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**REVIEWS and CRITIQUES
33 CLINICAL TRIALS RELATED TO
ARTIFICIAL FOOD COLORS
and
ADHD in CHILDHOOD and RELATED PROBLEM BEHAVIORS**

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(Chronological Order)

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REVIEWS and CRITIQUES

(trials conducted prior to 1982)

(Conners et al, 1976)

Conners CK, Goyette CH, Southwick DA, lees JM, and Andrulonis PA. Food additives and hyperkinesis: A controlled double-blind experiment. Pediatrics, 1976 (August), 58 (No.2): 154-166.

In one of the first controlled clinical trials designed to test the effects of the Feingold diet (eliminated artificial colors, flavors and natural salicylates) on the behavior of hyperactive children Conners et al (1976) recruited 15 children aged 6-12 years who were diagnosed with hyperkinetic reaction of childhood and conducted a single cross-over dietary challenge study comparing the Feingold diet with a matched placebo diet. Parents were given lists of approved foods for use in making the appropriate diet for each challenge period but were not told which diet they were preparing (i.e., blinded to treatment). It should be noted, however, that no specific procedure was used to verify the effectiveness of the parents' blinding, for example, parents and children were not asked whether they could differentiate the F/KP and placebo diets. After several weeks of pre-treatment/baseline, each child was given either the Feingold diet (also referred to as the Feingold/Kaiser-Permanente or F/KP diet) or a matched placebo diet for 4 weeks and then crossed over to the other diet for an additional 4 weeks. The order in which the two diets were given (i.e., F/KP first and Placebo second, or Placebo first and F/KP second) was determined randomly for each child. The outcome measures to determine treatment related changes in behavior consisted of: (1) parent and teacher behavioral ratings, using the standard Conners Parent/Teacher Hyperactivity Questionnaire, and (2) a Global Behavior Assessment by the principle investigator based on interviews with parents and teachers and their behavior ratings.

Study Results: The analysis of the whole study group's data showed significantly improved behavior on the F/KP diet relative to control based primarily on teacher behavior ratings with some support from the global behavior assessment but no confirmation from parent behavior ratings which detected no significant overall diet related changes in behavior. However, the teacher's finding of a F/KP diet effect was not consistent due to an inexplicable treatment x order effect in which the F/KP diet improved behavior was seen primarily when the placebo diet was given first and the F/KP diet second but was less apparent when the F/KP diet was given first. Viewing the data for individual subjects revealed that both the teacher and parent behavioral ratings agreed that 4 to 5 of the 15 subjects showed improved behavior on the F/KP diet, but, as noted for the overall group effect, the diet effects for these individual "responders" were also not consistent due to the treatment x order effect.

Study Assessment and Evaluation: Although the authors conclude that the F/KP diet, free of most natural salicylates, artificial flavors and artificial colors, reduces the perceived hyperactivity of some children with hyperkinetic impulse disorder, the findings on which this conclusion is based are inconsistent and equivocal. The small number of subjects, the uncertain effectiveness of the blinding, the absence of objective behavior outcome measures, the discrepancy between teacher and parent behavioral ratings in detecting an overall group effect, and the inexplicable treatment x order effect which mitigated the significance of not only the teacher reported group effect but also the parent and teacher agreement in identifying 4 to 5 individual children who responded positively to the F/KP diet, lessen the confidence in the reliability and biological significance of the study's findings. The reviewer concludes that this

study provides no credible evidence of any effects of the Feingold diet on childhood hyperactivity.

(Harley et al, 1978: Phase I)

Harley JP, Ray RS, Tomasi L, Eichman PL, Matthews CG, Chun R, Cleeland CS and Traisman E. Hyperkinesis and food additives: testing the Feingold hypothesis. *Pediatrics*, 1978 (June), 61(6):818-28 (I)

In an effort to test the behavioral effects of the Feingold diet on childhood hyperactivity using objective laboratory and classroom outcome measures, in conjunction with subjective parent-teacher ratings, Harley et al (1978) recruited 46 male hyperactive children (36 school aged at 6-13 years and 10 pre-school aged at 3-6 years) to conduct a double-blind dietary crossover study comparing the Feingold diet with a matched control diet. Throughout the study all of the food (experimental F/KP diet and control diet) for each family was provided weekly by the investigators. Parents, teachers and study personnel were blinded to treatment condition. Efforts were made to verify blinding effectiveness at the end of the study by asking parents if they could identify when they were on the F/KP experimental diet; none of the parents were able to respond correctly. Procedurally, after an initial 2 week baseline on normal diet, the children were randomly assigned to one of two diet conditions (control or F/KP). They were given their initially assigned diet for 3 - 4 weeks (the study was run in three phases and duration of diet exposure varied across phases), then crossed over to the other diet for an additional 3 - 4 weeks. In addition to a neurological and physical examinations, the primary behavioral outcome measures for school aged subjects included: (1) Conners parent and teacher behavioral ratings, (2) classroom and laboratory/activity observations by trained observers, and (3) neuropsychological evaluations. Observation data and neuropsychological test data were collected for hyperactive subjects and corresponding matched controls. Behavioral measure for pre-school subjects included: parent behavior ratings; laboratory/activity observations; and neuropsychological evaluations (the latter data for preschool subjects was not presented but was discussed).

Study Results: The physical exams of all 46 subjects were normal. Neurologically, 14/36 school aged and 2/10 preschool boys had positive findings. In the analyses of the entire dataset for school aged subjects parent behavioral ratings showed a significant overall F/KP diet effect with improvement in hyperactive behaviors. But this diet effect was not consistently seen due to a significant diet x order effect in which the F/KP related improved behaviors were apparent only when subjects were exposed to the control diet first and the F/KP diet second. The significance of the parent detection of a F/KP diet effect was further mitigated by the fact that teacher behavior ratings did not confirm any diet related changes in group behavior and that, while classroom/laboratory observations and neuropsychological testing discriminated between hyperactive and control children, there was no significant or convincing evidence of an overall effect of the F/KP diet on these behavioral measures. In the analyses of the dataset for preschool subjects parent ratings showed a significant group F/KP diet effect of improved behavior but, in contrast to the school aged subjects, there was no confounding diet x diet order effects. Still, the significance of the parental finding of a F/KP diet effect in preschoolers was mitigated by the lack of confirmation of any diet related effects based on either objective measure of laboratory/activity setting observations or neuropsychological testing (teacher behavior ratings were not collected for preschool subjects). The overall assessment of the dataset for individual school aged subjects did reveal an agreement between parent and teacher behavior ratings

identifying 4 of the 36 children as being responsive to the dietary conditions with behavior improving on the F/KP diet. However, as noted for the overall diet effect based on the parent ratings alone, the diet x diet order effect also mitigated the significance of the F/KP diet effects in these 4 subjects. No mention is made by the authors as to whether the other more objective outcome behavioral measures these 4 children showed any confirmatory diet related changes.

Study Assessment and Evaluation: There are several caveats mitigating the reliability and biological significance of the parental ratings in school aged boys showing a significant F/KP diet related improvement in hyperactive behavior. These caveats include a significant diet x diet order effect and an absence of confirmation with teacher ratings or various objective behavioral test measures (neuropsychological evaluation, classroom observations, and laboratory observations), all of which showed no corresponding F/KP diet related group changes in behavior. There was an agreement between parent and teacher ratings that 4/36 children showed improved behavior on the F/KP diet. However, the significance of this is limited by the confounding diet x diet order effect and the absence of confirmation of any comparable diet related changes based on objective laboratory/activity observations and extensive neuropsychological testing. Also, in the preschool subjects the significance of the parental ratings showing a significant diet effect is also mitigated by the small sample size and the lack of confirmation of a F/KP diet effect based on objective measures of laboratory/activity setting observations and a battery of neuropsychological tests. In view of the questionable reliability and biological significance of the findings, this controlled double-blind dietary cross-over study does not provide any credible support for the contention that the Feingold diet significantly affects disruptive behaviors in either school aged or preschool aged hyperactive children or that the hyperkinetic disorder is triggered or exaggerated by artificial food colors/flavors and salicylates. It is questionable whether these study findings would even support the suggestion that a small subset of hyperactive children may be responsive to the Feingold diet. .

(Goyette et al, 1978)

Goyette CH, Connors CK, Petti TA and Curtis LE. Effects of Artificial Colors on Hyperkinetic Children: A Double-Blind Challenge Study. Psychopharmacology Bulletin, 1978, 14 (No.2): 39-40.

Goyette et al (1978) were among the first clinical investigators to conduct a clinical study involving a double-blind challenge with putative offending agents to address the suggestion that common food additives, specifically artificial colors, may cause or exacerbate behavioral symptoms associated with the hyperactivity disorder of children. Two separate challenge trials were conducted. Trial 1, using a double 2-week crossover design over an 8 week period, tested 16 children (average age of 8.3 years, range 4.7-11.8; sexes not specified) with a behavioral and clinical diagnosis of hyperkinesis and carefully screened prior to the challenge phase to show significant symptom reduction when placed on the F/KP elimination diet under non-blinded conditions. The challenge vehicle was a chocolate cookie with/without a 13.5 mg mixture of all approved artificial food colors ($\frac{1}{2}$ the average daily intake). While being maintained on an additive-free diet (not specified in the publication but assumed to be the F/KP diet eliminating artificial colors and flavors and salicylates), each child ingested two cookies per day, one after breakfast and one after lunch (total daily color challenge of 27 mg). Color challenge and placebo cookies were each given in 2-week alternating sequences over an 8 week period. Children were randomly assigned to one of two challenge sequences (ABAB or BABA). Behavioral outcome measures included: (1) parent and teacher behavior ratings (3 times per week using Connors

hyperkinesis index) and (2) a visual motor tracking task conducted 1 to 2 hours after ingestion of color challenge/placebo materials (frequency not specified and not clear whether testing was conducted after the first or second daily cookie). Trial 2 was a basic repeat of Trial 1 with the following differences: (1) a daily parent behavior rating for a specific 3-hour period after ingestion of a single cookie at supper was the only outcome measure obtained; (2) younger children were recruited; and (3) a single 2-week crossover design was used. A total of 13 children were tested (mean age of 6 years, range 3.4-10.2; sexes not specified), including 8 diet-responsive, hyperkinetic children (“*criterion*” group) and 5 children with borderline diet response or borderline hyperactivity (“*borderline*” group). Both Trial 1 and 2 were described as being “double blind” but no description of blinding procedures was given other than use of a placebo and random assignment to challenge sequence. Also, no efforts were described for determining whether any of the study participants were in fact blind to treatment condition.

Trial 1 Results: All subjects were clearly identified in open trial, i.e. non-blind, as responders to the elimination diet (parents rated a 57 percent hyperactive symptom reduction and teachers a 34 percent reduction). However, double-blind challenge with color challenge and placebo revealed no significant challenge effects based on any outcome measure (parent rating, teacher rating, tracking task). The tracking task, however, did suggest a non-significant trend for performance deficits with exposure to the color challenge but not with placebo. Inspection of the individual tracking data suggested that younger subjects tended to show a more noted response to the color challenge and retesting of several younger subjects with color challenge alone suggested that effects tend to occur at 1 hour but not 2 or 3 hours after challenge.

Trial 2 Results: Overall, the 13 subjects in Trial 2 showed a 45 percent mean reduction in behavior problems on the open (non-blinded) F/KP elimination diet (relative change in symptoms between “*criterion*” and “*borderline*” children was not specified). A significant color challenge effect was found within 3 hours after ingestion of a single challenge cookie (i.e. ½ average daily intake) without any differences between the color response of the “*criterion*” and “*borderline*” groups of subjects and without any challenge x order effect.

Study Assessment and Evaluation: In Trial 1 parent/teacher ratings and a tracking task showed that hyperactive children, who were identified in open trial as definite diet responders, exhibited no significant adverse behavioral response to repeated daily challenge with a mixture of artificial colors at levels equivalent to the average daily adult intake when tested under double-blind placebo controlled challenge conditions. The suggestion of a trend for performance deficit in the tracking task, particularly in the younger children, was not significant. The investigators suggested that the parent and teacher behavioral ratings failed to detect a color challenge effect because those ratings which were conducted 3 times per week may have been insensitive due to the long time span of the observation periods compared to a possible short duration of the color challenge effect. This rationale for dismissing these parent/teacher behavioral ratings as insensitive is inconsistent with the fact that the parent and teacher ratings assess behavioral changes throughout the day; the teacher ratings, in particular, reflect the children’s behavior during the periods shortly after ingestion of each cookie at breakfast and lunch. In Trial 2, which used slightly younger subjects than Trial 1, significant group color challenge effects were detected based on parent ratings specifically conducted within a 3-hour period after ingestion of a single cookie at supper. According to the investigators, this particular finding suggests that artificial food color do act to impair and disrupt behavior of hyperkinetic children and firmly establish that artificial colors may be particularly disruptive to younger children. In the absence of any other behavior outcome measure to confirm a treatment related behavioral effect and without a clear description of the blinding procedures and verification of the effectiveness of parent blinding, the reliability of the trial 2 findings of a significant color challenge effect on

behavior based only on parent ratings remains questionable. Also, there is limited support for the conclusion regarding younger children being more sensitive, since in Trial 1 the suggestive trend of a treatment effect particularly in younger children was not statistically significant and in Trial 2 there was no difference in the response to color challenge between the two groups of children one with an average age of 5 and the other slightly older children with an average age of 7 (which is actually close to the average age of 8.3 years for the "older" children in Trial 1). Consequently, overall this study does not provide credible evidence for a significant treatment effect of artificial food colors in F/KP diet sensitive hyperkinetic children. Whatever benefit the children may have appeared to obtain from the open F/KP elimination diet was apparently not attributable to the elimination of artificial food colors alone but possibly to some other food component(s) that were eliminated in the F/KP diet.

(Harley et al, 1978: Phase II)

Harley P, Matthews CG and Eichman P. Synthetic Food Colors and Hyperactivity in Children: Double-Blind Challenge Experiment. *Pediatrics*, 1978, 62: 975-983. (II)

Harley et al (1978: Phase I) conducted an earlier double-blind dietary crossover study (Phase I) comparing the Feingold/Kaiser-Permanente diet, eliminating artificial colors and flavors and salicylates, with a matched control diet to determine whether the disruptive behaviors of hyperactive children can be improved with the F/KP elimination diet. These same investigators conducted the present Phase II study (Harley et al, 1978: Phase II) designed to select those children who showed the best response to the F/KP diet in Phase I and then to challenge them repeatedly with specified amounts of artificial food colors while being maintained on the F/KP diet. Nine male hyperactive children (mean age 9.3 years), identified as F/KP diet "responders" were selected from the Phase I study (Harley et al, 1978: Phase I). One non-hyperactive control subject was matched with each hyperactive subject on sex, grade, and academic ability. The challenge vehicles were candy bars and cookies with/without a 13.5 mg mixture of all approved artificial food colors (two items were ingested daily throughout the challenge period of the study resulting in a daily color challenge of 27 mg, which the investigators considered the "average" daily intake of artificial colors). A panel of judges determined the color/placebo cookies to be indistinguishable. All study participants were blinded to placebo/challenge code and post-study interviews showed that none of the parents or children could distinguish between the placebo and color challenge items (effectiveness of blinding appeared to be reliably established). Procedurally, the subject and his family were started on the elimination diet during the second 2 weeks of a 4 week baseline period prior to the challenge phase of the study (placement on the elimination diet appeared to be non-blinded). While being maintained on the elimination diet, the challenge phase was conducted over a 9 week period during which subjects were randomly assigned to one of two double cross-over challenge sequences with daily challenges (placebo/2 wks, color/2 wks, placebo/2 wks, color/3 wks or colors 2 wks, Placebo 2 wks, Colors 2 wks, Placebo 3 wks). Behavioral outcome measures included: (1) parent (mother and father) and teacher behavior ratings (2 times per week during baseline and challenge), (2) classroom behavioral ratings by trained observer (2 times per week during baseline and challenge), and (3) neuropsychological testing (at end of baseline and of challenge weeks 2, 4 and 9).

Study Results: During the initial period of baseline with subjects on their 'regular diets' (investigators provided no information about composition of the hyperactive children's regular diet), parent and teacher behavior ratings and classroom observer data showed an expected significant difference between the hyperactive and matched control subjects (hyperactives with

more disruptive behavior). It is significant to note that these same outcome measures found that behaviors of the hyperactive and control subjects were still significantly different during the latter part of baseline when all of the hyperactive subjects and their families were placed openly (non-blinded) on the Feingold elimination diet, indicating the perception by parents, teachers and classroom observers that the Feingold elimination diet had only a nominal, if any, effect on the behavior of the hyperactive children who were selected as being the best diet responders in the earlier Phase I study. Analysis of the group challenge data from parent and teacher ratings, classroom observer data, and neuropsychological testing revealed no significant color challenge effects. Graphic analysis of the behavioral profiles from parent and teacher ratings and classroom observer data for each individual hyperactive subject showed that only 1 of the 9 hyperactive subjects displayed a behavioral profile of parent ratings and classroom observer data that even approximated a significant color challenge effect of increased hyperactive behaviors. Teacher behavior ratings did not appear to support a color challenge effect in that single subject and the neuropsychological test data apparently were not considered in the individual profile graphic analysis.

Study Assessment and Evaluation: The results from this study suggest three conclusions related to the proposed association between artificial color and problem behaviors related to ADHD (hyperactivity of childhood). **(1)** This study provides little support for any substantial or consistent beneficial effects of the Feingold elimination diet on the behavior of hyperactive children. When hyperactive children were assessed while on their 'regular diet', their behavior was, as expected, more disruptive than the control (non-hyperactive) children. But when the hyperactive children were placed openly on the Feingold elimination diet, behavioral assessments found their behaviors remained significantly worse than matched control children. Although the Feingold diet was given under non-blinded conditions, it is significant that parents, teachers and classroom observers perceived the behavior of hyperactive children on the Feingold diet still worse than that of controls, even though these hyperactive children were specifically selected for this study on the basis of being identified as among the best Feingold diet positive responders from an earlier diet study. With the caveat that these observations were made under non-blinded conditions, these results provide no confirmation of any or consistent significant beneficial effect of Feingold's elimination diet on the behavior of hyperactive children. **(2)** This study does not support or confirm the hypothesis that artificial food colors cause or trigger problem behaviors related to hyperactivity of childhood. The overall results of this study based on multiple outcome measures (parent, teacher, classroom observer ratings, and neuropsychological testing) showed no adverse behavioral effects of daily ingestion of a 27 mg mix of artificial colors (considered the average daily intake of artificial colors) by hyperactive children for up to 21 consecutive days and, consequently, do not support or confirm Feingold's hypothesis, based on his clinical observations, (Feingold, 1975) that ingestion by hyperactive children of even minute amounts of foods containing synthetic flavors, colors and salicylates "causes a recurrence of the complete behavioral pattern within two to four hours which persists for one to four days". **(3)** Certain results from this study may be viewed as possibly suggesting that a small subgroup of hyperactive children may be intolerant to artificial food colors and respond with a limited range of behavioral response. In the assessment of behavioral profiles for each individual subject, 1 of 9 subjects exhibited a behavioral profile, based on parent ratings and classroom observer data, which reportedly (data not shown in this paper) suggested disruptive behavior related to color challenge. Although teacher behavior ratings did not support this suggested color challenge effect (and neuropsychological test data were not included in the individual assessments), these results may still be viewed as possibly suggesting that a small subgroup of hyperactive children may be intolerant to artificial food colors and respond with

limited range of behavioral response.

(Levy and Hobbes, 1978)

Levy F and Hobbes G. Hyperkinesis and Diet: A Replication Study. American Journal of Psychiatry, 1978, 135 (No.12): 1559-1560.

A single crossover challenge study of tartrazine was conducted using 7 hyperkinetic children (6 male, 1 female; average 5.2 years old) who were identified as being responsive to an open Feingold/KP elimination diet based on at least a 25% decrease in the Conners behavior ratings conducted by the mothers. (8 subjects were recruited but 1 subject dropped out of the study.) The challenge vehicle consisted of cookies each with/without 1 mg tartrazine (Yellow #5) (four cookies were ingested on each challenge day resulting in tartrazine challenge of 4 mg). Procedurally, all subjects were maintained on a F/KP elimination diet throughout the 4-week challenge period. Subjects were randomly assigned to a placebo or color challenge group and received 4 scheduled cookies daily for 2 weeks. Subjects were then crossed over to the opposite challenge group for a additional 2 weeks of testing. The parents provided the only outcome measures which consisted of a Conners parent behavior rating conducted daily during the 3-hours immediately after challenge and a parent global behavioral assessment at the end of each 2-week challenge period. Other than the use of placebo cookies, no other measures to blind the challenge study were described.

