PRO 07/09/01 **20 SEPTEMBER 2007**

RECENT DEVELOPMENTS ON FOOD COLOURINGS AND ADDITIVES

Executive Summary

1. This paper updates the Board concerning recent developments on food colourings and additives. In July 2006 the European Commission published proposals to consolidate and simplify existing harmonised legislation on food additives; these proposals form part of the Agency's simplification plan. Meanwhile the EU is currently undertaking a re-evaluation of all existing additives and following the European Food Safety Authority's opinion the food colour E128 Red 2G has been suspended from food use. The Agency has published research it has funded on the possible effects of certain food colours and a preservative on children's behaviour (the "Southampton study").

2. The Board is asked to:

- **note** progress on Commission proposals to consolidate existing legislation;
- **comment** on the steps taken so far in response to publication of the Southampton study; and
- **advise** on whether there are further actions that the Agency should take.

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PRO 07/09/01 **20 SEPTEMBER 2007**

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Issue

1. There is significant public interest in the use of additives in general as well as in the use of some individual additives. In the 2006 Consumer Attitudes Survey, food additives/preservatives were the most mentioned spontaneous response to a question on food issues of concern. The European Community already has harmonised legislation on food additives (including sweeteners and colours) and flavourings dating from 1989 and 1988 respectively. Currently there are 319 additives permitted for food use in the EU of which 42 are colours. In July 2006 the European Commission published a package of proposals to consolidate and simplify the existing legislation as well as introducing legislation to harmonise the control of enzymes. As the new proposals are for co-decision Regulations they have been discussed in both Council and the European Parliament. The proposal on additives continues to be based on the principles noted in paragraph 4 below, but there is on-going discussion in Council and Parliament as to whether these should be strengthened.

2. The EU is undertaking a re-evaluation of all existing additives and, following the European Food Safety Authority's opinion, the food colour E128 Red 2G has been suspended from food use. Following previous Government funded work, research funded by the Agency on the possible effects of certain food additives on children's behaviour has just been published.

Strategic Aims

3. We seek to ensure all permitted food additives are safe for use, there is a technological need for their use, and that consumers can make informed choices about the additives they consume.

Background

4. The Agency consulted publicly on the Commission proposals in autumn 2006 and the UK Government position was agreed in early February 2007. The proposals are about the framework for the control of additives, flavourings and enzymes and not about the desirability, or otherwise, of authorising any individual substance for its use in food. The proposal on additives continues to be based on the principles that:

    - an additive should not, on the basis of the scientific evidence available, pose a safety concern to the health of consumers at the level of use proposed;

- there should be a reasonable technological need that cannot be achieved by other economically and technologically practicable means;

- use does not mislead the consumer.

EU negotiations on the Commission proposals

5. Under the German Presidency in the first half of 2007, the Council reached a general agreement to a common approach for the proposals on food additives, enzymes and a common authorisation procedure; and there was a large measure of agreement concerning the flavourings proposal. Meanwhile, following discussion in committee, the Parliament had its first reading of all four proposals in July. On-going discussion will pave the way for the Council to reach political agreement on a common position and for a second reading in Parliament.

Review of all existing additives and action taken on Red 2G

6. The European Commission has recently submitted a report to the Parliament and Council on the progress of the re-evaluation of food additives[1]. It notes the legal requirement for additives to be kept under continuous observation and re-evaluated whenever necessary in the light of changing conditions of use and new scientific information, notes the outcomes of some recent re-evaluations, and notes a general re-evaluation of all currently authorised food additives under existing EU legislation has commenced with a review of colours.

7. Having re-evaluated the food colour E128 Red 2G as part of this programme, the European Food Safety Authority published its opinion which showed that Red 2G may have the potential to damage the genetic material in cells and cause cancer in animals. The Agency held a scoping meeting with stakeholders to gain intelligence on the UK use of this colour. It had been permitted only in certain types of sausages and burgers, and that use has now been suspended across the EU. We will continue to consult with stakeholders on a case by case basis as further opinions are published.

Research on the possible effect of some additives on children's behaviour

8. There has been a longstanding suggestion that some artificial food colours and/or the preservative sodium benzoate may affect children's behaviour, particularly in relation to hyperactivity and Attention Deficit Hyperactivity Disorder (ADHD). A previous study commissioned in 1997 by the Ministry of Agriculture, Fisheries and Food[2] examined the effects of a mixture of certain artificial food colours and the preservative sodium benzoate on the behaviour of three-year old children. The results of that study were reviewed by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) in

[1] COM(2007) 418 final, Brussels 18.7.2007
[2] The Isle of Wight study.

2001/2002, who considered that the findings were inconclusive as the effects that had been observed were only evident in the parental reports of behaviour and were not confirmed by the independent assessments made in a clinical setting. A further limitation was the observed "placebo effect" seen in some children when they were given the control drink not containing the test additives. COT concluded it was not possible to reach firm conclusions about the clinical significance of the observed effects in this study.

9. The Agency commissioned further research in this area in 2004 after seeking advice from independent experts on the limitations of the design of the first study and possible experimental design improvements[3]. The primary hypothesis tested by the researchers in this new study was that, compared with a placebo, mixtures of certain artificial food colours with the preservative sodium benzoate increase the mean level of hyperactive behaviour of children drawn from the general population. The study also sought to address a number of other research questions:

    - whether genetic differences moderated any observed effect,
    - whether there were effects in both pre-school and older children,
    - whether any response to the additive mixtures was related to initial levels of hyperactive behaviour as scored on a hyperactivity behaviour scale, and
    - whether any response was seen via teacher ratings, direct observations of behaviour and computer based test performance as well as via parental ratings.

10. The researchers employed a double blind placebo controlled randomised cross-over food challenge to investigate the effect of two different mixtures of additives[4], compared with a placebo, on the behaviour of children of both sexes and two age groups, 3 year olds and 8 to 9 year olds. A scientific paper summarising the findings of the study was recently published in The Lancet and a copy is attached as Annex I to this paper.

11. The study showed an association between certain mixtures of artificial food colours and sodium benzoate preservative and an increase in the mean level of hyperactivity in children from the general population. The increases in mean levels of hyperactivity observed were small relative to normal variation between children, and 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.

[3] The research cost £750,000.

[4] Each mixture contained the preservative sodium benzoate and four food colours. Mix A contained tartrazine (E102), ponceau 4 R (E124), sunset yellow (E110) and carmoisine (E122), whilst Mix B contained quinoline yellow (E104), allura red AC (E129), sunset yellow (E110) and carmoisine (E122).

