CENTER FOR Science IN THE Public Interest

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**Comments from**
**Center for Science in the Public Interest (CSPI)**
**On the**
**OEHHA Public Review Draft:**
**Health Effects Assessment:**
**Potential Neurobehavioral Effects of Synthetic Food Dyes in Children**

**November 2020**

These comments are intended to supplement the comments that were submitted by CSPI in conjunction with other health-based organizations and physicians and researchers (see Attachment 1).

The following numbered comments are intended to make additional points and respond to some criticisms of OEHHA's draft report by the International Association of Color Manufacturers (IACM) provided on its website. IACM's members include large food companies as well as manufacturers and marketers of color additives.

1. There is sufficient evidence linking synthetic food dyes and neurobehavioral effects

IACM claims that "the assertions made in the draft report linking all FD&C colors with possible negative health or behavior effects are based on insufficient evidence."[1]

This assertion flies in the face of the more than 200 relevant studies and reviews examined by OEHHA, including 27 clinical trials in children. Indeed, it is very rare for *any* human evidence to be available on the safety or toxicity of substances intentionally added to food, much less clinical trials, the "gold standard" for establishing causality. FDA does not require clinical trials or other human studies for food or color additives, and only recommends them "if indicated by available data or information."[2] FDA's guidance states, "However, petitioners may elect to perform such studies in certain circumstances, such as when the proposed additive will be consumed by humans at relatively high levels..."[3] In CSPI's experience, few elect to perform expensive clinical trials on substances added to food. The exceptions are for major nutrients such as fats or sugar or their substitutes (e.g., related to effects on weight, weight gain, or other metabolic parameters, or on gastrointestinal tolerance in the case of individual sugar alcohols or poorly absorbed sugars).

In addition to the 27 clinical trials meeting OEHHA's inclusion criteria, the majority of which found that children reacted behaviorally to synthetic food dyes, OEHHA also included numerous animal toxicology studies in its evaluation, as well as studies relevant to understanding the mechanisms by which synthetic food dyes might cause neurobehavioral effects.

On top of the 230[1] studies related to human, animal, and mechanistic evidence it considered, OEHHA examined 283 assays in a sophisticated high-throughput assay evaluation that examined the extent to which dyes interact with specific molecular targets underlying neurological processes, or that otherwise trigger pathways suspected to lead to neurotoxicity.

All three streams of evidence—human, animal, and mechanistic—support OEHHA's conclusion that "food dyes may cause or exacerbate neurobehavioral problems in some children."

2. The evidence supporting OEHHA's conclusions that synthetic dyes cause or exacerbate neurobehavioral problems in children is consistent across evidence streams

IACM asserts that, "[t]he studies evaluated in the OEHHA risk assessment do not indicate consistent or strong associations between any food dyes and hyperactivity or neurologic effects in children."

In fact, what is so striking about the OEHHA assessment—the first systematic review and integrated assessment ever conducted on synthetic food dyes—is how consistent findings are across evidence streams (human, animal, mechanistic). The findings in multiple evidence streams reinforce and strengthen OEHHA's conclusion that synthetic food dyes can cause or exacerbate neurobehavioral problems in children. As an example, we provide more detail on FD&C Yellow No. 5, below (Appendix 1).

3. Positive associations between synthetic food dyes and neurobehavioral effects are consistently reported in children.

The majority of studies in children actually do report evidence of an association between synthetic food dye exposure and neurobehavioral outcomes.

Furthermore, as reported by OEHHA, studies using validated measures for assessing outcomes (generally higher-quality studies) were more likely to find positive associations than studies that did not use validated measures for assessing outcomes (70.6% vs. 50%), as were newer studies (conducted after 1990) compare to older studies (83.3% vs. 57.9%). This increases the confidence that the reported positive associations are real. Also, not surprisingly, studies that used larger numbers of participants tended to report positive associations more frequently than smaller studies (66.7% of larger studies (N>100) and 85.7% of large studies (20-100) % vs. 53.3% of small studies (<20)), although this difference was not statistically significant. Other aspects that OEHHA examined, such as location of the study (e.g., United States or another country), or the use of multiple dyes vs. one dye only, were not strongly related to whether a study reported an association, according to OEHHA.

