Studies on Dyes

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


"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."


"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 amaranth 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 colon DNA damage at very low doses. [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."


"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."

"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."  

"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."

"...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."

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.

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

"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.


   "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."


   "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.


   "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."


   "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."


   "...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."


   "...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.

**BLUE**

FD&C Blue No. 1 - Brilliant Blue
FD&C Blue No 2 - Indigo Carmine


"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."

17. **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 . . ."


"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."


"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."


"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**


"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."


"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."


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."


". . . 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 . . . "


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

"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. ..."


"... 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...."


"...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."


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."


"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. . ."