Overview and Evaluation
of
Proposed Association Between
Artificial Food Colors and Attention Deficit Hyperactivity Disorders (ADHD) and Problem Behaviors in Children

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I Introduction

The present report addresses the project assignment (#2008-31) to conduct a critical review and analysis of peer-reviewed clinical trials, identified through a comprehensive literature search (ORNL), related to the consumption of food additives and problem behaviors in children, such as the attention deficit hyperactivity disorders (ADHD). This review focused on those trials that related to artificial food colors, since food color was the type of additive most commonly investigated in relation to possible adverse effects on children's behaviors, such as ADHD, and was also the basic focus of a citizen's petition to the FDA (dated June 3, 2008). However, many of the trials reviewed in this report also involved artificial flavors, natural salicylates, preservatives, other additives, and general food items in conjunction with artificial colors.

As requested in the project assignment, a thorough search of the literature from 1982 to the present was conducted and in-depth reviews of the identified relevant clinical publications (17 clinical trials) were carried out. In addition, 16 other relevant publications of clinical trials conducted prior to 1982 (between 1976 and 1981) were identified, most of which were also referenced in the citizen's petition to the FDA. For purposes of completeness and continuity, these pre-1982 clinical trials were also included in this review.

As noted in the attached *Bibliography*, this report was based on an in-depth review and analysis of a total of 33 clinical trials with supplemental information obtained from 17 animal/laboratory studies and 51 background documents, including various reports, commentaries, reviews, and meta-analyses. A summarized outline of the 33 clinical trials in chronological order is presented in *Table 1* (the 16 pre-1982 clinical trials are identified by grey highlighting of the Author/Year in column one of the Table) and the detailed review of each is presented in the attached *Reviews and Critiques* section (and file) of this report.

II Background

Among the variety of problematic behaviors in children that are particularly disruptive in the home and school environment is a spectrum of behavioral disorders characterized by a pattern of behavior deficits including inattention, impulsivity, disinhibited behavior, and overactivity. Of course, similar types of behaviors are seen in most children in the general population (Conners, 1970a: *Conners, 1970b; Pollock, 1991; Stevenson, 2006; Taylor, 1984*), but when these behaviors are determined by professional judgment to occur in a developmentally and situationally inappropriate manner, persist over a prolonged period of time and at a high level of severity, and possibly be associated with learning disabilities, they may be considered part of a pathological state of hyperactivity (Pollock, 1991), commonly referred to as attention deficit hyperactivity disorder of childhood (ADHD) (*Diagnostic and Statistical Manual III* of the American Psychiatric Association). Other terms used include hyperactivity syndrome of childhood, hyperkinetic syndrome, hyperkinesis, minimal brain dysfunction, attention deficit disorder, and conduct disorder, although some distinctions may exist between these terms (NIH Consensus, 1983; Pollock, 1991; Ribon and Joshi, 1982; Schab and Trinh, 2004; Schauss, 1984; Silbergeld and Anderson, 1982; Taylor, 1984). ADHD is generally estimated to occur in approximately 3 – 10% of the population of school children (Banergee et al, 2007; National Institutes of Health, 1998) with a male preponderance of about 4:1 (Pollock, 1991).

Many possible factors have been suggested to be involved, singly or synergistically, in the etiology of childhood ADHD syndromes, including environmental, genetic, allergic/immunologic, psychosocial, and dietary (Arnold, 1999; Banerjee et al, 2007; Cormier and Elder, 2007; Cruz and Bahna, 2006; NIH, 1982; Pollock, 1991; Stevenson, 2006;Taylor, 1984; Wender, 1986). Focused interest in the association between diet and hyperactivity and other problem behaviors in children was stimulated in 1973, when an hypothesis was put forward that certain food additives, specifically artificial food colors and flavors and natural salicylates, may cause or exaggerate a number of childhood behavior disorders, such as hyperkinesis (ADHD) and learning disabilities (Feingold, 1973 and 1975). Based on clinical observations, Feingold claimed that some children with problem behaviors, including hyperactivity and learning disabilities, showed notable improvement when given a defined diet excluding foods with artificial colors, flavors and natural salicylates and that dramatic deterioration of behavior occurred when the children were exposed to foods with even small amounts of those additives, particularly the artificial colors. Feingold's elimination diet rapidly became a popularized treatment for hyperactivity among the general public, even though the scientific community continued to question the validity of Feingold's uncontrolled anecdotal clinical observations (Wender, 1986). The need for sound scientific study to assess the validity of Feingold's claims that foods with artificial colors and other additives can cause or trigger ADHD types of problem behaviors in susceptible children was evident and resulted in a series of peer-reviewed clinical trials being conducted over the last 35 years, along with a vast array of related published reports, reviews, analyses, and commentaries. A total of 31 clinical trials, fairly representative of those conducted over this period of time and considered relevant to an assessment of the proposed association between artificial food colors and problem behaviors in children, were included in this project review.

III Criteria for Review and Assessment of Clinical Trials

In the review and assessment of each clinical trial, it was deemed important to identify all study findings, both positive and negative, and to consider any relevant experimental factors, particularly limitations, and inconsistencies or uncertainties in the data that may impact on the credibility, reliability and interpretability of the reported study findings. Further, in the interpretation of results efforts were made to distinguish between statistically significant and clinically relevant findings since some outcome measures may reveal statistical differences between treatment and control that may be within normally accepted standards. To this end, the general criteria used for assessing the trials and interpreting the findings included due consideration of the following (Krummel et al, 1996; NIH, 1982; Schab and Trinh, 2004; Wender, 1986):

- homogeneity of sample
- randomization to treatment
- crossover designs with subjects serving as own control
- counterbalanced treatment/challenge order
- double-blind/placebo-controlled challenges
- placebo and challenge indistinguishable
- *verification of effectiveness of blinding particularly for behavioral raters
- appropriate control outcome measurements
- age-appropriate outcome measures
- use validated measures (i.e. detect behavior differences/sensitive to treatment)

- *confirmatory sources of outcome data (parents, teachers, testing, etc.)

* Two of the above factors were considered by this reviewer to be particularly important in the evaluation and assessment of the study findings, specifically *confirmatory sources of outcome data* and *verification of effectiveness of blinding*. The importance of the factor *confirmatory sources of outcome data* was related to determining how much weight should be given to this factor in assessing reliability of study findings? Is a positive effect based on one source of data sufficient to conclude a reliable finding? The difficulty in making this determination was compounded by the fact that some trials used only one source of data, while other trials used multiple sources. Out of 33 clinical trials, 11 used only a single source of outcome data to assess treatment related effects on behavior and 8 of those showed positive effects; no other source of data was included in those studies to provide any confirmation of the positive findings. The remaining 22 clinical trials used multiple sources of outcome data but in 8 of those trials only one of the multiple sources of data showed positive treatment effects and no confirmation or support of a treatment effect was provided by the other sources of data in each of those 8 trials. Lipton and Mayo (1983) considered treatment related effects detected by one outcome measure (i.e., parent rating) to be suspect if there was no confirmation by other observers or objective tests. Similarly, Stevenson (2007) considered the study results reported by Batemen et al (2004) as somewhat equivocal, since they only related to the parent ratings. In making the determination of weighting and level of confidence in the present review, this reviewer adopted a conservative approach and in most cases considered any findings that were based solely on one source of data to be suggestive, but not conclusive evidence, of a treatment effect. Less confidence was given to the reliability of a single source finding when other sources of assessing behavioral change were used in the same study and provided data showing no confirmation or supportive evidence of any treatment related effects. It should be noted that many of the trials (14/33) cited in the present review reported treatment related effects based only on a parental rating outcome measure (either as the only source outcome measure used or the only outcome measure to detect a treatment effect with no confirmation by other behavioral measures). Consequently, since only a single source outcome measure detected an effect, a lowered weighting/level of confidence was generally assigned to these study findings. However, it should be clear that this lower weighting/less confidence was not selectively directed at the use of parental ratings but at the use of a single source outcome measure of behavioral change. Parental ratings under controlled clinical conditions are viewed as uniquely sensitive and as providing relevant information in the assessment of treatment related changes in behavior (Schab and Trinh, 2004), although it is considered preferable if they could be supported with observations by independent observers or the use of standardized tests (Stevenson, 2007). The main issue in assigning the weightings/confidence levels in this review was confirmation of a treatment related finding across outcome measures within the same study.

The importance of *verifying the effectiveness of the blind* stems from the widely acknowledged tenet that in any clinical therapeutic study the use of appropriate blinding procedures is essential to preclude placebo effects and observer bias from influencing the study results. In non-blinded studies the participants are typically aware of the subjects' treatment conditions and, consequently, the possible influence of placebo effects and observer bias lessens confidence in the reliability of those study findings. Double-blind studies, however, are considered more reliable, since various efforts are made to prevent (blind) the researchers, observers and subjects from knowing when the active or placebo treatments are administered (CSPI, 2008)**.** However, even in double-blind studies, it is possible that the blinding procedures used in a particular study

may not be completely adequate or effective. Therefore, it is important that the effectiveness of the blinding procedures be assessed in each study. Without such assurance of the effectiveness of the blinding, the reliability of the study's findings may be considered questionable.

IV Summary Reviews/Assessments of Clinical Trials

An in-depth review and analysis for each of the 33 clinical trials included in this report is contained in the attachment, *Reviews and Critiques*. Those reviews are presented in that attachment in the chronological order in which they were published. This section of the report will present a brief summary and assessment of the findings from each of the clinical trials. *Table 1* is provided as a reference outline of each trial (in chronological order), color-coded† to identify those trials that used the Feingold diet, other diets, color challenges or food item challenges as the experimental treatment and to indicate for each trial whether treatment effects were found for each outcome measure used in that study.

The 33 clinical trials reviewed in this report are separated into two general groups based on a notable difference in one particular feature of the experimental design, that is, the nature of the experimental treatment. Group I consists of 26 clinical trials (color-coded pink† in Table 1) that are characterized by the use of an experimental treatment consisting of either the Feingold diet (eliminating foods with artificial colors, flavors and natural salicylates) or challenges with select artificial food colors (Conners et al, 1976; Harley et al, 1978 PI; Harley et al, 1978 PII; Goyette et al, 1978; Rose, 1978; Levy and Hobbes, 1978; Levy et al, 1978; Mattes and Gittelman-Klein, 1978; Williams et al, 1978; Conners, 1980; Conners et al, 1980; Swanson and Kinsbourne, 1980; Weiss et al, 1980; Mattes and Gittelman-Klein, 1981; Adams, 1981; Spring et al, 1981;Salamy et al, 1982; Thorley, 1984; David, 1987; Rowe, 1988; Wilson and Scott, 1989; Pollock and Warner, 1989; Sarantinos et al, 1990; Rowe and Rowe, 1994; Batemann et al, 2004; McCann et al, 2007). This group of clinical trials assessed the validity of Feingold's hypothesis relating artificial colors and ADHD and other problem behaviors in susceptible children. The first fourteen of these trials (Conners et al, 1976 to Spring et al, 1981 as listed above; also color-coded grey† in Table 1) were conducted prior to 1982, at which time the National Institutes of Health convened a Consensus Panel to review the status of information regarding defined diets (Feingold diet) and childhood hyperactivity (NIH, 1982). Subsequent to that NIH Consensus Panel, ten additional clinical trials (Salamy et al, 1982 to McCann et al, 2007, as listed above) were conducted to investigate further the adverse behavioral effects of artificial colors in children. Group II consists of 7 clinical trials (color-coded blue† in Table 1) that are characterized by the use of an experimental treatment consisting of either a foods elimination diet (also referred to as 'oligoantigenic' and 'few foods' diet that excludes all foods, additives and food components assumed to provoke adverse behavioral reactions in certain children) or challenges with specific provoking food items (Egger et al, 1985; Kaplan et al, 1989; Egger et al, 1992; Carter et al, 1993; Boris and Mandell, 1994; Schmidt et al, 1997; Uhlig et al, 1997). The use of this type of experimental treatment in studies, all of which were conducted after the NIH Consensus Panel (NIH, 1982), focused on a broader array of substances by assessing adverse effects of food itself in hyperactive and problem behavior children and reflected the growing opinion that it may be more likely that any adverse food related behavioral changes in hyperactive children are caused by an individual intolerance to various foods or food components (which could include color additives), rather than by a singular involvement of color or other additives (Arnold, 1999; Bishop, 1983; Courmier and Elder, 2007; Robinson and Ferguson, 1992; Stare et al,

1980;Young, 1997). The two different experimental treatments in these two groups of clinical trials may reflect either two different views or one evolving view of the relationship between food/colors and ADHD and other problem behaviors in children.

All of the clinical trials were designed to be conducted under double-blind, placebo-controlled conditions using one of two basic types of experimental procedures - "*diet crossover*" which focused on determining under controlled scientific conditions whether a particular defined diet was an effective dietary treatment for reducing problem behaviors in children, particularly those associated with ADHDs, and "*specific challenge*" which focused on determining whether exposure to a particular food item, excluded from the defined diet, could trigger or exaggerate adverse behavioral changes in the test population of children (Bishop, 1983).

Group I Trials (n=26)

As noted above, the focus of these trials was to assess the validity of Feingold's hypothesis relating artificial colors and ADHD and other problem behaviors in susceptible children. Of the 26 trials in this group, 2 were "*diet crossover trials*" assessing the effectiveness of the Feingold diet, eliminating artificial food colors, flavors and natural salicylates, in improving the behavior of hyperactive children. The remaining 24 trials were "*specific challenge trials*" which assessed the adverse behavioral effects of artificial food colors (either mixtures or single colors), occasionally in conjunction with a food preservative, in children diagnosed ADHD, with problem behaviors, or from the general population. Although Feingold's hypothesis implicated artificial colors, flavors, natural salicylates, and other additives, clinical investigations focused initial attention primarily on artificial food colors, since colors were specifically mentioned by Feingold as being of primary concern and since colors represented a relatively smaller group of chemicals to deal with in comparison to the food flavors and were thought to be more easily masked (blinded) than artificial flavors in the challenge studies. The implied intent, however, seemed to be that eventually other categories of food additives would be investigated. With the exception of several artificial color studies that also included one or two preservatives as part of the experimental treatment, no systematic clinical investigation of adverse behavioral effects of flavors or natural salicylates has yet been carried out.

Typically, the procedure for the Group I "*diet crossover trial*" involved randomly assigning a group of children to either a Feingold diet or a matched placebo diet for a defined period of time, then crossing them over to the other diet condition for an equivalent period of time and evaluating the behavioral responses to both diet conditions. For the "*specific challenge trial*" all subjects were maintained on the Feingold diet for a variable period of time prior to and throughout the study period. Each child was given a color challenge (i.e., a blend of colors or an individual color) in some delivery item that masked the presence of the color (e.g. cookies, cupcake, drink, or capsule), and a matched placebo treatment in randomized fashion for a defined period of time or on random days. A variable washout period was used between challenge periods. Behavioral responses were measured at specified times for both treatment conditions. It should be noted that challenge studies provide information for only one component part of the Feingold diet (e.g. artificial colors) and should not be interpreted as evidence of effectiveness for the diet as a whole. In the recruitment of subjects for the "specific challenge" trials, many trials (18/22 cited below) included efforts to maximize detection of behavioral

effects in the controlled challenge study by including a specific selection criterion that the children had to be reportedly sensitive to the Feingold diet under non-blind conditions, showing marked behavioral improvements on the open Feingold diet and exhibiting immediate and dramatic deterioration of behavior after ingesting even small amounts of prohibited foods containing artificial food colors and other additives (Harley et al, 1978- Phase II; Goyette et al, 1978; Rose, 1978; Levy and Hobbes, 1978; Levy et al, 1978; Mattes and Gittelman-Klein, 1978; Conners, 1980; Conners et al, 1980; Weiss et al, 1980; Mattes and Gittelman-Klein, 1981; Adams, 1981; Spring et al, 1981; Salamy et al, 1982; David, 1987; Rowe, 1988; Wilson and Scott, 1989; Pollock and Warner, 1989; Rowe and Rowe, 1994)

Individual Trial Summary Reviews (Group I)

- In the first controlled crossover trial of the Feingold diet **(Conners et al, 1976)** the teacher behavior ratings showed an overall behavioral improvement for a group of 15 school age diagnosed hyperkinetic children on the Feingold diet but the biological significance of this finding was confounded by an inexplicable treatment x order effect in which the improved behavior was seen only when the placebo diet was given first. Also, the parent ratings showed no diet related changes in behavior. Viewing the data for individual subjects indicated that teacher and parent ratings did agree that 4 to 5 of the 15 subjects showed improved behavior on the Feingold diet. Since the significance of even this observation was also confounded by the treatment x order effect, overall this trial provided no evidence that Feingold's diet improves behavior of hyperkinetic children.

