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PREFACE

The monographs contained in this volume were prepared at the seventy-fourth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 14–23 June 2011. These monographs summarize the data on selected food additives and contaminants reviewed by the Committee.

The seventy-fourth report of JECFA has been published by the World Health Organization as WHO Technical Report No. 966. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by temporary advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by M. Sheffer, Ottawa, Canada.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

ALUMINIUM-CONTAINING FOOD ADDITIVES (addendum)

First draft prepared by

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1. EXPLANATION

Aluminium can occur in food as a result of its natural occurrence in the environment, contamination from various sources, leaching from food contact materials and the use of aluminium-containing food additives.

Various aluminium compounds were evaluated by the Committee at its thirteenth, twenty-first, twenty-sixth, twenty-ninth, thirtieth, thirty-third and sixty-seventh meetings (Annex 1, references *20*, *44*, *59*, *70*, *73*, *83* and *184*). At its thirteenth meeting, the Committee established an acceptable daily intake (ADI) "not specified" for sodium aluminosilicate and aluminium calcium silicate (Annex 1, reference *20*). At its twenty-sixth meeting, the Committee established a temporary ADI of 0–0.6 mg/kg body weight (bw) for sodium aluminium phosphate (Annex 1, reference *59*). At its thirtieth meeting, the Committee noted concerns about a lack of precise information on the aluminium content of the diet and a need for additional safety data. The Committee extended the temporary ADI of 0–0.6 mg/kg bw expressed as aluminium to all aluminium salts added to food and recommended that aluminium in all its forms should be reviewed at a future meeting (Annex 1, reference *73*).

The Committee evaluated aluminium as a contaminant at its thirty-third meeting, placing emphasis on estimates of consumer exposure, absorption and distribution of dietary aluminium and possible neurotoxicity, particularly

the relationship between exposure to aluminium and Alzheimer disease. The Committee established a provisional tolerable weekly intake (PTWI) of 0–7.0 mg/kg bw for aluminium, and a consolidated monograph was produced (Annex 1, reference *84*). The Committee concluded that there was no need to set a separate ADI for the food additives sodium aluminium phosphate basic or sodium aluminium phosphate acidic, because the PTWI included aluminium exposure arising from food additive uses.

At its sixty-seventh meeting, the Committee re-evaluated aluminium used in food additives and from other sources and concluded that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than those used in establishing the previous PTWI (Annex 1, reference *186*). The Committee noted that the lowest lowest-observed-effect levels (LOELs) for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50–75 mg/kg bw per day. The Committee selected the lower end of this range of LOELs (50 mg/kg bw per day) and established a PTWI of 1 mg/kg bw by applying an uncertainty factor of 100 to allow for interspecies and intraspecies differences and an additional uncertainty factor of 3 for deficiencies in the database, notably the absence of no-observed-effect levels (NOELs) in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The PTWI applied to all aluminium compounds in food, including food additives. The previously established ADIs and PTWI for aluminium compounds were withdrawn. The Committee noted that the PTWI was likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing food additives. The Committee also noted that dietary exposure to aluminium is expected to be very high for infants fed on soya-based formula. The Committee noted a need for:

- further data on the bioavailability of different aluminium-containing food additives;
- an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioural end-points using relevant aluminium compounds;
- studies to identify the forms of aluminium present in soya-based formula and their bioavailability.

Aluminium-containing food additives were re-evaluated by the Committee at its present meeting, as requested by the Codex Committee on Food Additives (CCFA). The Committee was asked to consider all data necessary for safety evaluation (bioavailability, developmental toxicity and multigeneration reproductive toxicity) and data on actual use levels in food. In addition, the Committee was asked to consider all data necessary for the assessment of safety, dietary exposure and specifications for aluminium lactate and potassium aluminium silicate, which had not been evaluated previously by the Committee for use as food additives. Potassium aluminium silicate is mined from natural sources and then further purified for use as a carrier substrate for potassium aluminium silicate–based pearlescent pigments. Potassium aluminium silicate–based pearlescent pigments are produced by reaction of potassium aluminium silicate with soluble salts of titanium and/or iron followed by calcination at high temperatures. The pigments can be produced with

a variety of different pearlescent colour effects depending upon particle size and the combination of titanium dioxide and/or iron oxide deposited on the potassium aluminium silicate.

The Committee received submissions from a number of sponsors, including unpublished studies of bioavailability and toxicity and a review of the scientific literature. Additional information was identified from the scientific literature. No information was received on the forms of aluminium present in soya-based infant formula.

Additional information was identified by searching PubMed for [aluminium and bioavailability] and [aluminium and neurotox*], focusing on studies likely to provide information on dose–response relationships.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

The bioavailability of a single dose of aluminium ammonium sulfate was assessed in groups of four male (302–379 g) and four female (236–265 g) fasted Crl:CD (SD) rats in a study that was compliant with good laboratory practice (GLP). Aluminium ammonium sulfate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw and intravenously at 2 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. Four of the top-dose animals (one male and three females) died and were replaced by additional animals. The cause of death in these animals is unclear. The bioavailability was calculated from the 24-hour area under the concentration versus time curve (AUC) values to be 0.039% in males and 0.061% in females dosed with aluminium ammonium sulfate at 300 mg/kg bw and 0.048% in males and 0.067% in females dosed at 1000 mg/kg bw (Sunaga, 2010a). If it is assumed that these doses were expressed as aluminium ammonium sulfate, the oral doses of aluminium would be 33 and 110 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium ammonium sulfate was assessed in groups of four male (267–293 g) and four female (183–198 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium ammonium sulfate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.008% in males and 0.003% in females dosed with aluminium ammonium sulfate at 300 mg/kg bw and 0.006% in males and 0.023% in females dosed at 1000 mg/kg bw. The

maximum concentration (C_{max}) and AUC values increased in a dose-related manner between groups. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010a) led the author to conclude that repeated administration resulted in decreased absorption of aluminium ammonium sulfate (Sunaga, 2010b). If it is assumed that these doses were expressed as aluminium ammonium sulfate, the oral doses of aluminium would be 33 and 110 mg/kg bw, respectively.

The bioavailability of a single dose of aluminium lactate was assessed in groups of four male (296–330 g) and four female (190–217 g) fasted Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium lactate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.067% in males and 0.164% in females dosed with aluminium lactate at 300 mg/kg bw and 0.161% in males and 0.175% in females dosed at 1000 mg/kg bw (Sunaga, 2010c). If it is assumed that these doses were expressed as aluminium lactate, the oral doses of aluminium would be 27 and 91 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium lactate was assessed in groups of four male (253–272 g) and four female (187–211 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium lactate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.009% in males and 0.007% in females dosed with aluminium lactate at 300 mg/kg bw and 0.043% in males and 0.044% in females dosed at 1000 mg/kg bw. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010c) led the author to conclude that repeated administration resulted in decreased absorption of aluminium lactate. The AUCs for the high-dose group were about 10–15 times greater than those for the low-dose group. The author considered the exceedance of the dose ratio to be due to disappearance of aluminium in blood at an early stage in the low-dose group and bimodal transition of serum aluminium concentrations in the high-dose group (Sunaga, 2010d). If it is assumed that these doses were expressed as aluminium lactate, the oral doses of aluminium would be 27 and 91 mg/kg bw, respectively.

The bioavailability of a single dose of aluminium sulfate was assessed in groups of four male (297–335 g) and four female (195–224 g) fasted Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium sulfate dissolved in physiological saline was administered by oral gavage at 600, 1000 and 2000 mg/kg bw and intravenously at 1 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. All of the top-dose animals, except for one female, died. The bioavailability was calculated from the 24-hour AUC values to be 0.046% in males and 0.064% in females dosed with aluminium sulfate at 600 mg/kg bw and

0.053% in males and 0.069% in females dosed at 1000 mg/kg bw (Sunaga, 2010e). If it is assumed that these doses were expressed as aluminium sulfate, the oral doses of aluminium would be 95 and 158 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium sulfate was assessed in groups of four male (247–270 g) and four female (184–213 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium sulfate dissolved in physiological saline was administered by oral gavage at 600, 1000 and 2000 mg/kg bw or intravenously at 1 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. Dosing at 2000 mg/kg bw was discontinued as a result of deaths and loss of body weight. The bioavailability was calculated from the 24-hour AUC values to be 0.012% in males and 0.035% in females dosed with aluminium sulfate at 600 mg/kg bw and 0.012% in males and 0.052% in females dosed at 1000 mg/kg bw. The C_{max} and AUC values increased in a dose-related manner between groups. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010e) led the author to conclude that repeated administration resulted in decreased absorption of aluminium sulfate in male rats. The C_{max} and AUC values increased in a dose-related manner between groups (Sunaga, 2010f). If it is assumed that these doses were expressed as aluminium sulfate, the oral doses of aluminium would be 95 and 158 mg/kg bw, respectively.

The oral bioavailability of aluminium compounds has been examined in studies using the long-lived radionuclide ^{26}Al as a tracer. Test animals were given food containing ^{26}Al orally, while a concurrent dose of ^{27}Al was given through intravenous infusion. The extent of oral absorption or bioavailability was determined by comparing the AUCs for aluminium given via the two routes. Compared with an experimental design in which a rat receives the two doses at different times, this method reduces variability by concurrently determining the AUCs from the oral and intravenous doses in the rat.

The bioavailability of acidic sodium aluminium phosphate incorporated into biscuits was examined in male Fischer 344 rats (322 ± 32 g, mean ± standard deviation [SD]). Biscuits were prepared with baking powder containing 25% sodium aluminium phosphate acidic, which is typical for baking powder. Five rats (which had been conditioned to eat biscuits containing sodium aluminium phosphate acidic) in each of two groups were given 1 g biscuit containing 1% or 2% sodium aluminium phosphate acidic that had ^{26}Al incorporated at known concentrations. The rats were concurrently intravenously infused with ^{27}Al at a dose of 100 µg/kg bw per hour (potassium aluminium sulfate was continuously infused from 14 hours prior to 60 hours after oral dosing) to produce an estimated aluminium concentration of 500 µg/l in the blood plasma to provide the ^{27}Al dose. Two control rats simultaneously received biscuits containing 1.5% sodium aluminium phosphate acidic, and one rat received an intragastric administration of 1 ml water, without ^{26}Al. Blood was withdrawn 1 hour prior to and up to 60 hours after oral dosing. The peak serum ^{26}Al concentration was increased by approximately 160-fold to 1840-fold above mean pretreatment values. Peak serum ^{26}Al concentrations in the 1% sodium aluminium phosphate acidic and 2% sodium aluminium phosphate acidic

groups occurred at 4.2 hours and 6 hours, respectively. The oral bioavailability was calculated to be 0.11% ± 0.11% and 0.13% ± 0.12% (mean ± SD), respectively, for the biscuits containing 1% and 2% sodium aluminium phosphate acidic, and the difference for the two groups was not statistically significant (Yokel & Florence, 2006). The authors reported that these results were significantly different from their previously reported bioavailability data for aluminium absorption from water (0.28% ± 0.18% for 1%; 0.29% ± 0.11% for 2%) using a similar method (Yokel et al., 2001; Zhou & Yokel, 2006).

In a similar experiment to determine the bioavailability of sodium aluminium phosphate basic in processed cheese, groups of six male Fischer 344 rats (272 ± 11 g, mean ± SD) were fed cheese containing 1.5% and 3% ^{26}Al incorporated into sodium aluminium phosphate basic. Three control animals received either processed cheese containing 2.5% sodium aluminium phosphate basic or intragastric administration of 1 ml of water without ^{26}Al. One rat was intravenously infused with ^{27}Al at a dose of 100 µg/kg bw per hour from 14 hours prior to 60 hours after oral dosing to produce an estimated aluminium concentration of 500 µg/l in the blood plasma to provide the ^{27}Al dose. This dose is below the transferrin binding capacity for aluminium (~1350 µg/l) and therefore will ensure that the chemical species are the same (aluminium–transferrin) for both ^{27}Al and ^{26}Al. Blood was withdrawn 1 hour prior to and up to 60 hours after oral dosing. Peak serum ^{26}Al concentrations were at least 200-fold above pretreatment values and occurred at 8.0 and 8.6 hours in the 1.5% and 3% sodium aluminium phosphate basic groups, respectively. The oral bioavailability was calculated to be 0.10% ± 0.07% and 0.29% ± 0.18%, respectively, for 1.5% and 3% sodium aluminium phosphate basic (Yokel, Hicks & Florence, 2008).

The same laboratory investigated aluminium bioavailability from a tea infusion using tracer ^{26}Al. ^{26}Al citrate was injected into tea leaves, providing about 0.65 mg aluminium (similar to the inherent quantity in tea leaves), and an infusion was prepared that contained 50 Bq (71.3 ng) ^{26}Al per millilitre. A similar infusate was prepared with non-^{26}Al-containing aluminium citrate. The infusions were given intragastrically to male Fischer 344 rats (312 ± 5 g, mean ± SD), which also received a concurrent intravenous ^{27}Al infusion. The oral bioavailability, estimated from the AUC, was 0.37% ± 0.26%. Compared with previous results, this was similar to that from water (0.28%), but significantly greater than that from sodium aluminium phosphate acidic in biscuits (0.12%) (Yokel & Florence, 2008).

A study was conducted to compare the bioavailabilities of different ^{26}Al-labelled aluminium compounds in groups of six female Sprague-Dawley rats. As oral doses, the amounts of aluminium administered were 1.47 ng ^{26}Al:50 mg ^{27}Al as citrate, 1.24 ng ^{26}Al:50 mg ^{27}Al as chloride, 1.77 ng ^{26}Al:50 mg ^{27}Al as nitrate, 2.44 ng ^{26}Al:50 mg ^{27}Al as sulfate (as solutions), 12.2 ng ^{26}Al:17 mg ^{27}Al as hydroxide, 17.9 ng ^{26}Al:23 mg ^{27}Al as oxide, 0.46 ng ^{26}Al:10 mg ^{27}Al as sodium aluminium phosphate acidic, 0.31 ng ^{26}Al:10 mg ^{27}Al as sodium aluminium phosphate basic and 0.60 ng ^{26}Al:27 mg ^{27}Al as sodium aluminosilicate (as suspensions in 1% carboxymethyl cellulose); and 0.96 ng ^{26}Al in 414 mg FD&C Red 40 aluminium lake, 2.4 ng ^{26}Al:26 mg ^{27}Al powdered pot electrolyte and 1.4 ng ^{26}Al:6.9 mg ^{27}Al as aluminium metal (mixed with honey and placed on the back of the tongue). Bioavailability was

assessed by comparing the amount of ^{26}Al remaining in the carcass after 7 days with that remaining 7 days after intravenous injection of 0.19 ng ^{26}Al as citrate. The 7-day time span was to ensure that all ingested aluminium had been cleared from the gastrointestinal tract and that the initial rapid clearance phase had been exceeded. The absorbed fraction was less than 0.3% for each of the different compounds. For the soluble aluminium compounds, it ranged from 0.05% to 0.2% (aluminium nitrate, 0.045%; aluminium chloride, 0.054%; aluminium citrate, 0.078%; aluminium sulfate, 0.21%). The absorbed fractions of sodium aluminosilicate and FD&C Red 40 aluminium lake were similar (0.12% and 0.093%, respectively). Uptakes of the other insoluble compounds were slightly lower (powdered pot electrolyte, 0.042%; aluminium hydroxide, 0.025%; aluminium oxide, 0.018%). Uptake of sodium aluminium phosphate acidic, sodium aluminium phosphate basic and aluminium metal could not be fully quantified because it was below the limit of detection (LOD); however, based on 50% of the LOD, uptake was reported to be <0.024%, <0.015% and <0.015%, respectively. The author noted that these results were consistent with those of human volunteer studies (Priest, 2010).

In an investigation of whether citrate, maltolate and fluoride significantly influence oral aluminium bioavailability, male Fischer rats were given intragastrically 1 ml of solution containing 37 Bq ^{26}Al (65 nmol total aluminium) as the Al^{3+} ion or as complexes with [^{14}C]citrate, [^{14}C]maltolate or fluoride, with concurrent ^{27}Al intravenous infusion. The aluminium bioavailability was estimated to be 0.29% ± 0.11%, 0.61% ± 0.31%, 0.50% ± 0.25% and 0.35% ± 0.10% from the ion, citrate, maltolate and fluoride, respectively. These differences were not statistically significant (Zhou, Harris & Yokel, 2008).

The solubility of six pigments consisting of potassium aluminium silicate (mica) coated with iron(III) oxide (0–56%) and/or titanium dioxide (0–52%) was investigated in model systems using simulated gastric (pH 1.231) and intestinal (pH 6.714) fluids, as an indicator of bioavailability, in a study conducted in compliance with GLP. The pigments were Candurin® Red Lustre, Candurin® Apple, Candurin® Silver Fine, Candurin® Gold Lustre and Candurin® Fudge. Aluminium silicate, iron(II) silicate and iron oxide were used as reference substances. The test materials were incubated with the simulated fluids for 2 hours at 37 °C, then insoluble material was separated by filtering, and the dissolved aluminium and iron were measured by atomic absorption. In the simulated gastric fluid, the solubility of aluminium from aluminium silicate (control) was 0.11%; for the six pigments, it ranged from 0.041% to 0.3%. In the simulated intestinal fluid, the solubility of aluminium from aluminium silicate (control) was 0.016%; for the six pigments, it ranged from 0.000 37% to 0.005%. The solubility of iron ranged from 0.0001% to 0.11% in the gastric fluid and from 0.000 33% to 0.024% in the intestinal fluid (St Laurent, 2006). In a data submission, it is argued that particles with a mean size greater than or equal to 5 µm are not taken up by the Peyer's patches and that lipophilic particles are taken up to a greater extent than hydrophilic particles; therefore, it is highly unlikely that insoluble potassium aluminium silicate particles in the range of 5–150 µm with a hydrophilic surface are absorbed by the gastrointestinal tract (Merck, 2010).

A wide variability in absorption was observed in a study in human volunteers. Four healthy males were given a capsule containing 960 mg aluminium hydroxide

(333 mg aluminium) with a drink of citrate and citrus juice to enhance absorption. Peak aluminium concentrations in serum were 31, 49, 426 and 766 µg/l in the four individuals. In five patients with Alzheimer disease given a 3-fold higher amount of aluminium hydroxide, the peak concentrations were 56–1447 µg/l (Molloy et al., 2007). The reported results of this study are insufficient to allow bioavailability to be estimated.

(b) Distribution

Groups of eight pregnant Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (70 mg/kg bw per day expressed as aluminium) on gestational days (GDs) 0–16. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated. Significantly higher levels of aluminium were detected in the blood, brain and placenta of the mothers and in the brains of fetuses compared with control animals. Additionally, groups of five lactating rats were given oral doses of aluminium chloride of 0 or 345 mg/kg bw (70 mg/kg bw per day expressed as aluminium) from days 0 to 16 postpartum. Following necropsy on day 20, higher levels of aluminium were detected in the brains of the pups of aluminium-treated animals, demonstrating transfer through the milk. Lesser increases in tissue aluminium levels were observed following co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or reduced glutathione (GSH) at 100 mg/kg bw every other day throughout the period of aluminium dosing (Sharma & Mishra, 2006).

The relative distribution of aluminium was investigated in a GLP-compliant study with repeated oral administration of aluminium citrate, sulfate, nitrate, chloride and hydroxide to Sprague-Dawley rats. The animals were maintained on low-aluminium feed (9 µg/kg) and water (2 µg/l) for 17 days prior to and during dosing. The aluminium salts were administered by gavage to groups of five male (142–203 g) and five female (127–172 g) rats at doses corresponding to 30 mg/kg bw per day, expressed as aluminium, for 7 or 14 days. Control animals received deionized water. Appearance, body weights and feed and water consumption were monitored during the study. Blood samples were taken on day 8 or 15, prior to autopsy, when samples of brain, liver, kidney, spinal cord, spleen and bone were collected for analysis of aluminium.

There were no overt signs of toxicity or differences in body weight or water consumption. Feed consumption was significantly higher in the females dosed with aluminium nitrate compared with controls, but did not differ for other treatment groups. The concentrations of aluminium in most tissues were lower after 14 days of dosing than after 7 days, indicating that the major impact on the levels was the aluminium exposure prior to introduction of the low-aluminium diet and drinking-water for the study and that dosing with the different aluminium salts had a minimal effect. Aluminium citrate showed some evidence of systemic exposure, with concentrations higher than those of controls in the kidney and bone of males after 7 days and in the kidney of females after 14 days. Spinal cord concentrations of aluminium were higher in every group (including controls) after 14 days compared with 7 days (Dziwenka & Semple, 2009).

(c) Excretion

No new data on excretion were identified. Studies reviewed previously by the Committee have shown that urine is the primary route of excretion of absorbed aluminium in experimental animals and in humans. Initial half-lives of 2–5 hours have been reported in rats, mice, rabbits and dogs after intravenous administration and less than 1 day in humans. Multiple half-lives have been reported in different studies and species for a later, slower phase of elimination, varying with the tissue and generally increasing with the duration of sampling. EFSA (2008) concluded that although retention times for aluminium appear to be longer in humans than in rodents, there is little information allowing for extrapolation from rodents to humans.

2.1.2 Effects on enzymes and other parameters

Aluminium levels and enzymatic stress markers have been assessed in amyloid beta peptide transgenic mice, an animal model of Alzheimer disease, after oral aluminium exposure for 6 months. Amyloid beta peptide transgenic (Tg2576) and C57BL6/SJL wild-type mice 5 months of age were fed a diet containing aluminium lactate. The nominal aluminium concentration was 1000 mg/kg feed, but the actual level was 370 mg/kg feed, equal to 3.41 and 54 mg/kg bw per day in the control and treated mice, respectively. Aluminium levels were determined in the hippocampus, cerebellum and cortex, in addition to a suite of oxidative stress markers (GSH, oxidized glutathione, copper–zinc superoxide dismutase [SOD], glutathione reductase, glutathione peroxidase, catalase and thiobarbituric acid reactive substances [TBARS]), with and without co-exposure of the animals to deferoxamine. The highest levels of aluminium were observed in the hippocampus of both wild-type and transgenic mice. SOD activity was significantly decreased in the hippocampus of the aluminium-treated wild-type mice compared with the control wild-type mice. Glutathione reductase activity was significantly increased in the cortex of aluminium-treated wild-type mice compared with control wild-type mice (Esparza, Garcia & Gomez, 2011).

Groups of eight pregnant or five lactating Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (0 or 70 mg/kg bw per day, expressed as aluminium) on GDs 0–16 or on days 0–16 postpartum, respectively. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated (see also section 2.1.1(a)). Animals exposed to aluminium showed a number of indicators of oxidative stress in the brains of the mothers and in some instances also in the brains of the fetuses and sucklings. These were significant decreases in the levels of GSH, glutathione reductase, glutathione peroxidase, catalase, SOD and acetylcholinesterase and increases in the levels of TBARS and glutathione-*S*-transferase (GST). These effects were decreased by co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or GSH at 100 mg/kg bw orally every other day throughout the period of dosing (Sharma & Mishra, 2006).

Groups of 10 male rabbits (1000–1100 g, strain not specified) were given aluminium chloride at 20 mg/l in drinking-water for 3 months alone or in combination with subcutaneous administration of melatonin, either for 15 days following or

simultaneously with the administration of aluminium chloride. A control group ($n = 5$) was included. The water intake was monitored weekly, and the aluminium chloride exposure was estimated at about 5–6.6 mg/day (approximately 1–1.3 mg/kg bw per day, expressed as aluminium). The aluminium contents of the diet and control tap water were not reported. After necropsy, the levels of malondialdehyde (MDA) and 4-hydroxyalkenal (4-HDA) (indicators of lipid peroxidation) and SOD activity were measured in the brain. The levels of MDA and 4-HDA were significantly increased, and SOD activity was decreased. These changes were lower in the groups treated with melatonin (as an antioxidant and free radical scavenger). The concentration of aluminium in the brain tissue was significantly increased in the aluminium-treated rabbits, and this change was also ameliorated by melatonin (Abd-Elghaffar, El Sokkary & Sharkawy, 2007).

Aluminium chloride was administered in the drinking-water for 6 months to male Wistar rats (young, 4 months; aged, 18 months; 10 animals in each treatment and control group), providing a dose of 50 mg/kg bw per day, expressed as aluminium. The aluminium content of the diet was not reported. When compared with controls, aluminium-treated rats showed a significant increase in electrophysiological activity. Histological examinations of hippocampal sections showed a decreased cell count in the CA1 and CA3 hippocampal fields with disorganized neurons that showed strong cytosolic staining in the aluminium-treated rats. The aluminium-treated rats showed oxidative stress–related damage to lipids (increased TBARS), decreased sodium–potassium adenosine triphosphatase (ATPase) activity, increased cytosolic protein kinase C activity and a significant decrease in the activity of SOD (Sethi et al., 2008). Curcumin administration by gavage (30 mg/kg bw per day) attenuated the changes (Sethi et al., 2009).

Aluminium chloride was administered in drinking-water to male Wistar rats (180–200 g; seven per group) at 100 mg/kg bw per day for 42 days. Additional groups of rats received concomitant doses of curcumin (30 and 60 mg/kg bw orally as a solution in 0.5% carboxymethyl cellulose 1 hour after aluminium chloride administration). No information was provided on levels of aluminium in food or control drinking-water. The animals were sacrificed on day 43, and biochemical indicators of oxidative stress were assessed in brain tissue. Aluminium chloride treatment resulted in a significant increase in brain levels of MDA and of nitrite (an indicator of nitric oxide production) and a decrease in GSH levels compared with controls. The treated rats also had a marked decrease in GST, SOD, catalase and acetylcholinesterase activities. The concentrations of aluminium were significantly increased in both the hippocampal and cortical areas of the brains of rats treated with aluminium chloride. Curcumin administration attenuated the changes in biochemical parameters and the increased aluminium concentration in the hippocampus, but not in the cortex (Kumar, Dogra & Prakash, 2009).

2.2 Toxicological studies

2.2.1 Acute toxicity

The Committee was provided with acute toxicity data on pigments consisting of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide

Table 1. Acute oral toxicity of pigments consisting of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide, titanium dioxide and myristic acid

Pigment	Species: number of each sex	Route	LD_{50} (mg/kg bw)	Reference
Iriodin® Ti 100K 68–76% mica 24–32% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color B Ti 100K 46–54% mica 46–54% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color Dy Ti 100K 51–65% mica 33–42% TiO_2 2–7% Fe_2O_3	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color G Ti 100K 46–50% mica 50–54% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color R Ti 100K 52–58% mica 42–48% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color Y Ti 100K 54–62% mica 38–46% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Colibri Red-brown 47–57% mica ≤3% TiO_2 43–50% Fe_2O_3	Rat: 10M + 10F	Oral gavage	>16 000	Von Eberstein (1975)
Iriodin® 502 C 63 58% mica 40% TiO_2 2% myristic acid	Rat: 5M + 5F	Oral gavage	>5 000	Heusener & Von Eberstein (1988)[a]
Iriodin® Ti 100K 68–76% mica 24–32% TiO_2	Dog: 2M + 2F	Oral gavage	>6 400	Von Eberstein (1971)

F, female; LD_{50}, median lethal dose; M, male

[a] Conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 401 in compliance with GLP.

(Fe_2O_3), titanium dioxide (TiO_2) and myristic acid. No lethality was reported at the maximum tested doses, corresponding to an aluminium concentration of greater than 2000 mg/kg bw (Table 1).

2.2.2 Short-term studies of toxicity

Two subchronic toxicity studies were conducted with potassium aluminium silicate (mica) coated with iron(III) oxide and/or titanium dioxide. According to the submission to the Committee, the formula of potassium aluminium silicate is $KAl_2[AlSi_3O_{10}](OH)_2$, which contains 20% aluminium based on atomic weights.

Iriodin® Ti 100K (69–75% mica, 25–31% titanium dioxide) was administered in the diet at a concentration of 0, 5000, 10 000 or 20 000 mg/kg to groups of 15 male (approximately 150 g) and 15 female (approximately 130 g) Wistar rats for 14 weeks, equivalent to 0, 500, 1000 and 2000 mg/kg bw per day as Iriodin® Ti 100K or approximately 0, 75, 150 and 300 mg/kg bw per day as aluminium. Five rats of each sex per group were followed up for a 10-week treatment-free recovery period. Clinical signs were checked daily, and feed consumption and body weights were recorded weekly during the study period. Blood and urine were sampled during and at the end of the study for haematological and biochemical analyses. At autopsy, the weights of 10 organs were recorded. Histopathological examinations were performed on a large range of organs of animals of all dose groups (five rats of each sex at 13 weeks, and three rats of each sex at the end of the recovery period). There were no treatment-related changes in any of the parameters recorded. Some histopathological findings were slightly increased in the treated animals, including fatty degeneration of the liver, hyperplasia of Kupffer cells and siderosis in the Kupffer cells and kidney. The authors considered that the siderosis could not be directly related to the test material, as the test material did not contain any iron, and haematological findings did not provide an indication that the siderosis could be related to phagocytosis of senescent or damaged erythrocytes. They concluded that there were no differences between the control and test groups and that the dietary concentration of Iriodin® Ti 100K of 20 000 mg/kg was the no-observed-adverse-effect level (NOAEL) (equivalent to about 300 mg/kg bw per day, expressed as aluminium) (Jochmann, 1972; Kramer & Broschard, 2000a).

Four potassium aluminium silicate–based pigments and a “placebo” (potash mica) were investigated in a subsequent study conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408, with the exception that ophthalmic examinations were not conducted. Potash mica (composition not provided, but assumed to be 100%), Iriodin® Ti 100 Color RY K (52.86% mica, 40.53% titanium dioxide, 6.6% iron(III) oxide), Iriodin® Colibri Red-brown K (52.84% mica, 1.87% titanium dioxide, 45.29% iron(III) oxide), Iriodin® Colibri Blue-green K (47.46% mica, 42.73% titanium dioxide, 9.81% chromium oxide) and Iriodin® Colibri Dark Blue (48.1% mica, 46.7% titanium dioxide, 5.2% Berlin blue) were administered in the diet at a concentration of 50 000 mg/kg to groups of 20 male (mean weight 183 g) and 20 female (mean weight 162 g) Wistar rats for 13 weeks. The control group consisted of 40 male and 40 female rats. Half of the animals from each group were maintained on untreated diet for a 2-month recovery period. These test material dietary concentrations were equal to doses of 3931 and 4370 (male and female), 3952 and 4466, 3983 and 4391, 3995 and 4418, and 3856 and 4362 mg/kg bw per day, respectively, equivalent to 786 and 875, 418 and 472, 421 and 464, 379 and 419, and 371 and 420 mg/kg bw per day, respectively, expressed as aluminium. Clinical signs were checked daily, and

feed consumption and body weights were recorded weekly during the study period. Blood and urine were sampled from half of the animals during and at the end of the study for haematological and biochemical analyses. At autopsy, organ weights were recorded. Histopathological examinations were performed on organs of all animals of all dose groups. Diarrhoea was reported in some treated animals during the 1st week, and soft faeces were observed occasionally throughout the treatment period, but not during the recovery period. Feed consumption was higher than that of controls in all treatment groups, including the potash mica group, as a result of the lower nutritional composition of the diets. Body weight gains did not differ between dose groups, except for some statistically significant slight increases at some time points in the females treated with Iriodin® Colibri Blue-green K and Iriodin® Colibri Dark Blue, which were not considered to be of biological significance. No other treatment-related effects were reported. The authors reported that no toxicological effects were observed in rats treated with pearlescent pigments at doses up to about 4000 mg/kg bw per day (Kieser, 1982; Kramer & Broschard, 2000b).