Study Results: In a placebo controlled single crossover challenge study, parent behavior ratings and global assessments revealed no significant adverse effects of daily challenge with 4 mg tartrazine for 2 weeks on behaviors of 7 hyperactive children, identified as responsive to the Feingold/KP elimination diet.

Study Assessment and Evaluation: There are several limitations to this study, including a small sample size of 7 children, no description of efforts to blind the parents to treatment conditions (other than the use of a placebo control), and use of a single source (parents) for outcome measures with no other outcome measures to confirm the parent findings. These caveats notwithstanding, the study provided no evidence that tartrazine at 4 mg per day for 2 weeks has any adverse effect on the behavior of hyperactive children (6 m, 1 f; 5.2 years old) who were reported to be responsive to the Feingold elimination diet.

(Levy et al, 1978)

Levy F, Dumbrell S, Hobbes G, Ryan M, Wilton N and Woodhill JM. Hyperkinesis and Diet: A Double-Blind Crossover Trial With A Tartrazine Challenge. Medical Journal of Australia, 1978 (January), 1 (No.2): 61-64.

Levy et al (1978) used 20 clinically diagnosed hyperkinetic children (19 male, 3 female; 4-8 yrs) to conduct a single crossover challenge study of tartrazine (Yellow #5). (22 children were recruited but 2 children did not complete the challenge portion of the study.) Responsiveness to a Feingold/KP elimination diet was assessed in open trial prior to the challenge portion of this study. The challenge vehicle was a biscuit with/without 1 mg tartrazine (five biscuits were ingested on each challenge day resulting in tartrazine challenge of 5 mg). It should be noted that the "second batch" of color challenge biscuits differed in color from the placebo biscuits, thereby compromising the blind for whatever portion of the study the second batch of color challenge biscuits were used; but it is not clear what portion of the study was actually involved.

Procedurally, following a 2-week pretest baseline period (regular diet), all subjects were placed on the F/KP elimination diet over a 4-week period and remained on that diet throughout the rest of the study. After the 4 weeks of diet initiation, half of the subjects were then assigned (random assignment assumed) to a placebo group and half to a color challenge group each receiving their appropriate 5 biscuits daily for a 2-week period. For the next period of 2 weeks, the children were crossed to the opposite treatment group. After completion of the challenge portion of the study, all children were then continued on the F/KP diet for a final 4-week washout period. Multiple outcome measures were taken at the end of each of the test periods described above, including: Conners behavior ratings completed by the mother, teachers and clinicians (psychiatrist and psychologist), and a series of objective psychological response tests to assess activity/motility, attention, impulsivity, perceptual-motor functioning, memory, intelligence and maze performance. It should be noted that the mothers and teachers were asked to rate the child over the 24-hour period before scoring. Blinding efforts included use of placebo biscuits, masking the color difference between placebo and color challenge biscuits, and use of a coded treatment schedule. No efforts to verify effectiveness of the blind were described, for example by asking the parents if they could differentiate the placebo and challenge biscuits.

Test Results: At the end of the first four weeks of open treatment with the F/KP diet, the mothers' behavioral ratings indicated a significant positive diet effect but these ratings were made under non-blind conditions and there was no confirmation of any diet effect based on the teacher or clinician behavior ratings on a majority of the series of objective psychological tests (the singular exception being a diet related improvement of maze performance, but this effect was not maintained in other study analyses). In the primary analysis of data for the full sample of 20 test subjects, there were no significant adverse effects of daily challenge with 5 mg tartrazine over a 2-week period based on any outcome measure including parent and teacher behavior ratings and any of the series of objective psychological tests. In order to determine whether the effects of diet and of tartrazine challenge might be amplified in the more hyperactive children, post-hoc analysis of the data was conducted for a selected subset of 16 children who were initially rated by teachers as exhibiting higher levels of hyperactivity (i.e. Conners scores \geq 17). This analysis yielded the same general pattern of negative results as that for the full sample indicating that sensitivity to diet and tartrazine challenge was not affected by level of hyperactivity. Additional post-hoc analysis was also carried out to investigate the ability of a select subgroup of mothers (parents) to observe adverse behavioral effects of tartrazine challenge. For this analysis a subgroup of 13 children was selected based on their being suspected as responsive to the F/KP diet, specifically rated by mothers under non-blind conditions as exhibiting a 12 to 15% reduction in problem behaviors when placed openly on the diet. Analysis of the mothers' behavior ratings for these children during the placebo/challenge period did indicate a significant adverse tartrazine challenge effect. However, no other confirmatory outcome measure, for example teacher ratings, was included in this particular post-hoc analysis.

Study Assessment and Evaluation: The report of positive improvements in the hyperactive childrens' behavior when placed openly on a F/KP elimination diet is of questionable credibility in view of the fact that, as the investigators point out, it was based solely on the mothers' ratings under non-blinded conditions and was not supported either by teacher ratings or by most of the objective psychological tests. The primary analyses of data for the full sample of 20 hyperactive children showed that daily challenges with 5 mg tartrazine for a 2 week period under placebo controlled conditions did not "trigger" any adverse behavioral effects. Special analysis of the study data for the more hyperactive children showed that the level of hyperactivity does not affect the amplitude of response or sensitivity to the effects of diet or tartrazine challenge.

However, when special analysis of only the mothers' ratings during challenge was conducted for a subgroup of children who reportedly exhibited a 12 to 25% behavioral improvement on the open F/KP elimination diet, there was a significant adverse tartrazine effect. However, the Reviewer considers there to be little, if any, confidence in the reliability or biological relevance of this finding, since the reported diet response of these selected children is based exclusively on mothers' ratings which are not considered credible (see comments above), the preparation of one batch of the challenge biscuits may have compromised the study blinding to some unknown extent, and other confirmatory outcome measures (teacher and clinician ratings and objective tests) were not included in this special analysis. Overall, this study provides no credible evidence of any significant adverse effects of daily exposure to 5 mg tartrazine for 2-weeks in hyperactive children.

(Mattes and Gittelman, 1978)

Mattes J and Gittelman-Klein R. A Crossover Study of Artificial Food Colorings in a Hyperkinetic Child. American Journal of Psychiatry, 1978, 135 (No.8): 987-988.

Mattes and Gittelman (1978) designed a placebo controlled double-blind clinical challenge study to maximize the likelihood of demonstrating a food color additive diet effect in one male child (10 years) diagnosed as hyperkinetic and whose parents reported definite behavioral improvement on the Feingold elimination diet and dramatic worsening of behavior, when the diet was violated, with restlessness and irritability lasting from hours to days. The challenge vehicle was a Nutrition Foundation cookie with/without a mix of commonly used artificial colors at a level 1/5 the average daily intake. The cookies, provided by the Nutrition Foundation, were made to be indistinguishable in taste and appearance. The study was conducted in two phases both with the child being continued on the Feingold elimination diet: (1) Dose-Ranging Phase – on each of 2 consecutive weeks, the child was randomly determined to receive either placebo or color cookies in progressively greater numbers each week up to six cookies per day; and (2) Multiple Crossover Challenge Phase – a 10-week double-blind multiple crossover challenge in which the child ate 3 cookies a day for two days each week, Wednesday and Thursday. Within each week the same type cookie was eaten on both days and the type of cookie each week was randomized (coin toss). The outcome measures consisted of daily behavior ratings (Conners brief questionnaire) by the mother and teacher daily during the dose-ranging phase and weekly during the multiple crossover phase. Also, at the end of each week, the child, mother and teacher were asked to guess whether that week's cookies were color or placebo.

Study Results: In the dose-ranging phase the mother's ratings showed a behavioral response to the color cookies (but not the placebo) starting after two cookies and a more exaggerated response after five cookies. The mother's ratings went from a baseline value of zero to a score of 15 (the usual cutoff score for hyperactivity) but restlessness and irritability were the predominant behaviors noted. The teacher's ratings, however, did not show any behavioral changes. Based on the mother's ratings during dose-ranging, three cookies per day were selected as adequate for the multiple crossover challenge phase of the study. In the multiple crossover challenge phase, neither the mother's nor the teacher's behavior ratings detected any adverse effects of challenge. The teacher and child were equally unable to guess reliably the type of cookie used each week (5/10 correct for both), but the mother was able to guess correctly 8/10 weeks (in guessing, the mother focused on irritability and fidgetiness as the main effects of the active/color cookies).

Study Assessment and Evaluation: The reviewer concurs with the authors' conclusion that the

results of this study do not support the contention that artificial food colors are instrumental in inducing significant changes in hyperactive symptomatology in a diagnosed hyperkinetic child even with parental anecdotal reports of marked positive response to the Feingold diet and dramatic behavioral deterioration with diet violation. The reviewer agrees with the investigators' view that the severe reaction reported by the mother, but not the teacher, during the Dose-Ranging phase was apparently not related to the active/color cookies, since this reaction did not occur with the same dosage during the Multiple Crossover phase. The investigators suggested that, since the mothers could correctly guess the type of cookie being used each week during the Multiple Crossover phase of this study, some aspects of the child's behavior may have been affected by the color challenge cookies but that this effect appeared to involve types of behavior, such as irritability, that are not typically associated with hyperkinesis and apparently not detected with the typical rating scales used for hyperactive children. This rationale of the authors is not consistent with the fact that the mother's behavioral ratings, but not the teacher's, did detect behavior responses in the Dose-Ranging phase of this study including irritability and restlessness (fidgetiness) (*see note below). Alternatively, it is possible that the mother was able to correctly guess which cookie was used each week in part because the blinding may not have been completely effective. However, with a single subject being used in this study, it is difficult to extrapolate the significance of this study's results. [*This may have been one of the earliest suggestions by investigators that adverse effects of artificial food colors may possibly involve exaggeration of behaviors, such as irritability, which are not typically associated with childhood hyperactivity. Over subsequent years, this concept evolved into one of the shifts in some clinical studies from a focus targeted on artificial food colors as possible triggers of criteria-specific hyperactive symptomatology to a wider focus on artificial food colors more as provoking general behavioral disturbance (Bateman et al, 2004; McCann et al, 2007; Schab and Trinh, 2004; Weiss et al, 1980).]

(Rose, 1978)

Rose TL. The Functional Relationship Between Artificial Food Colors and Hyperactivity. J Appl Beh Anal, 1978 (Winter), 115 (No.4): 439-446.

Rose (1978) conducted a placebo controlled double-blind challenge study of a single artificial food color, tartrazine (Yellow 5), using a within-subjects design with a sample of hyperactive children (n=2) who were anecdotally reported by parents to be behaviorally responsive to the Feingold/KP elimination diet. Two diagnosed hyperactive children (female; 8 years) diagnosed with hyperactivity and maintained on the F/KP diet for at least 11 months as the apparently exclusive means of behavioral management were recruited for this study. The challenge vehicle was an oatmeal cookie with/without 1.2 mg tartrazine (FDC Yellow 5) (i.e., 0.05 mg/kg bw). The cookies were tested by 2 panels of adults and submitted to other testing verifying that placebo and color challenge cookies could not be differentiated. Throughout the study observers, children and parents were blind to treatment, but the coordinating experimenter was not blinded and neither parents nor children were specifically asked to verify whether they could tell the difference between color and placebo cookies. Procedurally, with the F/KP elimination diet being maintained throughout, the study was conducted over a period of 6 weeks with the following weekly treatment sequence: baseline, placebo treatment, color challenge, placebo treatment, color challenge, placebo treatment. After the 1-week baseline, subjects received one

cookie/day with breakfast throughout the remainder of the study. The active color challenge consisted of a single tartrazine cookie given on only one day during each of the two color challenge weeks. The sole behavioral outcome measure was the daily assessment by trained observers at school of specific behaviors characteristic of the hyperactive syndrome, including on-task duration, out-of-seat duration/frequency, and physical aggression frequency; these behaviors were monitored at approximately 3 hours after cookie ingestion.

Study Results: Visual inspection of the graphed data for each subject showed apparent exaggeration of hyperactive behaviors (increased out-of-seat behavior and decreased on-task behavior) associated with ingestion of the tartrazine containing cookie for both children. A randomization test showed these behavioral changes to be significant treatment related (tartrazine) effects. Treatment related changes in the frequencies of physical aggression were far less conclusive. Dietary infractions (a chocolate bar, tomatoes, a commercial poultry food item) produced similar behavioral changes. When dietary infractions did occur, the parents' observations of their children's behavior were reported to correlate perfectly with the behavioral data obtained by the independent observer. However, there is no information as to whether the parents' observations correlated with the observers behavioral evaluations for the double-blind placebo controlled challenges.

Study Assessment and Evaluation: The data from this study do appear suggestive of a functional relationship between ingestion of tartrazine on the behavior of two hyperactive diet-responsive children based on behavioral evaluations by independent observers in a school environment. However, the absence of any other outcome measure (e.g., parent ratings) to verify the observers' findings and the uncertainty regarding verification of effectiveness of the blinded conditions for the parents and children limits confidence in the reliability of these study results and the use of only 2 children as the study sample makes it difficult to extrapolate the significance of these suggestive findings.

(Williams et al, 1978)

Williams JI, Cram DM, Tausig FT and Webster E. Relative Effects of Drugs and Diet on Hyperactive Behaviors: An Experimental Study. *Pediatrics*, 1978, 61: 811-817.

Williams et al (1978) designed a clinical challenge study to focus on the relative effects of a diet free of artificial food colors, flavors and major preservatives in comparison to stimulant medication in managing hyperactivity in children. The study was conducted as a double-blind challenge trial comparing the effects of stimulant medication versus a mixture of artificial food colors in a heterogeneous group of children with hyperactivity or problem behaviors maintained on a modified Feingold diet [*Feingold's *modified* diet focused more on colors, flavors and preservatives and less on salicylates as substances most likely to trigger the hyperactive response (Feingold, 1975)]. The 26 children (24 male, 2 female) recruited for the study were 6 to 14 years old, clinically assessed as hyperactive* based on parent and teacher reports, and responsive to medication for hyperactivity. [* Data collected during the study indicated that 7 children were apparently not hyperactive. Consequently, the study sample was behaviorally heterogeneous consisting of 19 hyperactive and 7 non-hyperactive children but with problem behaviors responsive to stimulant medication.] Unlike many other challenge studies involving artificial food colors, responsiveness to the Feingold elimination diet was not a criterion for admittance into this study. Two active treatments and two appropriate placebo/vehicle control treatments were used. The active treatments were: (1) chocolate cookies each with a mixture of nine artificial colors at a level of 1/2 the average daily intake of artificial colors (challenge was two

cookies per day) and (2) stimulant medication at dosages used prior to the study (challenge was two pills per day). Specific efforts were made to maintain the double-blind conditions of the study, including use of appropriate placebos, random assignment to treatment, challenge items provided daily by investigators, and having all study personnel and raters blinded to all treatments. Procedurally, the study period covered 14 weeks starting with a 1-week baseline with children on regular diets. During the next 5 weeks, all families were started on the modified Feingold diet which was then maintained throughout the rest of the study period. The primary challenge phase of the study was conducted over the next 4 weeks. On each challenge day subjects received a pairing of cookie (either placebo or color) and medication (either drug or vehicle) treatments. There were 24 possible complete sets of such paired-treatments. Each child was randomly assigned to one of those sets and rotated through the four paired challenge treatments (e.g., color cookie + drug, placebo cookie + drug, color cookie + drug vehicle, placebo cookie + drug vehicle), one paired-treatment each week. Subjects received either two color or placebo cookies and either two drug or placebo pills daily on the first 4 days of each week, i.e. Monday, Tuesday, Wednesday, Thursday). Generally, cookies as well as pills were taken in the morning and afternoon. This primary challenge period was followed by 2 weeks of preliminary data analysis to identify the two best treatment conditions for each child and then finally by an additional 2 weeks during which the children were rechallenged using only the two best treatments. The outcome measures consisted of behavior ratings by parents and teachers on treatment days (Conners 11-item checklist: parents daily, teachers alternate days), at the end of each treatment week (Conners 40-item checklist: parents and teachers), and at beginning and end of study (Conners 96-item checklist: parents only). Least square analyses were conducted for pairwise comparisons of means for checklist ratings.

Study Results: Based on grouped data analyses of the parent and teacher behavior ratings, the overall color challenge effects on the children's behavior were mixed. While the teacher ratings did reveal a significant main treatment effect with the color challenge adversely affecting the children's behavior, there was no confirmation from the parent ratings which found no significant main behavioral effects of the color challenge. In contrast to the inconsistent color challenge effects, both the teacher and parent behavior ratings found clearly significant main effects for stimulant medication in improving behavior, even though 7 of the subjects had such low overall behavior rating scores that they probably were not hyperactive. None of the data were not analyzed separately with and without the non-hyperactive children included. Based on analysis of data for individual subjects and a *criterion challenge response* defined as at least a 33% decrease in rating scores on placebo cookies compared to color cookies, parent ratings identified 3 *criterion responders* and teacher ratings identified 5 *criterion responders*. But, these findings were inconclusive since there was no agreement between the teacher and parent lists of responding subjects. Behavioral ratings were not conducted during the initial baseline period, when the subjects were still on their regular diets. Since no comparative behavioral data was available for children on regular diet and on the modified Feingold diet, no assessment can be made of the extent to which the modified Feingold diet may have affected (improved) the hyperactive/problem behaviors of the test subjects. Also, the results from the final 'rechallenge' phase of testing in this study using the 'two best treatment' were not presented.

Study Assessment and Evaluation: One general comment is a clarification in that the investigators' repeated reference to "diet effects" in presenting and discussing the study results is inaccurate and confusing, since the design of this study did not specifically assess the effects of "diet" itself on behavior. No comparison of behavioral scores (parent or teacher rated behaviors) was made between the baseline behaviors, when subjects were on 'normal' diet, and the study behaviors, when all subjects were maintained on the modified Feingold diet. Rather, this study

tested only the behavioral effects of challenge treatment (stimulant medication and cookies with artificial colors) in children being maintained on the modified Feingold diet throughout the study period. In terms of analysis of the data for the whole study group, while the effects of stimulant medication in improving the behavior of the test subjects were clearly significant, the overall effects of artificial color challenge on behavior were inconsistent. Teacher behavior ratings detected overall significant color challenge effects with worsening of hyperactive/problem behaviors but parent ratings provided no confirmation of any treatment related effects. Also, the results from analysis of data for individual subjects appeared to be inconclusive in that teacher and parent ratings each identified several children (5 by teacher and 3 by parent) as color responders but there was no agreement between parents and teachers as to which children were the responders. The investigators appeared to consider these differences between parent and teacher findings as real rather than perceived based on their view that the teacher ratings were more sensitive than the parent ratings due in part because the children received their color challenges in the morning and at noon and any color effects on behavior would have been more apparent within several hours after challenge during school hours but less apparent by the time the children returned home. This suggestion of an abbreviated time frame for color effects on behavior are not consistent with anecdotal reports by Feingold and parents that children on the Feingold elimination diet (free of artificial colors and flavors) exhibit dramatic behavioral deterioration lasting hours to days after even minor dietary infractions. Several additional limitations to this study were identified by the investigators: (1) behavioral checklists were the only outcome measure used, although two different sources of ratings (parent and teacher) were obtained; (2) the treatment of only 4 days limits confidence in inferring long term treatment effects; and (3) marked variations in rating scores possibly related to the 7 non-hyperactive children. The latter issue of variability could have been addressed by analyzing the parent/teachers ratings for the color challenges with/without the data from the 7 non-hyperactive children. 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 suggestive but inconclusive and not supportive of an effect of artificial colors on hyperactive and problem behaviors in children.

(Conners et al, 1980)

Conners CK, Goyette CH and Newman EB. Dose-Time Effect of Artificial Colors in Hyperactive Children. *Journal of Learning Disabilities*, 1980 (November), 13(No.9): 48-52.

Conners et al (1980) conducted this challenge study to determine whether hyperactive children who appear to react to artificial food colors could show a pharmacologic dose-time effect by using more sensitive laboratory tests and observations than used previously. Nine diagnosed hyperkinetic/attention deficit children (5 male, 4 female; 5-10.5 years), previously reported by teachers and/or parents under non-blind conditions to exhibit marked behavioral improvement on the Feingold elimination diet and to show notable behavioral sensitivity to artificial colors, were recruited for this study. The challenge vehicle was chocolate cookies each with/without 15 mg of a mix of artificial colors (challenge consisted of 2 cookies/day). Procedurally, the study was conducted under double-blind conditions in 2 sessions at 1-2 week intervals, one session using color challenge cookies and the other session placebo cookies. The order in which sessions were

run was randomized for each subject (4 subjects given a color challenge session first and placebo session second, and 5 subjects tested with placebo first and color second. Each session consisted of baseline measurements, followed by a one-time consumption of 2 cookies (total color challenge was 30 mg) and then retesting at 45, 90, 135 and 180 min after challenge. Outcome measures at baseline and each test interval included subjective behavior ratings by the experimenter and objective tests including actometer readings, activity chair measures, and performance of a paired-associate attention/learning task. The paired-associate learning task was selected on the presumption that it would be relatively practice-free from session to session.