- The only other controlled crossover trial of the Feingold diet was conducted by **Harley et al (1978: Phase I)** who included independent classroom observations and objective laboratory testing in addition to parent and teacher behavior ratings to assess behavioral change. This trial tested 46 hyperactive children (36 school age and 10 pre-school age). Analysis of the data for the 36 school age children showed inconsistent findings, similar to Conners et al (1976), except that here the behavioral improvements on the Feingold diet were noted by the parent ratings but were not confirmed by either the teacher ratings or the other outcome measures used in this study. The biological significance of the parent finding was also confounded by an inexplicable treatment x order effect. This also confounded the observation that parents and teachers agreed on a diet response in 4 of the 36 children. Analysis of the data for the 10 pre-school children also showed inconsistent findings with significant behavioral improvement on the Feingold diet being noted by the parent ratings but no confirmatory diet effect being found with either the classroom observation or laboratory testing (teacher ratings were not available for the pre-school children). In contrast to the school age children, the parent rated diet change in behavior of the pre-school children was not confounded by a treatment x order effect. Overall, in view of the inconsistent findings and the confounding treatment x order effect, this study provides no credible support for the contention that the Feingold diet affects disruptive behaviors in either school age or pre-school hyperactive children. It is questionable whether these findings would support the suggestion that a small subpopulation of younger, pre-school age hyperactive children may be responsive to the Feingold diet.

- In one of the first uses of the specific challenge design to assess the adverse behavioral effects of artificial colors, **Goyette et al (1978)** conducted a two-part trial. In Trial 1, 16 hyperactive children (4.7-11.8 years, mean age 8.3), prescreened to be 'diet responsive'

(behavioral improvement on open, i.e., non-blind, Feingold diet), exhibited no significant behavioral response to a daily cookie challenge with 27 mg mixed colors for two 2-week periods based on multiple outcome measures (parent rating, teacher rating, laboratory tracking test). A non-significant trend of performance deficit related to color challenge was noted for the younger subjects at 1 but not 2 or 3 hours after treatment. Trial 2 was a repeat of Trial 1 except that: a parent rating focused on the 3 hours after treatment was the only outcome measure; daily challenge was 13 mg mixed color for 1 week; and 13 younger children (3.4-10.2 years, mean age 6) were recruited, 8 "criterion" diet responsive/hyperactive (3.4-8.4 years, mean age 5.3) and 5 "borderline" diet responsive/hyperactive (3.7-10.2 years, mean age 7). Significant color challenge effects were found based on the parent ratings and, notably, without any differences in color responses between the 'criterion' and 'borderline' groups. In the absence of any other outcome measure to confirm the parent finding and without any verification of effectiveness of blinding, the reliability of the Trial 2 findings are considered questionable. Also, there seems to be limited support for the conclusion that artificial colors may be particularly disruptive to younger children or that they are more sensitive to artificial colors. The suggestive trend in Trial 1 of a treatment effect on tracking performance in younger subjects is not significant (and not supported by the parent and teacher ratings) and in Trial 2, the limitations of these findings notwithstanding, the 'criterion' children (mean 5.3 years) were younger than the 'borderline' group (mean age 7 years), yet there was no difference between these two groups in their color responses. Overall, this study does not provide credible evidence of an effect of artificial colors on behaviors of reported diet responsive hyperactive children. Note that a replication of this study by Conners (1980) detected no significant adverse effects of daily challenge with 26 mg mixed colors and the investigator concluded that the effects reported by Goyette et al (1978) were most likely due to chance and not biologically relevant.

- **Harley et al (1978 :Phase II)** conducted color challenge trial, as the second phase of an earlier diet crossover trial, using the best 9 diet responding hyperactive subjects from that earlier study. There was some question whether these children still appeared to demonstrate appreciable behavioral improvement when placed on the open Feingold diet immediately prior to the challenge phase of this study. A daily challenge with 27 mg mixed color in candy bars/cookies over 2 periods of 2 or 3 weeks each produced no significant behavioral changes based on multiple outcome measures (parent, teacher and trained classroom observer ratings and neuropsychological testing). There was a tenuous, but suggestive observation, that the parent rating and observer rating profile 1 of the 9 subjects may have approximated that of a color challenge effect, but this was not supported by the teacher ratings (individual neuropsychological data were not inspected). This study does not support the hypothesis that artificial food colors cause or trigger hyperactivity behaviors of childhood.

- **Levy and Hobbes (1978)** conducted one of the first color challenge studies using a single artificial color, tartrazine, as the treatment. Parent ratings and global assessments revealed no significant adverse behavioral effects of daily challenge with 4 mg *tartrazine* in cookies for 2 weeks on behaviors of 7 hyperactive children, identified as responsive to the Feingold/KP elimination diet. Within the context of the limitations for this study, that no blinding procedures were described and only one source (parent) of outcome determination was used, this study provides no evidence that tartrazine has any adverse effect on behavior in hyperactive children who are reportedly responsive to the Feingold diet.

- **Levy et al (1978)** conduct a challenge study of tartrazine (Yellow 5) in 20 hyperkinetic children. Responsiveness to the Feingold diet was determined in open trial prior to the challenge study. In group analysis there was no significant adverse behavioral effect of daily challenge with 5 mg tartrazine in biscuits for 2 weeks based on multiple outcome measures (mother, teacher and clinician ratings, and a series of objective psychological tests of activity, attention, impulsivity, perceptual-motor function, memory, intelligence and maze performance). Additional post-hoc analyses determined that the level of hyperactivity (prior to study) did not amplify the children's sensitivity to diet (i.e., response to open treatment with Feingold diet) or to tartrazine challenge. Additional post hoc analysis was conducted on a subgroup of children specifically rated by mothers under non-blind conditions as diet-responders, i.e. exhibited significant reductions in problem behaviors when placed openly on the Feingold diet. Analysis of the challenge data for these children did show a significant adverse tartrazine challenge effect based on mother ratings. However, no other confirmatory outcome measure was included in this particular post-hoc analysis. The blinding of this study may have been compromised since some of the color challenge biscuits differed in color from the placebo biscuits. Considering that the main analyses of the study data showed no significant color challenge effect based on multiple outcome measures, that the blinding in this study was compromised to some indeterminable extent, that the singular treatment effect was based on post hoc analyses of only one source of outcome measure (mother ratings) with no other outcome measures analyzed to support this finding, there is, overall, little, if any, confidence in the reliability or biological relevance of this study's finding of a treatment related effect or in its value to suggest that artificial colors have any effect on hyperactivity behaviors in childhood.

- The controlled challenge trial reported by **Mattes and Gittelman (1978)** using a mix of common artificial colors is significant for two reasons. This was the first attempt to individualize dosage of the challenge material to the sensitivity of the subject and this may have been one of the earliest suggestions that adverse effects of artificial colors may be associated with general behavior disturbances such as irritability and restlessness/fidgetiness, which are not typically characteristic of childhood hyperactivity. The single subject used in this trial was diagnosed hyperkinetic who reportedly showed improved behavior on the open Feingold diet and dramatic deterioration of behavior after eating prohibited food, with restlessness and irritability lasting hours to days. The initial "dose-ranging" phase of this trial was conducted under apparently blinded conditions to determine an appropriate behaviorally effective daily dosage level of the color mix to be used in the subsequent challenge phase. Although parents and teachers rated the child's behavior during the "dose-ranging", only the parent ratings were able to detect any color related change in behavior (the mother's Conner rating score went from a baseline of 0 to15, the cutoff index for hyperactivity). During the subsequent 10 week double-blind multiple crossover challenge phase, the child was given cookies with approximately 18 mg mixed colors on two days per week randomized with placebo weeks. Neither parent nor teacher ratings were able to detect any adverse effects of color challenge on behavior. However, the mother was able to guess correctly which treatment was given for 8/10 weeks, focusing on irritability and fidgetiness as the main changes. In view of the inconsistencies between the mother's ratings during dose-ranging and challenge testing, her ability to "guess" correctly most of the weekly treatments, and the fact that the teacher detected no color related change in behavior at anytime during dose-ranging

or challenge testing, the findings from this study are not readily interpretable and provide no credible support of an association between artificial colors and hyperactivity behaviors in children.

- **Rose (1978)** conducted a challenge study of the artificial color, tartrazine (Yellow 5), using a within-subject design with a sample of 2 hyperactive children reported by parents to be behaviorally responsive to the Feingold diet and maintained on that diet for behavioral management. Over a period of 5 weeks, children were given one oatmeal cookie daily. On one day during each of two different weeks (weeks 2 and 4) children were given the color challenge of a single cookie with 1.2 mg tartrazine. The sole analyzed outcome measure, provided by a trained observer, was an assessment in the school environment of specific behaviors characteristic of the hyperactive syndrome. While considerable efforts were made to ensure the blinding conditions, no actual verification of the effectiveness of the blind for the observer, parents and children was carried out. The analyses showed that for each subject there was a significant increase of hyperactive behaviors associated with the tartrazine challenge. Without the availability of other outcome measures to confirm or support these findings and some uncertainty about the effectiveness of the blinding, the study is viewed as suggestive of a functional relationship between tartrazine and hyperactive behavior in children reportedly responsive to the Feingold diet. Although with this limited sample of 2 children it is difficult to extrapolate the significance of these findings.

- **Williams et al (1978)** designed a rather complex challenge study to focus on the relative effects of a diet free of artificial food colors, flavors and major preservatives (i.e., a modified Feingold diet) compared to stimulant medication (methylphenidate) in managing hyperactivity in children. As a point of clarification, however, this study did not specifically assess the effects of "diet" on behavior. Rather, the study conducted was a double-blind challenge trial assessing the behavioral effects of stimulant medication and a mixture of artificial food colors, administered separately and in combination, in a heterogeneous group of 24 hyperactive/problem behavior children who were all maintained on a modified Feingold diet throughout the study. Prior reported responsiveness to the modified Feingold diet was not a criterion in subject selection for this study, but responsiveness to stimulation medication was a criterion. Over a period of 4 weeks each child was challenged on 4 days each week with a different combination of stimulant medication and mixed colors (26 mg in chocolate cookies) or their respective placebo treatments. Based on analysis of the parent and teacher ratings (standard Conners questionnaires), there were clearly significant effects (behavioral improvement) for the stimulant medication challenges but the effects for the artificial color challenges were inconsistent. Teacher ratings revealed a significant adverse behavioral effect of color challenge, but this was not confirmed by the parent ratings which found no significant effects. In the post hoc analysis of the rating data for individuals, the parent ratings identified 3 "criterion responders", i.e. subjects showing a 33% or greater behavioral deterioration with color challenge compared to placebo, and the teacher ratings identified 5 "criterion responders". But, these findings were inconclusive since there was no agreement between the parent and teacher lists of responders. Overall, in view of the clear effect noted by both parents and teachers of stimulant medication in improving hyperactive/problem behaviors, this reviewer views the disparity between the parent and teacher findings, in terms of the main findings of color challenge effects and the identification of different color responsive children, as definitely problematic for

interpretation of the color challenge findings and considers the study to be somewhat suggestive but inconclusive and not supportive of an effect of artificial colors on hyperactive and problem behaviors in children.

- **Conners (1980a)** conducted a challenge study of the behavioral effects of artificial colors to replicate a prior study by Goyette et al (1978) but with a larger group of 30 diet responsive hyperkinetic children. Based on parent and teacher ratings, there were no significant adverse behavioral effects of challenge with 26 mg mixed colors administered daily for two alternating 1-week periods in hyperkinetic diet responsive children. Since these negative effects produced no confirmation of the color challenge effects reported in Goyette et al (1978), this reviewer agrees with the conclusion that the effects reported by Goyette et al (1978) were most likely due to chance and not biologically relevant.

- **Conners et al (1980)** attempted to determine whether hyperactive children who appear to be sensitive to artificial food colors can be shown to exhibit a time dependent response to those colors using sensitive laboratory tests and observations. The 9 hyperactive children selected for the double-blind challenge trial were reported to exhibit under non-blind conditions marked behavioral improvement on the Feingold diet and notable behavioral sensitivity to foods with artificial colors. While maintained on the Feingold diet, each child was randomly given 2 challenge sessions at 1-2 week intervals, one session using color cookies (30 mg mixed colors in chocolate cookies) and the other session placebo cookies. Each session consisted of baseline measurements, followed by the appropriate treatment and then retested at 45, 90, 135 and 180 min after challenge. Testing consisted of behavior ratings by the experimenter and objective tests including actometer readings, activity chair measures, and a paired-associate attention/learning task. No significant adverse effects of the color challenge were found with any of the test measures. The investigators suggested several factors that may have minimized or confounded the detection of any significant color challenge effects, including the low detection power of the test with small numbers of subjects, the use of an ineffectively low level of artificial colors (30 mg), the increased levels of activity with ingestion of both color and placebo cookies masking any specific effect of the color, and particularly the marked practice effect for the paired-associated task across sessions. The suggestion that these factors may explain why treatment effects were not detected seem inconsistent with the fact that all of the children in this study were selected specifically based on teacher and/or parent reports, although non-blinded, of marked behavioral changes with the Feingold diet and notable sensitivity to artificial colors, and not merely subtle laboratory changes. This selection criterion was intended to enhance the ability to detect a real treatment related effect if there was such an effect. Within this context, it seems clear that this study failed to demonstrate any significant effects with in-depth testing at specified intervals up to 3 hours after challenge with a 30 mg mixture of artificial food colors in hyperkinetic children who were reportedly diet responsive and color sensitive.

- Attributing the poor response in previous studies to insufficiently low levels of colors, **Swanson and Kinsbourne (1980)** designed a challenge study to assess the effects of larger challenge dosages of artificial food colors in a hospital setting. This trial involved 20 children diagnosed hyperactive (average Conners score 16.2) who were responsive to stimulant medication (*hyperactive set*) and 20 children with problem behaviors not rated hyperactive (average Conners score 12.3) and who were reported to have adverse effects to stimulant

medication (*non-hyperactive set*); neither set was identified as diet responsive but all children were maintained on the Feingold diet prior to and during the challenge period. On one of two test days, ten children from each *set* were given a single capsule with 100 mg blended colors and the remaining ten children from each *set* were given a capsule with 150 mg blended colors; a placebo sugar capsule was given on the other day. The outcome measures included paired associate learning testing (PAT) which was assessed three times within 3.5 hours of treatment and Conners behavior ratings assessed two times each test day. Significant color related decrements in PAT performance were found following both dosage levels of 100 and 150 mg but only for the *hyperactive set* of children and not for the *non-hyperactive set.* There was no difference in effect between the two treatment levels of color, indicating no dose-response at least at these levels. This apparent effect of color challenge on performance of PAT was not supported by the Conners behavioral ratings which were described as (no data presented) showing no differences between the color challenges and placebo treatment. However, the reported color effect on PAT performance in the hyperactive children may be questionable due to an inexplicably unusual placebo performance specifically of the hyperactive children (the placebo performance of the hyperactive children improved across test intervals but the placebo performance of the non-hyperactive children deteriorated across intervals). Confidence in the findings of a color challenge effect in the pair-associate test may be further limited by an uncertainty about the effectiveness of the blind in this study, since the use of simple sugar capsules as placebo may not adequately blind for the presence of 100/150 mg color and no procedure was described to verify that the study personnel or children were effectively blinded to treatment. Also, the Conners ratings provided no supportive evidence of any behavioral effects of the color challenge. The investigator's suggestion that the objective PAT performance is more sensitive than the subjective Connors behavior ratings for documenting adverse effects of color on behavior seems inconsistent with the fact that the investigators considered the Conners score sensitive enough to identify the study populations of subjects in this study as *hyperactive* or *non-hyperactive* and that other investigators have found significant treatment effects with parent/teacher behavior ratings but not with objective testing (Harley et al, 1978; Egger et al, 1985; Carter et al, 1993; Batemann et al, 2004). Overall, in view of the questionable effectiveness of the blinding in this study, the absence of supportive evidence of any color related changes based on the Connors ratings, and the inexplicable difference in placebo performances of the paired-associate test by hyperactive and non-hyperactive children, there is minimal confidence in the reliability of the reported findings and this reviewer considers this study to be inconclusive and to provide no credible or suggestive support for a proposed association between hyperactivity or other problem behaviors and artificial food colors in children.