The findings of the above study were subsequently re-evaluated and confirmed (Hellmann & Broschard, 2005). The focus of this study was on the pigments containing approximately 50% potassium aluminium silicate carrier, and therefore the doses would correspond to about 400 mg/kg bw per day, expressed as aluminium. The “placebo” was potash mica without associated pigments, for which the dose expressed as aluminium was about 800 mg/kg bw per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

Potassium aluminium silicate (mica) coated with titanium dioxide was tested in a combined oral chronic toxicity and carcinogenicity study in Fischer 344 rats, conducted in compliance with GLP. The test material was a 1:1 blend of two pigments with overall composition of 72% potassium aluminium silicate and 28% titanium dioxide and was administered at a concentration of 0, 10 000, 20 000 or 50 000 mg/kg in the diet to groups of 10 male and 10 female rats for 52 weeks and to groups of 50 male and 50 female rats for 130 weeks. At the start of the study, male rats weighed 104–166 g and female rats weighed 91–125 g. Body weights, feed consumption and gross signs of toxicity were recorded weekly during the first 14 weeks and then once every 4 weeks. Ophthalmic examinations were conducted before the study and then at weeks 52 and 104. Haematology, clinical chemistry, urinalysis, organ weights and gross and microscopic evaluations were reported.

There were no treatment-related findings in the 52-week study, except for test material coloration of the faeces in the top-dose group. In the carcinogenicity study, there were no differences in survival up to week 102, although survival of low-dose females was significantly lower than that of controls at termination. Mean body weights of high-dose males and mid- and high-dose females were significantly lower than those of controls at week 25, but not at the end of the study. The incidence of mononuclear cell leukaemia in male rats was 10/17, 10/16, 13/16 and 22/25, respectively, at 0, 10 000, 20 000 and 50 000 mg/kg diet, and the increased incidence in the high-dose group was ascribed to the greater survival compared with the other dose groups. There were no other treatment-related findings. The authors concluded that titanium dioxide–coated potassium aluminium silicate did

not produce toxicological or carcinogenic effects at dietary concentrations up to 50 000 mg/kg diet. This is equivalent to 2500 mg/kg bw per day of the test material or 360 mg/kg bw per day of aluminium (Pence & Osheroff, 1987; Bernard et al., 1990).

2.2.4 Genotoxicity

The Committee was provided with genotoxicity data on pigments composed of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide and titanium dioxide. The preparations were negative for bacterial mutagenicity in the presence and absence of S9 and in an in vivo rat bone marrow micronucleus test (Table 2).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

The reproductive toxicity of aluminium sulfate was investigated in a GLP-compliant study conducted according to OECD Test Guideline 416. Aluminium sulfate was dissolved in ion exchange water at 0, 120, 600 or 3000 mg/l. Groups of 24 male and 24 female Crl:CD (SD) rats (F_0 generation) were administered the aluminium sulfate from 5 weeks of age for 10 weeks prior to mating, during mating and gestation, when the parental males were culled, and, for the females, through weaning. Litters were normalized to eight pups on postnatal day (PND) 4. At weaning, 24 males and 24 females were selected to serve as the F_1 generation and were administered the aluminium sulfate for 10 weeks prior to mating, during mating and gestation, and, for the females, through weaning, as for the F_0 generation. The calculated mean exposures to aluminium sulfate during the treatment period were 8.6, 10.7, 14.4 and 15.3 mg/kg bw per day in the 120 mg/l group, 41.0, 50.2, 71.5 and 74.2 mg/kg bw per day in the 600 mg/l group and 188, 232, 316 and 338 mg/kg bw per day in the 3000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The mean exposures to aluminium from the test substance were 1.36, 1.69, 2.27 and 2.41 mg/kg bw per day in the 120 mg/l group, 6.47, 7.92, 11.28 and 11.7 mg/kg bw per day in the 600 mg/l group and 29.7, 36.6, 49.8 and 53.3 mg/kg bw per day in the 3000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The aluminium concentration of the unsupplemented water was less than 5 mg/l, and the aluminium content of the batches of diet used throughout the study was in the range of 25–29 mg/kg. The mean aluminium exposures from the diet were 1.6, 1.9, 2.2 and 2.3 mg/kg bw per day in the F_0 males, F_1 males, F_0 females and F_1 females of each group, respectively. The F_0 and F_1 parental generations were monitored for mortality, behaviour, body weights, feed and water consumption and reproductive performance. Spontaneous locomotor activity was assessed in 10 males and 10 females randomly selected from the F_1 generation at 4 weeks of age. Learning was assessed in a multiple T-maze in 10 males and 10 females randomly selected from the F_1 generation at 6 weeks of age. F_1 and F_2 pups were assessed for gross abnormalities, anogenital distance on PND 4 and developmental milestones. One male and one female pup per litter were tested for reflex responses on PNDs 5, 8 and 18. At autopsy, organs were weighed

Table 2. Genotoxicity of pigments composed of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide and titanium dioxide

Test system	Test object	Test material	Dose	Results	Reference
In vitro					
Reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537	Mica pigment mix (63.5% mica, 26.4% TiO_2, 10.1% Fe_2O_3)	5–5000 µg/plate	Negative	Utesch (2006)
Reverse mutation[a]	*Escherichia coli* WP2uvrA	Mica pigment mix (63.5% mica, 26.4% TiO_2, 10.1% Fe_2O_3)	5–5000 µg/plate	Negative	Utesch (2006)
In vivo					
Micronucleus formation[b]	Male Wistar rat bone marrow	Candurin® Honeygold (36–52% mica, 42–52% TiO_2, 6–12% Fe_2O_3)	2000 mg/kg bw, orally	Negative[c]	Utesch (2000)

S9, 9000 × *g* rat liver supernatant

[a] In the presence and absence of Aroclor-induced rat liver S9 mix.

[b] Killed at 24 and 48 hours.

[c] No change in the proportion of polychromatic erythrocytes.

and stored. Histopathological examination was conducted on reproductive organs of parental animals of the high-dose and control groups and on animals for which abnormal findings were recorded.

Water consumption was significantly decreased compared with controls in males and females of all treatment groups in a concentration-dependent manner. This was attributed to avoidance of the drinking-water because of the low pH (pH 3.57–4.20). In the 3000 mg/l groups, body weights, body weight gains and feed consumption were significantly decreased compared with controls in the F_0 males and females for up to 3 weeks after the start of administration, and feed consumption was significantly decreased in the F_1 generation. In the F_0 and F_1 females, there was a dose-related decrease in feed consumption during the 3rd week of lactation, which was statistically significant at 600 and 3000 mg/l. Deaths occurred in one F_1 male in each of the 120 and 3000 mg/l groups and in one F_0 female in the 600 mg/l group; these were not considered to be treatment related.

The females showed no significant effects of treatment on the estrous cycle, and there were no differences reported for copulation, fertility index, gestation index, precoital interval, gestation length, number of implantations, number of pups delivered or delivery index. There were no significant differences between groups for sperm parameters except for a significant decrease in absolute (but not relative) number of sperm in the 3000 mg/l F_0 males. In the F_1 and F_2 pups, there were no treatment-related differences in malformations, sex ratio or viability on PND 0, 4 or 21. At 3000 mg/l, the F_1 male and female pups had a significantly lower body weight on PND 21, and a similar, but not statistically significant, trend was seen in the F_2 pups. Body weights of F_1 and F_2 male and female pups at 3000 mg/l were significantly lower than those of controls at autopsy (PND 26). No significant differences were reported for age at completion of pinna unfolding, age at incisor eruptions, age at eye opening or anogenital distance in the F_1 and F_2 male pups or in the F_1 female pups. In the F_2 female pups, the completion time of pinna unfolding was significantly lower in the 600 mg/l group. The F_1 male pups showed no significant treatment-related differences in the time of preputial separation. In F_1 female pups, vaginal opening was significantly delayed in the 3000 mg/l group (mean ± SD: 31.4 ± 1.7 days vs 29.5 ± 2.1 days in control), although body weights at the time of vaginal opening were not significantly different.

No significant treatment-related differences were reported for righting reflex (PND 5), negative geotaxis reflex (PND 8) or mid-air righting reflex (PND 18) in the F_1 or F_2 pups, in locomotor activity assessed in F_1 males and females at 4 weeks or in the learning outcomes assessed in F_1 males and females at 4 and 6 weeks. There were no treatment-related macroscopic observations in the F_0 or F_1 parental generations at autopsy. In F_0 males, the absolute and relative liver weights and the absolute spleen weights were significantly decreased at 3000 mg/l relative to controls. In the F_1 males, the only statistically significant changes in organ weights were decreased absolute adrenal weight at 3000 mg/l and decreased absolute testis weight at 600 mg/l. There were no statistically significant differences in organ weights in F_0 or F_1 females. Histopathological examination revealed no treatment-related changes in the liver or spleen or in the reproductive organs.

In the F_1 and F_2 pups, absolute and, in some cases, also relative weights of liver and spleen were significantly lower at 3000 mg/l than in controls, but the organs showed no histopathological abnormalities. Absolute weights of thymus, kidneys, testes, epididymides, ovaries and uterus and relative thymus weights were also lower than those of controls, and relative brain weights were significantly higher in high-dose pups than in controls. These findings were considered to be secondary to the decreased body weights. Other findings were not dose related and were considered not to be treatment related. The authors concluded that, based on the retardation of sexual development in the F_1 females, attributed to inhibition of growth, and decreased body weight gain and liver and spleen weights in the F_1 and F_2 offspring, the NOAEL was 600 mg/l aluminium sulfate in the drinking-water, corresponding to 41.0 mg/kg bw per day (Fujii, 2009; Hirata-Koizumi et al., 2011a).

Expressed as aluminium, the reported NOAEL from this study equates to 6.47 mg/kg bw per day from the test substance plus at least 1.6 mg/kg bw per day from the diet—i.e. a total of about 8 mg/kg bw per day. The lowest-observed-adverse-effect level (LOAEL) from this study would be equivalent to a total of approximately 31 mg/kg bw per day, expressed as aluminium. However, in view of the clear treatment-related effects on fluid consumption and feed consumption of F_0 and F_1 dams during the 3rd week of lactation, it is not possible to ascertain whether the observations reported in the pups were a direct effect of the aluminium sulfate or due to decreased milk production by the dams, affecting pup weight on PNDs 21 and 26. In addition, grip strength was not measured, which limits comparison with the results of the studies used by the Committee in establishing the PTWI at its sixty-seventh meeting and with the study of Semple (2010) (see section 2.2.6).

The reproductive toxicity of aluminium ammonium sulfate was also investigated in a GLP-compliant study conducted according to OECD Test Guideline 416. Aluminium ammonium sulfate was dissolved in ion exchange water at 0, 50, 500 or 5000 mg/l. Groups of 24 male and 24 female Crl:CD (SD) rats (F_0 generation) were administered the aluminium ammonium sulfate from 5 weeks of age for 10 weeks prior to mating, during mating and gestation, when the parental males were culled, and, for the females, through weaning. Litters were normalized to eight pups on PND 4. At weaning, 24 males and 24 females were selected to serve as the F_1 generation and were administered the aluminium ammonium sulfate for 10 weeks prior to mating, during mating and gestation, and, for the females, through weaning, as for the F_0 generation. The calculated mean exposures to aluminium ammonium sulfate during the treatment period were 3.78, 4.59, 6.52 and 6.65 mg/kg bw per day in the 50 mg/l group, 33.5, 41.8, 58.6 and 61.9 mg/kg bw per day in the 500 mg/l group and 305, 372, 500 and 517 mg/kg bw per day in the 5000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The mean exposures to aluminium from the test substance were 0.430, 0.522, 0.742 and 0.757 mg/kg bw per day in the 50 mg/l group, 3.81, 4.76, 6.67 and 7.04 mg/kg bw per day in the 500 mg/l group and 34.7, 42.3, 56.9 and 58.8 mg/kg bw per day in the 5000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The aluminium concentration of the unsupplemented water was less than 5 mg/l, and the aluminium content of the batches of diet used throughout the study was in the range of 22–29 mg/kg. The mean aluminium exposures from the diet were 1.6,

1.8, 2.2 and 2.4 mg/kg bw per day in the F_0 males, F_1 males, F_0 females and F_1 females of each group, respectively.

The F_0 and F_1 parental generations were monitored for mortality, behaviour, body weights, feed and water consumption and reproductive performance. Spontaneous locomotor activity was assessed in 10 males and 10 females randomly selected from the F_1 generation at 4 weeks of age. Learning was assessed in a multiple T-maze in 10 males and 10 females randomly selected from the F_1 generation at 6 weeks of age. F_1 and F_2 pups were assessed for gross abnormalities, anogenital distance on PND 4 and developmental milestones. One male and one female pup per litter were tested for reflex responses on PNDs 5, 8 and 18. At autopsy, organs were weighed and stored. Histopathological examination was conducted on reproductive organs of parental animals of the high-dose and control groups and on animals for which abnormal findings were recorded.

Water consumption was decreased compared with controls in males and females of all treatment groups in a concentration-dependent manner. The decrease was statistically significant at 500 and 5000 mg/l in males and females of the F_0 and F_1 generations as well as at 50 mg/l in the F_0 males and at some times during treatment for the F_0 and F_1 females. These changes were attributed to avoidance of the drinking-water because of the low pH (pH 3.45–4.38).

There were no significant differences compared with controls in body weight, body weight gain or feed consumption in the 50 and 500 mg/l groups, except for a reduction in feed consumption in the F_0 females during the 1st week of treatment. At 5000 mg/l, body weight was decreased in the F_0 and F_1 males for up to 2 weeks after the start of administration; body weight gain and feed consumption were also decreased at this time in the F_0 males, but not in the F_1 males. In the females at 5000 mg/l, body weights were decreased during the first 1 or 2 weeks of treatment in both the F_0 and F_1 generations and after 3 weeks of lactation in the F_0 generation. Body weight gains were decreased during the first 1 or 2 weeks of treatment in both the F_0 and F_1 generations and after 3 weeks of lactation in the F_1 generation. Feed consumption was decreased in the F_0 females during the 1st week of treatment and during the 2nd and 3rd weeks of lactation in both F_0 and F_1 dams. One F_1 male in the 500 mg/l group died, which was not considered to be treatment related.

The females showed no significant effects of treatment on the estrous cycle, and there were no differences reported for copulation, fertility index, gestation index, precoital interval, gestation length, number of implantations, number of pups delivered or delivery index. There were no significant differences between groups for sperm parameters.

In the F_1 and F_2 pups, there were no treatment-related differences in malformations, sex ratio or viability on PND 0, 4 or 21. Decreased body weights were reported in the F_1 male and female pups at 5000 mg/l, but not in the lower dose groups. F_1 male pups had a significantly lower body weight on PND 21, F_1 female pups on PNDs 14 and 21, and F_2 male and female pups on the day of autopsy (PND 26). No significant differences were reported for age at completion of pinna unfolding, age at incisor eruptions, age at eye opening or anogenital distance in the F_1 and F_2 male and female pups. The F_1 male pups showed no significant

treatment-related differences in the time of preputial separation. In F_1 female pups, vaginal opening was significantly delayed in the 5000 mg/l group (mean ± SD: 32.3 ± 1.8 days vs 30.2 ± 2.1 days in control), although body weights were not significantly different at the time of vaginal opening.

No significant treatment-related differences were reported for righting reflex (PND 5), negative geotaxis reflex (PND 8) or mid-air righting reflex (PND 18) in the F_1 or F_2 pups, in locomotor activity assessed in F_1 males and females at 4 weeks or in the learning outcomes assessed in F_1 males and females at 4 and 6 weeks of age.

There were no treatment-related macroscopic observations in the F_0 or F_1 parental generations at autopsy. In the F_0 and F_1 males, there were no treatment-related changes in organ weights at 50 or 500 mg/l. At 5000 mg/l, absolute pituitary gland weight was significantly lower and relative kidney weight was significantly higher than those of controls in the F_1 males, but not the F_0 males. In the females, there was an apparent dose-related decrease in absolute pituitary weight in both the F_0 and F_1 generations, which was significantly different from control only at 5000 mg/l. Relative kidney weight was significantly increased at 500 and 5000 mg/l in the F_0 generation and at 5000 mg/l in the F_1 generation, but it did not show a dose-related trend. Absolute thymus weight in the high-dose F_1 females was significantly lower than that of control. There were no other statistically significant changes in organ weights. Histopathological examination revealed no treatment-related changes in the reproductive organs.

In the F_1 and F_2 male and female pups, there was an apparent dose-related decrease in absolute and relative thymus weights, which was significantly different from control at 500 and 5000 mg/l in the F_1 females, but only at 5000 mg/l in the other groups. Histological examination was not conducted on the thymus. Absolute and, in some cases, also relative weights of liver and spleen in the F_1 and F_2 pups were significantly lower at 5000 mg/l than in controls, but the organs showed no histopathological abnormalities. Absolute weights of kidneys, adrenals, testes and epididymides of the F_1 and F_2 male pups at 5000 mg/l were also lower than those of controls, whereas relative brain and kidney weights were significantly higher in high-dose pups than in controls. Changes in organ weights other than the thymus, liver and spleen were inconsistent in the female pups, with absolute weights of the adrenals and uterus significantly lower and relative weights of brain and kidney significantly higher in the F_1 pups and absolute weights of the ovary and uterus significantly lower and relative weights of brain, kidney and adrenals significantly higher in the F_2 pups. These findings were considered to be secondary to the decreased body weights. Other findings were not dose related and were considered not to be treatment related. The authors concluded that, based on the retardation of sexual development in the F_1 females, attributed to inhibition of growth, and decreased body weight gain and liver, spleen and thymus weights in the F_1 and F_2 offspring, the NOAEL was 500 mg/l aluminium ammonium sulfate in the drinking-water, corresponding to 33.5 mg/kg bw per day (Fujii, 2010; Hirata-Koizumi et al., 2011b).

Expressed as aluminium, the reported NOAEL from this study equates to 3.81 mg/kg bw per day from the test substance plus at least 1.6 mg/kg bw per day

from the diet—that is, a total of about 6 mg/kg bw per day. The LOAEL from this study would be equivalent to a total aluminium dose of approximately 35 mg/kg bw per day. However, in view of the clear treatment-related effects on fluid consumption and feed consumption of F_0 and F_1 dams during the later stages of lactation, it is not possible to ascertain whether the observations reported in the pups were a direct effect of the aluminium ammonium sulfate or due to decreased milk production by the dams, affecting pup weight on PNDs 21 and 26. In addition, grip strength was not measured, which limits comparison with the results of the studies used by the Committee in establishing the PTWI at its sixty-seventh meeting and with the study of Semple (2010) (see section 2.2.6).

(b) Developmental toxicity

The effects of oral exposure to aluminium and prenatal stress on the neurobehavioural performance of the offspring at 1 year (adult) and 2 years (old age) were studied in Sprague-Dawley rats. Aluminium exposure was in the form of aluminium nitrate in the drinking-water at concentrations providing aluminium doses of 50 (n = 15) and 100 (n = 21) mg/kg bw per day. Citric acid (355 and 710 mg/kg bw per day for the rats exposed to aluminium at doses of 50 and 100 mg/kg bw per day, respectively) was added to the drinking-water to increase the availability of aluminium. Basal aluminium levels in the diet and drinking-water were not reported. A subgroup in each category was subjected to restraint stress (2 hours per day on GDs 6–20) (n = 4 for aluminium exposure of 50 mg/kg bw per day and n = 5 for aluminium exposure of 100 mg/kg bw per day). Control animals (no exposure, no restraint, n = 17; restraint only, n = 11) were also used. The offspring continued to receive the aluminium exposure during lactation and the experimental period (1 or 2 years). Body weight and fluid intake were monitored weekly. Behavioural tests were conducted 1 and 2 years after birth. At the end of 1 and 2 years, there was no significant difference in the general motor activity (open-field test) between the controls and the exposed animals (with or without prenatal restraint). However, there was a difference in the spatial learning and retention tests (water maze test), with the animals of the lower-dose group (50 mg/kg bw per day, with or without restraint) performing better than animals of the high-dose group. Also, the 1-year-old rats (adult) performed better than the 2-year-old rats (old) in the water maze. Aluminium levels in the brains at the end of 2 years were elevated in the rats exposed to 100 mg/kg bw per day, but not 50 mg/kg bw per day. Animals that had the same exposure but were subject to prenatal restraint stress did not have high levels of aluminium in the brain (even though the behavioural parameters were not different), indicating that the prenatal stress prevents aluminium accumulation (Roig et al., 2006).

Groups of eight pregnant Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (70 mg/kg bw per day, expressed as aluminium) on GDs 0–16. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated. Body weights were monitored daily. The animals were necropsied on day 18, for the collection of uteri, maternal blood and brain and fetal brain. Body weight gain was significantly reduced in the aluminium-treated dams. The numbers

of corpora lutea and implantation sites, placental weight, crown–rump length and fetal weight were also reduced significantly. There were no gross or skeletal malformations in the fetuses, but there was a significant reduction in ossification of the parietal and caudal bones. These effects were, to some extent, ameliorated by co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or GSH at 100 mg/kg bw every other day throughout the period of aluminium dosing (Sharma & Mishra, 2006).

The embryotoxic effects of aluminium chloride were studied in Sprague-Dawley rats. Groups of pregnant rats (240–250 g, n = 10) were given 0 or 50 mg aluminium chloride orally by gavage on GDs 1–3 (preimplantation) or GDs 4–6 (during implantation). This dose was approximately 200 mg/kg bw per day, expressed as aluminium chloride, or 40 mg/kg bw per day, expressed as aluminium. The exposure to aluminium from diet and drinking-water was not estimated. The animals were necropsied on day 20. The group exposed to aluminium chloride on GDs 1–3 showed a significantly increased number of resorptions (7.8% compared with 0% in the controls). Dosing on GDs 4–6 resulted in significantly reduced pregnancy rates and numbers of viable fetuses and significantly increased numbers of resorptions. Measures of maternal toxicity were not reported (Bataineh, Bataineh & Daradka, 2007).

A combined repeated-dose toxicity study on aluminium chloride basic with reproduction and developmental toxicity screening was conducted according to OECD Test Guideline 422, in compliance with GLP. Aluminium chloride basic consists of 17.0% aluminium oxide, 9.0% aluminium and 19.9% chlorine in aqueous solution. Groups of 10 male and 10 female Wistar rats were dosed by oral gavage with aqueous solutions of aluminium chloride basic at 0, 40, 200 and 1000 mg/kg bw per day (0, 3.6, 18 and 90 mg/kg bw per day, expressed as aluminium). Males were dosed for 28 days—that is, for 2 weeks prior to mating, during mating and up to termination. Females were dosed for 2 weeks prior to mating, during mating, through gestation and up to at least 3 days of lactation, comprising a total of 37–53 days. The aluminium content of the diet was not reported. The following observations were recorded: clinical signs, functional observations, body weights, feed consumption, haematological and clinical chemistry analyses, organ weights, and macroscopic and microscopic findings, with a particular focus on reproductive organs. Reproductive parameters included mating, fertility, conception, gestation duration, gestation index, percentage of live pups, postnatal loss, pup weights, sex ratio and clinical and behavioural signs during at least 4 days of lactation.

At the top dose, lower body weights and feed intake were reported in females during the first 3 weeks of the study, which the authors considered not to be of toxicological significance. At autopsy, there were signs of local irritation in the stomach in both sexes treated at the top dose, supported by histopathological observations of mild to moderate subacute inflammation of the glandular stomach. No other treatment-related changes were reported. The author considered that 1000 mg/kg bw per day was the NOAEL for systemic effects and 200 mg/kg bw per day was the NOAEL for local irritation (Beekhuijzen, 2007).

2.2.6 Special studies

(a) Neurotoxicity and neurobehavioural studies

Female transgenic (Tg2576) and wild-type mice were exposed for 6 months to aluminium lactate in the diet. The nominal concentration was 1000 mg/kg feed as aluminium, but the actual level was 370 mg/kg feed, equal to 3.41 and 54 mg/kg bw per day, expressed as aluminium, in the control and treated mice, respectively. General motor activity was evaluated using an open field, whereas spatial learning and memory were assessed in a water maze. No effects on general motor activity were found, whereas the open-field test showed an increased number of rearings in Tg2576 mice compared with the wild-type mice. Differences in learning were noted in the water maze acquisition test, in which aluminium-treated Tg2576 mice showed more difficulties in learning the task than aluminium-exposed wild-type mice (García et al., 2009).

The effect of chronic exposure to aluminium chloride on the function of the vestibulo-ocular reflex was examined in a study in which the vestibulo-ocular reflex was analysed to detect changes of the post-rotatory nystagmus main parameters with exposure to aluminium. Wistar rats (395–486 g) were subdivided into three groups (n = 90 each, 30 of which were used as controls, numbers of males and females unclear). Each group was further divided into animals of three different ages (3, 10 and 24 months). All the animals were given standard rat diet (mean aluminium levels 5.5 ± 0.1 µg/ml), and the water used to prepare the drinking solutions had aluminium levels of 40 ± 1 ng/ml. The control animals were given water with added sodium chloride (0.125, 0.25 and 0.5 g/l), whereas the exposed animals had aluminium chloride (0.5, 1 and 2 g/l) added to their drinking-water for 90 days (aluminium doses of 11.1 ± 2.5, 21.5 ± 4.2 and 43.1 ± 11.4 mg/kg bw per day, respectively, for the three groups). The vestibulo-ocular reflex was recorded every 10 days and at the end of the 90 days; the aluminium concentrations of the whole blood and (after necropsy) brain were measured in half of the animals, and immunohistochemistry studies were carried out on the remainder. There was no correlation between aluminium levels in the blood or in different compartments of the brain with animal age. However, there was a dose-related increase in the aluminium concentrations of the brainstem–cerebellum and the telencephalon. Post-rotatory nystagmus analysis was carried out with regard to onset latency, duration, frequency and amplitude of single jerks. Only animals with the highest aluminium exposure (43.1 ± 11.4 mg/kg bw per day) showed a significant impairment in all age groups, as shown by delayed onset latency, drastic reduction of its duration, jerk frequency and jerk amplitude. Immunohistochemical analysis of the brains of animals in the highest exposed group showed no difference in the number and shape of astrocytes and no amyloid deposits, regardless of age (Mameli et al., 2006).

In a pilot study, six male Wistar rats were fed a low-aluminium diet, providing 0.36 mg/kg bw per day twice weekly from age 5 months to age 16 months and then given aluminium chloride at 20 mg/l (as aluminium) in the drinking-water, providing a total aluminium dose of 1.52 mg/kg bw per day from food and water. From the age of 5 months, the rats were tested weekly in a T-maze task until the

end of their lifespan (averaging 29.8 months). Two of the six rats had significantly lower memory scores in “old age” compared with “middle age” (ages not defined) and exhibited “soft signs of dementia”, such as repetitive behaviour, indecision and inability to concentrate. Sections of brain were processed with the Walton bright field/fluorescent stain for aluminium; hippocampal neurons from the brains of all six rats showed varying extents of aluminium accumulation, with greater accumulation in the two rats showing memory deficits, whereas untreated rats aged 6 months were judged to be aluminium negative by this method. The author suggested that the two rats showing memory deficits absorbed more aluminium and were more susceptible to aluminium toxicity. However, there were no control animals in the study (Walton, 2007).

In a subsequent larger study, male Wistar rats were fed twice weekly on a restricted amount of low-aluminium diet from age 6 months to age 12 months, and then groups were given aluminium chloride at 0, 2 and 20 mg/l, expressed as aluminium, in the drinking-water, providing a total aluminium dose of approximately 0.4, 0.5 and 1.7 mg/kg bw per day from feed and water (group sizes of 13, 12 and 12, respectively). The dietary restriction was intended to reduce the rats’ weight to approximately 85% of the free-feeding weight, and typically the rats ate the feed in the first 2–3 days and had a day or more with no feed. The rats were tested weekly in a T-maze task until the end of their lifespan. In addition, gait characteristics were assessed once between 28 and 30 months. At necropsy, levels of γ-glutamyltranspeptidase, creatinine and aluminium were measured in serum, and brain sections were stained with a stain for aluminium developed by the author (modified Walton stain). Of the rats surviving to at least 28 months, 0/10 in the low-dose group, 2/10 in the intermediate-dose group and 7/10 in the high-dose group showed significantly lower performance in old age (>24 months) than in middle age (12–24 months). The rats with impaired performance had significantly higher serum aluminium levels and more aluminium in the entorhinal cortex cells of the brain. The author concluded that ingestion of aluminium at a dose of 0.5 mg/kg bw per day or more throughout most of adult life led, in old age, to a slowly progressing condition that impaired cognitive function in susceptible rats (Walton, 2009).

This study is difficult to interpret given the unusual feeding regime and the inconsistency with other studies that have not reported similar findings at much higher doses of aluminium. In addition, a submission received by the Committee noted 1) that the examiner was aware of the animals’ treatment group while assessing the cognitive outcomes, 2) that misclassification of exposure resulting from differences in individual animals’ feed and water consumption could not be excluded, 3) uncertainty regarding effects from frequent repeated administration of a neurobehavioural test in the same animals and 4) the lack of external validation of the method of staining for aluminium.

The effect of aluminium chloride on short- and long-term memory was examined in the offspring of lactating Wistar rats given aluminium chloride in their drinking-water at doses of 0, 200, 400, 600 and 800 mg/kg bw per day for 2 weeks. The pups were weaned at 37 days, and on day 45, they were trained in passive avoidance response, then tested for short- and long-term memory 2 and 30 days after the training. Two criteria were considered: latency in entering a dark chamber

(step-through latency) and the time spent in the dark chamber. Drinking-water consumption was not reported. There were no statistically significant differences from controls at aluminium chloride doses up to 600 mg/kg bw per day. Offspring of dams dosed at 800 mg/kg bw per day exhibited a decrease in step-through latency at both 2 days and 30 days after training, but not in the time spent in the dark chamber (Ali, Vostacolaee & Rahim, 2008).

Groups of 10 male rabbits (1000–1100 g, strain not specified) were given aluminium chloride at 20 mg/l in drinking-water for 3 months alone or in combination with subcutaneous administration of melatonin, either for 15 days following or simultaneously with the administration of aluminium chloride. A control group ($n = 5$) was included. The water intake was monitored weekly, and the aluminium chloride exposure was estimated at about 5–6.6 mg/day (approximately 1–1.3 mg/kg bw per day, expressed as aluminium). The aluminium contents of the diet and control tap water were not reported. After necropsy, the brains of the animals were subject to neuropathological examination. Atrophy and apoptosis of the neurons in the cerebral cortex and hippocampus, associated with neurofibrillary degeneration and argyrophilic inclusion, Schwann cell degeneration and nerve fibre demyelination, were reported in the aluminium-treated rabbits. These effects were lower in the groups treated with melatonin (as an antioxidant and free radical scavenger) (Abd-Elghaffar, El Sokkary & Sharkawy, 2007).

The effects of aluminium exposure on the glial system and behaviour of Wistar rats were examined in a study involving administration of aluminium chloride at 3 g/l in the drinking-water to adult (3-month-old) rats for 4 months or to female rats (n = 10) during gestation and lactation and then to their offspring until they were 4 months old. Two control groups (n = 5 each) of 7- and 4-month-old pups were also examined. No information was provided on the aluminium content of the food. Effects on the glial system were evaluated using immunohistochemistry for glial fibrillary acidic protein. Glial fibrillary acidic protein labelling and the numbers of astrocytes were increased in the brains of aluminium-treated rats compared with controls. Both groups of aluminium-treated rats showed significantly reduced locomotor activity compared with controls. The rats exposed in utero also exhibited significantly increased time in the lit compartment of a dark/light box (indicating increased anxiety), which was not seen in the rats exposed only as adults (Erazi, Sansar & Ahboucha, 2010).

