# Studies on Dyes

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**RED**

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FD&C Red No. 40 - Allura Red
FD&C Red No. 3 - Erythrosine

1. **Short-term erythrosine B-induced inhibition of the brain regional serotonergic activity suppresses motor activity (exploratory behavior) of young adult mammals.**Dalal A, Poddar MK.*Pharmacology, Biochemistry, and Behavior,* 2009 Jun;92(4):574-82. Epub 2009 Mar 3. MedLine

   "Previous studies showed that repeated ingestion of erythrosine B [Red 3] (artificial food color) developed behavioral hyperactivity, but nothing is known about its single administration effect as well as the neurochemical (s) involvement. ... **The degree of erythrosine-induced inhibition of both MA** [motor activity] **and brain regional serotonergic activity was dosage dependent**. ... Altogether these results suggest that a single higher dosage of erythrosine (10-200 mg/kg, p.o.) may reduce MA **by reducing serotonergic activity** with modulation of central dopaminergic activity depending on the brain regions."

2. **DNA damage induced by red food dyes orally administered to pregnant and male mice**. Tsuda S, et al, *Toxicol Sci* 2001, May;61(1):92-9 MedLine

   "We determined the genotoxicity of synthetic red tar dyes (amaranth - Red 2, allura red - Red 40, acid red - #106, new coccine - No. 18) currently used as food color additives in many countries, including Japan. …The assay was positive in the colon 3 hours after the administration of ama-ranth and allura red and weakly positive in the lung 6 hours after the administration of amaranth. Acid red did not induce DNA damage in any sample at any sampling time. …The 3 dyes induced DNA damage in the colon starting at 10 mg/kg. …6.5 mg/10 ml of new coccine induced DNA damage in colon, glandular stomach, and bladder….the 3 azo additives we examined induced c**olon DNA damage at very low doses.** MedLine

3. **Reproductive and neurobehavioral toxicity study of erythrosine (Red 3) administered to mice in the diet.** Tanaka T, *Food Chem Toxicol* 2001 May;39(5):447-54 MedLine

   "Erythrosine was given in the diet to provide levels of 0 (control), 0.005, 0.015 and 0.045% from 5 weeks of age of the F(0) generation to 9 weeks of age of the F(1) generation in mice, and selected reproductive and neurobehavioral parameters were measured. . .In movement activities of **exploratory behaviour**, several parameters were significantly changed in the high dose group, and those effects were dose-related in adult females in the F(0) and F(1) generations and in male offspring in the F(1) generation."

4. **Estrogenic and DNA-damaging activity of Red No. 3 in human breast cancer cells**. Dees C, et al, *Environ Health Perspect* 1997 Apr;105 Suppl 3:625-32 MedLine

   "Exposure to pesticides, dyes, and pollutants that mimic the growth promoting effects of estrogen may cause **breast cancer**. …Red No. 3 increased binding of the ER from MCF-7 cells to the estrogen responsive element. Consumption of Red No. 3, which has estrogenlike growth stimulatory properties and may be genotoxic, could be a significant risk factor in human breast carcinogenesis."

5. **A study on the reproductive toxicity of erythrosine (Red No. 3) in male mice**. Abdel Aziz AH, et al, *Pharmacol Res* 1997 May:35(5):457-62 MedLine

"The potential adverse effects of erythrosine (ER FD&C Red No. 3) on the spermatogenesis process were investigated in adult mice. . . sperm count as well as the percentage of motile sperms were significantly inhibited by about 50% and 57% respectively. Moreover. . .it increased the incidence of sperms with abnormal head by about 57% and 65% respectively. The induced **increase in sperm abnormalities** could enhance the spermatogenetic dysfunction and germ cell mutagenicity. These findings indicate that ER with used doses has a potential toxic effect on spermatogenesis in mice and in turn, it may affect its testicular function and reproductive performance." MedLine

6. **Developmental toxicity and psychotoxicity of FD and C red dye No 40 (allura red AC) in rats**. Vorhees, CV, et al, *Toxicology* 1983;28(3):207-17 MedLine ||Full Text

   "Adult Sprague-Dawley rats were fed diets containing FD and C red dye No. 40 for 2 weeks and were then bred. The diets were continued for the females throughout gestation and lactation and were provided continuously to the offspring thereafter. Red 40 significantly reduced reproductive success, parental and offspring weight, brain weight, survival, and female vaginal patency development. Behaviorally, Red 40 produced substantially decreased running wheel activity, and slightly increased post-weaning open-field rearing activity. Overall, R40 produced **evidence of both physical and behavioral toxicity** in developing rats at doses up to 10% of the diet."

