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ALUMINIUM
(addendum)
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ALUMINIUM FROM ALL SOURCES, INCLUDING FOOD ADDITIVES (addendum)

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1. EXPLANATION

1.1 Introduction

Various aluminium compounds were evaluated by the Committee at its thirteenth, twenty-first, twenty-sixth, twenty-ninth, thirtieth and thirty-third meetings (Annex 1, references *20*, *44*, *59*, *70*, *73* and *83*). At its thirteenth meeting, an acceptable daily intake (ADI) 'not specified' was established for sodium aluminosilicate and aluminium calcium silicate (Annex 1, reference *20*). 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 set a temporary ADI of 0–0.6 mg/kg bw expressed as aluminium for all aluminium salts added to food, and recommended that aluminium in all its forms should be reviewed at a future meeting.

In the evaluation made by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*), emphasis was placed 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 set a provisional tolerable weekly intake (PTWI) of 0–7.0 mg/kg bw for aluminium, including food additive uses. This was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing sodium aluminium phosphate (SALP) acidic at a concentration of 3% for 189 days, equivalent to approximately 110 mg/kg bw aluminium. A consolidated monograph was produced (Annex 1, reference *84*).

Aluminium was re-evaluated by the Committee at its present meeting, as requested by the Codex Commission on Food Additives and Contaminants (CCFAC) at its Thirty-seventh Session (Codex Alimentarius Commission, 2005).

The Committee was asked to consider all data relevant to the evaluation of the toxicity and intake (including bioavailability) of aluminium used in food additives and from other sources, including SALP. CCFAC asked that the exposure assessment cover all compounds included in the Codex General Standard for Food Additives (GSFA).

Two documents were particularly important in the evaluation made by the Committee at its present meeting: the International Programme on Chemical Safety (IPCS) *Environmental Health Criteria* document on aluminium (WHO, 1997) and a report by the United Kingdom (UK) Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) on a water pollution incident that occurred in Cornwall, England in 1988 (COT, 2005). The Committee used those assessments as the starting point for its evaluation and also evaluated other data in the scientific literature relating to aluminium compounds. No original toxicological data on aluminium-containing food additives were submitted.

1.1.1 General considerations on exposure

Aluminium is the third most abundant element and a major constituent of the earth's crust, where it is present as Al^{3+} in combination with oxygen, fluorine, silicon and other constituents, and not in the metallic elemental state. It is released to the environment both by natural processes and from anthropogenic sources. It is naturally present in varying amounts in most foodstuffs, and concentrations in food crops are influenced by geographical region. Use of aluminium and aluminium compounds in processing, packaging and storage of food products, and as flocculants in the treatment of drinking-water may contribute to its presence in drinking-water and foods. A number of aluminium salts are used as food additives (see section 6.1.4). In general, the foods that contain the highest concentration of aluminium are those that contain aluminium additives (WHO, 1997).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

The mechanism of gastrointestinal absorption of aluminium is complex and has not yet been fully elucidated (WHO, 1997). The extent to which aluminium is absorbed depends upon the amount of the chemical species present in the gut lumen, in the blood and in the interstitial fluid. Aluminium species may be modified in the gut before absorption. Absorption will also be influenced by complexing ligands (e.g. citrate, lactate) and competing ions (e.g. iron, silicon). Some authors have suggested that acid digestion in the stomach would solubilize the majority of ingested aluminium to the monomolecular species. This would then be converted to the aluminium hydroxide as pH is neutralized in the duodenum. The solubility of Al^{3+} is lowest at neutral pH. Most of the substance is expected to precipitate in the

intestine, making it unavailable for uptake, with subsequent faecal excretion. Aluminium complexes, particularly in the presence of carboxylic acids such as citrate, are thought to improve solubility in the intestine and hence increase aluminium available for intestinal uptake (Reiber et al., 1995; Yokel & McNamara, 2001).