Female Wistar rats (180 ± 4 g, n = 7) were given aluminium nitrate in drinking-water at a concentration of 0 or 80 mg/l for 90 days. No information was provided on the actual dose or the aluminium content of the food. Body weights were recorded weekly; motor activity in an open-field test and memory in a novel object recognition task were examined once every fortnight alternately. Brain aluminium concentration was evaluated at the end of the study. Body weights of treated rats were significantly lower than those of controls in weeks 12 and 13. There were no statistically significant differences in motor activity throughout the study. Treated rats exhibited a significant deficit in the recognition memory test in weeks 8 and 10 compared with the controls. There was no significant difference in the concentration of aluminium in the brain (Azzaoui, Ahami & Khadmaoui, 2008).

Aluminium chloride (100 mg/kg bw per day, expressed as aluminium) was given orally to rats for 6 weeks. On the 3rd week (21st day) and 6th week (42nd day) of the study, various behavioural tests (Morris water maze and elevated plus maze task paradigms) and locomotion (photoactometer) were conducted to evaluate cognitive performance. The rats were killed on the 43rd day following the last behavioural test. The aluminium treatment resulted in poor retention of memory in the Morris water maze and elevated plus maze task paradigms (Prakash & Kumar, 2009).

Aluminium toxicity and possible protection due to antioxidant effects of curcumin were studied in male Wistar rats (180–200 g; seven per group). Aluminium chloride was administered in drinking-water at 100 mg/kg bw per day for 42 days. Additional groups of rats received concomitant doses of curcumin (30 and 60 mg/kg bw orally as a solution in 0.5% carboxymethyl cellulose 1 hour after aluminium chloride administration). No information was provided on levels of aluminium in feed or control drinking-water. Behavioural studies were carried out on the 21st and 42nd days following training on day 20, to evaluate memory and locomotion. Aluminium chloride–treated rats showed a significant cognitive impairment in a spatial navigation task and significant memory impairment in an elevated plus maze task. No significant differences in locomotor activity between treated rats and controls were observed. Rats treated with curcumin showed improved cognitive performance and memory retention compared with those treated with aluminium chloride alone (Kumar, Dogra & Prakash, 2009).

The effects of aluminium on spatial learning and neurogenesis were studied in the transgenic mouse (Tg2576) model of Alzheimer disease. Groups (n = 7–8) of 5-month-old male Tg2576 mice and wild-type control mice were fed normal chow diet supplemented with aluminium lactate at 0 or 1000 mg/kg (0 or 101 mg/kg as aluminium, according to the authors) for 120 days. No information was provided on the content of aluminium in the chow or drinking-water. During the 4th month of treatment, activity in an open-field test and learning in a water maze were evaluated. The mice were then injected intraperitoneally with 5-bromo-2′-deoxyuridine at 100 mg/kg bw per day for 2 consecutive days and sacrificed 1 and 28 days after the last injection in order to study hippocampal cell proliferation and differentiation. In general, the aluminium-treated mice of both genotypes drank more water and ate less feed throughout the study. Although some differences were observed between the genotypes, this study did not demonstrate consistent effects due to the aluminium. The major observation was that in the Morris water maze, aluminium impaired learning and memory in the wild-type mice, but not in the transgenic mice. Aluminium treatment did not affect motor activity in either transgenic or wild-type mice (Ribes et al., 2008).

Aluminium chloride was administered in the drinking-water to male Wistar rats (young, 4 months old; aged, 18 months old; 10 animals in each treatment and control group) for 6 months, providing an aluminium dose of 50 mg/kg bw per day. The aluminium content of the diet was not reported. Cognitive outcomes were determined using the open-field test (locomotor activity: horizontal [ambulation] and vertical [rearing]), defecation index (number of faecal boluses) and the Morris water maze. The cognitive tests were administered at the end of exposure, just prior

to sacrifice. The open-field test showed a statistically significant detrimental effect of age on both horizontal and vertical activity, but no statistically significant effect of aluminium treatment on these parameters. Aluminium exposure was associated with an increase in faecal index in both age groups, with a larger increase in the young animals. Results of the Morris water maze were reported as mean latency to reach a hidden platform. On day 1, the young aluminium-treated rats required significantly longer than the young controls, whereas the aged aluminium-treated rats showed mean latencies similar to those of the aged controls. By day 4, the difference between the young aluminium-treated rats and young control rats had diminished. The difference between the aged aluminium-treated rats and aged control rats increased from day 1 to day 4, with the aluminium-treated rats showing a decreased ability to learn over the 4-day period (Sethi et al., 2008).

The developmental and chronic neurotoxicity of aluminium citrate was investigated in Sprague-Dawley rats in a study conducted according to GLP with a design based on OECD Test Guideline 426. Aluminium citrate was administered in drinking-water to groups of pregnant rats, commencing on GD 6, at concentrations aiming to deliver aluminium doses of 30, 100 and 300 mg/kg bw per day, based on an expected water intake of 120 ml/kg bw per day. Two control groups received either sodium citrate solution (27.2 g/l), the molar equivalent of the high-dose aluminium citrate, or plain water. The concentration of aluminium in the diets was 7–8.5 ng/ml, which would have contributed less than 1 µg/kg bw per day. After delivery, 20 litters per dose group were selected for the study, and the litters were culled to four males and four females. One male and one female per litter were assigned to one of four milestone groups designated for neurobehavioural testing on PNDs 23, 64, 120 and 364. Weaned pups received the same treatment as the dams. Actual doses were near or above target in the dams. Observations in the dams included water consumption, body weight, a functional observational battery, morbidity and mortality. Actual doses were one third to one half of the target doses in the pups for most of the 1-year treatment period owing to lower than expected fluid consumption. Observations on the pups included body weight twice weekly, fluid consumption weekly and a functional observational battery on all pups several times before weaning and twice weekly on the 1-year group until sacrifice. Motor activity, startle response and performance in a T-maze test and the Morris water maze test were assessed at various times. At each sacrifice time, half of the pups of each group were processed for neurohistopathological examination, and the other half were subjected to a regular necropsy followed by brain weight measurement, clinical chemistry, haematology, and collection of tissues and blood for measurement of aluminium and other metals.

There were no consistent effects of aluminium citrate on the dams, except for increased fluid consumption at the low and middle doses. The most notable treatment-related effect observed in the offspring was renal damage (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi), most prominently in the male pups. Higher mortality and significant morbidity, apparently due to urinary tract pathology, were observed in the male pups in the high aluminium citrate dose group, leading to termination of this group on day 98. Thus, the high-dose group was likely to be close to the maximum tolerated dose. Effects seen at the middle dose included urinary tract lesions, lower body weight in the males at PND 120

compared with controls, elevated fluid consumption in males and females, and an exaggerated response to tail pinch and narrower foot splay in the females. Apart from the urinary tract pathology, the most consistent and dose-related effect was decreased hindlimb and forelimb grip strength in both male and female pups. No consistent treatment-related effects were observed in ambulatory counts (motor activity) in the different cohorts. No significant effects were observed in the tests for learning or memory. None of the lesions seen on histopathological examination of brain tissues of the day 364 group were reported as treatment related, and, as these were also seen in the control group, the lesions were likely due to ageing. Tissue levels of aluminium were generally dose related, with the level in the bone showing the strongest association. Levels in blood were higher than those in the tissues. Of the central nervous system tissues, the highest level was in the brainstem. Overall, the authors concluded that the study indicated a LOAEL of 100 mg/kg bw per day and a NOAEL of 30 mg/kg bw per day (Semple, 2010; Poirier et al., 2011).

Identification of the LOAEL and NOAEL in this study is complicated by the decreasing fluid consumption and uncertainty regarding the critical exposure period. In the low-dose group, the achieved dose was about 40 mg/kg bw per day in the 1st week post-weaning, decreasing to 30 mg/kg bw per day (target dose) by week 5, and was about 15–45% of the target dose from post-weaning week 13 onwards. In the mid-dose group, the achieved dose was about 190 mg/kg bw per day in the 1st week post-weaning, decreasing to 100 mg/kg bw per day (target dose) by week 7, and was about 25–50% of the target dose from post-weaning week 15 onwards.

2.3 Observations in humans

The last evaluation by the Committee (Annex 1, reference *186*) considered all data relevant to the toxicity of and exposure to aluminium used in food additives and from other sources. A number of epidemiological studies were reviewed, most of them focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some studies suggested an association between consumption of aluminium in water and Alzheimer disease, but such an association was not confirmed in other studies. Only one of these studies assessed the ingestion of bottled water, whereas the remaining studies relied on concentrations of aluminium in water supply as a measure of exposure. None of them accounted for the ingestion of aluminium in foods, a potentially important confounding factor, as the aluminium in drinking-water represents a minor source of oral exposure. There was minimal information about the potential neurotoxic effects of aluminium in food, and the studies of the use of antacids did not demonstrate an association with neurological conditions. There were also a few case reports of adults and a child with normal kidney function who experienced skeletal changes attributed to frequent use of aluminium-containing antacids, considered to induce phosphate depletion. In summary, no pivotal epidemiological studies were available for the risk assessment in the previous evaluation.

The European Food Safety Authority (EFSA) also provided a scientific opinion on the safety of aluminium from all dietary sources (EFSA, 2008). EFSA

evaluated the neurotoxicity in patients undergoing dialysis in which insufficiently purified water was used and there was parenteral exposure to high concentrations of aluminium, as well as the potential role of aluminium in the etiology of Alzheimer disease and its association with other neurodegenerative diseases. However, these hypotheses remain controversial. There were very few specific toxicological data for food additives containing aluminium, and the available studies had a number of limitations and did not allow any dose–response relationships to be established.

Since the last evaluation by the Committee (Annex 1, reference *186*), a few epidemiological studies have been published on the association between exposure to aluminium and Alzheimer disease, dementia and other neurological outcomes, mainly among subjects exposed through drinking-water, but also in people following ingestion of antacids, children exposed from parenteral nutrition and workers with potential occupational exposure. Some of these studies were already included in the EFSA evaluation (EFSA, 2008), as well as in the recent report by Risk Sciences International (RSI, 2010).

2.3.1 Biomarkers of exposure

No studies on biomarkers of aluminium exposure were found.

2.3.2 Biomarkers of effects

No studies on biomarkers of aluminium effects were found.

2.3.3 Clinical observations

(a) Case reports

A case of possible relevance was reported for a woman who had been acutely exposed to high aluminium concentrations in drinking-water as a result of an accidental discharge of aluminium sulfate into the local mains water supply in Cornwall, England. Fifteen years later, the woman, by then aged 58 years, was referred for investigation of deterioration of her mental state; she continued to deteriorate and died within 1 year, and an autopsy was performed. A rare form of sporadic early-onset β-amyloid angiopathy in cerebral cortical and leptomeningeal vessels and in leptomeningeal vessels over the cerebellum was identified. A few neurofibrillary tangles (NFT) were observed in the cortex and hippocampus. In addition, high concentrations of aluminium were found coincident with the severely affected regions of the cortex. According to the authors, a causative role for aluminium in the development of the observed neuropathology cannot be concluded, although the association between high brain aluminium levels and unusual neuropathology deserves further investigation (Exley & Esiri, 2006).

Another case was reported for a 20-year-old woman who fell into a coma with anisocoria and left spastic hemiparesis after respiratory infection; her condition was slowly progressive and developed into a vegetative state. Brain imaging showed massive abnormal signals in the white matter. Electron spectroscopic imaging of biopsied brain tissue confirmed that the electron-dense deposits were associated with aluminium accumulation in the myelin sheath. The probable source

of exposure to aluminium was unknown. Myelin is known to easily become a primary target of aluminium toxicity, because aluminium binds to transferrin and is taken into oligodendrocytes, and this may have contributed to aluminium-induced toxicity (Itoh et al., 2008).

(b) Aluminium in brain and Alzheimer disease

A few studies have reported the presence of aluminium in brain tissue, often associated with neuropathological features of Alzheimer disease. One study assessed the localization of aluminium in corticolimbic neurons of six patients with autopsy-confirmed Alzheimer disease and six non-demented controls. All pyramidal neurons in these specimens appeared to exhibit at least some degree of aluminium staining. On the basis of their staining patterns, all pyramidal neurons could be classified into two stages: progressive increase of nuclear aluminium (often accompanied by granulovacuolar degeneration with granules that stain for aluminium) or formation of NFT in regions of aluminium-rich cytoplasm, especially in brain tissue of patients with Alzheimer disease. Given that the NFT in human neurons always developed in conjunction with cytoplasmic aluminium, it was hypothesized that aluminium may play a role in their formation (Walton, 2006). In a subsequent study, hippocampal cells from the brains of five patients with confirmed Alzheimer disease and five non-demented controls were examined. Mature NFT were observed in all the Alzheimer disease cases and three of the controls. NFT stained for both aluminium and hyperphosphorylated tau. Overall, the results showed co-localization of aluminium and hyperphosphorylated tau in an Alzheimer disease–vulnerable region of the brain (Walton, 2010).

In another study, the brains of patients with Alzheimer disease were examined using transmission electron microscopy energy-dispersive X-ray spectroscopy. The results from this study demonstrated the presence of aluminium in amyloid fibres in the cores of senile plaques located both in the hippocampus and in the temporal lobe (Yumoto et al., 2009).

Amyloid plaques and NFT are prominent neuropathological hallmarks of Alzheimer disease. Both the accumulation of aluminium in senile plaques, most of them consisting of aggregates of β-amyloid peptides, and the development of NFT in the presence of aluminium provide some support to the association between Alzheimer disease and the presence of aluminium in the brain. However, the coincidental observation of these neuropathological features and aluminium in the brain cannot confirm the causal role of aluminium in Alzheimer disease.

2.3.4 Epidemiological studies

A randomized controlled trial assessed the acute effects of oral ingestion of a common aluminium compound on neuropsychological function. The study included three groups: 16 patients with a diagnosis of possible or probable Alzheimer disease and other dementias (scores 26–10 on the Standardized Mini-Mental State Examination [MMSE]); 17 age-matched controls (patients' caregivers); and 10 younger volunteers (family members, hospital employees). Aluminium hydroxide gel with citrate or placebo was administered over 3 consecutive days in a crossover

design, with a 3-week washout period between the two 3-day test sessions. The dose was adjusted for each individual in order to attain serum aluminium levels of 50–150 µg/l. A neuropsychological test battery was administered to the subjects on day 1 and again on day 3, at time 0 and 90 min after ingestion of the study preparation. Thirty-eight out of the 55 participants completed the protocol. There were no significant differences in neuropsychological test battery scores between active and placebo for any of the individual tests or for any of the groups examined. The mean concentration of aluminium in serum on day 3, 90 min after ingestion, was 294 µg/l (95% confidence interval [CI] 181–407 µg/l); a quarter of the subjects were within the targeted range of 50–150 µg/l, whereas 66% were above this range. The absence of neuropsychological effects after short-term exposure at elevated levels of aluminium in serum was unexpected. In some cases, the levels of aluminium in serum far exceeded about 60–200 µg/l, at which aluminium has been associated with cognitive effects in dialysis dementia (Molloy et al., 2007).

(a) Aluminium in drinking-water and Alzheimer disease, dementia and cognitive disorders

The relationships between aluminium and silica in drinking-water and the risk of cognitive decline, dementia and Alzheimer disease in elderly people were investigated in two cohorts in the regions of Gironde and Dordogne, south-west France. The cohort PAQUID (Personnes Âgées Quid) is a prospective population-based cohort of 3777 elderly subjects, aged 65 years or older at recruitment (1988–1989), followed regularly up to 2004 and at a 10-year follow-up. The Aluminium-Maladie d'Alzheimer (ALMA+) cohort included 400 subjects, aged 75 years and over at entry in 1999; thus, these subjects were expected to be comparable with the subjects seen at the 10-year follow-up of the PAQUID cohort. As the methods to assess the exposure and outcomes were very similar, the subjects of both cohorts were pooled for this analysis. Exposure to aluminium and silica in drinking-water at the geographical level was based on the information on tap water chemical analysis provided by the sanitary administration for 91 drinking-water areas (77 for PAQUID and 14 for ALMA+). For each area, a weighted mean of all measures of aluminium and silica was computed using results of analyses of drinking-water carried out by the sanitary administration. For the evaluation of the subjects' past exposure (at the geographical level), the history of the water distribution network over the previous 10 years was taken into account. Exposure at the individual level used information from a dietary questionnaire, which contained specific questions about the daily consumption of tap water and bottled water, including the brand, combined with the bottled water composition provided by the respective distributing companies. Assessment of intellectual functioning was based upon the MMSE score. Cases of dementia were detected by a two-step procedure: first, all participants underwent an interview and a psychometric evaluation with a trained psychologist who systematically completed a questionnaire designed to fulfil standardized criteria for dementia; then, subjects positive for these criteria were examined by a senior neurologist who confirmed the diagnosis. Subjects with a diagnosis of dementia at recruitment were excluded from the analysis. In total, 1925 subjects were available for the analysis of cognitive function, performed using a random effects linear regression model. The mean exposure to aluminium from drinking-water was 0.025 mg/day,

96% supplied by tap water. Cognitive decline was greater in subjects with a high daily aluminium exposure (≥0.1 vs <0.1 mg/day, $P = 0.001$). The interaction between aluminium exposure and time was not significant when the demented subjects were excluded, suggesting that cognitive decline with time was related to daily aluminium exposure only when it is associated with a dementia process. During the 15-year follow-up, 461 subjects out of 1677 were diagnosed with dementia or Alzheimer disease; the association with aluminium or silica exposure was assessed by the Cox proportional hazards model. The risk of dementia or Alzheimer disease was associated with daily exposure to aluminium: relative risk (RR) of 2.26 (95% CI 1.00–5.07) for aluminium exposure greater than or equal to 0.1 mg/day versus less than 0.1 mg/day or RR of 1.28 (95% CI 1.05–1.58) for a 0.1 mg/day increase (as a continuous variable). When aluminium exposure was categorized by quartiles, only the highest level (>0.1 mg/day) was significantly associated when compared with the bottom quartile (RR = 2.34, 95% CI 1.03–5.32). Contrary to aluminium, silica was inversely associated with dementia or Alzheimer disease (RR = 0.88, 95% CI 0.79–0.99, for a 10 mg/day increase). All these estimates were adjusted for age, sex, educational level, wine consumption and place of residence; the estimates for aluminium and silica exposure were mutually adjusted as well. Although the prospective design must be considered as a strength of the study, there is limited power owing to the low number of subjects with the highest level of exposure; only 13 subjects, which included 6 cases, had aluminium exposure greater than or equal to 0.1 mg/day. Although the estimates were adjusted for several potentially confounding factors, the possibility of residual confounding cannot be excluded, mainly due to the lack of information on aluminium exposure from foods, thought to contribute approximately 95% of oral exposure (Rondeau et al., 2009).

A previous analysis of the PAQUID cohort was carried out among 292 subjects, including 55 Alzheimer disease cases, who provided a blood sample at the 10-year follow-up visit. Logistic regression was used to assess the potential risk of Alzheimer disease associated with aluminium in drinking-water and carrying the C2 allele in the transferrin gene. Transferrin is the major transport protein for aluminium, and deficient binding of aluminium to transferrin may increase the unbound aluminium, which could cross the blood–brain barrier. The risk of Alzheimer disease was not associated with either C2 carrier status or interaction between the C2 allele and aluminium exposure. However, aluminium exposure modified the risk of Alzheimer disease associated with apolipoprotein E (ApoE) ε4; carriers of ε4 with aluminium exposure in drinking-water less than 0.1 mg/day had an odds ratio (OR) of 5.98 (95% CI 2.13–16.8) compared with an OR of 2.72 (95% CI 0.99–7.43) among ε4 carriers with an aluminium exposure in drinking-water greater than or equal to 0.1 mg/day (Rondeau et al., 2006).

The association of Alzheimer disease with aluminium in drinking-water was assessed in the Canadian Study of Health and Aging cohort. After a 10-year follow-up, 490 Alzheimer disease incident cases were ascertained among the 7155 subjects recruited in 1991–1992. Exposure assessment was based on residential history collected from the subjects and data on aluminium concentrations from water treatment plants. A statistically significant association between the risk of Alzheimer disease and aluminium was found using Cox models with aluminium as the continuous variable; no association was observed in analyses with a

categorical variable or after adjustment for autocorrelation in a two-stage model. This study provides equivocal evidence that higher aluminium drinking-water levels are associated with increased risk of Alzheimer disease (Boom, 2008).

Finally, an ecological study was carried out in the Biga Peninsula, located in north-west Turkey, in order to evaluate the potential of aluminium to influence cognitive function. The Kirazli region was included in the study, as it has water supplies characterized by high acidic content and aluminium levels. At the time of the study, 73 people out of the 201 inhabitants agreed to participate. The control group consisted of 164 subjects selected from the 921 inhabitants of another region in the same province. A neurology specialist administered an MMSE and performed a neurological examination, and a blood sample was collected from each participant. All of the subjects obtained their drinking-water from the groundwater networks in their regions. Water samples collected in both regions revealed a much higher concentration of aluminium in Kirazli (13–16 mg/l) than in the control region (0.005–0.010 mg/l); there were also much higher levels of iron, manganese, lead and zinc. Despite the differences in aluminium levels in their water supplies, no statistically significant difference was detected between the serum levels of aluminium of participants living in the two regions. No statistically significant difference was detected in the distribution of MMSE scores or the presence of neuropathy at examination between the two regions (Bakar et al., 2010).

(b) Dementia and aluminium in haemodialysis patients

Dementia may occur in the course of dialysis; however, the risk factors for dementia of uraemic patients remain unclear. One cross-sectional study aimed to determine the difference in nutritional status and the contents of several plasma elements in haemodialysis patients with or without dementia. Forty-five haemodialysis patients were divided into two groups: 25 patients without dementia and 20 patients with dementia. In addition, a control group of 20 healthy volunteers was included. Thirteen non-dementia patients and 11 patients with dementia were treated with aluminium hydroxide for controlling phosphate levels. Dementia patients had significantly higher levels of plasma aluminium (11.7 vs 7.79 µg/dl), and both groups had higher levels than controls (3.17 mg/dl). Dementia patients also had significantly higher levels of iron, copper and magnesium and lower levels of zinc. Furthermore, dementia patients also had a significant increase in the levels of MDA, an indicator of lipid peroxidation, and MDA was positively correlated with levels of aluminium and magnesium and with the copper/zinc ratio. On the basis of these findings, the authors concluded that chronic haemodialysis may lead to significant changes in the serum that increase the susceptibility of uraemia patients to oxidative stress and inflammation, which could be associated with the development of dementia (Guo et al., 2009).

(c) Oral exposure to aluminium and bone health

Low bone formation and patchy osteomalacia have been observed in patients on dialysis and those who are on total parenteral nutrition. Standard solutions for parenteral nutrition of infants contain significant aluminium concentrations; a randomized trial was conducted to compare their long-term effects on bone health

with those from solutions specially sourced for low aluminium content. The trial aimed to test the hypothesis that neonatal exposure to aluminium in standard parenteral nutrition solutions results in reduced bone mass during adolescence. In total, 227 preterm infants were recruited in Cambridge and Norwich, England: 112 were assigned to receive standard aluminium (SA) solution and 115 to low-aluminium (LA) solution. Data were collected for the neonatal course, and the participants were invited for follow-up after 15 years; 59 out of the 177 participants eligible for follow-up were actually seen, 26 fed with SA solutions and 33 with LA solutions. Among the 118 subjects not seen at the 15-year follow-up visit, 48 were untraceable, 48 did not reply to the invitation and 22 declined to attend; the distribution of the drop-outs was very similar in both groups. Dual-energy radiograph absorptiometry was used to measure bone mineral content (BMC), bone area and bone mineral density (BMD) of the lumbar spine, hips and whole body. Mean aluminium exposure was significantly higher for infants fed with SA solutions compared with those fed with LA solutions (means 21.3 and 3.0 µg/kg bw per day, respectively). Most bone density measurements tended to be higher in the LA group, but the only statistically significant differences were observed for lumbar spine BMC (mean ± SD: 44.9 ± 8.8 g vs 39.8 ± 6.5 g, $P = 0.02$) and lumbar spine bone area (40.5 ± 5.4 cm^2 vs 37.8 ± 3.7 cm^2, $P = 0.03$). The increase in lumbar spine BMC seemed to be attributable to a concomitant increase in bone size in the LA group, as no difference between groups in lumbar spine BMC was seen after adjusting for height, weight and lumbar spine bone area. For lumbar spine BMD, the means ± SD for the LA and SA groups were, respectively, 1.10 ± 0.12 g/cm^2 and 1.05 ± 0.15 g/cm^2 ($P = 0.17$). In a non-randomized analysis with the total aluminium exposure from parenteral nutrition as a continuous variable, aluminium exposure from parenteral nutrition was not a significant predictor of BMC at any site, after adjusting for relevant neonatal variables. However, when aluminium exposure was categorized using the median exposure (55 µg/kg bw) as a cut-off, children with high exposure had significantly lower (7.6%) hip BMC. The mechanism for long-term effects of aluminium on bone health is unclear. A direct toxic effect seems unlikely, because bone tissue will have been replaced more than once by age 15 years. Aluminium exposure might modify the responsiveness of bone such that, for example, children who are exposed to more aluminium form less bone for a given level of mechanical stimulus. This could explain the apparent site-specific effects. The long-term clinical significance of the observed effects of early aluminium exposure on bone mass at 15 years cannot currently be quantified, although these subjects were only 5–8 years from attaining peak bone mass (Fewtrell et al., 2009).

A case–control study was carried out in Upsala, Sweden, to examine whether the aluminium content of bone differs between controls and hip fracture cases with and without dementia, in particular in patients with Alzheimer disease. Cases were 103 patients with hip fracture (81 women, 22 men, mean age 73 years), among whom 49 had a diagnosis of dementia, including 16 with Alzheimer disease. The control group consisted of 69 patients (33 women and 36 men, mean age 58 years) admitted to the hospital for arthroplasty because of osteoarthrosis of the hip or because of high-energy femoral or tibial fracture. During the operations, bone biopsies from the trabecular bone of the proximal femur or tibia were taken with an aluminium-free instrument and were then introduced into an inductively coupled

mass spectrometer for measurement of their content of aluminium. All samples contained aluminium, at concentrations ranging from 58 to 13 300 ng/g dry weight. There was an exponential increase in the aluminium content of bone with age, with a statistically significant quadratic term of age in a model that included age in continuous form. No significant differences were detected in sex- and age-adjusted mean log-transformed aluminium contents between the controls and the hip fracture cases with dementia ($P = 0.72$) or without dementia ($P = 0.33$). When bone aluminium content was categorized by quartiles, there was no association with the risk of hip fracture once adjusted for age and sex. The most important finding in this study is the sharp increase in the aluminium content of bone with increasing age, but there was no association between this content and the risk of hip fracture, which is the most serious consequence of osteoporosis. Hip fracture cases with dementia showed aluminium concentrations in trabecular bone that were similar to those of hip fracture cases without dementia (Hellström et al., 2005).

2.3.5 Occupational exposure to aluminium

The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence that certain exposures occurring during aluminium production cause cancer. Pitch volatiles, containing mainly polycyclic aromatic hydrocarbons, have fairly consistently been suggested in epidemiological studies as being possible causative agents. There is no evidence of increased cancer risk in non-occupationally exposed persons, and IARC did not implicate aluminium itself as a human carcinogen (IARC, 1984).

A few studies have addressed the potential effects of occupational exposure to aluminium on reproductive and neurobehavioural outcomes. One survey was carried out in a North American aluminium smelter plant where several adverse pregnancy outcomes had been reported. The participation rate for the survey was 85%: 621 workers participated, out of a total of 730 eligible, including 515 males and 106 females. Working in the laboratory was significantly associated with the occurrence of congenital anomaly. The congenital anomalies reported were three cases of trisomy (9, 18 and 21) as well as two renal, two musculoskeletal, one ocular, one cardiovascular and one genitourinary anomaly. The mean air concentration of aluminium in the laboratory was 2.8 mg/m^3 (range 0.07–8.3 mg/m^3, median 1.1 mg/m^3), far below the threshold limit value set by the American Conference of Governmental Industrial Hygienists of 10 mg/m^3. Given the diverse anomaly types, the absence of other adverse outcomes and the low exposure levels, the authors concluded that there is little evidence to suggest that the excess of congenital anomalies was due to workplace factors (Sakr et al., 2010).

A review with meta-analysis has summarized the evidence regarding the potential impact of occupational aluminium exposure on cognitive and motor performance. The final sample consisted of nine studies with 449 exposed and 315 reference subjects. Exposure originated from welding, smelting or electrolysis. Mean urinary aluminium concentrations ranged from 13 to 133 mg/l, with mean exposure duration ranging from 4.7 to 19.2 years. Six different neuropsychological tests were considered, and 10 performance variables were analysed: 7 of the variables pertained to aspects of attention, 2 to motor performance and 1 to constructional

performance. Almost all overall effect sizes indicated poorer performance of the exposed group, but a statistically significant result was found only for the digit symbol test (difference between the mean scores in exposed and control group –0.43, 95% CI –0.77 to –0.08). Performance of this test was negatively related to urinary concentrations of aluminium. Although this result suggests that the test for which a significant result was obtained might be a potential screening instrument for measuring aluminium-related changes in performance, 1 significant effect size out of 10 analyses could be a chance result. Uncertainties remain with respect to confounding, as the extent of confounding that has to be taken into account for at least some of the tests cannot be determined (Meyer-Baron et al., 2007).

The potential neurotoxic effects of aluminium have been analysed in two parallel longitudinal studies of aluminium welders in Germany. In the first study, 44 male aluminium welders in train and truck industries were compared with 37 assembly workers from the same enterprises; in the second study, 97 male aluminium welders in the automobile industry were compared with 50 non-exposed construction workers. In both studies, four examinations took place during a 4-year period; exposure was monitored by means of measurements of aluminium concentrations in the environment, as well as in urine and plasma of workers. The assessment of neurobehavioural performance included a questionnaire for the recording of neurotoxic symptoms and a number of psychological tests exploring different functional domains and premorbid intelligence. The aluminium welders who had been working in these industries for an average of 15 years showed no significantly increased symptom levels compared with the control group. The analyses revealed neither a correlation between biomonitoring and performance variables nor a significant difference between aluminium-exposed workers and controls in the performance courses during the 4-year period (Kiesswetter et al., 2007, 2009).

3. DIETARY EXPOSURE

3.1 Introduction

At the present meeting, the Committee was asked, based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010), to evaluate the safety of aluminium-containing food additives, including aluminium ammonium sulfate, aluminium lakes of colouring matters, aluminium potassium sulfate, aluminium powder, aluminium silicate, aluminium sulfate (anhydrous), calcium aluminium silicate, sodium aluminium phosphate acidic, sodium aluminium phosphate basic and aluminium lactate.

Owing to their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. At its present meeting, the Committee was asked to evaluate the safety of potassium aluminium silicate–based pearlescent pigments based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010). This aluminium-containing food additive has not previously been evaluated by the Committee. All data necessary for the assessment of dietary exposure to the substance, including information on actual use levels, was

requested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in a July 2010 call for data.

At its present meeting, the Committee, following its call for data, received submissions from:

- the International Council of Grocery Manufacturer Associations (ICGMA, 2010) on the current use levels for aluminium sulfate (International Numbering System [INS] 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate acidic (INS 541(i)) and aluminium lakes of colour;
- Food Standards Australia New Zealand (FSANZ, 2011) on dietary exposure to aluminium compounds in food, including additives, from its twenty-third Total Diet Study;
- Brazil (Aparecida, 2009) on the usages of aluminium-containing food additives and estimates of dietary exposure using the maximum permitted levels of these food additives.

Those submissions were complemented by a review of data from the literature published since the last JECFA evaluation in 2007 (accessible via Scopus or Medline as of 20 May 2011) from Europe (EFSA, 2008), the United Kingdom (Rose et al., 2010) and China, Hong Kong Special Administrative Region (SAR) (Wong et al., 2010).

