COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

STATEMENT ON RESEARCH PROJECT (T07040) INVESTIGATING THE EFFECT OF MIXTURES OF CERTAIN FOOD COLOURS AND A PRESERVATIVE ON BEHAVIOUR IN CHILDREN

1.	The COT was asked by the Food Standards Agency to review the results of a research project investigating the effect of two mixtures of certain artificial food colours together with the preservative sodium benzoate on behaviour in children. The study had been carried out by researchers at the University of Southampton and was funded by the Food Standards Agency. The research had been submitted for publication and the COT was provided with three draft scientific manuscripts and a commentary that had been written by the researchers, for review. The research was subsequently published as a single paper in the Lancet[1].

2.	The Committee was grateful for the advice of a number of external experts which informed its discussion of this research. These were Prof. Eric Taylor and Prof. Emily Simonoff of the Institute of Psychiatry, Ms Eleanor Allan of the University of Reading Statistical Services Centre, and Prof. Ian Kimber as Programme Advisor to the Agency's T07 Food Allergy & Intolerance Research Programme, under which this project was commissioned.

Background

3.	Hyperactivity, is a term that is somewhat ill defined but is used by most people to mean overactivity. To others it is associated additionally with inattention and impulsivity. Inattention, impulsivity and hyperactivity occurring together, and to a significant degree, comprise a behavioural disorder which adversely affects children's function at home and in school. This disorder is known as Attention Deficit Hyperactivity Disorder (ADHD) or Hyperkinetic Disorder (HKD). ADHD typically has onset in early childhood and is characterised by specific patterns of behaviour[2]. A review of international studies by Swanson et al. in 1998 suggested that the condition affects 5-10% of school age children[3]. In the UK, the best estimate of prevalence in children is 2.4%, based on data from a survey of 10,000 nationally representative children in the 1999 British Child and Adolescent Mental Health Survey[4]. The aetiology of the disorder is thought to be multifactorial, with both genetic (heritable) and environmental factors reported to be involved (the latter including for example, prematurity[5], institutionalised upbringing[6], and maternal smoking during pregnancy[7]).

4.	The COT had considered the results of a previous research study known as 'the Isle of Wight Study'[8], on the effect of food colours and a preservative on behaviour in children and issued a statement on that research in 2002 (statement available at http://www.food.gov.uk/science/ouradvisors/toxicity/statements/cotstatements2002/cotfoodadditives). The Committee had reservations about interpretation of the findings in

view of some aspects of the study design. The Committee noted that the results were consistent with published reports of behavioural changes occurring in some children following consumption of particular food additives. However, it was not possible to reach firm conclusions about the clinical significance of the observed effects. There had been a large placebo effect which had limited the ability to interpret and make generalisations about the results. In addition, statistically significant effects on behaviour had been observed only via parental reports of their children's behaviour, and had not been evident in the objective assessments that had been performed by independent researchers in a clinical setting.

5.   Subsequently, the Food Standards Agency set up an *ad-hoc* Working Group of independent experts to consider the feasibility of further research on this subject and to advise on study design. The recommendations of this *ad-hoc* Working Group were published in 2003 and the Agency commissioned a new study via open competition in 2004, incorporating the design changes that had been recommended by the *ad-hoc* Working Group. It was the results of this new study that the COT was asked to review.