There are indications that the toxicokinetics of aluminium are dose-dependent and since high doses have been administered in many studies, the results of these studies, with respect to their relevance to humans, should be interpreted with caution. It should also be considered that accurate quantification of aluminium absorption has proved difficult. One reviewer highlighted the fact that, in the past, the absence of an appropriate radioisotope compromised the reliability of studies. Measurement of blood concentrations appears to be a poor indicator of aluminium absorption and, while urinary excretion appears to provide a better estimation of aluminium absorption, it offers no information about retention in tissues such as bone. It has been suggested that measurement of the ^{26}Al radioisotope by high-energy accelerator mass spectrometry (AMS) offers a more accurate measurement of aluminium levels, since the lower limit of detection (10^{-18} g) allows physiological concentrations of aluminium to be quantified (Drueke, 2002).

A study investigated the influence of the chemical species of aluminium on uptake using the Caco-2 model of gastrointestinal absorption in vitro. Flux across and uptake into Caco-2 cells was investigated for the aluminium ion, and for aluminium citrate, maltolate, hydroxide and fluoride, at a concentration of 8 mmol/l. The flux of aluminium fluoride was dramatically increased at 2 h compared with that of the other aluminium species. This was associated with a reduced transepithelial electrical resistance (TEER) which indicates opening of tight junctions between cells resulting in increased paracellular flux, possibly as a result of toxicity caused by fluoride and/or aluminium. The permeability of all aluminium species highly correlated with a marker of paracellular diffusion, with the exception of aluminium hydroxide (possibly owing to its poor solubility). Calcium (1.25 mmol/l) is required in the culture medium to maintain tight junction integrity and its absence greatly increased aluminium flux across the monolayer. Kinetic studies indicated that uptake of aluminium species into cells was probably the result of passive diffusion. The use of the ^{26}Al radioisotope at 2 µmol/l, a concentration more relevant to drinking-water, showed that uptake and flux of aluminium were not significantly different among the aluminium species. Approximately 0.015% of the aluminium in the uptake medium fluxed across the monolayer, while about 0.75% remained associated with the cells, corresponding to an intracellular aluminium concentration of 5 µmol/l (Zhou & Yokel, 2005).

Rat small intestine was perfused with a buffered (pH 7) solution of aluminium sulfate (93 µmol/l). Of the total aluminium perfused, only 62.2 ± 6.1 (standard deviation, SD) % was recovered from the effluent and 35.1 ± 5.8 (SD) % was recovered from the mucus and mucosa, predominantly in the distal third. Since ultrafiltration experiments had indicated that only 14.3 ± 1.3 (SD)% of a freshly prepared perfusate was able to pass through an ultrafilter, the authors suggested that a proportion of the mucosal aluminium is likely to be colloidal/particulate (Powell et al., 1994).

In studies of rat intestinal perfusion, it was found that aluminium uptake was reduced by various paracellular pathway blockers and a sodium transport blocker. Aluminium uptake from calcium-supplemented medium was increased and the authors suggested this might be owing to decreased resistance to paracellular flux in the absence of calcium (Provan & Yokel, 1988a). Follow-up work suggested aluminium might interact with the calcium uptake pathway since various calcium-channel blockers reduced aluminium uptake. Conversely calcium-channel activators increased aluminium uptake (Provan & Yokel, 1988b).

Studies of rat intestinal perfusion with aluminium chloride (20 mmol/l) found that increasing concentrations of sodium chloride (0–120 mmol/l) did not affect aluminium uptake. However, increasing concentrations of calcium chloride (0–10 mmol/l) were associated with a reduction in aluminium absorption (van der Voet & de Wolff, 1998). The effect of aluminium on radiolabelled calcium (^{45}Ca) uptake was investigated in cultured chicken enterocytes. Increasing concentrations of aluminium lactate (0–150 µmol/l) resulted in approximately 50% reduction in ^{45}Ca uptake, although the effect of aluminium does not appear to be sensitive to calcium-channel activators. Similarly, an isolated intestinal loop experiment in rats showed that the presence of aluminium chloride at 50 mg Al/kg bw[1] resulted in a significant reduction in calcium uptake (Orihuela et al., 2005a).