A submission of data for the exposure assessment was provided to JECFA by a sponsor on 30 November 2010 (Merck, 2010). The exposure data provided by the sponsor were not for potassium aluminium silicate itself, but rather for the actual potassium aluminium silicate–based pearlescent pigment.

3.2 Use levels of the additives in food

3.2.1 Aluminium-containing food additives in the Codex General Standard for Food Additives

As shown in Table 3, three of the additives are currently listed in Table 3 of the Codex General Standard for Food Additives (GSFA) for use in most food categories at good manufacturing practice (GMP) levels; however, the Codex Alimentarius Commission revoked the Table 3 status for these three additives based on the recommendation of the Forty-third Session of CCFA (FAO/WHO, 2011).

All of the aluminium-containing food additives except aluminium lactate have previously been evaluated by the Committee.

(a) Current status of aluminium-containing food additives in the Codex General Standard for Food Additives

Because of their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. The current (adopted, draft and proposed draft) provisions made for aluminium compounds in Tables 1 and 2 of the Codex GSFA are reported in Table 4. In addition to the revocation of the GMP status of three aluminium additives in Table 3 of the GSFA, the Forty-third Session of CCFA recommended discontinuation or revocation of all aluminium provisions

Table 3. Aluminium-containing food additives in the Codex General Standard for Food Additives

INS	Additive name	Relevant tables
523	Aluminium ammonium sulfate	GSFA Tables 1 and 2
541(i)	Sodium aluminium phosphate acidic	GSFA Tables 1 and 2
541(ii)	Sodium aluminium phosphate basic	
554	Sodium aluminosilicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)
556	Calcium aluminium silicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)
559	Aluminium silicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)

that are listed without numerical use levels in Tables 1 and 2 of the Codex GSFA (highlighted in the final column of Table 4).

(b) Current use levels made available to the Committee by the International Council of Grocery Manufacturer Associations

The ICGMA (2010) submitted information on current use levels for aluminium lakes of colour, aluminium sulfate (INS 520), sodium aluminium phosphate acidic (INS 541(i)) and sodium aluminosilicate (INS 554) (Table 5).

3.2.2 Potassium aluminium silicate

The aluminium portion of the dietary assessment provided here refers only to aluminium from the food additive use of the potassium aluminium silicate–based pearlescent pigment and does not refer to aluminium as a contaminant present in foods as consumed. The contaminant portion of aluminium exposure has previously been assessed by the Committee at its sixty-seventh meeting in 2007 (Annex 1, reference *184*).

Potassium aluminium silicate (mica) is used as a carrier substrate for titanium dioxide and/or iron oxide. Potassium aluminium silicate is not intended to be placed on the market as such, but only when coated with the food colours titanium dioxide and/or iron oxide. In the European Union (EU), E555 potassium aluminium silicate is approved as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment) (Directive 95/2/EC as amended by Directive 2003/114/EC). In the

Table 4. List of all aluminium provisions in the Codex General Standard for Food Additives: adopted (Step 8), draft (Step 6) and proposed draft (Step 3)[a]

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
Aluminium ammonium sulfate (INS 523)						
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	350	6	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	150	6	3	—	—
04.1.2.7	Candied fruit	200	6	8	2001	—
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine or soya bean sauce	500	6	3	—	—
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine or soya bean sauce	35	6	8	2003	—
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5	200	6	8	2001	—
04.2.2.7	Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soya bean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3	500	6	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
06.2	Flours and starches (including soya bean powder)	500	6	3	—	—
06.2.2	Starches	GMP	6 & 26	6	—	Discontinue
06.4.1	Fresh pastas and noodles and like products	470	6	3	—	—
07.1.2	Crackers, excluding sweet crackers	10 000	29	3	—	—
07.1.3	Other ordinary bakery products (e.g. bagels, pita, English muffins)	10 000	29	3	—	—
07.1.4	Bread-type products, including bread stuffing and bread crumbs	10 000	29	3	—	—
07.1.5	Steamed breads and buns	10 000	29	3	—	—
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6	3	—	—
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	10 000	29	3	—	—
08.3.2	Heat-treated processed comminuted meat, poultry and game products	5	6	3	—	—
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	1 500	6	3	—	—
09.2.4	Cooked and/or fried fish and fish products, including molluscs, crustaceans and echinoderms	200	6	8	2001	—
09.3	Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	1 500	6	3	—	—
10.2	Egg products	30	6	8	2001	—
10.4	Egg-based desserts (e.g. custard)	380	6	8	2003	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	500	6	3	—	—
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	500	6	3	—	—
Sodium aluminium phosphate acidic (INS 541(i)) and sodium aluminium phosphate basic (INS 541(ii))						
01.6.1	Unripened cheese	670	6	3	—	—
01.6.4	Processed cheese	35 000	29	6	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	2 000	6	6	—	—
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	2 000	6	6	—	—
04.1.2.9	Fruit-based desserts, including fruit-flavoured water-based desserts	2 000	6	6	—	—
05.1.1	Cocoa mixes (powders) and cocoa mass/cake	2 000	6 & 72	6	—	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	350	29	3	—	—
06.2	Flours and starches (including soya bean powder)	3 600	6	3	—	—
06.2.1	Flours	45 000	29	6	—	—
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	2 000	6	6	—	—
06.6	Batters (e.g. for breading or batters for fish or poultry)	1 600	6	6	—	—
07.1	Bread and ordinary bakery wares	2 000	6	6	—	—
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types)	2 000	6	6	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)	2 000	6	6	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	15 300	29	6	—	—
08.3.3	Frozen processed comminuted meat, poultry and game products	360	6	3	—	—
09.2.2	Frozen battered fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms	190	6 & 41	6	—	—
09.2.4.3	Fried fish and fish products, including molluscs, crustaceans and echinoderms	600	6	3	—	—
10.4	Egg-based desserts (e.g. custard)	2 000	6	6	—	—
12.5.2	Mixes for soups and broths	2 000	6 & 127	6	—	—
12.6.3	Mixes for sauces and gravies	2 000	6 & 127	6	—	—
16.0	Composite foods: foods that could not be placed in categories 01–15	190	6	6	—	—
Sodium aluminosilicate (INS 554)						
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	20 000	6	3	—	—
01.3	Condensed milk and analogues (plain)	20 000	6	3	—	—
01.4.4	Cream analogues	20 000	6	3	—	—
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.8.1	Liquid whey and whey products, excluding whey cheeses	20 000	6	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	20 000	6	3	—	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.3	Breakfast cereals, including rolled oats	20 000	6	3	—	—
06.4.3	Pre-cooked pastas and noodles and like products	2 0000	6	3	—	—
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	20 000	6	3	—	—
06.6	Batters (e.g. for breading or batters for fish or poultry)	20 000	6	3	—	—
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
11.1.2	Powdered sugar, powdered dextrose	10 000	6 & 174	3	—	—
11.1.2	Powdered sugar, powdered dextrose	15 000	56	8	2006	—
12.1.1	Salt	20 000	6	3	—	—
12.1.1	Salt	GMP		8	2006	Revoke
12.1.2	Salt substitutes	10 000		6	—	—
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue
14.1.4.3	Concentrates (liquid or solid) for water-based flavoured drinks	10 000	6 & 174	3	—	—
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	120	6	3	—	—
Calcium aluminium silicate (INS 556)						
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.1	Unripened cheese	10 000	6 & 174	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	265	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.1	Whole, broken or flaked grain, including rice	GMP	—	6	—	Discontinue
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6 & 174	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
11.1.2	Powdered sugar, powdered dextrose	15 000	6 & 56	3	—	—
11.1.2	Powdered sugar, powdered dextrose	15 000	56	8	2006	—
12.1.1	Salt	20 000	6	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
12.1.1	Salt	GMP	—	8	2006	Revoke
12.1.2	Salt substitutes	10 000	—	6	—	—
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue
14.2.3	Grape wines	GMP	—	6	—	Discontinue
Aluminium silicate (INS 559)						
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.1	Unripened cheese	10 000	6	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.1	Whole, broken or flaked grain, including rice	GMP	—	6	—	Discontinue
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6 & 174	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
12.1.1	Salt	10 000	6	3	—	—
12.1.2	Salt substitutes	10 000	—	6	—	—
12.2.1	Herbs and spices	GMP	51	3	—	Discontinue
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue

[a] GMP aluminium provisions were recommended for discontinuation at the Forty-third Session of CCFA.

[b] Relevant GSFA notes:

Note 3 Surface treatment.
Note 6 As aluminium.
Note 26 For use in baking powder only.
Note 29 Reporting basis not specified.
Note 41 Use in breading or batter coatings only.
Note 51 For use in herbs only.
Note 56 Provided starch is not present.
Note 72 Ready-to-eat basis.
Note 127 As served to the consumer.
Note 174 Singly or in combination: sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).
Note 177 For use in sliced, cut, shredded or grated cheese only.
Note 179 For use in surface treatment of sausages.

Table 5. Current use levels of aluminium-containing food additives submitted by the International Council of Grocery Manufacturer Associations

(a) Aluminium lakes of colour

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	500	—	Powder, unreconstituted
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	2.4–6.7	—	—
02.1.2	Vegetable oils and fats (e.g. popcorn oil)	1.2–215.5	—	—
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5 (example – salsa)	600–20 000	80–4000 mg/kg as Al supplied	—
05.2.2	Soft candy (e.g. yoghurty coating around fruity-flavoured chewy centres)	—	5–200 mg/kg as Al supplied	—
05.3	Chewing gum	Up to 2000	—	—
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	1.2–58.5	—	Icings, frostings
06.3	Breakfast cereals, including rolled oats (e.g. mini-wheats)	—	30 mg/kg as Al supplied	—

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	800–8000	160–1600 mg/kg as Al supplied	—
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types) (e.g. cookies, pop-tarts)	290–1270	3–85 mg/kg as Al supplied	Pop-tarts
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins) (e.g. pancakes, waffles, snack bars)	—	1–50 mg/kg as Al supplied	Pancakes, waffles, snack breakfast bars
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	1.2–215	—	Cake mixes
12.2.1	Herbs and spices	900–10 000	125–1500 mg/kg as Al supplied	—
12.2.2	Seasonings and condiments	100–15 000	15–2900 mg/kg as Al supplied	—
12.6.2	Non-emulsified sauces (e.g. ketchup, cheese sauce, cream sauce, brown gravy)	39.7–2000	20–300 mg/kg as Al supplied	Cheese sauce
12.6.3	Mixes for sauces and gravies	100–95 000	15–1300 mg/kg as Al supplied	Meat marinade, as reconstituted
14.1.4.3	Concentrates (liquid or solid) for water-based flavoured drinks	—	1.6–14 mg/kg as Al (3–26 mg/kg as Al_2O_3)	These levels correspond to the reconstituted concentrate as consumed by the consumer (Note 127)[b] (If not reconstituted, then 148 mg/kg as Al_2O_3)

Table 5 (contd)

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes) (e.g. chips, snacks)	300–800	100–400 mg/kg as Al supplied	Popcorn, snacks, trail mix, etc.
		300–48 000 (chips)	40–7200 mg/kg as Al supplied	

[a] Note 6: As aluminium.
[b] Note 127: As served to the consumer.

(b) Aluminium sulfate (INS 520)

Food category No.	Food category name	Comments to CCFA (Note 6)		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium	Justification
10.2	Egg products	400–600	60–95 mg/kg as Al	Emulsifier: protein coagulation suppressant (crystallization inhibitor). The aluminium binds with egg proteins to help maintain protein solubility during the pasteurization/ heating process.

(c) Sodium aluminium phosphate acidic (INS 541(i); Function: acidity regulator, emulsifier, raising agent, stabilizer, thickener)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.1	Bread and ordinary bakery wares	2000	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in baking mixes for bread (Food category 07.1.1) and pizza crust (Food category 07.1.6), for example.
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types)	2000	Note 6	6	200	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 2220 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 190 mg/kg. This is used in cookies and pop-tarts.
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)	2000	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in muffins, French toast, filled sweet rolls, waffles, Cinnabon, pancakes and baked wafers.

Table 5 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	15 300	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/ raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in baking mixes for muffins, cakes and pancakes, for example.

[a] Note 6: As aluminium.
[b] Step 6: Draft.

(d) Sodium aluminosilicate (INS 554; Function: anticaking agent)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	20 000	Note 6	3	100	Note 6	Sodium aluminosilicate is used in dry mix hot chocolate at levels of 1000 mg/kg on the basis of the whole compound and 57 mg/kg on the basis of aluminium.

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
01.3	Condensed milk and analogues (plain)	20 000	Note 6	3	1000	Note 6	Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are necessary for beverage whiteners (Food category 01.3.2), including non-dairy creamer powder and coffee whitener powder.
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	Notes 6 & 174	3	1000	Note 6	Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are necessary for milk/cream powder analogues (e.g. soya oil powder), and levels of 5000 mg/kg on the basis of the whole compound (or 285 mg/kg on the basis of aluminium) are necessary for dairy-based creamers (e.g. milk powder and cream powder).
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	Notes 6 & 174	3	1500	Note 6	Sodium aluminosilicate is an anticaking agent that helps prevent components from adhering to each other. To ensure flowability for the cheese powder and to prevent clumping, a maximum level of 25 000 mg/kg on the basis of the whole compound (or 1425 mg/kg on the basis of aluminium) is being recommended.
07.1.6	Mixes for bread and ordinary bakery wares	10 000	Notes 6 & 174	3	500	Note 6	Levels of use range from 0.1% to 0.6% (6000 mg/kg on the basis of the whole compound or 342 mg/kg on the basis of aluminium) and are necessary to prevent clumping and ensure flowability.

Table 5 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	Note 6	3	1500	Note 6	To ensure flowability and prevent clumping, levels of 20 000 mg/kg on the basis of the whole compound (or 1140 mg/kg on the basis of aluminium) are necessary.
12.2.2	Seasonings and condiments	30 000	Notes 6 & 174	3	2000	Note 6	Sodium aluminosilicate is required as an anticaking agent in seasonings to prevent clumping and improve flowability. Levels of 30 000 mg/kg on the basis of the whole compound (or 1710 mg/kg on the basis of aluminium) are necessary.
12.5.2	Mixes for soups and broths	10 000	Notes 6 & 174	3	1000	Note 6	Sodium aluminosilicate is required as an anticaking agent in these mixes to prevent clumping and improve flowability. Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are sufficient to carry out the intended function.
12.6.3	Mixes for sauces and gravies	10 000	Notes 6 & 174	3	1500	Note 6	Sodium aluminosilicate is required as an anticaking agent in these mixes to prevent clumping and improve flowability. Levels of 20 000 mg/kg on the basis of the whole compound (or 1140 mg/kg on the basis of aluminium) are sufficient to carry out the intended function.

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	120	Note 6	3	120	Note 6	Snacks frequently have seasoning mixtures applied to them to create new flavours of products. These seasonings must flow to properly adhere to the product. Sodium aluminosilicate is needed as an anticaking agent in these seasonings to prevent components from adhering to each other and then not adhering to the snack product. Necessary levels to achieve this function are 2000 mg/kg on the basis of the whole compound (or 114 mg/kg on the basis of aluminium).

[a] Note 6: As aluminium.
Note 174: Singly or in combination: sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).
[b] Step 3: Proposed draft.

USA, pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide are approved for use as a colour additive at levels up to 1.25% in cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and capsules, and chewing gum (USFDA, 2006). Potassium aluminium silicate–based pearlescent pigments are proposed to be used in confectionery, chewing gums and beverages at usage levels ranging from a minimum of 0.02% up to a maximum of 1.25% (Table 6).

3.3 Estimates of dietary exposure

3.3.1 Aluminium-containing food additives

In its previous evaluation (Annex 1, reference *186*), the Committee considered only consumer exposure to aluminium in the diet; occupational exposure and other routes or commodities were not considered. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact material and food additives. When dietary exposure was expressed on a kilogram body weight basis, a standard body weight of 60 kg for an adult was considered by the Committee, unless otherwise specified.

The Committee at its sixty-seventh meeting estimated mean exposure of the adult population from the overall diet, including additives, to range from 14 to 280 mg/week, expressed as aluminium.

In its conclusion, the Committee confirmed previous evaluations made by the Committee in which dietary exposure, particularly through foods containing aluminium compounds used as food additives, was found to represent the major route of aluminium exposure for the general population, excluding persons who regularly ingest aluminium-containing drugs.

(a) Screening by the budget method

The “budget method” is used to assess theoretical maximum daily dietary exposure. The budget method has been used as a screening method in assessing food additives by JECFA (FAO/WHO, 2001) and for assessments within the EU Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998).

The method relies on assumptions regarding 1) the level of consumption of foods and of non-milk beverages, 2) the level of presence of the substance in foods and in non-milk beverages and 3) the proportion of foods and of non-milk beverages that may contain the substance. More specifically:

- The levels of consumption of foods and beverages considered are maximum physiological levels of consumption—i.e. the daily consumption of 0.1 litre/kg bw of non-milk beverages and the daily consumption of 100 kcal/kg bw from foods (equivalent to 0.05 kg/kg bw based on an estimated energy density of 2 kcal/g). In a person with a body weight of 60 kg, these levels correspond to the daily consumption of 6 litres of non-milk beverages and 3 kg of food (FAO/WHO, 2009).

Table 6. Proposed use levels for potassium aluminium silicate–based pearlescent pigments

Food categories Products coloured with titanium dioxide / iron oxide pearl effect colours based on potassium aluminium silicate (mica) as carrier	Corresponding food categories from the Codex GSFA	Reported use levels
Liquorice Pearl colours applied only on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–1.0%
Panned products (including hard sugar, chocolate and soft panned products) Pearl colour is applied only on the product surface	5.1 Cocoa products and chocolate products including imitations and chocolate substitutes	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.3–1.0%
Vermicelli, hundreds & thousands, nonpareils Pearl colour is applied only on the product surface	5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	Minimum efficacious level: 0.2% Maximum level: 1.25% Standard usage level: 0.4–1.0%
Jelly gums (transparent gums) Pearl colour is added to the product mass	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Jelly gums (non-transparent gums) (i.e. wine gums) Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Marzipan Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Hard boiled candies/lollipops Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%

Table 6 (contd)

Food categories Products coloured with titanium dioxide / iron oxide pearl effect colours based on potassium aluminium silicate (mica) as carrier	Corresponding food categories from the Codex GSFA	Reported use levels
Pearl colour is added to the product mass		Minimum efficacious level: 0.04% Maximum level: 1.25% Standard usage level: 0.06–0.8%
Compressed sweets (including lozenges & pastilles) Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.4–1.0%
Frostings Pearl colour is added only to the frosting	5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	Levels refer to the final product on which the frosting or coating is applied: Minimum efficacious level: 0.4% Maximum level: 1.25% Standard usage level: 0.6–1.0%
Chewing gums Pearl colour is applied on the product surface	5.3 Chewing gum	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.3–1.0%
Beverages Beverages should be transparent in order to be suited for the application of pearl effect colours; they can be either carbonated or non-carbonated	14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	Minimum efficacious level: 0.02% Maximum level: 0.5% Standard usage level: 0.08–0.3%
Beverages Water-based beverages have to be transparent in order to be suited for the application of pearl effect colours; they can be either carbonated or non-carbonated	14.1.4 Water-based flavoured drinks, including “sport”, “energy” or “electrolyte” drinks and particulated drinks	Minimum efficacious level: 0.02% Maximum level: 0.5% Standard usage level: 0.08–0.3%

Table 7. Theoretical maximum daily exposure to aluminium-containing food additives included in the Codex General Standard for Food Additives and for which uses have been identified in the International Council of Grocery Manufacturer Associations submission

INS	Food additive name	Maximum concentration submitted by ICGMA (mg/kg)[a]	Theoretical maximum daily exposure to aluminium (mg/kg bw per day)
—	Aluminium lakes of colour	Solid food: 4000 Liquid food: 14	25
520	Aluminium sulfate	95	0.6
541(i)	Sodium aluminium phosphate acidic	1000	6.2
554	Sodium aluminosilicate	1500	9

[a] Maximum level of use expressed on the basis of aluminium.

- The level present in foods is assumed to be the highest maximum level of the additive reported in any representative category, respectively, for foods and for beverages for all aluminium-containing food additives with current GSFA provisions.
- The proportion of, respectively, solid foods and beverages that may contain the substance is set generally at 12.5% and 25%. For these compounds, there are no provisions for non-milk beverages; therefore, the overall theoretical maximum daily exposure to each aluminium-containing food additive is calculated from the potential exposure from solid foods only.

Table 7 summarizes the maximum concentrations submitted by ICGMA for aluminium-containing food additives in the GSFA that are taken into account in the budget method for calculating the theoretical maximum daily dietary exposure to these additives. As non-milk beverages were not proposed as a food use category in ICGMA submissions except for aluminium lakes of colour, only solid foods were taken into account in the budget method calculation. The maximum levels of use provided by ICGMA were expressed both for the whole compounds and on the basis of aluminium. For the purpose of the evaluation, which refers to aluminium exposures, solely the maximum levels of use expressed on the basis of aluminium were used.

The Committee noted that no actual usage data (GSFA provisions or data submissions) were submitted for aluminium ammonium sulfate (INS 523), sodium aluminium phosphate basic (541(ii)), aluminium silicate (INS 559), aluminium powder or aluminium potassium sulfate (INS 522). As no uses were reported in the GSFA provisions for some of these food additives and no actual use levels were submitted by ICGMA for the same additives, the Committee concluded that these compounds have no usages in food categories currently reported and did not perform budget method calculations for these compounds.

The Committee noted that for other compounds evaluated at this meeting for which actual use levels were defined by ICGMA, the theoretical maximum dietary exposure based on the budget method is greater than the PTWI of 1 mg/kg bw for all aluminium-containing food additives except aluminium sulfate. Detailed assessment of the dietary exposure was therefore needed for those aluminium-containing food additives with theoretical maximum dietary exposures higher than the PTWI when used in solid foods and beverages.

(b) Concentrations of aluminium in foods and beverages and estimated national dietary exposures

Since the last aluminium evaluation (Annex 1, reference *186*), the Committee has reviewed new publications or submissions from Australia (FSANZ, 2011), Brazil (Aparecida, 2009), China (Wu, 2011), China, Hong Kong SAR (Wong et al., 2010), Europe (EFSA, 2008), Japan (Aung, Yoshinaga & Takahashi, 2006), Spain (González-Weller et al., 2010), the United Kingdom (Rose et al., 2010) and the USA (Saiyed & Yokel, 2005).

(i) Australia

The Australian permissions for use of aluminium-containing food additives are set out in Standard 1.3.1 – Food Additives of the Australia New Zealand Food Standards Code. The forms of aluminium-containing food additives of interest that are permitted in the Code, generally at GMP levels within each specified Australia New Zealand Food Classification System food group, are sodium aluminium phosphate (INS 541), potassium aluminium silicate (INS 555), aluminium silicate (INS 559), calcium aluminium silicate (INS 556), aluminium lakes of colouring matters and aluminium (INS 173). There are currently no permissions in Standard 1.3.1 – Food Additives to use the following forms of aluminium-containing food additives: aluminium ammonium sulfate, aluminium potassium sulfate, aluminium powder, aluminium sulfate, sodium aluminium phosphate and aluminium lactate.

FSANZ submitted the results of a dietary exposure assessment for aluminium from naturally occurring and food additive sources. Concentration data in food as consumed were analysed as part of the twenty-third Australian Total Diet Study (to be published in 2011; FSANZ, 2011). The dietary exposure assessment used food consumption data from two Australian National Nutrition Surveys: the 2007 Australian Children's Nutrition and Physical Activity Survey for children aged 2–16 years, which included two 24-hour recalls for all respondents, and the 1995 National Nutrition Survey for those aged 17 years and above, which included one 24-hour recall for all respondents.

Estimated dietary exposures to aluminium at the mean and 90th percentile (mg/day) were provided for all populations using median contaminant concentrations. Highest concentration levels were found in cereal products (from 2.2 mg/kg in breakfast cereal to 41.2 mg/kg in bread up to 108 mg/kg in chocolate cake; fish (from 19 to 25 mg/kg); and meat products (from 1.3 mg/kg in chicken to 15.7 mg/kg in beef sausages).

The estimated daily dietary exposure to aluminium for adult consumers was 0.04 mg/kg bw at the mean and 0.07 mg/kg bw for high-level consumers (90th percentile). For children, estimated daily dietary exposure to aluminium ranged from 0.03 mg/kg bw in teenagers (13–16 years) to 0.07 mg/kg bw in toddlers (2–5 years) at the mean and from 0.06 mg/kg bw to 0.15 mg/kg bw for the same two age groups of high-level consumers, respectively.

The major foods contributing to overall dietary exposure were tea for adults (35%); cakes, muffins and puddings (23% in adults to 38% in young children); white, multigrain, wholemeal and rye breads (13% in toddlers); and sausages and frankfurters (7% in toddlers).

(ii) Brazil

Aluminium-containing food additives authorized in the Mercosur harmonized list include sodium aluminium sulfate (INS 521), potassium aluminium sulfate (INS 522), aluminium ammonium sulfate (INS 523), sodium aluminium phosphate acidic (INS 541(i)), sodium aluminium phosphate basic (INS 541(ii)), sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).

Among 1081 products investigated in the Aparecida (2009) study, only 2.8% presented aluminium salts on their labels. Among identified additives, sodium aluminosilicate (INS 554) and sodium aluminium phosphate acidic (INS 541(i)) were declared as anticaking and leavening agents, respectively.

The contribution of food additives as a source of aluminium in the Brazilian diet was estimated based on the consumption of foods that may contain aluminium salts combined with the maximum permitted levels of these additives. When appropriate, information provided by the industry as well as provisions under discussion in the Mercosur were used in the calculations. Consumption data were inferred from a household economic survey or taken from the Nutrition Facts label. Products for which aluminium-containing food additives are allowed were first identified from regulations in force, and then the list of ingredients used in each product was checked at the web site of a supermarket. The exposure to these additives from the consumption of condiments, seasonings, salt and mixes for soup, cereal products, bakery products and cookies and the exposure to aluminium lakes of colour from confectioneries (pastilles) were estimated using the theoretical maximum intake approach. The dietary exposures were calculated for both adults and children.

Estimated exposures to aluminium from sodium aluminosilicate corresponded to 47% and 95% of the PTWI of 1 mg/kg bw established by JECFA for aluminium from all sources for adults and children, respectively. In these estimates, table salt was the main contributor to the exposure to aluminium (20.8 mg/week).

Regarding sodium aluminium phosphate acidic, the exposure of children to aluminium corresponded to 1.38 mg/kg bw per week, with bread contributing 50% of this exposure (24.1 mg/week). For adults, exposures up to 2.9 mg/kg bw per week were observed in regions where the consumption of flour is high. Among all foods analysed in this study, cake was identified as the major potential source of exposure of children to aluminium (up to 15.7 mg/kg bw per week), followed by

confectioneries, which can contribute up to 1.8 mg of aluminium per kilogram of body weight per week. Although these results may be overestimates, owing to the conservative approach undertaken, it should be emphasized that the estimates did not take account of other dietary sources of aluminium, such as drinking-water, natural occurrence and migration from food contact materials (e.g. containers, cookware, utensils and packaging).

The authors recommended that regulatory agencies adopt measures to reduce the exposure of the population to aluminium, including the revision of the present legislation towards reducing or banning permitted use levels of aluminium salts. To allow a more realistic exposure estimate, food industries should provide data on the actual use levels of aluminium-containing additives while seeking alternative additives to replace them.

(iii) China

China submitted levels of aluminium in foods using the fourth (2007) Chinese Total Diet Study samples (Wu, 2011). Aluminium concentrations were obtained for 144 composite diet samples collected in 2007 from 12 provinces in China. Samples were analysed using the inductively coupled plasma mass spectrometry (ICP-MS) technique. The LOD was 2.0 µg/kg. The highest contributing food groups together with the mean aluminium concentrations in those groups were aquatic foods from Fujian (42.7 mg/kg), cereals from Hebei (39.0 mg/kg), cereals from Heilongjiang (27.6 µg/kg) and potatoes from Liaoning (26.9 µg/kg). The highest contributing food items together with the mean aluminium concentrations in those items were fritters from Heilongjiang (1242 mg/kg), Hebei (1025 mg/kg) and Liaoning (956 mg/kg), jiang doufu from Hebei (2423 mg/kg), starch noodles from Hebei (681 mg/kg), steamed bread from Guangxi (332 mg/kg) and Shanghai (208 mg/kg), wheat noodles from Hubei (301 mg/kg) and cake from Sichuan (260 mg/kg).

China submitted estimates of dietary exposure to aluminium based on the results of the 2007 Chinese Total Diet Study (Wu, 2011). Aluminium concentrations of 144 food composites from 665 food samples prepared as consumed were used in the calculations. Dietary exposure calculations were performed using a deterministic method, combining mean aluminium concentrations from the food group composites with their associated food consumption. Concentration values reported below the reporting limits were assigned a concentration equal to one half the LOD. In 2007, the fourth Chinese Total Diet Study included 4320 persons and covered four baskets from 12 provinces, municipalities and autonomous regions in mainland China. The average exposure estimate for the whole population was 13.0 mg/day, with a range between 3 and 19 mg/person per day. Main food contributors to total exposure were cereals (72.7%), vegetables (9.8%) and potatoes (5%). The average aluminium exposure by age group in China ranged between 2.7 mg/kg bw per week for those older than 65 years and 5 mg/kg bw per week for children 2–7 years of age. The 90th percentile aluminium dietary exposure ranged between 4.4 and 10 mg/kg bw per week for the same population groups, respectively.

In China, aluminium-containing food additives are allowed to be used in the process of making wheat and starch products, which makes this the main contributor to the overall exposure. The purpose of adding the aluminium-containing

food additives while making cereal products is to give the final products a bulky appearance and soft texture. With respect to the estimated data from the Chinese Total Diet Study, only about 10% of residents who loved eating fritters or starch noodles might have a risk of higher aluminium exposure.

(iv) China, Hong Kong Special Administrative Region

An assessment of dietary exposure to aluminium for the population of Hong Kong SAR was published by Wong et al. (2010). In this study, 256 individual food samples were collected from various locations in Hong Kong SAR for aluminium testing. Basically, for packaged products, only food items labelled with aluminium-containing food additives in the ingredient list were selected for the testing of aluminium, given that most of food samples were analysed in ready-to-eat form.

High aluminium levels were found in steamed bread/buns/cakes (mean: 100–320 mg/kg), some bakery products, such as muffins, pancakes/waffles, coconut tarts and cakes (mean: 250, 160, 120 and 91 mg/kg, respectively) and jellyfish (ready-to-eat form) (mean: 1200 mg/kg). The authors concluded that results demonstrated that aluminium-containing food additives have been widely used in these food products.

Estimates of dietary exposure were made using the average consumption data of the corresponding food type analysed from the Hong Kong SAR adult dietary survey of 1995. The average dietary exposure to aluminium from packaged food consumption products reported in the national dietary survey was estimated to be 0.60 mg/kg bw per week for a 60 kg adult, corresponding to 60% of the PTWI of 1 mg/kg bw.

The main dietary food additive source was “steamed bread/buns/cakes”, which contributed 0.4 mg/kg bw per week to the total exposure, followed by “bakery products” and “jellyfish”, with 0.1 mg/kg bw per week, respectively.

The authors noted that the estimation did not include the exposure to aluminium from natural food sources, food contact materials or other sources (e.g. drinking-water) and indicated that even if aluminium is unlikely to cause adverse health effects for the general population, the risk to some populations who regularly consume foods with aluminium-containing food additives cannot be excluded.