12. It was important for the findings of this research to go through a rigorous peer review process involving other experts in the field, to ensure that the quality of the science has been properly scrutinised, and for it to be published in a scientific journal. The peer review process is the standard mechanism for establishing the validity and quality of the science. As part of this peer review process, the Agency again sought the advice of the COT.

13. The COT considered the full results of this study (not all of which are presented in the Lancet paper) and produced a statement of their views, which has been published on the COT's website and which can be found at Annex II to this paper. In their Statement, the COT concluded 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. 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. The timing and duration of any effect would need to be addressed by further research."

14. The use of food additives is controlled under EU harmonised legislation[5]. That means that a Member State can take unilateral action to suspend use of an additive only when permitted to do so by EU law. The additives legislation permits a Member State to take unilateral action where it considers, on the basis of new information, that the use of an additive endangers human health. Having considered the COT opinion of the scientific evidence we concluded that criterion is not met. Consideration of the scientific evidence also suggested there was currently no case for requiring the restriction or withdrawal of the additives in question under more general EU food safety legislation. A summary of the applicable EU legislation is attached at Annex III.

15. However this is important scientific evidence that needs to be considered by the EU. We therefore forwarded the evidence to the European Food Safety Authority as the first step to initiating appropriate pan-EU risk management measures. We have asked EFSA to consider this as a matter of urgency and the Chief Executive has written to the European Commission urging them to take swift action at EU level once EFSA's advice has been received.

16. On the basis of this research we have changed our advice to consumers: if a child shows signs of hyperactivity or ADHD then eliminating the colours used in the Southampton study from their diet might have some beneficial effects. This advice now appears on the FSA website.

[5] Council Directive on the approximation of the laws of the Member States concerning food additives authorised for use in foodstuffs intended for human consumption, 89/107/EEC.

17. We held an initial scoping meeting with the UK food industry to discuss the research findings and its implications. Representatives from manufacturing and retail organisations told the Agency that there was already a trend within industry towards finding alternatives to the colours used in the study. Representatives also highlighted some technical challenges in developing these alternatives.

18. In addition, we invited a number of public interest organisations to attend a briefing meeting ahead of the publication of the research to ensure they were fully informed about this study.

19. On Thursday 13 September we held further meetings both with industry and with public interest groups. We asked industry what actions they were taking in light of this new research especially how they intended to help customers who wished to avoid products containing these colours. Industry noted the widespread trend since 2003 to move away from artificial colours in food and drink products – especially those aimed at children. Where companies had reformulated without artificial colours they were already using front of pack 'flashes' (eg 'Contains no artificial colours'). The industry have agreed to our suggestion that we set up a web page which will provide information about what industry is doing together with details of companies websites and customer careline numbers so that parents are able to follow the FSA's advice. They also accepted the FSA offer to host a joint meeting with public interest groups to discuss what further actions can be taken to provide practical help to consumers

20. At the meeting with public interest groups they stated it was unreasonable to place the burden of avoiding these artificial colours on consumers. A significant proportion of food is sold loose (without labelling) or is bought by children themselves. The group view was that these colours should be banned and the FSA advice should have been extended to all children. Stakeholders considered other options would be front of pack labelling, more widespread communication especially to those who may not have access to the internet, naming companies not providing details of specific colour use in products, and working with small companies to ensure that they are aware of the research. The public interest groups responded positively to the idea of a joint meeting with industry.

Board Action Required

21. The Board is asked to:

- **note** progress on Commission proposals to consolidate existing legislation;

- **comment** on the steps taken so far in response to publication of the Southampton study; and

- **advise** on whether there are further actions that the Agency should take.

Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomised, double-blinded, placebo-controlled trial

Donna McCann, Angelina Barrett, Alison Cooper, Debbie Crumpler, Lindy Dalen, Kate Grimshaw, Elizabeth Kitchin, Kris Lok, Lucy Porteous, Emily Prince, Edmund Sonuga-Barke, John O Warner, Jim Stevenson

Summary

Background **We undertook a randomised, double-blinded, placebo-controlled, crossover trial to test whether intake of artificial food colour and additives (AFCA) affected childhood behaviour.**

Methods **153 3-year-old and 144 8/9-year-old children were included in the study. The challenge drink contained sodium benzoate and one of two AFCA mixes (A or B) or a placebo mix. The main outcome measure was a global hyperactivity aggregate (GHA), based on aggregated z-scores of observed behaviours and ratings by teachers and parents, plus, for 8/9-year-old children, a computerised test of attention. This clinical trial is registered with Current Controlled Trials (registration number ISRCTN74481308). Analysis was per protocol.**

Findings **16 3-year-old children and 14 8/9-year-old children did not complete the study, for reasons unrelated to childhood behaviour. Mix A had a significantly adverse effect compared with placebo in GHA for all 3-year-old children (effect size 0·20 [95% CI 0·01–0·39], p=0·044) but not mix B versus placebo. This result persisted when analysis was restricted to 3-year-old children who consumed more than 85% of juice and had no missing data (0·32 [0·05–0·60], p=0·02). 8/9-year-old children showed a significantly adverse effect when given mix A (0·12 [0·02–0·23], p=0·023) or mix B (0·17 [0·07–0·28], p=0·001) when analysis was restricted to those children consuming at least 85% of drinks with no missing data.**

Interpretation **Artificial colours or a sodium benzoate preservative (or both) in the diet result in increased hyperactivity in 3-year-old and 8/9-year-old children in the general population.**

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Introduction

Artificial food colours and other food additives (AFCA) have long been suggested to affect behaviour in children.[1] Ben Feingold made his initial claims of the detrimental effect of AFCA on childhood behaviour more than 30 years ago.[2] The main putative effect of AFCA is to produce overactive, impulsive, and inattentive behaviour—ie, hyperactivity—which is a pattern of behaviour that shows substantial individual differences in the general population. Children who show this behaviour pattern to a large degree are probably diagnosed with attention-deficit hyperactivity disorder (ADHD). Despite the failure of early studies[3] to identify the range of proposed adverse affects, a recent meta-analysis[4] of double-blinded, placebo-controlled trials has shown a significant effect of AFCA on the behaviour of children with ADHD. The possible benefit in a reduction in the level of hyperactivity of the general population by the removal of AFCA from the diet is less well established. Evidence from our previous study on the Isle of Wight has suggested adverse effects on hyperactivity, measured by parental ratings for 3-year-old children on a specific mix of additives.[5] These findings needed replication on 3-year-old children, and to establish whether the effects could be seen with a wider range of measures of hyperactivity. The present community-based, double-blinded, placebo-controlled food challenge was designed to extend the age range studied to include 8/9-year-old children to determine whether the effects could also be detected in middle childhood.