- In consideration of the possibility that behavioral responses to artificial colors may be specific to individual children, the challenge study conducted by **Weiss et al (1980)** was the among the first to include parental rating of selected behaviors targeted for each child as one of the primary outcome measures of treatment effect. The 22 subjects in this trial were non-hyperactive children with behavioral problems, whom parents reported to be responsive to open (non-blinded) treatment with a modified Feingold diet on which the children were maintained throughout this study. On 8 randomly selected days over a 77 day study period, children were challenged with a soft drink containing a 35 mg blend of artificial colors. Matched placebo drinks were given on all other days. Behavioral assessments were made by the parents' targeted ratings, Conners ratings, continuous records of aversive behaviors, and global estimates of target behaviors. Data for each child was analyzed separately (i.e.,

considered individual experiments). Under conditions designed to maximize detection of adverse effects of artificial colors, i.e., use of assessments including individualized targeted behavior ratings and use of subjects with problem behaviors who reportedly improved on a modified Feingold diet, a dramatic adverse response to color challenge was found in one of the 22 children tested. The remaining 21 children showed no convincing adverse reaction to the 35 mg color challenges, including one child showing only a very limited and inconsistent response. Any conclusions from these results should reflect the facts that the detection of treatment effects was based on only one source of behavioral assessment, the parents' observations, and that there was no specific validation of the effectiveness of the blind for the parents or children (e.g., asking them if they could differentiate the two treatment items based on some physical characteristic). In the absence of any other source of behavioral assessment to confirm or support the parents' assessments, confidence in the significance of the study findings is limited. While the findings do not provide definitive evidence, these findings may be viewed as suggesting that a small subgroup of children with problem behaviors who reportedly improve on a modified Feingold diet may be intolerant to artificial color additives, exhibited by adverse behavioral effects. Since all of the children in this study were reported to have improved on a modified Feingold diet, although in non-blinded conditions, and most of these children showed no sensitivity to artificial color additives, this would suggest that intolerances to various foods or food ingredients, other than colors, are of more significance in provoking or aggravating problem behaviors. This in fact is reflected in the investigators' conclusion that "…. colors, and perhaps other agents excluded by elimination diets, can provoke disturbed behavior in children".

- **Mattes and Gittelman (1981)** incorporated several elements in the design of this crossover challenge study to maximize the likelihood of detecting any adverse behavioral effects of the color challenges in a group of children heterogeneous for hyperactivity (6 ADHD/ADD and 5 problem behaviors or history of hyperactivity). Specifically, (1) this study included only children reported by non-blinded parents to show marked behavioral improvement on the Feingold diet and to deteriorate quickly and dramatically with exposure to foods with artificial colors, (2) made efforts to exclude children responding to the placebo cookie alone, and (3) used high dosages of artificial colors as challenge (levels of the blended colors increased daily during the one challenge week from 13 mg/day to 78 mg/day). Based on an extensive battery of subjective (parent, teacher and psychologist behavior ratings, and psychiatric evaluation) and objective (laboratory test of distractibility) behavioral measures, there were no overall significant differences between placebo and the incremental color challenge. Viewing the data for individual subjects, parent ratings did indicate that six children did show some color challenge effects. However, this was not confirmed or supported by any of the other behavioral ratings/evaluations (teachers, psychologist, psychiatrist) all of which showed no treatment related differences in the behavior of any of the children, making the clinical relevance of the parent ratings highly questionable. The results of this study indicate that artificial food colors, even at relatively high exposure levels, have limited, if any, adverse effects on the behavior of most school-age children with hyperactivity or other behavioral problems who are claimed by parents to be markedly sensitive to these agents. Assuming some level of credibility to the non-blind reports of behavioral improvement of the children in this study when they were placed the Feingold and of dramatic behavioral deterioration with exposure to foods with artificial food colors, the basically negative results from this study might suggest that some foods or food ingredients,

other than artificial colors, may be more significantly associated with provoking or aggravating problem behaviors in some hyperactive or problem behavior children.

- **Adams (1981)** conducted a double-blind, placebo-controlled challenge trial to investigate whether an *infraction* of the Feingold diet under controlled clinical conditions exaggerates hyperactive behaviors in children. The trial included a total of 18 hyperactive children who were reported to be responsive to the Feingold diet and sensitive to infractions of the diet. The challenge *infraction* was an iced chocolate cupcake with a glass of lemonade both made from commercial mixes with added artificial red/yellow colors (and vanilla flavor) to produce an "artificial snack" containing 26.3 mg mixed food colors in addition to other unidentified additives; the placebo ("natural snack") cupcake and lemonade were made with color-free natural ingredients. Parents and observer was blinded to which snack was given and, while the effectiveness of this blind was not verified, there was no indication that integrity of outcome measures was affected. While being maintained on the Feingold diet, each child was randomly exposed to both the "artificial snack" and the "natural snack", one each on two separate days (interval between days was not specified). On each test day, the children ate their assigned snack 3 to 4 hours before being tested with a battery of objective behavioral tests of activity, memory, attention and motor skills. As a second source of behavioral assessment, on each test day the parents also rated the degree of change in their child's behavior during the 3 to 4 hour period after the snack was consumed (no specific parental questionnaire was described). Data for individual subjects were not analyzed. The results of the group data analyses showed no significant main or interactive effects of the "artificial snack" *infraction* on any of the battery of objective behavioral tests or on the parental rating of degree of change in behavior. Although data for individual subjects were not analyzed statistically, the investigator did mention that only small or slight changes in behavior were noted for any given child. Although the effectiveness of the blinding for the parents and children was not verified in this study, there was no reason to believe that the blinding may have been compromised or otherwise affected the credibility of this trial's results. Overall, the primary finding from this trial was that, despite the very select nature of the hyperactive diet-responsive subjects, no significant overall adverse behavioral effects resulted in these children from a single *infraction* of the Feingold diet, based on a battery of objective tests and subjective parental ratings. Since the only artificial ingredients identified in the food items used as the dietary *infraction* included 26.3 mg mixed artificial red/yellow colors and an unknown amount of artificial vanilla flavor, it may be concluded only that this study's findings provide no evidence of an association between ingestion of these particular artificial ingredients and behaviors associated with hyperactivity in a select group of school-aged hyperactive children. While this study does not disprove the possibility that there may be behavioral effects produced by some food substances in some susceptible children, it may lend support to the view that, if diet is related in some way to problem behaviors, this effect is less reliably demonstrated than parents predict and may possibly call into question the reliability of a parent's belief of what substances negatively affect their children's behavior.

- **Spring et al (1981)** conducted a controlled challenge trial to test the Feingold hypothesis, recruiting only hyperactive children (n=6) already on the Feingold diet whose mothers reported a diet-related reduction of hyperactivity symptoms. Additionally, to ensure that any treatment related findings were causal and not coincidental effects, a replication procedure similar to the original experimental procedure was also carried out. The active challenge item

was a chocolate cookie containing a 13 mg blend of eight approved artificial food colors; subjects received two cookies per test day, resulting in a total daily color challenge of 26 mg. Placebo cookies contained no artificial colors. Independent testing confirmed blinding of challenge cookies. With the Feingold diet continued, children were given two cookies daily, one before school and one after school, on Tuesday, Wednesday and Thursday during each of six weeks (primary experimental period). Active challenge cookies were given during two of those six weeks. Three randomly selected children received active challenged cookies during weeks 1 and 4; the remaining three children received active challenge cookies during weeks 3 and 6. Placebo cookies were given during the other weeks. On each cookie day, both the mother and teacher were asked to rate their child's behavior using two outcome measures. One measure was an abbreviated 10-item modified version of the Conners hyperactivity rating scale, which the investigators verified as a valid measure of hyperactive behavior but for which the score level that indicated hyperactivity was unclear. For the second outcome measure (global behavior judgment) the respondents (mother and teacher) were asked to guess which treatment the child received based on their assessment of the child's general behavior on that day. Each of the 6 subjects constituted a single subject experiment and analysis of the grouped data was not conducted. Subsequent to the original trial, the investigators conducted a replication procedure that was similar to the original trial but collected only the mother's rating data (hyperactivity rating and global behavior judgment). When the data from the primary experiment were analyzed with the two active challenge periods combined, the results indicated that only two of the six subjects appeared to respond to the active challenge. For one of these two subjects (Subject A), the only indication of a treatment effect was the mother's hyperactivity ratings which were significantly correlated with active challenge but which were not confirmed by the teacher's ratings. Subsequently, it was revealed that a home event occurred that may have influenced the mother's ratings. Consequently, the investigators essentially dismissed the findings for Subject A since they considered it likely that the relationship between the mother's rating of increased adverse behaviors for Subject A and the active color challenge may have been coincidental rather than causal. For the second apparent responding subject (Subject E), significantly accurate guesses were made only by teacher but were not confirmed by the mother. However, the daily hyperactivity ratings for Subject E showed that both the mother's and teacher's ratings were significantly correlated with the active challenge. The relevance of these latter correlations became suspect when the hyperactivity ratings data for Subject E were viewed separately for each week of the study period, since the ratings by both the mother and the teacher appeared (to this reviewer) to be inconsistent between challenge periods and the raters did not appear to agree on which challenge period the change in behavior occurred. The absence of any significant effect of the active color challenge on the behavior of Subject E was confirmed in the replication procedure, based on the mother's daily hyperactivity ratings and global behavior judgment (daily guesses about treatment). In assessing the overall findings of this study it should also be noted that, although the effectiveness of blinding was not verified for parents, teachers or children, there was no indication that the credibility of the outcome measures was in any way affected. Overall, this reviewer's assessment concludes that this study's findings provide no consistent or credible evidence that hyperactive behavioral effects were elicited or exaggerated by repeated challenge with 26 mg mixed artificial food colors during two separate weeks in a small sample of hyperactive boys. It is possible that these children, selected for their reported sensitivity to the Feingold diet, could have been responsive to food elements in that diet other than artificial colors.

- **Salamy et al (1982)** designed a single challenge clinical trial using physiological measures of autonomic nervous system (heart rate) and central nervous system (electroencephalogram) activity for evaluating the effects of the artificial food color, Red 40. Four hyperactive children who were reported to be responsive to the Feingold diet and four non-hyperactive sibling controls were maintained on the Feingold diet during this study. Over two days children were randomly treated with a commercial drink colored with Red 40 (level of Red 40 or other additives in the drink were not specified) and a matched placebo. There was no evidence of significant physiologic changes (HR or EEG) attributable to the artificial color/additives treatment in either the hyperactive or control subjects. But there was a striking difference between the two groups of subjects, with a relative absence of physiological changes in the control subjects but an abundance of changes in the hyperactive children during both testing sessions unrelated to treatment. The hyperactive children seemed to be more labile physiologically, responding generally to the experimental situation. Even though these results were negative, the absence of information regarding the level of Red 40 used for the challenge does not allow the possibility to be dismissed that the level used may have been too low to elicit physiologic responses particularly with the few numbers of subjects tested.

- **Thorley (1984)** conducted a double-blind clinical challenge study designed to maximize detection of any behavioral and cognitive response to color challenge, including such features as a high challenge dosage level of 92 mg of blended colors, optimized testing using a battery of sensitive subjective and objective outcome measures, use of a residential setting for better control, and a relatively homogenous population of intellectually retarded children with inattentive/hyperactive behavior. The 10 subjects, none of whom were identified as diet responsive, were maintained on a modified Feingold diet and given a cocoa drink daily throughout the 28 day study period. Each child was randomly selected and given the color challenge (92 mg blended color in a cocoa drink) on 2 consecutive days. Outcome measures included teacher ratings which included a devised individual rating scale of most problematic behaviors, care staff ratings, psychometric testing with mazes and paired associate learning test, and actometer recordings. No significant color related effects were found for any outcome measure. even though there may have been some question regarding the effectiveness of blinding and the appropriateness of analyzing the data using uncorrected repeating t-testing, the absence of any adverse effect of a high dosage (92 mg) of artificial colors in a study designed to maximize detection of behavioral and cognitive responses indicates that it would be unlikely that intellectually retarded children as a whole would show adverse effects to artificial food colors.

- **David (1987)** conducted a double-blind challenge study using high dosages of tartrazine (50 mg and 250 mg) and benzoic acid (50 mg and 250 mg) to verify parent reports that their children showed immediate adverse behavioral reactions (within two hours) to foods with additives, particularly tartrazine and benzoic acid. The 24 children in this study were heterogeneous with problem behaviors related to hyperactivity and were maintained on elimination diets that avoided food additives and any other suspect foods. Twelve were inpatients in a general pediatric ward and 12 came to the ward as outpatients. Each child was challenged with a drink containing 50 mg tartrazine and two hours later challenged with a second challenge drink containing 250 mg tartrazine. Subsequently, but on a separate day, all subjects were challenged with drinks containing the same two dosages of benzoic acid

following the same procedure. Apparently, no randomized treatment order was used, either between tartrazine and benzoic acid challenge periods or between each of their dosages. The outcome measures consisted of recorded observations by a parent and the nursing staff, but no specific or structured scoring system was used and it was not specified how or whether data were statistically analyzed. Parents of outpatient children knew when challenge days occurred but parents of inpatient children and nursing staff did not. Based on recorded observations by parents and nursing staff, none of the children was reported to show any treatment related change in behavior following the tartrazine or benzoic acid challenges. Unfortunately, there were several rather prominent limitations and weaknesses in the design and conduct of this study. These included the facts that the outcome measure consisted of unstructured observations, randomized treatment conditions were not used, half of the parents were not blind to active challenge days, and data were neither presented nor statistically analyzed. While these issues raise questions about the sensitivity and reliability of this study for detecting little more than obvious treatment related effects, this study was able to show that there was no confirmation of the parents' anecdotal reports of immediate and obvious adverse behavioral reactions to foods specifically attributable to either tartrazine or benzoic acid. It may be possible that those parental reports, although anecdotal, of a history of obvious adverse behavioral changes in these children may have been associated with some food items or components other than tartrazine and benzoic acid.