(v) Europe

In its scientific opinion on the safety of aluminium from dietary exposure, EFSA (2008) concluded that most unprocessed foods typically contain less than 5 mg of aluminium per kilogram. Higher concentrations (mean levels 5–10 mg/kg) were often found in breads, cakes and pastries (with biscuits having the highest levels), some vegetables (with mushrooms, spinach, radishes, swiss chard, lettuce and corn salad having the highest levels), glacé fruits, dairy products, sausages, offal, shellfish, sugar-rich foods, baking mixes and a majority of farinaceous products and flours. Foods with very high mean concentrations included tea leaves, herbs, cocoa and cocoa products, and spices. No analytical studies in Europe have focused on the aluminium content of food that contains permitted aluminium-containing food additives.

Total dietary exposure to aluminium from all sources has been estimated from duplicate diet studies (Germany, Hungary, Italy, the Netherlands and Sweden) and market basket and total diet studies (Finland, France and the United Kingdom). Mean dietary exposure from water and food in non-occupationally exposed adults showed large variations between the different countries and, within a country, between different surveys. It ranged from 1.6 to 13 mg of aluminium per day, corresponding to 0.2–1.5 mg/kg bw per week in a 60 kg adult. Children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the group with the highest potential exposure to aluminium per kilogram body weight. Large individual variations in dietary exposure to aluminium can occur. In young people, the potential estimated exposure at the 97.5th percentile ranged from 0.7 mg/kg bw per week for children aged 3–15 years in France to 2.3 mg/kg bw per week for toddlers (1.5–4.5 years) and 1.7 mg/kg bw per week for those aged 4–18 years in the United Kingdom. The main contributors to overall mean dietary exposure were cereals and cereal products (50% in the United Kingdom and 20% in France), vegetables (20% in France) and beverages (30% in the United Kingdom).

Because of the design of the human dietary studies and the analytical methods used, which determine only the total aluminium content in food (from duplicate diet studies or total diet studies), and not the individual aluminium compounds or species present, it is not possible to conclude on the specific sources contributing to the aluminium content of a particular food, such as the amount inherently present, the contributions from use of food additives and the amounts released to the food during processing and storage from aluminium-containing foils, containers or utensils. Therefore, these contributions may also partly reflect the use of aluminium-containing food additives that are permitted for use (e.g. in some bakery products and aluminium from food colours used as aluminium lakes). Thus, a detailed breakdown by exposure source was not possible in the EFSA evaluation, but the panel nevertheless recognized that the major route of exposure to aluminium for the general population was through food.

(vi) Japan

The duplicate diet samples published by Aung, Yoshinaga & Takahashi (2006), including drinking-water, snacks and beverages, were collected from 33 households in November and December 2000 on 7 consecutive days. Nineteen out of 33 households were located in the city centre; the remaining households were in the suburb regions of Tokyo Metropolitan Area.

Exposures to aluminium were calculated for the individual subjects by multiplication of the weights of foods by the concentrations of aluminium in the samples and expressed in milligrams per kilogram of fresh material. The daily exposure ranges were calculated by averaging 7-day duplicate diet composites. The weekly average exposures to aluminium were estimated to be 2.85 mg/kg bw in children 3–6 years of age and 1.37 mg/kg bw in adults 28–40 years of age.

(vii) Spain

A dietary exposure assessment for aluminium in a Spanish population (Canary Islands) was published by González-Weller et al. (2010). The aim of

this study was to analyse the aluminium content in foods and beverages most commonly consumed by the population of the Canary Islands and to determine the dietary exposure to this metal throughout the Canary Islands as a whole and in each of the seven islands (Gran Canaria, Lanzarote, Fuerteventura, Tenerife, La Palma, La Gomera and El Hierro). Four hundred and forty samples collected over 28 months (2006–2008) in different shopping malls and representing the foods most commonly bought and consumed by the population of the Canary Islands were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). The highest average aluminium contents were found in vegetables (squash, carrots, bubang, cabbage, watercress, spinach: 27.5 mg/kg for the whole group), fruits (bananas: 32.8 mg/kg; peaches, pears, plums: 9.7 mg/kg), sweet cakes (14.2 mg/kg), viscera (11.2 mg/kg), red meat (9.3 mg/kg), poultry and rabbit (6.4 mg/kg), potatoes (5.9 mg/kg) and pastries (muffins, croissants, doughnuts, other bakery products: 5.7 mg/kg). Other food groups were generally found to contain aluminium at levels below 5 mg/kg.

The estimated average total exposure to aluminium for the population of the Canary Islands was 10.2 mg/day. In all of the islands, fruits and vegetables were found to be the food groups that contributed the most to the total average dietary exposure to aluminium.

(viii) United Kingdom

An assessment of dietary exposure to metals and other elements in the 2006 United Kingdom Total Diet Study and some trends over the last 30 years was published by Rose et al. (2010). The foods making up the 20 groups were bought from retail outlets in 24 randomly selected towns throughout the United Kingdom. The food samples were prepared and cooked according to normal consumer practices. Equal quantities of samples from each town were mixed for each food group to obtain the national composite samples. These composite samples for each food group were homogenized and supplied frozen at –20 °C for laboratory analysis.

Consumption data from the 1999 and 2002 British National Diet and Nutrition Survey were used to estimate dietary exposures for individuals in the general population who eat average amounts of each food group (i.e. consumers with mean consumption) and those who eat significantly more than average amounts (i.e. consumers with high-level, 97.5th percentile consumption). Total consumer dietary exposures are derived from an average of the individual consumer's exposure patterns with regard to individual foods.

In the 20 food groups of the 2006 Total Diet Study samples, most groups had aluminium concentrations lower than or similar to those reported in the 2000 Total Diet Study, the exceptions being bread, meat products and other vegetable groups. The miscellaneous cereals group had the highest concentration of aluminium (17.5 mg/kg, range from 4.8 to 78 mg/kg).

The population dietary exposure to aluminium was 5.4 mg/day, which was higher than the estimates from the 2000 Total Diet Study and the 1997 Total Diet Study (4.7 and 3.4 mg/day, respectively).

The estimated daily dietary exposure to aluminium was 0.07 mg/kg bw for adult consumers at the mean and 0.14 mg/kg bw for high-level consumers (97.5th percentile). For toddlers (1.5–4.5 years old) and young people (4–18 years old), estimated daily dietary exposure to aluminium was 0.19 mg/kg bw and 0.12 mg/kg bw at the mean and 0.35 mg/kg bw and 0.25 mg/kg bw for high-level consumers (97.5th percentile), respectively. The authors noted that estimates of high-level dietary exposure of toddlers, young people, the elderly and vegetarians exceeded the PTWI of 1 mg/kg bw by up to 2.4-fold. The major foods contributing to overall dietary exposure of the total population were miscellaneous cereals (flour, buns, cakes, pastries, chocolate biscuits and other biscuits, 42%), beverages (tea, instant coffee, cocoa, concentrated and ready-to-drink soft drinks, 34%) and bread (white, wholemeal, brown, 7%).

(ix) United States of America

A survey of the aluminium content of some foods and food products containing aluminium-containing food additives in the USA was published by Saiyed et al. (2005). The primary objective was to focus on processed food products in the USA and to determine the aluminium content of the selected foods that contain aluminium as an approved food additive.

Approximately 95 single-sample purchases were made locally during 2003–2004. The products purchased were nationally available brands and house brands that are presumed to be available nationally. They were selected to be representative of products that did, or did not, have added aluminium, according to the Nutrition Facts label. The purchase locations were varied, but as all the products were national brands or house brands of national chains, it was assumed that the products would be the same if purchased elsewhere. Some were known to contain aluminium from their list of ingredients. The aluminium-contributing ingredients, according to the products' Nutrition Facts labels, were sodium aluminium phosphate (INS 541) and sodium aluminosilicate (INS 554).

Frozen pizza that listed sodium aluminium phosphate as an additive generally had about 200–750 mg of aluminium per kilogram cheese. The cheese from frozen pizza products that did not list aluminium as an additive and ready-to-eat pizza had only a few milligrams of aluminium per kilogram, similar to a natural cheese, which contains about 0.5–3 mg of aluminium per kilogram.

The crust (grain product/bread) of frozen pizza that did not list aluminium as a food additive and of ready-to-eat pizza contained about 12 mg aluminium per kilogram, whereas a frozen pizza listing aluminium as an additive (sodium aluminium phosphate) in the crust had about 200 mg aluminium per kilogram. The high concentration of aluminium in the crust of the pizza containing aluminium as an additive is consistent with baked goods representing the largest source of aluminium in the typical diet.

The authors noted that the finding of considerable amounts of added aluminium in grain-based foods, particularly those containing self-rising flour, was in agreement with other publications. The very high aluminium concentration in baking powder is also consistent with previous reports of 20 000–34 000 mg aluminium

per kilogram. Considerable aluminium concentrations were found in many pancake and waffle mixes, frozen products and ready-to-eat products, up to 1200, 600 and 1200 mg/kg, respectively.

Exposure to aluminium from the labelled serving size of each food product was calculated. Food product aluminium content ranged from less than 1 to 27 000 mg/kg. Cheese in a serving of frozen pizzas had up to 14 mg of aluminium, from sodium aluminium phosphate basic, whereas the same amount of cheese in a ready-to-eat restaurant pizza provided 0.03–0.09 mg. Many single-serving packets of non-dairy creamer had 50–600 mg aluminium per kilogram as sodium aluminosilicate, providing up to 1.5 mg aluminium per serving. Many single-serving packets of salt also had sodium aluminosilicate as an additive, but the aluminium content was less than in single-serving non-dairy creamer packets. Sodium aluminium phosphate acidic was present in many food products, pancakes and waffles. Baking powder, some pancake and waffle mixes, some frozen products and ready-to-eat pancakes provided the most aluminium of the foods tested, up to 180 mg/serving.

The authors concluded that many products provide a significant amount of aluminium compared with the typical exposure of 3–12 mg/day reported from dietary aluminium studies conducted in many countries.

(x) Summary of national estimates of exposure

For aluminium-containing food additives under re-evaluation, a tentative estimate of dietary exposure from food additive sources has been made, taking into account previous assessments and other publications or submissions reviewed by the Committee at the current meeting. The Committee noted, from the report of its sixty-seventh meeting and from an EFSA scientific opinion, that the range of estimates was mainly based on dietary exposure calculated with the total diet study method, which takes into account water consumption. It is known from the literature that the main sources of migration of aluminium into food are from the use of cookware or aluminium utensils. It is also known that the design of total diet studies generally tries to control any bias of additional contamination that may result from the use of containers, cookware or utensils containing aluminium during the preparation and storage of food as consumed.

The Committee noted that estimates of the contribution to overall mean dietary exposure from all sources (including natural sources, water consumption, food contact materials and food additives) were in the range of 10–140 mg/week in adult populations (0.2–2.3 mg/kg bw per week as aluminium, assuming a body weight of 60 kg; Table 8). Major contributors to these estimates were cereals and cereal-based food products, with a proportion of 20–90%, depending on the country, equivalent to an exposure of approximately 2–120 mg/week (0.03–2 mg/kg bw per week as aluminium, assuming a body weight of 60 kg).

This assessment is consistent with previous evaluations made by the Committee in which cereal products were considered as potentially high contributors to dietary aluminium exposure. The Committee also noted from its review that high levels of the actual uses of aluminium-containing food additives were reported for cereals and cereal-based products, in particular for sodium aluminosilicate (INS 554)

Table 8. Estimated ranges of mean exposure of the adult population to aluminium from different dietary sources

Country/region	Estimated mean exposure (mg/person per week)	
	From food additives used in cereals and cereal-based products	From overall diet, including natural sources, water consumption, food contact materials and food additives
JECFA[a]	—	14–280
JECFA[b]	2–124	11–136
Australia	4	17
Brazil	40–70	—
China	4–124	23–136
China, Hong Kong SAR	30	36
Europe (EFSA)	2–46	11–91
Japan	—	84
USA	24–30	60

[a] Estimated ranges from the sixty-seventh meeting of the Committee (Annex 1, reference *186*).
[b] Estimated ranges from the data reviewed at this meeting.

and sodium aluminium phosphate acidic (INS 541(i)). Based on this, the Committee concluded that aluminium from the consumption of cereals and cereal-based products could reasonably be assumed to be mainly from food additive sources.

The Committee noted that the estimated dietary exposures related to average adult populations and that high dietary exposures (e.g. 90th or 95th percentile) are generally assumed to be 2 times higher than the reported average. It also noted that children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the highest potential exposure to aluminium per kilogram of body weight.

(c) International estimates of dietary exposure

The Committee considered it inappropriate to use the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets to calculate international estimates of dietary exposure because the aluminium-containing food additives are present mainly in prepared foods and not in staple foods.

3.3.2 Potassium aluminium silicate

(a) Annual poundage of the additive introduced into the food supply

The sponsor indicated that annual poundage data for the worldwide use of potassium aluminium silicate–based pearlescent pigments are not available. The

Table 9. Theoretical maximum daily exposure to potassium aluminium silicate–based pearlescent pigments for which uses have been identified by the sponsor

Food additive name	Food type	Concentration range submitted by sponsor (mg/kg)			Ranges of theoretical maximum daily exposure (mg/kg bw per day)		
		Minimum	Standard	Maximum	Minimum	Standard	Maximum
Potassium aluminium silicate	Solid food	1 000	10 000	12 500	6	60	78
	Liquid food	200	3 000	5 000	10	150	250
	Total	—	—	—	16	210	328

sponsor stated that it sells Candurin® pigments worldwide to food companies and has no information on sales volumes in different countries.

The usage of potassium aluminium silicate–based pearlescent pigments in food products in many countries is directly connected to the regulatory status of the food colours titanium dioxide and iron oxide. Furthermore, the pigments cannot be used in all foods, as there are certain technical restrictions regarding the application of the pigments within the group of permitted food products (e.g. stability and transparency). Based on these factors, potassium aluminium silicate–based pearlescent pigments represent a small portion of the global food colour market (estimated by the sponsor to be less than 0.2% in 2009).

(b) Screening by the budget method

As noted above in section 3.3.1(a), the budget method is used to assess theoretical maximum daily dietary exposure. The method relies on assumptions regarding 1) the level of consumption of foods and of non-milk beverages, 2) the use level of the substance in foods and in non-milk beverages and 3) the proportion of foods and of non-milk beverages that may contain the substance. For a person with a body weight of 60 kg, the levels of consumption are assumed to be 6 litres of non-milk beverages and 3 kg of food per day. The level of the additive used in foods is assumed to be the highest maximum level of the additive reported in any representative category, respectively, for foods and for beverages for which usage data were provided. The proportion of solid foods and beverages that may contain the substance is, respectively, 12.5% and 50%.

Table 9 summarizes the theoretical maximum daily exposure to potassium aluminium silicate–based pearlescent pigments for which uses have been identified by the sponsor. Three scenarios were considered by the Committee, based on the minimum efficacious level, the standard usage level and the maximum level reported by the sponsor for the products in which potassium aluminium silicate–based pearlescent pigments may be used.

The Committee noted that when potassium aluminium silicate–based pearlescent pigments are used in solid and liquid foods, the theoretical maximum

daily exposure based on the budget method would give rounded estimates ranging from 20 mg/kg bw per day (when using the reported minimum efficacious use level) up to 330 mg/kg bw per day (when using the maximum proposed use level).

(c) National estimates of dietary exposure

(i) Europe

According to Directive 95/2/EC as amended by Directive 2003/114/EC (see Attachment 01 for a consolidated version of Directive 95/2/EC including the amendments according to Directive 2003/114/EC), potassium aluminium silicate is allowed for use as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment). Regulation (EC) 1333/2008 (Attachment 02) repeals Directive 95/2/EC. However, according to Articles 30 and 34, this directive will remain in force during a transitional period until the European Community list of food additives has been established. The food additives will be entered in the relevant Annexes of Regulation (EC) 1333/2008 after a review of their compliance with Article 6.

Exposure estimates for European consumers have been made using the uses in food of potassium aluminium silicate–based pearlescent pigments at maximum use levels of the resulting pigments of 0.5% in beverages up to 1.25% by weight in solid foods proposed by the sponsor with the use of summary statistics (average all population and 95th percentile consumers only) from available EU food consumption data and following the rules of calculation defined by the EFSA Panel on Food Additives and Nutrient Sources Added to Food.

For children (aged 1–14 years, weighing 16–54 kg), anticipated exposures have been estimated by the Committee, based on summary statistics (average all population and 95th percentile consumers only) from detailed individual food consumption data from 11 European countries (Belgium, Cyprus, Czech Republic, Finland, France, Germany, Greece, Italy, the Netherlands, Spain and Sweden) provided by the EXPOCHI ("Individual food consumption data and exposure assessment studies for children") consortium (Huybrechts et al., 2011). As the United Kingdom is not part of the EXPOCHI consortium, estimates for children (aged 1.5–4.5 years) in the United Kingdom were made with the use of detailed individual food consumption data (United Kingdom National Diet and Nutrition Survey, 1992–1993) available from the Union of European Soft Drinks Associations (UNESDA) report (Tennant, 2008).

As the United Kingdom population is considered to be one of the highest consumers of soft drinks in Europe, it was decided to select the United Kingdom population as representative of EU consumers for potassium aluminium silicate exposure estimates for adults from an earlier report provided by UNESDA (Tennant, 2008).

For children, the data from the EXPOCHI countries and the United Kingdom data were used to calculate the mean and high-level exposures to potassium aluminium silicate–based pearlescent pigments using proposed maximum use levels. High-level exposure (95th percentile consumers only) was based on the assumption that an individual might be a high-level consumer of one food category

and an average consumer of the others. This approach has been tested several times by the EFSA Panel on Food Additives and Nutrient Sources Added to Food in each re-evaluation of food colours, and the results were in agreement with the exposure figures obtained by computer analysis using raw individual food consumption data. Therefore, this approach was preferred for the calculations based on the maximum proposed use levels in order to avoid excessively conservative estimates.

When considering the proposed maximum use levels, the dietary exposure to potassium aluminium silicate–based pearlescent pigments in European children ranged from 10 to 116 mg/kg bw per day at the mean and from 40 to 323 mg/kg bw per day at the 95th/97.5th percentile. The main contributors to the total anticipated exposure to potassium aluminium silicate–based pearlescent pigments (>10% in all countries) were non-alcoholic flavoured drinks (20–70%) and fine bakery wares (13–79%). Confectionery accounted for more than 10% of exposure in two European countries (1–20%).

For the United Kingdom adult population, the mean estimated dietary exposure to potassium aluminium silicate–based pearlescent pigments was 28 mg/kg bw per day, and the estimated dietary exposure for high-level consumers (97.5th percentile) was 86 mg/kg bw per day. The main contributors to the total anticipated exposure to potassium aluminium silicate–based pearlescent pigments (>10%) were non-alcoholic flavoured drinks (74%) and fine bakery wares (16%).

(ii) United States of America

The United States Food and Drug Administration (USFDA) performed an estimate of daily exposure to potassium aluminium silicate–based pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide for consumers aged 2 years or more and children 2–5 years of age (USDA, 2006b). This estimate incorporated the maximum permitted use level of the potassium aluminium silicate–based pearlescent pigment in food of 1.25% by weight. The estimate included the categories of food for which titanium-containing potassium aluminium silicate–based pearlescent pigments are permitted for use as colour additives in the USA: cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and gelatine capsules, and chewing gum. The Committee noted that beverages were not included as a proposed use by the sponsor when the USFDA made its evaluation.

Estimates of dietary exposure for consumers aged 2 years or more were 0.43 g/person per day at the mean and 0.86 g/person per day at the 90th percentile. Estimates of dietary exposure for children 2–5 years of age were 0.38 g/person per day at the mean up to 0.76 g/person per day at the 90th percentile (USDA, 2006b). In these estimates, gelatine candies and desserts prepared from gelatine powders and breakfast cereals were the major sources of the dietary exposures to the pigments (USFDA, 2005).

(iii) Conclusion

The Committee concluded that anticipated dietary exposure in the general population from the use of potassium aluminium silicate–based pearlescent

Table 10. Summary of anticipated exposure to potassium aluminium silicate–based pearlescent pigments in children and adult populations

	Exposure (mg/kg bw per day)			
	All (2+ years)* (USA)[a]	Children (2–5 years)* (USA)[a]	Adult (18+ years)*** (EU)	Children (1–14 years)**, *** (EU)
Mean exposure[b]	35	135	28	10–116
Exposure at the 90th*, 95th** or 97.5th*** percentile[b]	70	270	86	40–323

[a] For the USA population, estimates are based on assuming a 60 kg body weight for the general population and a 15 kg body weight for toddlers. At the time of the USFDA evaluation, beverages were not included in those estimates; the Committee provided an estimate for the USA population taking into account an average consumption of beverages of 330 ml (one can) per day.

[b] Assuming all processed foods and beverages contain colour added at maximum proposed use levels. Maximum use level in products coloured with titanium dioxide/iron dioxide pearl effect colours based on potassium aluminium silicate as the carrier of 0.5% in beverages up to 1.25% by weight in solid food.

pigments at the maximum proposed use levels (0.5% in beverages and 1.25% by weight in solid food) would range from 10 mg/kg bw per day at the mean to 323 mg/kg bw per day for consumers with a high dietary exposure. The Committee noted that in these conservative estimates, non-alcoholic flavoured drinks are the major contributor, from 20% up to 70%, to overall dietary exposure.

The estimates presented in Table 10 are for exposure to the potassium aluminium silicate–based pearlescent pigments themselves. In order to consider the contribution of the potassium aluminium silicate–based pearlescent pigments to aluminium exposure, the exposure values must first be converted to an aluminium basis. Taking into account a maximum level of 90% potassium aluminium silicate in the potassium aluminium silicate–based pearlescent pigments and the fact that potassium aluminium silicate is composed of 20% aluminium by weight (potassium aluminium silicate, $KAl_2[AlSi_3O_{10}](OH)_2$; relative molecular mass of 398), this corresponds to an aluminium exposure from potassium aluminium silicate–based pearlescent pigments of 1.8 mg/kg bw per day up to 58 mg/kg bw per day.

The Committee recognizes that its estimates could be considered as being conservative, as it is assumed that all processed foods and beverages contain the colour added at the maximum proposed use levels.

(d) International estimates of dietary exposure

The Committee considered it inappropriate to use the GEMS/Food consumption cluster diets to calculate international estimates of dietary exposure because potassium aluminium silicate is present mainly in prepared foods and not in staple foods.

4. COMMENTS

4.1 Toxicological data

As recommended by the Committee at its sixty-seventh meeting, new studies had been conducted on the bioavailability of aluminium compounds. The new data indicated that absorption of aluminium following the ingestion of various aluminium compounds by rats is generally in the region of 0.01–0.3% and support the assumption that the more water-soluble aluminium compounds are generally more bioavailable. As a result of limitations in the sensitivity of the analytical methods, inter-animal variation and methodological differences between studies, including the administered doses, it is not possible to draw firm conclusions on quantitative differences in absorption between different compounds. There are indications that there are sex differences in absorption in rats and that the proportion of the dose absorbed is lower following repeated administration than following single administration. The reported absorptions of the food additives for which data were available (sodium aluminium phosphate acidic, sodium aluminium phosphate basic, sodium aluminosilicate, aluminium sulfate, FD&C aluminium lake, aluminium metal, aluminium ammonium sulfate) are within the overall range of 0.01–0.3% in rats. A possible exception relates to potassium aluminium silicate–based pearlescent pigments. These products are marketed in particulate form. The solubility of the particulates is very low, and therefore it is likely that the bioavailability is lower than for other aluminium-containing food additives. However, direct data to support a conclusion that aluminium is appreciably less available from these pigments than from other aluminium compounds were not available.

In studies reviewed previously by the Committee, absorption of aluminium in human volunteers was within the same range as that in rats, with some indication of increased absorption in the elderly. The absorption can be modified by substances in foods that bind to the aluminium ion, such as citrate, which increases absorption, and phosphate, which forms an insoluble aluminium salt, thereby decreasing absorption. The newly available data indicate that absorption in humans is likely to vary widely, but did not support an estimation of bioavailability.

New studies in rats have confirmed that absorbed aluminium is able to cross the placental barrier into the fetus and then into the fetal brain and that it is also transferred to the offspring via lactation. The new studies have also confirmed that administration of a number of aluminium salts to rats can result in increased concentrations of aluminium in bone, kidney and spinal cord. About 90% of Al^{3+} in plasma is bound to transferrin, and about 10% to citrate. Cellular uptake is thought to occur from the aluminium bound to transferrin by transferrin receptor–mediated endocytosis.

No new data on excretion were identified. Studies reviewed previously by the Committee have shown that urine is the primary route of excretion of absorbed aluminium in experimental animals and in humans. Initial half-lives of 2–5 hours have been reported in rats, mice, rabbits and dogs after intravenous administration and less than 1 day in humans after intravenous administration. In different studies and species, multiple half-lives have been reported, arising from slower rates of elimination from different tissues.

Based on the available data relating to the absorption, distribution and elimination of aluminium from a variety of different aluminium compounds, the Committee concluded that there was no basis for deriving a chemical-specific adjustment factor for either interspecies or intraspecies differences in toxicokinetics.

As recommended by the Committee at its sixty-seventh meeting, new multigeneration reproductive and developmental toxicity studies incorporating neurobehavioural end-points had been conducted.

The multigeneration reproductive studies conducted with aluminium sulfate and aluminium ammonium sulfate administered to rats in the drinking-water did not provide evidence of reproductive toxicity. The major developmental effects observed in both studies were delayed maturation of the female offspring, decreased body weight gain and changes in some organ weights. These effects are likely to have been related to the reported decrease in maternal fluid and feed consumption. Thus, it is not possible to attribute the findings to a direct effect of the aluminium. No effects on motor activity or learning ability were observed in these studies.

The available developmental toxicity studies include two published studies involving dosing of aluminium chloride by oral gavage to pregnant rats. These studies provided evidence of fetotoxicity, but it was unclear if the findings were secondary to maternal toxicity. There were no effects on pregnancy outcome in a developmental study of aluminium chloride basic.

Cognitive deficits were observed in a number of new studies of neurotoxicity and neurobehavioural end-points. Most of these studies have limitations for use in risk assessment, such as administration of only one high dose level, failure to consider aluminium content in the diet, lack of assessment of other forms of toxicity and assessment of only a limited number of outcomes. The lowest aluminium dose linked with cognitive effects was 0.5 mg/kg bw per day administered to rats as aluminium chloride in the drinking-water, which was reported to be associated with impaired memory in old rats. In this study, the rats were given a restricted amount of feed twice weekly in order to reduce the rats' weight to approximately 85% of the free-feeding weight and hence prolong their lifespan. Typically, they ate the feed in the first 2–3 days and had a day or more with no feed. Whereas impaired cognitive function in old age is a potentially relevant observation, the impact of the restricted feeding regimen used in this study is unknown, and impaired cognitive function has been observed in other studies only at much higher levels of exposure, albeit in younger animals. The Committee therefore concluded that the results of this study require independent verification and were not suitable for use in the risk assessment.

In a developmental and chronic neurotoxicity study of aluminium citrate administered to rats in drinking-water, the major treatment-related effects were renal damage (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi) and reduced grip strength, but not cognitive impairment, in the pups. Renal damage was not observed in a control group of rats given sodium citrate at the molar equivalent of the high-dose aluminium citrate, demonstrating that the effect was not due to the citrate ion. Dosing with both aluminium citrate and sodium citrate resulted in a significant increase in fluid consumption compared with control

animals. The NOAEL and LOAEL for these effects were at target aluminium doses of 30 and 100 mg/kg bw per day. However, because the aluminium citrate was administered in the drinking-water, the actual dose was influenced by the water consumption, which varied in the different stages of the study. Mean doses at the NOAEL were 10–14% below target during gestation, up to 50% above target during lactation, up to about 30% above target in the weaned pups for the first few weeks, but then 15–45% of target for the remainder of the study. At the LOAEL, the mean dosage level was approximately at target during gestation, up to 90% above target during lactation and the first few weeks post-weaning, and then 25–50% of target for the remainder of the study. Hence, if the effects in the pups were mediated in utero, the NOAEL is slightly overestimated; conversely, however, if the effects were mediated during lactation or the first few weeks after weaning, the NOAEL is underestimated. As the effect on grip strength was more pronounced in younger animals, exposure in utero and/or during lactation is likely to be more important than exposure during the later stages, when exposure was decreased due to decreased fluid consumption. The Committee concluded that, taking into account the greater bioavailability of aluminium from aluminium citrate than from other aluminium compounds, it was appropriate to assume that the NOAEL was 30 mg/kg bw per day. In view of the uncertainty regarding the doses at different times of this study as a result of changes in water consumption, the Committee decided not to model the dose–response data.

The Committee received a submission specifically on potassium aluminium silicate–based pearlescent pigments. No effects were observed in subchronic or chronic toxicity studies at doses of the test material up to 2500 mg/kg bw per day, equivalent to 360 mg/kg bw per day as aluminium, but no studies were available regarding reproductive or neurobehavioural effects.

Most epidemiological studies reviewed addressed the potential neurotoxicity of aluminium in drinking-water or antacids, by means of different designs: experimental, prospective cohort or case–control studies or ecological studies. The results of these studies were controversial; some of the drinking-water studies showed an association of aluminium with dementia or Alzheimer disease, whereas others reported an absence of neuropsychological effects measured in several ways. None of these studies took into account the ingestion of aluminium in food. The coincidental observation of neuropathological features of Alzheimer disease and aluminium in brain reported in some cases does not demonstrate a causal role of aluminium in Alzheimer disease. Occupational exposure to aluminium does not seem to have an impact on cognitive performance, motor performance or adverse reproductive outcomes in exposed workers. Although recent studies do not definitively rule out a positive association between aluminium in drinking-water and Alzheimer disease, the information available remains inconsistent and does not support a causal association. Neonates who were exposed to aluminium from solutions for parenteral nutrition had reduced lumbar spine and hip bone mass in adolescence. However, in elderly people, the aluminium content in bones was not associated with increased risk of hip fractures. There was no information from the epidemiological literature about the potential effects of oral exposure to aluminium in food. Given these limitations, no pivotal epidemiological studies are available for risk assessment.

4.2 Assessment of dietary exposure

Owing to their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. At its present meeting, the Committee was asked to evaluate the safety of potassium aluminium silicate–based pearlescent pigments based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010). This aluminium-containing food additive has not previously been evaluated by the Committee.

Potassium aluminium silicate (mica) is used as a carrier substrate for titanium dioxide and/or iron oxide. Potassium aluminium silicate is not intended to be placed on the market as such, but only when coated with the food colours titanium dioxide and/or iron oxide. In the EU, E555 potassium aluminium silicate is approved as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment) (Directive 95/2/EC as amended by Directive 2003/114/EC). In the USA, pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide are approved for use as a colour additive at levels up to 1.25% in cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and capsules, and chewing gum (USFDA, 2006a). Potassium aluminium silicate–based pearlescent pigments are proposed to be used in confectionery, chewing gums and beverages at usage levels ranging from a minimum of 0.02% up to a maximum of 1.25%.

The Committee noted that no actual usage data were submitted for aluminium ammonium sulfate (INS 523), sodium aluminium phosphate basic (541(ii)), aluminium silicate (INS 559), aluminium powder or aluminium potassium sulfate (INS 522). Currently used aluminium-containing food additives are aluminium sulfate (INS 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate acidic (INS 541(i)) and aluminium lakes of food colour.

At the sixty-seventh meeting, the Committee considered only consumer exposure to aluminium in the diet; occupational exposure and other routes or commodities were not considered. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact materials and food additives. The potential range of exposure to aluminium from dietary sources reviewed at the sixty-seventh meeting by the Committee was 14–280 mg/week (see Table 8 in section 3.3.1).

