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

STATEMENT ON FOOD ADDITIVES AND DEVELOPMENTAL NEUROTOXICITY

Background

1.   The European Food Safety Authority (EFSA) has been asked to review the food additives currently permitted within the EU in order to determine whether full re-evaluation is required. The COT has been invited to contribute to the re-evaluation process, with a particular focus on neurotoxicity, because there have been recent developments in the toxicological approaches in this area.

2.   The additives reviewed by the COT have previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food (SCF). However, a number of the evaluations, particularly those for the colours, were conducted more than 20 years ago. It is appropriate therefore to consider whether the potential for neurotoxicity and developmental effects was taken into account in setting the acceptable daily intake (ADI) for certain food additives and whether significant new data of relevance to neurodevelopmental effects have emerged since the ADI was set. It should be noted that the original data for many of these assessments were submitted directly to the committees concerned and due to the passage of time are unlikely to be available except in a summary form.

3.   The additives considered were quinoline yellow (E104), sunset yellow (E110), carmoisine (E122), Ponceau 4R (E124), indigo carmine (E132), brilliant blue (E133), sodium benzoate (E211), sulphur dioxide (E220), monosodium glutamate (E621), acesulfame K (E950), aspartame (E951) and saccharin (E954).

4.   The data for most of the colouring agents are limited and the original assessments were conducted more than 20 years ago, with few details of the studies reviewed being included in the reports. There are some new data on some of the colours that may be of relevance when considering whether these substances have neurotoxic potential.

5.   Developmental neurotoxicity studies are most useful where there is potential for significant pre- or post-natal exposure of the developing brain. They are particularly likely to be needed if the test agent is known to have neurotoxic potential in adults. The best tests are specially devised developmental neurotoxicity screens for learning and behaviour, and quantitative or semi-quantitative morphological examinations of a range of brain structures[1]. In the absence of these, a standard Functional Observational Battery in the F_1

generation as young adults, and general morphological observations on the brain based on at least 3 cross-sectional levels would be a substitute that should detect gross effects, though this would be of low sensitivity. If these measures are absent, the simple ability to feed and mate, and a normal appearance of the brain at 1 or 2 sectional levels in a standard two generation study do provide some reassurance, but may not be very sensitive indices of developmental neurotoxicity. However, it has been noted that for some pesticides, the NOAELs and LOAELs were not significantly lower in specific developmental tests than in standard toxicology tests (developmental toxicity, multi-generation toxicity, acute and short-term toxicity)[2].

Summary of data available

Quinoline Yellow (E104)

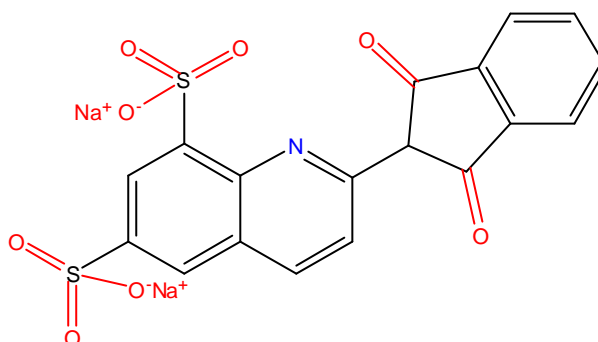6. Quinoline yellow is a synthetic, yellow food colouring (see Figure 1).

Figure 1. Quinoline yellow (disodium 2-(1,3-dioxo-2-indanyl)-6,8-quinolinesulphate)

7. Quinoline yellow was evaluated by the SCF[3] and by JECFA[4]. The toxicity data assessed by the committees included acute and short-term studies, long-term studies in the rat and mouse and multigeneration, reproduction and teratology studies.

8. Both the SCF and JECFA derived an ADI from a two-generation study in which OFI mice were fed diet containing up to 1% quinoline yellow. A No Observed Adverse Effect Level (NOAEL) of 1% of the diet (equivalent to 1000 mg/kg bw/day) was observed and an ADI of 0-10 mg/kg bw/day established. Neurotoxicity was not specifically investigated in the study. However, histological examination of a number of tissues including the brain, optic nerve, and spinal column did not reveal any abnormalities. Brain weights were also unaffected by treatment.

9. A subsequent study by Osman *et al.*[5] reported that quinoline yellow could inhibit both true (acetyl) and pseudo (butyryl) cholinesterase (ChE) enzymes in human erythrocytes and plasma *in vitro*. A dose dependent decrease in the activity of pseudo and true ChE activity of up to 53% and 87% respectively was reported. Blood acetylcholinesterase inhibition greater than 20% is generally

considered to be adverse, whereas inhibition of pseudo-ChE by itself is not usually considered adverse. The authors did not comment on the *in vivo* relevance of the concentrations tested.

10.   COT view: A biologically relevant reduction in ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. These symptoms have not been reported in any of the available animal studies. Structurally, quinoline yellow would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via lactation.   The two-generation study provided limited reassurance that no gross effects occurred since the parental animals were able to feed and rear a second generation. Adverse histological effects were not observed in the brain or in other nervous tissues, but details of the histopathology were not available. It is unclear what observations were made of the animals' behaviour.

Sunset Yellow (E110)

11.   Sunset yellow is a synthetic, yellow azo dye that is used as a food colouring (see Figure 2).
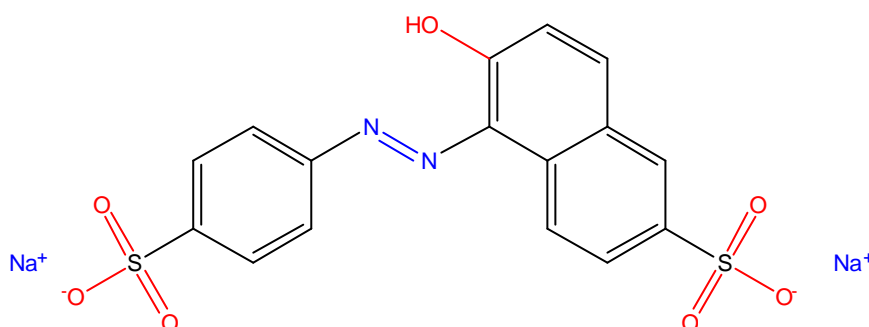

Figure 2.   Sunset yellow (disodium 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalene-sulfonate)

12.   Sunset yellow was evaluated by JECFA[6] and the SCF[3]. The toxicity data assessed by the committees included acute and short-term studies, multi-generation, reproduction and teratology studies and long-term studies in the rat, mouse and dog.