- **Rowe (1988)** conducted a controlled challenge clinical trial to assess the behavioral effects of two artificial colors, tartrazine and carmoisine, in 8 children who were suspected hyperactive and who reportedly showed improved behavior in an open trial (Phase I of this study) of the Feingold diet and reacted adversely (commonly described as extreme irritability, restlessness and sleep disturbance) to the ingestion of foods containing additives, particularly the red and yellow artificial food colors. All children were maintained on Feingold diet and received one capsule daily during the 18 week study. Each child, in a random sequence, was given daily capsules with 50 mg tartrazine or 50 mg carmoisine each for one week on 2 randomly determined occasions (i.e., a total exposure of 2 weeks for each color) with 2 or 3 weeks washout between each color week. Parents and children were blind to challenge sequence but verification of effectiveness of blind was not determined. Data for each child were analyzed separately. Based on the sole outcome measure of daily parent behavior ratings, using a targeted frequent symptoms checklist for each child, only two (one hyperactive, the other non-hyperactive with problem behaviors) of the 8 subjects were identified as showing significant adverse behavioral responses to both the 50 mg tartrazine and 50 mg carmoisine challenges. Their responses (increased activity, irritability, low frustration tolerance, sleep disturbance, short attention span) began within hours after initiating each color challenge treatment and continued for several days to several weeks after the last of each color challenge treatment week. Both children were atopic, but symptoms did not relate to color challenges. Attempted teacher ratings were incomplete. Confidence in the reliability of these positive findings is limited by the absence of any other source of behavioral measures to corroborate the parental ratings and the lack of attempt to verify the effectiveness of the blind for parents and children. While not conclusive, these findings do suggest that a small subgroup of children with problem behaviors heterogeneous for hyperactivity may be adversely affected by the artificial colors, tartrazine and carmoisine, and that this subgroup of color intolerant children may not be limited to those with ADHD. Interestingly, the common behaviors parents emphasized as being associated with open ingestion of foods with colors, and possibly other intolerant food substances, were extreme "irritability", "restlessness" and

"sleep disturbance" rather than those associated with "attention deficits" (suggested by Mattes and Gittelman, 1978). The author viewed this as suggesting that the inclusion of children in clinical trials on the basis of 'attention deficit' alone may miss some color reactors. The six children who did not react to the controlled color challenges were reported in open (non-blind) trial to be diet responsive and to react adversely to foods containing food additives. It is possible that part of these apparent responses to diet may have been associated with intolerance to certain food items/components eliminated from the Feingold diet other than artificial colors. This suggests a broader view of the possible behavioral impact of artificial food colors, and possibly other food intolerances, from only children with ADHD and related behaviors to children with more general types of problem behaviors, including but not limited to those associated with ADHD.

- **Wilson and Scott (1989)** conducted a controlled clinical challenge trial to assess food additive intolerance in 19 non-hyperactive children reported to be responsive to open (non-blind) treatment with a color-free diet and with a definite but anecdotal history of adverse reaction (respiratory, dermatologic, and/or behavioral) to artificial yellow color. For most children color intolerance was verified in open (non-blind) challenge prior to the study. All subjects were maintained on an additive-free diet throughout the study. Two types of challenge drinks were use: a color challenge aide drink with 8.5 mg tartrazine and 8.5 mg sunset yellow, and a preservative challenge aide drink with 12.5 mg sodium metabisulphite and 55 mg sodium benzoate. The placebo treatment was an aide drink with B-carotene coloring to match the challenge treatments. The initial challenge stage of the double-blind trial consisted of three 14-day phases with each of the three experimental treatments randomly assigned double-blind to the three phases for each child. During each phase one bottle of the same type of drink was consumed daily for 12 days followed by a 2-day washout period. If behavioral symptoms appeared to deteriorate during that initial stage, a repeat challenge stage was offered and an additional repeat stage was offered if those results were inconclusive. The assessment of behavioral response was rather basic and limited to two types of subjective unstructured assessments by parents: a daily diary of symptom scores and an overall assessment at the end of each challenge phase. The results were assessed for individuals and not for the group as a whole. However, no statistical analyses of the data were apparently made. Four of the 19 subjects had a pre-study history of adverse behavioral reaction to yellow color but none of these four showed any adverse effects with the controlled color challenge. Among the remaining 15 children only 3 showed any adverse effects of challenge and only one of these 3 "responders" developed adverse behavioral symptoms that were associated with the preservative challenge but not with the color challenge. Confidence in the reliability of these study results, however, is lessened by the questionable accuracy and sensitivity of the limited and unstructured behavioral assessment using a very limited, poorly defined and non-validated scoring system, and the conduct of this assessment only by the parents with no other source of assessment to confirm the parental observations. Additional limitations include no apparent verification of the effectiveness of the blinding for the parents and children (blinding of drinks with tartrazine is reportedly very difficult due to the intense coloration of the dye), and no description of statistical analyses of the data. Even considering these caveats, this study does suggest that anecdotal reports of definite adverse behavioral (and other) intolerance to artificial colors, specifically yellow colors, are not clearly evident when tested under controlled clinical conditions.

- **Pollock and Warner (1990)** conducted a placebo controlled artificial color challenge trial of children specifically identified by parents as exhibiting food additive intolerance, whose behaviors reportedly improved on a diet excluding food/color additives and returned (primarily poor concentration and excess fidgetiness) shortly after consuming even small amounts of food additives. The 19 children used for the study, who were heterogeneous for hyperactivity but with problem behaviors, were maintained on their additive-free diets throughout the 7 week trial period. On 2 separate weeks children were administered a daily gelatin capsule containing a 125 mg blend of artificial food colors; placebo capsules with lactose were given during all other weeks. Parents were blind to treatment order but were not asked whether they could differentiate the color and placebo capsules. Two parent-based outcome measures were used: (1) a daily Conners behavior rating supplemented for somatic/allergy symptom assessment and (2) a weekly overall behavior assessment. The parent daily behavior ratings showed significantly enhanced problem behaviors during the color challenge weeks compared with placebo weeks and no treatment order effects. However, it should be noted that these were very small differences between the color and placebo weeks. The daily somatic/allergy scores showed no treatment related differences, indicating that the changes in behavior scores were not related to or secondary to somatic symptoms. Additional analyses showed no difference in behavior ratings between week days 1 and 7, indicating that the treatment related effects of food color intolerance began occurring after the first challenge and did not result in cumulative effects. However, the absence of any treatment related effects on behavior based on the parents' overall assessments at the end of each week provides no supportive confirmation for the color effects seen with the parent daily behavioral ratings. Unfortunately, the use of only parents' observation of behavior to measure treatment related effects does not enable independent sources of information to help resolve this inconsistency. While this inconsistency may reflect a questionable reliability of one of the measures, it seems more likely that the very small treatment effect was simply not detectable in the weekly assessment. However, since the claim by parents of being able to detect behavioral deterioration after consumption of even small amounts of food additives was a criterion for entry into the study, the fact that the parents weekly assessment could not detect an overall behavioral change even after daily challenges with a high dosage level of 125 mg artificial colors and that rather small behavioral differences were revealed in the parent daily ratings suggest that there is little substantial or functionally (biologically) relevant association between artificial colors and problem behaviors in children reported to be food additive intolerant.

- **Sarantinos et al (1990)** conducted a placebo controlled challenge trial of two artificial food colors, tartrazine and sunset yellow, on the behavior of children diagnosed with attention deficit disorder (ADD). *(It should be noted that only an abstracted summary of this study was available for review).* Of the 13 ADD children recruited for this study, 9 were considered responsive to a color-free diet and 4 were considered uncertain diet responders. Several children were reported to be intolerant to multiple food items and, oddly, two were claimed to be behaviorally intolerant to orange juice, which was the vehicle used in this study. The children were maintained on a color-free diet during the study and assigned to one of two treatment groups. Over the 28-day study period, the children in Group 1 (n=6-7) were challenged with an orange drink containing only 10 mg tartrazine on 6 random days. The children in Group 2 (n=6 or 7) were also given color challenges on 6 random days but on 3 days the orange drink contained 10 mg tartrazine and on the 3 other days the orange drink

contained 10 mg sunset yellow. On all non-color challenge days, children received a placebo drink. The only source of outcome measure was the parent behavior ratings using two rating scales (Conners scale and Rowe Behavioural Inventory scale). No procedure to verify effectiveness of blinding for the parents was described. One unidentified subject did not complete the trial. Based on repeated measures analysis of variance of the parent behavioral ratings, 2 of the 6 or 7 ADD children in Group 2 showed significant adverse changes in behavior (i.e., increased irritability, impulsivity, restlessness, and sleep disturbance) associated with both tartrazine and sunset yellow challenges. Both children were atopic and, among those whom parents had reported to be responsive to color-free diets, one child was intolerant to multiple food items whose mother could correctly identify color or placebo days (25/28 times but unclear whether this was based on child's behavior or broken blind). It was not stated whether either of these two responders were the 2 children reported to be intolerant to orange juice. Although not specifically stated, it appears that the 6 or 7 children in Group 1 did not show any significant response to their tartrazine challenges, thereby providing no confirmation or support of the responses noted in the two Group 2 children. Overall, based the absence of other independent outcome measures to corroborate the parental ratings, the inconsistency in findings between Group 1 and Group 2 children, the uncertainties regarding the effectiveness of blinding and the limited details about the experimental design, there are considerable questions about the confidence in reliability and biological significance of the findings, precluding any credible interpretation of these findings with regard to the possible association of the artificial colors, tartrazine and sunset yellow, and adverse behavioral effects in children with ADD.

- **Rowe and Rowe (1994)** uniquely conducted a double-blind, placebo controlled, repeated measures clinical challenge trial providing a full dose response assessment of tartrazine (Yellow 5) to establish whether there is an association between this artificial color and behavioral change in children with suspected hyperactivity. The first stage of this study was an open trial to identify children suspected of hyperactivity who might be sensitive to artificial colors based on parents reporting behavioral improvement on a color-free diet and deterioration with ingestion of foods containing artificial colors. Behaviors consistently reported related to irritability, restlessness, and sleep disturbance (behaviors not typically characteristic of hyperactivity syndrome) (also reported in Rowe, 1988). A total of 34 children with suspected hyperactivity, categorized as *likely* diet responders (23) or *uncertain* diet responders (11), and 20 children without problem behaviors participated in the challenge stage of this trial. All subjects were maintained on a color-free diet and given one double capsule (children $\geq$6 years) or packaged orange juice (children <6 years) on each morning of the 21-day study period. Over 6 random days, each child was administered (in capsule or orange juice) all six dosages of tartrazine (1, 2, 5, 10, 20, and 50 mg) once with one dosage per day in random order, except that the 20 and 50 mg dosages were administered toward the end of the study period. A placebo washout period of at least 2 days separated each challenge day for each child. Other than use of specially designed double capsules with an inner capsule surrounded by lactose, no description was given as to blinding procedures or to verification of effectiveness of blinding. Daily parent ratings provided the only outcome measures of behavioral change. The primary rating scale was a behavioral inventory devised by the investigators and validated to discriminate between color and placebo ingestion. A parent Conners rating was also included for comparative purposes only but not analyzed for treatment effects. The parent ratings identified a total of 24 of the 54 children tested as showing consistent behavioral reaction to the tartrazine challenges, significant at all six

dosage levels in clearly dose related fashion with amplitude and duration of effect increasing with increasing dosage. Beyond 10 mg there appeared to be a ceiling effect in magnitude but higher doses increased duration of effect. Most tartrazine reactors were among the children categorized as "likely diet responder" (19/23), while only few tartrazine reactors were in the "uncertain diet responder" group (3/11) and even fewer among the control subject with unknown diet sensitivity (2/20). There were no significant sex or age related factors and, while all tartrazine reactors were atopic but none showed an allergic reaction to tartrazine, it was not stated whether the nonreactors showed similar results. The principle behavioral features reported to be associated with tartrazine exposure, based on the devised behavior inventory, were irritability, restlessness, and sleep disturbance. It is possible that the devised behavior inventory rating may underestimate changes in attention behaviors. The two basic experimental issues that potentially affect the confidence in the reliability of the behavioral findings in this study include the adequacy of the study blind and the use of parent ratings as the sole measure of behavioral change. With regard to blinding, while the study was conducted under double-blind conditions, there was no apparent effort to determine whether the difference between placebo and color capsules and orange juice were adequately blinded or to verify the effectiveness of the blinding procedures for the parents and children. However, with the results showing clear dose response effects, it seems likely that the integrity of the blind was adequately maintained. With regard to the use of parental ratings as the sole outcome measure in this study, confidence in the reliability of study findings would certainly be strengthened if other measures of behavior were also used to corroborate the parental findings. However, in view of the fact that the parent ratings were able to discern significant incremental differences in behavior and in clear dose related fashion, there appears to be sufficient confidence in the reliability of these study findings based solely on the parental behavioral ratings. Overall, these findings do indicate that intolerance to tartrazine can cause adverse behavioral effects, not necessarily associated with hyperactivity, in selected subgroups of diet sensitive children with problem behaviors and possibly in a small subgroup of control children without problem behaviors and unknown diet sensitivity.

- **Bateman et al (2004)** designed a double blind, placebo controlled challenge trial to determine whether food additives can cause hyperactive behaviors in preschool children sampled from the general population with and without hyperactivity and with and without atopy (allergic sensitivities). The 277 preschool children, who completed the challenge trial, were not diagnosed ADHD but were assessed for hyperactivity based on parent behavior ratings and were designated hyperactive (HA) or non-hyperactive (non-HA). All children were further assessed for atopy (allergy sensitivity) and identified as either atopic (AT) or non-atopic (non-AT). They were then sorted into four test groups: HA/AT (36), HA/non-AT (75), non-HA/AT (79), and non-HA/non-AT (87), each group having slightly more males than females. The children were not reported diet responsive but were placed on a diet free of artificial colors and preservatives throughout the 4 week study period. During two weeks of that period each child received a daily drink of either the placebo fruit juice or the challenge fruit juice containing a 20 mg blend of artificial colors plus 45 mg sodium benzoate (one type of treatment per week determined randomly). Two weekly behavioral outcome measures were used: (1) a clinically based aggregate test hyperactivity score (ATH), validated as distinguishing between hyperactive and non-hyperactive preschool children at baseline, and (2) an aggregate parent hyperactivity rating score (APHR). Analyses of variance and co-variance were used to analyze the data. Based on grouped data analyses, APHR (parent ratings) scores showed an overall significantly greater increase in hyperactivity behaviors for

the active color/benzoate challenge than for the placebo with no difference in response between the groups of children categorized HA and non-HA or between the AT and non-AT groups. Curiously, both placebo treatment and color/benzoate challenge increased levels of hyperactivity behaviors, but a statistically greater increase in hyperactivity behaviors occurred overall during the active color/benzoate challenge week. Group analysis of the ATH (clinical measures) scores, however, showed no significant behavioral effects associated with the color/benzoate challenges. The fact that the ATH score provided no confirmation or support for the APHR findings is particularly relevant in assessing the significance of the ATH findings, since validation of the APHR scoring was based on distinguishing between hyperactive and non-hyperactive preschool children at baseline. An additional issue to be considered in evaluating these contradictory findings is the effectiveness of the blind for the parents. While efforts were made to maintain the blinding conditions for this study, it was reported that approximately half of the parents could correctly identify the treatment but it was not clear whether this was due to behavioral changes in the children or to detectable differences between placebo and challenge drinks. In view of the contradictory findings between the two primary outcome measures, together with the uncertain effectiveness of the blind, there is limited confidence in the reliability of this study's findings. This study provides no evidence that food additives cause hyperactivity in preschool children from the general population. At best this study may suggest that non-allergy related intolerance to artificial food color additives and benzoic acid may occur in some children with or without hyperactivity related behavior and that this intolerance may be associated with only limited behavioral changes.