For the evaluation of potassium aluminium silicate–based pearlescent pigments as a new food additive, the Committee evaluated an anticipated dietary exposure assessment based on food consumption data from the EU and the USA with the maximum proposed levels of use of potassium aluminium silicate–based pearlescent pigments. The Committee concluded that anticipated dietary exposure in the general population from the use of this food colour at the maximum proposed use levels (0.5% in beverages and 1.25% by weight in solid food) would range from 10 mg/kg bw per day at the mean to 323 mg/kg bw per day for consumers with a high consumption of non-alcoholic beverages. When converted to an aluminium basis, this corresponds to an aluminium exposure from potassium aluminium silicate–based pearlescent pigments of 1.8 mg/kg bw per day up to 58 mg/kg bw per day.

The Committee recognizes that its estimates are conservative, as it is assumed that all processed foods and beverages contain the colour added at the maximum proposed use levels. The Committee noted that non-alcoholic flavoured drinks are the major contributor in these estimates, accounting for 20–70% of overall dietary exposure.

For other aluminium-containing food additives under re-evaluation, a tentative estimate of dietary exposure from food additive sources has been made, taking into account previous assessments and other publications or submissions reviewed by the Committee at the current meeting. The Committee noted, from the report of its sixty-seventh meeting and from an EFSA scientific opinion, that the range of estimates was mainly based on dietary exposure calculated with the total diet study method, which takes into account water consumption. It is known from the literature that the main sources of migration of aluminium into food are from the use of cookware or aluminium utensils. It is also known that the design of total diet studies generally tries to control any bias of additional contamination that may result from the use of containers, cookware or utensils containing aluminium during the preparation and storage of food as consumed.

The Committee noted that estimates of the contribution to overall mean dietary exposure from all sources (including natural sources, water consumption, food contact materials and food additives) were in the range of 10–140 mg/week in adult populations (0.2–2.3 mg/kg bw per week as aluminium, assuming a body weight of 60 kg; see Table 8 in section 3.3.1 above). Major contributors to these estimates were cereals and cereal-based food products, with a proportion of 20–90%, depending on the country, equivalent to a dietary exposure of approximately 2–120 mg/week (0.03–2 mg/kg bw per week as aluminium, assuming a body weight of 60 kg).

This assessment is consistent with previous evaluations made by the Committee in which cereal products were considered as potentially high contributors to dietary aluminium exposure. The Committee also noted from its review that high levels of the actual uses of aluminium-containing food additives were reported for cereals and cereal-based products, in particular for sodium aluminosilicate (INS 554) and sodium aluminium phosphate acidic (INS 541(i)). Based on this, the Committee concluded that aluminium from the consumption of cereals and cereal-based products could reasonably be assumed to be mainly from food additive sources.

The Committee noted that the estimated dietary exposures related to average adult populations and that high dietary exposures (e.g. 90th or 95th percentile) are generally assumed to be 2 times higher than the reported average. It also noted that children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the highest potential exposure to aluminium per kilogram of body weight.

5. EVALUATION

The new data submitted to the Committee and available in the published literature addressed some of the research needs identified previously, including

studies of bioavailability and reproductive, developmental and neurobehavioural effects.

The absorption of aluminium compounds is generally in the region of 0.01–0.3%. Soluble aluminium compounds appear to be more bioavailable, but it is not possible to draw conclusions on quantitative differences in the overall toxicokinetics of different aluminium-containing food additives or between experimental animals and humans.

The recent evidence did not show effects of aluminium on reproductive outcomes. The new studies support previous observations of neurodevelopmental effects in experimental animals, but there continues to be a lack of consistency regarding the reported effects, and there are some limitations to all of the studies. Most of the studies involved administration of aluminium compounds in drinking-water, rather than in the diet.

At its current meeting, the Committee noted that the new data did not substantially change the LOAEL range of 50–75 mg/kg bw per day, but one of the studies also provided a NOAEL of 30 mg/kg bw per day. This NOAEL was identified from a study in which aluminium citrate was administered in drinking-water. Aluminium citrate is more soluble than many other aluminium compounds and is likely to be more bioavailable from drinking-water than from food. The Committee concluded that the NOAEL of 30 mg/kg bw per day was an appropriate basis for establishing a PTWI for aluminium compounds. Because long-term studies on the relevant toxicological end-points had become available since the sixty-seventh meeting, there was no longer a requirement for an additional safety factor for deficiencies in the database. The Committee therefore established a PTWI of 2 mg/kg bw from the NOAEL of 30 mg/kg bw per day by applying a safety factor of 100 for interspecies and intraspecies differences. The previous PTWI of 1 mg/kg bw was withdrawn.

The data submitted on aluminium lactate and potassium aluminium silicate–based pearlescent pigments were insufficient to demonstrate that these food additives differ from other forms of aluminium in their bioavailability or toxicity. The PTWI applies to all aluminium compounds in food, including food additives. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI.

The Committee concluded that, for adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI of 2 mg/kg bw. Estimates of dietary exposure of children to aluminium-containing food additives, including high-level dietary exposure, can exceed the PTWI by up to 2-fold.

For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, the Committee noted that anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI of 2 mg/kg bw.

Therefore, the Committee recommended that provisions for food additives containing aluminium included in the GSFA should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg bw as aluminium from all sources.

There is a need for convincing data to demonstrate that aluminium is not bioavailable from potassium aluminium silicate–based pearlescent pigments.

No data were available to identify the forms of aluminium present in soya-based formula and their bioavailability. Such studies were requested at the sixty-seventh meeting and are still required.

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PONCEAU 4R (addendum)

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1. EXPLANATION

Ponceau 4R (Chemical Abstracts Service No. 2611-82-7), also known as Cochineal Red and New Coccine, is a synthetic food colour. Ponceau 4R consists essentially of trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Ponceau 4R was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). Ponceau 4R was previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-seventh meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*). At its eighth meeting, the Committee did not establish an acceptable daily intake (ADI) for Ponceau 4R because of inadequate toxicological data but recognized that some long-term feeding studies were available. At its thirteenth meeting, the Committee reviewed these data and established a temporary ADI of 0–0.75 mg/kg body weight (bw) based on a no-observed-effect level (NOEL)[1] of 150 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because the Committee noted the absence of suitable information on the metabolism and kinetics of Ponceau 4R and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered an additional long-term feeding study in mice that had become available and revised the temporary ADI to 0–0.125 mg/kg bw based on a NOEL of 25 mg/kg bw per day (this NOEL assumed 500 mg/kg in the diet to be equivalent to 25 mg/kg bw per day). The current method of calculating administered dose from a concentration of test material present in the feed would yield an equivalent dose of 75 mg/kg bw per day (FAO/WHO, 2009). At that meeting, the Committee reiterated the need to review more studies on metabolism and reproduction and a long-term feeding study in a non-rodent species.

At the twenty-second and twenty-fifth meetings, the Committee extended the temporary ADI on the understanding that the data requested at the eighteenth meeting would become available for review. At the twenty-seventh meeting, the Committee reviewed new data on metabolism, a long-term study in rats that had been exposed in utero and through lactation, a multigeneration feeding study and a teratogenicity study. The Committee noted that the long-term study in rats showed no adverse effects in the kidneys and had a NOEL of 500 mg/kg bw per day based on reduced body weight gain at higher doses. The results of this study in rats and a reconsideration of the severity of the renal effects observed in the long-term study in mice led the Committee to establish an ADI of 0–4 mg/kg bw. The ADI was derived by applying a 100-fold safety factor to the higher NOEL from the mouse dietary study, which was equivalent to 375 mg/kg bw per day.

At its present meeting, the Committee based its evaluation on data previously reviewed together with a limited number of published studies that had become available since the twenty-seventh meeting. The new data included a

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL). This NOEL would now be considered a NOAEL.

reproduction study in mice that measured several neurological end-points, studies on genotoxicity and biochemical enzyme activity, and studies on additive intolerance. The Committee took note of the content of a recently completed review of Ponceau 4R by the European Food Safety Authority (EFSA).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

No new information was available on the absorption, distribution and excretion of Ponceau 4R.

2.1.2 Biotransformation

Singh, Das & Khanna (1997) compared the rate of formation of aromatic amines from four red azo dyes—namely, Amaranth, Carmoisine, Fast Red E and Ponceau 4R (all at 37.5 µmol/l)—by gastrointestinal microbes under anaerobic conditions with that obtained using hepatic microsomes. The caecal suspension exhibited higher azo reductase activity compared with the hepatic microsomal fraction using each of the four azo dyes. Caecal microbes showed maximal azo reductase activity when Ponceau 4R was used as a substrate, followed by Fast Red E and Carmoisine, whereas the activity was least with Amaranth. Similarly, maximum hepatic microsomal azo reductase activity was observed with Ponceau 4R as the substrate, followed by Fast Red E and Carmoisine, whereas the least activity was observed with Amaranth. Caecal flora possessed almost a 17-fold higher capability to degrade Ponceau 4R and Fast Red E colorants compared with the hepatic microsomal fraction.

2.1.3 Effects on enzymes and other biochemical parameters

To investigate the inhibition of the activities of human phenolsulfotransferase-P (PST-P), phenolsulfotransferase-M (PST-M) and monoamine oxidase A and B by eight food colours, including Ponceau 4R and Sunset Yellow FCF, each colour was tested separately at a concentration of 1, 5 or 25 µmol/l using conventional in vitro testing protocols. The substrates used for the enzymes were phenol for PST-P, tyramine for PST-M and [^{14}C]tyramine for both monoamine oxidases. At a concentration of 25 µmol/l, Ponceau 4R and Sunset Yellow FCF completely inhibited PST-P activity. However, at 5 and 1 µmol/l, the extent of inhibition was 39% and 11%, respectively, for Ponceau 4R and 55% and 17%, respectively, for Sunset Yellow FCF. Ponceau 4R and Sunset Yellow FCF had little to no inhibitory effect on PST-M or monoamine oxidase activities at a concentration of 25 µmol/l (Gibb, Glover & Sandler, 1987).

Kuno & Mizutani (2005) investigated the influence of Ponceau 4R (New Coccine) on the activities of phase I and phase II drug-metabolizing enzymes (cytochrome P450 [CYP] 2A6, uridine diphosphate glucuronosyltransferase [UGT] 1A6 and 2B7) derived from bovine liver microsomes. Their findings indicated that Ponceau 4R is neither a substrate nor an inhibitor of the enzymes studied.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Ponceau 4R.

2.2.2 Short-term studies of toxicity

No new information was available from short-term studies of the toxicity of Ponceau 4R.

2.2.3 Long-term studies of toxicity and carcinogenicity

No new information was available from long-term studies of the toxicity and carcinogenicity of Ponceau 4R.

2.2.4 Genotoxicity

The genotoxicity of Ponceau 4R is summarized in Table 1.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

Ponceau 4R admixed in the diet was fed to Crj: CD-1 mice at concentrations of 0 (control), 1200, 2400 or 4800 mg/kg (equal to 0, 212, 423 and 819 mg/kg bw per day; average for both sexes combined) from 5 weeks of age in the F_0 generation to 9 weeks of age in the F_1 generation. Feed consumption data indicated no significant difference between controls and those groups consuming Ponceau 4R. Mice were weighed on days 0, 2, 4, 7, 14, 21, 28 and 30 during the pre-mating phase. Females were paired 1:1 with males and separated after 5 days. Dams were allowed to deliver and rear their offspring in solitude. Pups were weighed on postnatal days (PNDs) 0, 4, 7, 14 and 21. Functional and behavioural parameters, such as surface righting (PNDs 4 and 7), negative geotaxis (body righting on an inclined plane; PNDs 4 and 7), cliff avoidance (PND 7), swimming behaviour (PNDs 4 and 14) and olfactory orientation (PND 14), were measured in all F_1 pups during PNDs 0–21. On PND 49, all pups performed in a multiple water T-maze daily for 3 consecutive days.

There was no adverse effect of Ponceau 4R on litter size, litter weight or sex ratio at birth. The average body weight of male and female offspring was increased significantly in the high-dose group at PNDs 0, 4 and 21. In behavioural developmental parameters, surface righting at PND 4, but not at PND 7, was affected significantly in the high-dose group in male offspring. Other variables measured showed no consistently significant adverse effect on either sex in the lactation period. In multiple water T-maze performances in the F_1 generation, the time taken was significantly longer in the middle-dose and high-dose groups in males compared with the controls, and those effects were significantly dose related ($P < 0.01$). It was concluded that the no-observed-adverse-effect level (NOAEL) was 1200 mg/kg in the diet (approximately 205 mg/kg bw per day) for maze learning by males in the F_1 generation (Tanaka, 2006).

Table 1. Genotoxicity of Ponceau 4R

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	Up to 10 000 µg/ml, ±S9	Negative	Cameron et al. (1987)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Cameron et al. (1987)
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Up to 5000 µg/ml (liquid culture method), ±S9	Negative	Longstaff et al. (1984)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535 and TA1537	Up to 5000 µg/plate, ±S9	Negative	Ishidate et al. (1984)
	S. typhimurium TA1535 and TA1538 and *Escherichia coli* WP2uvrA	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Haveland-Smith & Combes (1980)
Chromosomal aberration	Chinese hamster fibroblast line	Up to 1000 µg/ml, –S9, 24 h and 48 h incubation	Positive	Ishidate et al. (1984)
DNA repair	Rat hepatocytes	Up to 0.1 mmol/l, 4 h incubation	Negative	Kornbrust & Barfknecht (1985)
In vivo				
DNA repair	Hepatocytes taken from oral gavage–dosed Sprague-Dawley rat	300 mg/kg bw	Negative	Kornbrust & Barfknecht (1985)
Comet assay	ddY mouse (oral gavage); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	1–2000 mg/kg bw with 3 h exposure or 2000 mg/kg bw with 24 h exposure	Positive: colon, >10 mg/kg bw; stomach, bladder, >100 mg/kg bw; kidney, liver, lung, bladder, 2000 mg/kg bw for 24 h	Tsuda et al. (2001); Sasaki et al. (2002)
	ICR mouse or F344 rat (oral gavage dosed); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	Mouse; 1 or 10 mg/kg bw with 3 h exposure Rat; 10 mg/kg bw with 3 h exposure	Positive: mouse, colon at 10 mg/kg bw Negative: rat	Shimada et al. (2010)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant from rat liver

(b) Developmental toxicity

No new information was available on the developmental toxicity of Ponceau 4R.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical signs attributed to food intolerance often involve recurrent urticaria or angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Mikkelsen et al., 1978; Ibero et al., 1982). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria or angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Weber et al., 1979; Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang et al., 1994).

Veien & Krogdahl (1991) reported a case of a 24-year-old woman who responded with development of leukoclastic vasculitis (i.e. inflammation of small blood vessels) after a placebo-controlled oral challenge with 50 mg of Ponceau 4R.

2.3.2 Clinical trials

Bateman et al. (2004) investigated the behavioural effects on 3-year-old children (n = 277) of ingesting a high-dose azo food dye mixture containing Sunset Yellow FCF, Tartrazine, Carmoisine and Ponceau 4R (5 mg of each) and 45 mg sodium benzoate in a double-blind, placebo-controlled study. The children were classified as having hyperactivity (HA) (using two different activity scales: emotionality, activity and sociability; and Weiss–Werry–Peters) or not, with or without atopy (AT) (i.e. positive skin prick test with a number of known protein allergens), in a 2 × 2 group design (AT/HA, non-AT/HA, AT/non-HA, non-AT/non-HA). Over a 4-week period, the children received either the azo dye mixture with fruit juice or placebo (fruit juice only) on the 2nd and 4th weeks. The children's behaviour was assessed by research psychologists using validated tests and by the parents. Using assessments made by the parents, there were significant reductions in hyperactive behaviour during the withdrawal phase. Furthermore, there were significantly greater increases in hyperactive behaviour during the active period compared with the placebo period. These effects were not influenced by the presence or absence of previously diagnosed hyperactivity or by the presence or absence of atopy. However, there were no significant differences detected based on objective interactive testing by psychologists in the clinic.

A follow-up study was conducted to further investigate the association of ingestion of a mixture of food colour additives and sodium benzoate with hyperactive behaviour in children. The hypothesis was tested using a community-based, double-blind, placebo-controlled randomized crossover food challenge in which two groups of children aged 3 (n = 153) and 8 or 9 years (n = 144) received one of two mixtures

of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions plus sodium benzoate) were those tested in the Bateman et al. (2004) study, whereas mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel global hyperactivity aggregate (GHA) measure, which comprised an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- to 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

In order to investigate the hypothesis that the children's behaviour reported in the McCann et al. (2007) study was influenced by allelic variation in a number of genes that have previously been implicated in ADHD (Thapar et al., 1999; Swanson et al., 2000; Kuntsi & Stevenson, 2001), buccal swabs were collected for genotypic analyses of cellular deoxyribonucleic acid (DNA). The genes studied were from the dopamine (dopamine transporter [DAT1], dopamine D4 receptor [DRD4]

and catechol *O*-methyl-transferase [COMT]), adrenergic (adrenergic receptor alpha 2A [ADRA2A]) and histamine (histamine *N*-methyl-transferase [HNMT]) neurotransmitter systems. The genotype analysis involved the detection of single nucleotide polymorphisms (two in HNMT, one in COMT, one in DRD4 and one in ADRA2A) in the genes. There was evidence that the HNMT T939C and the DRD4 4rs740373 polymorphisms correlated to the overall GHA score in the 3-year-old children. However, there was no significant relationship of the polymorphisms to the GHA scores in the 8- and 9-year-olds (Stevenson et al., 2010).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Ponceau 4R. The Committee received a submission from EFSA concerning dietary exposure to Ponceau 4R that was a part of its re-evaluation of the safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Ponceau 4R is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, Ponceau 4R is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Ponceau 4R made using Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Ponceau 4R is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Ponceau 4R (E 124) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

Table 2. Maximum permitted use levels of Ponceau 4R in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks	50
Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Confectionery Fine bakery wares Edible ices Desserts including flavoured milk products Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products Sobrasada Chorizo sausage	200
Pre-cooked crustaceans Salchichon	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion of beverages and solid food that could contain the additive (25%) was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Ponceau 4R, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children, assuming that the maximum level in beverages was 50 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(50 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for children aged 1.5–4.5 years in the United Kingdom were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and maximum permitted levels of use as specified in the EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Ponceau 4R exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the adult food consumption data for the maximum permitted levels population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.3 to 2.5 mg/kg bw per day, whereas those at the 95th percentile ranged from 0.7 to 6.7 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 1.4 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 3.5 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (14%) and fruit wines, cider and perry (13%).

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

The tier 3 approach employed by EFSA used maximum reported Ponceau 4R use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Ponceau 4R for European children ranged from 0.3 to 2.4 mg/kg bw per day at the mean and from 0.7 to 6.2 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.3 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 3.3 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.4 mg/kg bw per day at the mean and 1.0 mg/kg bw per day at the 97.5th percentile. As in the tier 2 estimates above, for adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (52%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (16%).

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Ponceau 4R in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Ponceau 4R were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Ponceau 4R in survey foods were used. Both detected and “non-detect” results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical concentrations of Ponceau 4R in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Ponceau 4R. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Ponceau 4R was 0.15 mg/day, with a 90th percentile exposure of

Table 3. EFSA dietary exposures to Ponceau 4R

	Exposure (mg/kg bw per day)		
	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		8.1[a]
Maximum permitted levels			
- Mean exposure	0.5	1.4	0.3–2.5
- Exposure at the 95th or 97.5th percentile	1.1	3.5	0.6–6.7
Maximum reported use levels			
- Mean exposure	0.4	1.3	0.3–2.4
- Exposure at the 95th or 97.5th percentile	1.0	3.3	0.7–6.2

[a] For children (age range not specified).

0.45 mg/day. The highest subpopulation mean was 0.22 mg/day for 13- to 18-year-olds. The highest subpopulation 90th percentile exposure was 0.62 mg/day, also for 13- to 18-year-olds. The highest estimates made using the maximum colours scenario were 1.17 mg/day at the mean and 2.90 mg/day at the 90th percentile, both for the 13- to 18-year-old subpopulation. The main contributors to dietary exposure were ice cream and ice confections, cakes, muffins and pastries, and soft drinks.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Ponceau 4R calculated by EFSA were much higher than those of FSANZ. The Committee concluded that this was due to EFSA's use of maximum permitted and maximum reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 6 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Ponceau 4R, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.02 mg/kg bw per day, was a more realistic dietary exposure estimate because of the extensive post-market analyses used in its preparation.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*) with recently published data.

Table 4. FSANZ dietary exposures to Ponceau 4R using the mean colours scenario

Population group	Mean exposure		90th percentile exposure	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.12	0.01	0.38	0.02
6–12 years old	0.21	0.01	0.56	0.02
13–18 years old	0.22	<0.01	0.62	0.01
19–24 years old	0.17	<0.01	0.49	0.01
25+ years old	0.13	<0.01	0.38	0.01
2+ years old	0.15	<0.01	0.45	0.01

The absorption of ingested Ponceau 4R is limited. After Ponceau 4R is anaerobically reduced by microflora in the gastrointestinal tract, small amounts of its metabolites, in the form of the free sulfonated aromatic amines, naphthionic acid and 7-hydroxy-8-amino-naphthalene-1,3-disulfonic acid, reach the systemic circulation. Ponceau 4R does not accumulate in tissues. Almost all of an orally administered dose is excreted in urine and faeces within 72 hours, with the majority (90%) being present in faeces.

Repeated-dose feeding studies of short and long duration revealed no adverse findings. In 90-day studies, NOAELs of 500 mg/kg bw per day in rats and 300 mg/kg bw per day in pigs were reported. For long-term daily exposure, the NOAELs were 375 mg/kg bw per day in mice and 500 mg/kg bw per day in rats.

There was no evidence of carcinogenicity in long-term feeding studies in rats at doses up to 1500 mg/kg bw per day and in mice at doses up to 1875 mg/kg bw per day. Despite a recent report of a comet assay showing evidence of DNA damage in the colon and bladder at 10 mg/kg bw and in the stomach at 100 mg/kg bw, there was no evidence of any neoplasia in the stomach, bladder or colon of mice in the carcinogenicity studies. The authors of the comet assay study noted that a histopathological examination did not reveal any treatment-related effects in the colon, bladder or glandular stomach. No mutagenic or cytotoxic effects were found when Ponceau 4R was tested in a range of in vitro experiments.

Reproduction studies revealed no adverse effects of Ponceau 4R in the feed at doses equivalent to 1250 mg/kg bw per day in the rat and up to 205 mg/kg bw per day in a neurobehavioural study in mice. Adverse neurobehavioural findings among mouse pups were inconsistent. Teratogenicity studies in mice at oral gavage doses up to 100 mg/kg bw per day (the highest dose tested) and in rats at 4000 mg/kg bw per day did not reveal any adverse effects.

Urticarial and vasculitic reactions have been reported in humans following exposure to Ponceau 4R. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with

better control conditions are available, no conclusion on idiosyncratic responses to Ponceau 4R could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Ponceau 4R prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Ponceau 4R calculated by EFSA were much higher than those of FSANZ (0.02 mg/kg bw per day at the 90th percentile for children). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimate of 6 mg/kg bw per day for children should be considered in the safety assessment for Ponceau 4R in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that the data do not indicate a need to revise the existing ADI of 0–4 mg/kg bw for Ponceau 4R. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children estimated by the more realistic FSANZ approach was 0.5% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Ponceau 4R does not present a health concern.

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QUINOLINE YELLOW (addendum)

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1. EXPLANATION

Quinoline Yellow is a synthetic food colour. It is prepared by sulfonating either 2-(2-quinolyl)-1,3-indandione (unmethylated variety) or a mixture containing about two thirds 2-(2-quinolyl)-1,3-indandione and one third 2-[2-(6-methyl-quinolyl)]1,3-indandione (methylated variety). It consists essentially of sodium salts of a mixture

of disulfonates, monosulfonates and trisulfonates of the above compounds and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Quinoline Yellow was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). Toxicological data related to Quinoline Yellow were previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-eighth meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*). At its eighth meeting, the Committee did not establish an acceptable daily intake (ADI) for Quinoline Yellow because of inadequate toxicological data. At its thirteenth meeting, the Committee reviewed the available data and established a temporary ADI of 0–1 mg/kg body weight (bw) based on a no-observed-effect level (NOEL)[1] of 500 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because of data gaps. In particular, the Committee noted the absence of suitable information on the metabolism and kinetics of Quinoline Yellow and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered a suitable long-term feeding study in rats. Using the results of that study, the Committee established a temporary ADI of 0–0.5 mg/kg bw based on the absence of any adverse effects at the highest tested dose of 50 mg/kg bw per day. The Committee reiterated its desire to review a three-generation reproduction study that was in progress, more information on metabolism and a long-term feeding study in a non-rodent species.

At its nineteenth meeting in 1975 (Annex 1, reference *38*), the Committee was informed that there were two types of Quinoline Yellow: non-methylated Quinoline Yellow and partially (30%) methylated Quinoline Yellow. The Committee indicated that data generated using either source could be used to define the toxicological hazard associated with Quinoline Yellow. At its twenty-second meeting, the Committee reviewed a three-generation reproduction study in rats but did not amend the temporary ADI. At its twenty-fifth meeting, the Committee was advised that two major studies were nearing completion and decided to extend the temporary ADI that it had established at its eighteenth meeting until the twenty-eighth meeting.

At the twenty-eighth meeting, the Committee reviewed new data on metabolism and a long-term repeated-dose study in mice that had been exposed to Quinoline Yellow in utero and through lactation. The Committee established an ADI of 0–10 mg/kg bw based on a NOEL of 10 000 mg/kg in the diet (equivalent to a range of 1000–1500 mg/kg bw per day) in the long-term study in mice.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since the twenty-eighth meeting. No new unpublished toxicological studies were submitted following a public call for data. The Committee took note of the content of a recently completed review of Quinoline Yellow by the European Food Safety Authority (EFSA).

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[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL). This NOEL would now be considered a NOAEL.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

No new information was available on the absorption, distribution, metabolism and excretion of Quinoline Yellow or on its effects on enzymes and other biochemical parameters.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Quinoline Yellow.

2.2.2 Short-term studies of toxicity

No new information was available from short-term studies of the toxicity of Quinoline Yellow.

2.2.3 Long-term studies of toxicity and carcinogenicity

Two additional unpublished long-term chronic toxicity and carcinogenicity studies with a reproductive toxicity phase, carried out in the rat and in the mouse by Biodynamics Laboratories Inc. in 1980–1981, were summarized by SCCNFP (2004). These two studies were not included in the previous evaluation by the Committee.

In the first study, groups of mice (60 of each sex) were fed Quinoline Yellow at dietary levels of up to 50 000 mg/kg (equivalent to approximately 7500 mg/kg bw per day) for 23–24 months. No adverse toxic effects were observed, and no evidence of carcinogenicity was noted (SCCNFP, 2004).

In the second study, to assess the effects of chronic daily exposure to Quinoline Yellow in albino (CD) rats (60 of each sex per group), Quinoline Yellow was admixed in the diet at 0, 300, 1000, 5000 or 20 000 mg/kg (equivalent to 0, 15, 50, 250 and 1000 mg/kg bw per day, respectively) and fed ad libitum for 30 months. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation. A second dietary exposure study (70 of each sex per group) with Quinoline Yellow at a concentration of 50 000 mg/kg in the diet (equivalent to 2500 mg/kg bw per day) was initiated after the United States Food and Drug Administration (USFDA) concluded that the 20 000 mg/kg diet level in the first study did not achieve the maximum tolerated dose (MTD). After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation. The reproductive aspects of this study are described under section 2.2.5. For the chronic phase, offspring (70 of each sex) were selected randomly from each of the treated and control groups. Tissues from rats were prepared and sectioned for histopathological examination.

Lower body weights compared with controls were observed at Quinoline Yellow dietary levels of 20 000 and 50 000 mg/kg. The weights of the kidneys,

adrenals, spleen, thyroid, uterus and ovaries were reduced in the absence of any histopathological lesions at the same dose levels. No treatment-related effects were described at Quinoline Yellow dietary levels of 5000 mg/kg, equivalent to 250 mg/kg bw per day (SCCNFP, 2004). According to SCCNFP (2004), the USFDA derived a no-observed-adverse-effect level (NOAEL) of 1000 mg/kg bw per day from this study. However, in the absence of the original study data, the Committee was unable to independently verify the effects of Quinoline Yellow at 20 000 mg/kg diet on body weight. The effects of in utero exposure on reproductive toxicity are reported under section 2.2.5.

2.2.4 Genotoxicity

The genotoxicity of Quinoline Yellow is summarized in Table 1.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

To assess the effects of chronic daily exposure to Quinoline Yellow in albino (CD) rats (60 of each sex per group), Quinoline Yellow was admixed in the diet at 0, 300, 1000, 5000 or 20 000 mg/kg (equivalent to 0, 15, 50, 250 and 1000 mg/kg bw per day, respectively) and fed ad libitum for 30 months in an unpublished study conducted by Biodynamics Laboratories Inc. in the early 1980s and reviewed by SCCNFP (2004). To permit in utero exposure, F_0 parental rats were fed for 2 months prior to mating and then continuously thereafter. A second dietary exposure study (70 of each sex per group) with Quinoline Yellow at 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 20 000 mg/kg dietary level in the first study did not achieve the MTD. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation.

The pups of the F_0 dams were reported to have reduced survival coupled with lower weight gains during lactation at Quinoline Yellow dose levels of 5000 mg/kg in the diet (equivalent to 250 mg/kg bw per day) and above, although no other treatment-related effects on reproductive parameters were noted. The NOAEL for this study is considered to be 50 mg/kg bw per day, based on the available summarized information reported in SCCNFP (2004).

(b) Developmental toxicity

No information on the developmental toxicity of Quinoline Yellow was available.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical signs attributed to food intolerance often involve recurrent urticaria/angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many

Table 1. Genotoxicity of Quinoline Yellow

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	118–3800 µg/ml, ±S9	Negative	Wollny (2000)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	33–5000 µg/plate, ±S9	Negative	Wollny (1999)
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Up to 500 µg/ml (spot test method), ±S9	Negative	Blevins & Taylor (1982)
In vivo				
Micronucleus	NMRI mouse (5/sex)	500, 1000 or 2000 mg/kg bw	Negative	Honarvar (2003)

S9, 9000 × *g* supernatant from rat liver

of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Juhlin, 1981). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria/angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Weber et al., 1979; Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang, 1994).

2.3.2 Clinical trials

The hypothesis that ingestion of mixtures of certain food colours and sodium benzoate increases the hyperactive behaviour of children was investigated using a community-based, double-blind, placebo-controlled, randomized crossover food challenge in which two groups of children aged 3 (n = 153) and 8 or 9 years (n = 144) received one of two mixtures of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions, plus sodium benzoate) and mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions, plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel global hyperactivity aggregate (GHA) measure, which comprised

an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- to 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

In order to investigate the hypothesis that the children's behaviour reported in the McCann et al. (2007) study was influenced by allelic variation in a number of genes that have previously been implicated in ADHD (Thapar et al., 1999; Swanson et al., 2000; Kuntsi & Stevenson, 2001), buccal swabs were collected for genotypic analyses of cellular deoxyribonucleic acid (DNA). The genes studied included genes from the dopamine (dopamine transporter [DAT1], dopamine D4 receptor [DRD4] and catechol *O*-methyl-transferase [COMT]), adrenergic (adrenergic receptor alpha 2A [ADRA2A]) and histamine (histamine *N*-methyl-transferase [HNMT]) neurotransmitter systems. The genotype analysis involved the detection of single nucleotide polymorphisms (two in HNMT, one in COMT, one in DRD4 and one in ADRA2A) in the genes. There was evidence that the HNMT T939C and the DRD4 4rs740373 polymorphisms correlated to the overall GHA score in the 3-year-old children. However, there was no significant relationship of the polymorphisms to the GHA scores in the 8- and 9-year-olds (Stevenson et al., 2010).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Quinoline Yellow. The Committee received a submission from EFSA concerning dietary exposure to Quinoline Yellow that was a part of its re-evaluation of the

safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Quinoline Yellow is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, it is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Quinoline Yellow made using Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Quinoline Yellow is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Quinoline Yellow (E 104) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Quinoline Yellow, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children assuming that the maximum level in beverages was 100 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(100 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 10 + 3.125 = 13.1 mg/kg bw per day

Table 2. Maximum permitted use levels of Quinoline Yellow in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Edible ices Desserts including flavoured milk products	150
Fine bakery wares Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Confectionery Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for United Kingdom children aged 1.5–4.5 years were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and with maximum permitted levels of use as specified in EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Quinoline Yellow exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the adult food consumption data for the United Kingdom population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.8 to 3.5 mg/kg bw per day, whereas those at the 95th percentile were from 1.8 to 9.6 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 3.1 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 7.3 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.9 mg/kg bw per day at the mean and 2.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (13–41%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers) (14–29%) and desserts (including flavoured milk products) (17–62%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10–50% of exposure in four countries. Confectionery accounted for 11% of exposure in one country.