Study Results: No significant adverse effects of color challenge (30 mg of mixed colors) on behavioral of diet responsive hyperkinetic/attention deficit children were found for any of the dependent outcome measures. The children's level of activity appeared to increase with ingestion of both color and placebo challenges. The investigators speculate that this normal course of activity could mask any specific effects of color challenge. The paired-associate task showed a marked practice effect across sessions, which appeared to confound detection of any possible color challenge effects.

Study Assessment and Evaluation: In an attempt to explain the negative findings in this study, the investigators suggest 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) which greatly underestimates the more realistic levels of colors consumed by children, the increased levels of activity with ingestion of both color and placebo cookies, 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 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 a significant pharmacological dose-time effect for the 30 mg mixture of artificial food colors in hyperkinetic children.

(Conners, 1980a)

Conners CK. Artificial colors in the diet and disruptive behavior. In R Knights and D Bakker (Eds.) *Treatment of Hyperactive and Learning Disordered Children: Current Research*. Baltimore, University Park Press, 1980: 113-120.

This double crossover challenge study of the behavioral effects of artificial colors was conducted to replicate a prior study by Goyette et al (1978, II) but with a larger sample size of 30 children (assumably, all were diagnosed hyperkinetic). The challenge vehicle was a chocolate cookie with/without a 13 mg mix of all approved artificial food colors (challenge consisted of 2 cookies per day). Efforts to blind the study were inherent in the use of color and placebo cookies that were made to be indistinguishable but no apparent efforts were made to ensure the effectiveness of the blinding for parent and teacher raters. Procedurally, this study was conducted over a period of 10 weeks consisting of an initial 2-week baseline period (regular diet), a 3-week period during which all subjects were place openly (non-blind) on the F/KP elimination diet, and a final 4-week double crossover challenge period during which those children showing a 25% or greater reduction in hyperactive symptoms were maintained on the F/KP diet and randomly assigned to one of two sequences of weekly challenge conditions (color/placebo/color/placebo or

placebo/color/placebo/color). During each challenge week children received either 2 placebo cookies or 2 color cookies, daily. The outcome measures consisted of Conners behavior ratings conducted by the parents daily within 3 hours of ingestion of the challenge cookies at the dinner meal and by the teachers on school days with their ratings based on behaviors exhibited during the first school period of the day.

Study Results: There were no significant adverse behavioral effects of challenge with 26 mg mixed colors administered daily for two alternating 1-week periods in the group of 30 hyperkinetic diet responsive children based on either parent or teacher behavior ratings.

Study Assessment and Evaluation: This study, which was intended as a replication of Goyette et al (1978), detected no significant adverse effects in hyperactive children of daily challenge with 26 mg mixed artificial colors. Since these negative results provided no confirmation of the color challenge effects reported in Goyette et al (1978), the reviewer concurs with the conclusion that the effects reported by Goyette et al (1978) were most likely due to chance and not biologically relevant.

(Swanson and Kinsbourne, 1980)

Swanson JM and Kinsbourne M. Food dyes impair performance of hyperactive children on a laboratory learning test. *Science*, 1980 (March 28), 207: 1485-1487.

Swanson and Kinsbourne noted that a number of earlier studies found no behavioral effects of artificial color challenge, when up to 26 mg of food colors were used as the challenge. Since they suggested that the lack of response in those studies may have been due to an insufficient low level of colors, Swanson and Kinsbourne (1980) designed the present placebo-controlled challenge study to assess the effects of larger challenge doses of artificial food colors in a hospital setting. The investigators tested 40 children (36 boys, 4 girls; average age of 10 years) who were referred to the hospital with problem behaviors suggesting hyperactivity. For 20 of these children the diagnosis of hyperactivity was confirmed (average Conners score of 16.2) and stimulant medication produced a favorable response (*hyperactive set*). The other 20 children were considered not to be hyperactive (average Conners score 12.3, which is below the criterion of 15 for a diagnosis of hyperactivity) and reported to have adverse response to stimulant medication (*non-hyperactive set*). None of the children were previously identified as responsive to the Feingold diet. The challenge item was a capsule containing either 100 mg or 150 mg of a blend of 9 artificial food colors; placebo capsules contained sugar. Ten (10) children from each study group, i.e. hyperactive and non-hyperactive, were randomly assigned to either the 100 mg or the 150 mg color challenge group. All medications were stopped prior to the study. Procedurally, all children were maintained on the Feingold diet for the 3 days prior to testing and also the 2 days during testing. In the morning of each of the 2 test days the children received either one placebo or color capsule (po), with the placebo/color order counterbalanced across subjects. On each day of testing the primary outcome measure, a paired-associate learning test (PAT) was administered 0.5 hour prior to treatment and again at 0.5, 1.5 and 3.5 hours after treatment. An additional outcome measure, the Conners behavior rating, was completed two times on each test day (neither the timing of the rating relative to treatment nor the identity of the rater was specified).

Study Results: Challenge with both 100 and 150 mg of a mixture of artificial food colors significantly impaired paired-associate test performance (decreased attention) relative to placebo treatment but only in the hyperactive set of children. Performance was not affected by color challenge relative to placebo treatment in the non-hyperactive children. The color effect in the

hyperactive children was significant at all post-challenge test intervals (0.5, 1.5 and 3.5 hours post challenge), indicating a rapid onset effect which increased in magnitude over time. Also, there was no significant difference in effect between the 100 mg and 150 mg color challenge levels, indicating no dose-response effect at these levels of treatment. The apparent effect of color challenge on performance of the paired-associate test was not supported by the findings of the Conners behavior ratings which were described as showing no differences between the color challenge and placebo treatment. Since none of the Conners rating data were presented nor were the analyses of these data described, it is assumed that the Conners ratings showed no overall color challenge effects and no effects in either group of children. Also, without the availability of the Conners rating scores it is not known whether maintenance of the children on the Feingold diet was effective in moderating the problem behaviors of either the hyperactive or non-hyperactive group of children during the study.

Study Assessment and Evaluation: The singular significant finding of this study was that challenge with a mixture of artificial food colors (both 100 and 150 mg levels) resulted in a significant deterioration in performance of a paired-associate test in a group of hyperactive children when compared with their placebo control performance. This color effect was not seen in the group of non-hyperactive children. However, the reported color challenge effect in the hyperactive children may be a questionable treatment effect and may possibly be an artifact due to an inexplicably unusual placebo performance 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. Blinding procedures appeared to be limited to the use of sugar in capsules as placebo, random assignment of subjects to challenge dose group, and randomized order of placebo/challenge treatments but it is unclear whether the study personnel were blinded to treatment. Actually, the use of simple sugar capsules as placebo may not have adequately blinded either the children or study personnel to the colored challenge capsules. Also, no procedure was described to verify the blinding. In addition to the paired-associate test, the Conners behavior rating was another outcome measure used in this study and the results from this outcome measure provided no supportive evidence of any behavioral effects of the color challenge. The investigators attempted to dismiss the lack of effect with the Conners ratings by suggesting that the objective laboratory paired-associate learning test is more sensitive than the subjective Conners behavior ratings for documenting adverse effects of color on behavior. This argument seems inconsistent with the fact that these same 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). The absence of any dye-related changes in the Conners ratings in this study suggests that whatever, if any, effect the dyes may have on the PAT performance in the hyperactive children does not include eliciting an overall exaggeration of the hyperactive or other problem behaviors in either group of children. Overall, the questionable effectiveness of the blinding in this study, the absence of supportive evidence of any color related changes based on the Conners ratings, and the inexplicable difference in placebo performances of the paired-associate test by hyperactive and non-hyperactive children lessen confidence in the reliability of the reported color related changes in paired-associate test performance in the hyperactive children. In view of these limitations and caveats the reviewer considers the findings of this study to be inconclusive and to provide no credible support for the proposed association between hyperactivity or other problem behaviors

and artificial food colors.

(Weiss et al, 1980)

Weiss B, Hicks William J, Margen S, Abrams B, Caan B, Citron LJ, Cox C, McKibben J, Ogan D and Schultz S. Behavioral Responses to Artificial Food Colors. Science, 207 (March), 1980: 1487-1489.

Weiss et al (1980) conducted a clinical challenge study to determine if behavioral sensitivity (adverse effects) to artificial food color additives could be demonstrated in a controlled trial. It is notable that diagnosed hyperkinetic children were not the target population for this study. This study used 22 non-hyperactive children with problem behaviors (15 male, 7 female; 2.5 – 7 years), whom parents reported as responsive to open (non-blinded) treatment with an elimination diet that excluded artificial colors and flavors. In the design of this study these investigators were among the first to include parental rating of selected behaviors targeted for each child as one of the primary outcome measures in an effort to maximize detection of behavioral responses to artificial colors that may be specific to individual subjects. The challenge consisted of a soft drink with a 35 mg blend of 7 artificial food colors plus cranberry coloring. Placebo soft drinks contained a combination of caramel/cranberry coloring but no artificial colors. The two drinks were determined to be indistinguishable by sight, smell, taste, or stain color. Throughout the study all children were maintained on a version of the modified Feingold diet that excluded artificial colors, flavors, select spices and extracts, the preservatives BHA and BHT, and for some children designated fruits and vegetables. Procedurally, the study was conducted as a double-blind challenge over a period of 11 weeks with each child serving as its own control. At a specified time on each of the 77 days, each child consumed a bottle of soft drink. On 8 days distributed randomly for each child among weeks 3 through 10 of the study period, each child received the color challenge drink. No parent or member of the study team knew which child was being challenged on any given day. Parental ratings/observations comprised the main outcome measures. Prior to the study the each set of parents selected a 10-item checklist of target behaviors that served as individualized response criteria for their child throughout the 11-week experimental period. The checklist consisted of 7 aversive behaviors associated with dietary infractions and 3 positive (“good”) behaviors. On each day of the study, the parents completed the target behavior rating two times, the first within 3.5 hours of treatment and the second at a later time. Twenty-four hours after each treatment parents also made a global estimate of each target behavior, recorded dietary infractions, kept sleep data, rated the day as a whole, commented on any deviations from daily routine, counted during the day occurrence of any of the 7 aversive behaviors, and completed the 10-item Conners Parent/Teacher questionnaire. The investigators did not consider this a “group” experiment but 22 separate experiments. Accordingly, statistical analyses were conducted on each child’s data separately; no group data analyses were conducted.

Study Results: Only 1 of the 22 test subjects, a 3 year old girl, appeared to show a dramatic adverse response to the color challenge (significant worsening of 5 of the 7 aversive behaviors, the Conners behavior ratings, and all global behavioral measures). During the study, the comments made by the mother of this child showed that she was apparently able to identify correctly five of the eight color challenge days, which the investigators attributed to the mother’s ability to discriminate the response to color. Although various procedures were used to blind the study, there was no indication that the parents or children were asked to verify that they could not tell the difference between the placebo and color challenge drinks. The remaining 21

subjects showed no convincing sensitivity to the color challenge, including one 3-year old male reported to exhibit an inconsistent increase in 2 aversive behaviors primarily on three of the eight challenge days but no treatment related changes in any of the other parental measures including the Conners ratings.

Study Assessment and Evaluation: This study reported that one out of 22 test subjects exhibited a dramatic adverse behavioral response to challenge with a 35 mg mixture of artificial colors. A second subject with only limited and inconsistent behavioral changes showed no convincing sensitivity to the color challenge. The investigators asserted that these data strengthen the evidence that "modest doses of synthetic colors, and perhaps other agents excluded by elimination diets, can provoke disturbed behavior in children.". However, the reviewer considers this conclusion to be clearly overstated. Some perspective must be given to the conclusions to account for the fact that the vast majority of subjects in this study, 21 of the 22 non-hyperkinetic children with problem behaviors selected for their reported positive response to diets free of artificial colors and flavors, did not show any convincing evidence of intolerance or any other type of adverse response to the color challenge. In this context one responder suggests a rare occurrence of adverse effect. Also, although notable efforts were made to maximize detection of treatment related behavioral effects by using individualized targeted behavioral ratings, the conclusions should reflect the fact that confidence in the relevance of the study findings is limited by the fact that the detection of treatment effects was based exclusively on only one source of behavioral assessment, the parents' observations. Use of only parents for assessing behavioral change in the children and not including for example trained observers or teachers does not allow for independent confirmation of any treatment related effects in the home or other settings. In the absence of any other source of outcome measures, it is difficult to assess the reliability of the parental reports of adverse behavioral changes as true treatment effects. Even accepting the parental findings as suggestive of color challenge effects, the more accurate conclusion would be that these data may strengthen the possibility that a small subgroup of children with problem behaviors that reportedly improve on a diet that eliminates artificial colors and flavors may exhibit an intolerance to modest doses of artificial food colors responding with adverse behavioral changes. Assuming some credibility to the parents reported improvements in problem behaviors when the children were placed on an elimination diet (e.g. Feingold), it is notable that 21 out of 22 of these children showed no convincing evidence of a significant sensitivity to the color challenge. This might suggest that, while a very small sub-population of children with problem behaviors may be intolerant to food colors, there may be various foods or food ingredients, other than colors, that are of possibly more significance in provoking or aggravating problem behaviors. In fact, a part of the conclusion by Weiss et al (1980) does state that " colors, and perhaps other agents excluded by elimination diets, can provoke disturbed behavior in children".

(Mattes and Gittelman, 1981)

Mattes J and Gittelman R. Effects of Artificial Food Colorings In Children With Hyperactive Symptoms: A Critical Review and Results of a Controlled Study. Archives of General Psychiatry, 1981, 38:714-718.

Mattes and Gittelman (1981) conducted a double-blind placebo controlled crossover challenge study to assess the behavioral effects of artificial food colors in a group of children heterogeneous for hyperactivity. Several elements were incorporated into the experimental design to maximize the likelihood of demonstrating any behavioral effects of the food colors.

Specifically, (1) the study included only children maintained on the Feingold diet because, as reported by parents, their behavior improved markedly on the diet and deteriorated quickly and dramatically with exposure to artificial food colors, (2) efforts were made to exclude placebo responders, and (3) high dosages of colors were used. This study was conducted using a group of 11 children (6 male, 5 female; 4-13 years) who were heterogeneous for hyperactivity (6 ADHD/ADD and 5 other problem behaviors or history of hyperactivity) and FKP diet responsive. The challenge items were cookies with/without a 13 mg blend of food colors (children were given an increasing number of cookies/day to a maximum of 6/day, i.e. a maximum color challenge of 78 mg). Placebo and color cookies were made to be indistinguishable in taste and appearance (Nutrition Foundation). All study personnel involved in evaluation and care of children were blind to treatment. Also, teacher, parent, psychiatrist and child were asked to guess the type of cookie at the end of each trial week (although not specified, it is assumed this was an additional means of gauging behavioral status and not to verify effectiveness of blinding conditions). Throughout the study, all children were continued on the same Feingold elimination diets they used previously. Procedurally, the study was conducted over a period of 5 weeks starting with a one week baseline. The second study week was a non-blind trial period of daily ingestion of placebo cookies to identify any children reacting adversely to placebo and exclude them from further study (two of 13 children originally recruited for the study were thusly eliminated). The third and fifth weeks of study were the experimental weeks during which the children were randomly assigned to receive either placebo or color cookies daily for an entire week each. Interposed between these two experimental weeks was a one-week (fourth study week) washout period without cookies. Each experimental week consisted of one cookie on the first day (i.e., a color challenge of 13 mg) and an additional cookie each day to a maximum of six cookies on the last two days (i.e., a maximum color challenge of 78 mg/day). Cookies were given at breakfast, lunch and after school. Several subjective and objective outcome measures were used including parent and teacher behavior ratings (Conners rating scale; a special scale developed to assess hyperactive symptoms; global severity of illness); psychiatric evaluation including overall change and a Children's Diagnostic Scale; and a laboratory test of distractibility with a psychologist's rating of behavior during the testing. All observations and testing were conducted during baseline and at the end of placebo week and each experimental week. The distractibility test was given 1.5 hours after ingestion of cookies. To evaluate timing of any behavioral reactions to challenge, teachers and parents also completed a brief Conners rating 3 and 5 days after the initiation of each type of cookie. At end of each experimental week, each child also completed a Brief Conners Scale and rated overall change and global severity.

Study Results: Three of the children failed to ingest the maximum 6 cookies per day. One child simply refused and two children consumed only four cookies due to severe behavior reactions one involving color cookies and the other placebo cookies. Overall, none of the behavioral measures, i.e. ratings, evaluations or laboratory distractibility testing by parents, teachers, children, psychologists, or psychiatrists, showed any significant differences between placebo and artificial color treatment. The absence of treatment related effects was also reflected in the fact that none of the participants guessed beyond chance the type of cookie being used in either treatment week. Repeated analyses of the study data excluding the data for children not diagnosed as hyperactive did not alter the negative results. Based on assessment of the data for individual subjects, the investigators reported that six children showed some difference between placebo and color cookie treatment but that the parents were the only raters who reported noticing this difference. All others (teachers, psychiatrists, psychologists) noted minimal if any differences, providing no confirmation of the responses noted by the parents.

Study Assessment and Evaluation: This double-blind placebo controlled challenge study using a variety of subjective and objective behavior outcome measures was designed specifically to maximize the likelihood of detecting significant adverse behavioral effects of artificial food colors in a heterogeneous group of children with ADHD/ADD or other behavioral problems, which reportedly improved on the Feingold elimination diet and dramatically deteriorated with exposure to artificial food colors. The results of this study showed that no significant adverse behavioral effects were produced by daily challenge to a blend of artificial colors for one week at dosages up to 78 mg per day. The reviewer concurs with investigators' conclusion that the results of this study indicate that artificial food colors do not adversely affect the behavior of school-age children with hyperactivity or other behavioral problems who are claimed by parents to be markedly sensitive to these agents. As noted in other reviews in this report, it may be assumed that there is some level of credibility to the parents reported improvement in problem behaviors when the children were placed on an elimination diet (e.g. Feingold) and dramatic behavioral deterioration with exposure to foods with artificial food colors. This suggested diet effect together with the fact that none of the subjects showed adverse response specifically to artificial colors under controlled challenge conditions 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 children.

(Adams, 1981)

Adams W. Lack of Behavioral Effects From Feingold Diet Violations. Perceptual and Motor Skills, 1981, 52: 307-313.

Adams (1981) conducted a clinical double-blind, placebo-controlled challenge trial to investigate whether an *infrac*tion of the Feingold diet exaggerates behaviors often associated with hyperactivity in children. To minimize equivocal findings, this trial was designed to: (1) select hyperactive subjects who were reported to be behaviorally responsive to the Feingold diet and sensitive to violations of the diet and (2) evaluate treatment effects using primarily objective measures of activity, memory, attention and motor skills, in addition to parental ratings of behavioral change. A total of 18 hyperactive children were recruited for this trial (15 male/3 female, mean age of 7.6 years ranging from 4.4 to 11.6 years). The parents agreed that a positive diet effect was detectable in their child for at least three months and subjectively reported to have witnessed a prior negative effect following a diet violation using substances the same as or similar to those used as "challenges" in this controlled trial. The challenge item ("artificial snack") consisted of a chocolate cupcake with icing and a glass of lemonade both made from commercial mixes to which additional artificial red and yellow colors and artificial vanilla flavor were added. While the investigator stated that each serving of the "artificial snack" contained a total of 26.3 mg artificial food colors, only red (#3; #40) and yellow (#5; #7) colors were identified as being included. (There was a discrepancy in the publication's Table 2 between the stated total content per serving as being 26.3 mg and the total amount of itemized colors used which was 18.8 mg.) The amount of added artificial vanilla flavor was not specified and the other ingredients in the commercial mixes were not identified. The placebo ("natural snack") iced chocolate cupcake and lemonade were made with color/flavor-free natural ingredients consistent with the Feingold diet. While the parents were aware that one snack was "artificial" and the other "natural", neither the parents nor the observer knew which snack was given. However, the effectiveness of this blinding of the parents was not verified. Throughout the study, all children were continued on the same Feingold elimination diet they used previously.

Procedurally, each child was tested on two separate days with the placebo “natural snack” being given on one day and the challenge “artificial snack” on the other. The interval between test days was not specified. The children were randomly assigned to the placebo/challenge (n=9) or the challenge/placebo treatment order (n=9). On the first test day each child was given the scheduled snack 3 to 4 hours prior to the actual testing session. At the conclusion of that test session, the child was given an appointment for the second test session along with the snack for that session. In each test session the outcome measures consisted primarily of a battery of objective tests assessing overall activity level (activity room with multiple toys and games), fine-motor skill (drawing, handwriting), gross-motor skill (hopping, ball bouncing, bean-bag throwing), short-term memory (numerical and visual), and language (picture vocabulary). On each test day the parents also provided a checklist rating of their child’s observed behavioral changes during the 3 to 4 hour period after the snack was consumed (no specific parental questionnaire was described). Within-groups analyses of variance and Chi-square analyses were performed. Data for individual subjects were not analyzed.