Study design for the new research

6.   The primary hypothesis tested by the researchers was that mixtures of certain artificial food colours with the preservative sodium benzoate compared with a placebo increase the mean level of hyperactive behaviour of children drawn from the general population. With a minimum target sample of 80 children, the study had 80% power at alpha = 0.05 to identify an effect size of 0.32 standard deviation units (SDU) in the mean score on a hyperactive behaviour scale for the active compared with the placebo periods of the food challenge.
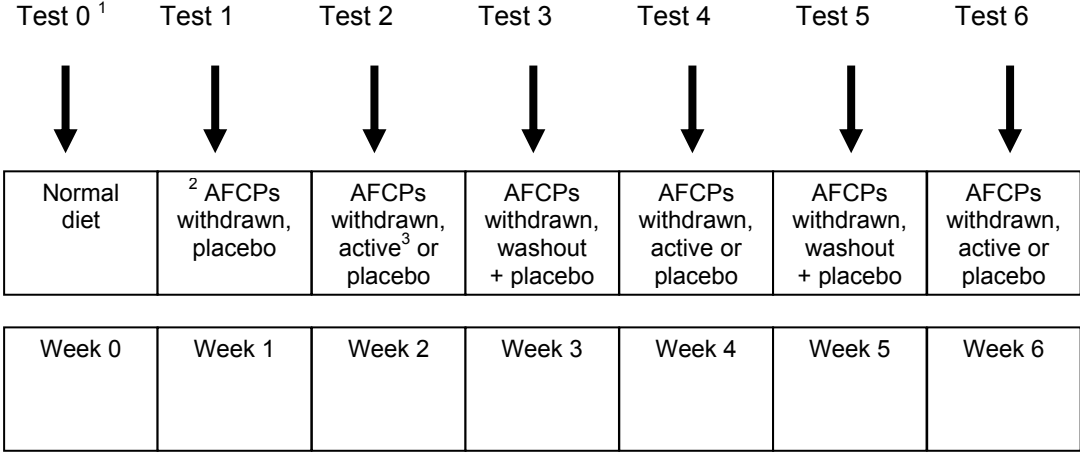
7.   Secondary research questions addressed: whether genetic differences moderate any observed effect; whether there are effects in both pre-school and older children; whether any response to the additive mixtures is related to initial levels of hyperactive behaviour as scored on a hyperactive behaviour scale: and whether any response is seen via teacher ratings, direct observations of behaviour and computer based test performance as well as via parental ratings.

8.   The researchers employed a double blind placebo controlled randomised cross-over food challenge to investigate the effect of two different mixtures of additives on the behaviour of children of both sexes and in two age groups. Children who took part in the study were selected from families volunteering from, in the case of 3 year olds, nurseries, day nurseries, preschools, playgroups and, in the case of 8 to 9 year olds, schools, in the Southampton area. Although there was a degree of self-selection in that families volunteered to take part in the study, the children that were recruited to the study (153 aged 3 years and 144 aged 8 to 9 years) from those who volunteered (n=209 and 160, respectively), were selected to represent the full range of behaviour in the general population, from normal through to high level hyperactivity. However, children who were on medication for ADHD or for whom it was considered by the researchers that the additive challenge could compromise medical treatments being given for other conditions, were excluded from the study.

9.   The families were given instructions that the children should maintain, for the duration of the study, a diet that excluded the artificial food colours used in the trial and sodium benzoate used as a preservative. Compliance with the diet was monitored by

means of a diary which parents completed to indicate the level of consumption of the challenge drinks and compliance with the diet over the study period. The outline design of this sub-acute challenge trial, which formed the main part of the study, is shown in the following diagram:

Fig. 1 Design of the double blind placebo controlled food

Test 0 [1]	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6
Normal diet	[2] AFCPs withdrawn, placebo	AFCPs withdrawn, active[3] or placebo	AFCPs withdrawn, washout + placebo	AFCPs withdrawn, active or placebo	AFCPs withdrawn, washout + placebo	AFCPs withdrawn, active or placebo
Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6

[1] 'Test': assessment of children's behaviour
[2] AFCPs (Artificial Food Colours and Preservatives) withdrawn: exclusion from the diet of those artificial food colourings and of the preservative sodium benzoate, which were used in the active mixtures
[3] 'Active': either of two specific mixtures of food colours and sodium benzoate

10.   During the 6 week challenge, children received batches of drinks on a weekly basis, one drink to be consumed on each day. Instructions to parents were that the challenge drinks should be consumed at home so that compliance could be monitored. During the wash-out weeks (weeks 1, 3 and 5) all children received a placebo drink of mixed fruit juices. During the challenge weeks (weeks 2, 4 and 6 in Fig. 1), the drinks that children received were either the placebo, or a drink of juices of identical appearance and taste containing one or other of the two additive mixtures. The order of receipt of the three drink types (Mix A, Mix B or placebo) across the three challenge weeks was allocated at random. Blinding tests conducted at the beginning and part way through the study established that two independent panels of 20 adults of similar age to the parents of the children in the study could not distinguish between the active and placebo drinks, but blinding was not assessed in children. Behaviour was assessed in each week of the study to avoid a perceived difference in treatment, but data deriving from weeks 1, 3 and 5 (the washout weeks) were not included in the analyses.