7. **Neurotransmitter Release from a Vertebrate Neuromuscular Synapse Affected by a Food Dye**. Augustine G, Levitan H, *Science Magazine*, March 28, 1980, Vol. 207, pp. 1489-90 MedLine ||Full Text

   ". . .FD&C No. 3 . . .produced an **irreversible, dose-dependent increase in neurotransmitter release**. . . These results suggest that erythrosine might provide a useful pharmacological tool for studying the process of transmitter release, but that **its use as a food additive should be re-examined**."

8. **Erythrosine B inhibits dopamine transport in rat caudate synaptosomes.** Lafferman JA, Silbergeld EK, *Science* 1979 205:410-412 MedLine ||Full Text

   Erythrosin B is a member of a class of fluorescein dyes that are suggested to elicit hyperkinesis when ingested by susceptible children. We found that erythrosin B inhibits dopamine uptake . . . Erythrosin B also decreased nonsaturable binding of dopamine to the synaptosome membrane. The inhibitory action of erythrosin B on dopamine uptake is consistent with the hypothesis that erythrosin B can act as a **central excitatory agent able to induce hyperkinetic behavior**.

9. 

   **YELLOW**

10. 
    FD&C Yellow No. 5 - Tartrazine
    FD&C Yellow No. 6 - Sunset Yellow

11. **Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet.**Tanaka T., *Food Chem Toxicol.* 2005 Aug 5 (epub ahead of print)
    MedLine ||Full Text

    "Tartrazine was given in the diet . . . and selected reproductive and neurobehavioural parameters were measured. In movement activity of exploratory behaviour in the F(0) generation, number of vertical activity was significantly **increased** ...The average body weight . . .was significantly **increased** . . . In behavioural developmental parameters, surface righting . . . was significantly **accelerated** . . . Cliff avoidance at PND 7 was significantly **accelerated** . . . Negative geotaxis at PND 4 was significantly **delayed** . . . number of movement showed a significant tendency to be affected . . . Nevertheless, . . . the actual dietary intake of tartrazine is presumed to be much lower. It would therefore

appear that the levels of actual dietary intake of tartrazine is **unlikely to produce any adverse effects in humans**."

Note: in the face of these clear adverse effects on mice, he concluded that tartrazine is not a problem for humans. And his basis for such a conclusion? Simply that we would theoretically eat less than the mice did.

12. **Immumological aspects of the common food colorants, amaranth and tartrazine.** Koutsogeorgopoulou L, et al, *Vet Hum Toxicol* 1998 Feb;40(1):1-4 MedLine

    "We described . . . the cytotoxic and **immunosuppressive effects** of food colorants such as amaranth and tartrazine. . . The results showed clear immunosuppressive effects from the 2 substances tested, although the concentrations chosen for this study provide to be non-cytotoxic."

13. **Reproductive and neurobehavioral effects of Sunset Yellow FCF administered to mice in their diet**. Tanaka T, *Toxicol Ind Health* 1996 Jan-Feb;12(1):69-79 MedLine

    "Selected reproductive and neurobehavioral parameters were measured in mice given the color additive Sunset Yellow [*FD&C Yellow #6*] FCF in the diet. The additive was given at levels of 0 (control), 0.15, 0.30, and 0.60%, from five weeks of age in the F0 generation to nine weeks of age in the F1 generation. There were few adverse effects on litter size, weight, or sex ratio. Average body weight . . . was significantly increased . . . In the neurobehavioral parameters, **swimming direction was significantly affected in a dose-related manner** in male and female offspring . . . Also in the early lactation period, **surface righting and negative geotaxis were significantly affected in male offspring** in the middle-dose group, and **swimming head angle was significantly affected in female offspring** in a dose-related manner. The dose levels of Sunset Yellow FCF in this study **did produce some adverse effects in reproductive and neurobehavioral parameters.**