Methods

Participants

Figures 1 and 2 present details of recruitment and participation in the study, for 3-year-old and 8/9-year-old children, respectively. The study sample was drawn from a population of children aged between 3 years and 4 years, 2 months, registered in early-years settings (nurseries, day nurseries, preschool groups, playgroups) and from children aged between 8 and 9 years attending schools in Southampton, UK. To ensure that the study sample included children from the full range of socioeconomic backgrounds, schools were recruited based on the number of children receiving free school meals (an index of social disadvantage). The distribution of the percentage of children receiving free meals in the schools taking part indicated the proportions for the city as a whole. To further check on how representative the sample was, teachers completed a hyperactivity questionnaire[6] for all 3-year-old and 8/9-year-old children.

Parents who returned an expression of interest form were contacted by phone and a home visit arranged. On this visit, a research assistant and the study dietitian, provided full information about the study and its dietary

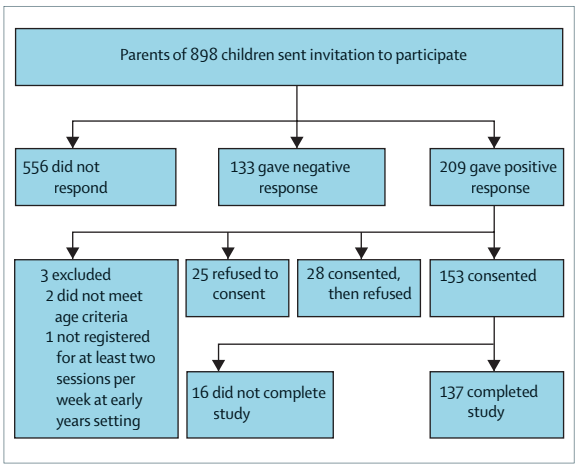

Figure 1: **Enlistment of 3-year-old participants**

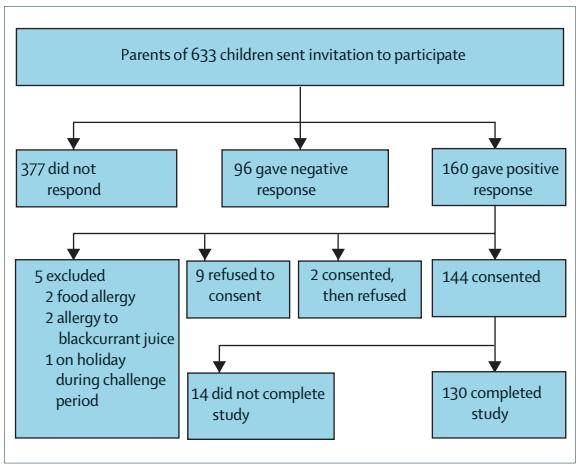

Figure 2: **Enlistment of 8/9-year-old participants**

implications, and written informed consent was obtained. The study dietitian also obtained a report based on 24-h recall by the parent of the child's pretrial diet, which allowed an assessment of baseline levels of the number of foods containing additives consumed by the child in the previous 24 h. The study was approved by the local research ethics committee (reference no 04/Q1702/61) and written informed consent was obtained from parents. Participating early-years settings received £250 and each school £500 as a contribution towards school funds for the benefit of all children.

Study design and challenge protocols

The study design and challenge protocols for both ages were similar. Children were entered into this study with a within-subject crossover between two active mixes (A and B) and a placebo drink.

The two active mixes differed both in the quantities of additives and the specific additives included. Mix A was similar to the active challenge used in the Isle of Wight study,[5] and mix B was selected to indicate the current average daily consumption of food additives by 3-year-old and 8/9-year-old children in the UK.[7] Both mixes included sodium benzoate, which had been included in the challenge on the Isle of Wight study and in previous studies.[8,9]

Mix A for 3-year-old children included 20 mg of artificial food colourings (5 mg sunset yellow [E110], 2·5 mg carmoisine [E122], 7·5 mg tartrazine [E102], and 5 mg ponceau 4R [E124, Forrester Wood, Oldham, UK]) and 45 mg of sodium benzoate [E211, Sigma Aldridge, Gillingham, UK]). Active mix B included 30 mg of artificial food colourings (7·5 mg sunset yellow, 7·5 mg carmoisine, 7·5 mg quinoline yellow [E110], and 7·5 mg allura red AC [E129]) and 45 mg of sodium benzoate.

Mix A amounts for 8/9-year-old children were multiplied by 1·25 to account for the increased amount of food consumed by children at this age. Therefore, mix A included 24·98 mg of artificial food colourings (6·25 mg sunset yellow, 3·12 mg carmoisine, 9·36 mg tartrazine, and 6·25 mg ponceau 4R) and 45 mg of sodium benzoate. Active mix B included 62·4 mg of artificial food colourings (15·6 mg sunset yellow, 15·6 mg carmoisine, 15·6 mg quinoline yellow, and 15·6 mg allura red AC) and 45 mg of sodium benzoate.

Doses for mixes A and B for 3-year-old children were roughly the same as the amount of food colouring in two 56-g bags of sweets. For 8/9-year-old children, the dose for mix A was equal to about two bags of sweets a day and for mix B about four bags of sweets a day.

After a week on their typical diet (week 0: baseline diet), the artificial colours to be used in the challenges and sodium benzoate were withdrawn from their diet for 6 weeks. Over this period when challenge with active or placebo drinks were given, additive withdrawal continued (week 1: withdrawal period but receiving placebo; weeks 2, 4, and 6: challenge with randomisation to two active periods and one placebo period; weeks 3 and 5: washout continuing on placebo). During this period, 3-year-old children received the challenge and washout-placebo drinks on a weekly basis and consumed mixed fruit juices (placebo or active) at home (300 mL/day for 3-year-old children, 625 mL/day for 8/9-year-old children), provided in identical sealed bottles. At the beginning of the study, children were assigned by the study administrator by a random-number generator to receive one of six possible sequences of placebo, active mix A, or active mix B challenges across weeks 2, 4, and 6.