11.   Behaviour was assessed using a range of different measures, including assessments by parents in the home, and by teachers and independent observers in a classroom setting. For the older children only, behaviour was additionally assessed via a computer-based attention task. For each individual measure, behaviour was scored using standardised and validated hyperactive behaviour assessment tools. Parents and teachers were asked to rate each child's behaviour over the previous week and independent assessors observed each child for 3 separate periods each week. Ratings of behaviour from each of the individual measures (teacher, parent, independent observer and computer task) were combined, un-weighted, to give an overall weekly Global Hyperactivity Aggregate (GHA) score of each child's level of

hyperactive behaviour. This GHA measure of behaviour was a novel metric devised by the researchers to derive an overall outcome measure that combined both subjective and objective behavioural measures.

12.   During the food challenge trial, DNA from buccal swabs collected from all children participating in the challenge was subjected to genotype analyses. The aim was to determine whether allelic variation in certain genes that have previously been implicated in ADHD influenced any observed effects of the food colour and benzoate preservative test mixtures on the children's behaviour. The genes studied included genes from the dopamine neurotransmitter system (gene catechol-o-methyltransferase, polymorphism COMT Val108Met), from the adrenergic neurotransmitter system (gene ADRA2A, polymorphism ADRA2A C1291A), and from the histamine neurotransmitter system (gene HNMT, polymorphisms HNMT T939C and HNMT Thr105lle).

13.   The primary analysis of the data from the main 6 week repeat dose challenge trial was on an intention-to-treat basis (i.e. including data obtained from the whole cohort), and was based on use of the GHA as the primary outcome measure. The researchers also carried out a number of additional *post-hoc* analyses on the data. These included analysis of the GHA data for a sub-set of the subjects (approximately 80% of total) who had consumed ≥ 85% of the drinks. This was a pragmatic level chosen to represent the equivalent of full consumption on 6 out of 7 days in a challenge week. A further *post-hoc* analysis of the GHA data based on another sub-set of the subjects who had consumed ≥ 85% of the drinks and for whom there were no missing data, was also conducted. Finally, the researchers conducted some analyses on the data relating to the disaggregated behaviour measures (i.e. analysis of the behaviour scores from the parental assessments, teacher assessments, independent observer assessments and from the computerised test of attention, separately) for the sub-set who had consumed ≥ 85% of the drinks and subsequently for the whole cohort. All of these analyses used data from behaviour assessments made in the baseline week (Week 0) and in weeks 2, 4 and 6 of the food challenge.

14.   Details of the identity and dose of the additives in the challenge mixtures are given in Table 1. The doses were determined by the researchers based on the amount of the additives to be administered per child per day. Both additive mixtures administered to both age groups contained the same amount of sodium benzoate. For the colours, the amounts in Mix A given to 3 year olds were identical to those used in the previous (Isle of Wight) study. For 8 to 9 year olds the amounts of the colours in Mix A were increased by 25% to reflect the greater food intake by these older children. For Mix B for 8 to 9 year olds, the amounts of the colours in the mixture reflected what a child could reasonably consume in a day and were based on average consumption of foods containing colours with the assumption that the colours were included in those foods at their maximum permitted levels. Constraints regarding the maximum concentration of additives in the test drinks, which could not exceed the regulatory limits, meant that, for 3 year olds to consume equivalent amounts of Mix B colours to the older children, they would have been required to consume a 500ml drink on a daily basis. This was not regarded as feasible by the researchers and was considered likely to affect compliance adversely. Therefore, the volume of Mix B in the daily drink given to the 3 year olds was kept at 300ml which necessitated a consequential reduction in amounts of the Mix B colours that could be administered to this age group as shown in Table 1.

15.   For the purposes of the COT evaluation and comparison with the Acceptable Daily Intake (ADI), the doses are also expressed on a mg/kg body weight (bw) basis in Table 1. These were calculated using average body weights for the two age groups obtained from UK National Diet and Nutrition Survey data[9,10], because the actual body weights of the children in the study were not recorded. On a mg/kg bw basis the younger children received higher doses of the additives in Mix A, whereas for Mix B the doses were comparable across the age groups.