- **McCann et al (2007)** conducted a controlled challenge trial to test whether artificial food colors and additives (AFCA) affected childhood behaviors in a general population of preschool and school age children and in part to replicate a previous study (Bateman et al, 2004). The study sample of 153 preschool (3-4 years) and 144 school age (8-9 years) children were recruited from the general population, with neither group being diagnosed hyperactive or having special problem behaviors. The challenge material was a fruit juice drink containing one of two different color blends plus sodium benzoate (Mix A and B). Two sets of each Mix were used, one containing lower levels of colors (20 mg Mix A and 30 mg Mix B) to challenge the preschool children and the second set containing higher levels of colors (25 mg Mix A and 62 mg Mix B) to challenge the school age children. All challenge mixes contained 45 mg sodium benzoate. After baseline, children were maintained on a diet excluding artificial colors and sodium benzoate throughout the remaining 6 weeks of the study. During each of 3 different weeks, children were given daily treatments with either Mix A, Mix B or placebo fruit juice, with one type of treatment per week; the order of treatment across weeks was determined randomly for each child. While all study participants were blind to treatment and other efforts were made to ensure the blinding of the study, effectiveness of blind for parents and children was not verified. The sources of outcome behavioral measures included: (1) teacher ratings, (2) parent ratings; (3) trained observer classroom observations; and (4) a computerized continuous performance task was used as an additional objective source measure only for school age children. However, for purposes of determining whether behaviors were affected by either of the color/benzoate treatments, the data from all sources of behavioral measures were combined for each age group separately into a single composite Global Hyperactivity Assessment score (GHA) which was then analyzed to identify any significant treatment related behavioral effects. Based on differences in the composite GHA scores for the full sample of children, the young preschool children

responded to Mix A challenge with significantly elevated hyperactivity scores but did not respond to Mix B challenge. Contrarily, the older school aged children responded to Mix B challenge with significantly elevated hyperactivity scores but did not respond to Mix A challenge. These differences in response to the two mixtures of colors/benzoic acid seem to be a puzzling inconsistency and the investigators even acknowledge the need for additional study" to establish whether the age-related differences seen in the present study can be replicated". While the specific nature of the behavioral effects are not clear (none of the component outcome behavior measures were described), whatever behavioral changes may have occurred were apparently of rather low magnitude (effect size of 0.18). This would suggest that the type of treatment effects reported in this study, even though the investigators referred to increases in levels of "hyperactivity", were not the disruptive excessive hyperactivity behaviors of ADHD but more likely the type of overactivity exhibited occasionally by the general population of preschool and school age children. However, due to the study weaknesses and caveats of this study, particularly the puzzling inconsistency in response by the two age groups of children and the rather small treatment related changes, the reported effects appear to be equivocal and of uncertain biological relevance. This study does not provide credible evidence of deleterious effects of artificial food colors and additives on children's behavior nor does it lend any support for the contention that food additives exacerbate hyperactive behaviors (inattention, impulsivity, overactivity) in children.

Group I: Summary Conclusions

Pre-1982 Clinical Trials (Summary Conclusions)

In attempting to summarize the conclusions that may be drawn from the Group I studies, particular note should be given to an NIH Consensus Development Conference on Defined Diets and Childhood Hyperactivity convened in 1982 to assess the status of extant information relating defined diets (specifically, the Feingold diet) and hyperactivity disorders in children (NIH, 1982). At that time, at least 16 controlled clinical trials (reviewed in this project report and color-coded grey† in Table 1), two diet crossover trials and 14 specific challenge trials, had been conducted to assess the Feingold hypothesis. Based on the committee's review of those trials and other information, the NIH consensus report alluded to differences and inadequacies in the designs of the controlled clinical trials making analysis difficult, but concluded that the available studies "*did indicate a limited positive association between 'the defined diets' and a decrease in hyperactivity...that involved only a small proportion of patients; furthermore, the decreases in hyperactivity were not observed consistently. Studies indicated that some hyperactive children on a defined diet experienced an increase in hyperactivity when given moderate doses of artificial food colors.....by only a small group of patients and the increase was not consistently reported by teachers, parents, and other observers*". This highly qualified conclusion fairly represents this reviewer's assessment of the information from the Group I clinical trials conducted prior to 1982 and refers to several of the same types of problematic and confounding issues identified in this reviewer's evaluation of most of those same early clinical studies. These issues include varying degrees of inconclusive or equivocal findings (Swanson and Kinsbourne, 1980), only a small percentage of responders under controlled conditions (Conners et al, 1976; Harley et al, 1978; Weiss et al, 1980), inconsistent or unconfirmed reports of treatment effects between different sources of behavior assessment (Goyette et al, 1978; Rose, 1978; Spring et al, 1981; Swanson

and Kinsbourne, 1980; Weiss et al, 1980;Williams et al, 1978; Mates and Gittelman, 1981) or due to inexplicable treatment order effects (Conners et al, 1976; Harley et al, 1978), and questionable effectiveness of blinding conditions (Conners et al, 1976; Harley et al, 1978; Goyette et al, 1978; Levy et al, 1978; Levy and Hobbes, 1978; Conners, 1980; Swanson and Kinsbourne, 1980; Weiss et al, 1980). In view of these caveats, the treatment related findings from these pre-1982 studies were generally considered by this reviewer as only suggestive of limited beneficial effects of Feingold's diet in hyperactive children and a limited association between artificial colors, even at high daily dosages of 78 to 150 mg mixed colors (Swanson and Kinsbourne, 1980; Mattes and Gittelman, 1981), and adverse behaviors in a small subgroup of children with hyperactivity or other problem behaviors. Subsequently, in 1983 a meta-analysis of trials, including those reviewed at the 1982 NIH Consensus Conference, focused on the Feingold hypothesis, particularly as it related to the effectiveness of his defined diet in treating childhood hyperactivity (Kavale and Forness, 1983). These authors concluded that their analysis of trials up to that time point provided no support for the Feingold hypothesis and that his dietary treatment was of questionable effectiveness, producing only slight improvements in behavior of hyperactive children with little substantive changes to the basic elements of attention and learning.

Post-1982 Trials (Summary Conclusions)

Following the 1982 NIH Consensus Conference and up to the present time, at least 10 additional clinical trials dealing with the association between food colors and problems behaviors related to hyperactivity of childhood were conducted (Table 1). In summarizing the conclusions that may be drawn from these 10 post-1982 clinical trials, it should be noted that, comparable to the pre-1982 trials, many of these trials were found to have varying limitations to their experimental designs which resulted in varying levels of confidence in the reliability of their study findings. Among the study limitations were included: use of unstructured non-validated rating systems (David, 1987; Wilson and Scott, 1989), non-blinded study personnel responsible for behavior ratings (David, 1987) or effectiveness of blinding not verified or questionable (David, 1987; Thorley, 1984; Rowe, 1988; Wilson and Scott, 1989; Pollock and Warner, 1990; Sarantinos et al, 1990; Rowe and Rowe, 1994; Bateman et al, 2004; McCann et al, 2007), use of single source for behavioral outcome measures not confirmed by other sources of measurement (Rowe, 1988; Wilson and Scott, 1989; Pollock and Warner, 1990; Sarantinos et al, 1990; Rowe and Rowe, 1994), no randomized assignment to treatment (David, 1987), and no statistical analysis (David, 1987; Wilson and Scott, 1989) or incomplete presentation of data (David, 1987; Wilson and Scott, 1989; Sarantinos et al, 1990; McCann et al, 2007). Across trials, the reported findings of adverse reactions to color additive challenge were mixed (4 negative, 3 inconsistent, 3 positive), as were the levels of confidence in those various trial results. Among the four challenge trials showing no adverse responses to color challenge, there was reasonable confidence in the results from one trial showing no adverse behavioral or cognitive effects of 92 mg mixed colors in retarded children (Thorley, 1984), moderate confidence in another showing no effect of Red 40 on heart rate or EEG activity a hyperactive children (Salamy et al, 1982), and low confidence in two trials with tartrazine, sunset yellow and preservatives (David, 1987; Wilson and Scott, 1989). For the three trials showing inconsistent or inconclusive color effects, there was reasonable confidence in the results of one trial showing inconsistent but very small behavioral effects of daily 125 mg mixed color challenge in problem behavior children (Pollock and Warner, 1990), moderate confidence in one trial with inconsistent behavioral effects with 2 mixes of 20-30 mg artificial colors in preschool and school age children from the general population (McCann et al, 2007), and limited confidence in one trial with inconsistent small

behavioral effects and uncertain effectiveness of parent blinding with 20 mg mixed color challenge in pre-school children from the general population (Bateman et al, 2004). And, among the three trials associating adverse behavioral effects with artificial color challenge, there was reasonable confidence in one study showing clear dose response behavioral effects at each challenge dose of tartrazine from 1 to 50 mg primarily in problem behavior children but also in several children without behavioral problems (Rowe and Rowe, 1994), moderate confidence in one trial reporting 2 children (one hyperactive, the other non-hyperactive with problem behaviors) responding to 50 mg tartrazine or carmoisine with adverse behaviors that may or may not be characteristic of hyperactivity (Rowe, 1988), and low confidence in the third trial reporting several children responding to intermittent challenge with 10 mg tartrazine and sunset yellow (Sarantinos et al, 1990).

Generally, the various reported findings across these 10 reviewed post-1982 portion of Group I trials, suggests that certain susceptible subgroups of problem behavior children with and without ADHD and, possibly, certain susceptible children from the general population without particular behavioral problems may exhibit a unique intolerance to artificial food colors resulting in typically small to moderate adverse behavioral changes which may not necessarily be characteristic of the ADHD syndromes. A relatively recent meta-analysis of clinical trials dealing with artificial food colors and hyperactivity in children (Schab and Trinh, 2004) was conducted and considered the relevant artificial food color challenge studies conducted through 2004, including most of the Group I trials reviewed in this project report. Their analyses across all trials found small but significant treatment effects for the color challenges, suggesting an association between ingestion of artificial food colors and hyperactivity behaviors. Their secondary analyses also implicated artificial food colors more in provoking general behavioral disturbances than hyperactive symptomatology and suggested that sensitivity to artificial food colors may not be limited to those children with clear-cut hyperactive syndromes.

For many of the color challenge trials the children recruited for that study were specifically selected in part because of their reported diet responsiveness, showing improvement in their hyperactive and problem behaviors on an open (non-blinded) defined diet (usually Feingold's diet) which at least excluded artificial food colors, and their dramatic deterioration of behavior after exposure to foods containing artificial colors. However, in the controlled challenge phase of testing, typically few, certainly not all, of the subjects were found to exhibit adverse behavioral reactions to the controlled color challenges. Speculatively, for those subjects who did not respond to the controlled color challenges, part of their reported diet responsiveness and dramatic sensitivity to foods with artificial colors (to the extent that non-blinded reported responses were real) may have been associated with a sensitivity or intolerance to certain food items/components other than artificial colors (Stare et al, 1980). This possibility of sensitivity or intolerance to a broader spectrum of food substances, other than artificial colors, is the focus of the Group II trials also reviewed in this project.

Group I Trials: General Conclusion

The focus of the 26 Group I trials was to assess the validity of Feingold's hypothesis relating food additives and ADHD and other problem behaviors in susceptible children. The clinical trials were typically designed as double blind, placebo controlled studies either to assess the effectiveness of the Feingold diet as a dietary treatment of ADHD or to test the role specifically

of artificial colors in causing or provoking adverse behaviors related to ADHD or other problem behaviors in children. Various caveats in the study designs and uncertainties in the study results make interpretation of the study findings difficult and limited in scope. Within this context, the results from the Group I controlled clinical trials, overall, suggest the conclusion that certain subgroups of children with problem behaviors that may or may not be related to ADHD/ADD and, possibly, certain children from the general population without particular behavioral problems may exhibit a unique intolerance to artificial food colors resulting in typically small to moderate adverse behavioral changes which may not necessarily be characteristic of the ADHD syndromes.

Group II Trials (n=7)

As noted previously, these controlled trials focused on a broader array of substances by assessing the role of food intolerance, i.e. adverse effects of food itself, in hyperactive and problem behavior children. Of the 7 trials in this group, 2 were "diet crossover trials" both of which used a particular type of elimination diet (i.e., oligoantigenic diet, few foods diet, and Alberta Children's Hospital diet) that excludes all foods, additives, including artificial colors, and food components assumed to provoke adverse behavioral reactions in certain children and assessed the effectiveness of these diets in improving the behavior of hyperactive or problem behavior children. The remaining 5 trials were "*specific challenge trials*" which assessed the adverse behavioral and other effects of various suspected provoking food items (pre-identified for individual subjects under non-blind conditions) in children diagnosed ADHD or with problem behaviors. The consideration in these Group II trials of a broad range of food substances that may be associated with hyperactivity appears to be responsive to the concern expressed in the 1982 NIH Consensus Report that "controlled challenge studies have primarily involved the administration of food dyes in children" and "do not appear to have addressed adequately the role of diet in hyperactivity".

Typically, the procedure for Group II "*diet crossover trials*" was basically the same as for the Group I trials, i.e. randomly assigning a group of children to either a defined elimination diet or a matched placebo diet for a specified period of time, then crossing them over to the other diet condition for an equivalent period of time and evaluating the behavioral responses to both diet conditions. The "specific challenge trials" were typically conducted in three phases: Phase I – identify a study group of children who reportedly show improved behavior when placed on an open (non-blind) food elimination diet; Phase II – methodically re-introduce foods/components to the 'food elimination diet' in non-blinded fashion to tentatively identify specific provoking food items for individual subjects (foods not producing a reaction were kept in the diet on which the child was maintained throughout the subsequent challenge phase of testing); Phase III – conduct double blind, placebo controlled challenge testing with one or more suspect provoking food items to verify and assess their adverse behavioral effects under controlled conditions.

Individual Trial Summary Reviews (Group II)

- Based on the possibility that some type of food intolerance (food allergy) may play a role in causing or aggravating the hyperkinetic syndrome in children, **Egger et al (1985)** conducted one of the first trials to treat problem behavior children with an oligoantigenic diet. In a clinic setting 76 children, considered atypical hyperactive many with neurological disorders,

allergic conditions and other physical conditions, were placed on an open (non-blinded) oligoantigenic diet and 62 (82%) were reported to show improved behavior. Skin-prick testing showed that atopy status (allergic sensitivity) did not differ between the children who did and did not respond to the diet, but IgE levels were higher in the responders. Twenty-eight of the "food intolerant" children (average age approximately 7 years) were tested in open re-introduction trials to identify specific foods/components to which they were sensitive. While all children appeared to react to more than one food/component, the most common suspect provoking food ingredients were benzoic acid and tartrazine. The varieties of food items subsequently used as challenges included cow milk, orange juice, wheat, tinned food, tartrazine (150mg) in capsule, or benzoic acid (150mg) in capsule. Eight of the 28 subjects received either tartrazine or benzoic acid as their challenge. Placebo items were matched to blind the challenge. All participants were blind to order of challenge and staff members found no difference in taste between active and placebo treatments, but families were not assessed to verify the effectiveness of blinding. In the double blind, placebo controlled phase of this trial, each child was randomly assigned to receive either his/her suspect provoking food item or placebo for 1 to 2 weeks and an additional 1 to 2 weeks with the opposite treatment, with at least a 2-week washout period between treatments. Multiple outcome measures were used, including parent Conners behavioral ratings, overall behavior assessments by parents, clinician, and psychologist, and a battery of psychological tests. Under these controlled challenge conditions, the overall behavior assessments by parents, psychologist and clinician linked better behavior with the placebo challenge significantly more often than with the suspect provoking food challenge. Parent Conners behavior ratings also showed significant treatment effects, with the suspect provoking food challenge eliciting more hyperactive behavior than placebo. While group analyses linked the provoking food items with adverse behavioral changes, graphic representation of some of the challenge data showed that approximately 6 children did not respond adversely to their challenge. It is not known with what food items these children were challenged. In contrast to the subjective rating and assessment measures, none of the objective psychological tests showed any significant adverse behavioral effects with the suspect provoking food challenge, although non-significant trends did generally indicate placebo behavior better than active challenge. Since significant adverse effects of provoking food items was corroborated by several sources of outcome measures (parents, clinician and psychologist), it seems likely that the failure to detect treatment related effects with the objective psychological test in this study may have been due to the possibility that those tests were not sensitive enough to detect either the type or magnitude of behavior changes specifically associated with food intolerance. Although there is some uncertainty regarding validation of the effectiveness of the blind for the parents and children, this study does provide sufficiently reliable findings to indicate that some children with problem behaviors may be intolerant to a variety of food items, including but certainly not limited to artificial food colors or preservatives.