13.   JECFA established an ADI of 0-2.5 mg/kg bw/day on the basis of a NOAEL of 2% in the diet (equivalent to 500 mg/kg bw) in a 7-year feeding study in dogs and a NOAEL of 1% in the diet (equivalent to 500 mg/kg bw) in long term rat studies.

14.   The SCF also established an ADI of 0-2.5 mg/kg bw/day on the basis of a NOAEL of 250 mg/kg bw in a long term feeding study (the precise duration is not stated) in dogs though this was not the same one as that used by JECFA).

15.   A summary of a three-generation reproductive study in rodents[7] was available to both JECFA and the SCF. The actual doses used (1, 10, 30 and 100 times the ADI) were not reported although it was noted that no doses in excess of

1000 mg/kg bw/day were used. No effects on reproductive performance were reported through to the F_{2b} generation. Neurotoxicity does not appear to have been specifically investigated in this study.

16. The Registry of Toxic Effects of Chemical Substances (RTECS) report prepared by the National Institute for Occupational Safety and Health (NIOSH) listed sunset yellow as having behavioural effects in two rodent studies including coma and effect on seizure threshold. However, both were acute (LD_{50}) studies in which very high doses of sunset yellow were administered by intraperitoneal (i.p.) injection, convulsions and coma occurring prior to death in some animals. The report is based on studies[8] which additionally reported that doses of up to 6g/kg bw in mice and 10/kg bw in rats were without adverse effects other than slight diarrhoea.

17. Tanaka (1996)[9] investigated the effect of sunset yellow on selected reproductive and neurobehavioural parameters in rats. Sunset yellow was administered in the diet at dose levels of 0, 243, 493 and 999 mg/kg bw/day during gestation, the middle dose being approximately equivalent to the NOAEL identified by JECFA and used to derive the ADI.

18. There were no adverse effects on litter size, weight or sex ratio. Some changes in the body weights and survival of offspring in some groups were reported but these were not dose related. A range of neurobehavioural parameters was then measured in the F_1 generation. Of these, swimming direction on post-natal day (PND) 4 was significantly affected in the mid and top dose group males and in females in all dose groups. Swimming head angle was also affected on PND 4 in the mid and top dose group females. Treatment effects on water T-maze performance were also reported, but with no clear pattern. The authors concluded that sunset yellow had some adverse effects on reproductive and neurobehavioural parameters. It was not possible to determine a NOAEL from this study.

19. Osman et al. (2002)[5] reported that sunset yellow could inhibit both true and pseudo-ChE enzymes in human erythrocytes and plasma in vitro in a concentration dependent manner with inhibition of pseudo-ChE and true ChE of 43% and 70% respectively at the highest concentration used.

20. Osman et al. (2004)[10] reported that in rats fed 4 mg/kg bw sunset yellow or its metabolite sulphanilic acid, pseudo ChE enzymes in plasma were inhibited by 23 and 14.5% respectively and true-ChE in red blood cells by 14.5 and 30.6%. The authors did not report whether any clinical symptoms were apparent. In studies with human blood in vitro, sulphanillic acid was reported to result in dose related inhibition of pseudo and true ChE activity of up to 50%.

21. COT view: A biologically relevant reduction in brain ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. Of these symptoms, only diarrhoea has been reported in the available animal studies (see paragraph 16). Structurally, sunset yellow would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via

lactation. Potential adult neurotoxicity was only seen at lethal doses of sunset yellow, given by i.p. injection. The three-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear two further generations.

22. Sunset yellow is the only colour of those evaluated here that has undergone specific neurodevelopmental assessment. The Tanaka study had multiple endpoints increasing the possibility of confounding, and gave mixed results. Sunset yellow appears to have had an effect on swimming behaviour at 4 days of age, though this did not persist at later ages. In contrast treatment appeared to enhance maze learning at 7 weeks of age but this may have been due to poor performance in the controls.

Carmoisine (E122)

23. Carmoisine (azorubine, E122) is a synthetic, red azo dye that is used as a food colouring agent (see Figure 3).
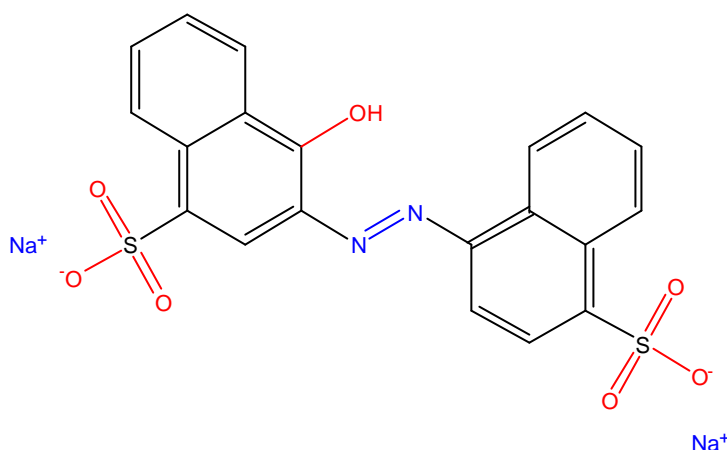

Figure 3. Carmoisine (disodium 4-hydroxy-3-(4-sulfonato-1-naphthylazo)-1-naphthalene-sulfonate)

24. Carmoisine was evaluated in 1983 by JECFA[11] and the SCF[3]. The animal studies assessed included acute and short-term studies, multigeneration, reproduction and teratology studies and long-term studies in the rat and mouse. Both JECFA and the SCF derived an ADI of 0-4 mg/kg bw/day on the basis of a NOAEL equivalent to 400 mg/kg bw/day in a 1-year study in rats[12].