Table 1: Composition of the food additive challenge mixtures used in research project T07040

Name of Additive (E number)	ADI [1] (mg/kg bw)	Mix A 3 year olds mg/day (mg/kg bw/day) [2]	Mix B 3 year olds mg/day (mg/kg bw/day) [2]	Mix A 8 to 9 year olds mg/day (mg/kg bw/day) [3]	Mix B 8 to 9 year olds mg/day (mg/kg bw/day) [3]
Tartrazine (E102)	7.5	7.5 (0.50)	0	9.36 (0.30)	0
Ponceau 4 R (E124)	4	5.0 (0.33)	0	6.25 (0.20)	0
Sunset Yellow (E110)	2.5	5.0 (0.33)	7.5 (0.50)	6.25 (0.20)	15.6 (0.50)
Carmoisine (E122)	4	2.5 (0.17)	7.5 (0.50)	3.12 (0.10)	15.6 (0.50)
Quinoline yellow (E104)	10	0	7.5 (0.50)	0	15.6 (0.50)
Allura Red AC (E129)	7	0	7.5 (0.50)	0	15.6 (0.50)
Total colouring per day (mg)		20	30	25	62.5
Volume of drink given daily (ml)		300	300	625	625
Concentration of colour in mg/L		66.7	100	40	100
Sodium benzoate (E211)	5	45 (3)	45 (3)	45 (1.45)	45 (1.45)

[1]   The ADI is an estimate of the amount of a substance in food or drink, expressed on a body weight basis, that can be ingested daily over a lifetime by humans without appreciable health risk.
[2]   Based on average body weight of 15kg for a 3 year old [ref 9]
[3]   Based on average body weight of 31kg for an 8 year old [ref 10]

16.   The researchers also included a 'proof of principle' acute challenge to explore the possibility of demonstrating short term changes in hyperactive behaviour immediately post challenge. This comprised a double blind cross-over acute challenge study in a sub-set of two groups of 15 of those 8 to 9 year old boys who were considered to have responded or not responded to the additives in the 6 week sub-acute challenge trial. Mix B or placebo was administered and the children's behaviour was then assessed over a three hour period using independent observer ratings and the specific computer based attention task.

Differences in the study design compared with the previous Isle of Wight study

17.   The design of the new study had incorporated the following key changes compared with that of the previous study conducted on the Isle of Wight. A drink was administered to children daily throughout the 6 week challenge period, including the

initial withdrawal period, with the aim of reducing the placebo effect that had been observed in the previous study. A second, older group of children (8 to 9 year olds) was included, in addition to conducting the trial on 3 year old children as in the Isle of Wight study. A second mixture of additives (referred to as Mix B) was included with a different combination and amount of food colours from that administered to children in the Isle of Wight study (referred to as Mix A). The inclusion of an older group of children and of a second mixture of food colours and sodium benzoate at levels that were reflective of what an average child could consume in a day was in line with the recommendations of the *ad-hoc* Working Group.

18.   Behaviour was assessed using a wider range of measures than had been used in the Isle of Wight study. Teacher and independent observer assessments were conducted in a normal classroom setting and aggregated with the parental ratings (and for the older children only, with the results of the computer-based attention task), into the GHA score. This GHA score was the primary outcome measure for the study. It was formulated by the researchers to enable incorporation of both the objective assessments of behaviour (collected in a real life setting), and the subjective assessments of behaviour, into a single outcome measure, in order to address a concern raised in relation to the previous study that effects had only been detectable via the parental assessments and not by the more objective assessments of behaviour performed in the clinic.

Results

19.   The results presented in this section are based on the statistical analyses carried out by the researchers in which the effects of certain possible confounders were adjusted for within the analysis. The factors controlled for were: week during the study; sex; base-line GHA; number of additive containing foods consumed per day in the pre-trial diet; maternal educational level and social class.

20.   Table 2. summarises the results of the primary data analysis on the GHA scores (on the whole cohort), and also the results of the *post-hoc* analysis performed on the sub-group which consumed ≥ 85% of the drinks and for whom there were no missing GHA data.