- **Kaplan et al (1989)** designed a crossover dietary trial to assess the effects of a special foods elimination diet (Alberta Children's Hospital Diet, ACH) specifically in preschool hyperactive male children in day-care with sleep problems and/or allergy-type somatic symptoms. Twenty-four preschool ADHD children (3.5 – 6 years), reported by parents to have sleep or physical problems participated in this study. No stimulant medication was used during the study. The experimental diet, ACH, eliminated artificial food colors, flavors, preservatives, MSG, chocolate, caffeine, and milk, dairy products, and natural salicylates for some children, and decreased simple sugars. An apparent equivalent diet was used as

placebo. Extensive efforts were carried out to ensure effectiveness of the blind. All children were given the placebo equivalent diet for 3 weeks and the experimental ACH diet for 4 weeks, with these two diet treatments given in randomized counter-balanced order across subjects. While multiple sources of behavioral outcome measures were planned, including parent ratings/records (included Conners rating scale supplemented with individualized problem behaviors identified by parents), day-care worker ratings (Conners rating scale not individualized), independent observer records, and laboratory psychometric testing, only parent ratings/records were completed. Day-care worker ratings were only partially completed (complete data for 10/24 subjects) and the other two planned measures were terminated due to technical difficulties or discontinued due to subjects being deemed "untestable", respectively. Other non-behavioral testing (blood chemistry/hematology/basic physical) was completed. Statistical analysis of data was conducted using repeated measures multivariate and univariate ANOVA with Tukey's method of multiple comparisons. Based on the parent Conners behavior ratings without the individualized item, there was a small but statistically significant improvement of the children on the ACH diet (mean score 10.8) compared with the equivalent placebo diet (13.1). Evaluation of the parent Conners scores for individual children revealed that not all children were equally responsive to the ACH diet. Compared with the equivalent placebo diet, 14 (68%) of the 24 children showed some behavioral improvement on the ACH diet, with 9 showing at least a 25% improvement and 5 showing a milder improvement. The remaining 10 children (42%) were unresponsive to dietary intervention. The parent ratings of the individualized problem behaviors and the sleep records showed comparable overall improvement on the ACH diet (the numbers of individual responders were not identified). The limited the day-care worker ratings data showed marginal but non-significant diet related differences in behavior. Also, the physical signs/symptoms, clinical or nutritional measures showed no reliable diet related differences. Overall, since the day-care worker ratings, although completed on less than half of the subjects, did not confirm a significant diet related effect and since no other source of outcome behavioral data was available to resolve this inconsistency, the diet related findings in this study are considered inconclusive but suggestive that a special food elimination ACH diet may result in some level of behavioral and sleep improvement in some hyperactive pre-school children. While this suggests that some hyperactive children may be intolerant to some dietary elements, it is unknown what specific food item(s) or component(s) might be involved.

- Based on the premise that food intolerance may be associated with adverse behaviors in certain ADHD children through an allergic/immunologic mechanism, **Egger et al (1992)** designed a placebo controlled clinical challenge trial to determine whether hyperkinetic children can be desensitized to the adverse effects of provoking foods using an enzyme-potentiated desensitization treatment (EPD). In initial non-blinded phases of this study 116 out of 185 hyperkinetic children (63%) were reported to show improved behavior on an oligoantigenic diet and, subsequently, specific provoking foods/components were identified for each child based on recurrence of hyperkinetic behaviors or other associated physical symptoms. Chocolate, artificial colors, cow milk, egg, citrus, wheat, beet sugar and nuts were among the most common provoking food items. Forty of these food intolerant children participated in the double-blind, placebo-controlled trials and were maintained on an oligoantigenic diet throughout the study period. Half of the children received injections of either placebo/buffer solution or an EPD solution of mixed food antigens (multiple foods, additives, colors, and preservatives). Both solutions were reported to be "colourless" and

parents and children were blind to treatment. The effectiveness of the experimental treatment was determined by reintroducing provoking foods individually and assessing the children's response. Parents maintained a record of hyperactivity behaviors and other symptoms (e.g., headache, abdominal discomfort, bloating, and diarrhea). Treatment effectiveness was based on two measures: number of provoking foods given before parents stopped food reintroduction when adverse symptoms occurred and whether parents rated treatment as successful in preventing or reducing symptoms from one or more provoking foods. Statistical analyses used the Fisher's exact test. Atopy and IgE levels were determined. Based on the measure of parents stopping the reintroduction of provoking foods, adverse response to provoking food items occurred less frequently in the EPD treated group than in the placebo group. Specifically, 15 of the 20 children given EPD treatment were able to eat previously identified provoking food items without adverse reactions, but only 7 out of 20 placebo treated children were able to do so. Also, more parents of children treated with EPD than those treated with placebo thought the treatment was successful. While both behavioral and physical symptoms were reportedly used to assess response to provoking food items, the physical symptoms, such as abdominal discomfort, bloating and diarrhea, were usually the first to appear but no description of behavioral effects was included. These results were not related to atopic status or IgE levels in these children. Several issues involving experimental design weaknesses affect confidence in this study's finding. One issue is the questionable masking of the difference between the placebo and EDP solutions which is central to the study blind and the reliability of the subjective parental observations. The presence of 6 different food colors in the EDP solution seems to be inconsistent with the EDP solution being described as "colourless". Any notable difference in coloration between the placebo and EPD solutions would make effective blinding of the parents and children to treatment questionable and introduces a possible unintentional assessment bias. A second study limitation is the fact that no other source of information was available to confirm or support the parent detection of adverse response to the provoking foods items. A third issue involves the fact that no structured or validated rating system was used to assess the behavioral changes and it is unclear what types of adverse behavioral effects occurred. In view of these caveats, particularly the questionable masking of treatment and the use of parent reports as the sole outcome source, there is limited confidence in the reliability of the study data. Overall, however, the reported findings may be viewed as suggestive that intolerance to a variety of food items, associated with physical, and possibly behavioral, symptoms in some hyperkinetic children, may be lessened by a process of desensitization which further suggests some immunologic process.

- In an effort to determine whether anecdotal reports of food intolerance associated with ADHD can be verified under controlled clinical conditions, **Carter et al (1993)** conducted a double blind, placebo controlled clinical trial testing the effects of a "few foods" elimination diet in hyperactive children. The "few foods" diet is analogous to the oligoantigenic diet in that both eliminate every food or additive assumed to provoke behavioral reactions in certain children. In the open (non-blind) phase of testing 59 out of 78 of the ADHD children tested (76%), many of whom were already on some restricted diet, were found to be food intolerant based on their reported behavioral improvement on the "few foods" elimination diet, which was continued throughout the duration of this study. In the open reintroduction phase a large number of food items were identified that provoked an adverse behavioral response sometimes with physical symptoms. Nineteen of the diet responsive ADHD children completed the main controlled challenge phase of testing. Most children were challenged

with a series of more than one provoking food item, each disguised in a tolerated food which served as the placebo. Artificial colors (daily maximum of 26 mg) were given in capsules (glucose as placebo). The number of subjects challenged with various food items was not specified. Sufficient measures were taken to ensure blinding of study. Each child was challenged with a suspect provoking food item or the placebo each daily for one week in randomized order with a two-week washout period between active and placebo treatments. The multiple outcome measures included parent and psychologist ratings and 2 laboratory tests (paired associate/learning and familiar figures/impulsiveness). The parent ratings showed statistically significant deterioration of behavior with the provoking food item challenges compared with the placebo, but the magnitude of the behavioral change was small and the behaviors most affected related to irritability rather than attention deficit. The psychologist ratings also showed challenge with the provoking food items to significantly increase behavioral response, primarily fidgetiness. The laboratory test of impulsiveness also showed significant adverse food challenge effects but the learning task was unaffected. While all of the treatment related behavioral changes were in the direction of negative effects, the small magnitude of these changes makes their biological relevance unclear. The possibility of synergistic or additive effects from multiple provoking food items represents a data gap and should be addressed in subsequent studies. Notably also, the types of behaviors elicited related more to irritability and fidgetiness than on attention deficit, suggesting behavioral changes not necessarily associated only with the hyperactivity syndrome. Overall, this study presents credible findings indicating that parent anecdotal reports of a selected group of ADHD children showing improved behavior in a non-blinded trial of a "few foods" elimination diet can be confirmed to some extent in controlled clinical trial. These findings also support the conclusion that some ADHD children may have intolerances to various foods/components, including artificial food colors and additives, which may cause or exaggerate adverse problem behaviors which may or may not be related to the hyperactivity syndrome. However, with the use of general food items in this study it is not possible to identify specific food chemicals that may be causing the adverse effects.

- **Boris and Mandel (1994)** focused on the role dietary components may play in ADHD of childhood by conducting a double blind, placebo controlled food challenge trial to determine whether reported responses of ADHD children to suspect provoking food items were reproducible under controlled test conditions. Prior to the controlled challenge phase of the study, non-blinded testing was conducted to identify ADHD children who showed improved behavior on an open food elimination diet, comparable to the 'few foods'diet, (19 out of 26 children responded to diet with responders having parent mean Conners score of 25 prior to diet and 9.6 on diet) and who responded adversely to certain food items. More atopic children than non-atopics responded to the elimination diet, contradicting an earlier study which found no involvement of atopy (Egger et al, 1985). Sixteen of these "diet responders" (7.5 years) then completed the controlled food challenge trial. Each child was challenged with only the most provoking food item for that child, given as 5 grams powdered food or 100 mg blended artificial colors masked in a lentil soup or cranberry sauce. Provoking challenge items included milk (5), colors (4), corn (3), wheat (2), soy (1), and oranges (1). All study personnel were blind to treatment but effectiveness of blinding for parents and children was not verified. Note that no subjects reacted to preservatives in open challenge. During a 7-day experimental period, days 1, 2, and 7 were designated placebo days for all subjects. The food challenge and placebo treatment were randomly administered on days 3, 4, 5 and 6 with 13 subjects receiving food challenge two times, 2 subjects three times, and 1

subject one time. All other week days were placebo. Based on the parent behavior ratings (the sole outcome measure in this study), there was an overall significant increase in hyperactivity scores with the provoking food challenges (mean Conners score of 18.1) compared with placebo treatments (mean Conners score of 8.2). However, inspection of the graphed data show that only 11 of the 16 subjects actually responded to the provoking food challenge. The challenge food items for the challenge responders and non-responders were not identified. It should be pointed out that the noted difference in hyperactivity scores between the original (pre-study) diet period and the provoking food challenge period (mean Conners scores of 25 and 18.1, respectively) may be more apparent than real, since the blinding and treatment conditions under which the two ratings were conducted were completely different. Two experimental limitations of this study affect the confidence in the findings. Since the parent rating was the only measure of behavioral response, without any other sources of information to confirm or support the parent ratings, confidence in the reliability of these study findings is limited. A second limitation involves the study blinding which is critical to the integrity and reliability of any controlled study, particularly when subjective single source outcome measures are used. Although study participants were not informed of the challenge order and the placebo and food challenges could not be distinguished by taste, the effectiveness of the blind specifically for the parents and children was not validated leaving an uncertainty about the adequacy of the study blind. Overall, in view of these limitations, the confidence in the reliability of the study findings and their relevance is unclear. The findings may at least be considered suggestive that intolerance to various food components may cause or exaggerate hyperactive behaviors to a moderate degree in some ADHD children. The use of general food items does not allow specific food chemicals, which might be involved, to be even tentatively identified.

- **Schmidt et al (1997)** conducted a controlled diet crossover trial to examine the effectiveness of an oligoantigenic diet as a possible treatment for ADHD/Disruptive Behavior Disorder children and uniquely compared the diet with stimulant medication treatment. The study participants were an unselected sample of 49 children (6-12 years) hospitalized for treatment of ADHD/Conduct Disorder Behavior. The two dietary treatments were a restrictive oligoantigenic diet with beverages of apple juice and water, and a placebo diet of common food items with daily beverages containing 50 mg mixed artificial colors. All personnel were blind to treatment and diets designed so that differences in appearance would not allow identifying the experimental diet (this was not verified). All 49 children were tested with the experimental diet, but only 36 were additionally tested non-blinded with stimulant medication (methylphenidate, 0.4 mg/kg, po) for comparative purposes. After a 4-day baseline, children were given the oligoantigenic or placebo diets each for 9 days, with treatment order randomized. After a washout period of 9 days, children were treated with stimulant medication. Outcome measures consisted of both behavioral ratings (teacher rating in classroom and trained observer ratings at play and in laboratory during performance testing) and laboratory performance testing (continuous performance, paired associate learning, and activity). Notably, treatment effects were based only on the ratings data and did not include results from the objective laboratory testing. Blood levels of IgE were also measured but showed no difference between experimental diet and baseline conditions. Group level analysis showed significantly lower problem behaviors on the experimental diet compared with placebo but based only on the observer ratings at play and in the laboratory. The teacher ratings in the classroom showed no significant diet related differences in behavior. Analysis of rating data for individual subjects showed significantly lower problem

behaviors for 22/49 children (45%) in the laboratory setting and 21/49 children (43%) in the play setting. Twelve children (24%) were considered clinically significant "responders" (i.e.25% improved behavior on two measures). In the non-blind testing of methylphenidate treatment, 16/36 children (44%) were considered "responders". Out of 36 children who received both diet and drug treatment, 31% improved on drug only, 8% on diet only, and 14% on both. The findings in this study appear to be inconclusive due to the apparent discrepancy between observer and teacher ratings. Although several objective laboratory tests were conducted, none of those data were presented or analyzed and provide no information with which to resolve this discrepancy. In addition to the inconsistent findings between the two sources of behavioral assessment, the parents and trained observers, there are two other issues which may affect confidence in the results of this study. One issue involves the fact that no efforts were made to verify the effectiveness of the blinding for the children, i.e. whether or not they could actually tell which of the two diets was the restrictive oligoantigenic diet. The other issue is that, even though objective laboratory tests of behavior were actually conducted, none of those data were presented or analyzed. The investigators dismissed the data because they viewed these objective test data as less comprehensive, confounded by differences in motivation, insensitive to subtle treatment effects, providing redundant information, and not suitable for assessing response in a clinically relevant sense due to ceiling effects. The inclusion of objective laboratory tests to supplement the behavioral ratings was a feature viewed as adding credibility to the study by providing confirmatory evidence of treatment related effects. Dismissing these test results *post hoc*, without even presenting the data, lessens the ability to interpret the study findings that were presented. Overall, considering the limitations noted above, the results of this study are considered inconclusive and should be viewed only as suggestive of a possible limited beneficial effect of the oligoantigenic diet in certain children with a profile of disruptive behaviors including ADHD and Conduct Disorder or that food intolerance may exaggerate some adverse behaviors in a small group of select children with ADHD/Conduct Disorder.

- **Uhlig et al (1997)** designed a clinical challenge trial, using a special technique of EEG topographical mapping, to investigate whether changes in brain electrical activity occur in food sensitive ADHD children exposed to provoking foods. The 12 children (6-15 years) completing all phases of this study were outpatients from a special diet clinic and were diagnosed ADHD. In initial open (non-blind) trials, behavior ratings by parents and teachers showed improved behavior in these children on an oligoantigenic (few foods) diet and suspect food items provoking the return of adverse behaviors were identified for use in the challenge phase of this study. EEG mapping of each child was carried out under both conditions of "consuming" and "avoiding" provoking foods. For the "avoiding" condition, children simply maintained the oligoantigenic diet for 14 days before EEG mapping. For the "consuming" condition, children ate provoking foods daily for at least 5 days prior to and on the day of EEG mapping. During the EEG sessions two investigators also conducted Conners behavioral ratings. Notably, other than one investigator being blind to treatment order during evaluation of EEG recordings, no other study personnel particularly including parents and children were reported as being blinded to treatment during this study. Conners scores were significantly higher for all children under the "consuming" provoking foods condition compared with the "avoiding" condition (it should be noted that two other children who showed no challenge response to the provoking foods were excluded from the study). Significant EEG differences were also found between diet conditions, primarily in the fronto-temporal regions of the brain with few changes in the parieto-occipital areas. Relative to the

"avoiding" diet condition, when children consumed provoking food items, the percentages of fast beta-1 frequency electrical activity increased in frontal areas of the brain in conjunction with abnormal behavior ratings. The relative power spectra of all recordings while consuming provoking food items were significantly higher than during avoidance of the provoking food items. Unfortunately, in an otherwise well-conducted electrophysiological study, the absence of additional sources of behavioral measures to confirm the investigators' ratings lessens confidence in the reliability of the findings. Further, the critical absence of any apparent blinding conditions introduces the possibility of an unintentional influence on the behavior of the children and confounds interpretation of the EEG results and associated behavioral ratings. The children may have behaved differently not because of the diet but because they or their parents knew which diet they were receiving, which in turn could have affected the behavioral ratings and the EEG mapping. Also, the exclusion from the study of two subjects specifically because "there was no change in behaviour during the crossover study" is questionable. Overall, due primarily to the absence of blinding conditions and the use of a single source measure of behavioral changes, the results from this study cannot be interpreted as evidence of an association between exposure to "provoking" foods (food intolerance) and specific EEG changes or behavioral rating changes but may be suggestive of such an association. However, the results do appear to show credible evidence associating specific electrical activity changes in the frontal brain region with increased hyperactivity behaviors (indicated by increased Conners rating scores).