In its handbook on conducting health assessments using systematic review and evidence integration, the National Toxicology Program considers "unexplained inconsistency" as a factor that can decrease confidence in the results.[4] However, as indicated above, OEHHA's analysis reveals that inconsistencies seen between human studies can at least in part be explained by well

[1] There were 207 unique cites related to human, animal, or mechanistic evidence, and 23 studies listed in Table 2.2 that did not meet OEHHA's inclusion criteria.

known factors such as quality of the studies, size of the studies, and the size of the doses used.

Additionally, the largest and best conducted studies were two randomized, cross-over, double blinded clinical trials funded by the United Kingdom of mixtures of azo dyes plus benzoate.[2] As OEHHA reports, these studies addressed many limitations of earlier study designs, used several validated outcome measures for hyperactivity, and included both children with and without hyperactivity. In the first study there were significant ($P<0.001$) reductions in hyperactive behavior during the withdrawal phase of the study, and significantly greater ($p<0.02$) increases in hyperactive behavior during the challenge period compared to the placebo period. The effects were not influenced by the presence or absence of hyperactivity or of atopy.

The second study validated the statistically significant effects on hyperactivity seen in the first study on three-year old children and extended the results to older children (8-9 years old). As OEHHA notes, the statistically significant effects in three-year old children (effect size 0.20; 95% C.I. 0.01-0.39) were greater for those who consumed at least 85% of the juice containing the dye dose and had no missing data (effect size 0.32; 95% C.I. 0.05-0.60) compared to placebo. Statistically significant effects were also seen in eight and nine year olds who consumed at least 85% of the juice, compared to placebo. Thus, the effects were replicated in three large samples of children (n=277, 140, 136).[5,6]

4. No biases or other factors invalidate the positive associations reported

It is true that not every study conducted on food dyes for neurobehavioral effects found effects. This is not at all unusual and is generally the case when reviewing evidence on any topic. Studies differ in many ways (e.g., number of subjects, age of subjects, sensitivity of subjects, dose of dyes, when exposure occurred, for how long, outcome measured, method used to measure the outcome), and all studies have limitations. OEHHA carefully examined these and other important features of the studies (e.g., adequacy of blinding, adequacy of placebo) to evaluate whether certain factors or biases might explain the results.

Regarding the evidence from clinical trials in children, OEHHA concludes, "However, after extensive analyses we were unable to identify any clear set of biases or other factors that invalidated the positive associations reported in the current epidemiological literature."

While all of the human studies evaluated exposures to children, animal studies evaluated exposures in adult animals, animals exposed in-utero, and animals exposed from before birth through adulthood. In addition to differences in the timing and duration of exposure, different animal studies used different doses; different species; different behavioral tests with different sensitivities that measuring different behaviors in different ways; and measured effects at different times in the lifespan of the animal. Therefore, it should not be expected that results should be completely consistent.

Importantly, studies that find no effects (a minority of the studies) do not outweigh studies that find effects. As noted above, OEHHA reported that studies using validated measures were more

[2] One dye in one of the mixes (mix B) was not an azo dye (Quinoline Yellow). Quinoline Yellow is approved in the United States as D&C Yellow 10 and only permitted in drugs and cosmetics, not food. Benzoate was included since some early studies (e.g., Egger et al, *Lancet* 1985;325: 540-545) included it as part of the challenge.

likely to report a positive association. Also, since individual susceptibility varies, studies finding effects may have included children more susceptible to the effects of synthetic dyes than studies that did not. Furthermore, several meta-analyses have found that the pooled overall effect size is statistically significant in the direction of adverse effects.

5. OEHHA's conclusions are consistent with those of other independent reviewers

OEHHA's assertions are fully in line with other independent reviews, including three meta-analyses[7,8,9], a review on behalf of the European ADHD Guidelines Group,[10] a review using the Oxford Center for Evidence-Based Medicine guidelines,[11] and others.[12,13,14,15]

6. The association between food dyes and neurobehavioral effects is significant

As noted previously, IACM asserts that, "The studies evaluated in the OEHHA risk assessment do not indicate consistent or strong associations between any food dyes and hyperactivity or neurologic effects in children."