Table 2: Summary of analysis of changes in GHA scores following challenge with Mix A or B compared with placebo, for the whole cohort (primary analysis) and a sub-group consuming ≥ 85% of the challenge drinks and no missing data (*post-hoc* analysis)

		Mix A	Mix B
whole sample (primary analysis)	3 year olds (n = 140)	0.20 (0.01 to 0.39)*	0.17 (-0.03 to 0.36)
	8 to 9 year olds (n = 136)	0.08 (-0.02 to 0.17)	0.12 (0.03 to 0.22)*
≥ 85% consumption and no missing GHA data (*post-hoc analysis*)	3 year olds (n = 73)	0.32 (0.05 to 0.60)*	0.21 (-0.06 to 0.48)
	8 to 9 year olds (n = 91)	0.12 (0.02 to 0.23)*	0.17 (0.07 to 0.28)*

*Statistically significant (at $p < 0.05$)
Scores are expressed as mean SDU with 95% confidence intervals in parentheses

21. The researchers found a statistically significant increase in the level of hyperactive behaviour, as measured by the GHA scores, when the children were challenged with Mix A compared with the placebo in the whole group of 3 year olds. The mean increase was 0.20 SDU (95% CI 0.01 to 0.39 SDU), n = 140. In the whole group analysis for the 8 to 9 year old children, the mean increase was 0.08 SDU (95% CI -0.02 to 0.17 SDU), n = 136 which was not statistically significant. The slightly lower numbers of children included in the analysis ('n'), compared with the numbers originally recruited (detailed in paragraph 8) reflect that a few children from each age group dropped out of the study after the trial had started. Drop-outs occurred for a variety of reasons, including parental pressure of work or other commitments, medical reasons, behaviour related to the child or inadequate juice consumption. No differences were found in terms of age, gender or marital status of parents between those who dropped out and the resulting cohort.

22. The results of the whole group analyses for Mix B were rather more consistent across age groups although here, too, statistical significance was reached in only one of the age groups. A statistically significant increase in the GHA scores was reported for the 8 to 9 year olds (mean increase = 0.12 SDU, 95% CI 0.03 to 0.22 SDU). For 3 year old children, the mean change in behaviour score was of similar magnitude (0.17 SDU), but with a wider 95% confidence interval (-0.03 to 0.36 SDU).

23. Similar changes in the mean GHA scores were seen in the *post-hoc* analysis of the subgroup consuming 85% or more of the drinks and for whom there were no missing data. For Mix A, the mean increases compared with the placebo were 0.32 SDU (95% CI 0.05 to 0.60 SDU) in the 3 year olds and 0.12 SDU (95% CI 0.02 to 0.23 SDU) in the 8 to 9 year olds, both of which were statistically significant increases. For Mix B, the mean increases compared with the placebo were 0.21 SDU (95% CI -0.06 to 0.48 SDU) in the 3 year olds and 0.17 SDU (95% CI 0.07 to 0.28 SDU) in the 8 to 9 year olds. Here the increase was statistically significant only in the case of the 8 to 9 year olds.

24. The observed increases in the GHA scores were not statistically significantly modified by sex, pre-trial level of hyperactive behaviour, additive content of the children's pre-trial diet, maternal education level or maternal social class.

25. Based on consideration of the subgroup of children who had consumed ≥ 85% of the challenge drinks, the researchers found that the observed increases in the GHA scores with Mix A in 3 year olds and 8 to 9 year olds and with Mix B in 8 to 9 year olds were statistically significantly associated with differences in genotype, specifically with two genetic polymorphisms thought to impair histamine clearance (histamine N-methyltransferase, HNMT Thr105lle and/or HNMT T939C).

26. In their draft final technical report[11] the researchers presented a *post-hoc* analysis of the disaggregated behaviour measures in the subgroup consuming 85% or more of the challenge drinks. Table 3 summarises the results of these analyses. The only statistically significant changes were in the parental measures for Mix A in 3 year olds and for Mix B in 8 to 9 year olds. Changes in the other measures (teacher assessments, independent observer assessments or computer based performance task) were mostly in the same direction, but were not statistically significant and the mean differences were very small.