A masked testing by two independent panels of 20 young adults showed that the active and placebo juice drinks could not be differentiated. When asked if the mix contained additive, 16 (40%), 21 (52%), and 26 (65%) adults responded positively for mix A, mix B, and placebo, respectively. We recorded no significant differences between these proportions (Friedman test, χ^2=4·412, df=2). Therefore, no reliable differences were seen between the look and taste of the drinks. The only difference in the composition of the placebo and active

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mixes was the presence of the AFCA in the active mix with some variation in the proportions of the fruit juices to ensure matching colour and taste for the placebo and active drinks. The child's family and the research team were masked to the challenge allocation. The study administrator assigned the challenge sequence and assisted in the preparation and packaging of juice drinks that were then delivered by the masked research team to homes every week, when questionnaires and other forms were obtained and dispensed. Parents completed a daily diary of juice consumption and compliance with the diet over the study period. Parents also recorded a mistake event when a child consumed a portion of food containing the artificial colours or sodium benzoate. Any bottles containing juice not consumed in the previous week were obtained, returned to the study office, and measured to help validate, if possible, parental reports of juice consumption by children.

Global hyperactivity aggregate (GHA)

Three measures of behaviour were used to calculate GHA for 3-year-old children, with an additional measure for 8/9-year-old children. First, the abbreviated ADHD rating scale IV (teacher version)[6] was used. A total score was obtained for ten of the 18 items (inattentive=5, hyperactive=5) in this questionnaire, which was completed to describe the frequency of the specific behaviours displayed over the past week, for every week of the study. Parent behaviour was the second measure, by use of the abbreviated Weiss-Werry-Peters (WWP) hyperactivity scale,[10] which has been used in several studies to assess hyperactivity.[11,12] Interparent agreement is good for ratings of childhood behaviour (r=0·82).[13] Parents rated their child's behaviour during the previous week for seven items previously used (switching activities; interrupting or talking too much; wriggling; fiddling with objects or own body; restless; always on the go; concentration),[4] from which we obtained a total score. For 8/9-year-old children, we used an abbreviated ADHD rating scale IV (parent version)[14] to measure parent behaviour, whereby a ten-item questionnaire was completed by parents every week.

A third measure was the classroom observation code,[15] which assesses the occurrence of 12 mutually exclusive behaviours during structured didactic teaching and during periods of independent work under teacher supervision. To develop this measure, the behaviours had been selected to indicate components of ADHD that are shown in the classroom. After observers (psychology graduates) were given extensive training, the inter-rater reliability of the classroom observation code, tested before and during the study, exceeded 0·87. Children were observed for 24 min every week (three observation sessions of 8 min each) and a total weekly mean score was derived from the total score over every session. The code was slightly modified for 3-year-old children, since preschool children in the UK are not usually given structured or didactic teaching sessions and tend to engage in activities rather than in tasks. Observation took place over a range of activities and the off-task category in the code was scored when the child switched activities.

A fourth measure for 8/9-year-old children was the Conners continuous performance test II (CPTII),[16] a test using visual stimuli of 14-min duration and is widely used to assess attention and the response inhibition component of executive control. We used four scores (SE of reaction time, % of commission errors, d´ [discriminability index], and β) to derive a weekly aggregate score. This subset of indicators from the CPTII has been shown to be highly correlated with the ADHD rating scale.[17]

The GHA was developed to measure individual differences in hyperactivity using different sources (teacher, parent ratings, direct observation, and a computerised test) and covering the components of hyperactivity (overactivity, impulsivity, and inattention). Weekly scores for every child were standardised to time 0 at baseline (T0). Weekly standardised (z) aggregate scores were calculated as: (score minus mean score at T0) divided by SD at T0. The GHA was an equally weighted aggregate of the weekly z-scores, and calculated only when at least two (or three for 8/9-year-old children) of

	3-year-old children in total sample analysis (n=153)	8/9-year-old children in total sample analysis (n=144)
Racial background		
White	126 (82%)	130 (90%)
Other	15 (10%)	14 (10%)
Missing data	12 (8%)	..
Marital status		
Married/partner	127 (83%)	115 (80%)
Single/separated/divorced/widowed	26 (17%)	29 (20%)
NSSC (father)		
Higher occupations	34 (22%)	37 (26%)
Intermediate occupations	26 (17%)	18 (13%)
Lower occupations	51 (33%)	44 (31%)
Never worked/long-term unemployed	4 (3%)	7 (5%)
No father present	26 (17%)	29 (20%)
Missing data	12 (8%)	9 (6%)
NSSC (mother)		
Higher occupations	31 (20%)	38 (26%)
Intermediate occupations	18 (12%)	26 (18%)
Lower occupations	66 (43%)	32 (22%)
Never worked/long-term unemployed	26 (17%)	32 (22%)
Missing data	12 (8%)	16 (11%)
Mother's education		
School attendance up to age 16 years (no qualifications or certificates below "A" level)	53 (35%)	60 (42%)
"A" levels	61 (40%)	42 (29%)
University degree/postgraduate qualification	27 (18%)	27 (19%)
Missing data	12 (8%)	15 (10%)

Data are n (%). NSSC=national statistics social class.[20] Higher=managerial and professional. Intermediate=self-employed. Lower=routine work. "A" levels=pre-university, school examinations in the UK.

Table 1: **Characteristics of parents of children enlisted in study**

	Mix A		Mix B		Placebo	
	n	mean (SD)	n	mean (SD)	n	mean (SD)
3-year-old children						
Entire sample (n=140)	131	−0·11 (1·03)	134	−0·14 (1·03)	129	−0·32 (1·11)
≥85% consumption (n=130)	104	−0·11 (1·03)	108	−0·15 (1·07)	99	−0·39 (1·07)
Complete case (n=73)	73	−0·14 (1·04)	73	−0·26 (1·05)	73	−0·44 (0·98)
8/9-year-old children						
Whole sample (n=136)	132	0·25 (0·97)	133	0·33 (1·10)	127	0·19 (1·03)
≥85% consumption (n=119)	104	0·26 (0·93)	112	0·32 (1·09)	103	0·19 (1·04)
Complete case (n=91)	91	0·27 (0·92)	91	0·35 (1·08)	91	0·19 (1·06)

Table 2: **Mean GHA scores for 3-year-old and 8/9-year-old children by challenge type**