25. In a four-generation rat study using doses up to 2% in the diet, there were no adverse effects on fertility, viability or lactation indices[13]. Rats from the 3_b generation were subsequently exposed to dietary concentrations of carmoisine up to 2% for 1-year. There were no adverse effects on bodyweight gains, urinalysis, haematology, gross pathology or histology (the latter did not include brain tissue).

26. Two additional multi-generation reproduction studies of carmoisine were also available to the committees, in which no treatment related adverse effects were apparent. However, neurological effects were not specifically investigated in any of the multigeneration reproduction studies.

27.	The RTECS report listed carmoisine as having behavioural effects in rodent studies including somnolence, coma and convulsions or effect on seizure threshold. The report is based on an LD_{50} study[14] where coma and convulsions often preceded death following i.p. administration of high doses of carmoisine. However it was also reported that oral doses of up to 8g/kg bw in mice and 10/kg bw in rats were without adverse effect other than slight lethargy.

28.	Since the evaluation of carmoisine by JECFA and SCF, two additional studies have been published. In a two-generation reproduction study[15] rats were fed up to 1200 mg/kg bw/day carmoisine, the F_1 generation being exposed for 110-115 weeks. No adverse clinical effects or effects on behaviour or reproductive performance were reported in the F_0 generation. Mortality in the F_1 generation was not affected by treatment. The tissues sampled for histopathology included brain, sciatic nerve and spinal cord and no adverse effects or differences in tumour incidence were reported. Neurotoxicity was not specifically investigated in this study.

29.	Osman *et al.* (2004)[10] reported that carmoisine inhibited both true and pseudo-ChE enzymes in human erythrocytes and plasma in a dose-dependent manner with up to 50% inhibition of both pseudo and true ChE produced at the highest concentrations of carmoisine tested. Inhibition of these enzymes was reversible. Naphthionic acid, a metabolite of carmoisine, was also able to inhibit both pseudo and true ChE *in vitro*.

30.	Osman *et al.* (2004)[10] also reported that in rats fed 4 mg/kg bw carmoisine or napthionic acid for up to 7 days pseudo-Che was inhibited by 15.1 and 18.5% respectively. True ChE was inhibited by 27.8% in the animals fed carmoisine but was unaffected in those given napthionic acid. The authors did not mention whether any adverse clinical effects were apparent.

31.	COT view. A biologically relevant reduction in brain ChE activity would be clearly visible in animal studies. A 70% decrease in activity would result in cholinergic activity such as increased salivation, urination and defecation. Symptoms of this type were not reported in any of the available animal studies. Structurally, carmoisine would be unlikely to cross the mature blood-brain barrier but the developing brain could be exposed via lactation. Potential adult neurotoxicity was only seen at lethal doses, given by i.p. injection. The three-generation study provides some reassurance that no gross effects occurred since parental animals were able to feed and rear through two further generations.

Ponceau 4R (E124)

32.	Ponceau 4R is a synthetically produced azo dye that is used as a red colouring for foodstuffs (see Figure 4).
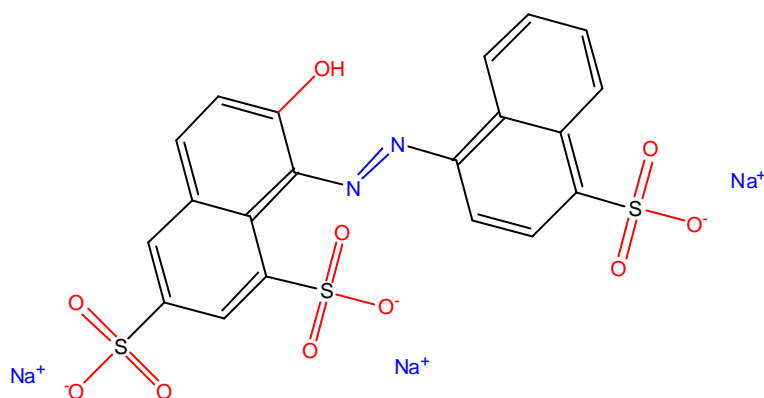
Figure 4. **Ponceau** **4R** **(trisodium** **2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate)**

33.   Ponceau 4R was evaluated in 1983 by JECFA[11] and the SCF[3]. The animal studies assessed included acute and short-term studies, multigeneration, reproduction and teratology studies and long-term studies in the rat and mouse.

34.   Both Committees identified a NOAEL equivalent to 375 mg/kg bw/day from a long term mouse study, adverse renal effects being reported at higher doses, this was used to derive an ADI of 0-4 mg/kg bw/day.

35.   Ponceau 4R had no adverse effects in a 3-generation reproductive study in rats at dietary concentrations of up to 1250 mg/kg bw/day. There was no effect on the incidence of pre- and post-implantation losses or the weight or appearance of foetuses. The postnatal development of offspring as judged by survival, bodyweight and "developmental milestones" was not affected by treatment. There were no treatment related differences in histopathology (tissues not specified) between the control and F_3 animals.

36.   The RTECS report stated that Ponceau 4R had behavioural effects in rodent studies. The report is based on an LD_{50} study[16] where coma and sometimes convulsions preceded death following i.p. administration of high doses of Ponceau 4R. However, it was further reported that oral doses of up to 8 g/kg bw in mice and rats were without adverse effect other than slight lethargy.

37.   Following evaluation of Ponceau 4R by JECFA and the SCF, two additional studies have been identified. Brantom *et al.* (1987)[17] conducted a three-generation reproduction study in rats fed up to 1250 mg/kg bw/day Ponceau 4R in the diet. There were no treatment related clinical effects or deaths during the study. No differences in bodyweight, food or water intakes were observed. Fertility of treated dams and viability of litters was unaffected by treatment. Some differences in litter development were apparent in the F_2 but not the F_3 generation. For the F_3 generation, two additional responses, the righting response and the startle response, were assessed but were not affected by treatment. Some changes in organ weights were reported in the F_3 animals but there were no treatment related histopathological findings.