In fact, OEHHA evaluated the magnitude of the association using established criteria in each of the clinical trials in children that reported an association.[3] Of the 16[4] out of 25 challenge studies that reported a statistically significant positive association, the magnitude could be measured in 13, and in 12 of those, the magnitude exceeded OEHHA's established criteria (the remaining one was borderline). In the remaining 3 studies, OEHHA found the magnitude of the statistically significant positive association to be unclear.

The criteria used by OEHHA (20% or 0.20 effect size) is roughly the same as the effect size seen in the UK studies and obtained in the meta-analyses on dyes. While this effect size may not be considered large from a statistical standpoint, the biological significance should not be underestimated.

For example, subtle yet highly significant biological effects are seen in a large (N=11,640) study that followed children with behavioral problems of various causes for nine years using a reliable

[3] The OEHHA draft report explains: "If an association was identified, we evaluated whether the mean difference was greater than 20%, whether an effect >20% was seen in any individual, or whether the standardized effect size was >0.20. An effect size of 20% is close to the minimal effect size detectable with sufficient statistical power ($\beta$=0.80, $\alpha$=0.05, paired sample test) for the parent portion of the Conners test in a study with 44 participants, the average size of the studies meeting our inclusion criteria (we used the average test-retest correlation of 0.70 reported by Nigg et al., 2012 and the mean score of 12.86 (standard deviation=6.39) from Harley et al., 1978b in these power calculations). A standardized effect size of 0.20 is also close to that reported for synthetic food dyes in the meta-analysis by Nigg et al. (J. T. Nigg et al. 2012). This criteria is similar to the "Large magnitude" criteria used by National Toxicology Program risk of bias tool (NTP Office of Health Assessment and Translation 2019) and the "Strength of the association" criteria used in the causal inference methods of Bradford Hill (Bradford Hill 1965). We acknowledge that the specific criteria we use here are somewhat arbitrary. However, effect size is an important component for evaluating causality since small effect sizes (mean differences close to 0) are more likely to be due to relatively small degrees of confounding or other bias than larger effect sizes (Axelson 1978). In addition, large effect sizes may be real, but not statistically significant because sample sizes were too small. Our evaluations of effect size are not meant to imply that all small effect sizes are due to confounding or bias or that all large effect sizes are real. Rather we used this criterion only to help identify results that might be especially prone to bias or confounding, and to identify effects that might be real but for which sample sizes were too small for statistical significance. We did not use this criterion as our sole indicator of causality."

[4] In one of these 16 studies, OEHHA rated the statistical significance of the association as "likely".

and well-validated measure of behavior; it found that each 1-point increase in inattention at age 7 was associated with a 6-7% increased risk of failing to gain five good grades on a standardized test taken by students aged 15-16 in England, Wales, Northern Ireland and other British territories.[16]

7. IACM and its members have a financial interest in the continuing use of synthetic food dyes and in opposing OEHHA's conclusions

IACM and its members have a clear financial interest in the continued use of synthetic food dyes. Synthetic food dyes are cheaper than using fruits and vegetables to provide color to food, or colorings derived from fruits vegetables.[17] According to the most recent annual letter by the Executive Director of IACM posted on the IACM website, "IACM maintains five strategic objectives that drive all the work that we do."[18] The five strategic objectives include "Protect and expand the worldwide uses of colors."[19]

Additional Recommendations

In addition to the recommendations contained in the joint comments by CSPI and others previously submitted (see Attachment 1), we offer the following additional recommendations.

***Convey Conclusions Clearly and Unambiguously; Avoid or Clarify use of "May"and "Some"***

The risk characterization section of the draft report (Chapter 7) states (p. 247),

> "Based on multiple streams of evidence, the FD&C synthetic food dyes cause or exacerbate neurobehavioral problems in children (see Chapter 5, Hazard Identification.)"

CSPI fully supports this conclusion and recommends that this way of stating the conclusion be used throughout the report.

We are concerned that some readers will misinterpret the conclusion in the Executive Summary, due to use of the term "may" and "some." That conclusion states,

> "The scientific literature indicates that synthetic food dyes may cause or exacerbate neurobehavioral problems in some children."

We recommend that "may" and "some" be deleted.