Study Results: No significant group differences between placebo and the “artificial snack” challenge treatments were found with any of the battery of objective tests or with the parental rating of changes in behavior. Also, no significant interaction effects, including age-related changes, were noted. 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.

Study Assessment and Evaluation: Hyperactive children were specifically selected for this study based on their parents’ anecdotal reports of notable behavioral improvement on the Feingold diet (eliminating artificial food colors, flavors, and natural salicylates) and behavioral deterioration with violations of that diet. Despite the very select nature of this group of children, an infraction of the Feingold diet under controlled clinical conditions did not produce any overall significant deterioration in a variety of objectively measured behaviors often associated with hyperactivity and did not result in any negative changes in behavior detectable by the parents. The investigator considered and dismissed two possible reasons for the absence of significant group treatment effects in this study, i.e. age and amount of color in the “artificial snack” challenge. The investigator appeared to consider it unlikely that more pronounced effects might have been noted in this study if younger children had been tested, since no age-related interactions were found in the analysis of the data. Even though the total amount of food color used in the artificial challenge (26.3 mg) was somewhat lower than the average daily intake of colors for children (as estimated at the time of this study), the parents in this study assured the investigator that their children would show adverse behavioral changes if only part of the “artificial snack” challenge were consumed. It also appears that this trial focused more on verifying the possible adverse effects of an *infraction* to the Feingold diet, rather than specifically the possible adverse effects of artificial colors. While the colors represented a principle component of the “artificial snack”, the iced cupcake/lemonade challenge contained added artificial vanilla flavor and were made from commercial mixes which undoubtedly contained other unspecified ingredients that were also considered *infractions* of the Feingold diet. An additional factor that may affect the results of a controlled challenge trial is the blinding of the study participants, since ineffective blinding may introduce an unintentional bias in behavioral assessments. 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 adversely affected the credibility of this trial’s results.

Overall, the primary finding from this trial was that no significant overall adverse behavioral

effects in a group of hyperactive children resulted from a single *infractio*n 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 (iced chocolate cupcake and lemonade) used as the dietary *infractio*n 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 some negative behavioral effects produced by some artificial food substances or other food components or ingredients in some susceptible children, it does lend support to the views that, if diet is related in some way to problem behaviors, this effect is (1) 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, (2) involves fewer children than originally postulated, and (3) has a less dramatic and pervasive effect on attentional and motor performance than originally believed.

(Spring et al, 1981)

Spring C, Vermeersch J, Blunden D and Sterling H. Case Studies of Effects of Artificial Food Colors on Hyperactivity. The Journal of Special Education, 1981, 15 (Number 3): 361-372.

Spring et al (1981) conducted a double-blind, placebo-controlled double-crossover clinical trial to test the Feingold hypothesis that artificial food colors cause hyperactivity in some children. To maximize the probability of demonstrating an unequivocal causal relationship between food colors and hyperactivity, the investigators recruited only children who were already on the Feingold diet and 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. Six male children (8 to 13 years), all considered hyperactive and several with learning problems and neurological signs, were included in the trial. Although no mention was made of a formal diagnosis of hyperactivity, mothers used a hyperactivity scale and rated their child's behavior before starting the Feingold diet at scores ranging from 23 to 27 and after starting the Feingold diet at scores ranging from 12 to 15. This rating of behavior before the diet was based on the mothers' recollections, since the approximate time before this study that these children were on the Feingold diet ranged from 7 to 32 months. The test materials were chocolate cookies (Nutrition Foundation). Placebo (control) cookies contained no artificial colors. Each active challenge cookie contained a 13 mg mix of all eight approved artificial food colors and, since subjects received two cookies each test day, the total daily challenge was 26 mg. A pilot study with college students confirmed that the placebo and challenge cookies could not be discriminated by sight or taste. The Feingold diet was continued throughout the 8-week primary trial and the subsequent 6-week replication procedure. Procedurally, the initial 2 weeks of the primary trial were baseline with no cookies administered but hyperactivity ratings taken as scheduled (see below) for both weeks. The subsequent 6 weeks constituted the primary experimental period during which two cookies were given daily on Tuesdays, Wednesdays and Thursdays of each week, one cookie before school and one cookie after school. Active challenge cookies only were given during two of these experimental weeks and placebo cookies only during the other four experimental weeks. The children were randomly assigned to one of 2 groups. The three children in Group 1 received the active challenge cookies during experimental

weeks 1 and 4, and the three children in Group 2 were challenged during experimental weeks 3 and 6. On each day that a child ate cookies, 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 with an internal assessment and concluded was a valid measure of hyperactive behavior. However, since this was a modified Conners scale, the score level that indicated hyperactivity was unclear. The second outcome measure was a global behavior judgment whereby the respondent (mother or teacher) was to guess whether the child had received the active challenge or the placebo based on the respondent's assessment of the child's general behavior on that day. Both ratings were collected by telephone each day but it is not clear whether reporting times for mothers and teachers were standardized to a defined period of time after ingestion of the before-school and after-school cookies. Diet diaries were also collected on test days to assess nutrient intakes on the Feingold diet but differences appeared to reflect individual food preferences. Each of the 6 subjects constituted a single subject experiment. The hyperactivity ratings data were analyzed by computing point-biserial correlations separately for mothers' and teachers' ratings. The global behavior judgment (daily guesses) data were analyzed with Fisher exact-probability tests. Analysis of the grouped data was not conducted. Subsequent to the original trial, a replication procedure was carried out to confirm any treatment related findings. This replication procedure was similar to the original trial, except that baseline lasted only 1 week, cookies were given for 5 weeks with active challenge cookies being given during treatment weeks 2 and 4, and only the mother's rating data (hyperactivity rating and global behavior judgment) were collected.

Study Results: When the data were analyzed with both active challenge periods combined, the global behavior judgment measure in which mothers and teachers guessed which treatment their children received revealed that significantly accurate guesses were made only by one subject's (Subject E) teacher. The guesses of Subject E's mother, as well as the guesses of the remaining subjects' mothers and teachers were not significantly accurate. Analyses of the daily hyperactivity ratings across both challenge periods showed Subject E's mother and teacher ratings of increased hyperactive behaviors to be significantly correlated with active challenge (although, as noted below in the *Study Assessment and Evaluation* section, when the rating data were viewed for each week, the ratings by both mother and teacher were found to be inconsistent between challenge periods and the raters did not agree on which challenge period the change in behavior occurred). Additionally, the overall hyperactivity ratings of Subject A's mother, but not his teacher, were also significantly correlated with color challenge. Data for the remaining subjects indicated little relationship between overall daily hyperactivity ratings and daily treatment.

For a more in depth assessment, the hyperactivity ratings data for Subjects A and E were viewed separately for each week of the study period (these data presented graphically in Figure 2 of Spring et al, 1981). Both Subject A and E were challenged during experimental week 3 (first challenge period) and week 6 (second challenge period). With weeks viewed separately the ratings of Subject A's mother inconsistently showed a peak increase in adverse behaviors during the first challenge period but not during the second challenge period. Furthermore, it was revealed that during the first challenge period a home event occurred that may have influenced the mother's rating and, consequently, the investigators considered it likely that the relationship between the peak increase in adverse behaviors of Subject A and the first color challenge may have been coincidental rather than causal. The weekly hyperactivity scores for Subject E showed, what the investigators viewed as, peaks in the teacher's ratings during both challenge periods, which coincided with a peak in this subject's mother's ratings but only during the

second challenge period. Based on their assessment of these data, the investigators considered it reasonable to (tentatively) regard the positive relationship between Subject E's diet challenges and his increase in adverse behavior as causal. (As will be discussed in the *Study Assessment and Evaluation* section below, this reviewer considers that this interpretation of the study data does not reflect an adequate assessment of the mean weekly hyperactivity ratings for Subject E.)

Acknowledging the possibility of a coincidental relationship between food colors and hyperactivity for Subject E, the investigators conducted a replication procedure with this subject. The replication procedure, based only on the mother's daily hyperactivity ratings and global behavior judgment (daily guesses about treatment), failed to confirm any significant effect of the active (color) challenge for Subject E.

Study Assessment and Evaluation:

In the analyses of the mothers' and teachers' daily hyperactivity ratings and global behavior judgments (guesses about treatment) with data for both active challenge periods combined, only 2 (Subjects A and E) of the 6 reportedly diet-sensitive subjects initially appeared to show any indication of an adverse treatment related effect. For Subject A, with both active challenge weeks combined the singular significant indication of a possible treatment effect was in the mother's increased hyperactivity ratings. However, when these data were viewed for each week individually, the mother's hyperactivity ratings were actually shown to be inconsistently increased only during the first active challenge period but not during the second active challenge period. Furthermore, due to an undisclosed home event the apparent association between the behavioral changes and color challenge reported for Subject A was considered coincidental and not causal, thereby dismissing the presence of any treatment related effects for Subject A. For Subject E, with both active challenge weeks combined both the mother and teacher reported significantly increased adverse behaviors in the daily hyperactivity ratings but only the teacher's global behavior judgment (guess) about treatment was significantly accurate. When the hyperactivity ratings data for Subject E was viewed separately for each week of the study period, the ratings by both mother and teacher were found to be inconsistent between challenge periods and the raters did not agree on which challenge period the change in behavior occurred. The mother's ratings showed a peak in adverse behaviors during the second challenge period but no changes in the ratings during the first challenge period. Contrarily, Subject E's teacher's ratings showed a peak in adverse behaviors during the first challenge period but, in the opinion of this reviewer, showed no adverse changes in rated behavior during the second challenge period. The latter opinion, which conflicts with the investigators' interpretation, is based on the fact that the teacher's hyperactivity rating score (approximately 8) for Subject E during the second challenge period was lower than the placebo week 2 hyperactivity score (approximately 10). Consequently, this reviewer does not consider the teacher rating during the second challenge period to indicate a treatment related change. The absence of any treatment effects on the behavior of Subject E was confirmed in the replication procedure. In assessing the overall findings of this study it should also be noted that a reasonable effort was made to confirm the blinding of the challenge and, although the effectiveness of blinding was not specifically verified for parents, teachers or children, there was no indication that the participants held negative or biased attitudes about the Feingold hypothesis or that the credibility of the outcome measures was in any way affected.

Overall, in deference to the investigators' conclusion that this study (primarily based on the primary trial data for Subject E) provides equivocal support for Feingold's hypothesis, 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 exposure to

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.

(trials conducted from 1982 to 2007)

(Salamy et al, 1982)

Salamy J, Shucard D, Alexander H, Peterson D, Braud L. Physiological changes in hyperactive children following the ingestion of food additives. *Int J Neurosci.* 1982 (May), 16(3-4):241-6.

Salamy et al (1982) conducted a study to explore a method for assessing dietary interventions based on each individual's response irrespective of group performance. Their approach used a single case experimental design based on physiological measures of autonomic nervous system (heart rate or HR) and central nervous system (electroencephalogram or EEG) activity for evaluating the effects of food additive infractions of the Feingold elimination diet in hyperactive children. The study was conducted on a total of 8 children (5-11 years). Four of the children were previously diagnosed hyperactive and reported to be responsive to the Feingold diet. The other 4 subjects were non-hyperactive sibling controls. All children were currently being maintained on the Feingold diet and were continued on this diet throughout this study. The challenge item containing artificial additives was a lemon flavored commercial drink colored with Red 40 (amounts of additives including Red 40 were not specified). The placebo was a home-made lemonade drink colored with beet juice. Procedurally, the double-blind placebo controlled randomized crossover study was conducted on two separate test days one week apart. On each test day baseline physiological recordings (HR, EEG) were taken for 10 minutes. Immediately after baseline, children were given their pre-assigned placebo or color drinks orally through a straw from an opaque container and served along with popcorn. Neither the investigators nor the children knew which drink was given on either test day. Following administration of the drink, children played for one hour, after which physiological measurements were again recorded for 10 minutes. (The parents of two hyperactive children reported marked behavioral changes after the children left the laboratory. Even though it was not stated which treatment (placebo or color) these children had just received, the investigators gave these children an additional day of testing to determine whether a post treatment period longer than one hour may be necessary to observe treatment effects. In this retest physiological recordings were obtained four hours after a double dose of the color drink, but the double-blind design was abandoned for this additional test, confounding the reliability of these retest results.)

Study Results: Based on measures of HR and EEG, there was no evidence of significant physiologic changes (EEG or HR) specifically attributable to the color/artificial additives treatment in either hyperactive or control subjects. The most striking finding was a relative absence of physiological changes seen in the control subjects but an abundance of physiologic changes in the hyperactive children under both placebo and color/additive treatment conditions. The hyperactive children appeared to be influenced by the placebo as readily as they were by the drink containing color/additives. Thus there appeared to be a difference between groups (hyperactive vs sibling control) but not between treatment conditions (color/additives vs placebo). These findings suggest that the hyperactive children compared with controls were simply more labile physiologically, responding generally to the experimental situation.

Study Assessment and Evaluation: The data showed no reliable evidence of significant physiologic changes (EEG or HR) specifically attributable to the color/artificial additives

treatment in any of the 4 hyperactive or 4 control children. However, while suggestive, this negative finding should be considered inconclusive. One of the more apparent reservations about the reliability of this negative finding is that no information was given in this study about the levels of Red 40 or other artificial additives present in the active treatment. The possibility that the levels of color/additives used may have been too low to elicit any physiologic response cannot be dismissed. Additionally, confidence in these results is also limited by the fact that only one recording session was made with each experimental treatment, i.e. placebo and color/artificial additives, for each test subject. Variability in physiologic responses between and within subject to any treatment is expected. Confidence in the detection of real treatment related physiologic effects or in the determination of no treatment related effects relies on repeated testing of the experimental treatment to replicate the physiological response or verify the absence of a treatment related effect. Consequently, the inconclusive findings from this study do not provide sufficient evidence to conclude that there were no treatment related physiologic effects of challenge with Red 40/artificial additives in hyperactive or control children.

(Thorley, 1984)

Thorley G. Pilot study to assess behavioural and cognitive effects of artificial food colours in a group of retarded children. *Dev Med Child Neurol*, 1984 (February), 26(1):56-61.

Based in part on Thorley's views (1980) that some clinical studies do suggest that subgroups of hyperactive children may benefit most from additive-free diets (Goyette 1978; Williams et al ,1978; Swanson and Kinsbourne, 1980), that adverse effects of artificial colors are more apparent at higher, than lower, doses (Swanson and Kinsbourne, 1980) and that only small numbers of "normal" children, i.e. w/o psychiatric disorder, show adverse behavioral effects after color challenge (Rose 1978, Weiss 1980), Thorley (1980) conducted a clinical challenge study designed to maximize detection of behavioral and cognitive response to color challenge. The following features were incorporated into this study: (1) use of high challenge dose levels, i.e. 2 times the estimated daily intake; (2) use of an optimum post-challenge time for testing; (3) use of a 2-week washout period and no cross-over challenges to minimize the confound of long-lasting behavioral effects of colors; (4) use of sensitive measures; (5) use of a residential setting to allow control of study conditions; and (6) use of a relatively homogenous population with all children intellectually retarded. The study was conducted in a residential school using 10 intellectually retarded children (8 male, 2 female; 12 years average age) exhibiting inattentive/hyperactive behavior. The children were not previously identified as diet responders. The challenge material was a cocoa drink with/without a 91.8 mg blend of 16 approved artificial food colors (UK) (twice the estimated daily intake). Although a placebo control was used and all participants were blinded to challenge (children, teachers, care staff, and psychologist), there was no determination made of the effectiveness of the blinding procedures. Procedurally, the experimental design was a placebo controlled double blind challenge study (without cross-over) conducted over a period of 28 days with children maintained on an elimination diet (modified Feingold diet) free of all colors, flavors and preservatives throughout the study. The initial 14 days of the study was a "washout period" during which the elimination diet was started and children were 'habituated' to the placebo cocoa drink (assumed this was given daily during the "washout period"). This was followed by a 14 day "challenge period" in which 2 randomly selected children at a time received the color challenge drink for 2 consecutive days at 9:30 AM and all other children received the placebo cocoa drink. Outcome measures consisted of multiple

subjective and objective testing, including: (1) teacher behavior ratings in morning (Conners Teacher Scale and a Devised Individual Rating Scale of 5 most problematic behaviors); (2) Care Staff behavior rating (Conners Parent Scale) at meal-time and recreation times; and (3) Psychologist psychometric testing one hour after challenge (Porteus Mazes for visuomotor skills and Paired Associates learning test for short-term verbal memory) and actometer motor activity. The data for each measure were simply summed to arrive at group scores for the baseline condition (2 weeks prior to challenge) and the color challenge condition (2 days of color challenge). The two sets of data for each parameter were simply compared using uncorrected multiple t-testing.

Study Results: Although the study was designed to maximize detection of any behavioral or cognitive response to artificial color challenge, there were no significant treatment related effects found for any behavioral or psychometric test measure in a group of intellectually retarded children at a high challenge dosage of 91.8 mg of artificial food colors, which is twice the estimated daily intake. There was no information presented to determine whether the elimination diet, relative to the regular diet, may have affected the disruptive behavior of the children.

Study Assessment and Evaluation: Since no convincing adverse effect of artificial colors was found in a study designed to maximize detection of behavioral and cognitive responses to challenge with artificial food colors, the Reviewer agrees with the investigator's conclusion that this study seems to indicate that it would be unlikely that retarded children as a whole would show gross adverse effects to artificial food colors. 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, there appeared to be no apparent effect of these factors on the study results.

(Egger et al, 1985)

Egger J, Carter CM, Graham PJ, Gumley D and Soothill JF. Controlled trial of oligoantigenic treatment in the hyperkinetic syndrome. Lancet. 1985 (Mar 9), 1(8428):540-5

Based on the possibility that food allergy may play a role in causing or aggravating the hyperkinetic syndrome in children, Egger et al (1985) conducted one of the first multistage studies to treat overactive (hyperactive) children with a diet that contains few varieties of foods (oligoantigenic diet), to identify in open trial possible provoking foods in those children with improved behavior, and then to test the effects of those presumptive provoking foods in a randomized, placebo-controlled, crossover challenge trial. The subjects in this study were atypical hyperactive children, either diagnosed hyperkinetic or exhibiting problem behaviors including overactivity, and many with neurological disorders, allergic conditions, or other associated physical conditions. All subjects were in a special clinic to assess and treat overactive children by dietary means. Of the 76 children placed on an open (non-blinded) oligoantigenic diet, 62 (82%) were found to be "food intolerant" with noted improved behaviors on the diet. Thirty-one of these "food intolerant" children (23 males, 8 females; average age 7 years) were included in open re-introduction trials (one test food per week) to identify provoking foods and 28 completed the double-blind placebo controlled challenge trials with each child being challenged with one of their provoking foods. In the open re-introduction all children reacted to more than one food but the most common food ingredients causing reactions were benzoic acid and tartrazine but no child was sensitive to these food additives alone. The varieties of food used as challenge items in the double-blind trials included cow milk, orange juice, wheat, tinned food,

tartrazine (150mg) in capsule, or benzoic acid (150mg) in capsule (8 subjects received either tartrazine or benzoic acid). Placebo items, respectively, included soya, sheep milk, or goat milk; blackcurrant, apricot or pineapple juice; rice or oats; and ascorbic or calcium tartrate. Staff members verified no difference in taste between active and placebo treatments and all study participants were blind to the order of challenge, but no verification of blinding effectiveness for the families was carried out. Procedurally, the double-blind challenge phase of this study was conducted over a period of 4 – 6 weeks during which children were maintained on their individual accepted oligoantigenic diet. Each child was given either his/her incriminated food item or placebo for 1 to 2 weeks and an additional 1 to 2 weeks with the opposite treatment; there were at least 2 weeks of washout between treatments. The order in which each child received the incriminated food or placebo was randomly determined. The outcome measures included: (1) parent behavior ratings completed daily (Conners abbreviated scale); (2) parents and clinician made overall behavior assessments at end of each treatment period; and (3) psychologist conducted overall behavior assessments and psychological testing at end of each treatment period (included actometer measures, matching familiar figures, Porteus maze, and intelligence assessments – note that not all tests were done by all children). (Skin prick testing and serum Ig E level determinations were made during the initial open testing of the oligoantigenic diet and not during the challenge phase of testing).