Table 3: Summary of disaggregated analysis of changes in behaviour measures assessed following challenge with Mix A or B compared with placebo, based on subgroup consuming ≥ 85% of the challenge drinks

	Mix A		Mix B	
	3 year olds	8-9 year olds	3 year olds	8-9 year olds
Parental score	0.49 (0.09-0.89)*	0.03 (-0.10 to 0.16)	0.36 (-0.04 to 0.76)	0.13 (0.00 to 0.25)*
Teacher score	0.03 (-0.11-0.16)	-0.01 (-0.12 to 0.09)	0.08 (-0.05 to 0.21)	0.01 (-0.09 to 0.11)
Classroom observation score	0.10 (-0.07-0.27)	0.08 (-0.07 to 0.22)	-0.01 (-0.18 to 0.16)	0.05 (-0.09 to 0.19)
Computer-based task score	N.D.	0.08 (-0.16 to 0.32)	N.D.	0.20 (-0.04 to 0.43)

*Statistically significant (at $p < 0.05$)
Analyses were conducted on the data for the subgroup consuming ≥ 85% of the challenge drinks. The different measures focus on differing aspects of hyperactive behaviour in differing contexts.
Scores are expressed as mean SDU with 95% confidence intervals in parentheses
N.D.: not determined

27.   Subsequent analysis by the researchers of the disaggregated measures for the entire cohort indicated a smaller increase in the mean parental score for Mix A in the 3 year olds, which was not statistically significant ($p = 0.058$). For the entire cohort of 8 to 9 year old children, the increases in mean parental scores and associated confidence intervals for Mix A and Mix B were similar to those seen in the ≥ 85% consumption subgroup analysis.

28.   No statistically significant differences in hyperactive behaviour were found in the acute challenge study, which was conducted on a sub-set of the older children, using Mix B only, with assessments based on independent observer ratings and computer-based tasks, but not parental or teacher observations.

Committee discussion

Design of study T07040

29.   The Committee noted the changes that had been made to the design of the study compared with the previous Isle of Wight Study, which had improved the statistical power of the study to be able to detect behavioural effects. The administration of a drink daily throughout the challenge trial largely overcame the placebo effects that had been a major concern of the previous study design.

30.   The dose levels of the individual additives in the two food challenge mixtures were relevant to dietary intake levels of these additives in these age groups of children, and were below the respective ADIs. The fact that the researchers had used, in one of the mixtures (Mix A) the same combination of additive colours and a preservative at the same dose as was used in the Isle of Wight study, enabled comparison with the results of that previous study. The addition of a second challenge mixture into the study design (Mix B) consisting of a combination of additive colours and a preservative more commonly found in children's foods at the time the present study was commissioned,

and at higher dose levels to represent higher intake levels, represented a further improvement to the study design.

31.   However, the Committee noted some limitations in the study design and analysis. The timing of the assessments of behaviour in relation to the administration of the drinks appeared to be based on an assumption that any effects would be long-lasting. The time of day the drink was to be consumed was not defined in the instructions to parents and therefore it might not have been optimal for relatively transient effects to be observed. Recording of the children's body weights would have allowed a more accurate assessment of the administered doses, and comparison with effects in individual children. The initial exclusive use of the GHA in the primary analysis did not allow assessment of the relative contributions of the parental and other more objective measures of behaviour, although results from analyses of the disaggregated measures were provided subsequently by the researchers. Analysis of the GHA scores in the wash-out weeks of the study would have provided useful information on intra-individual variability over time.

The findings of the study

32.   The study showed increases in the levels of children's hyperactive behaviour when they were challenged with combinations of particular food colours together with sodium benzoate, compared with a placebo. However, the increases were not consistently statistically significant for the two mixtures or in the two age groups.

33.   Based on the primary outcome for the whole unselected cohort, there was an increase in the mean GHA score associated with both mixtures compared with the placebo, for both age groups, which reached statistical significance for Mix A in the 3 year old children and for Mix B in the 8 to 9 year olds. For Mix A, the dose was slightly higher for the 3 year olds than for the 8 to 9 year olds when expressed on a body weight basis, which might have contributed to the difference in the magnitude of the increase in the GHA. For Mix B, there was no difference in dose between age groups, when expressed on a body weight basis. Influence of dose between the mixtures is more difficult to assess as two of the four food colours in each mixture were different.