14. **Synthetic Food Coloring and Behavior: A Dose Response Effect in a Double-Blind, Placebo-Controlled, Repeated-Measures Study**, Rowe KS, Rowe KJ, *Journal of Pediatrics* November 1994 Vol. 135, pp.691-8 MedLine ||Full Text

    "This study demonstrated a functional relation between the ingestion of a synthetic food color (tartrazine) and **behavioral changes** in 24 atopic children, aged 2 to 14 years, with marked reactions being observed at all six dosage levels of dye challenge."

15. **Controlled Trial of Oligoantigenic Treatment in the Hyperkinetic Syndrome**, Egger J, et al, *The Lancet* March 9, 1985 MedLine || Full Text

    "76 selected overactive children were treated with an oligoantigenic diet. 62 improved, and a normal range of behaviour was achieved in 21 of these. Other symptoms such as headaches, abdominal pain, and fits, also often improved…. Artificial colorants (Yellow No. 5) and preservatives were the commonest provoking substances, but no child was sensitive to these alone."

16. **The influence of the chemical additive tartrazine on the zinc status of hyperactive children: A double-blind placebo-controlled study.** Ward NI, et al. *J Nutr Med*;1(1). 1990 51-58 MedLine

    "...Tartrazine induces a **reduction in serum and saliva zinc**concentrations and an increase in urinary zinc content with a corresponding deterioration in behaviour/emotional responses of the hyperactive children but not the controls."

17. **Assessment of chemical factors in relation to child hyperactivity.** Ward NI, *Journal of Nutritional & Environmental Medicine (Abingdon)*; 7 (4). 1997. 333-342. MedLine ||Full Text

    "...**Only hyperactive children showed a significant reduction in blood serum zinc levels and an increase in urinary zinc output** following the consumption of E102 [tartrazine] and E110 [sunset yellow]. . . For the 23 children

who consumed a tartrazine beverage there were increased levels of overactivity (n = 18 children), aggressive (n = 16) and/or violent (n = 4) activity, poor speech (n = 2), poor coordination (n = 12), and the development of asthma and/or eczema (n = 8). Most of these were severe or moderate changes. Only one control child showed minor behavioural responses to tartrazine."

18.

**BLUE**

19. FD&C Blue No. 1 - Brilliant Blue
    FD&C Blue No 2 - Indigo Carmine
20. **Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury**. Peng et al, *Proceedings of the National Academy of Sciences of the United States of America*, July 28, 2009, Vol 106 (30) 12489-12493. MedLine || Full Text

"Importantly, BBG is a derivative of a commonly used blue food color (FD&C blue No. 1), which crosses the blood-brain barrier. Systemic administration of BBG may thus comprise a readily feasible approach by which to treat traumatic SCI [*spinal cord injury*] in humans."

21. **Toxicity of Food Drug and Cosmetic Blue No. 1 dye in critically ill patients.**Lucarelli MR, Shirk MB, Julian MW, Crouser ED., *Chest.* 2004 Feb;125(2):793-5. MedLine || Full Text

    " Food Drug and Cosmetic Blue No. 1 dye (FD&C Blue No. 1) is commonly added to enteral nutrition formulations in order to facilitate the detection of gastric aspirate in tracheal secretions of critically ill patients. However, reports of systemic blue dye absorption and associated adverse outcomes are emerging. We report two cases of abnormal systemic absorption of FD&C Blue No. 1 in critically ill patients who subsequently died of refractory shock and metabolic acidosis. Risk factors and mechanisms of FD&C Blue No. 1 toxicity are discussed, and alternate approaches to gastric aspiration detection in critically ill patients are considered."