Study Results: In double blind challenge, 'overall behavior assessments' by parents, psychologist and clinician (all $p < .001$) linked better behavior with the placebo challenge significantly more often than with the active food challenge. No significant order effects occurred. Parent behavior ratings using the Conners scale showed similar trends with active provoking food challenge eliciting more hyperactive behavior than placebo ($p < .01$). However, while there were no significant order effects for the parent ratings, data analyses were complicated by a significant treatment order interaction involving the placebo values for the two treatment orders. In contrast to the behavioral ratings/assessments, none of the objective psychological tests (psychologist ratings, actometer measures, match familiar figures, and Porteus maze) showed any significant differences between active provoking food challenge and placebo, although non-significant trends did generally indicate placebo behavior better than active challenge. Atopy status did not differ between the diet responders and non-diet responders.

Study Assessment and Evaluation: : In open trials of the oligoantigenic (few foods) diet 62 out of 76 (82%) overactive/atypical hyperkinetic children appeared to show improved behaviors, suggesting possible food intolerance. Atopy (allergic sensitivity) was not different between the diet responders and non-responders, but IgE levels were higher for the diet responders. Multiple provoking food items were identified in open trials. All children appeared to react to more than one food and the most common food ingredients causing reactions were benzoic acid and tartrazine, but no child was sensitive to these food items alone. Select incriminated food items were used to test 28 responders under double-blind challenge conditions. Overall assessments by multiple sources including parents, psychologist and clinician, linked various provocative food items with increased hyperkinetic/overactive behaviors in children and the parents' daily behavior ratings also indicated the same association. In the presentation of results graphic representation of some of the behavior data indicates that several children (approximately 6) did not respond to the provoking food challenge. However, the investigators did not specify what provoking food items these 'non responding' children received. So it is unclear whether any of these "non-responders" were among the 8 children challenged double-blind with either tartrazine or benzoic acid. Also, there appeared to be an inexplicable but not significant difference in placebo values between the treatment order P/A (lower placebo values) and the treatment order

A/P (higher placebo values). However, this appeared to have no effect on the challenge related behavioral effects. In contrast to the subjective measures, a battery of objective psychological tests, including an actometer measure of motor activity, showed no confirmation of any provoking food challenge effect on hyperactive behaviors. 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 and preservatives.

(David, 1987)

David TJ. Reactions to dietary tartrazine. Archives of Disease in Childhood, 1987, 62: 119-122.

David (1987) conducted a double-blind challenge study of selected children in a hospital setting to verify observations by parents of a definite history of an immediate adverse behavioral reaction (within two hours) to a food additive, particularly tartrazine and benzoic acid. This study used 24 children (19 male, 5 female; 2-12 years) with problem behaviors related to hyperactivity who were maintained on elimination diets that avoided food additives and any other suspect foods. Although the extent to which the elimination diets diminished problem behaviors appeared to be variable among the children, all parents reported the children to exhibit obvious adverse behavioral reaction within two hours of any lapse of the elimination diet. For all subjects parents reported tartrazine to be the additive that caused the most severe adverse reaction; benzoic acid was reported to cause similar adverse effects but in fewer subjects. Twelve of these subjects were inpatients in the general pediatric ward and 12 came to the ward as outpatients. The challenge items consisted of either orange juice or blackcurrant (Ribena) drinks with/without 50 mg and 250 mg tartrazine (Yellow 5) and separate drinks with/without 50 mg and 250 mg benzoic acid. All drinks (in this study) were taken through a straw because of the characteristic staining of the skin around the mouth after drinking solutions containing tartrazine. The investigator noted the difficulty of disguising tartrazine, particularly at high concentrations which give a characteristic appearance to food and drink that is quite obvious even in highly colored food or drink. The investigator considered capsules as not suitable because of the obvious bright color of those containing tartrazine. Parents of the outpatients knew on which days challenges were to be performed, but for the inpatients neither the parents nor the nursing staff knew on which days the challenges were to be performed. Procedurally, all children were maintained on their special diets throughout the study. Each child was given the placebo vehicle drink daily throughout the period of admission. The duration of this placebo treatment was not specified. On the first experimental day a member of the medical staff gave each child a challenge drink with 50 mg tartrazine and behavior was monitored for at least two hours. A second challenge drink with 250 mg tartrazine was given at least two hours after the first

challenge and again behavior was monitored for at least two hours after treatment. It is not clear whether placebo treatments were also given on challenge days. Subsequently, but on a separate day, all subjects were challenged with the two dosages of benzoic acid drinks following the same procedure as was used for the tartrazine challenges. It is not clear how many days were allowed between tartrazine and benzoic acid treatments and whether placebo treatments were given on those intervening days. 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 each child being observed after placebo treatment and active challenges by a parent and by the nursing staff, each of whom were asked to keep a timed record of any change in the child's behavior. No specific or structured scoring system was used and the duration of observation was not specified. No procedures for statistical analysis of the data were described.

Study Results: No child (inpatient or outpatient) was reported to show any change in behavior as noted either by the parents or the nursing staff after administration of placebo or a single oral treatment with 50 mg or 250 mg of either tartrazine or benzoic acid. However, only a summary description of these findings were presented. No itemized data were presented and no data analyses reported.

Study Assessment and Evaluation: This double blind challenge study using rather high dosages of 50 and 250 mg tartrazine and benzoic acid did not appear to produce any observable adverse behavioral reactions in children who were reported to have a definite history of immediate adverse behavioral reactions to these food additives. However, several prominent limitations and weaknesses in the design and conduct of this study should be noted including the facts that the measure of behavioral change was based solely on unstructured observations by parents and nursing staff, that randomized treatment conditions were not used, that half of the parents were not blind to active challenge days, and that itemized observation data were neither presented nor statistically analyzed. These factors raise questions about the sensitivity and reliability of this study in detecting more than overt treatment related effects. Within the context of these caveats this study, even at high challenge dosages of tartrazine and benzoic acid, provides no support for the parents' anecdotal reports of immediate and obvious adverse behavioral reactions specifically attributable to these food additives. Since all of the children in this study were on food elimination diets that avoided a variety of food items, it may be possible that the previous parental reports of obvious adverse behavioral responses in these children may have been associated with an intolerance to food items other than tartrazine and benzoic acid. However, this study cannot exclude the possibility that there may be some subgroup of children who have more specific or subtle behavioral effects from ingestion of additives such as tartrazine and benzoic acid.

(Rowe 1988)

Rowe KS. Synthetic food colourings and 'hyperactivity': a double-blind crossover study. Aust Paediatr J, 1988 (April), 24(2):143-7.

Rowe (1988) conducted a two-phase clinical study designed (Phase I) to identify a group of children who were suspected hyperactive and reportedly improved in an open trial of the Feingold diet and (Phase II) to assess the relationship between certain behavioral characteristics and ingestion of two commonly implicated artificial food colors, tartrazine (Yellow 5) and red carmoisine. In Phase I, 55 heterogeneous children, both with and without suspected "hyperactivity", were tested in an open (non-blinded) trial of the Feingold diet. While 40 of those children (72%) appeared to show behavioral improvement on the diet (26 due to a possible placebo effect), only 14 (25%) were suspected of reacting adversely to the ingestion of foods

containing additives, particularly the red and yellow artificial food colors. From this latter group of suspected reactors 9 children (7 males, 2 females; 3-15 years) were selected for Phase II double-blind testing. The challenge items were colorless capsules with or without 50 mg of either tartrazine (Yellow 5) or red carmoisine in an inner (capsule) surrounded by lactose. The placebo inner capsule contained lactose. All children were maintained on their Phase I additive-free diets throughout the study. Procedurally, one capsule was given to each subject daily at breakfast for the entire study period of 126 days (18 weeks). After variable placebo lead-in periods of 3, 4 or 5 weeks, tartrazine and carmoisine capsules were each given daily for one week on 2 separate occasions (i.e., a total exposure of 2 weeks for each color) with placebo washout periods of 2 or 3 weeks between each week of color challenge. The sequence of color challenge varied for each subject (assumed randomly). Parents, subjects and the data collector were blind to this challenge sequence. The behavioral outcome measure consisted only of a daily behavior rating completed by the parents, using a "frequent symptoms" 8-item checklist. (Attempts to obtain teacher behavior ratings were incomplete and did not provide a source of corroboration for any treatment effects.) The "frequent symptoms" checklist was compiled from behaviors commonly reported by parents as being associated with exposure to foods containing artificial colors. Data for each child were analyzed separately.

Study Results: One of the 9 children recruited for this study did not complete the challenge phase of testing. Two of the remaining 8 children who were heterogeneous for hyperactivity, claimed to be responsive to the Feingold diet, and suspected of reacting adversely to foods with synthetic additives (commonly red/yellow colors) were shown by parental ratings ('frequent symptom' behaviors) to show significant adverse behavioral responses to double-blind challenge with either 50 mg tartrazine or 50 mg carmoisine. One color responder was a 7-year old non-hyperactive female and the other was a 12-year old hyperactive male. Both responded with increased activity, irritability, low frustration tolerance, sleep disturbance and short attention span. Their responses began within hours after initiating each color challenge treatment and continued until 3-4 days (female) and 3.5 weeks (male) after the last of each color challenge treatment. Both children were atopic, but their symptoms of asthma and allergic rhinitis were not exacerbated by the food colors. The data for the remaining 6 children yielded results which were neither consistent nor statistically significant.

Study Assessment and Evaluation: Two (one hyperactive, one non-hyperactive) out of 8 children heterogeneous for hyperactivity with problem behaviors, responsive to the open trial with the Feingold diet and claimed to be behaviorally reactive to foods with artificial additives (commonly red/yellow colors) were shown by parental ratings ('frequent symptom' behaviors) to respond adversely to controlled double-blind challenge with either 50 mg tartrazine or 50 mg carmoisine. 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 (teacher ratings were incomplete) and by the lack of attempt to verify the effectiveness of the blind for parents and children. At best, the study's finding of 2 possible color responders may suggest that a poorly characterized small subpopulation of children with behavioral problems may be intolerant to certain artificial food colors. Since the six children who did not respond to the controlled color challenges were reported in Phase I to have improved on the Feingold elimination diet and to have reacted to foods containing artificial colors, it may be possible that these Phase I behavioral responses may have been associated with some intolerance to food items/components eliminated from the Feingold diet other than artificial colors. Interestingly, the investigator points out that only one of these two possible color responders originally showed any signs of "attention deficit" behavior and that the common features of behavior associated with ingestion of food colors, as described by parents, emphasized extreme "irritability", "restlessness" and "sleep disturbance"

rather than those associated with “attention deficits” (implying rating scales designed to focus on hyperactivity related behaviors may not be sensitive to the range of other behaviors more typically exhibited by color responders). The author viewed this as suggesting that the behavioral dimensions associated with the ingestion of synthetic food colors require careful identification, since the inclusion of children in clinical trials on the basis of ‘attention deficit’ alone may miss some color reactors. This viewpoint effectively broadens the suggested behavioral impact of artificial food colors 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. Within this context, while this study does not support a causal association between artificial colors and ‘hyperkinetic disorder’ (ADHD) in children, the study results in fact suggest that the small subpopulation of children showing intolerance to artificial food colors may not be limited to those with ADHD.

(Kaplan et al, 1989)

Kaplan BJ, McNicol J, Conte RA and Moghadam HK. Dietary replacement in preschool-aged hyperactive boys. *Pediatrics*, 1989 (January), 83 (No.1): 7-17.

Kaplan et al (1989) designed a crossover dietary replacement study in hyperactive children to address several issues raised by previous research by including only preschool-aged boys with sleep problems and/or allergy-type somatic symptoms and notably using a replacement diet involving a broad range of food substances rather than targeting one class of substance. Twenty-four (24) pre-school male children (3.5 – 6 years) in day-care met the criteria of being diagnosed ADHD (during screening, children averaged more than 3 SD above the age-related mean on the parents Conners rating scale) and reported by parents to have sleep or physical problems. No stimulant medication was used during the study. The experimental diet was the Alberta Children’s Hospital elimination diet (ACH) which eliminated artificial food colors, flavors, preservatives, MSG, chocolate, and caffeine, and decreased simple sugars; for some children the diet also eliminated milk and dairy products (15 children) and naturally occurring salicylates (4 children), for which the families reported possible problems. A placebo diet that matched the regular baseline diet was designed to appear *equivalent* to the experimental diet. Also, if the parent identified a food item (e.g., apples or carrots) to which the parent thought their child reacted, that item was eliminated from both the experimental ACH and placebo equivalent diet. Extensive efforts were carried out to ensure that the parents were effectively blind to treatment conditions and could not differentiate the placebo and experimental diets. The effectiveness of the blinding was verified in a post-study debriefing session with the parents. Procedurally, this study was conducted over a 10 week period consisting of an initial 3 weeks of baseline on regular diet, which was intended to determine normal eating patterns, to determine persistence of problem behaviors, and to obtain baseline data for outcome measures. This was followed by the dietary crossover phase of 3 weeks of placebo/equivalent diet and 4 weeks of ACH diet with these two diet treatments given in randomized counter-balanced order across subjects. All foods during dietary phase were prepared and delivered to families once each week. The outcome measures included: (1) daily measures by parents, including the Conners 10-item behavior rating with up to 4 additional individualized troublesome behavior items identified by parents, sleep measures, physical signs/symptoms, and food diary; (2) daily measures by day-care workers, including the Conners 10-item behavior rating; (3) a planned additional daily observation by independent observers which was terminated due to technical difficulties; and (4) laboratory testing conducted three times, once near the end of each test phase, consisting of clinical

measures/nutritional status (blood chemistry/hematology/basic physical) and planned psychometric testing (motor, sensory, attention, cognitive/learning/memory). It should be noted that the psychometric testing was not completed because of the investigators found the children to be “untestable” reportedly due in part to the nature of their ADHD behaviors and also to selection of inappropriate age-related tests. Statistical analyses of data from the last two weeks of each phase of study was conducted using repeated measures multivariate and univariate ANOVA with Tukey's method of multiple comparisons.

Study Results: The parent Conners behavior ratings showed a significant group effect for the ACH diet with the children's mean score (10.8) being significantly lower (i.e., improved) than their mean scores for either the placebo/equivalent diet (13.1) or baseline diet (14.5). Assessment of the parental data for individual children revealed that not all children were equally responsive to the ACH elimination diet but that 14 (68%) of the 24 children showed some behavioral improvement (9 showing at least a 25% improvement in behavior and 5 showing a milder improvement) on the ACH elimination diet as compared with the placebo equivalent diet. The remaining 10 children (42%) were unresponsive to dietary intervention. No placebo effects (baseline versus placebo/equivalent diet) or treatment x order effects were found. Analyses of the grouped data for the parent ratings of the individualized problem behaviors showed comparable significant improvement in behavior during the ACH diet compared with the placebo/equivalent diet (the number of individual responders was not identified). The parent sleep records also found that the ACH diet resulted in significantly shorter sleep latency (i.e., improved sleep measure) as compared with the placebo/equivalent diet treatment (the number of individual responders was not identified). However, the day-care worker daily ratings, using the same Conners 10-item behavior questionnaire that parents used, showed marginal but no confirmatory significant diet related differences in the children's behavior. Also, there were no reliable diet related effects for physical signs/symptoms or nutritional status. The incomplete psychometric testing precluded any meaningful statistical analysis of that data.

Study Assessment and Evaluation: In a group of 24 pre-school hyperactive children with sleep problems, parent ratings and recordings revealed a significant overall improvement in behavior and sleep when the children were on the ACH elimination diet compared with the placebo/equivalent diet. When the parent ratings data were viewed for each child, 9 children showed at least a 25% improvement in behavior and 5 children showed a milder improvement when being fed the ACH elimination diet compared with the placebo/equivalent diet. Although the investigators analyzed the ACH elimination data against both the baseline and placebo/equivalent diet data, the baseline diet is not the appropriate control for the ACH diet treatment. To assess diet related effects, the ACH elimination diet data should be compared with data from the proper diet control which is the placebo/equivalent diet. The remaining 10 children showed no diet related changes in the parent ratings. With the apparently effective procedures in this study for blinding the parents to treatment conditions, the parent-based detection of significant behavioral and sleep improvement in some of the children with the ACH elimination diet appears to be reliable. However, the day-care workers using the same behavior rating scale as the parents did not confirm a significant diet related change in behavior, although these day-care worker results were based on somewhat limited data. Unfortunately, the other two planned sources of behavioral outcome data, i.e. the psychometric testing and the behavior ratings by independent observers, were discontinued. Speculatively, since the investigators noted that psychometric testing could not be completed in part because of the children's ADHD behavior, this may suggest that the nature or scope of the effects associated with the ACH elimination diet apparently was not sufficiently effective in improving the children's behavior to enable their completion of the psychometric testing. But, the investigators did also note the difficulty in

selecting tests acceptable to this age-group of children. No diet related effects were found with any of the clinical or physical measurements conducted. 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.

(Wilson and Scott, 1989)

Wilson N and Scott A. A double-blind assessment of additive intolerance in children using a 12 day challenge period at home. *Clinical and Experimental Allergy*, 1989, 19: 267-272.

Wilson and Scott (1989) designed a clinical double-blind challenge study to assess food additive intolerance under placebo controlled conditions. The study was conducted with 19 children (11 male, 8 female; 2-13 years) who were non-hyperactive but with a definite history of adverse reaction (respiratory, dermatologic, and/or behavioral) to artificial yellow color. (Note that 29 children were initially recruited and started on test but only 19 of these children completed the primary phases of this study) All subjects were reported to be responsive to a color-free diet and for most children color intolerance was verified in open (non-blind) challenge at home prior to the study. Four of the 19 subjects had a history that included adverse behavioral reaction to yellow color. The challenge items were administered in a bottled aide drink (250 ml of Lucozade with B-carotene coloring). Three types of drink, identical in appearance and flavor were used: (1) a color challenge with 8.5 mg tartrazine and 8.5 mg sunset yellow; (2) a preservative challenge with 12.5 mg sodium metabisulphite and 55 mg sodium benzoate; and (3) control/placebo aide. All drinks were reportedly identical in appearance and flavor but no procedures were described to verify the effectiveness of this blinding for parents or children. Procedurally, all children were started on an additive-free diet prior to the study and maintained on that diet throughout the period of testing. The initial study trial consisted of three 14-day phases with a different challenge drink for each phase. During each phase one bottle of the same type of drink was consumed daily at any time of day for 12 days. This was followed by a 2-day washout period after which the child was seen in the clinic (procedures in the clinic were not described). The three types of drinks were allocated double-blind in random order to each phase of testing. At the end of this initial study trial, the response to each type of drink was assessed with blind still maintained. If during one or two phases of that initial trial there was a suggestion that a deterioration in symptoms occurred, the child was invited to repeat the trial using the same protocol but with drinks recoded and the order randomized double-blind. A third trial (three 7-day phases with drinks taken for 6 days in each phase) was also offered if results were inconclusive. The outcome measures to assess treatment effects were admittedly more detailed and objective for asthma than for either dermatologic or behavioral effects. The assessment of behavioral responses was basically limited to two types of subjective unstructured assessments both by parents: (1) a daily diary of relevant symptoms with a simple scoring system ('daily maximum = 2'), and (2) an overall assessment at the end of each challenge phase with parents recording simply whether the child's symptoms were "better/worse/no difference". A deterioration in behavior was defined by a parent's assessment of "worse" confirmed by an increase in the total symptom score. When these differed, the result was considered

"inconclusive". The children were reported "seen in the clinic" after each challenge phase but no further description was given of whether or what clinical evaluations were conducted. The results were analyzed for individuals and not for the group as a whole. For each subject a total symptom score was calculated for each phase and a comparison was made between the three challenge phases of each trial. However, no statistical analyses of the data were apparently made. **Study Results:** Out of 19 children, who were claimed to have a history of adverse effects from exposure to artificial yellow colors and definite benefit from the exclusion of artificial food colorings in their diets, only 3 showed any positive association between food color or preservative additives and adverse symptoms when challenged daily for 12 days under double-blind controlled conditions. Only 1 of these 3 "responders" developed adverse behavioral symptoms and that was in response to the preservative challenge but not to the color challenge. Interestingly, behavioral changes were not part of this child's prior history of adverse responses to artificial yellow color but this child had never previously been given food or drink with preservatives. The other two "responders" exhibited other symptoms without behavioral changes, one child developing urticaria when challenged with tartrazine/sunset yellow drinks and the other child developing intermittent asthma and abdominal pain with both the color and preservative challenge drinks. Notably, among the remaining 16 children who showed no adverse effects of challenge under double-blind conditions were the 4 subjects who were claimed to have had a definite prior history of adverse behavioral responses to artificial yellow color. **Study Assessment and Evaluation:** None of the 19 children with a definite history of adverse effects from exposure to artificial yellow colors, including 4 with a history of adverse behavioral reactions, showed any adverse behavioral effects of challenge with combined tartrazine and sunset yellow under placebo controlled double-blind conditions. One child did exhibit an adverse behavioral response to the preservative challenge. Confidence in the study results, however, is somewhat limited in part by the questionable accuracy and sensitivity of the means used for detecting behavioral responses to the challenges. The assessment of behavior was totally subjective and unstructured with a very limited and poorly defined nonvalidated scoring system (behaviors scored as 1 or 2 and overall assessments as 'better, worse, no difference') and conducted only by the parents with no other source of assessment to confirm the parental observations. Although the investigators point out that the same criteria would have been used by the parents in the initial alleged association between additives and behavioral disturbance, this is hardly an appropriate basis for credible scientific assessment. Additional limitations in this study 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 more controlled clinical conditions.