38.   In a subsequent two-generation study[18] rats were fed Ponceau 4R at doses up to 1250 mg/kg bw/day. There were no treatment-related effects of Ponceau 4R

in the F_0 generation. The F_1 generation received the same dietary concentrations for up to 114 or 188 weeks. Higher brain weights were reported in the top dose male rats, these changes became significant when adjusted for bodyweight. Degeneration of the granular layer neurones in the cerebellum was also reported in females at the highest dose (3/53 examined compared to 0/96 in the controls). The authors did not comment on this finding, but noted a number of statistically significant lesions were observed which were age related and confined to one sex. A NOAEL of 500 mg/kg bw/day was identified, comparable to that used to establish the ADI. Neurotoxicity was not specifically reported in either of these studies.

39. <u>COT view</u>. Structurally Ponceau 4R would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. The three-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear two further generations. Neurotoxic symptoms have been reported in adults but only at lethal doses, given i.p. However, of more concern are the effects on cerebellar granule cells in the two generation study since, although the effect was only seen at the highest dose, the qualitative measure used may have been insufficiently sensitive to detect neuronal loss at a lower dose.

Indigo carmine (E132)

40. Indigo carmine (indigotine) is a synthetically produced blue food colouring (see Figure 5).

41. Indigo carmine has been evaluated by JECFA[19] and by the SCF[3]. The animal studies assessed included acute and short-term studies and teratology studies. Data on long-term studies in the rat and mouse were available in summary form.
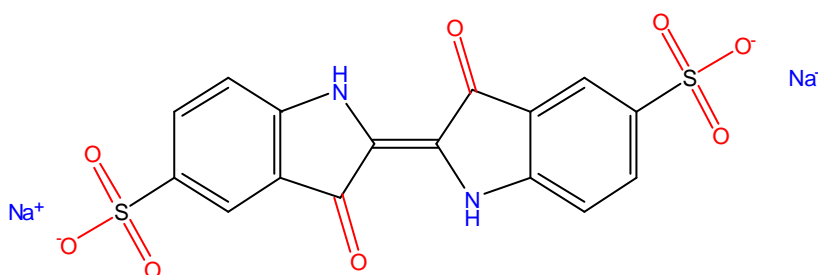

Figure 5. Indigo carmine (disodium 3-3' dioxo-2,2'bi-indolylidene-5-5' disulfonate)

42. Both Committees established an ADI of 0-5 mg/kg bw/day on the basis of a NOAEL equivalent to 500 mg/kg bw in a 2-year study in rats. JECFA reported that no adverse effects were noted on reproduction, gross or microscopic pathology. A similar study in mice did not indicate any adverse effects on histopathology. No neurotoxicity or multi-generation studies were available to JECFA or the SCF.

43. Since the evaluations were conducted an additional study has been published[20], which investigated the chronic toxicity and carcinogenicity of indigo carmine in rats treated with indigo carmine *in utero*. The F_0 generation received up to 2% indigo carmine in the diet prior to mating with no effects on bodyweight or mortality. Following mating, there were no treatment-related effects on fertility or pup viability. Animals from the F_1 generation received up to 2% indigo carmine in the diet for a maximum of 29-30 months. There were no treatment-related effects on mortality or on physical observations. Some changes in organ weights were measured but brain weights were unaffected. No treatment-related effects were apparent after histopathological examination of tissues including three sections of the brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) optic nerve, sciatic nerve and spinal cord. Overall, the authors reported that there was no evidence of toxicity or carcinogenicity. Neurotoxicity was not specifically investigated.

44. COT view. The two-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear a further generations. Histopathological examination of the brain and other nervous tissue did not reveal any adverse effects. There was no suggestion of potential to produce neurotoxicity in the adult at any dose. Structurally, indigo carmine would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation.

Brilliant blue (E133)

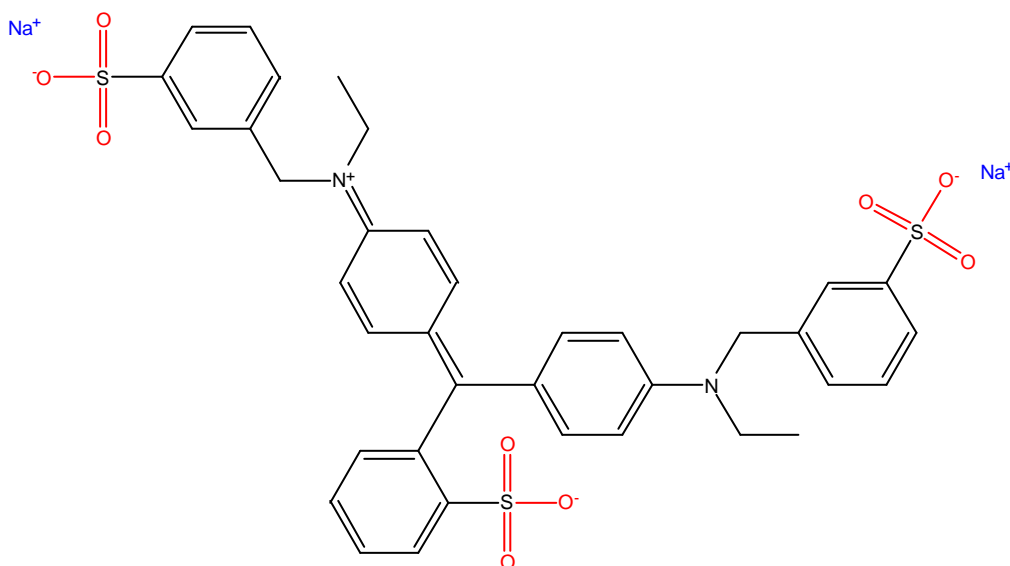45. Brilliant blue is a synthetic, blue food colouring (see Figure 6)

Figure 6. Brilliant blue (disodium **a**-[4-(N-ethyl-3-sulfonatobenzylamino)phenyl]-**a**-[4-(N-ethyl-3-sulfonatobenzylimino)cyclohexa-2,5-dienylidene] toluene-2- sulfonate

46. Brilliant blue has been evaluated by JECFA[21] and the SCF[3]. The animal studies assessed included acute and short-term studies, reproduction and teratology studies and long-term studies in the rat and mouse.