	Entire sample (n=140)	Group with ≥85% consumption (n=130)	Complete case group (n=73)
Model 1			
Intercept	−0·31 (−0·49 to −0·13)*	−0·33 (−0·53 to −0·13)†	−0·44 (−0·68 to −0·21)†
Challenge type			
Mix A *vs* placebo	0·20 (0·01 to 0·40)‡	0·24 (0·02 to 0·47)‡	0·31 (0·04 to 0·58)‡
Mix B *vs* placebo	0·16 (−0·04 to 0·35)	0·16 (−0·07 to 0·38)	0·19 (−0·08 to 0·46)
Model 2			
Intercept	−0·54 (−0·89 to −0·18)*	−0·51 (−0·92 to −0·11)	−0·58 (−1·08 to −0·09)‡
Challenge type			
Mix A *vs* placebo	0·20 (0·01 to 0·39)‡	0·28 (0·05 to 0·51)‡	0·32 (0·05 to 0·60)‡
Mix B *vs* placebo	0·17 (−0·03 to 0·36)	0·19 (−0·04 to 0·41)	0·21 (−0·06 to 0·48)
Week of study			
Week 2 *vs* week 6	0·15 (−0·05 to 0·34)	0·15 (−0·08 to 0·38)	0·19 (−0·08 to 0·46)
Week 4 *vs* week 6	0·17 (−0·03 to 0·36)	0·23 (0·00 to 0·46)‡	0·19 (−0·09 to 0·46)
Sex	0·18 (−0·10 to 0·45)	0·22 (−0·07 to 0·51)	0·05 (−0·31 to 0·40)
Baseline GHA score	0·46 (0·26 to 0·66)†	0·54 (0·31 to 0·76)†	0·36 (0·06 to 0·66)‡
Pretrial diet	0·08 (−0·02 to 0·19)	0·07 (−0·04 to 0·18)	0·09 (−0·04 to 0·23)
Maternal education level	−0·01 (−0·29 to 0·28)	−0·04 (−0·34 to 0·26)	−0·03 (−0·41 to 0·35)
Maternal social class	0·15 (−0·44 to 0·13)	−0·23 (−0·53 to 0·08)	−0·21 (−0·58 to 0·16)

Data are estimate (95% CI). *p<0·01. †p<0·001. ‡p<0·05. Complete case=≥85% consumption and no missing data. Model 1=challenge type alone. Model 2=challenge type with additional factors controlled.

Table 3: **General GHA estimates in linear mixed models during challenge period for 3-year-old children**

these behaviour scores were present for any week (one of which being for the classroom observation code) and averaged across the number of available scores. A high GHA indicates more hyperactivity.

Statistical analysis

Although the study designs for the two age groups were similar, the difference in composition of the GHA, and in the dose of AFCA used, meant that data from the two studies could not be analysed jointly. Therefore, we treated the studies as parallel but independent.

Linear mixed-model methods[18,19] in SPSS (version 14.0) were used to analyse data. Several possible covariates were thought to be significantly related to GHA (eg, sex). Two models were tested separately for each age for the effects on GHA in challenge weeks. Model 1 used the challenge type alone as a fixed effect testing for mix A against placebo and mix B against placebo. In model 2, in addition to challenge type, the effects of the following factors were adjusted for: week during study, sex, GHA in baseline week, number of additives in pretrial diet, maternal educational level, and social class. A compound symmetry covariance matrix provided the best-fit model for 3-year-old children and an unstructured covariance matrix for 8/9-year-old children. The study was powered to detect differences between the active and placebo periods and, accordingly in each case, the effects of mix A and mix B were compared with that of placebo. We anticipated that the additional controls on placebo effects would result in an effect size smaller than that achieved in the Isle of Wight study.[5] A sample of 80 children had 80% power at $\alpha=0\cdot05$ to identify an effect size of $0\cdot32$—ie, the magnitude of the difference in GHA mean score changes (SD). This value was lower than that achieved in the previous study ($0\cdot51$). We were uncertain about the number of children and families who would comply with the demands of a 7-week study, so we set a target of 120 children to reduce the effect of attrition on power, which was eventually exceeded in both age groups.

The analyses were replicated for the full sample, a high consumption group (≥85% consumption of drinks in any challenge week), and a complete case group (≥85% consumption in all challenge weeks and no missing GHA). The high consumption and complete case groups were included to determine whether non-compliance and the method of handling missing data affected the pattern of results. Analysis was per protocol.

This clinical trial is registered with Current Controlled Trials (registration number ISRCTN74481308).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 153 children (mean age 43·5 months [SD 4·5]) enlisted, 79 were boys (43·5 months [4·6]) and 74 were girls (43·4 months [4·3]). Table 1 provides parents' characteristics for the entire sample. We saw no significant differences in these background characteristics between groups assigned to receive the challenge drinks in different orders during each of the six periods. The proportion of children in each of five quintile ranges on the teachers questionnaire[6] was not significantly different for the sample or for the total population (n=898, χ^2 [4]=1·60).

16 (10%) 3-year-old children failed to complete the study. Age, sex, and marital status of the parents had no effect on study completion and children were no more likely to drop out during active challenge weeks than placebo. In only one case was this failure to complete related to problems with the child's behaviour. Of those