(Pollock and Warner, 1990)

Pollock I, Warner JO. Effects of artificial food colours on childhood behaviour. *Dis Child*, 1990 (January), 65(1):74-7.

Pollock and Warner (1990) conducted a placebo controlled challenge study to investigate children with food additive intolerance. The study involved 19 children (1 hyperkinetic, 1 retarded; 3-15 years) with problem behaviors who were selected specifically based on parents' observations that their behaviors improved on a diet free of food/color additives and returned (primarily poor concentration and excess fidgetiness) within an average of 2.5 hours after

consuming food additives. According to the parents, even small amounts of food additives, precipitated the recurrence of behavioral problems. (Note that 39 children were recruited for this study but only 19 completed all testing.) The challenge item was a gelatin capsule with/without 125 mg blend of artificial food colors (50 mg tartrazine, 25 mg sunset yellow, 25 mg carmoisine, 25 mg amaranth). The placebo contained lactose. The gelatin capsules were made opaque with iron oxide which, according to the investigators, effectively disguised the color additive capsules so that they could not be distinguished from the placebo capsules. The parents were told only that the capsules for each week contained either food additives or placebo, but parents were not asked whether they could differentiate the color and placebo capsules. Procedurally, all children were maintained on their food additive elimination diets throughout the test period. The 7 week double blind placebo controlled challenge study consisted of 2 separate weeks of color challenge and 5 weeks of placebo with 3 placebo weeks between each color challenge week. Children were randomly assigned to one of two treatment sequences (C P P P C P P or P P C P P P C) and received an single

appropriate capsule (color or placebo) daily. Two outcome measures, used to assess treatment effects, were both provided by the parents and consisted of: (1) a daily rating of the child's behavioral and somatic symptoms (Conners Hyperactivity Index supplemented with questions about symptoms associated with allergies) and (2) a weekly overall behavior assessment (recorded as improved/same/worse). Data were analyzed using the Wilcoxin rank tests.

Study Results: The parent daily behavior ratings were significantly higher (enhanced problem behaviors) for the color challenge weeks than for placebo weeks and there was no confounding treatment order effects. The daily somatic scores (associated with allergies) showed no differences between color and placebo challenge weeks, indicating that the changes in behavior scores were not related to or secondary to somatic symptoms. Additional analyses showed no carry-over of the color challenge effect into the washout week and also showed no difference in behavior ratings between week days 1 and 7 indicating that the behavioral changes occurred early in the challenge week and that the effects of food color intolerance do not result from a cumulative effect of food colors. Notably, however, the absence of any differences between color and placebo weeks in the parents' overall assessment of behavior at the end of each week provides no supportive confirmation for the color effects seen with the parent daily behavioral ratings.

Study Assessment and Evaluation: This study reported that a daily intake of 125 mg mixed artificial colors for 1 week, relative to placebo, can produce a small but measurable change in the parents daily rating showing an increase in problem behaviors for non-ADHD children. This effect was unrelated to the somatic (allergy) symptoms or atopic state of the children. However, the reliability of these findings, or at least their biological relevance, appear to be questionable due primarily to the fact that most of these parents were unable to confirm a color related worsening of behavior in these children in their weekly overall behavioral assessments. 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 the study provided no verification of the effectiveness of the blinding procedures for the parents, the investigators do suggest that any ineffective blinding of the parents seems unlikely since their weekly behavioral assessments might then have more accurately reflected the daily behavior ratings. Overall, since the claim by parents of being able to detect deterioration of behavioral after even consumption of 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 from daily challenge to 125 mg artificial colors, a rather high dosage, for a week (on two separate week trials) and the rather small change in behavior detected with the parents' daily

Connors assessment indicate that there is little substantial or functionally (biologically) relevant adverse effects of artificial colors on the behavior of children anecdotally identified as having some food intolerance.

(Sarantinos et al, 1990)

Sarantinos J, Rowe KS and Briggs DR. Synthetic Food Colouring and Behavioral Change In Children With Attention Deficit Disorder: A Double-Blind, Placebo Controlled (Challenge Study). Proc. Nutr. Aust., 1990, 15: 233.

(It should be noted that the review and evaluation of this clinical study will be limited since it is based only on an Abstract Summary of the study which contains few details of the experimental design, test group assignments, and test results. A full published report of this study was not available. Consequently, only a tentative assessment and evaluation of the study and results can be presented.)

Sarantinos et al (1990) conducted a placebo controlled challenge study to investigate the effects of two artificial food colors, tartrazine and sunset yellow, on the behavior of children diagnosed with attention deficit disorder (ADD). The investigators recruited 13 children (12 male, 1 female; 4-14 years) diagnosed with ADD and previously placed on a diet free of artificial food colors. Four to six children were reportedly intolerant to various foods, two of which were behaviorally intolerant to orange juice (the challenge vehicle used in this study). Nine parents considered their child improved on the diet, while 4 parents were uncertain about improvement. The challenge item was orange juice with/without 10 mg tartrazine or 10 mg sunset yellow. The orange juice drinks were consumed from an opaque pack using a straw. No procedures for determining or validating the effectiveness of the blind were described. All children were placed on color-free diet at least 6 weeks prior to study and remained on color-free diet throughout the 28-day study. Procedurally, the 13 children were assigned to one of two groups (report did not state whether assignment was randomized and did not specify distribution of subjects or composition of the two groups). Over the 28-day study period, the children in Group 1 (n=6 or 7) were challenged only with tartrazine on 6 random days (not specified whether all children were given color challenge on same days). The children Group 2 (n=6 or 7) were also given color challenges on 6 random days, tartrazine on 3 of those days and sunset yellow on the 3 other days. A 2-3 day washout (placebo) period was allowed between each challenge day. On all non-color challenge days, children received a placebo drink. As the outcome measure, behavior was assessed daily by parents only using both the Connors' Abbreviated Parental Scale and the Rowe Behavioural Rating Inventory Scale. Data were analyzed with repeated measures analysis of variance.

Study Results: Only 12 subjects completed the double-blind challenge trial but the report did not specify which one child was excluded. Both parental rating scales showed that 2 of the 6 or 7 ADD subjects in Test Group 2 showed significant adverse change in behavior (i.e., increased irritability, impulsivity, restlessness and sleep disturbance) associated with both color challenges (tartrazine and sunset yellow). 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 and the mother could correctly identify (25/28 times) whether the child received color or placebo (unclear whether guess was based on behavior or broken blind). Apparently, none of the 6 or 7 children in Group 1 responded to the challenge with tartrazine alone.

Study Assessment and Evaluation: Due to the absence of a full report for this study, there are numerous uncertainties regarding the study design, conduct and results. As a result, there are several aspects of this study as described in the Abstract Summary of this study which minimize

confidence in the reliability of this study's findings and confound interpretation of the study results. These include, for example: (1) 13 children were recruited for the study but only 6-7 children comprise each of the two treatment groups; (2) unknown composition of the two treatment groups, e.g., how many diet responders were in Group 1 and Group 2; (3) the questionable validity of using 2 subjects who were reported behaviorally intolerant to orange juice for this study in which orange juice was the challenge vehicle and placebo treatment; (4) the use of only parents to assess treatment effects without any other source of behavior assessment to confirm the parents' reported treatment effects; (5) the unclear effectiveness of the blinding for parents and children; (6) no actual data are presented in this abstract summary; and (7) questionable significance of no treatment effects in Group 1 with tartrazine challenge but significant treatment effects in Group 2 children who were also given tartrazine challenge randomly alternated on separate days with sunset yellow challenge. Overall, based on the information available, the absence of other behavioral measures to corroborate the changes in parental ratings and the uncertainties engendered in the absence of various details about the experimental design, the effectiveness of the blinding conditions and data analysis certainly raise 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.

(Egger et al, 1992)

Egger J., Stolla A and McEwen LM. Controlled trial of hyposensitization in children with food-induced hyperkinetic syndrome. *The Lancet*, 1992 (May), 339: 1150-1153.

Based on the premise, suggested by previous research (see reviews of Egger et al, 1985; Kaplan et al, 1989), that food intolerance is a possible cause of the hyperkinetic syndrome in some children and may involve some allergic/immunologic mechanism, Egger et al (1992) designed a placebo controlled clinical study to determine whether hyperkinetic children can be desensitized to the adverse effects of provoking foods using an enzyme-potentiated desensitization treatment (EPD). This study was conducted in four phases: Phase I – identify hyperkinetic children responsive to an open (non-blind) oligoantigenic diet (i.e., a very restrictive diet that eliminates every kind of food and food additive assumed to provoke adverse behavioral reactions in certain children); Phase II – non-blinded identification of specific provoking food items; Phase III – double blind, placebo controlled EPD treatment; and Phase IV – determine effectiveness of treatment by challenge with provoking food items. In Phase I, 185 children diagnosed with hyperkinetic syndrome (Conners score > 15) received an open (non-blind) oligoantigenic diet for 4 weeks. Based on parent and teacher non-blinded observations and a Conners score <15, 116 children (63%) reportedly improved on the diet and were entered into the food reintroduction phase of study. For Phase II, all 116 food intolerant children were maintained on their oligoantigenic diet and food items eliminated from that diet were sequentially reintroduced under non-blind conditions singly every 5 days. Identification of provoking (intolerant) food items was based on recurrence of hyperkinetic behavior (overactivity) or other associated symptoms (e.g., headache and recurrent abdominal symptoms such as discomfort, bloating, diarrhea) on at least three trials with that food item. In the experimental treatment Phase III, 40 of the food-intolerant hyperkinetic children (36 male, 4 female; 3-15 years/ average 9 years) agreed to take part in the double-blind, placebo-controlled trials. All children were maintained on an oligoantigenic diet and provoking foods were avoided during this period. Subjects were randomized to receive 3

intradermal injections of either placebo/buffer solution (n=20; Treatment Group 1) or EPD treatment solution (n=20; Treatment Group 2) at two-monthly intervals. The EPD treatment included mixed food antigens (multiple foods, additives, colors and preservatives). Only one principle investigator knew the key to the randomized EPD/Placebo treatments. Children and parents were not told which treatment was administered. The investigators stated that “placebo and active [EPD] treatments were both colourless solutions”, ostensibly blinding the children and parents to any apparent difference between the two treatment solutions (see comment regarding blinding of treatments in Study Assessment and Evaluation, below). The Phase IV assessment of the effectiveness of treatment was initiated three weeks after the third EPD injection, when provoking foods for each subject were reintroduced, one item at a time, while the children were still maintained on their oligoantigenic diets. For evaluating responses to the various provoking food items, the parents were asked to maintain a daily diary card of hyperactivity and other symptoms (e.g., headache and recurrent abdominal symptoms such as discomfort, bloating, diarrhea). As one measure of treatment effectiveness, parents were told to stop the food when food-related symptoms occurred and persisted for 24 hours. As a second measure of treatment effectiveness, at the end of introducing all provoking foods, parents were asked whether the treatment had been successful in preventing or reducing symptoms on eating one or more of the provoking foods. Statistical analyses (Fisher’s exact test) were conducted for (1) parents’ stopping the first provoking food given to the EPD and placebo groups, and (2) response to whether parents considered treatment successful. Skin tests were also conducted to identify atopic children and serum IgE was measured.

Study Results: A variety of food items were identified as provoking behavioral and associated symptoms; chocolate, artificial colors, cow milk, egg, citrus, wheat, beet sugar and nuts were among the most common provoking food items. Adverse response to provoking food items (based on analysis of the number of parents stopping the first provoking food reintroduction) occurred less frequently in the EPD treated group than in the placebo treated 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 continue eating provoking foods. Also, after all provoking food items were reintroduced, more parents of children treated with EPD than those treated with placebo, thought the treatment was successful. While these results depended on recurrence of any adverse responses, behavioral or associated symptoms, to the provoking food items, the abdominal symptoms, such as discomfort, bloating and diarrhea, were usually the first to appear (it is assumed but not specifically stated that hyperactive behaviors occurred subsequently, as well). Reaction to provoking foods tended to recur several months after completion of the study but tolerance to the provoking foods could be reinstated with additional EPD treatment. At the end of the trial, 16 of the children in the placebo/Group 1 were given EPD treatment and 15 developed tolerance to previously provoking foods (it was unclear whether this was conducted under non-blinded conditions). These study results were not related to atopic status or IgE levels in these children.

Study Assessment and Evaluation: This study provided some information about the proportion of hyperkinetic (hyperactive) children that may react adversely to some foods. Based on observations by parents and teachers under non-blinded conditions, approximately 63% (116/185) of children diagnosed with hyperkinetic syndrome appeared to be food intolerant, showing positive response to an oligoantigenic diet which eliminated provoking food items and showing adverse response to reintroduction of those provoking food items. When 40 of these apparent food intolerant hyperkinetic children were tested under double-blind, placebo controlled conditions to determine whether they could be desensitized (EPD treatment) to the adverse effects of the provoking food items, it was shown that more of the EPD treated children (15/20)

were able to eat previously identified provoking food items than placebo treated children (7/20). Several issues involving experimental design weaknesses affect the reliability and biological relevance of this study's finding: (1) One particular issue of concern involves the adequacy of the blinding of the experimental treatment which is central to the reliability of the subjective parental observations. The investigators stated that "placebo and active [EPD] treatments were both colourless solutions". The placebo was simply a colorless buffer solution. However, it is difficult to understand how the active (EPD) solution could have been colorless in view of the fact that the mixed antigens in that preparation included multiple food colors (tartrazine, chocolate brown, Ponceau 3R, erythrosine, green S, and Annatto). Any notable difference in coloration between the placebo and EPD solutions would make blinding of the parents and children to the treatment very difficult and introduces the possibility of unintentional confounding assessment bias. (2) A second study limitation which affects confidence in the reliability of the study findings, or at least their biological relevance, involves the fact that the parents were the only source for detecting response to provoking foods and assessing effectiveness of the desensitization treatment. In the absence of supporting data from sources other than parents, there was no corroboration of the parental based findings. And, a third issues involves the fact that (3) A final study issue involves the minimal assessment of specific behavioral changes during the double-blind, placebo controlled testing of the effectiveness of desensitization to the adverse effects of provoking food items. The effectiveness of treatment (placebo and EPD) was based on the parents' determination of whether reintroduction of foods should be stopped and on the end of trial parents' overall assessment of improvement in symptoms. Responses to the various provoking food items were evaluated by parents who were asked to maintain a daily diary card of hyperactivity and other symptoms, but no structured or validated rating system, e.g. Conners hyperactivity rating scale, was used to help characterize the scope of behavioral changes, even though Conners behavior ratings were conducted by parents in the initial non-blinded identification of responders to the oligoantigenic diet. The investigators do not even provide data identifying which food-related behavioral change or associated symptoms were used by the parents to decide whether the provoking food should be stopped. In fact no behavioral data are presented or analyzed to show whether any food-related behavioral changes even occurred during the re-introduction of provoking foods. And, beyond the statement that abdominal symptoms were typically the earliest signs of adverse response to provoking food items, no data for those associated symptoms were presented. Overall, the questionable confidence in the reliability of the study data and the minimal specific assessment of behavioral changes due to provoking food items limit the potential significance and interpretability of this study's findings relating food and hyperactive behaviors. In view of the caveats inherent in these study weaknesses the results of this study may be viewed only as suggestive that intolerance to a variety of food items may be causally associated with hyperkinetic behavior and symptoms in some children and that some food intolerant hyperactive children may be desensitized to the adverse effects of provoking food items.

(Carter et al 1993)

Carter CM, Urbanowicz M, Hemsley R, Mantilla L, Strobel S, Graham PJ, Taylor E. Effects of a few food diet in attention deficit disorder. Arch Dis Child, 1993 (November), 69(5):564-8.

A previous study suggested that foods and additives could affect hyperactive behavior adversely (Egger et al, 1985), based on the experimental design from that study Carter et al (1993)

conducted a multiphase study of the effects of a restricted food elimination (“few foods”) diet on ADHD children. The “few foods” diet is virtually identical to the oligoantigenic diet (Egger et al, 1985) both of which are very restrictive diets that eliminate every kind of food and food additive assumed to provoke behavioral reactions in certain children. The main purpose of the study was to determine whether anecdotal reports of food intolerance in ADHD children may be due to a 'placebo effect' of expectation and suggestion. The study was conducted in three phases: Phase I – identify ADHD who are food intolerant and improve behaviorally on a “few foods” diet; Phase II – open (non-blind) reintroduction of food items and additives to identify food components responsible for adverse reactions; and Phase III – experimental double-blind, placebo controlled re-introduction of incriminated foods and additives. Seventy-eight (78) ADHD children (69 male, 9 female; 3 – 12 years) were used for Phase I testing. Those children who were already on some special diet (43) were asked to stop those diets at least one week prior to this study. All children were then given an open (non-blind) restricted “few foods” diet for three to four weeks. The foods allowed were typically two meats, two carbohydrate sources, several root and green vegetables, bottle water, sunflower oil, and milk free margarine; for individual children the diet also avoided foods already suspected. Approximately 76% (59/78) of the ADHD children were found to be food intolerant based on their reported behavioral improvement on the diet. Forty-seven (47) of these “diet responders” were used for Phase II testing in which the “few foods” diet was continued and various foods and additives were openly reintroduced (non-blinded) at the rate of one per week to tentatively identify those that may elicit an adverse response. Food additives were usually given initially as mixtures in additive-containing foods. When adverse reactions occurred and colors were suspected, response to artificial colors was specifically tested using capsules with 6.5 mg mixed colors. A large number of foods were implicated during the open reintroduction phase. Most reactions to food included worsening of behavior sometimes accompanied by physical symptoms. For the Phase III double-blind placebo controlled challenge with provoking food items, 19 diet responsive ADHD children were used (23 children were started in Phase III but 4 did not complete testing). The active food challenges consisted of multiple food items, including artificial colors and preservatives, incriminated as provoking adverse behavioral responses in Phase II. Each provoking food item was selected to be adequately disguised in a food known to be tolerated (to act as an excipient and placebo). Artificial colors were generally given in capsules rendered opaque with iron oxide with each capsule containing 6.5 mg of mixed colors; the number of capsules given varied, but no child received more than 4 capsules or 26 mg daily. Glucose capsules were used as a placebo. Only the study director, who took no part in actual testing, knew the treatment code and everyone else remained blind to the order in which active and placebo foods were given. To ensure that the taste and appearance of the placebo and active food treatments were indistinguishable, on the first day of each test week the families were asked to guess whether they had been given the active or placebo food item; none were able to guess correctly beyond chance, verifying the effectiveness of the blind. Procedurally, all children were maintained on their “few foods” diet throughout the Phase III period of testing. Each incriminated food item or additive and its appropriate placebo were given daily each for one week (experimental period) with a two-week washout period between active and placebo treatments. The order in which incriminated foods and placebo were given was allocated randomly. Most of the children received more than one provoking food item. The outcome measures to assess behavior were taken at three time points: at entry to the experimental Phase III, at the end of each experimental week (placebo or food item). These measures included: (1) parent behavior ratings (Conners parent rating scale) and parent global rating of severity of behavior problems; (2) psychologist rating of hyperactive behavior (inattentiveness, restlessness, fidgetiness); (3) paired associate learning test; and (4) matching

familiar figures with a simplified form of testing for children under 6 years (impulsiveness). Most data were statistically analyzed using t-tests. The parent global ratings were tested by Fisher's exact probability test.

Test Results: Approximately 76% (59/78) of the ADHD children were found to be food intolerant based on their improvement on an open (non-blind) 'few foods' elimination diet. In the double blind phase of testing, parent ratings of behavioral change showed statistically significant deterioration of behavior following the incriminated food item challenge compared with the placebo, but the magnitude of this behavioral effect was shown to be rather small (Conners score of 8.8 for placebo and 13.9 for food item challenge which did not reach the conventional hyperactivity cutoff score of 15) and the types of behaviors rated by the parents as most affected suggested a possibly greater effect on irritability than on attention deficit. (The parent global ratings were described as favoring the placebo and showing fewer treatment failures. But, none of these data were presented and the actual meaning of these stated effects was not interpretable.) The psychologist ratings also showed significantly more hyperactive behavior, primarily fidgetiness, following the food item challenge than placebo, as did the matching familiar figures test of impulsiveness. Paired associate learning, however, was unaffected.