Saying "some" children implies that the neurobehavioral effects of synthetic food dyes are limited to a particular subpopulation of children. In fact, effects from synthetic dyes are not limited to children with food intolerances, ADHD, atopy, or any other identifiable subpopulation. OEHHA was not able to identify predictors for which children will experience behavioral problems from synthetic food dyes. The UK-funded studies were unable to identify any simple demographic or clinical attribute (e.g., gender, social class background, atopy) indicating which children would react. The UK team did identify certain genetic polymorphisms that appear to confer greater susceptibility to synthetic food dyes,[20] but further studies are needed to replicate the results, and it would not be practical to screen children for these genetic polymorphisms.

Furthermore, the term "some" is redundant. There is always a distribution of response to exposures. Some people experience an adverse reaction to a drug, while others tolerate it well.

Some people are exposed to COVID-19 and do not develop symptoms, whereas others develop symptoms, sometimes severe or fatal. Others do not even become infected. Some people develop lung cancer from smoking cigarettes, whereas others may smoke for many years without developing lung cancer. Yet we do not say that cigarette smoking causes lung cancer in "some" people. It is understood that the probability of developing an effect, disease, or health condition from an exposure will vary amongst individuals.

This is important to clarify since it corrects an earlier view put forward by FDA at its Food Advisory Committee in 2011 that the effects of synthetic food dyes on behavior "appear to be due to a unique intolerance to these substances and not to any inherent neurotoxic properties."[21] In fact, the best animal studies and mechanistic evidence clarify that dyes do have neurotoxic properties.

"May" should be deleted since some readers interpret "may" to mean that OEHHA has not been able to arrive at a firm conclusion regarding the association between food dyes and neurobehavioral problems in children, because the evidence is not sufficiently strong. In our view, this incorrect. Moreover, it is not consistent with the conclusion in the risk characterization section of the report cited above ("the FD&C synthetic food dyes cause or exacerbate neurobehavioral problems in children"). Because results are consistent across evidence streams, and include human evidence derived from 27 clinical trials, OEHHA has reached a firm conclusion, based on sufficient evidence, and this should be made clear.

In our view, the use of "may" in this context does not reflect doubt or uncertainty about whether or not there is an association between synthetic food dyes and neurobehavioral problems in children, but reflects the fact that the response of people varies and that not each and every person will experience neurobehavioral problems when exposed to synthetic food dyes. This is analogous to use of "may" on drug labels, for example, some antihistamines that state, "may increase drowsiness," not because there is doubt about whether antihistamines cause or increase drowsiness, but because responses between people may vary (and responses may vary even for the same person, depending on other factors).

As stated above, we recommend that OEHHA use the form of the conclusion in its risk characterization, i.e., "Based on multiple streams of evidence, the FD&C synthetic food dyes cause or exacerbate neurobehavioral problems in children." Alternatively, the word "may" in the Executive Summary conclusion could be replaced by "can." If OEHHA uses the term "may," it should clarify that "may" does not reflect the level of confidence in the results or the sufficiency of the evidence, but rather, is an acknowledgement that responses to dyes, just as to any type of exposure, from smoking to COVID-19 to medications, can vary. And, for the reasons stated above, we recommend that the word "some" before "children" be deleted.

***Consider the cumulative effect of color additives, taking into account chemically or pharmacologically related substances, though not at the expense of delaying the final report.***

Studies of synthetic food dyes have been conducted both on individual dyes and mixtures of dyes.[5] For example, FD&C Yellow No. 5 is the only dye that has been tested individually in

[5] In some studies, a preservative such as sodium benzoate was also included in the mixture.

humans. Studies of mixtures of synthetic food dyes have been conducted with the recognition that children are not exposed to dyes in isolation but rather are exposed to mixtures of synthetic food dyes from multiple sources, primarily food, drugs, and supplements, that may have interactions (e.g., synergistic or additive effects).

FD&C Yellow No. 5, Yellow No. 6, and Red No. 40 are all chemically related azo dyes. These three dyes comprise more than 90% of dyes certified for use in food in the United States. In many studies of mixtures of dyes in children, the amount of dye used was proportionate to the amount certified. Thus, these three azo dyes dominated many of the mixtures tested.