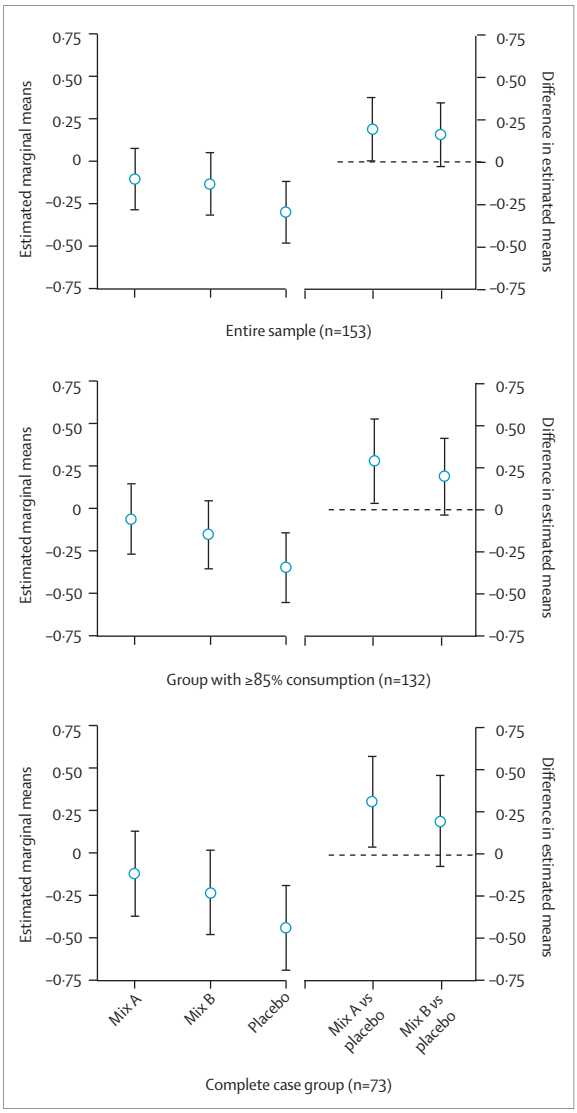

Figure 3: **Estimated marginal means by challenge type and difference in estimated means in GHA under model 2 for 3-year-old children**
Bars=95% CI. Dashed line=zero difference between mean GHA under active mix and mean GHA under placebo.

	Enitre sample (n=136)	Group with ≥85% consumption (n=119)	Complete case group (n=91)
Model 1			
Intercept	0·16 (−0·01 to 0·34)	0·09 (−0·09 to 0·27)	0·11 (−0·10 to 0·32)
Challenge type			
Mix A *vs* placebo	0·08 (−0·02 to 0·18)	0·12 (0·02 to 0·23)*	0·14 (0·03 to 0·24)*
Mix B *vs* placebo	0·12 (0·03 to 0·22)*	0·15 (0·05 to 0·25)†	0·17 (0·06 to 0·28)†
Model 2			
Intercept	0·02 (−0·22 to 0·26)	0·14 (−0·08 to 0·37)	0·14 (−0·12 to 0·39)
Challenge type			
Mix A *vs* placebo	0·08 (−0·02 to 0·17)	0·09 (−0·01 to 0·19)	0·12 (0·02 to 0·23)*
Mix B *vs* placebo	0·12 (0·03 to 0·22)*	0·15 (0·05 to 0·25)†	0·17 (0·07 to 0·28)†
Week of study			
Week 2 *vs* week 6	−0·11 (−0·21 to 0·00)*	−0·19 (−0·29 to −0·08)†	−0·20 (−0·32 to −0·09) †
Week 4 *vs* week 6	0·06 (−0·03 to 0·14)	0·04 (−0·06 to 0·13)	0·03 (−0·07 to 0·13)
Sex	0·16 (−0·03 to 0·35)	0·08 (−0·10 to 0·26)	0·11 (−0·09 to 0·31)
Baseline GHA score	0·78 (0·69 to 0·88)‡	0·79 (0·71 to 0·88)‡	0·79 (0·70 to 0·89)‡
Pretrial diet	0·04 (−0·02 to 0·10)	0·03 (−0·03 to 0·09)	0·02 (−0·05 to 0·09)
Maternal education level	−0·02 (0·20 to 0·16)	−0·02 (−0·19 to 0·15)	0·01 (−0·18 to 0·21)
Maternal social class	0·04 (−0·14 to 0·22)	−0·03 (−0·20 to 0·14)	−0·06 (−0·25 to 0·13)

Data are estimate (95% CI). *p<0·05. †p<0·01. ‡p<0·001. Complete case=≥85% consumption and no missing data. Model 1=challenge type alone. Model 2=challenge type with additional factors controlled.

Table 4: **General GHA estimates in linear mixed models during challenge period for 8/9-year-old children**

children lost to the study, 12 had a mean of 41% consumption in the first challenge week and data were missing for four children. 128 (93%) of the 137 children who completed the study consumed more than two-thirds of all drinks, of which 103 (80%) consumed 85% or more (ie, at least six of seven daily drinks per week). Only one of the remaining nine children drank less than 50% of placebo and active drinks during the study period. The occurrence of dietary infractions or mistakes by 3-year-old children was low (0=33% of children, 1–2=31%, 3–4=18·3%, >4=17%). Rate of infractions did not differ during active and placebo weeks.

117 (76%) 3-year-old children had complete GHA data over active and placebo weeks, 19 (12%) had two GHA scores, and one had one score. Of children who left the study, 12 provided one score, and four had missing data.

Table 2 shows the mean GHA scores under each of the three challenge types. For the challenge periods in weeks 2, 4, and 6, preliminary analyses had shown no effect of the type of challenge in the previous challenge period on the GHA; therefore, the washout periods had eradicated carry-over effects. Table 3 shows the results of the linear mixed-model analyses for 3-year-old children. For model 1 (the unadjusted effects of challenge type), all three samples had significant adverse effects of mix A on GHA compared with placebo. The higher GHA scores for mix B were not significantly greater than for placebo. Under model 2, with the effects of other factors controlled, the effect of mix A was significant for the entire sample (table 2, p=0·044), by contrast with that of mix B (p=0·093). When the analyses are restricted to those children with at least 85% juice consumption, the adverse effect of mix A on behaviour was still significant (p=0·016), but non-significant for mix B (p=0·098). The complete case groups showed the same pattern of results (mix A, p=0·020; mix B, p=0·131). Figure 3 shows estimated marginal mean scores after adjustment for factors in model 2.

Of 144 8/9-year-old children (mean age 106·3 months [SD 5·9]) enlisted to the study, 75 were boys (106·4 months [6·1]) and 69 were girls (106·1 months [5·8]). Table 1 provides parents' characteristics for the entire sample. We recorded no significant differences in these background characteristics between groups of children assigned to receive the challenge drinks in different