Study Assessment and Evaluation: Both parent and psychologist ratings and one of two objective psychological tests (impulsiveness) showed significant behavioral changes associated with food item challenges for the entire group of subjects. While these behavioral changes were generally in the direction of negative (worsening) effects, the small magnitude of these changes makes their biological significance unclear. Possibly, combined exposure to multiple provoking food items may be necessary to elicit an additive effect of a greater magnitude behavioral response. The possibility of additive effects should be addressed in subsequent studies. Notably, the types of behaviors rated by the parents showed a possibly greater effect on irritability than on attention deficit, suggesting behavioral changes not necessarily associated only with the hyperactivity syndrome. Unfortunately, the data for individual subjects were not assessed or presented to determine whether some of the children may have exhibited stronger responses and behavioral changes possibly more closely associated with attention deficits. Also, although most of the children did receive more than one provoking food item, it was not stated whether those children exhibited adverse responses to all challenge food items and whether those responses were similar in type and magnitude. In assessing the overall significance of these study findings two minor study weaknesses should be mentioned. First, even though food challenge was given for a whole week, families were asked to guess if they had been given the provocative or placebo food only on the first day of each challenge week. While there may be some uncertainty as to whether the effectiveness of the parents' blind was maintained throughout the test week, a reasonable effort was made verifying the effective blinding of parents to active food and placebo treatments. Second, while the use of uncorrected multiple t-testing may have increased the probability of false positives (i.e., erroneous findings), it is unlikely that many, if any, of the treatment effects in this study were false positives. These study weaknesses are considered minor with little impact on the reliability of the study findings of small but significant adverse behavioral effects of a variety of provoking food items, which were confirmed across several sources of measurement (parent, psychologist, objective testing). Overall, these findings do show that parents' reports of behavior change with a "few foods" elimination diet can be confirmed to some extent, in a selected group of ADHD children, by double blind, placebo controlled trial with multiple sources of valid testing. These findings also support the conclusion that some ADHD children have an intolerance to a variety of food items, including but not limited to artificial food colors and additives, which may cause or exaggerate adverse problem behaviors but which may or may not be related to the hyperactivity syndrome. However, the use of general

food items in this study makes it difficult to identify specific food chemicals that may be causing the adverse effects.

(Boris et al, 1994)

Boris M and Mandel FS. Foods and additives are common causes of the attention deficit hyperactive disorder in children. *Ann Allergy*, 1994 (May), 72(5):462-8.

The stated purpose of this study was to determine whether dietary components play a role in attention deficit hyperactivity disorder (ADHD) in children. ADHD children showing improved behavior on an open (non-blind) multiple food elimination diet (Phase I) and responding adversely to certain food components in an open challenge (Phase II), were subsequently tested in a double blind placebo controlled food challenge (DBPCFC) to determine whether the responses to suspect provoking food components were reproducible under controlled test conditions (Phase III).

Phase I - Twenty-six children with ADHD were placed on an open (i.e. not blinded) multiple food elimination diet for 2 weeks to identify children that appear to show behavioral improvement on the elimination diet. This diet eliminated any components that consisted of dairy products, wheat, corn, yeast, soy, citrus, egg, chocolate, peanuts, and artificial colors and preservatives. Prior to and at the conclusion of the 2 week elimination diet phase, the parents completed a Conners Parent Rating Scale-48 (CPRS-48) which included a subset of questions used in this study as the quantitative index of hyperactivity. A score of 15 or greater was taken to identify hyperactive children. Nineteen or 73% of the 26 children appeared to show improved behavior (mean hyperactivity score of 9.6) on the open elimination diet, as compared with their original diets (mean hyperactivity score of 25). Significantly more of the 19 “diet responders” were atopic than of the 7 “non-diet responders”.

Phase II - The 19 “diet responders” were then given open (i.e. not blinded) food challenges over the next month. The parents challenged their child with one restricted diet item every two days; reactions to a diet item were retested to confirm the reaction. All 19 children reacted to multiple diet items during the open challenge.

Phase III – The 19 “diet responders” were then enrolled in a 7-day DBPCFC trial, but only 16 completed the entire trial (11 male, 5 female; 7.5 years). Each set of parents were asked to select the most reactive food or agent that appeared to induce the recurrence of symptoms in their child during open challenge (Phase II) and that one item was used to challenge that child during the DBPCFC. Challenge items (number and sex of subjects) included the following: milk (2m, 3f), colors (4m), corn (3m), wheat (2f), soy (1m), and oranges (1m). Note that none of the subjects responded to preservatives in open challenge. The challenge material consisted of 5 grams of the powdered food or 100 mg of a blend of food colors. To control for the taste and color of the challenge items, either lentil soup or an apple cranberry sauce was used as the excipient and as the placebo vehicle. Seven dieticians tasted the vehicles with each of the challenge items and could not distinguish a difference in taste. However, the parents and children were not asked to verify whether they could differentiate the placebo from the challenge item. Procedurally, all children were continued on the elimination diet throughout double-blind testing. Over the 7-day challenge period, days 1, 2, and 7 were designated placebo days for all subjects. On days 3, 4, 5 and 6, the food challenge and placebo treatments were randomly administered with 13 subjects receiving food challenge two times, 2 subjects receiving food challenge three times, and 1 subject receiving food challenge one time. Both the parents and primary investigators were blinded to the challenge order during this 7-day period. The study was carried out in the home environment during a school vacation. As the sole outcome measure, the parents completed a

Conners Parent Rating Scale-48 (CPRS-48) which included a subset of 10 questions used in this study as the quantitative index of hyperactivity. The parent ratings were completed prior to the study while children were still on their original diets, at the end of the open elimination diet period, and daily during the 7-day challenge period. Statistical analyses of behavioral data were conducted primarily using the repeated measures analysis of variance and repeated measures analysis of covariance..

Study Results: Nineteen or 73% of the 26 ADHD children given an open (non-blind) elimination diet appeared to show marked behavioral improvement, as compared with their original diets (mean hyperactivity scores of 9.6 and 25, respectively). A significantly greater number of atopic subjects (i.e., history of allergic manifestations) than non-atopic subjects were among the “diet responders” (15 atopics and 4 non-atopics responded to the elimination diet). The 16 “diet responders” that completed the double-blind, placebo controlled challenge trial collectively showed significantly elevated hyperactivity scores with the food challenges (mean Conners score of 18.1) as compared with the placebo treatments (mean Conners score of 8.2). However, inspection of the graphed Conners scores showed that only 11 of the 16 subjects actually responded to the active food challenges. Five (5) subjects did not respond to their active food challenge under blinded conditions even though they were reported to show improved behaviors when switched from their original diet to the open elimination diet and to show marked adverse behavioral effects when given provoking food items in open (non-blinded) challenge. In the double blind challenge, the authors do not identify what food items these 5 non-responding subjects were receiving. Nor is it known whether the 4 children that received the artificial colors as their food challenge did or did not respond. No information was presented as to whether the elimination diet or the exposure to provoking food items affected the atopy/allergic status of the children

Study Assessment and Evaluation: In open (non-blind) diet and challenge trials, 19 or 73% of 26 ADHD children were reported to be food intolerant, showing behavioral improvement with a multiple foods elimination diet and adverse behavioral changes elicited by various food components (reacting to at least three or more different food items). Interestingly, in this study 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” were subsequently tested in a double blind, placebo controlled challenge trial and 11 children were confirmed to be food intolerant. Based on parental ratings, these children exhibited adverse behavioral responses (elevated hyperactivity scores) to challenge with previously identified provocative food items; the other 5 children did not react to the food challenges. The investigators also noted a substantial difference in hyperactivity scores between the original (pre-study) diet period and the active food challenge days (mean Conners hyperactivity scores of 25 versus 18.1, respectively). They suggested that this showed multiple food diets to have a greater adverse effect on the behavior of hyperactive children than a single item food challenge based on the fact that only one provoking food was used in the challenge but the children were reactive to multiple provoking foods in their regular diet. While conceptually it seems plausible that exposure to multiple provoking food items might be expected to have a greater (additive) adverse affect than challenge with a single provoking food item, in this instance, however, the difference between the two scores of hyperactivity may be more apparent than real since the conditions under which the two ratings were conducted were completely different. The parental rating of the hyperactive behaviors prior to the study (with the regular diets) was conducted under non-blinded conditions (i.e., they knew that their child’s hyperactivity was not being treated in any manner) and, consequently, they may have had a biased perception of their child exhibiting a greater level of hyperactive behavior. However, when the parents rated the behaviors after a

blinded challenge treatment, their uncertainty may have resulted in a more conservative (lower score) assessment of their child's behavior.

As noted previously, the significant food related challenge effects in this study were based on parental ratings (Conners hyperactivity index) as the only measure of behavioral response. However, in the absence of other confirmatory outcome measures, confidence in these findings is limited. A more reliable assessment of the relevance of the parental findings could be made if other measures of behavior were included, e.g. clinical evaluation and teacher evaluation. Furthermore, although the parents and primary investigators were blinded to the challenge order during the testing period and several dieticians were unable to distinguish the taste of the active food item versus the placebo treatments, there is still an uncertainty about the adequacy of the study blind, since no specific effort was made to determine whether parents/children were able to distinguish the two treatment items. Verification of the effectiveness of the blinding conditions, particularly in a study that uses subjective parental evaluation as the sole measure of treatment related behavioral effects, is critical for the integrity and reliability of any findings in a controlled challenge study. Overall, in view of the uncertainty about the effectiveness of the blinding for the parents and children and particularly in conjunction with the fact that no other measures of behavioral change were used to verify the parental reports of adverse behavioral response to provocative food challenges, the confidence in the reliability of the study findings showing adverse behavioral effects of challenge with various provoking food items is limited and the relevance of these observed behavioral changes is unclear. The findings may at least be considered suggestive that intolerance to various food components, possibly including but not limited to artificial colors, may cause or exaggerate hyperactive behaviors in some ADHD children. While the use of general food items in this study makes it difficult to even tentatively identify specifically the food elements (component) that are causing the adverse effects, none of the subjects were reported to have responded to preservatives in open challenge.

With regard to the 73% of ADHD children who responded to the open elimination diet, the present study also showed that ADHD children who were atopic (history of allergic symptoms) were more likely to respond to the elimination diet than those children who were non-atopic, suggesting a possible association between allergy/immune function and reported food intolerance in ADHD children. This contradicted the results from another study (Egger et al, 1985) in which atopy was equal in diet responders and non-responders. Although the present study did not report whether the subjects exhibited allergic reactions to the challenge food items, one possibility suggested by these non-blind observations is that the reportedly responsive subjects were somehow immunologically sensitive to the provoking food items and that the negative behavioral changes were secondary to or an associated consequence of the "allergic" or immunological response. In lieu of an "allergic"/immunological role, the investigators suggest the possibility that the challenge items could be having a more direct adverse effect on the nervous system. Based on the information presented in this study it is not possible to determine which of these alternative interpretations may be more feasible.

(Rowe and Rowe, 1994)

Rowe KS, Rowe KJ. Synthetic food coloring and behavior: a dose response effect in a double-blind, placebo-controlled, repeated-measures study. J Pediatr, 1994 (November), 125(5 Pt 1):691-8

The objective of this challenge study was to establish whether there is an association between

tartrazine, an artificial food color, and behavioral change in children referred for assessment of suspected hyperactivity. Uniquely, a full dose response assessment of tartrazine was used as the study design. The first stage of this study identified children who might be sensitive to artificial colors. For this, 200 children with problem behaviors (suspected but not diagnosed Hyperactive) were given a 6-week open trial of a diet free of artificial colors. The parents of 150 or 75% of the 200 reported behavioral improvement with the diet but deterioration in behavior with ingestion of foods containing artificial colors. The primary behaviors consistently reported related to irritability, restlessness, and sleep disturbance (behaviors not typically characteristic of hyperactivity syndrome). Thirty-four (34) of these “diet responders” and 20 children without problem behaviors given the color-free diet for 6 weeks participated in the double-blind, placebo controlled, repeated measures challenge study (38 male, 16 female; 2-14 years @ average 7 years). The “diet responders” were categorized by parents as likely diet responders (23) or uncertain diet responders (11). For children ≥ 6 years of age the challenge item was a double capsule containing either lactose (placebo) or one of six dosages of tartrazine (yellow 5) in the inner capsule surrounded by lactose. For children < 6 years the challenge item was packaged orange juice with a straw (250 ml) with/without one of six dosages of tartrazine. The level of tartrazine used were: 1, 2, 5, 10, 20, and 50 mg (the latter two highest dosages were administered toward the end of the test period). Other than use of specially designed capsules, no description was given as to blinding procedures or to verification of effectiveness of blinding. Procedurally, all children were maintained on a color-free diet and given one capsule (or orange juice) on each morning of the 21-day study period. For each child the tartrazine dosages were administered randomly once each on a different day, except that the 20 and 50 mg dosages were administered toward the end of the study period. The design allowed for a placebo lead-in period of 3 days and a placebo washout period of at least 2 days between each day of tartrazine challenge for each child. The outcome measures for behavioral assessment consisted of two ratings scales completed by the parents daily throughout the study at the end of each 24-hour period to ensure that the sleep period was included. The two rating scales were: (1) a 30-item behavioral rating inventory, which was devised by the investigators based on a composite analysis of the clinical histories of 50 suspect reactors and was validated to discriminate between color and placebo ingestion, and (2) the Conners 10-item parent/teacher questionnaire, included for comparative purposes only (none of the Conners rating data were statistically analyzed for treatment effects). Atopic histories and allergic reactions to allergens and tartrazine were also obtained. Standard parametric and non-parametric statistical techniques were used to analyze the data.

Study Results: In the initial stage of this study, 150 or 75% of the 200 children with problem behaviors (suspected but not diagnosed Hyperactive) were reported by parents to be adversely responsive to foods with artificial colors, showing primarily increased irritability, restlessness, and sleep disturbance. Under double-blind challenge conditions, the parent behavioral inventory ratings identified a total of 24 of the 54 children tested as showing consistent behavioral reaction to the tartrazine challenges in clearly dose related fashion with amplitude and duration of effect increasing with increasing dosage level. Beyond 10 mg there appeared to be a ceiling effect in magnitude but higher doses increased duration of effect. The tartrazine challenge reactors included 19 of the 23 “likely diet responders”, 3 of the 11 “uncertain diet responders”, and 2 of the 20 control subjects. When the mean parent behavioral ratings were used to analyze the differences between placebo and tartrazine challenge, significant differences were found at all six dosage levels. There were no sex related (male/female) or age related (2-6 years/7-14 years) differences between the tartrazine reactors and non-reactors. All tartrazine reactors, including the 2 control subjects, were atopic but none showed an allergic reaction to tartrazine. However, it was not stated whether the non-reactors were atopic or had an allergic reaction to tartrazine. The

behavioral changes were independent of tartrazine's known allergenic effects.

Study Assessment and Evaluation:

This parental behavioral ratings in this study revealed a dose related functional relationship between the artificial food color tartrazine (Yellow 5) and adverse behavioral changes in 24 of 54 children at dosage levels of tartrazine ranging from 1 mg to 50 mg. These tartrazine reactors included 19 of 23 diet-responsive children with behavioral problems (suspected but not diagnosed hyperactive), 3 of 11 uncertain diet-responsive children also with problem behaviors (suspected but not diagnosed hyperactive), and 2 of 20 control subjects (not diet responsive and no problem behaviors). There were no sex-related or age-related (preschool versus school aged) differences in these tartrazine effects. The investigators reported that the main behavioral features associated with the double-blind tartrazine exposure, as rated by parents using the devised Behavioral Rating Inventory, were irritability, restlessness, and sleep disturbance, but apparently not behaviors associated with hyperactivity such as inattention. Tabulations of these specific rating data were not presented in the study report. It is possible that the devised rating system itself may have inadvertently favored reporting of those three behaviors over the reporting of attention related behaviors, since there were more rating elements for irritability (11), sleep disturbance (9) and restlessness (4), than for attention span (3). Some suggestion that attention may have been affected to some extent by the tartrazine treatment is provided by the graphic presentations of rating data for two reactive subjects based on the Conners hyperactivity index, in which attention behavior is a core element, showing apparent dose related effects of tartrazine. Unfortunately, the parental ratings with the Conners index were not tabulated either in this report. Since the Behavioral Rating Inventory was not properly validated and without presentation and analysis of all of these rating elements, the question of whether treatment behaviors related to inattention may have occurred but were inadvertently underestimated cannot be resolved.

There are two primary experimental issues which could potentially affect the confidence in the reliability of the basic findings from this study, one involving the adequacy of the study blind and the other involving the fact that the parent ratings provided the only measure of behavior to assess treatment effects. With regard to blinding, while the investigators do state that the study was conducted under double-blind conditions (treatments provided to the parents in dated sealed envelopes) and that a special double capsule was used to administer treatment to the older (≥ 6 years) children and orange juice with a straw was used as the vehicle for the younger children, there was no apparent effort to determine whether the capsules and orange juice adequately blinded the various dosages of tartrazine or to verify the effectiveness of the blinding procedures for the parents and children. However, with results showing clear dose response effects across a range of multiple dosage levels of tartrazine it seems likely that the integrity of the blind was adequately maintained in this study. With regard to the use of parental ratings as the sole outcome measure in this study, confidence in the reliability of study findings would 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 across the dosage levels of tartrazine challenge, 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 tartrazine can cause in dose-related fashion across challenge doses of 1 to 50 mg adverse behavioral effects (consistently including irritability, restlessness and sleep disturbances and questionably associated with hyperactivity) in a subgroup of select children with problem behaviors who are suspected hyperactive and reportedly affected by diet.

(Schmidt et al, 1997)

Schmidt MH, Mocks P, Lay B, Eisert HG, Fojkar R, Fritz-Sigmund D, Marcus A and Musaeus B. Does oligoantigenic diet influence hyperactive/conduct-disordered children - a controlled trial. *European Child & Adolescent Psychiatry*, 1997, 6: 88-95.

A placebo controlled, double-blind dietary crossover study was conducted to examine the effectiveness of an oligoantigenic diet (eliminates all foods/additives assumed to provoke adverse behaviors in some children) in children diagnosed with ADHD/Disruptive Behavior Disorder, using multiple outcome measures of behavioral change. The dietary effects were also compared with the effects of stimulant medication treatment. Participants in this study consisted of an unselected sample of in-patients newly admitted to a hospital facility for treatment of hyperactive/disruptive behavior disorder. A total of 49 children (47 male, 2 female; 6 – 12 years) specifically diagnosed with ADHD and/or Conduct Disorder participated. The experimental diet was a very restrictive *oligoantigenic* diet that eliminates every kind of food and food additive (e.g. artificial colors) assumed to provoke behavioral reactions in certain children with beverages confined to apple juice and mineral water. The placebo control diet included common food items and ingredients, including food additives, assumed to be antigenic and also included daily beverages containing tartrazine (20 mg; FDC Yellow 5), quinoline yellow (10 mg), new cocchine (10 mg) and carmoisine (10 mg). This placebo diet was specifically designed to control for the confounding effects of ‘positive attention’ in that the children were able to detect the difference in appearance between the experimental (oligoantigenic) and placebo diets, but were not supposed to be able to identify the experimental diet. It was not specified whether or not the children were actually able to identify the experimental diet. All personnel involved in rating behaviors were blind to diet treatments. All 49 children were tested with the experimental diet, but only 37 were additionally tested under non-blind conditions with stimulant medication for comparative purposes only. The stimulant medication was methylphenidate (0.4 mg /kg, orally); no placebo was specified since raters were not blind to medication treatment. Procedurally, each subject went through the following phases of test: *Phase 1 /Pre-Study Baseline I* (4 days) - subjects were entered/acclimated to in-patient ward with standard food and assessment procedures (data not used for study); *Phase 2 / Diet Crossover Testing* (18 days @ 9 days per diet condition) - subjects were randomly assigned in pairs to the diet order of oligoantigenic/placebo or placebo/oligoantigenic and each diet was given for 9 days with two fixed assessment points at day 3 (T1) and day 8 (T2); *Phase 3 / Washout Baseline II* (9 days) – assessments were conducted once after at least 3 of the 9 washout days but diet condition (placebo control or regular clinic) was not specified; and *Phase 4 Methylphenidate Daily Treatment* (≥ 5 days determined individually) - assessments were made in the morning of days 3-5 of drug treatment, 1.5 hours after drug ingestion, but diet condition was not specified. Outcome measures included both behavior ratings and objective laboratory testing. The behavior ratings were conducted in three standardized situations: in classroom, rated by teachers using the established widely used Conners Parent/Teacher 10 item rating scale; at play in the ward, rated by trained observers using a specially constructed 18 item behavior checklist; and during performance testing, rated by trained observers using a specially constructed 14 item behavior checklist. The laboratory testing consisted of two performance tests (continuous performance task or CPT, and paired associate learning task or PAT) and a continuous measure of activity (actometer). Blood levels of total IgE were determined once during baseline and once during the experimental diet testing. It should be noted that for purposes of data analysis the response definition (i.e., treatment effect) in this study was based only on the ratings data and did not include analysis of data from the objective laboratory testing. Since there were no significant

differences in behavior ratings between T1 and T2, these two assessment points were averaged for each child, and since there was no treatment order effect for the behavior ratings, these data for children of both crossover groups were pooled. Repeated measures analyses of variance were performed to analyze the data.