34.   The results of the *post-hoc* analyses of the GHA scores, carried out on data from a sub-set of the subjects, were broadly consistent with the primary analysis. The Committee noted that a subsidiary analysis of compliant subjects was a reasonable approach but it would have been preferable if criteria for selection of the sub-set had been defined in the original study protocol.

35.   Although not all risk estimates reached statistical significance, all showed a small increase in the mean GHA score associated with consumption of Mix A or Mix B. This does not automatically lead to the conclusion that the mixtures caused an increase in hyperactivity (see paragraph 44 below). It is unclear whether the differences in response to the mixtures by the different age groups were real or, in the case of Mix A, merely reflected differences in dose on a bodyweight basis. In addition, it was noted that the individual measures that contributed to the GHA scores differed between the two age groups (there was no continuous performance monitoring using the computer based task in the younger children).

36.   The researchers' findings of a significant increase in mean GHA score of 3 year old children associated with challenge with Mix A were consistent with the results reported in the previous Isle of Wight study in which the same food colours and sodium benzoate preservative mixture was used. The improvements to the protocol of the present study add weight to the previous findings.

37.   The size of the observed increase in mean GHA score (which encompassed parental, teacher and independent observer assessments) associated with consumption of Mix A in 3 year olds was smaller in the present study than was observed in the Isle of Wight study, in which the quoted effect size had been based solely upon parental ratings (mean increase 0.20 SDU compared with 0.51 previously).

38.   The *post-hoc* analyses of the disaggregated measures for both the whole cohort and the subgroup that had consumed ≥ 85% of the drinks, showed that the parental reports were the main contributor to the changes in the GHA score for the 3 year olds, as was seen in the Isle of Wight study. In the 8 to 9 year old children, the largest increases in hyperactive behaviour score for both mixtures were seen in the computer-based task. Parental reports were the only statistically significant discriminator of differences in children's behaviour on the challenge compared with the placebo, and, when the whole cohort is considered in the analysis, only in the case of Mix B in 8 to 9 year olds. When the same analysis was conducted on the ≥ 85% consumption subgroup, the differences in parental reports were statistically significant for both Mix B in 8 to 9 year olds and Mix A in 3 year olds.

39.   The researchers have suggested that parents may have been more sensitive to, or more exposed to, behavioural changes in their children in this study than the independent observers or teachers, because most of the challenge drinks were consumed at home after school. The timing of consumption of the drinks was a consequence of the design of the study, as children were instructed to consume the drinks at home rather than at school, so that compliance with consuming the challenge drinks could be monitored by the families and the researchers could be relatively certain that the child had consumed the challenge drinks, as intended. However there was some uncertainty as to whether the drinks were consumed in the morning prior to school or in the evening after school and this was recognised by the COT as a complicating factor in the interpretation of the results.

40.   There was no evidence of carry-over of effects on behaviour from each active challenge week to the next active challenge week (i.e. no evidence that behaviour in week 4 was influenced by the type of challenge (artificial colour and benzoate preservative mixture or placebo) in week 2, and behaviour in week 6 by the type of challenge in week 4). However, it is not possible to say whether behavioural changes persisted from the active challenge weeks into the wash-out weeks. The study design employed the wash-out weeks to minimise the likelihood of carry-over effects confounding behaviour during subsequent active challenge weeks, and not to test the duration of any effect of the mixtures. The one week washout was chosen by the researchers on a pragmatic basis, and was the period used in the previous Isle of Wight Study. In setting the length of this period account was taken of the burdens placed on families taking part in such studies and the recognition that both subject recruitment and retention might be compromised by the use of a longer wash-out period. The duration of exposure to the additive mixtures was only 7 days and

therefore it was not possible to determine whether longer term exposure would increase or decrease any potential effects on behaviour.