Group II Trials: General Conclusion

To summarize the conclusions from Group II trials, while all 7 Group II trials showed some findings indicative of food intolerance, 3 trials were inconclusive and there were varying levels of confidence in the findings for all trials. Among the 4 trials showing positive findings of food intolerance, there was a level of reasonable confidence in only one of those trials (Carter et al, 1993), and moderate or limited confidence in 2 trials (Boris et al, 1994; Egger et al, 1992) and low confidence in one trial (Uhlig et al, 1997). For the 3 inconclusive trials there was reasonable confidence in the findings for one trial (Egger et al, 1985), and moderate and low confidence for other two (Kaplan et al, 1989; Schmidt et al, 1997), respectively. Across all trials, the levels of confidence were affected by various study design limitations, similar to those in the Group I trials, including: use of unstructured non-validated rating systems (Egger et al, 1992), non-blinded study personnel responsible for behavior ratings (Uhlig et al, 1997) or effectiveness of blinding not verified or questionable (Egger et al, 1985; Egger et al, 1992; Boris et al, 1994; Schmidt et al, 1997), use of single source for behavioral outcome measures not confirmed by other sources of measurement (Egger et al, 1992; Boris et al, 1994; Uhlig et al, 1997), all/part of data not statistically analyzed (Schmidt et al, 1997) or incomplete presentation of data (Schmidt et al, 1997).

Collectively, these trials provided reasonably acceptable evidence (Carter et al, 1994; Egger et al, 1985) or suggestive findings (Boris et al, 1994; Egger et al, 1992; Kaplan et al, 1989; Schmidt et al, 1997; Uhlig et al, 1997) to conclude that certain children with ADHD or other problem behaviors may exhibit a unique intolerance to a variety of foods and food components, including artificial colors. While a general increase in hyperactive behaviors in ADHD/behavior problem children has been associated with food intolerance (Boris et al, 1994; Egger et al, 1985), there is some evidence that exposure of such children to various individual provoking food items may

specifically result in small but adverse behaviors associated more with irritability, fidgetiness, sleep problems, and impulsiveness, than attention deficit and learning deficiency which are related to the hyperactivity syndromes (Carter et al, 1993). Carter et al (1993) also suggested that subsequent investigations should consider the extent to which synergistic or additive effects may occur from exposure to multiple provoking food items. The 1982 NIH Consensus Conference report had also identified the need for further research on the synergistic effects of dietary components (NIH, 1982). While it has been suggested that food intolerance in ADHD children may involve some type of immunologic process, there are conflicting results regarding atopy and IgE (Boris et al, 1994; Egger et al, 1985; Egger et al, 1992; Schmidt et al, 1997), but the desensitization trial by Egger et al (1992) attributed desensitization induced tolerance to provoking foods as an immunological response most likely involving a non-IgE cellular response to antigen rather than an antibody mediated immunization**.**

V Overview of Animal/Laboratory Studies

A variety of animal laboratory studies have been conducted to determine whether there is any biological support for a possible links between artificial food colors and Hyperactivity. A fairly representative group of these studies are referenced as supportive information in this review (see attached *References: IIa. Animal/Laboratory Studies*). Early studies reported that the food color, erythrosine (Red No. 3), was shown *in vitro* to inhibit the uptake of neurotransmitters, specifically including dopamine, by nerve cells (Lafferman and Silbergeld, 1979; Logan and Swanson, 1979). However, subsequent experimental information found that effect was due to nonspecific interactions of erythrosine with many biological membranes rather than a specific neuronal effect, which made a link with Hyperactivity very difficult to discern (Mailman and Lewis, 1983). To help determine whether behavior may be affected by erythrosine, investigators also began conducting animal studies but these produced rather variable results, providing no clear evidence that erythrosine had any significant adverse effects on behavioral functions. Some of those early animal studies showed no behavioral effects of erythrosine in either developing or adult animals (Goldenring et al, 1981; Mailman et al, 1980), while others did report positive effects but often with no clear dose response or at high dose levels (see review in background bibliography by Silbergeld and Anderson, 1982). Subsequently, additional laboratory studies have been conducted related to erythrosine and other color additives. Among the 4 additional erythrosine studies, reviewed in this report, one showed that erythrosine does not appreciably penetrate the blood brain barrier in adult rats (developing animals not investigated) (Levitan et al, 1985); another showed that erythrosine does not affect activity in adult mice in the dark or under irradiated light (Galloway et al, 1986); and two developmental neurotoxicity studies, one with rats and one with mice, showed that there was no evidence of neurobehavioral toxicity in developing rats from dietary exposure to erythrosine up to the highest dietary level used of 1% (Vorhees et al, 1983) and only few minor behavioral milestone changes in male mice but only at the highest dietary level of 0.045% erythrosine (Tanaka, 2001). Based on the above animal/laboratory information, there appears to be no convincing evidence that can be extrapolated as being supportive of a link specifically between erythrosine and Hyperactivity in children.

The remaining animal/laboratory studies related to foods colors that were reviewed in this report

provided a variety of additional interesting information. *Two developmental neurotoxicity studies, one using rats and the other mice, assessed the effects of dietary exposure to Red 40 (allura red AC). The study with rats, using the higher dietary levels of 2.5 to 10%, found that all dietary levels of Red 40 produced both physical and behavioral toxicity in the developing rats (Vorhees et al, 1983). The study with mice, using lower dietary levels or Red-40 at 0.42% to 1.68%, found no effects on behavioral development at any dietary level and only limited effects on maze performance, of questionable relevance, only at the highest dietary level (Tanaka, 1994). *A developmental neurotoxicity study of amaranth (Red No. 2) was conducted in mice using dietary levels from 0.03% to 0.27% (the 0.03% dietary level is equivalent to a dose level of 50 mg/kg/day). All dietary levels of amaranth significantly affected several measures of behavioral development but no effects on maze performance and inconsistent changes in activity (Tanaka, 1992). *A single generation toxicity study with rats exposed *in utero* showed carmoisine at dose levels up to 1200 mg/kg/day delivered in the diet showed no overt behavioral effects but some general signs of toxicity, such as decreased body weights, starting somewhat at 400 mg/kg/day (Ford et al, 1987). *A combined reproductive and developmental neurobehavioral toxicity study was conducted in mice with dietary tartrazine (Yellow 5) at levels of 0.05 to 0.45%. Only the highest dietary level of tartrazine (0.45%) produced significant adverse effects on a few indices of behavioral development in developing mice (Tanaka, 2006). *A behavioral development study showed that postnatal injections of sulfanilic acid, a metabolite of azo dyes such as Yellow 5 (tartrazine) and Yellow 6, produce several significant behavioral changes in developing rats that are dissimilar to those effects of 6OHDA injection, which is considered an animal model of Hyperactivity, but these findings have little relevance to humans because of ADME differences (Goldenring et al, 1982) *A reproductive and neurobehavioral development study of *lac* dye, a natural color additive, was conducted in mice at dietary levels of 0.15 to 0.6%. There were variable statistically significant effects on behavioral development and function across all dietary levels in both sexes, most consistently at the highest dietary level of 0.6%, also occurring at the 0.3% dietary level, and occasionally at the lowest 0.15% dietary level; body weights of both sexes were significantly decreased toward the end of lactation with females noted as more affected than males (Tanaka, 1997). *A very interesting *in vitro* study with neuroblastoma cell cultures showed a potential *synergistic* neurotoxicity in inhibiting neurite outgrowth (an *in vitro* model of neuronal cell differentiation) with two combinations of food additives, specifically Brilliant Blue + MSG (l-glutamic acid) and Quinoline Yellow + aspartame (Lau et al, 2006).

In 1982, the NIH Consensus Conference (NIH, 1982) concluded that there was a need for epidemiological studies to include addressing possible genetic, developmental and environmental factors which may be causal and serve as predictors of effect, and animal studies to obtain basic relevant biological information. Thus far, based on the representative studies identified above, the primary contribution of the animal/laboratory studies in providing biological information linking artificial food colors and ADHD seems to have focused on identifying particular color additives that may have a potential for causing behavioral (neurotoxic) effects. Even in this regard additional laboratory testing in needed to better characterize neurotoxic potential of these chemicals, particularly at lower levels of exposure, and to determine whether sensitivity to their potential neurotoxicity may be modulated by genetic polymorphisms or by some synergistic interaction with other chemical substances. Hopefully, this type of information can at least help clinical investigators to prioritize color additives of interest and focus future clinical studies on the more suspect chemicals. However, laboratory investigations need to devote increasingly more attention to systematically exploring the possible biological processes that may underlie

links between food colors and ADHD or other related behavioral disorders of childhood and, as necessary, develop innovative new experimental approaches for chemical testing, for example developing an *in vivo* protocol to complement the *in vitro* demonstration of possible *synergistic* neurotoxicity (Lau et al, 2006).

VI Possible Biological Processes/Mechanisms

Attempts to identify the biological process(es) underlying the proposed relationship between artificial food colors and problem behaviors in children, such as ADHD, is complicated by the multitude of possible scenarios due to the broad array of basic questions about the nature of this proposed relationship. For example, are the colors acting through some toxic, physiologic, allergic or other immunologic process? Are the major behavioral effects caused by one particular color, by the combined action of multiple colors, or by some interaction, perhaps synergistic, with other component(s) in the food? Are these color effects associated with some factor(s) that predispose children to ADHD or other types of behavioral pathology, or could the color effects be associated with some predisposing factor(s) not necessarily related to behavioral disorders? Although many investigators have speculated about these various issues, most of these basic questions still remain largely unanswered.

The considerations of possible biological processes that may underlie a relationship between artificial food colors and problem behaviors, such as ADHD, in children have can be summarized into several broad categories:

Neurochemical

As noted previously, one of the earliest proposed biological mechanisms linking food color additives and hyperactivity involved a defective neurochemical process affecting synaptic availability of certain neurotransmitters, particularly dopamine. The basis for this original proposal was eventually considered inconclusive. However, a dopaminergic or other neurotransmitter involvement is still considered likely based on the view that altered dopaminergic neurotransmission may be involved in the pathophysiology of ADHD (Brookes et al, 2006; Sonuga-Barke, 2003). Since therapeutic treatments are known to positively modulate the dopaminergic system (Banergee et al, 2007), it seems logical that treatments (colors) that can trigger or exaggerate ADHD behaviors may possibly be expected to negatively modulate the dopaminergic system. Hopefully, investigators will take full advantage of the recent identification of several gene variants associated with susceptibility to ADHD that include dopamine receptor and dopamine transporter genes (Banerjee et al, 2007; Farone et al., 2001; Farone et al, 2005) to pursue productive investigations of the role of dopamine in the effects of colors on ADHD.

Many environmental factors can increase histamine release, including infections as well as many food items and certain artificial food colors. This, together with the frequent claim that food allergy/intolerance is a cause of hyperactivity has led to the suggestion that the current focus on a dopaminergic mechanism in ADHD needs to be extended to histamine (Stevenson et al, 2007a). Since genetic polymorphisms involving the histamine N-methyltransferase gene can impair histamine clearance and the histamine (H3) receptors are present in the brain, this provides a possible mechanistic basis for gene-food interactions associated with ADHD. Indeed, some tentative information that genetic variants related to histamine may modulate behavioral responses to artificial colors in

some children was suggested in a study report by Stevenson et al (2007).

Genetic Processes

The possibility that genetic processes may underlie the link between colors and ADHD stems from the fact that there is a strong genetic component for ADHD (Banerjee et al, 2007; Goodman and Stevenson, 1989; Stevenson, 2006). In addition to the genetic component, there appears to be a variety of interacting biological and environmental factors that may be associated with expression of the ADHD (Banarjee et al, 2007) and food may be one of those risk factors that may elicit or exaggerate, but not cause, hyperactive behaviors in some children (Cruz and Bahna, 2006; Mattes, 1983; NIH, 1982; Schab and Trinh, 2004; Wender, 1986). But it remains to be determined whether the genetic variants associated with ADHD may also modulate sensitivity to food additives or, generally, the development or expression of food intolerances. Some suggestion for this does come from results presented by Rowe and Rowe (1994) in which they reported that more hyperactive children reacted to color challenge than normal children, suggesting that the certain genetic elements associated with predisposing children to hyperactivity may also predispose some of those children to a sensitivity to food colors. Also, as noted earlier, there is some tentative information that genetic variants related to histamine, which itself may be associated with ADHD, may modulate behavioral responses to artificial colors in some children (Stevenson et al, 2007). However, as Schon and Trinh (2004) point out, the possible contribution of artificial colors in triggering the expression of ADHD must contend with the incongruity that the pattern of behaviors reported by Rowe and Rowe (1994) following the color challenge (although tartrazine only) differ from the behaviors associated with ADHD. A similar incongruity exists for food intolerance which is also reported to elicit behaviors that are not characteristic of ADHD (Carter et al, 1993). Additional systematic experimental studies are needed to provide more systematic information in this area.

Food Intolerance/Allergy/Immunologic

The adverse effects of artificial food color, although limited and affecting only a small group of children with problem behaviors, such as ADHD, have not been consistently associated with atopy and are now generally thought not to be caused by an allergic reaction mediated through an IgE mechanism (Bateman et al, 2004; MacGibbon, 1983; Pollock and Warner, 1990). The color effects are more likely to occur through some pharmacologic effects such as a non-IgE dependent histamine release (Bateman et al, 2004).

An observation was made that most of the children who are anecdotally reported to improve on the Feingold diet do not show adverse behavioral response to the controlled challenge with color additives which indicates that other factors in the diet, not the artificial food colors, are the key dietary variables for those children (Bishop, 1983; Stare et al, 1980). Investigators began broadening Feingold's original hypothesis to restrict not only food colors and flavors, but also any food items that were assumed or suspected of causing an adverse reaction and reported findings suggesting that multiple food items can provoke adverse behavioral reaction (Kaplan et al, 1989; Schmidt et al, 1997). Other investigators used challenge trials which suggested that some ADHD children have intolerance to a variety of food items, not limited to colors or additives, which may cause or exaggerate adverse behaviors. In an interesting food desensitization study, ADHD

children were desensitized which produced in these children a tolerance to food items that previously provoked adverse behavioral reactions (Eggers et al, 1992). The authors attributed this to an immunological response most likely involving a non-IgE cellular response to antigen rather than an antibody mediated immunization**.**

It should be noted that, although understanding these modes of action (neurochemical and genetic processes) will aid in filling the data gaps, technical limitations presently exist in connecting basic animal neurochemical anatomy/physiology or genetic/epigenetic factors with complex and subtle human behavioral characteristics.

VII Overall Evaluation & Interpretation of Available Information

The interpretation of and conclusions drawn from the studies reviewed in this report includes consideration of the differing dietary conditions (defined diet, food elimination diet), challenge items (diet, artificial color(s), provoking food items), and study population (ADHD, heterogeneous problem behaviors, general population). Overall interpretation of the significance of the reported findings is complicated by the methodological limitations in many of these studies affecting the level of confidence in the data and the occurrence of inconclusive or inconsistent findings, which in several trials consisted of small treatment effects for subjects many of whom were selected for their reported diet responsiveness prior to the study.