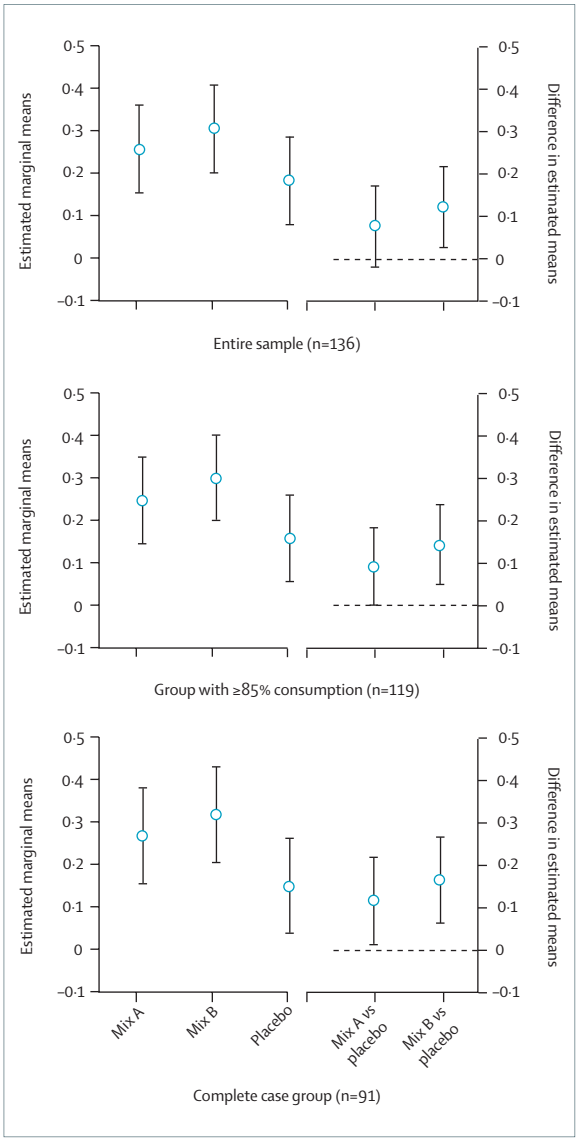

Figure 4: **Estimated marginal means by challenge type and difference in estimated means in GHA under model 2 for 8/9-year-old children**
Bars=95% CI. Dashed line=zero difference between mean GHA under active mix and mean GHA under placebo.

orders over each of the six periods. The proportion of children in each of five quintile ranges on the teachers questionnaire[6] was not significantly different for the sample and for the total population (n=663, χ^2 [4]=5·05).

14 (10%) 8/9-year-old children failed to complete the study; reasons for failure were unrelated to behavioural problems. Age, sex, and marital status of the parents had no effect on study completion and children were no more likely to drop out during active challenge weeks than placebo. Of those children lost to the study, two had a mean of 93% consumption in the first challenge week and data were missing for 12 children. Of the remaining children who completed the study, 98 (75%) consumed 85% or more of the drinks over the challenge weeks (at least six of seven daily drinks per week). Only seven of the remaining 28 children drank less than 50% of placebo and active drinks over the study period. The occurrence of dietary infractions or mistakes by 8/9-year-old children during the study period was low (0=25% of children, 1–2=41%, 3–4=19%, >4=16%). Rate of infractions did not differ during active and placebo weeks.

Of 125 8/9-year-old children, 114 (87%) had full GHA data during active and placebo weeks, six (4%) had two GHA scores, and five (3%) had one score; eight (6%) had no GHA scores. Table 2 also shows mean GHA scores for 8/9-year-old children for the entire sample, the group with at least 85% consumption, and the complete case sample. For the challenge periods in weeks 2, 4, and 6, preliminary analyses showed no effect of the type of challenge in the previous challenge period on the GHA, showing that the washout periods had eradicated carry-over effects. For model 1 (the unadjusted effects of challenge type) the effects of mix A and mix B were significantly greater than that of placebo, with the exception of the entire sample in which the effects of mix A versus placebo fail to reach significance (table 4). Under model 2, in which the effects of other factors were controlled, the effect of mix A for the entire sample was not significant (p=0·123) but mix B did have a significantly adverse effect compared with placebo (p=0·012). When the analyses are restricted to those children who consumed at least 85% juice, the adverse effect of mix A on behaviour remained non-significant (p=0·066) but was significant for mix B (p=0·003). The complete case groups showed significantly higher GHA scores than placebo for mix A (p=0·023) and mix B (p=0·001). Figure 4 shows the estimated marginal means score after adjustment for factors in model 2.

Discussion

In this community-based, double-blinded, placebo-controlled food challenge, we tested the effects of artificial food additives on children's behaviour and have shown that a mix of additives commonly found in children's food increases the mean level of hyperactivity in children aged 3 years and 8/9 years. Our complete case data has indicated that the effect sizes, in terms of the difference between the GHA under active mix and placebo challenges, were very similar for mix B in 3-year-old and 8/9-year-old children. For mix A, the effect for 3-year-old children was greater than for 8/9-year-old children. The effects for mix B were not significant for 3-year-old children because there was greater variability in the response to the active challenges than placebo in this age group. Thus, we recorded substantial individual differences in the response of children to the additives. For both age groups, no significant effect of social and demographic factors was seen on the initial level of GHA or in the moderation of the challenge effects. The moderating effects of genotype on the child's behaviour response to

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AFCA are examined in a separate paper (unpublished data).

The effect sizes reported in this study are similar to those calculated in the meta-analysis by Schab and Trinh.[4] They estimated the effects of AFCA on hyperactivity to be 0·283 (95% CI 0·079–0·488), falling to 0·210 (0·007–0·414) when the smallest and lowest quality trials were excluded. It should be noted that this meta-analysis included studies of hyperactivity in clinical samples, whereas the present study was done on children in the general population with the full range of degrees of hyperactivity. These effect sizes recorded by Schab and Trinh are smaller than those reported for stimulant treatment for ADHD in children, for which one meta-analysis[21] reported a range of effect sizes from 0·78 (0·64–0·91) by teacher report to 0·54 (0·40–0·67) by parent report. We report effect sizes that average at about 0·18. Children with ADHD are generally about 2 SD higher on hyperactivity measures than those without the disorder,[22] thus an effect size of 0·2 is about 10% of the behavioural difference between them.

This study provides evidence of deleterious effects of AFCA on children's behaviour with data from a whole population sample, using a combination of robust objective measures with strong ecological validity, based partly on observations in the classroom and ratings of behaviour made independently by teachers and by parents in the different context of the home and applying double-blinded challenges with quantities of additives equal to typical dietary intakes. It also replicates the effects of mix A previously reported on a large sample (n=277) of 3-year-old children,[5] although significant effects were only seen with parental ratings in that study.

The specific deleterious compounds in the mix cannot be determined for the present study and need to be examined in subsequent studies. The effect of artificial colours needs to be differentiated from the effects of preservatives in a 2×2 design. Further investigation would also need to establish whether the age-related difference seen in the present study can be replicated—ie, the effects of mix A being greater for 3-year-old children than for 8/9-year-old children. We examined the effects of additives on changes in behaviour during an extended period in a community-based, double-blinded, placebo-controlled food challenge. A weakness in this approach is the lack of control over when the challenges are ingested in relation to the timing of measures of hyperactivity. This study design also needs extensive resources to obtain multisource and multicontext measures of hyperactivity. We have completed a pilot study showing that changes in hyperactivity in response to food additives can be produced within about 1 h. Therefore, future studies could use more feasible acute double-blinded challenges undertaken in more controlled settings.