Section 706 of the Federal Food, Drug and Cosmetic Act codified under 21 U.S.C. § 379e states:

> "In determining, for the purposes of this section, whether a proposed use of a color additive is safe, the Secretary shall consider, among other relevant factors—
>
> (i) …
>
> (ii) the cumulative effect, if any, of such additive in the diet of man or animals, taking into account the same or any chemically or pharmacologically related substance or substances in such diet; …"

Furthermore, 21 CFR 70.11 states that in the absence of evidence to the contrary, color additives that cause similar or related effects should be considered to have additive toxic effects:

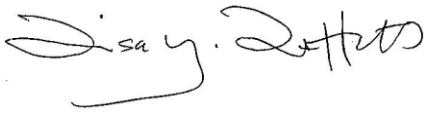
> Different color additives may cause similar or related pharmacological or biological effects, and in the absence of evidence to the contrary, those that do so will be considered to have additive toxic effects.

In characterizing the risks of dyes, OEHHA should develop appropriate reference levels based on neurobehavioral effects, and consider the cumulative effect of each dye, taking into account chemically or pharmacologically related substances, especially including other synthetic food dyes. At the very least, the cumulative effect of chemically related azo dyes should be considered. A number of resources may be useful in this regard.[22,23.24,25]

Given that there are no human trials on FD&C Red 40 and Yellow 6 individually, should there be any doubt as to whether those dyes cause or exacerbate neurobehavioral problems in children—despite the ample animal and mechanistic evidence on those dyes when tested individually, and the evidence from human and animal studies of the neurobehavioral effects of mixtures in which those dyes (plus FD&C Yellow No. 5) contributed the largest amounts to the mixtures—then the compelling evidence on FD&C Yellow No. 5 demonstrating neurobehavioral effects could be utilized for FD&C Red No. 40 and Yellow No. 6, since they are in the same chemical class (azo dyes). This is analogous to the decision FDA made to apply data demonstrating reproductive and developmental toxicity on PFOA to other long chain perfluorinated chemicals for which such data were unavailable.[26]

Thank you for considering our comments, and we appreciate your excellent report.

Sincerely,



Lisa Y. Lefferts, MSPH, Senior Scientist

Peter Lurie, MD, MPH, President

## Appendix I: A Closer Look at One Dye: FD&C Yellow No. 5

### Compelling human evidence indicates that FD&C Yellow No. 5 causes neurobehavioral effects in children

FD&C Yellow No. 5 (also called tartrazine) has been studied both in mixtures of dyes, and individually. It is the only dye that has been tested individually in human studies. There have been six clinical trials of FD&C Yellow 5/tartrazine in children. All but one of the studies found that the dye caused effects on behavior.

The largest of the six (Rowe and Rowe 1994) was a double-blinded placebo-controlled trial that tested multiple doses of Yellow 5 (0,1,2,5,10, or 20 mg) on children, over half of whom did not have behavioral problems, and used a validated behavior test. The behavior scores on days the children were given the dye challenge were significantly different than the scores on the days they were given a placebo. Furthermore, the more dye that was consumed, the worse the children scored. This kind of dose-response relationship is strong evidence of a true effect. The mean behavior score difference between the group of children who reacted to dyes and the group that did not was statistically significant at doses of 2 mg and higher.

### Children are exposed to FD&C Yellow No. 5 at levels that cause behavioral effects

For the study described above, OEHHA identifies 1 mg as the "No Observed Adverse Effect Level" (NOAEL). According to OEHHA, and using a reference body weight of 25.5 kg for the mean age of 7 years, 1 mg is equivalent to 0.04 mg/kg/day. Thus, 2mg is the "Lowest Observed Adverse Effect Level" (LOAEL). FDA and other agencies generally use the NOAEL from the study with the smallest NOAEL as a point of departure to derive an "Acceptable Daily Intake" (ADI), the amount of a substance that is considered safe to consume each day. In cases where a NOAEL is not available, the lowest LOAEL is generally used, in combination with the application of an additional safety or uncertainty factor.

By way of contrast, the FDA ADI for FD&C Yellow No. 5 is 5 mg/kg-bw/day. Thus the FDA ADI is 125 times higher than the NOAEL identified by OEHHA.