47.   In 1969, JECFA established an ADI of 0-12.5 mg/kg bw/day on the basis of a NOAEL equivalent to 2500 mg/kg bw/day in a two-year rat study. In 1983, the SCF established an ADI of 0-10 mg/kg bw/day on the basis of a NOAEL equivalent to 1000 mg/kg bw in rodent studies. In the SCF review, no compound related effects were reported in reproductive and teratology studies in the rat. Neurological effects were not specifically investigated in these studies. There are no additional details of the studies reviewed by the SCF.

48.   The RTECS report lists brilliant blue as having behavioural effects including convulsions and effects on seizure threshold in mice. This is taken from an LD_{50} study in which the effects were reported following i.p. administration of 4600 mg/kg, a lethal dose.

49.   Following the JECFA and SCF evaluations, one additional paper has been published, Borzelleca *et al.* (1990)[22] investigated the lifetime toxicity and carcinogenicity of brilliant blue in rats and mice exposed to brilliant blue *in utero*. In the rat study, the F_0 generation received up to 2% in the diet for 2 months prior to mating. There were no consistent treatment-related effects on fertility, gestation, parturition, lactation, pup survival through weaning or on the number of live or stillborn pups. The F_1 generation was also exposed to up to 2% brilliant blue for up to 111-116 weeks. Some effects on food consumption and mean bodyweights were reported in some dose groups and survival was significantly reduced in females at 2%. No treatment related clinical, haematological or urinalysis findings were reported. There were no effects on any of the organ weights measured including brain. In addition, there no treatment related gross or histological changes following examination of tissues including three sections of the brain (frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons) optic nerve, sciatic nerve and spinal cord. NOAELs of 2 % (1072 mg/kg bw/day) and 1% (631 mg/kg bw/day) for males and females respectively were identified.

50.   In the mouse study, brilliant blue was administered in the diet at concentrations of up to 5% for 104 weeks. There were no compound related adverse effects on general physical observations, food consumption, survival or on haematological parameters. Group mean bodyweights were slightly lower in treated mice at various intervals, but this was not consistent throughout the study. The NOAEL in the mouse study was 5% (7354 and 8699 mg/kg bw/day for males and females respectively). The same tissues were examined histologically as in the rat study. No adverse effects were apparent.

51.   <u>COT view</u>. Structurally brilliant blue would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. Potential adult neurotoxicity was only seen at lethal dose levels given by i.p. injection. The two-generation study provides some reassurance that no gross effects occurred since parental animals were able to feed and rear a further generation. Histopathological examination of the brain did not reveal any effects.

Sodium benzoate (E211)

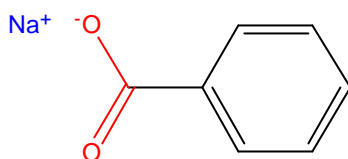52.   Sodium benzoate is used as a food-preserving agent (see Figure 7).

Figure 7. Sodium benzoate

53.   Sodium benzoate has been evaluated by the SCF[23] and JECFA[24]. Both Committees established an ADI of 0-5 mg/kg bw/day on the basis of the NOAEL of 500 mg/kg bw/day from a four-generation study in rats. There were no adverse effects on fertility or lactation, the only parameters investigated. Developmental studies were available in the mouse, rat, rabbit and hamster. Neurotoxicity was not specifically investigated in any studies considered in the JECFA or SCF reviews.

54.   The SCF evaluation noted that high, acute doses of sodium benzoate were associated with effects on the central nervous system in humans due to the disruption of acid-base balance, but were rapidly reversible. Such effects were not expected to occur at the level of the ADI. Sodium benzoate is detoxified via glycine conjugation and urinary excretion thus benzoate toxicity is reduced by glycine supplementation.

55.   No additional relevant studies have been identified since sodium benzoate was evaluated.

56.   <u>COT view</u>. The structure of benzoate suggests that it could penetrate the blood brain barrier or cross the placenta, possibly via the organic anion transporter, though metabolic studies with radiolabelled benzoate reviewed by the SCF and JECFA indicate that the vast majority is excreted with little accumulation in the organs. No evidence of neurotoxicity is apparent in the available data which includes a four-generation reproduction study. Effects on the central nervous system have been noted but this is due to the disruption to acid-base balance rather than specific neurotoxicity.

Sulphur dioxide (E220)

57.   Sulphur dioxide is used as a food-preserving agent. It has been evaluated by JECFA[25] and the SCF[23]. Both Committees established an ADI of 0-0.7 mg/kg bw/day based on a NOAEL of 70 mg/kg b.w. in long term studies in rats and pigs.

58.   In the rat study, the animals were treated for up to two years and over three generations. The brain was weighed and brain, spinal cord and femoral nerve examined histopathologically. No treatment related changes relevant to neurotoxicity were observed. The animals were bred for 2 further generations. There were no treatment-related decreases in mortality; body weight gain was

reduced in some of the treated animals in the F_1 and F_2 generations, but a dose-response relationship was not apparent. The number of F_{2a} young were reduced in the treated animals but this was not dose-related and did not occur in the F_{2b} litter.

59.   No additional relevant studies have been identified since sulphur dioxide was evaluated.

60.   COT view. Structurally sulphur dioxide would be unlikely to cross the mature blood-brain barrier or placenta but the developing brain could be exposed via lactation. The two-generation study provides limited reassurance that no gross effects occurred since parental animals were able to feed and rear a further generation. No neurotoxic effects were apparent in other animal studies. Limited histopathology in brain and other nervous tissue did not suggest any adverse effects.
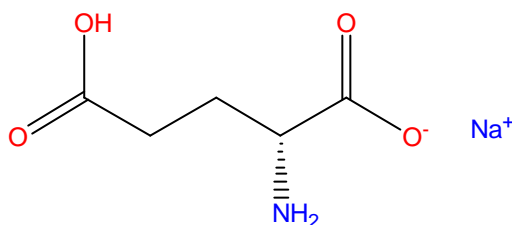
Monosodium glutamate (E621)

Figure 9. Monosodium glutamate

61.   Monosodium glutamate is a flavour-enhancing agent. It is a salt of L-glutamic acid, an amino acid representing approximately 20% of ingested protein.