Group I Trials: Assessment

The focus of the 26 Group I trials was to assess the validity of Feingold's hypothesis relating food additives and ADHD and other problem behaviors in susceptible children. The clinical trials were typically designed as double blind, placebo controlled studies either to assess the effectiveness of the Feingold diet as a dietary treatment of ADHD or to test the role specifically of artificial colors in causing or provoking adverse behaviors related to ADHD or other problem behaviors in children. Various caveats in the study designs and uncertainties in the study results make interpretation of the study findings difficult and limited in scope.

Pre-1982 Clinical Trials: Collectively, across the 16 pre-1982 trials, the numbers of trials reporting findings of either improved behavior on Feingold's diet or adverse reactions to color challenge were mixed: 2 positive, 8 inconsistent, and 6 negative. Based on an NIH Consensus Committee's review in 1982 of the extant information (including 16 trials conducted prior to 1982 and reviewed in this project), the committee report alluded to differences and inadequacies in the designs of the controlled clinical trials making analysis difficult, but concluded that the available studies "*did indicate a limited positive association between 'the defined diets' and a decrease in hyperactivity...that involved only a small proportion of patients; furthermore, the decreases in hyperactivity were not observed consistently. Studies indicated that some hyperactive children on a defined diet experienced an increase in hyperactivity when given moderate doses of artificial food colors.....by only a small group of patients and the increase was not consistently reported by teachers, parents, and other observers*". This highly qualified conclusion fairly represents this reviewer's assessment of the information from the Group I clinical trials conducted prior to 1982.

Post-1982 Clinical Trials: Collectively, across the 10 post-1982 trials, the numbers of

trials reporting findings of adverse reactions to color challenge were mixed: 3 positive, 3 inconsistent, and 4 negative. Among the 3 positive trials associating adverse behavioral effects with artificial color challenge, there was reasonable confidence in only one study showing clear dose response behavioral effects at each challenge dose of tartrazine from 1 to 50 mg primarily in problem behavior children but also in several children without behavioral problems (Rowe and Rowe, 1994), moderate confidence in one trial reporting 2 children (one hyperactive, the other non-hyperactive with problem behaviors) responding to 50 mg tartrazine or carmoisine with adverse behaviors that may or may not be characteristic of hyperactivity (Rowe, 1988), and low confidence in the third trial reporting several children responding to intermittent challenge with 10 mg tartrazine and sunset yellow (Sarantinos et al, 1990). There were mixed levels of confidence in the remaining trials reporting inconsistent findings (*reasonable* -Thorley, 1984; *moderate*-Salamy et al, 1982; *low*- David, 1987; *low*-Wilson and Scott, 1989) and negative findings (*reasonable*- Pollock and Warner, 1990; *moderate*- McCann et al, 2007; *limited*-Bateman et al, 2004). Particular note should be made of the dose response trial with tartrazine, conducted by Rowe and Rowe (1994) was considered to present some of the more reliable and significant findings. The clear dose related behavioral changes in response to tartrazine challenge across dosages of 1 to 50 mg did show evidence of adverse general behavioral effects primarily in suspect hyperactive children with problem behaviors (and also several control children). The behavioral effects elicited by the tartrazine challenges, however, involved irritability, fidgetiness and sleep problems which are not typically representative of hyperactivity related behaviors. Several other investigators also reported behavioral responses to color challenge that were not particularly characteristic of ADHD (Mattes and Gittelman, 1978; Rowe, 1988; Sarantinos et al, 1990); the citations reporting non-ADHD types of behavioral effects are highlighted in Table 1. Carter et al (1993) also reported similar types of non-hyperactive behavioral responses to provoking food challenges in their study group of ADHD children. The study by Rowe and Rowe (1994) is also notable for reporting that more of the children assessed hyperactive reacted to tartrazine color challenge than normal children. Given the fact that there is a strong genetic component to hyperactivity, more hyperactive than control subjects responding to the adverse behavioral effects of tartrazine suggests that the genetic elements predisposing children to hyperactivity may also predispose some of those children to sensitivity to tartrazine.

The results from all 26 Group I controlled clinical trials, overall, suggest the conclusion that certain subgroups of children with problem behaviors that may or may not be related to ADHD/ADD and, possibly, certain children from the general population without particular behavioral problems may exhibit a unique intolerance to artificial food colors resulting in typically small to moderate adverse behavioral changes which may not necessarily be characteristic of the ADHD syndromes.

Group II Trials: Assessment

Collectively, these trials provided reasonably acceptable evidence in 2 trials (Carter et al, 1994; Egger et al, 1985) or suggestive findings in 5 trials (Boris et al, 1994; Egger et al, 1992; Kaplan et al, 1989; Schmidt et al, 1997; Uhlig et al, 1997) to conclude that certain children with ADHD or other problem behaviors may exhibit a unique intolerance to a

> variety of foods and food components, including artificial colors. While a general increase in hyperactive behaviors in ADHD/behavior problem children has been associated with food intolerance (Boris et al, 1994; Egger et al, 1985), there is some evidence that exposure of such children to various individual provoking food items may specifically result in small but adverse behaviors associated more with irritability, fidgetiness, sleep problems, and impulsiveness, than attention deficit and learning deficiency which are related to the hyperactivity syndromes (Carter et al, 1993). Carter et al (1993) also suggested that subsequent investigations should consider the extent to which synergistic or additive effects may occur from exposure to multiple provoking food items. The 1982 NIH Consensus Conference report had also identified the need for further research on the synergistic effects of dietary components (NIH, 1982). While it has been suggested that food intolerance in ADHD children may involve some type of immunologic process, there are conflicting results regarding atopy and IgE (Boris et al, 1994; Egger et al, 1985; Egger et al, 1992; Schmidt et al, 1997), but the desensitization trial by Egger et al (1992) attributed desensitization induced tolerance to provoking foods as an immunological response most likely involving a non-IgE cellular response to antigen rather than an antibody mediated immunization**.**

One particularly confusing and contentious issue that has been raised by several clinical investigators and reviewers requires some attention primarily because it casts some doubt on the results from a number of clinical trials investigating artificial colors. The issue is whether the approximately 27 - 30 mg of mixed artificial colors, used in a number of clinical trials, represents a level of artificial color too low to enable detection of any treatment related effects (King, 1984; Rapp, 1982; Rimland, 1983; Rippere, 1983; Schab and Trinh, 2004). Rapp (1982) voiced the opinion that enough food coloring must be used in challenge to produce symptoms and further that the quantity should be tailored to the amount needed to cause an individual child to have symptoms, this to be determined prior to the challenge study. This latter suggestion was actually considered in two studies conducted by Mattes and Gittelman (1978, 1981). In the earlier study (Mattes and Gittelman,1978) the challenge dosage of artificial color was based on an initial dose-ranging trial with the test subjects and in the later study (Mattes and Gittelman,1981) incremental daily dosing up to 78 mg/day was used, and in both studies multiple outcome measures detected no significant color challenge effects (in the latter study parent ratings indicated that several children were responding to the color challenge but this was not supported by multiple other subjective and objective behavioral measures). There are several other observations that might suggest that levels of artificial color, within reason, may not necessarily be an important determinant of behavioral effects in studies assessing the association between food colors and problem behaviors such as hyperactivity of childhood. Several other observations are relevant. First, clinical challenge studies assessing behavioral effects using high doses of mixed artificial colors (Swanson and Kinsbourne, 1980; Mattes and Gittelman, 1981; Thorley, 1984; David, 1987; Rowe, 1988; Pollock and Warner, 1990) have generally shown no more dramatic, reliable or conclusive behavioral effects than studies using lower or moderate dose levels (Harley et al, 1978; Goyette et al, 1978; Rose 1978; Levy and Hobbes, 1978; Levy et al, 1978, Mattes and Gittelman, 1978;Williams et al, 1978, Conners, 1980; Conners et al, 1980; Weiss et al, 1980; Wilson and Scott, 1989; Sarantinos et al, 1990; Bateman et al, 2004; McCann et al, 2007). The only evidence of a dose response effect for any color was reported by Rowe and Rowe (1994) in which tartrazine reportedly affected behavior across all dosages from 1 to 50 mg with a ceiling effect (magnitude but not duration) above 10 mg. Second, in many clinical color challenge studies that recruit children who are responsive to the Feingold diet, parents commonly

report that their children exhibit rapid and dramatic deterioration of behavior with even minor infractions of the Feingold diet (Adams, 1981; Conners, 1980; Mattes, 1983; Mattes and Gittelman, 1978; Pollock and Warner, 1990; Rowe, 1988). Feingold even asserted that minute amounts of foods containing synthetic flavors, colors and salicylates is sufficient to cause a recurrence of the hyperactivity behavioral pattern within several hours which persist for up to several days (Feingold, 1975; Ribon and Johsi, 1982). These observations clearly suggest that dosage levels of artificial color, within reason, may not necessarily be an important determinant of behavioral effects in clinical trials assessing the association between food colors and problem behaviors such as hyperactivity of childhood.

Overall, the available information from all 33 trials does not support a causal relationship of either food intolerance in general or artificial food colors/preservatives in particular with ADHD or other problem behaviors in children. However, within the context of the caveats associated with these studies the findings do suggest that small subpopulations of susceptible children with ADHD and/or other problem behaviors, and possibly susceptible children from the general population, may exhibit similar behavioral reactions, not necessarily related to ADHD, to a variety of foods and food ingredients, not limited to artificial food colors and preservatives. The pattern of study findings, in particular the similarity in behavioral response to a wide variety of foods and food ingredients, including colors, suggests that these effects are not the result of inherently behavioral neurotoxic properties of the food chemicals but that the behavioral changes result from some unique food intolerance to which certain individual children may be predisposed, possibly associated with some genetic or epigenetic factors.
Consequently, a parsimonious assessment of the available information concludes that small subpopulations of susceptible children with ADHD or other problem behaviors and, possibly, certain susceptible children from the general population may be predisposed to a unique intolerance to a variety of foods and food ingredients, not limited to artificial food colors and preservatives, that may be associated with adverse behavioral responses, including non-hyperactive behaviors such as irritability, restlessness and sleep disturbances, and physical responses.

VIII Identification of Information Gaps/Suggested Additional Study

- Information which might throw some light on the mechanism(s) of production of adverse reactions could be obtained by studying differences in the genetic, immunological and pharmacological background of reactors and of non-reactors (GI factors would be of importance, too). Without this understanding of what happens in sensitive human subjects, it may not prove possible to develop an animals or *in vitro* models for predictive testing of new additives (MacGibbon, 1983). Additional clinical studies are needed not only to confirm/determine that adverse behavioral responses are elicited by artificial colors and intolerant foods in hyperactive and normal (non-hyperactive) children but also to compare the nature and extent of these responses between these two groups of children and to understand the mechanism underlying these behavioral responses. Do the responses between normal and hyperactive children differ in nature and/or severity? Are they exhibiting the same types of behavioral changes but of different magnitudes/severities? Are responses to colors and food items categorically different or are they similar enough to suggest both

represent food intolerance? Is there any identifiable genetic or polymorphic predisposition to the adverse behavioral responses? Are these behavioral changes due to some direct effect on the nervous system or are they secondary to some immunologic, allergenic, or other physiological effects of treatment? Subsequent studies should compare the behavioral responses of hyperactive children and of normal (non-hyperactive) children to artificial colors and to provoking food items. Specific physical and immunological assessments should be included in the study design and genetic analyses conducted.

- There are known genetic variants associated with ADHD (Faraone et al, 2001; Faraone et al, 2005; Goodman and Stevenson, 1989). But it is not known whether these proposed genetic variants may also modulate sensitivity to food additives or may be associated with the development or expression of food intolerances. Clinical experimental studies to provide this information should be conducted. Some tentative information that genetic variants related to histamine may modulate behavioral responses of children was suggested in a study report by Stevenson et al (2007). Species differences in toxicity, for example the significantly different neurotoxic potential of benzaldehyde between rats and mice (Kluwe et al, 1983), indicate genetic based differences in susceptibility. Can this be demonstrated for behavioral effects of other food chemicals? Correlative information should be developed in laboratory studies to determine whether genetic modulation of behavioral sensitivity to food additives/colors can be demonstrated in animal models.

- In the food intolerance study conducted by Carter et al (1993), various treatment related effects from challenge with various provoking food items were found, but the small magnitude of these effects make their biological relevance unclear. Other studies have reported similar findings of small treatment related (color challenge and provoking food challenge) adverse behavioral effects. Possibly, combined exposure to multiple types of provoking food items may be necessary to elicit an additive effect of a greater magnitude behavioral response, as suggested by Carter et al (1993). Although the diet crossover study (multiple provoking food items) by Kaplan et al (1989) was inconclusive, their findings did suggest appreciable effects in some of the children. A recent *in vitro* laboratory study has reported possible synergistic effects of several food additives (Lau et al, 2006). There is little systematic information about combination effects of chemical substances, particularly with regard to potential adverse behavioral effects. The possibility of synergistic or additive adverse behavioral effects from exposure to multiple provoking food items should be addressed in subsequent studies.

Considerations in Study Design

The design and conduct of any clinical trial to assess the relationship between artificial colors and problem behaviors, such as ADHD, in children must adhere to basic principles of experimental design and study conduct. The following represent the minimum issues that should be considered to maximize confidence in the reliability of the study findings:

- Homogeneity and characterization of sample
- Randomization to treatment
- Crossover designs with subjects serving as own control
- Counterbalanced treatment/challenge order

- Double-blind/placebo-controlled challenges
- Placebo and challenge indistinguishable
- Verification of effectiveness of blinding, particularly those rating behaviors
- Age-appropriate outcome measures
- Use of validated measures (detect behavior differences/sensitive to treatment effects)
- Individualized/target behavior checklists used by all raters in study
- Use of individualized ratings with other standard/validated ratings
- Multiple sources of outcome measures (e.g., parents, teachers, objective tests, etc.)
- Analysis of all data using appropriate statistical procedures
- All data should be presented at least in summary form (tables/graphs)
- Data interpretation should consider any inadvertent occurrences during study

IX Conclusion

Exposure to food and food components, including artificial food colors and preservatives, may be associated with adverse behaviors, not necessarily related to hyperactivity, in certain susceptible children with ADHD and other problem behaviors, and possibly in susceptible children from the general population. A parsimonious interpretation of findings from relevant clinical trials indicates that this food related triggering of problem behaviors is not due to an inherent neurotoxic property of the food or food components, including any of the artificial food colors and preservatives, but appears to result from a unique intolerance exhibited by certain predisposed children to a variety of food items and color additives. The etiology of this type of unique intolerance is unclear but may involve genetic or epigenetic factors.

X Comment

As a general observation, there seem to be two possible basic scenarios that could be operative whereby food additive/environmental chemicals would be associated with triggering adverse behaviors such as those related to ADHD or other behavioral disorders of childhood, or even in the general population. One "traditional toxicology" scenario is that certain chemicals may have inherent neurobehavioral toxicity properties which may directly or indirectly (e.g., endocrine or immunologic pathways) affect nervous system function resulting in behavioral deficits. This scenario may be addressed with reliable toxicological testing including adequate neurobehavioral toxicological evaluations as a routine component of the process of chemical safety assessment. The other "non-traditional toxicology" scenario is that the elicitation of problem behaviors by various common foods and food related chemicals may be due not to an inherent neurobehavioral toxic property of these food items and food related chemicals but to some unique hypersensitivity or intolerance in certain children stemming from some genetic/epigenetic/polymorphic related predisposition. This latter scenario of unique hypersensitivity can best be addressed by continuing efforts to understand the biomolecular factors that may predispose an organism to this type of unique disruptive behavioral response to otherwise non-neurotoxic chemical substances.

† The color-coding is for quick visual reference, but it is not required to access and interpret the data.