The tier 3 approach employed by EFSA used maximum reported Quinoline Yellow use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Quinoline Yellow for European children ranged from 0.45 to 2.0 mg/kg bw per day at the mean and from 1.1 to 4.1 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.8 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 4.3 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.2 mg/kg bw per day at the 97.5th percentile. The main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (10–39%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers) (14–60%) and desserts (including flavoured milk products) (14–57%). Confectionery accounted for 13–18% of exposure in two countries, and

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

Table 3. EFSA dietary exposure estimates for Quinoline Yellow

	Exposure (mg/kg bw per day)		
	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		13.1[a]
Maximum permitted levels			
- Mean exposure	0.9	3.1	0.8–3.5
- Exposure at the 95th or 97.5th percentile	2.1	7.3	1.8–9.6
Maximum reported use levels			
- Mean exposure	0.5	1.8	0.45–2.0
- Exposure at the 95th or 97.5th percentile	1.2	4.3	1.1–4.1

[a] For children (age range not specified).

surimi, sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 15% of exposure in one country.

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Quinoline Yellow in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Quinoline Yellow were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Quinoline Yellow in survey foods were used. Both detected and “non-detect” results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical

Table 4. FSANZ dietary exposures to Quinoline Yellow using the mean colours scenario

Population group	Mean exposure		90th percentile exposure	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.05	<0.01	0.15	0.01
6–12 years old	0.06	<0.01	0.14	0.01
13–18 years old	0.08	<0.01	0.15	<0.01
19–24 years old	0.15	<0.01	0.38	0.01
25+ years old	0.17	<0.01	0.48	0.01
2+ years old	0.13	<0.01	0.36	0.01

concentrations of Quinoline Yellow in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Quinoline Yellow. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Quinoline Yellow was 0.13 mg/day, with a 90th percentile exposure of 0.36 mg/day. The highest subpopulation mean was 0.17 mg/day, for those 25+ years of age. The highest subpopulation 90th percentile exposure was 0.48 mg/day, also for those 25+ years of age. The highest estimates made using the maximum colours scenario were 0.41 mg/day at the mean (for those 25+ years of age and older) and 1.10 mg/day at the 90th percentile (for the 19- to 24-year-old subpopulation). The main contributors to dietary exposure were cakes, muffins and pastries, and sweet biscuits.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Quinoline Yellow calculated by EFSA were much higher than those of FSANZ. The Committee concluded that this was due to EFSA's use of maximum permitted and maximum reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 4 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Quinoline Yellow, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.01 mg/kg bw per day, was a more realistic

dietary exposure estimate because of the extensive post-market analyses used in its preparation.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*) with recently published data.

The absorption of ingested Quinoline Yellow is between 3% and 4% in rats and dogs, with most being excreted unchanged in faeces. There is evidence that some of the absorbed Quinoline Yellow is excreted in bile. Quinoline Yellow does not accumulate in tissues, and 85–90% of the Quinoline Yellow absorbed from the gastrointestinal tract is excreted unchanged in the urine.

Repeated-dose feeding studies for 90 days in the rat showed an absence of adverse effects at dose levels up to 2500 mg/kg bw per day. Two-year feeding studies confirmed the absence of any treatment-related effects in mice, rats and dogs at doses equivalent to 1500, 500 and 50 mg/kg bw per day, respectively. The long-term feeding studies in rodents also indicated that Quinoline Yellow was not carcinogenic. This was consistent with an absence of any genotoxicity reported previously or in the new studies completed since the Committee last considered Quinoline Yellow.

No adverse effects on reproductive performance in rats over three generations were reported following dietary exposure to Quinoline Yellow at the highest tested dose of 50 mg/kg bw per day. Similarly, a comprehensive two-generation study involving 65 rats of each sex per group on test showed no adverse reproductive effects at Quinoline Yellow concentrations up to 10 000 mg/kg in the diet (equivalent to a dose range of 1000–1500 mg/kg bw per day).

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Quinoline Yellow. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Quinoline Yellow could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Quinoline Yellow prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary

exposure to Quinoline Yellow calculated by EFSA were much higher than those of FSANZ (0.01 mg/kg bw per day for children at the 90th percentile). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach was considered by the Committee to be more realistic for preparing long-term dietary exposure estimates. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that EFSA's 97.5th percentile estimate of 4 mg/kg bw per day for children should be considered in the safety assessment for Quinoline Yellow in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that there were no new data submitted that would provide a suitable basis on which to revise the existing ADI of 0–10 mg/kg bw for Quinoline Yellow. However, the Committee was aware of unpublished long-term studies in mice and rats with in utero exposure to Quinoline Yellow that had been completed by Biodynamics Laboratories in 1980–1981, but had not been submitted for evaluation. One of these studies was used by EFSA to establish its ADI for Quinoline Yellow. As the results of these studies in rodents might affect the existing ADI, the Committee established a temporary ADI of 0–5 mg/kg bw, incorporating an additional 2-fold uncertainty factor, pending submission of the Biodynamics Laboratories studies by the end of 2013. The previously established ADI of 0–10 mg/kg bw was withdrawn. The conservative exposure estimates were below the upper limit of the temporary ADI.

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SUNSET YELLOW FCF (addendum)

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1. EXPLANATION

Sunset Yellow FCF (Chemical Abstracts Service No. 2783-94-0) is a synthetic food colour. It is also known as Orange Yellow S, CI Food Yellow 3, FD&C Yellow 6 and C.I. 15985. Sunset Yellow FCF consists principally of the disodium salt of 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalenesulfonic acid and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Sunset Yellow FCF was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). The Committee was asked to evaluate all data necessary for the assessment of the safety, dietary exposure and specifications for Sunset Yellow FCF. Sunset Yellow FCF was evaluated by the Committee at its eighth and twenty-sixth meetings (Annex 1, references *8* and *59*). At its eighth meeting, the Committee considered that sufficient toxicological data were available to establish an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) for Sunset Yellow FCF. At the twenty-sixth meeting, the Committee considered new studies on long-term and reproductive toxicity and established an ADI of 0–2.5 mg/kg bw.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since Sunset Yellow FCF was last considered by the Committee. There were no new unpublished toxicological studies submitted following a public call for data. However, a comprehensive review of one unpublished long-term feeding study in mice and two in rats was provided by the United States Food and Drug Administration (USFDA). The Committee also took note of the content of a recently completed review of Sunset Yellow FCF by the European Food Safety Authority (EFSA).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

No new information was available on the absorption, distribution and excretion of Sunset Yellow FCF.

2.1.2 Biotransformation

In vitro studies comparing Sunset Yellow FCF reduction rates between bacterial suspensions derived from rat intestines and those derived from human faeces indicated that the rate was approximately 4–5 times greater in rats. There was very little difference in activity between the five tested male human faecal samples in spite of a considerable divergence in age, daily diets and living circumstances (Watabe et al., 1980).

Oral gavage administration of Sunset Yellow FCF at 200 and 1000 mg/kg bw to two groups of nine male Swiss mice showed that nearly all was recovered in the faeces within 24 hours. The main aromatic amine metabolite, sulfanilic acid,

was also present. The bacterial metabolic transformation rates appeared to be dependent on the administered dose level, with the biotransformation rate being higher at 200 mg/kg bw than at 1000 mg/kg bw (Poul et al., 2009).

2.1.3 Effects on enzymes and other biochemical parameters

To investigate the inhibition of the activities of human phenolsulfotransferase-P (PST-P), phenolsulfotransferase-M (PST-M) and monoamine oxidase A and B by eight food colours, including Sunset Yellow FCF and Ponceau 4R, each colour was tested separately at a concentration of 1, 5 or 25 µmol/l using conventional in vitro testing protocols. The substrates used for the enzymes were phenol for PST-P, tyramine for PST-M and [^{14}C]tyramine for both monoamine oxidases. At a concentration of 25 µmol/l, Sunset Yellow FCF and Ponceau 4R completely inhibited PST-P activity. However, at 5 and 1 µmol/l, the extent of inhibition was 55% and 17%, respectively, for Sunset Yellow FCF and 39% and 11%, respectively, for Ponceau 4R. Sunset Yellow FCF and Ponceau 4R had little to no inhibitory effect on PST-M or monoamine oxidase activities at a concentration of 25 µmol/l (Gibb, Glover & Sandler, 1987).

Kuno & Mizutani (2005) investigated the influence of Sunset Yellow FCF on the activities of phase I and phase II drug-metabolizing enzymes (cytochrome P450 [CYP] 2A6, uridine diphosphate glucuronosyltransferase [UGT] 1A6 and 2B7) derived from bovine liver microsomes. Their findings indicated that Sunset Yellow FCF is neither a substrate nor an inhibitor of the enzymes studied.

Osman et al. (2004) investigated the inhibitory effect of Sunset Yellow FCF and its major metabolite sulfanilic acid on cholinesterase activity in plasma and erythrocytes. For the in vivo study, Sunset Yellow FCF or sulfanilic acid was administered to groups of five male albino rats in the diet at 400 mg/kg for an unspecified duration. This concentration in the feed gave a final dose of 4 mg/kg bw per day, assuming a reported daily feed intake of only 2 g/day for 200 g rats, which seems remarkably low for ad libitum feed consumption (FAO/WHO, 2009). The usual feed consumption for a 200 g rat is around 10 times this value (i.e. 20 g/day). For a feed intake of 20 g/day, the dose for each rat would increase to 40 mg/kg bw per day.

Even though Sunset Yellow FCF and sulfanilic acid inhibited the acetylcholinesterase activity in rat erythrocytes by 14% and 31%, respectively, no clinical signs in rats were reported. In contrast, the cholinesterase activity in plasma was reduced by 23% and 13% for Sunset Yellow FCF and sulfanilic acid, respectively. It is known that rat plasma contains approximately equal proportions of acetylcholinesterase and butyrylcholinesterase, whereas human plasma contains almost exclusively one form of cholinesterase—namely, butyrylcholinesterase (García-Ayllón et al., 2006).

In a separate in vitro study, the cholinesterase inhibitory effect of Sunset Yellow FCF on human blood from 10 male volunteers was investigated; a 50% inhibition of activity required a concentration of 0.33 mmol/l (149 mg/l) in plasma and 0.24 mmol/l (108 mg/l) in erythrocytes. Similar results for cholinesterase inhibition in human plasma in vitro were reported by Osman et al. (2002). For sulfanilic acid,

a concentration of 0.77 mmol/l resulted in a modest enzyme activity reduction of 13% and 14% for plasma and erythrocytes, respectively. Given the uncertainty in the administered dose in rats and the high concentrations of Sunset Yellow FCF needed to elicit appreciable inhibition of cholinesterase activity in vitro, the effect on physiological cholinesterase activity levels is unlikely to contribute much to its hazard profile.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Sunset Yellow FCF.

2.2.2 Short-term studies of toxicity

(a) Mouse

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered in the diet to groups of 10 male and 10 female B6C3F1 mice at 0, 6000, 12 500, 25 000, 50 000 or 100 000 mg/kg for 12 weeks followed by 1 week of recovery with control diet only. Dose selection was based on an earlier 14-day repeated-dose dietary study that revealed no deaths or signs of toxicity in groups of five mice of each sex. In the main study, mice were observed twice per day and weighed weekly. Gross and histopathological examinations were performed on all animals.

Mean body weight gain was reduced by more than 10% among male mice at the 100 000 mg/kg dietary intake level and in females at all concentration levels tested in a dose-related manner from 12 500 to 100 000 mg/kg diet. Gross and histopathological examinations revealed no treatment-related lesions in male or female mice at any intake level. The concentrations selected for the chronic study were 12 500 and 25 000 mg/kg diet (NCI/NTP, 1982).

(b) Rat

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 10 male and 10 female F344 rats at a concentration of 0, 6000, 12 500, 25 000, 50 000 or 100 000 mg/kg in the diet for 12 weeks followed by 1 week of control diet only. Dose selection was based on an earlier 14-day repeated-dose dietary study that revealed no deaths or signs of toxicity in groups of five of each sex. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Gross and histopathological examinations were performed on all animals.

No animals died during the study. Reductions in mean body weight gain exceeding 9.8% were reported in male rats at intake levels of 25 000, 50 000 and 100 000 mg/kg. In female rats, similar reductions in mean body weight gain were reported at 12 500, 25 000, 50 000 and 100 000 mg/kg diet. Bone marrow hyperplasia was reported in all animals at 50 000 and 100 000 mg/kg diet. The

concentrations selected for the chronic study were 12 500 and 25 000 mg/kg diet (NCI/NTP, 1982).

Two different mixtures containing Tartrazine, Brilliant Blue FCF, Sunset Yellow FCF and Carmoisine in undefined, but different, ratios (mixtures A and B; purity not specified) were administered in their diet to six groups of 10 male albino rats. Each colour mixture was purchased from a local market and was added to the rat diet at a concentration aimed to achieve a daily dose of 800 mg/kg bw. The dosing duration was 30 days, 60 days or 60 days with a 30-day recovery period for each mixture separately. A number of haematological and clinical chemistry parameters were measured, such as haemoglobin, red blood cell and white blood cell counts, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein, albumin, globulin and albumin/globulin ratio, glucose, total lipids, triglycerides, cholesterol, cholesterol/high-density lipoprotein ratio, urea, creatinine, liver deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) concentration, thyroid hormones (triiodothyronine [T_3] and thyroxine [T_4]) and growth hormone. At the end of dosing, only kidney, liver and stomach sections were prepared for histopathological examination.

Effects considered to be treatment related because they increased in magnitude with duration and showed an apparent decline following the recovery period were elevated levels of serum total lipids, cholesterol, triglycerides, total protein, globulin and serum ALT. Haematological investigations demonstrated selective neutropenia and lymphocytosis in the absence of any significant changes in total white blood cell counts and significantly decreased haemoglobin concentrations and red blood cell counts. Eosinophilia was observed only in rats receiving mixture A. Histopathological examination revealed few adverse effects in the stomach. However, in the liver and kidneys, congested blood vessels and areas of haemorrhage were observed in rats receiving mixture B. A brown pigment deposition was observed in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. As there was no information available on either the purity or ratios of the various colours in the two mixtures, the relevance of these observations in defining the hazard of Sunset Yellow FCF is limited (Aboel-Zahab et al., 1997).

Sunset Yellow FCF (purity not specified) was administered orally by gavage to groups of 10 male albino rats. A 5 mg/kg bw dose was given daily for 30 days, after which five rats per group were killed, and the remaining five rats had a 2-week recovery period before sacrifice. Body weight was measured before and at the end of treatment. Clinical chemistry testing included AST, ALT, bilirubin, creatinine, urea, total protein, albumin, inorganic phosphorus and calcium. No necropsy or histopathology was undertaken.

Cage-side observations indicated that the Sunset Yellow FCF–treated rats appeared to be more aggressive, nervous and generally more active relative to controls. Small but statistically significant increases relative to controls were observed in AST activity (119% of control; $P < 0.05$) and ALT activity (113% of control; $P < 0.05$), indirect bilirubin (175% of control; $P < 0.05$) but not total bilirubin, and urea (132% of control; $P < 0.01$). Body weight (88% of control; $P < 0.01$), total protein (86% of control; $P < 0.05$) and serum globulin (67% of control; $P < 0.01$)

were significantly reduced. Apart from body weight (88% of control; $P < 0.01$), AST activity (114% of control; $P < 0.05$) and urea (111% of control; $P < 0.05$), all other levels returned to control levels at the end of the recovery period. The toxicological significance of these small magnitude changes in the very limited range of measured parameters is difficult to interpret. This, coupled with the very small number of test animals, limits the study's value in defining the hazard profile of Sunset Yellow FCF (Helal et al., 2000; Mekkawy et al., 2001).

Sunset Yellow FCF (purity not specified) in combination with sodium nitrate (143 mg/kg bw per day) was administered orally by gavage to groups of 10 male albino rats at 7 mg/kg bw per day. After daily dosing for 30 days, five rats per group were killed, and the remaining five were held for a 2-week recovery period before sacrifice. Body weight was measured before and at the end of treatment. Clinical chemistry testing included AST, ALT, calcium, glucose, cholesterol, T_3 and T_4, γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), AP, bilirubin, creatinine, urea, total protein, albumin, inorganic phosphorus and calcium. Haematology investigations included measuring haematocrit, haemoglobin concentration and white blood cell count. No necropsy or histopathology was undertaken.

Exposure to the mixture significantly decreased body weight, red blood cell count, haemoglobin, haematocrit, white blood cell count, serum inorganic phosphorus, serum protein and serum albumin. Significant increases were observed in serum glucose, T_3 and T_4, calcium, GGT, LDH, CPK, AP and cholesterol. After the 14-day recovery period, most biochemical and haematological parameters had recovered. In view of the presence of sodium nitrate in combination with Sunset Yellow FCF of undefined purity, it is not possible to ascertain the toxicological significance of these findings in defining the hazard of Sunset Yellow FCF (Helal, 2001).

Mathur et al. (2005a) purchased a yellow food colorant that was claimed to be Sunset Yellow FCF (purity not specified) from a local market in India and administered it to groups of 10 male Wistar rats in their diet at concentrations of 0, 5000 or 30 000 mg/kg (equivalent to 0, 250 and 1500 mg/kg bw per day) for 90 days. At sacrifice, only testes were collected, prepared and sectioned for light microscopy.

Histologically, the testes of the 5000 mg/kg diet group showed degenerative changes in some seminiferous tubules. Spermatogonia in the basal layer of seminiferous tubules were found to have an abnormal shape. Arrested maturation was observed in many tubules. Mature sperm were absent, but Leydig cells and Sertoli cells appeared to be normal. Testes of the rats treated with Sunset Yellow FCF at 30 000 mg/kg diet showed an increase in the degenerative changes. Necrotic areas appeared irregular, involving many tubules, and the affected tubules displayed extensive desquamation and sloughing off of almost all the seminiferous epithelium lining the basement membrane. Seminiferous tubules near the degenerated ones appeared normal. In most of the tubules, pycnotic spermatocytes at the germinal elements were seen. In some other tubules, pycnotic spermatogenesis was arrested at the spermatogonial or spermatocyte stage, whereas in a few tubules, transformation into spermatozoa could be seen. It was reported that Sertoli cells had virtually obliterated the lumen in some degenerating tubules, and they were highly

vacuolated. The Leydig cells and blood vessels appeared normal. The histological observations on testes revealed that almost 50% of the tubules displayed signs of toxicity. At both dose levels, the AP activity and cholesterol level in serum were significantly increased and the serum protein level was significantly decreased. The effect on serum AP activity amounted to +151% of control ($P < 0.001$) and +128% of control ($P < 0.001$) at the low and high dose levels, respectively.

As it is well known that an azo dye precursor such as 1,3-dinitrobenzene can induce testicular lesions very similar to those described in this study (Hess et al., 1988) and there is considerable uncertainty surrounding the identity and purity of the administered colorant, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

In another study, Mathur et al. (2005b) administered an uncharacterized sample of yellow food colorant, reported to be Sunset Yellow FCF, to male albino rats (10 per group) in their diet for 90 days at 0, 5000 or 30 000 mg/kg (equivalent to 0, 250 and 1500 mg/kg bw per day, respectively). The investigators reported significant and dose-related elevations in levels of total lipids and various lipid fractions. The maximum increase was seen in triglyceride levels, and the lowest increase was observed in cholesterol levels. The authors concluded that changes in lipid metabolism were caused by liver damage. However, given the uncertainties surrounding the identity and purity of the administered material, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

Another study that investigated the histological effects of a Sunset Yellow/Tartrazine mixture (no information on the ratio or purity reported) in Wistar rats (five of each sex per group) was reported by Ching et al. (2005). The colour mixture was orally administered by gavage to rats at doses of 500, 1000 or 2000 mg/kg bw on 3 consecutive days. Gross examination of tissues revealed marked ulcerative lesions and haemorrhage on the antra of stomachs of rats given the mixture at 2000 mg/kg bw, but only hyperaemia at 1000 mg/kg bw. At 1000 mg/kg bw, mild splenomegaly, hepatomegaly and enlarged pale kidneys were observed in the rats administered the colour mixture at 1000 or 2000 mg/kg bw by either oral or intraperitoneal injection. Histopathological examination of sections from the liver, kidney, spleen, stomach and ileum of rats treated with Sunset Yellow FCF revealed a variety of dose-related degenerative, inflammatory and proliferative lesions, which included necrosis, especially in the liver. Necrosis was observed in the kidneys, glomeruli and renal papillae as well as in splenic tissue. Given the uncertainties surrounding the identity and purity of the administered material and the small number of animals, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mouse

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 50 male and 50 female B6C3F1 mice at a concentration of 0, 12 500 (only 49 males per group) or 25 000 mg/kg in the diet for 103 weeks, and then

the animals were placed on a control diet for 1 week. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Gross and histopathological examinations were performed on all animals. Tissues examined included skin (abdominal), lungs and bronchi, trachea, bone, bone marrow (femur), thigh muscle, spleen, lymph nodes, thymus, heart, salivary glands, liver, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain, epididymis, eye and all tissue masses.

The mean body weights of male and female mice receiving Sunset Yellow FCF at a dietary concentration of 25 000 mg/kg were slightly lower (<10%) than those of the control animals throughout most of the study. However, the survival of male and female mice was similar between treated animals and controls (males: control 38/50 [76%], low dose 40/50 [80%] and high dose 33/50 [66%]; and females: control 38/50 [76%], low dose 35/50 [70%] and high dose 43/50 [86%]). An increased incidence of hepatocellular carcinomas was reported among males in the low-dose (46%) and high-dose (32%) groups relative to the control males (26%), but it achieved statistical significance ($P = 0.02$) only at the low dose. In contrast, no significant differences were observed in females. The increased incidence of hepatocellular carcinomas in male mice was not considered to be related to the test material because of the variability in tumour occurrence in control male B6C3F1 mice and because the incidence was not significantly increased in high-dose male mice. The investigators reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of Sunset Yellow FCF in B6C3F1 mice at doses up to 25 000 mg/kg diet (equivalent to 3750 mg/kg bw per day) (Huff, 1982; NCI/NTP, 1982). Although there was a body weight decrement among males and females at the highest tested dose compared with control animals, it was less than 10%, a value not considered to be biologically significant; the no-observed-adverse-effect level (NOAEL) for this study is therefore considered to be 25 000 mg/kg diet (equivalent to 3750 mg/kg bw per day).

Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) was administered to CD-1 COBS (ICR-derived) mice ad libitum at a dietary level of 5000, 15 000 or 50 000 mg/kg. Two separate groups were fed the control diet. Each group consisted of 60 males and 60 females that were randomly assigned. The study was terminated at 20 months for the male mice and at 23 months for the female mice by sacrificing the surviving animals.

The mortality rate was higher in male mice fed diets containing Sunset Yellow FCF at a dietary concentration of 50 000 mg/kg than in the male control groups ($P < 0.01$, life table analysis). Mean body weights of male mice of this dosage group were less than those of the pooled controls throughout the study, despite elevated feed consumption. In female mice, elevated feed consumption occurred for the group fed Sunset Yellow FCF at 50 000 mg/kg in the diet. Elevated feed consumption also occurred for the male mice fed Sunset Yellow FCF at 5000 and 15 000 mg/kg diet.

Complete histopathology was done on the controls and the high-dose groups. Histopathological examinations of all tissue masses and other gross changes

of an uncertain nature were done for mice in the two lowest dose groups. Gross postmortem examinations of treated mice revealed yellow to orange discoloration of the gastrointestinal tract by Sunset Yellow FCF.

The gross and microscopic examinations of tissues and organs of mice from this study revealed no adverse morphological changes that could be attributed to treatment with Sunset Yellow FCF. A detailed statistical analysis indicated that this colour additive had no effect on the incidence of any tumour type or on the time to tumour formation at concentrations up to 50 000 mg/kg diet. However, based on deaths and a body weight decrement relative to the controls at 50 000 mg/kg diet, the NOAEL for the study is 15 000 mg/kg diet (equivalent to 2250 mg/kg bw per day) (USFDA, 1986).

(b) Rat

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 50 male and 50 female F344 rats at a concentration of 0, 12 500 or 25 000 mg/kg in the diet for 103 weeks, followed by a control diet for 1 week. Ninety male and 90 female rats served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Gross and histopathological examinations were performed on all animals. Tissues examined were the same as those described for the mouse (see above).

The mean body weights of male rats at the highest concentration were slightly lower than those of the control animals throughout the study (<10%). The survival of male and female rats was similar between treated animals and controls (males: control 70/90 [78%], low dose 36/50 [72%] and high dose 38/50 [76%]; and females: control 66/88 [75%], low dose 40/50 [80%] and high dose 37/50 [74%]). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material, and no other effects were reported. Therefore, under the conditions of the 2-year bioassay, there was no clear evidence of carcinogenicity of Sunset Yellow FCF in F344 rats at doses up to 25 000 mg/kg diet (equivalent to 1250 mg/kg bw per day) (Huff, 1982; NCI/NTP, 1982). As the body weight decrement among males at the highest dose was less than 10%, a value not considered to be biologically significant, the NOAEL for this study is considered to be 25 000 mg/kg diet (equivalent to 1250 mg/kg bw per day).

To assess the effects of chronic daily exposure to Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) in albino (CD) rats (60 of each sex per group), Sunset Yellow FCF was admixed in the diet at 7500, 15 000 or 30 000 mg/kg (equivalent to 375, 750 and 1500 mg/kg bw per day, respectively), and the rats were fed ad libitum for 2 years. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Sunset Yellow FCF as administered to the parental generation. Although the survival of rats was shortened and the delivered pups had lower body weights relative to controls at a Sunset Yellow FCF dietary concentration of 30 000 mg/kg, a second dietary exposure study with a Sunset Yellow FCF concentration of 50 000 mg/kg in diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 30 000 mg/kg level in the

first study did not achieve the maximum tolerated dose (MTD) for the purpose of maximizing the potential to assess carcinogenicity. The reproductive aspects of this study are described under section 2.2.5. Two groups of controls were fed the plain diet in the first study, and one group of controls was included in the second study.

For the chronic phase, offspring (70 of each sex) were selected randomly from each of the treated and control groups. Interim sacrifice and necropsy of 10 rats of each sex per group were performed 1 year after the initiation of the long-term feeding study in the offspring. Tissues from all rats in the three control groups and the two high-dose groups (30 000 and 50 000 mg/kg diet) were prepared and sectioned for histopathological examination. Histopathological evaluations were performed on all tissue masses and gross lesions from animals in the two lowest dose groups.

Although feed consumption by all treated groups in both studies was generally increased, the body weights of rats fed the 50 000 mg/kg diet were lower compared with controls throughout the study. In the chronic phase, survival of F_0 males fed Sunset Yellow FCF at 50 000 mg/kg diet was decreased ($P = 0.01$). There were no treatment-related differences in haematology measurements, clinical chemistry measurements or urinalysis in either study. A slight increase in kidney weights in females treated with Sunset Yellow FCF at 50 000 mg/kg diet was observed at the 1-year interim sacrifice, but this was not associated with any histopathological abnormality. At the terminal sacrifice, females treated with Sunset Yellow FCF at dietary concentrations of 30 000 and 50 000 mg/kg had increased mean relative kidney weights ($P = 0.05$).

There was no evidence of a carcinogenic effect in male rats that could be attributed to treatment with Sunset Yellow FCF. However, in treated female rats, a higher incidence of adrenal medullary tumours was observed at Sunset Yellow FCF concentrations of 30 000 and 50 000 mg/kg diet. As a confirmatory step, the USFDA requested that additional histopathological sections be prepared from the preserved adrenal tissue blocks and subjected to scrutiny by a panel of pathologists. Adrenal glands from female rats of the two lowest dose groups not previously examined were also sectioned and submitted for review.

According to the USFDA panel of pathologists, the incidence of adrenal medullary tumours (phaeochromocytoma) in female rats was 12/68 (17.6%) in control 1A, 6/66 (9.1%) in control 1B, 7/66 (10.6%) in the 7500 mg/kg diet group, 9/64 (14.1%) in the 15 000 mg/kg diet group and 15/66 (22.7%) in the 30 000 mg/kg diet group. Prevalence statistical tests for high dose relative to combined controls and dose-related trend test yielded *P*-values of 0.054 and 0.022, respectively, for these incidences. (The prevalence analysis is a time-adjusted statistical test for comparing incidences of lesions considered to be non-lethal.) In the second study, the 50 000 mg/kg diet group and its control female group had medullary tumour incidences of 15/68 (22.1%) and 5/70 (7.1%), respectively. Utilizing the low control incidence in this study, the prevalence *P*-value for this comparison was 0.01.

The following considerations suggest that it is unlikely that a causal relationship exists between the occurrence of phaeochromocytoma and exposure to Sunset Yellow FCF:

- small magnitude of difference and absence of a dose–response relationship;
- absence of any precancerous lesions;
- morphological similarity of adrenal medullary lesions in treated and control rats;
- unaffected latency period (time to tumour onset);
- absence of a similar response in male rats;
- lack of concordance with other Sunset Yellow FCF carcinogenicity studies.

In the first rat study on Sunset Yellow FCF, no renal cortical tumours were reported in the high-dose group (30 000 mg/kg diet) or either of the two control groups of female rats. In the second study, five females in the 50 000 mg/kg diet group were reported to have a renal tubular adenoma. No renal cortical tumours were reported in the control groups of the first study, although one control female rat had a transitional cell tumour. In the second study, females in the group fed Sunset Yellow FCF at 50 000 mg/kg diet and the control group had a high incidence of chronic progressive nephrosis (also called old-rat nephropathy).

It is unlikely that these renal cortical tumours are related to Sunset Yellow FCF exposure because of a number of considerations, such as the following:

- A survey of more than 230 chemicals tested in chronic toxicity bioassays by the United States National Cancer Institute/National Toxicology Program showed that chemically induced neoplasia of the kidney occurred more commonly in males than in females (Kluwe et al., 1984). There is no reason to believe that Sunset Yellow FCF would be an exception to the general observation that male rats are more sensitive than female rats to the effects of renal cortical carcinogens.
- There were no malignant or precancerous renal tubular lesions observed in the female rats.
- If Sunset Yellow FCF were a carcinogen for rat kidneys, then at least some of the proliferative lesions observed in the study should have progressed to become malignant.
- There was no reduction in the latency period.
- There is an absence of corroborative evidence from other carcinogenicity studies.

Based on these considerations, it is considered unlikely that Sunset Yellow FCF is able to induce carcinogenic activity in rats at concentrations up to 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day). However, reduced body weight gain and poorer survival in rats were observed at a Sunset Yellow FCF dietary concentration of 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day). Hence, the NOAEL for the chronic phase of the study is 30 000 mg/kg diet (equivalent to 1500 mg/kg bw per day) (USFDA, 1986).