62.   Monosodium glutamate has been evaluated by JECFA[26] and by the SCF[27]. Both Committees established a group ADI 'not specified' on the basis of the data provided and since large intakes of glutamates are consumed in the normal diet.

63.   There is a large body of information regarding the neurological effects of MSG. The SCF stated that some studies demonstrated a strain dependant, variable vulnerability of the developing rat or mouse CNS to high levels of glutamate alone or in combination with other amino acids in massive doses but that no brain lesions occurred in mouse, rat and hamster studies in which the animals had ingested large doses of MSG in their diet. In addition, some of the acute effects in humans observed after ingestion of more than 3 g glutamate per person were also observed with other foods not containing glutamates. The symptoms are not specified by the SCF but high plasma glutamate levels are associated with nausea and vomiting[26]. Acute symptoms of burning, facial pressure and chest pains have also been reported[28].

64.   Since the evaluations were conducted, four additional neurotoxicity studies have been identified. Bawari *et al.* (1995)[29] reported that subcutaneous administration of MSG to rat pups resulted in significantly increased lipid peroxidation and catalase levels in the mid-brain region. A significant reduction in

total as well as non-protein SH groups was also reported. There were no effects in the frontal cortex. Similar findings were reported by Babu *et al.* (1994)[30]. Hsieh *et al.* (1997)[31] reported that subcutaneous administration of MSG resulted in sex-specific and area-specific changes in neuronal density (both number and volume) in rats.

65.   Jing *et al.* (1994)[32] injected pregnant mice s.c. with monosodium glutamate on alternate days until parturition. Memory and Y-maze spatial discrimination learning were damaged in the offspring of the high dose (2.5g/kg bw) MSG group compared to the low dose group (1g/kg bw). There was significant destruction of neurones in the arcuate nucleus and ventromedial nucleus of the hypothalamus exhibiting cytoplasmic swelling, nuclear pyknosis and a reduction in the number of neurones. MSG treatment affected the amount of ^{3}H-glutamate binding in the hypothalamus and hippocampus of the mice. It was also reported that MSG can increase the calcium ion concentration in individual neurones by inducing an influx of extracellular calcium and releasing intracellular calcium stores which could be responsible for the observed cell damage and behavioural changes.

66.   COT view. Assessment of MSG assumes that the sodium salt behaves in the same way as the glutamate derived from dietary protein breakdown. Glutamate is a neuroactive compound and can cross the blood-brain barrier and placenta. Hence there is potential for brain exposure at all developmental stages. However, glutamate is present in normal blood and brain, and brain levels are well controlled by active physiological regulation, so there is unlikely to be a hazard at realistic dose levels. Neurotoxic effects have been seen in animal studies but only at very high doses, often administered by s.c. injection. Such high doses would be expected to overcome physiological regulation of brain glutamate concentration. There is a substantial body of work investigating MSG at lower doses with no indication of any adverse effects, as well as extensive human exposure data.

Acesulfame K

67.   Acesulfame K (E950) is a synthetic sweetening agent (see Figure 10).
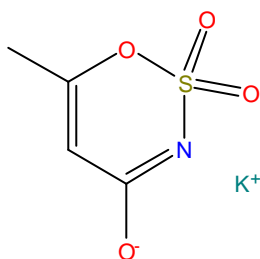

Figure 10. Acesulfame K (potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide.

68.   Acesulfame K has been evaluated by JECFA[33] and the SCF[34]. JECFA established an ADI of 0-15 mg/kg bw/day on the basis of a NOAEL equivalent to 1500 mg/kg bw/day in a chronic study in rats. In the SCF review, the dog was considered to be the most relevant species, due to greater kinetic similarities.

The NOAEL in the dog study was 900 mg/kg bw/day from which an ADI of 0-9 mg/kg bw/day was established.

69.   Neurotoxicity was not specifically investigated in a four-generation rat study at dietary concentrations of up to 3%. No adverse effects on fertility, pups per litter, birth weight and mortality during lactation were reported. Growth rate was slightly affected in the top dose animals of the F_0 and F_1 generations. No dose-related effects were found in a second reproduction study in which rats were treated with up to 3% acesulfame K in the diet. Tissues such as brain, spinal cord and sciatic nerve were examined histologically in a variety of studies, with no adverse effects being apparent.

70.   No additional relevant studies have been identified since acesulfame K was evaluated.

71.   <u>COT view</u>. Structurally acesulfame K would be unlikely to cross the mature blood-brain barrier or placenta to a significant extent but the developing brain could be exposed via lactation. However, the four-generation study provides reassurance that no gross effects occurred since parental animals were able to feed and rear subsequent generations. There was no suggestion of potential to produce neurotoxicity in the adult at any dose. The limited histopathology available from other animal studies did not indicate any adverse neurological effects.

Aspartame (E951)

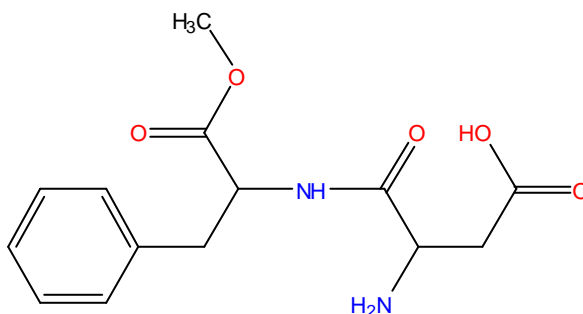72.   Aspartame (is a synthetic sweetener (see Figure 11).

Figure 11. Aspartame (6-methyl-1,2,3-oxathiazine-4(3H)--one-2,2-dioxide salt of L-phenylalanyl-2-methyl-L-a-aspartic acid.

73.   Aspartame has been evaluated by JECFA[35] and the SCF[36]. Both Committees derived an ADI of 0-40 mg/kg bw/day on the basis of a 104-week study in rats.

