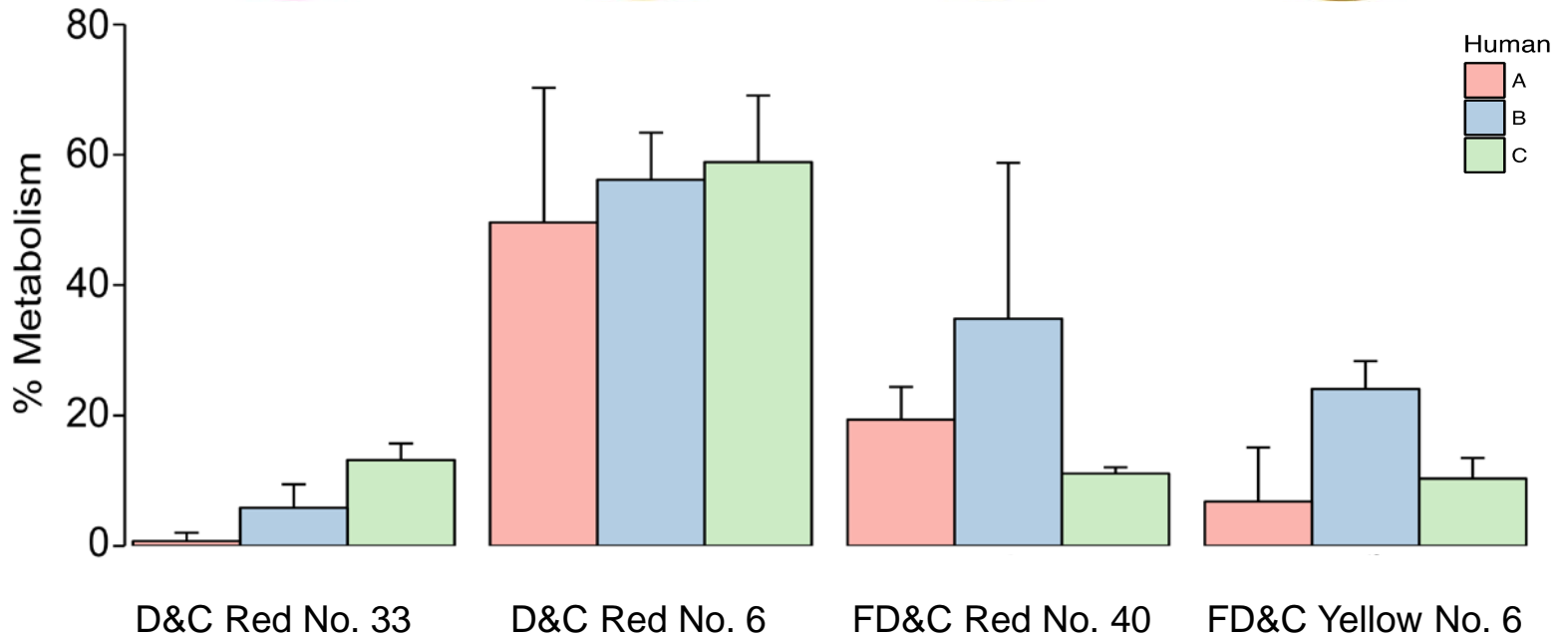
FD&C Red No. 40

FD&C Yellow No. 6

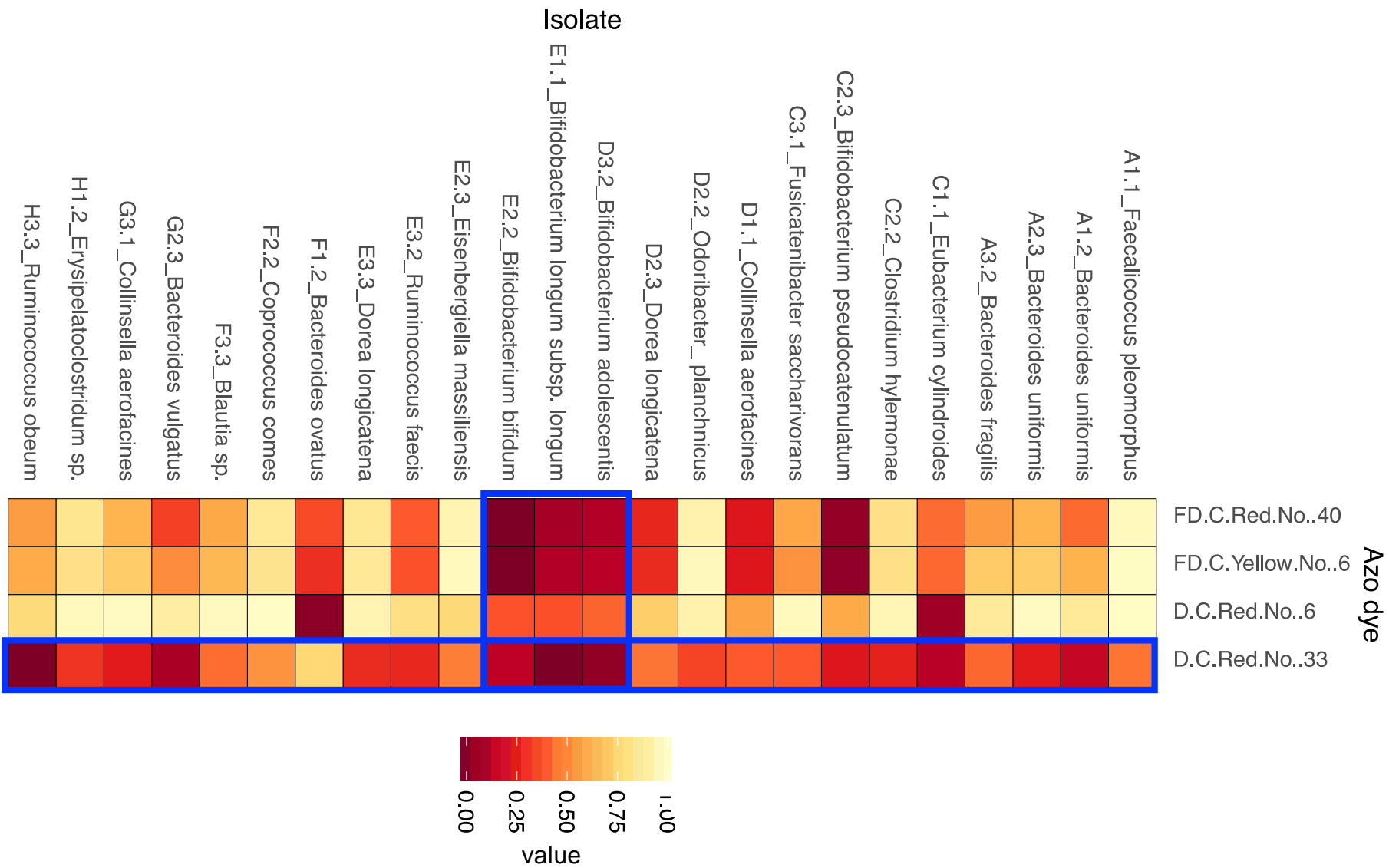
Agar plate



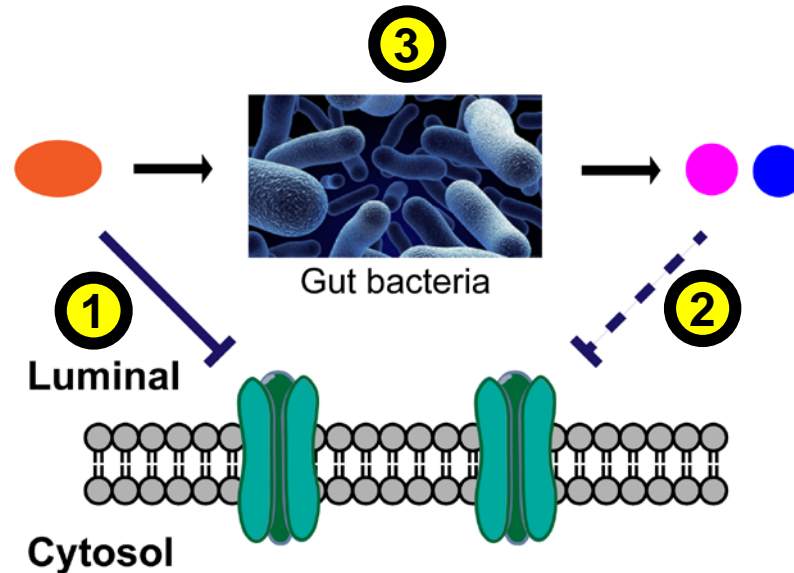
Culture medium



Variable Azo Dye Metabolism Mediated by 24 Bacteria Strains Isolated from Human Stool Samples



Azo Dyes Inhibit OATP2B1 Transport Activity and Gut Bacteria May Modulate These Effects Through Azo Reduction



- ① Azo dyes commonly added in food and drug products are inhibitors of intestinal influx transporter, OATP2B1.
- ② Azo dye metabolites are much less potent inhibitors of OATP2B1 compared with azo dyes.
- ③ Bacteria isolated from human stool samples show different capabilities in metabolizing azo dyes, e.g., *F. pleomorphus* avidly reduces four azo dyes tested vs. *B. bifidum* has poor reductive capability.

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