2.2.4 Genotoxicity

The genotoxicity of Sunset Yellow FCF is summarized in Table 1.

Table 1. Genotoxicity of Sunset Yellow FCF

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	L61178Y tk^+/tk^- mouse lymphoma cells	Up to 5000 µg/ml, ±S9	Negative (–S9) Weak positive, LOED 1000 µg/ml (+S9)	McGregor et al. (1988)
Reverse mutation	*Salmonella typhimurium* TA98, TA100	Pooled bile (0.2, 0.4, 0.6 ml/plate; ±S9) from 5 rats, 4 h after treatment with 1500 mg/kg bw by oral gavage	Negative	Wever et al. (1989)
		Pooled urine (0.2, 0.4, 0.6 ml/plate; ±S9) from 6 rats, three doses of 1500 mg/kg bw over 2 days		
		Pooled faecal extracts (0.2, 0.4, 0.6 ml/plate; ±S9) from 6 rats, three doses of 1500 mg/kg bw over 2 days	Negative (–S9) Positive (+S9)	
	S. typhimurium TA98, TA100, TA1535, TA1537	10–250 mg/plate, ±S9	Negative	Muzzall & Cook (1979)
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Up to 5000 µg/plate, ±S9 Metabolites sulfanilic acid and 4-amino-1-naphthalenesulfonic acid: both up to 5000 µg/plate	Negative	Chung, Fulk & Andrews (1981)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 5000 µg/plate, ±S9	Negative	Ishidate et al. (1984)
	S. typhimurium TA1535, TA1538; *Escherichia coli* WP2uvrA	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Haveland-Smith & Combes (1980)

End-point	Test system	Concentration	Result	Reference
Chromosomal aberration	Chinese hamster fibroblast line	Up to 6000 µg/ml, –S9 24 h and 48 h incubation	Positive	Ishidate et al. (1984)
	Chinese hamster ovary cells	Up to 5000 µg/ml, ±S9 8 h incubation without S9 and 2 h with S9	Negative	Ivett et al. (1989)
		Up to 5000 µg/ml, ±S9 25 h incubation without S9 and 2 h with S9	Negative	
In vivo				
Unscheduled DNA synthesis	NMRI, C3H and C57B1 mouse; Chinese hamster (all by oral gavage)	500, 1000, 1500 or 2000 mg/kg bw and 30 h exposure	Negative	Wever et al. (1989)
Micronucleus formation	CRH mouse or hooded rat (oral gavage) bone marrow cells	500, 1000 or 2000 mg/kg bw and 24 h or 48 h exposure	Negative	Westmoreland & Gatehouse (1991)
	Swiss mouse (oral gavage); colonic epithelial cells	200 or 1000 mg/kg bw and 24 h exposure	Negative	Poul et al. (2009)
	Chinese hamster bone marrow cells	1500 mg/kg bw and 30 h exposure	Negative	Wever et al. (1989)
Comet assay	ddY mouse (oral gavage); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	2000 mg/kg bw and 3 h or 24 h exposure	Negative	Sasaki et al. (2002)

LOED, lowest-observed-effect dose; S9, 9000 × *g* supernatant from rat liver

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

To assess the effects of chronic daily exposure to Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) in albino (CD) rats (60 of each sex per group), Sunset Yellow FCF was admixed in the diet at 7500, 15 000 or 30 000 mg/kg diet (equivalent to 375, 750 and 1500 mg/kg bw per day, respectively), and the rats were fed ad libitum for 2 years. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Sunset Yellow FCF as administered to the parental generation. Although survival of the rats was shortened and delivered pups had lower body weights at the Sunset Yellow FCF concentration of 30 000 mg/kg diet relative to the control, a second dietary exposure study with Sunset Yellow FCF at a concentration of 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 30 000 mg/kg dietary level in the first study did not achieve the MTD. Two groups of controls were fed the plain diet in the first study, and one group of controls was included in the second study.

Reduced pup weights occurred at 15 000, 30 000 and 50 000 mg/kg diet, and offspring survival was reduced in the groups treated with Sunset Yellow FCF at 30 000 or 50 000 mg/kg diet. The body weights of rats fed Sunset Yellow FCF at 50 000 mg/kg diet were lower throughout the study. However, the body weights of treated and control rats of the first study were generally comparable, despite lower pup weights in groups treated with Sunset Yellow FCF at 15 000 and 30 000 mg/kg diet relative to the controls. No other adverse effects were observed. Hence, the NOAEL for reproductive toxicity is 7500 mg/kg diet (equivalent to 375 mg/kg bw per day) (USFDA, 1986).

Sunset Yellow FCF (purity >85%) was administered to groups of Crj:CD-1 mice (10 of each sex) in their diet at a concentration of 0, 1500, 3000 or 6000 mg/kg diet in a two-generation reproduction study. Dosing commenced when mice in the F_0 generation were 5 weeks old and continued until mice in the F_1 generation were 9 weeks old. Feed consumption data indicated no significant difference between controls and those groups consuming Sunset Yellow FCF. The actual dose of Sunset Yellow FCF achieved in each group was approximately 250, 500 and 1000 mg/kg bw per day for non-lactating mice at dietary concentrations of 1500, 3000 and 6000 mg/kg diet, respectively. However, during lactation, the dose ranged up to approximately 890, 1650 and 3360 mg/kg bw per day, respectively. Mice were weighed on days 0, 2, 4, 7, 14, 21, 28 and 30 during the pre-mating phase. Females were paired 1:1 with males and separated after 5 days. Dams were allowed to deliver and rear their offspring in solitude. Pups were weighed on postnatal days (PNDs) 0, 4, 7, 14 and 21. Functional and behavioural parameters, such as surface righting (PNDs 4 and 7), negative geotaxis (body righting on an inclined plane; PNDs 4 and 7), cliff avoidance (PND 7), swimming behaviour (PNDs 4 and 14) and olfactory orientation (PND 14), were measured in all F_1 pups during PNDs 0–21. On PND 49, all pups performed in a multiple water T-maze daily for 3 consecutive days.

For F_1 pups, there were no significant differences observed in litter size, litter weight or sex ratio. During lactation, there were no dose-related changes in body weight or survival. Score frequencies for swimming direction at PND 4 (but not PND 14) were significantly depressed in both males and females, but were dose dependent only in females. The score for swimming head angle was also affected in a dose-dependent manner in females. Scores for surface righting at PND 7, but not at PND 4, and negative geotaxis at PND 4, but not at PND 7, were affected in males only at the middle dose (3000 mg/kg diet). These effects were not dose related. Several of the other measured functional and behavioural parameters differed from controls, but not in a manner that allowed the investigators to conclude that they were related to exposure to Sunset Yellow FCF. Hence, in the absence of any effects on the reproductive parameters and inconsistent neurological outcomes, it can be concluded that the NOAEL for this study is 6000 mg/kg in the diet (approximately 1000 mg/kg bw per day), the highest dose tested (Tanaka, 1996).

(b) Developmental toxicity

No information on the developmental toxicity of Sunset Yellow FCF was available.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical manifestations attributed to food intolerance usually include recurrent urticaria/angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Mikkelsen et al., 1978; Ibero et al., 1982; Schultz-Ehrenburg & Gilde, 1987; Wilson & Scott, 1989; Worm et al., 2000). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria/angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang et al., 1994).

2.3.2 Clinical trials

In 1990, Pollock and Warner conducted a study to investigate claims by parents that the behaviour of their 39 children aged between 3 and 15 years (mean 8.9 years) improved on a synthetic food additive–free diet, but deteriorated markedly with lapses from the diet. The children were recruited for the study from a paediatric allergy clinic and from a population survey of food additive intolerance. The trial consisted of a double-blind, placebo-controlled challenge with synthetic food colours. The food colours tested were a mixture of Tartrazine (50 mg), Sunset Yellow FCF (25 mg), Carmoisine (25 mg) and Amaranth (25 mg). Only 19 children completed the double-blind, placebo-controlled challenge study with artificial food colours. In these children, food colours were shown to have an adverse effect on a daily Conners' rating of behaviour, but most parents could not detect these changes.

This disparity between the results of the behaviour scores and the parents' weekly assessments is important when it is remembered that entry into the study was based on the parents' claim to be able to detect when their children had consumed food additives. It is important to acknowledge that the doses of food colours used in this trial were considerably greater than the amounts the children are likely to consume through food. The authors postulated that a pharmacological mechanism of food additive intolerance—namely, histamine release from basophils—was possible.

Bateman et al. (2004) investigated the behavioural effects on 3-year-old children (n = 277) of ingesting a high-dose azo food dye mixture containing Sunset Yellow FCF, Tartrazine, Carmoisine and Ponceau 4R (5 mg of each) and sodium benzoate (45 mg) in a double-blind, placebo-controlled study. The children were classified as having hyperactivity (HA) (using two different activity scales: emotionality, activity and sociability; and Weiss–Werry–Peters) or not, with and without atopy (AT) (i.e. positive skin prick test with a number of known protein allergens), in a 2 × 2 group design (AT/HA, non-AT/HA, AT/non-HA, non-AT/non-HA). Over a 4-week period, the children received either the azo dye mixture with fruit juice or placebo (fruit juice only) on the 2nd and 4th weeks. Children's behaviour was assessed by research psychologists using validated tests and by the parents. Using assessments made by the parents, there were significant reductions in hyperactive behaviour during the withdrawal phase. Furthermore, there were significantly greater increases in hyperactive behaviour during the active period compared with the placebo period. These effects were not influenced by the presence or absence of previously diagnosed hyperactivity or by the presence or absence of atopy. However, there were no significant differences detected based on objective interactive testing by psychologists in the clinic.

A follow-up study was conducted to further investigate the association of ingestion of a mixture of food colour additives and sodium benzoate with hyperactive behaviour in children. The hypothesis was tested using a community-based, double-blind, placebo-controlled, randomized crossover food challenge in which two groups of children aged 3 years (n = 153) and 8 or 9 years (n = 144) received one of two mixtures of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except for Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions plus sodium benzoate) were those tested in the Bateman et al. (2004) study, whereas mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel

global hyperactivity aggregate (GHA) measure, which comprised an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- and 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Sunset Yellow FCF. The Committee received a submission from EFSA concerning dietary exposure to Sunset Yellow FCF that was a part of its re-evaluation of the safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Sunset Yellow FCF is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, it is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Sunset Yellow FCF made using Global Environment Monitoring System – Food

Table 2. Maximum permitted use levels of Sunset Yellow FCF in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks	50
Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Confectionery Fine bakery wares Edible ices Desserts including flavoured milk products Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Sobrasada	135
Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Sunset Yellow FCF is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Sunset Yellow FCF (E 110) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Sunset Yellow FCF, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children, assuming that the maximum level in beverages was 50 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(50 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for United Kingdom children aged 1.5–4.5 years were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and with maximum permitted levels of use as specified in the EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Sunset Yellow FCF exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the

adult food consumption data for the United Kingdom population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.3 to 2.5 mg/kg bw per day, whereas those at the 95th percentile were from 0.7 to 6.7 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 1.4 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 3.5 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (14%) and fruit wines, cider and perry (13%).

The tier 3 approach employed by EFSA used maximum reported Sunset Yellow FCF use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Sunset Yellow FCF for European children ranged from 0.2 to 2.1 mg/kg bw per day at the mean and from 0.6 to 5.8 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.1 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 3.2 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.3 mg/kg bw per day at the mean and 0.9 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (60%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (18%).

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Sunset Yellow FCF in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Sunset Yellow FCF were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

Table 3. EFSA dietary exposures to Sunset Yellow FCF

	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		8.1[a]
Maximum permitted levels			
- Mean exposure	0.5	1.4	0.3–2.5
- Exposure at the 95th or 97.5th percentile	1.1	3.5	0.7–6.7
Maximum reported use levels			
- Mean exposure	0.3	1.1	0.2–2.1
- Exposure at the 95th or 97.5th percentile	0.9	3.2	0.6–5.8

[a] For children (age range not specified).

Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Sunset Yellow FCF in survey foods were used. Both detected and "non-detect" results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical concentrations of Sunset Yellow FCF in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Sunset Yellow FCF. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Sunset Yellow FCF was 1.12 mg/day, with a 90th percentile exposure of 3.46 mg/day. The highest subpopulation mean was 1.82 mg/day, for 13- to 18-year-olds and 19- to 24-year-olds. The highest subpopulation 90th percentile exposure was 5.42 mg/day, for 19- to 24-year-olds. The highest estimates made using the maximum colours scenario were 7.57 mg/day at the mean and 23.43 mg/day at the 90th percentile, both for the 19- to 24-year-old subpopulation. The main contributors to dietary exposure were soft drinks, savoury snacks, ice cream and ice confections, and cordial.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Sunset Yellow FCF calculated by EFSA were higher than those of FSANZ. The Committee concluded that this was

Table 4. FSANZ dietary exposures to Sunset Yellow FCF using the mean colours scenario

Population group	Mean		90th percentile	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.81	0.05	1.97	0.12
6–12 years old	1.30	0.04	3.79	0.12
13–18 years old	1.82	0.03	5.19	0.09
19–24 years old	1.82	0.03	5.42	0.08
25+ years old	0.93	0.01	2.95	0.04
2+ years old	1.12	0.02	3.46	0.06

due to EFSA's use of maximum permitted and reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 6 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Sunset Yellow FCF, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.12 mg/kg bw per day, would be a more realistic dietary exposure estimate because of the extensive post-market analyses used in its preparation. The Committee concluded that the use of the realistic assessment was appropriate.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8* and *59*) with recently published data.

Sunset Yellow FCF has a strongly anionic sulfonated moiety on the molecule, which limits its absorption from the gastrointestinal tract and results in excretion of greater than 95% of an orally administered dose in the faeces, with only about 3% absorbed as the parent compound. However, little of the ingested Sunset Yellow FCF present in faeces remains unchanged, with the extent of bacterial reduction of the azo group being dependent on the administered dose. Sunset Yellow FCF is metabolized by bacteria in the gastrointestinal tract to yield sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, which are absorbed and metabolized to various *N*-acetylated forms.

Dietary administration of Sunset Yellow FCF to rats at doses up to 2330 mg/kg bw per day for 96 days was reported to cause diarrhoea and distension of the caecum at doses equal to and above 1500 mg/kg bw per day. Diarrhoea was

also observed in dogs after repeated oral exposure to Sunset Yellow FCF at a dose equivalent to 1250 mg/kg bw per day, but no details on the duration of the study or the sex of the animals were available. At 2330 mg/kg bw per day, increased relative weights of the testes were observed in rats, but without any accompanying histopathological lesions.

In contrast, a feeding study in rats completed in 2005 reported degenerative changes in the testes after 90 days of administration of Sunset Yellow FCF at doses equivalent to 250 or 1500 mg/kg bw per day. However, in that study, the purity of the administered Sunset Yellow FCF, which was purchased at a local market in India, was unknown. The presence of impurities in the administered material may explain the lack of concordance with the absence of any testicular lesions in studies of longer duration (80–104 weeks) and at higher doses (up to 2500 mg/kg bw per day) when using Sunset Yellow FCF of known purity. A consistent adverse finding in repeated-dose feeding studies in mice and rats was reduced body weight gain (>10%) at doses in excess of 2250 mg/kg bw per day in adult mice and in excess of 1500 mg/kg bw per day in adult rats. Reduced body weight gain was also observed in dogs after 2–3 months at a dose of 1250 mg/kg bw per day, but not in pigs after 98 days at dietary concentrations equivalent to a dose of 1000 mg/kg bw per day.

Eight long-term studies previously reviewed by the Committee showed no evidence of carcinogenicity at concentrations in the feed equivalent to an oral dose of up to 3000 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The present review included five additional long-term repeated-dose studies that tested dietary concentrations of Sunset Yellow FCF equivalent to oral doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The absence of carcinogenicity in the long-term bioassays is consistent with the weight of evidence from a range of in vitro and in vivo genotoxicity tests reviewed at this meeting and at previous meetings, indicating that Sunset Yellow FCF is not genotoxic.

No adverse effects on reproductive performance in mice and rats have been reported following dietary exposure to Sunset Yellow FCF at doses up to 1000 mg/kg bw per day. However, reduced rat pup survival was observed in comprehensive studies at doses of 1500 and 2500 mg/kg bw per day, with reduced pup body weight at doses of 750 mg/kg bw per day and above. Dam body weight was affected only at the highest tested dose of 2500 mg/kg bw per day. The NOAEL for reduced pup body weight was 375 mg/kg bw per day.

Teratogenicity studies in rats and rabbits at oral gavage doses up to 1000 mg/kg bw per day (highest tested dose) did not reveal any compound-related adverse effects.

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Sunset Yellow FCF. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Sunset Yellow FCF could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value

of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Sunset Yellow FCF prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Sunset Yellow FCF calculated by EFSA were much higher than those of FSANZ (0.12 mg/kg bw per day for children at the 90th percentile). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimate of 6 mg/kg bw per day for children should be considered in the safety assessment for Sunset Yellow FCF in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that there were five additional long-term repeated-dose feeding studies that tested Sunset Yellow FCF at dietary concentrations equivalent to doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. One of these long-term studies in rats, which included in utero exposure, had a NOAEL of 375 mg/kg bw per day for reduced body weight among pups. On the basis of this NOAEL and the usual 100-fold uncertainty factor, the Committee established an ADI of 0–4 mg/kg bw (with rounding). The previous ADI of 0–2.5 mg/kg bw was withdrawn. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children, estimated by the more realistic FSANZ approach, was 3% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Sunset Yellow FCF does not present a health concern.

6. REFERENCES

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ANNEXES

ANNEX 1

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants,* Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
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10. *Specifications for identity and purity and toxicological evaluation of food colours.* FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
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19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
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ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

2-AAF	2-acetylaminofluorene
4-AP	4-aminopyridine
4-HDA	4-hydroxyalkenal
8-OH-dG	8-hydroxy-2′-deoxyguanosine
ADHD	attention deficit hyperactivity disorder
ADI	acceptable daily intake
ADRA2A	adrenergic receptor alpha 2A
AFB	aflatoxin B (e.g. AFB_1)
AIC	Akaike's information criterion
AIDS	acquired immunodeficiency syndrome
ALMA	Aluminium-Maladie d'Alzheimer
ALT	alanine aminotransferase
AP	alkaline phosphatase
ApoE	apolipoprotein E
ARfD	acute reference dose
AST	aspartate aminotransferase
AT	atopy
ATPase	adenosine triphosphatase
AUC	area under the concentration versus time curve
BEA	beauvericin
BMC	bone mineral content
BMD	benchmark dose; bone mineral density
BMDL	lower limit of the 95% confidence interval on the benchmark dose
BMR	benchmark response
Bt	*Bacillus thuringiensis*
bw	body weight
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CI	confidence interval
CIT	citrinin
C_{max}	maximum concentration
COMT	catechol *O*-methyl-transferase
ConA	concanavalin A
CPK	creatine phosphokinase
CYP	cytochrome P450
DAT1	dopamine transporter
DNA	deoxyribonucleic acid
DON	deoxynivalenol
DRD4	dopamine D4 receptor
EC	Enzyme Commission
EFSA	European Food Safety Authority

ELEM	equine leukoencephalomalacia
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
EU	European Union
F	female; filial generation
FAO	Food and Agriculture Organization of the United Nations
FB	fumonisin B (e.g. FB_1, FB_2, FB_3)
FB_T	total fumonisins
FC	fumonisin C
FFQ	food frequency questionnaire
FSANZ	Food Standards Australia New Zealand
GD	gestation day
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GGT	γ-glutamyl transferase
GHA	global hyperactivity aggregate
GLC	gas–liquid chromatography
GLP	good laboratory practice
GMP	good manufacturing practice
GSFA	Codex General Standard for Food Additives
GSH	glutathione (reduced)
GST	glutathione *S*-transferase
GSTP	placental glutathione *S*-transferase
HA	hyperactivity
HACCP	hazard analysis and critical control point
HFB	totally hydrolysed fumonisin B
HIV	human immunodeficiency virus
HNMT	histamine *N*-methyl-transferase
HPLC	high-performance liquid chromatography
HTLV	human T-cell lymphotropic virus
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
ICGMA	International Council of Grocery Manufacturer Associations
ICP	inductively coupled plasma
ID_{50}	median inhibitory dose
IFN	interferon (e.g. IFN-γ)
IG_{50}	median growth inhibitory concentration
Ig	immunoglobulin (e.g. IgA, IgE, IgG, IgM)
IL	interleukin (e.g. IL-1β, IL-2)
INCA-2	Second National Individual Survey on Food Consumption (France)
INS	International Numbering System
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LA	linoleic acid
LC	liquid chromatography
LD_{50}	median lethal dose
LDH	lactate dehydrogenase

LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOED	lowest-observed-effect dose
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LPS	lipopolysaccharide
M	male
MDA	malondialdehyde
ML	maximum level
MMSE	Mini-Mental State Examination
MON	moniliformin
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
NFT	neurofibrillary tangles
NIV	nivalenol
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NS	not specified
NTD	neural tube defect
NTP	United States National Toxicology Program
OA	oleic acid
OECD	Organisation for Economic Co-operation and Development
OES	optical emission spectrometry
OR	odds ratio
OTA	ochratoxin A
P	probability
PA	penicillic acid; palmitic acid
PAQUID	Personnes Âgées Quid
PCR	polymerase chain reaction
PH	partial hepatectomy
PHA	phytohaemagglutinin
PHA-P	phytohaemagglutinin P
PHFB	partially hydrolysed fumonisin B
PMTDI	provisional maximum tolerable daily intake
PND	postnatal day
PPARα	peroxisome proliferator-activated receptor alpha
PPE	porcine pulmonary oedema
PRRSV	porcine reproductive and respiratory syndrome virus
p-Si	poly-silicon
PST-M	phenolsulfotransferase-M
PST-P	phenolsulfotransferase-P
PTWI	provisional tolerable weekly intake
RNA	ribonucleic acid
RR	relative risk

RSD	relative standard deviation
RSD_r	relative standard deviation for within-laboratory repeatability
RSD_R	relative standard deviation for between-laboratory reproducibility
RT-PCR	reverse transcriptase polymerase chain reaction
S1P	sphingoid base 1-phosphate
S1PR	sphingoid base 1-phosphate receptor
S9	9000 × *g* rat liver supernatant
SAR	Special Administrative Region
SCOOP	Scientific Cooperation
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SOD	superoxide dismutase
SPE	solid-phase extraction
T_3	triiodothyronine
T_4	thyroxine
TBARS	thiobarbituric acid reactive substances
TCA	tricarballylic acid
TDI	tolerable daily intake
TLC	thin-layer chromatography
TNFα	tumour necrosis factor alpha
TOS	total organic solids
TUNEL	terminal deoxynucleotidyl transferase–mediated deoxyuridine diphosphate nick-end labelling
UGT	uridine diphosphate glucuronosyltransferase
UNESDA	Union of European Soft Drinks Associations
UPLC	ultra high-performance liquid chromatography
USA	United States of America
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
UV	ultraviolet
WHO	World Health Organization
w/w	weight per weight
ZEA	zearalenone

ANNEX 3

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 14–23 June 2011

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ANNEX 4

TOLERABLE AND ACCEPTABLE INTAKES, OTHER TOXICOLOGICAL INFORMATION AND INFORMATION ON SPECIFICATIONS

Food additives evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Aluminium-containing food additives (including new food additives potassium aluminium silicate and potassium aluminium silicate–based pearlescent pigments)	N, T[b]	**The Committee established a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight** based on a no-observed-adverse-effect level (NOAEL) of 30 mg/kg body weight per day and application of an uncertainty factor of 100. The PTWI applies to all aluminium compounds in food, including food additives. **The previous PTWI of 1 mg/kg body weight was withdrawn**. For adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI. Estimates of dietary exposure of children to aluminium-containing food additives, including high dietary exposures (e.g. 90th or 95th percentile), can exceed the PTWI by up to 2-fold. For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI. The Committee recommended that provisions for food additives containing aluminium included in the Codex General Standard for Food Additives should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg body weight as aluminium from all sources.

Annex 4 (contd)

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Benzoe Tonkinensis	N, T	**The Committee concluded that the available data were inadequate to establish an acceptable daily intake (ADI)** because of the variability in composition of Benzoe Tonkinensis and the inadequate characterization of the material tested. The margin of exposure between the conservative dietary exposure estimate of 0.2 mg/kg body weight per day and the NOAEL of 500 mg/kg body weight per day identified in a 90-day oral toxicity study in rats is 2500. Given this margin of exposure as well as the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, **the Committee concluded that Benzoe Tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.** The Committee also noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe Tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg body weight) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg body weight). The Committee further noted that benzoic acid, one of the major components of Benzoe Tonkinensis, is used as a preservative, but that Benzoe Tonkinensis has not been assessed for this use.
Glycerol ester of gum rosin (GEGR)	R, T	**The Committee withdrew the group ADI for GEGR and glycerol ester of wood rosin (GEWR) and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight,** pending the submission of the full reports of the 90-day toxicity studies on GEGR as well as additional compositional information on the GEWR from *Pinus elliottii*. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Glycerol ester of tall oil rosin (GETOR)	R, T	**The Committee was unable to complete the evaluation of GETOR** because additional data are required to characterize the GETOR in commerce. Validated methods for the determination of the substances considered in the specifications are also required. The above information should be submitted by the end of 2012.

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Glycerol ester of wood rosin (GEWR)	R, T	**The Committee withdrew the group ADI for GEGR and GEWR and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight,** applying an additional uncertainty factor of 2, because new information raises questions about the identity and composition of the product in commerce. Additional compositional information on the GEWR from *Pinus elliottii* to assess similarity with the GEWR from *Pinus palustris* is required. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Octenyl succinic acid (OSA) modified gum arabic	R	**The Committee deferred further evaluation of OSA modified gum arabic** pending the submission of data on its stability in food and on the extent to which it is hydrolysed in the gastrointestinal tract, to be provided by the end of 2013. The existing temporary ADI "not specified"[c] was retained.
Polydimethyl siloxane	M	**The Committee withdrew the temporary ADI of 0–0.8 mg/kg body weight and re-established the ADI of 0–1.5 mg/kg body weight,** originally established at the eighteenth meeting.
Ponceau 4R	R	**The Committee concluded that new data do not indicate a need to revise the existing ADI of 0–4 mg/kg body weight** and that dietary exposure to Ponceau 4R does not present a health concern.
Pullulan	R	Dietary exposure to pullulan as a dietary fibre could reach 1 g/kg body weight per day for children (2–5 years old) and 0.4 g/kg body weight per day for the general population (2 years of age and older). These estimates are 8 and 20 times lower, respectively, than the no-observed-effect level (NOEL) observed in the 90-day rat study evaluated previously. Gastrointestinal effects observed in humans should be taken into account when considering appropriate use levels. The Committee stressed that it assessed the safety of use and not the efficacy of pullulan used as a dietary fibre. **The Committee maintained the previously established ADI "not specified"[c] for the previously evaluated food additive uses.**
Pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*	N	**The Committee established an ADI "not specified"[c] for pullulanase from *B. deramificans* expressed in *B. licheniformis*** when used in the applications specified and in accordance with good manufacturing practice.

Annex 4 (contd)

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Quinoline Yellow	R, T	**The Committee established a temporary ADI of 0–5 mg/kg body weight,** incorporating an additional 2-fold uncertainty factor, pending submission of requested toxicological studies by the end of 2013. **The previously established ADI of 0–10 mg/kg body weight was withdrawn.** The conservative exposure estimates were within the range of the temporary ADI. Additional information on the composition of the product in commerce is required, in particular relating to the identity and purity of the unmethylated form of Quinoline Yellow.
Sunset Yellow FCF	M	**The Committee established an ADI of 0–4 mg/kg body weight and withdrew the previous ADI of 0–2.5 mg/kg body weight**. The Committee concluded that dietary exposure to Sunset Yellow FCF does not present a health concern.

[a] M, existing specifications maintained; N, new specifications prepared; R, existing specifications revised; T, tentative specifications.

[b] For potassium aluminium silicate and pearlescent pigments containing potassium aluminium silicate.

[c] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice—i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

Food additives considered for specifications only

Food additive	Specifications[a]
β-Apo-8′-carotenal	R
β-Apo-8′-carotenoic acid ethyl ester	R
β-Carotene, synthetic	R
Hydroxypropyl methyl cellulose	R[b]
Magnesium silicate, synthetic	R
Modified starches	R
Nitrous oxide	R
Sodium carboxymethyl cellulose	R
Sucrose monoesters of lauric, palmitic or stearic acid	R

[a] R, existing specifications revised.

[b] The Committee concluded that levels of propylene chlorohydrins up to the new limit of not more than 1 mg/kg for the sum of both isomers in hydroxypropyl methyl cellulose were not of toxicological concern.

Analytical methods for food additives in the Combined Compendium of Food Additive Specifications, Volume 4 (FAO JECFA Monographs 1, 2006)

Food additive	Method[a]
Colouring matters content by spectrophotometry	R, T

[a] R, existing method revised; T, tentative method.

Contaminants evaluated toxicologically

Cyanogenic glycosides

The Third Session of the Codex Committee on Contaminants in Food (CCCF) in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate.

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an acute reference dose (ARfD) for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

Derivation of the ARfD

Following review of a developmental toxicity study with linamarin, the Committee considered this study as suitable for establishing an ARfD. Benchmark dose (BMD) modelling of the data from this study provided a lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for linamarin of 85 mg/kg body weight for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. Although the study did not use dietary exposure, gavage dosing was considered relevant to establishing the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg body weight (equivalent to 0.09 mg/kg body weight as cyanide). This value was considered, when compared on a cyanide molar basis, to also be applicable to other cyanogenic glycosides. Therefore, the Committee recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg body weight. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

Derivation of the provisional maximum tolerable daily intake (PMTDI)

In a 13-week United States National Toxicology Program study not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest BMDL for a one standard deviation response ($BMDL_{1SD}$) of 1.9 mg/kg body weight per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 0.02 mg/kg body weight by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

Comparison of estimated dietary exposures with health-based guidance values and the impact of maximum limits (MLs) on dietary exposure

Estimated dietary exposures to total available hydrocyanic acid (HCN) were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 0.09 mg/kg body weight as cyanide equivalents was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 0.02 mg/kg body weight as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold for children and between 1- and 2-fold for adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded, which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give an HCN concentration of 2 mg/kg.

ARfD: 0.09 mg/kg body weight as cyanide (applies only to foods containing cyanogenic glycosides as the main source of cyanide)

PMTDI: 0.02 mg/kg body weight as cyanide

Fumonisins

For the current evaluation of fumonisins, the Committee reviewed all relevant studies performed on fumonisins since 2001.

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents either employing purified fumonisin B_1 (FB_1) or using *Fusarium verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as

a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 µg/kg body weight per day for megalocytic hepatocytes in male mice. Using an uncertainty factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 µg/kg body weight per day. As this was the same value as the previously established group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, this group PMTDI was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 µg/kg body weight per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material, because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 µg/kg body weight per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 µg/kg body weight per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 14.4 µg/kg body weight per day, whereas for consumers with high consumption, exposure would be up to 44.8 µg/kg body weight per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed Codex ML for the category "Corn/maize grain, unprocessed". The Committee noted that implementation

of the proposed MLs would result in rejection of 1–88% of "Corn/maize grain, unprocessed" and 4–57% of "Corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF.

Group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, of 2 µg/kg body weight was retained

This volume contains monographs prepared at the seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 14 to 23 June 2011.

The toxicological monographs in this volume summarize the safety data on a number of food additives: aluminium-containing food additives, Benzoe Tonkinensis, Ponceau 4R, pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*, Quinoline Yellow and Sunset Yellow FCF.

This volume also contains monographs summarizing the toxicological and dietary exposure data for the contaminants cyanogenic glycosides and fumonisins.

This volume and others in the WHO Food Additives Series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

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