41.   The results of the 'proof of principle' acute challenge study, on a limited number of the 8 to 9 year old children with Mix B, did not demonstrate a statistically significant association between administration of the food colour and sodium benzoate mixture and hyperactivity in this group, although there was a trend towards an effect (estimate = 0.66 (95% CI -0.06 to 1.38) p = 0.072) when "responders" were compared with "non-responders". It was noted that the end point used in this acute challenge was not the same as in the main study, and that it was restricted to a small selected sub-set of boys from the main study sample.

Relevance of the findings at the individual and population level

42.   The Committee was informed that, although small, the size of the reported effects on hyperactive behaviour could be of clinical relevance for individual children. The observed changes in behaviour did not obviously vary according to social or demographic factors, or to children's pre-trial level of hyperactive behaviour, pre-trial additive content of diet, or sex. The mean differences observed, if causal, could be clinically relevant.   The duration of effect would be an important additional consideration, which has not been elucidated by the current study. If there are real effects of this magnitude, but they are only transient, they would potentially be of less concern. The study measured mean differences in the GHA score in the study sample, which was selected to cover the full range of behaviour in the general population, from normal through to high level hyperactivity. However, as the selection of subjects was intentionally stratified across the behaviour scale, the study sample would not have been adequately representative of the wider population.

43.   Genetic factors are known to influence hyperactivity and ADHD[12,13]. The findings of the present study suggest possible differential sensitivity to the particular mixtures used in this study in relation to certain genetic polymorphisms. However, the increases in GHA scores were not limited to individuals with the specific polymorphisms measured in the study, and the observed associations between polymorphisms in the histamine N-methyltransferase gene and the difference in behaviour with Mix A in 3 year olds and Mix A and Mix B in 8 to 9 year olds compared to placebo, even if real and not merely chance effects, were not so strong that they could usefully be applied to identify at-risk groups or individuals. There were no associations between behaviour and the other genetic polymorphisms investigated in the study. These included genetic polymorphisms selected from the dopamine neurotransmitter systems, which have previously been implicated in ADHD[14].

44.   The findings did not provide any information on the likely biological mechanism for the observed differences in hyperactivity.   The Committee had previously considered the available data on the potential for neurotoxicity of a number of the food additives[15], including some of the colours that were used in the mixtures in the present study (quinoline yellow, sunset yellow, carmoisine, and ponceau 4R), and the preservative sodium benzoate. The limited toxicological databases that were available for the individual additives in the mixtures used in the present study did not provide positive neurotoxicological alerts at doses relevant to dietary consumption. It was considered unlikely that the colours concerned would cross the mature blood-brain barrier, although sodium benzoate might. In the absence of stronger evidence for an

underlying biological mechanism of toxicity, doubt remains as to whether the observed differences in behaviour were caused by the challenge mixtures. Despite the statistical significance of some of the associations, the possibility still exists that these could have arisen by chance. Furthermore, if the associations were causal, it is not possible to determine whether specific food additives within the mixtures were responsible, or whether the association depended on the combined action of the mixture. The study did not provide any information as to whether or not any associations seen would be specific for children.

Conclusions

45.   We consider that this study has provided supporting evidence suggesting that certain mixtures of artificial food colours together with the preservative sodium benzoate are associated with an increase in hyperactivity in children from the general population. If causal, this observation may be of significance for some individual children across the range of hyperactive behaviours, but could be of more relevance for children towards the more hyperactive end of the scales.

46.   We note that the increases in mean levels of hyperactivity observed in this study were small relative to normal inter-individual variation and that changes in behaviour were not evident in all children in any one group and were not consistent across age groups or across the different mixtures used in the study. Therefore it is not possible to draw conclusions on the implications of the observed changes at the population level. It is also not possible to extrapolate the findings to additives other than the specific combination in the mixtures used in this study.

47.   We conclude that the results of this study are consistent with, and add weight to, previous published reports of behavioural changes occurring in children following consumption of particular food additives.

48.   This research has not indicated any possible biological mechanism for the observations made, which might have provided evidence of causality or of the possible effects of individual additives or of other mixtures of additives.

49.   The timing and duration of any possible effects would need to be addressed by further research.

50.   Further analyses of data from this study may provide additional information on intra-individual variability and the extent of any carryover from the challenge weeks into the wash out weeks.

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