22. **2003: FDA Public Health Advisory: Reports of Blue Discoloration and Death in Patients Receiving Enteral Feedings Tinted With The Dye, FD&C Blue No. 1** FDA paper

    "Dear Health Care Professional: The Food and Drug Administration (FDA) would like you to be aware of several reports of toxicity, including death, temporally associated with the use of FD&C Blue No. 1 (Blue 1) in enteral feeding solutions. . . in vitro evidence that Blue 1 can be a mitochondrial toxin lends plausibility to the idea ... "

23. **Blue colon at autopsy.** Granville LA, Finch C., *Arch Pathol Lab Med*. 2001 May;125(5):599.
    MedLine || Full Text

    "FD&C Blue No. 1 was hypothesized to have caused refractory hypotension and metabolic acidosis in 2 patients who died. The Food and Drug Administration approved the blue food coloring based on experiments performed on healthy animals, which demonstrated the dye to be nonabsorbable. Now there are case reports of humans in which the dye may have been absorbed."

24. **Green colon: an unusual appearance at autopsy.** Boutilier RG, Murray SK, Walley VM. *Arch Pathol Lab Med.* 2000 Sep;124(9):1397-8.
    MedLine || Full Text

"Following ingestion of the water-soluble dye, it is apparently concentrated in the colon, the site of water reabsorption in the gastrointestinal tract. The concentration in the large bowel wall likely varies, depending on the amount administered. In any circumstance, it appears that the clinical use of this dye has a pathologic correlate at autopsy. "

25. **Systemic Absorption of Food Dye in Patients with Sepsis**, Maloney JP, Halbower AC, Fouty BF, Fagan KA, Balasubramaniam V, Pike AW, Fennessey PV, Moss M, *N Engl J Med* 2000 Oct 5;343(14):1047-8 MedLine ||Full Text

    " Autopsies of both patients revealed green or blue discoloration of the skin and internal organs, without gastrointestinal perforation. . . Blue dye no. 1, . . . reduces oxygen consumption by a factor of eight in mitochondrial preparations in vitro. . . . Although both patients had serious underlying illnesses, their condition was improving before they received the dye and turned color. . . .We encourage judicious use of this food dye in patients with sepsis or other illnesses associated with increased gastrointestinal permeability. "

## MIXED FOOD DYES

26. **Differential colon DNA damage induced by azo food additives between rats and mice.** Shimada C, Kano K, Sasaki YF, Sato I, Tsudua S, *Journal of Toxicological Sciences* 2010;35(4):547-54. MedLine ||Full Text

    " Azo dyes, amaranth, allura red and new coccine, which are currently used as food color additives in Japan, have been reported to cause colon specific DNA damage in mice. To examine species difference in the DNA damage between rats and mice, each of dyes was administered to male mice (1 and 10 mg/kg) and male rats (10, 100 and 1,000 mg/kg) by gavage. Brain, lung, liver, kidney, glandular stomach, colon, urinary bladder and bone marrow were sampled 3 hr (for mice) and 3, 6, 12 and 24 hr (for rats) after the treatment. The alkaline comet assay showed DNA damage in the mouse colon 3 hr after the administration of all of the dyes at 10 mg/kg. In rats, however, none of the dyes damaged DNA. Azo dyes should undergo metabolic reduction in the colon to be adducted to DNA. To determine transit time of the dyes to the colon after their administration, gastric emptying and intestinal transport in mice and rats were examined using brilliant blue FCF (BB) as an indicator. The half times of gastric emptying were 70 and 80 min for mice and rats, respectively; and about 60% of the BB was removed from the stomach 1 hr after the gastric intubation in both mice and rats. BB reached the mouse and rat colon 1 and 3 hr after the administration, respectively. Considering the wide dose range and sampling times well covering the transit time to the colon, rats may be insensitive to these azo dye-induced DNA damage."

27. **Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial.** McCann D, et al. *Lancet*, September 6, 2007 on line. MedLine ||Full Text

    "Artificial colours or a sodium benzoate preservative (or both) in the diet result in increased hyperactivity in 3-year-old and 8/9-year-old children in the general population."

28. **Synergistic Interactions Between Commonly Used Food Additives in a Developmental Neurotoxicity Test.**Lau K, et al. *Toxicol Sci*. 2006 Mar;90(1):178-87. MedLine ||Full Text

    Lau found that combining additives led to a much greater effect than expected on developing neurons. He said, "Inhibition of neurite outgrowth was found at concentrations of additives theoretically achievable in plasma by ingestion of a typical snack and drink."