Study Results: With the response definition based only on the ratings data, group level analysis showed significant diet effects with improved behaviors rated by trained observers in both the play setting and the laboratory testing setting for those children on the oligoantigenic diet (9 days) compared with the placebo diet. However, there was no confirmation of a diet related effect on behavior based on the teacher ratings of classroom behavior. Objective laboratory testing results were not presented. Analysis of the rating data for individual subjects revealed significant behavioral improvement on the oligoantigenic diet for 22 of the 49 children (45%) in the laboratory testing setting and for 21 of the 49 children (43%) in the play setting. Parenthetically, significant behavioral deterioration on the oligoantigenic diet was also found for 7 (4%) children in the play setting and 6 (12%) in the laboratory testing setting. In terms of children exhibiting clinically significant changes, defined as 25% improvement on two measures, 12 of the 49 children (24%) were identified as “responders” to the oligoantigenic diet, and 2 (4%) showing more problem behaviors. The non-blind assessment of the effects of methylphenidate treatment (≥ 5 days) was based on behavioral ratings in the play and laboratory testing settings only. Since the teacher ratings of classroom behavior did not reveal any diet effects, the investigators excluded classroom ratings from further group and individual analyses including those for the methylphenidate treatment. Therefore, the statement by the investigators that classroom ratings were not able to demonstrate the expected treatment effects of stimulant therapy is not completely accurate. Sixteen of 36 children (44%) with complete data showed clinically significant improvement in behavior (i.e., "responders") and 4 (11%) children showed deteriorated behavior with drug treatment. Overall, out of the 36 children who received both diet and methylphenidate testing, 11 (31%) improved on drug only, 3 (8%) improved on diet only, and 5 (14%) improved on both drug and diet. IgE levels, measured at baseline and under oligoantigenic diet condition, showed no differences between diet and baseline.

Study Assessment and Evaluation: In a sample of 49 children with ADHD/Conduct Disorder problem behaviors, 12 (24%) exhibited what was defined as clinically significant improvement in behavior when treated with an oligoantigenic diet for 9 days, based on behavior ratings by trained observers in two behavior settings (play and laboratory testing settings). But the teacher ratings in the classroom setting did not confirm any diet related effects on behavior. The investigators suggest that this negative finding in the classroom was the result of the teachers being highly experienced in dealing with psychiatric patients and that "biased ratings of a reduced range are, thus, rather to be expected". However, these results should be interpreted in light of the fact that the two behavioral rating checklists that were used in the play and laboratory testing settings were newly developed specifically for this study. While the investigators stated that on-site testing indicated both of these scales to be highly reliable and that significant correlation of both scales with activity measures appeared to support their validity, these newly devised rating scales have not been completely validated through use by other investigators. In contrast, the Conners behavioral scale used for the teacher rating in the classroom setting is a well-established, widely used, and validated rating scale. Although several objective laboratory tests were also conducted, unfortunately none of those data were presented or analyzed. Consequently, no additional information is available to corroborate any diet related effects. Regarding the relative effectiveness of diet and drug, although more patients appeared to respond to methylphenidate than to diet (44% and 24%, respectively), the level of improvement for those responders was comparable between both treatments. The absence of diet related differences in

IgE levels in this study do not support the notion that an IgE-related mechanism underlies the behavioral effects associated with food intolerance.

In addition to the inconsistent findings with significant diet related changes in behavior ratings by trained observers (play and laboratory testing settings) but no confirmation of diet effects in ratings by teachers (classroom setting), there are two other issues related to this study which significantly affect the confidence in the reliability of the study findings: (1) There is a possibility of a biasing influence that the clearly distinguished diets may have had on the behavioral ratings. No efforts were described to verify the effectiveness of the blinding, e.g. determine whether or not the children could actually tell which of the two diets was the experimental diet, whether they thought one of the diets should (adversely) affect their behavior, or whether the children told the raters which diet they were presently eating. (2) The response definition (i.e., treatment effect) in this study was based only on the behavioral ratings data and did not include data from the completed objective laboratory testing. The investigators considered the objective testing as less comprehensive, confounded by variables such as motivation, insensitive to subtle treatment effects, providing information already included in the behavioral ratings, not suitable for assessing response in a clinically relevant sense due to ceiling effects (CPT and PAT). It is unclear why these concerns were not considered relevant when these objective laboratory tests were included in and actually completed as part of the study design. The original inclusion of several objective laboratory tests in addition to the behavioral ratings was an experimental design feature that was viewed as adding credibility to the study by providing confirmatory evidence of treatment related effects. Simply dismissing these validly conducted test results *post hoc*, particularly without even presenting the data or their analyses, appears to be scientifically inappropriate and significantly lessens the confidence in the credibility of this study and in the completeness and reliability of the findings. Overall, considering the inconsistent behavioral findings (i.e., significant behavioral effects with ratings conducted in play and laboratory testing settings but no confirmation of behavioral effects with teacher ratings in the classroom), the use of incompletely validated behavioral checklists for ratings in play and laboratory test settings, the fact that objective laboratory tests (CPT, PALT and actometer readings) were collected but neither presented nor analyzed to help determine or confirm treatment related effects, together with the questionable effectiveness of the diet blinding conditions, these study results should be considered at best as only suggestive of a possible limited beneficial effect of the oligoantigenic diet in a small number of children with a profile of disruptive behaviors involving ADHD and Conduct Disorder. Alternatively expressed, this study may suggest that food intolerance may exaggerate some adverse behaviors in a small group of select children with ADHD/Conduct Disorder.

(Uhlig et al, 1997)

Uhlig T Merckenschlager A Brandmaier R Egger J. Topographic mapping of brain electrical activity in children with food-induced attention deficit hyperkinetic disorder. Eur J Pediatr, 1997 (July), 156(7):557-61.

A crossover clinical study was designed to investigate whether changes in brain electrical activity detectable by EEG topographical mapping occur in food sensitive ADHD children when exposed to provoking foods. The study was conducted in three phases - Phase I involved identifying ADHD children responsive to an oligoantigenic diet. The children participating in this phase were outpatients in a special dietary clinic and all were diagnosed ADHD with

Conners rating scores between 15 and 30 (6-15 years old; number and sex unspecified). For 3 weeks children received an open (non-blind) oligoantigenic (few foods) diet, which is a very restrictive diet that eliminates every kind of food and food additive assumed to provoke adverse behavioral reactions in certain children. Based on non-blind observations by parents and teachers and on Conners scores below 15 (the index level for ADHD), 71% of the children on diet showed improved behavior and were entered into the next phase of testing. Phase II involved the non-blind reintroduction of foods to identify specific provoking food items. With the children continuing on the oligoantigenic diet, eliminated foods were reintroduced one by one at the rate of one every 5 days. If hyperactive related behaviors did not occur that food item was incorporated into the diet. The provoking foods in order of prevalence were beet sugar, colors, wheat, cow milk, banana, egg, citrus, cacao, beef, pork, and oats. Phase III involved the experimental testing of provoking food items and EEG mapping. Fifteen children were entered into Phase III but only 12 completed testing. Procedurally, testing of each child was carried out both under “consuming” and “avoiding” provoking foods conditions. Eight children were tested in the order, “avoiding” then “consuming”; the other 4 children were tested in the reverse order, “consuming” then “avoiding”. For the “avoiding” condition, children avoided provoking foods (oligoantigenic diet) for 14 days before EEG mapping. For the “consuming” condition, children ate normal helpings of their provoking foods daily (regular diet) for at least 5 days prior to and on the day of EEG mapping. Between the avoidance and consumption of provoking foods a 1-week washout period was interposed, although it was not stated what diet the children were receiving during washout. Outcome measures consisted of EEG recordings for topographic mapping conducted in 20 minute sessions at the end of each diet treatment condition and Conners behavior ratings conducted daily and during EEG recording sessions. Notably, only one study investigator was stated as being blind to order of testing but behavioral assessments were conducted by two investigators, and no other study personnel, including parents and children, were described as being blinded to treatment. So, it appears that the study was conducted virtually without effective blinding. The Wilcoxon two sample test was used to analyze data.

Study Results: Conners scores were significantly higher while the children were consuming the provoking foods. No order effect was detected. Significant differences between recordings after consumption or avoidance of the provoking food items were seen in the fronto-temporal regions of the brain with few changes in the parieto-occipital areas. Percentages of fast Beta-1 frequency electrical activity increased in frontal areas of the brain in association with abnormal behavior (elevated Conners scores) following consumption of provoking food items. The relative power spectra of all recordings while consuming provoking food items were significantly higher than during avoidance of the provoking food items.

Study Assessment and Evaluation: Unfortunately, in an otherwise well-conducted study, the critical absence of any apparent blinding conditions introduces the possibility of an unintentional influence on the behavior of the children, confounding 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, the results from this study cannot be interpreted to indicate any association between exposure to “provoking” foods (food intolerance) and specific EEG changes or behavioral rating changes. 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).

(Bateman et al, 2004)

Bateman B, Warner JO, Hutchinson E, Dean T, Rowlandson P, Gant C, Grundy J, Fitzgerald C and Stevenson J. The effects of a double blind, placebo controlled artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. Arch Dis Child, 2004 (June), 89(6): 506-11.

A double blind, placebo controlled challenge study was designed to determine whether food additives can cause hyperactive related behaviors in a sample of preschool children from the general population with and without hyperactivity and with and without atopy (exhibit allergic reactions). The 277 children who completed the challenge study were recruited from the general preschool population (Isle of Wright, UK) (average age 3 years) were assessed for hyperactivity and evaluated for atopy. ADHD was not clinically diagnosed but children were assessed for hyperactivity using two scales, completed by parents, which rated inattention, overactivity, fidgetiness, and impulsivity. Those children above a defined threshold scoring level were designated hyperactive (HA) and represented a distinct group of hyperactive 3 year old children. A wider range of behavioral problems were also assessed using the Behavioral Checklist scale. Children were defined as atopic (AT) if they showed an allergic reaction in a skin prick tests. The 277 children were sorted into 4 study groups: 36 HA/AT (22 male, 14 female), 75 HA/non-AT (39 male, 36 female), 79 non-HA/AT (44 male, 35 female), and 87 non-HA/non-AT (46 male, 41 female). The challenge item was a 300ml drink of mixed fruit juices with/without 20 mg mix of artificial food colors (5 mg each of sunset yellow, tartrazine, carmoisine, ponceau 4R) plus 45 mg sodium benzoate. The placebo and color drinks were in identical sealed bottles of the same appearance. Preliminary testing by 34 adults showed that the drinks could not be accurately differentiated on blind testing. All rating team members and family were blind to treatment. At the end of the study period the parents were equally divided into those who did or did not correctly identify the drink order (meaning that approximately half of the parents did correctly identify the drink order). Procedurally, after baseline data collection, all children were placed on a diet free of artificial color and benzoic acid for four weeks. Weeks 1 and 3 were "washout" weeks with no treatments. During weeks 2 and 4 all children received daily drinks of either placebo fruit juice or color challenge fruit juice (one type treatment per week determined randomly). Drinks were to be taken at home over the course of the day. Apparently, no drinks were given on the other study weeks. The two primary behavioral outcome measures included: (1) weekly clinical tests analyzed as an Aggregated Test Hyperactivity score (ATH), reflecting task performance and psychologist recording of behavioral measures of inattention, activity, and impulsivity; and (2) weekly parent ratings of behaviors from the Weiss-Werry-Peters activity scale analyzed as an Aggregated Parental Hyperactivity Rating score (APHR). In a preliminary analysis the clinical testing, including the ATH score, was validated as reliably distinguishing the cognitive and behavioral differences between hyperactive and non-hyperactive preschool children at baseline. Analysis of variance and analysis of covariance was used for to analyze the data.

Study Results: APHR (parents ratings) showed an overall significantly greater increase in hyperactivity for the active color challenge than for the placebo but there was no interaction with either initial hyperactivity or atopy (i.e., based on parent behavior ratings, adverse behavioral response to color/benzoate challenge occurred in both hyperactive and non-hyperactive children and in both allergic and non-allergic children). Curiously, there were increased levels of hyperactivity behavior for both the placebo and color/benzoate challenge periods, but a greater

increase in hyperactivity during the active periods. However, no behavioral effects of color/benzoate challenge were found with the ATH/clinical measures.

Study Assessment and Evaluation: In a group of 277 preschool children from the general population with/without hyperactivity and with/without atopy, overall group analysis of parent ratings indicated that the children exhibited a significantly greater increase in hyperactivity behaviors when challenged daily for one week with 20 mg artificial colors and 45 mg benzoic acid than with placebo treatment (both active and placebo treatments increased hyperactive behaviors, but active challenge a greater significant affect). This effect was seen for both the hyperactive and non-hyperactive children and for both the atopic and non-atopic children. However, it is not known what percent of the children were reported to be responsive to the color/benzoate challenge. In contrast to the parental ratings, the clinical testing measures, previously validated in-house for distinguishing between hyperactive and non-hyperactive children, provided no confirmatory evidence of any behavioral changes associated with the color/benzoate challenge in any of the children. In evaluating these contradictory findings, consideration should be given to the fact that, while efforts were made to maintain the blinding of all study participants, the interviews at the end of the study period found that the parents were equally divided into those who did or did not correctly identify the challenge treatment order. This would indicate that approximately half of the parents may not have been completely blind to treatment conditions. There would be more confidence in the parental ratings if they could be supported by observations by independent observers or by the use of standardized tests. In view of the equivocal finding of treatment effects 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 support for the suggestion 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 behaviors and that this intolerance may be associated with only limited behavioral changes.

(McCann et al, 2007)

McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K, Kitchin E, Lok K, Porteous L, Prince E, Sonuga-Barke E, Warner JO, Stevenson J. Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial. Lancet, 2007 (November 3), 370 (9598):1560-7.

McCann et al (2007) conducted a controlled challenge study to test whether intake of artificial food colors and additives (AFCA) affected childhood behaviors in a general population of preschool and school aged children. This study was designed in part to replicate a previous study on preschool children, to extend the investigation to school aged children, and to use a wider range of behavior measures. Two age groups of children, 3-4 year-old (preschool) and 8-9 year old (school aged), from the general population in Southampton, UK were recruited for participation. The initial samples consisted of 153 preschool children (79 male, 74 female) and 144 school aged children (75 male, 69 female). Neither group was diagnosed hyperactive or identified as having appreciable problem behaviors. The challenge material was a fruit juice drink containing one of two different color mixes plus sodium benzoate (Mix A and B). Two sets of each Mix were used, each set consisting of a Mix with lower color levels and a Mix with

higher color levels (lower levels for challenging preschool children and higher levels for challenging school aged children). Mix A/preschool contained 20 mg mixed color (5 mg sunset yellow; 2.5 mg carmoisine; 7.5 mg tartrazine; 5 mg ponceau 4R) and 45 mg sodium benzoate. Mix B/preschool contained 30 mg mixed color (7.5 mg sunset yellow; 7.5 mg carmoisine; 7.5 mg quinoline yellow; 7.5 mg allura red AC) and 45 mg sodium benzoate. Mix A/school-aged contained 25 mg mixed colors (levels of same colors increased by 1.25 times; sodium benzoate remained at 45 mg). Mix B/school-aged contained 62 mg mixed colors (levels of each color increased by 2.08 times; sodium benzoate remained at 45 mg). The preschoolers received 300 ml juice/day and the school aged children 625 ml/day. The placebo was mixed fruit juice. Families and all members of research team were blind to treatment. To test the challenge blind, two panels of 20 adult subjects were tested and reportedly were not able to distinguish the color mixes and placebo. Parents and children were not asked to verify blinding.

Procedurally, the study was conducted as a within-subject crossover challenge between two active color/benzoate mixes (A and B) and a placebo drink. During the initial baseline week (W0) regular diets were used, but during the subsequent 6 study weeks (W1-W6) a 'special diet' was used from which the additives used in the challenges (artificial colors and sodium benzoate) were withdrawn and the following weekly schedule of treatment was followed for all children: W1- washout week with daily placebo, W2 - daily active challenge or placebo, W3 - washout week with daily placebo, W4 - daily active challenge or placebo, W5 - washout week with daily placebo, W6 - daily active challenge or placebo. Challenge weeks were randomized for 2 active challenges (Mix A and Mix B) and 1 placebo treatment. Three sources of outcome behavioral measures were used for both the 3 year old and the 8/9 year old groups: (1) a weekly teacher ADHD rating, (2) a weekly parent hyperactivity rating (Weiss-Werry-Peters scale for preschool children and an ADHD rating scale IV for school aged children), and (3) a weekly trained observer classroom observation rating. One additional objective source measure, used for the school aged children only, was a weekly assessment of attention and response inhibition with the Conners continuous performance test. A single Global Hyperactivity Score (GHA) was calculated as a composite score of all test measures and used to assess individual differences in behavior based on the collective information from the different sources of outcome measures (teacher, parent, trained observers, and computerized laboratory test) covering various components of hyperactivity (overactivity, impulsivity, inattention). Because of differences between the testing of preschool and school aged children in the composition of the GHA scores and in the levels of colors used, the data for the two age groups could not be analyzed jointly. So, for purposes of data analysis the studies with the two age groups were treated as parallel but independent. Linear mixed-model methods were used to analyze the data and potential confounding factors were controlled. The data analyses were conducted for the full sample of children (the principle analysis for this study's findings) and replicated for a high consumption group (>85% consumption of drinks in any challenge week) and a complete case group (high consumption and no missing GHA scores). The latter two analyses were intended to determine whether non-compliance and the method of handling missing data affected the pattern of results.

Study Results: Based on differences in the composite GHA scores for the full sample, 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. In terms of the magnitude of the behavioral changes, the effect sizes in this study averaged at about 0.18. The specific nature of the treatment related behavioral effects, generally referred to as increases in the mean level of hyperactivity, were not described. Also, other than the composite GHA scores, the investigators did not present the results for any of the component

outcome measures, i.e. teacher scores, parent scores, trained observer scores, and computerized laboratory testing.

Study Assessment and Evaluation: The fact that significant behavioral effects were found for the preschool children with Mix A but not Mix B and for the school aged children with Mix B but not Mix A seems to be a puzzling inconsistency. The investigators even acknowledge the need for additional study "to establish whether the age-related differences seen in the present study can be replicated". Also, the specific nature of the behavioral effects were not described, other than being generally referred to as increases in the mean level of hyperactivity. The children used in this study were from the general population and were not specifically diagnosed as ADHD. Consequently, the behaviors measured in this study, even though treatment effects were referred to by the investigators as increases in levels of "hyperactivity", were probably not the disruptive hyperactivity behaviors of ADHD but more likely the type of hyperactivity exhibited occasionally by the general population of preschool and school aged children. Furthermore, the magnitude of whatever behavioral changes may have been associated with the active challenges appeared to be rather small, with the effect sizes in this study averaging about 0.18 which is approximately equivalent to less than 10% of the difference between children with ADHD and without that disorder. Other than the composite GHA scores, the investigators did not present the results for any of the component outcome measures, i.e. teacher scores, parent scores, trained observer scores, and computerized laboratory testing, to enable assessing whether the various source measures were equally sensitive in detecting a treatment related change in behavior. In fact, information obtained by this reviewer from an earlier critique of the final report for this study showed that the component parent ratings appear to be one of the primary sources detecting treatment effects in both age groups of children but that the teacher and trained observer ratings were less able to detect significant treatment effects. It might have been more likely for teachers to have observed challenge related effects on behavior since many but not all children drank the juice challenge in the morning at breakfast and the children might have been expected to exhibit effect, in any occurred, during the school day. Unfortunately, this study appeared to lack control over when the challenges were ingested relative to timing of measures of behavior. The computerized testing in the older children did appear to detect some challenge related effects but there were unanticipated general performance decrements with repeated testing ostensibly attributed to boredom with that testing paradigm. The prominence of parental ratings, but not other clinical test measures, in detecting treatment related behavioral effects was also reported in the study by Bateman et al (1994). Overall, the primary study findings are suggestive of low level behavioral effects of a one week exposure to AFCAs on behavior in 3/4 year old children (Mix A) and 8/9 year old children (Mix B), based solely on parental ratings. However, due to the study weaknesses and caveats described above, particularly the puzzling inconsistency in response by the two age groups of children to both active challenges 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.