The present findings, in combination with the replicated evidence for the AFCA effects on the behaviour of 3-year-old children, lend strong support for the case that food additives exacerbate hyperactive behaviours (inattention, impulsivity, and overactivity) in children at least up to middle childhood. Increased hyperactivity is associated with the development of educational difficulties, especially in relation to reading, and therefore these adverse effects could affect the child's ability to benefit from the experience of schooling.[23] These findings show that adverse effects are not just seen in children with extreme hyperactivity (ie, ADHD),[4] but can also be seen in the general population and across the range of severities of hyperactivity. Our results are consistent with those from previous studies and extend the findings to show significant effects in the general population. The effects are shown after a rigorous control of placebo effects and for children with the full range of levels of hyperactivity.

We have found an adverse effect of food additives on the hyperactive behaviour of 3-year-old and 8/9-year-old children. Although the use of artificial colouring in food manufacture might seem superfluous, the same cannot be said for sodium benzoate, which has an important preservative function. The implications of these results for the regulation of food additive use could be substantial.

Contributors

JS, JOW, and ES-B participated in the conception and design of the study. The Food Standards Agency assisted with the design of the study. DMC directed the execution of the study. AB, AC, DC, LD, EK, LP, and EP undertook assessments of the children and helped to develop the observational methods employed in the study. KG supervised and KL executed the nutritional aspects of the study in relation to the preparation of suitable challenge drinks and advice on diet for parents. DMC and JS analysed the data and wrote the manuscript with input from all the authors.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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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.

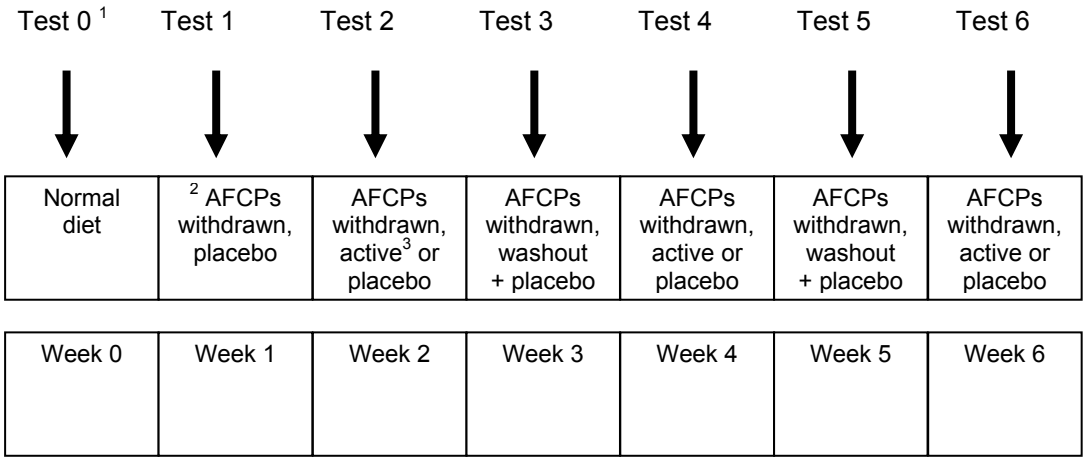
7. Secondary research questions addressed: whether genetic differences moderate any observed effect; whether there are effects in both pre-school and older children; whether any response to the additive mixtures is related to initial levels of hyperactive behaviour as scored on a hyperactive behaviour 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



[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 Thr105Ile).

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 Thr105Ile 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.

COT statement 2007/04
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ANNEX III

FOOD ADDITIVES: LIMITS ON POWERS OF MEMBER STATES TO TAKE UNILATERAL ACTION

Powers under specific additives legislation

The framework legislation for food additives is Council Directive 89/107/EEC as amended. Article 4 allows member states a limited discretion to take unilateral action where new information casts doubt on the safety of permitted additives:

> *Where a Member State, as a result of new information or of a re-assessment of existing information made since this Directive, or the comprehensive directive referred to in Article 3, was adopted, has detailed grounds for considering that the use of additives in food, although it complies with this Directive or any list drawn up under Article 3,* ***endangers human health****, that Member State may temporarily suspend or restrict application of the provisions in question in its territory. It shall immediately inform the other Member States and the Commission thereof and give reasons for its decision.*

For the power to apply, there must be detailed evidence to support a view that the additive in question "endangers human health".

In such a case the Commission must, in consultation with other member states decide whether or not to amend the EU legislation. Any national suspension or restriction is therefore temporary and requires the endorsement of the Commission before it can be made permanent.

Powers under Regulation (EC) 178/2002 where food is unsafe

Article 14 of Regulation 178/2002 prohibits the placing on the market of any food which is unsafe. "Unsafe" is defined as injurious to health or unfit for human consumption.

In determining whether any food is unsafe, regard is had among other things to the information provided to the consumer, including information on the label, about avoiding adverse effects from the food.

Food which complies with specific Community food safety provisions is deemed to be safe, but where there are reasons to suspect a **food may be unsafe despite conformity with Community food safety laws**, the authorities in member states may take appropriate action to restrict the placing of the food on the market or require its withdrawal.

It is for food businesses to withdraw or recall a food where it is considered to be unsafe (Article 19). If a food business fails to take the required action either on its own initiative or on the recommendation of the FSA and/or local authorities, the local authorities may take enforcement action, including prosecution and detention or seizure of the unsafe food.

Emergency measures under Regulation 178/2002

A member state can request the Commission to take emergency measures where food **is likely to constitute a serious risk to human health.** If a request is made and the Commission does not take action, the member state may adopt interim protective measures. The Commission then has to extend, amend or abrogate those measures, which remain in force until it does so.

In such a case an order can be made under section 13 of the Food Safety Act 1990. This empowers the Secretary of State to make an emergency control order where he or she believes that the carrying out of commercial operations with respect to food involves or may involve "**imminent risk of injury to health**". The purpose of the power is to enable the SoS to take emergency action in a situation where the danger is too serious or widespread for it to be dealt with effectively by the local food authorities.