74.   The 2002 SCF review considered neurological symptoms in particular detail. They concluded that despite targeted animal studies, no consistent effect of aspartame on neurotransmitters or their precursors had been observed. Human studies indicate that there were no changes in behaviour, cognition, mood or learning associated with aspartame nor was it more likely to be associated with

headaches than placebo. No additional relevant studies have been identified since the most recent SCF evaluation.

75.   COT view. Aspartame has been extensively studied in both humans and animals, with no indication of any adverse effects being apparent from either aspartame or its breakdown products (which include the natural amino acid phenylalanine). Only doses high enough to increase plasma phenylalanine well beyond the normal level would have the potential to be neurotoxic. As well as conventional animal studies, the data available include behavioural studies in animals and assessment of reported human side effects, including in potentially sensitive individuals none of which indicate neurotoxic potential.

Saccharin (E954)

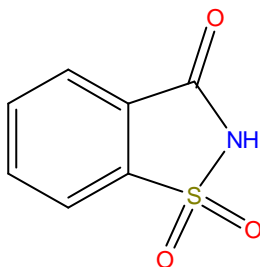76.   Saccharin is a synthetic sweetening agent (see Figure 12).

Figure 12. Saccharin (3-oxo-2,3-dihydrobenzo[d]isothiazol-1,1-dioxide)

77.   Saccharin has been evaluated by JECFA[37] and the SCF[38]. Both Committees derived an ADI of 0-5 mg/kg bw on the basis of a NOAEL equivalent to 500 mg/kg bw/day for bladder tumours in a 2-generation feeding study in the rat.

78.   Neurological effects were not specifically investigated in young rats exposed to saccharin from parturition (up to 5% in the diet) or in two multi-generation reproductive studies (up to 7.5% in the diet). However, no adverse effects on reproductive parameters were apparent.

79.   No additional relevant studies have been identified.

80.   COT view. Structurally saccharin would be unlikely to cross the mature blood-brain barrier or placenta to a significant extent but the developing brain could be exposed via lactation. However, the available two-generation reproduction studies do not indicate any gross effects. No evidence of neurotoxicity is apparent from other animal studies. The limited histopathology available did not indicate any adverse neurological effects.

Summary and Discussion

81.   Neurotoxic symptoms have been reported in acute studies for some of the additives but these have been LD50 studies using very high doses administered ip and may not be relevant to assessing the use of these colours as food additives.

82.   For most of the substances there were insufficient data to fully assess developmental neurotoxicity. Gross neurotoxic effects would be apparent in conventional animal studies but developmental and behavioural effects can be much more sensitive than other end points, for example, as with studies of lead and mercury toxicity. However, certain factors would indicate whether there was need for concern and thus assist in prioritising the additives for review. These could include chemical structure, indicating whether the compound could be likely to cross the blood brain barrier or the placenta, and the neurotoxic potential in adults since almost all neurodevelopmental toxins in children are also neurotoxins in adults. It was agreed that a tiered approach should be taken in assessing these additives, considering both the potential for adult toxicity and potential routes of exposure to establish whether significant exposure could occur *in utero* or via lactation.

83.   Quinoline yellow, sunset yellow (and its metabolite sulphanillic acid) and carmoisine (and its metabolite naphthionic acid) have been reported to inhibit pseudo and true cholinesterase activity *in vitro*. Sunset yellow and carmoisine have also been reported to reversibly inhibit cholinesterase following administration to rats. The significance of these data is currently unclear and cholinergic symptoms have not been observed in conventional animal studies even after high dose and/or chronic exposure.

84.   Sunset yellow has been assessed for effects on a variety of neurobehavioural endpoints at doses in the region of the NOAEL used to derive the ADI. Some non-persisting effects on behaviour were observed.

85.   Indigo carmine and brilliant blue have not been specifically investigated for neurotoxicity. Chronic toxicity and reproductive studies were available for the original evaluations, and some additional studies have been conducted since. No overt effects on behaviour and no effects on fertility or reproductive parameters have been reported. This suggests that gross neurotoxic effects would have been detected. Although more subtle behavioural or neurodevelopmental effects have not been investigated, the available studies do not provide any indications of neurotoxic potential, or that the ADI is inadequate.

86.   Ponceau 4R has not been specifically investigated for neurotoxicity. Chronic toxicity and reproductive studies were available for the original evaluations, and some additional studies have been conducted since. No overt effects on behaviour and no effects on fertility or reproductive parameters have been reported. A conventional two-generation study however, gave clear evidence of neuronal loss, and hence of developmental neurotoxicity, at a high dose. The possibility that a quantitative evaluation might have shown more subtle effects at lower doses cannot be excluded.

87.   Sodium benzoate, sulphur dioxide, acesulfame K and saccharin were evaluated more recently and no new relevant studies have emerged since. Overall the data do not provide indications of concern for neurotoxicity.

88.   For MSG and aspartame, where concerns have been expressed, neurotoxicity has been considered in more detail by the SCF and JECFA. There are several additional studies on MSG which indicate damage to nervous tissue but these have required administration of sufficiently high MSG doses to overcome physiological control of brain glutamine and the relevance of this route to its use as a food additive is unclear.

Conclusions

89.   In summary, the Committee concluded that there was no evidence of properties that would suggest a potential to produce developmental neurotoxicity for acesulfame K, brilliant blue, indigo carmine, saccharin, sodium benzoate or sulphur dioxide. There is some equivocal evidence suggesting the potential to produce developmental neurotoxicity at very high dose levels for carmoisine, sunset yellow and quinoline yellow. There was actual evidence suggesting the potential to produce developmental neurotoxicity at very high doses for aspartame, monosodium glutamate and Ponceau 4R. For no agents was there any evidence suggesting the potential for developmental neurotoxicity at current acceptable daily intakes. Therefore, although direct evaluations of developmental neurotoxicity were mostly absent and would, in principle, be desirable, the available data did not suggest that further investigations of any of these agents would be a priority given current dietary intakes.