29. **The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children.**, Bateman B et al, *Archives of Disease in Childhood*. 2004 Jun;89(6):506-11 MedLine ||Full Text

". . . there were significantly greater increases in hyperactive behaviour during the active than the placebo period based on parental reports. . . .CONCLUSIONS: There is a general adverse effect of artificial food colouring and benzoate preservatives on the behaviour of 3 year old children . . . "

30. **Synergistic effects of food colors on the toxicity of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) in primary cultured rat hepatocytes.** Asida H, et al. *J. Nutru Sci V8itaminol.* 2000. 46 130-136. MedLine || Full Text

    "These results suggest that the daily intake of artificial food colors may impair hepatic functions such a gluconeogenesis and ureogenesis, when dietary carcinogens are exposed to the liver cells."

31. **Physiological effects of some synthetic food colouring additives on rats.** Aboel-Zahab H, et al., *Boll Chim Farm* 1997 Nov;136(10):615-27 MedLine

    " Three different synthetic chocolate colourant agents (A, B and C) were administered to healthy adult male albino rats for 30 and 60 day periods to evaluate their effects ... Ingestion of colourant C (brown HT and indigocarmine) significantly decreased rat body weight, serum cholesterol and HDL-cholesterol fraction, while, T4 hormone, liver RNA content, liver enzymes (S. GOT, S. GPT and alkaline phosphatase), total protein and globulin fractions were significantly elevated. Significant increases were observed in serum total lipids, cholesterol, triglycerides, total protein, globulin and serum transaminases in rats whose diets were supplemented with chocolate colours A and B (sunset yellow, tartrazine, carmoisine and brilliant blue in varying concentrations). ... haemoglobin concentrations and red blood cell counts were significantly decreased in the rats who were administered food additives A and B. ... Congested blood vessels and areas of haemorrhage in both liver and renal sections were revealed in those rats who were given colourants B and C. ..."

32. **Effect of organic synthetic food colours on mitochondrial respiration.** Reyes FG, Valim MF, Vercesi AE. Food Additives and Contaminants. 1996 Jan;13(1):5-11 MedLine

    " ... The compounds tested were: Erythrosine, Ponceau 4R, Allura Red, Sunset yellow, Tartrazine, Amaranth, Brilliant Blue, Blue, Fast Red E, Orange GGN and Scarlet GN. All food colours tested inhibited mitochondrial respiration ...This inhibition varied largely, e.g. from 100% to 16% for Erythrosine and Tartrazine respectively, ...This effect was dose related .... "

33. **Food Additives are Common Causes of the Attention Deficit Hyperactive Disorder in Children**. Boris M, Mandel F, *Annals of Allergy*, May 1994. MedLine ||Full Text

    ". . .this double-blind, placebo-controlled food challenge study supports the role of dietary factors in ADHD (including dyes). Through a simple elimination diet symptoms can be controlled."

34. **Behavioral responses to artificial food colors**, Weiss B, et al., *Science*, March 28, 1980 207:1487-1488 MedLine ||Full Text

    Twenty-two young children on an elimination diet were challenged intermittently with a blend of seven artificial colors in a double-blind trial. Parents' observations provided the criteria of response. One child that responded mildly to the challenge and one that responded dramatically were detected. The latter, a 34-month-old female, showed a significant increase in aversive behaviors. **These results further confirm previous controlled studies.**"

    Note:

    - The children were **not** diagnosed as hyperkinetic (hyperactive).
    - 35.26 mg of mixed colors were used as the "challenge" in this study. Compare to 150 mg in one Tb green ketchup. Note also that when a challenge does not provoke worse behavior, it does not mean that the **diet** did not "work" but that the **challenge** did not "work."

35. **Food Dyes Impair Performance of Hyperactive Children on a Laboratory Learning Test**, Swanson J, Kinsbourne

M, *Science*, March 238, 1980, Vol. 207. pp. 1485-7. MedLine ||Full Text ||

"The performance of the hyperactive children on paired-associate learning tests on the day they received the dye blend was impaired relative to their performance after they received the placebo, but the performance of the non-hyperactive group was not affected by the challenges. . ."

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