Significantly, the mean one-day exposure for children of all age groups examined by OEHHA exceeds the 2 mg LOAEL under a typical exposure scenario, except for children under 2 years; for those children, the mean one-day exposure under a high-exposure scenario exceeds 2 mg/day.

### Animal and mechanistic evidence support the conclusion that FD&C Yellow No. 5 causes neurobehavioral effects in children

Several older animal studies[27,28,29] found minimal effects on behavior or learning in rats exposed starting in utero to doses below those known to be toxic at that time, but they had significant weaknesses that make interpretation difficult.[6]

[6] For example, in the 1977 study by Sobotka, advancing clinging responses in exposed pre-weaned infants were noted (no effects reported in post weaning behavioral tests), but no data was provided on pregnancy outcomes or on

More recent animal studies with more robust study designs have identified a relationship between Yellow No. 5 treatment in rodents and neurobehavioral outcomes. Gao and colleagues found in a 2011 study that exposure to Yellow No. 5 affected activity in rats and learning and memory in mice.[30] The investigators used doses around and below the NOAEL (500 mg/kg/day) that FDA used to establish an ADI (of 5 mg/kg/day) for FD&C Yellow No. 5, administered by gavage for 30 days, beginning as juveniles. (Gavage dosing more closely mimics dosing in human challenge studies, compared to dosing consumed in feed or water over the day). Rats treated at doses of 250 and 500 mg/kg/day showed a statistically significant increase in activity in terms of horizontal movement ($p<0.05$) and vertical activity (rearing) ($p<0.01$) compared to controls. In mice, doses of 300 and 700 mg/kg/day interfered with learning, as measured by a water maze and a step avoidance task, compared to controls. These findings were also statistically significant. The NOAEL was determined to be the low dose of Yellow No. 5 in both the rat and mouse experiments, 175 and 125 mg/kg/day, respectively, lower than the NOAEL of 500 mg/kg/day FDA relied on to establish an ADI.

The Joint Expert Committee for Food Additives (JECFA) of the World Health Organization and Food and Agriculture Organization stated that the small numbers of animals per dose group (10 animals/dose group) precluded the use of the Gao studies in mice and rats in its evaluation.[31]

However, the use of a smaller than desirable number of animals biases a study towards the null hypothesis because it decreases the power of a study to detect effects; it does not bias a study away from the null hypothesis and justify discounting effects. Furthermore, it should be noted that the FDA ADI of 5 mg/day was established in 1969 and is based on a NOAEL of 500 mg/kg-bw/day derived from a study in dogs that used only 4 animals/dose group.

Most notably, a 2017 study by Rafati et al. performed an experiment in 70 young adult rats (10/group) using low doses (0, 5, and 50 mg/kg/day) of Yellow No. 5 (the low dose was equivalent to the FDA ADI).[32] This study found that treated rats administered Yellow No. 5 daily for 7 weeks via gavage (both doses combined) required significantly more days to learn a maze compared to controls ($p<0.01$), and more errors were seen in treated groups ($p<0,01$). The investigators concluded that 5 mg/kg/day was the lowest observed adverse effect level (LOAEL) of Yellow No. 5, equivalent to FDA's ADI. This study is notable for testing and finding effects at such low doses. If this LOAEL was used to derive an ADI it would be between 100 and 1,000 times lower than FDA's ADI.

Interestingly, this experiment design also included the use of an antioxidant, vitamin E, which was administered to a group alongside Yellow No. 5, as oxidative stress is identified in the mechanistic literature to be a potential mechanism of Yellow No. 5 neurotoxicity.[33-37] The

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behavioral tests for control animals, there was no statistical analysis of results, and the results were not litter-based. Two studies on FD&C Yellow No. 5 by the Tokyo Metropolitan Institute of Public Health in 2006 and 2008 by Tanaka et al., a 2-generation and three-generation study, respectively, indicated for example treatment effects on juvenile males as measured by number of movements in the 2-generation study. However, due to deaths in dams, offspring, and some dams that did not become pregnant, there were sometimes only seven or eight animals per dose group, and the studies were insufficiently powered. Furthermore, some of the behavioral results seen may have been influenced by dam and litter loss when pups in all litters were pooled for testing, and litter-based statistics were not performed.

antioxidant mitigated most of the effects of Yellow No. 5, supporting this mechanistic hypothesis.

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