COT statement 2006/02
January 2006

REFERENCES

1.   Makris, S, Raffaele, K, Sette, W, Seed, J. (1998). A Retrospective Analysis of Twelve Developmental Neurotoxicity Submitted to the USEPA Office of Prevention, Pesticides, and Toxic Substances.

2.   JMPR (2002).   Pesticides Residues in Food-2002- Joint FAO/WHO meeting on Pesticide Residues. Studies of Developmental Neurotoxicity and their use in establishing acute references doses and acceptable daily intakes. http://www.inchem.org/documents/jmpr/jmpmono/2002pr15.htm

3.   SCF (1983). Reports of the Scientific Committee for Food. 13th Series.

4.   WHO (1984). World Health Organization. WHO Food Additives Series, 19.

5.   Osman MY, Sharaf IA, el-Rehim WM, el-Shharkawi AM (2002). Synthetic organic hard capsule colouring agents: in vitro effect on human true and pseudo-cholinesterases. *British Journal of Biomedical Sciences*. 59:212-217.

6.   WHO (1982). World Health Organization. WHO Food Additives Series, 17.

7.   Pierce E.C., Agersborg,H.P.K., Borzelleca, J.F., Burnett, C.M., Eagle, E., Ebert, A.G., Kirschman, J.C., Scala, R.A. (1974). Multi-generation studies with certified colors in rats. Abstract XIII. *Toxicology and Applied Pharmacology*, 136: 259.

8.   Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967a). Acute (rat and mouse) and short-term (rat) toxicity studies on sunset yellow FCF. *Food and Cosmetic Toxicology*, 5:747-754.

9.   Tanaka T (1996). Reproductive and neurobehavioural effects of sunset yellow FCF administered to mice in the diet. *Toxicology and Industrial Health*. 12:69-79.

10.   Osman MY, Sharaf IA, Osman HMY, El-Khouly ZA, Ahmed EI (2004). Synthetic organic food colouring agents and their degraded products: effects on human and rat cholinesterases. *British Journal of Biomedical Sciences*. 61:128-132.

11.   WHO (1983). World Health Organization. WHO Food Additives Series, 18.

12.   Holmes, P.A., Pritchard, A.B., Kirschman, J.C. (1978a). A one year feeding study with carmoisine in rats. *Toxicology*, 10:185-193.

13.   Holmes, P.A., Pritchard, A.B., Kirschman, J.C. (1978b). Multigeneration reproduction studies with carmoisine in rats. *Toxicology*, 10:169-183.

14.   Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967b). Acute (rat and mouse) and short-term (rat) toxicity studies on carmoisine. *Food and Cosmetic Toxicology*, 5:179-185.

15.   Ford GP, Stevenson BI, Evans JG (1987). Long term toxicity study of carmoisine in rats using animals exposed in utero. *Food Chemical Toxicology*. 25:919-925.

16.   Gaunt I.F., Farmer, M., Grasso, P., Gangolli, S.D. (1967c). Acute (rat and mouse) and short-term (rat) toxicity studies on Ponceau 4R. *Food and Cosmetic Toxicology*, 5:187-194.

17.   Brantom PG, Stevenson BI, Ingram AJ (1987). A three-generation reproduction study of ponceau 4R in the rats. *Food Chemical Toxicology*. 25:963-968.

18.   Brantom PG, Stevenson BI, Wright MG (1988). Long-term toxicity study of ponceau 4R in rats using animals exposed in utero. *Food Chemical Toxicology*. 25:955-962.

19.   WHO (1974). World Health Organization. WHO Food Additives Series, 6.

20.   Borzelleca JF, Hogan GK, Koestner A (1985). Chronic toxicity/carcinogenicity study of FD&C Blue No.2 in rats. *Food Chemical Toxicology*. 23:551-558.

21.   WHO (1969). World Health Organization. WHO FAO Nutrition Meetings Report Series 46a.

22.   Borzelleca JF, Depukat K, Hallagan JB (1990). Lifetime toxicity/carcinogenicity studies of FD&C Blue No.1 (Brilliant Blue FCF) in rats and mice. *Food Chemical Toxicology*. 28: 221-234.

23.   SCF (1994). Reports of the Scientific Committee for Food. 35th Series.

24.   WHO (1996). World Health Organization. WHO Food Additives Series, 37.

25.   WHO (1998). World Health Organization. WHO Food Additives Series, 42.

26.   WHO (1987). World Health Organization. WHO Food Additives Series, 22.

27.   SCF (1991). Reports of the Scientific Committee for Food. 25th Series.

28.   Gosselin, R.E., R.P. Smith, H.C. Hodge. (1984) Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams and Wilkins, II-407.

29.   Bawari M, Babu NG, Ali MM, Misra UK (1995). Effect of neonatal monosodium glutamate on lipid peroxidation in adult rat brain. *NeuroReport*. 6: 650-652.

30.   Babu GN, Bawari M, Ali MM (1994). Lipid peroxidation and antioxidant status of circumventricular organs of rat brain following neonatal monosodium glutamate. *Neurotoxicology*. 15: 773-778.

31.   Hsieh, Y., Hsu, C., Lue, S., Hsu, H., Peng, M. (1997). The neonatal neurotoxicity of monosodium L-glutamate on the sexually dimorphic nucleus of the preoptic area in rats. *Developmental Neuroscience*, 19: 342-347.

32.   Jing G, Juan, W, Xiaoning Z Weining Z, Yitu Z, Zuxuan Z. Effects of monosodium glutamate passing through the placental barrier on the structures and functions of specific areas of the brain in infant mice. *Acta Physiologica Sinica*. 48:44-51, 1994. [English translation of Chinese original]

33.   WHO (1990). World Health Organization. WHO Food Additives Series, 28.

34.   SCF (2000). Opinion of the SCF expressed on 9th March 2000.

35.   WHO (1981). World Health Organization. WHO Food Additives Series, 15.

36.   SCF (2002). Opinion of the SCF expressed on 4th December 2002.

37.   WHO (1993). World Health Organization. WHO Food Additives Series, 32.

38.   SCF (1995). Opinion of the SCF expressed on 2nd June 1995.