Aluminium was detected in samples of whole blood, urine and tissue from rats treated with aluminium lactate at 12 mg/kg bw, but not 1 mg/kg bw, by oral gavage (Wilhelm et al., 1992).

Groups of 10 male Wistar rats received either deionized water or drinking-water supplemented with aluminium chloride (5 or 20 mgAl/kg bw per day) for 6 months. The animals were placed in metabolic cages for 6 days before, during (third month) and at the end of the study, for measurement of water consumption and diuresis (balance study). Absorption was reported to be 6.1 and 5.8 µg/kg bw per day, in the groups receiving doses of 5 and 20 mg Al/kg bw per day respectively (Somova & Khan, 1996).

In studies reported by the World Health Organization (WHO, 1997), the relationship between solubility of various aluminium compounds and absorption was examined in Sprague-Dawley rats given aluminium compounds orally at a dose of 1.2 mmol (35 mg Al) per kg bw. Aluminium absorption, measured by urinary excretion, largely mirrored solubility. Urinary excretion of 0.015% of the administered dose was detected with aluminium hydroxide, with excretion being twice that for aluminium chloride and aluminium lactate. Inclusion of citrate resulted in an increase in excretion of 50–100 times. The validity of using urinary excretion as a marker of absorption was assessed in isolated intestinal loop experiments, which supported the excretion data (Froment et al., 1989a). Further work indicated that absorption was likely to occur in the proximal small intestine. A mechanism was proposed whereby potent calcium chelation by aluminium citrate compromises tight

[1] The abbreviation 'Al' is used in expressing dietary concentrations or doses as aluminium rather than as the administered aluminium compound, and for total aluminium content in food, or its associated dietary exposure when the nature of the aluminium compounds present is unknown.

junction integrity, leading to enhanced paracellular absorption (Froment et al., 1989b).

The ^{26}Al radioisotope was used to assess gastrointestinal absorption, tissue retention and urinary excretion of dietary aluminium in the presence and absence of citrate in rats. Groups of 20 rats were given 3.8 ng of ^{26}Al and 63 ng of ^{27}Al by oral gavage in 400 µl of deionized water, with either 20 mg of citric acid or weak hydrochloric acid of similar pH. Urine was collected throughout the study and two animals per dose group were killed at 0.5, 1, 1.5, 2, 4, 6, 8, 120, 360 and 720 h after gavage. ^{26}Al present in plasma reached a peak of 0.01% of the administered dose 1 h after gavage, with a modest but statistically significant increase in aluminium absorption in the presence of citrate (Jouhanneau et al., 1997).

A contemporary study also using the ^{26}Al radioisotope, given to fasted rats as either aluminium hydroxide or citrate, found fractional absorption to be 0.1% and 5% respectively (Schonholzer et al., 1997).

Groups of at least four guinea pigs were fed one of six diets twice per week for 3 weeks: sponge cake and orange juice (1 : 1); sponge cake and water (1 : 1); bread and jam and tea (2 : 1 : 1); bread and jam and water (2 : 1 : 1); orange juice; or tea (available instead of drinking-water and in the absence of diet for 2 h per day). The total amount of aluminium ingested from these test diets was 44 000, 37 000, 300, 230, 5, and 240 µg respectively. Each 24 h test-diet period was preceded and followed by an 8 h fast, with guinea-pig chow being consumed ad libitum at all other times. Control animals ate guinea-pig chow only, consuming approximately 2800 µg of aluminium. The sponge cake contained SALP acidic, and the test diets with sponge cake contained substantially more aluminium than the other test diets.

Aluminium concentrations were measured in brain, kidney and bone (femur) by inductively coupled plasma-mass spectrometry (ICP-MS), and in upper intestinal contents by size exclusion chromatography (SEC) coupled to ICP-MS. Aluminium concentrations in the femurs of animals fed sponge cake, with or without orange juice, were significantly higher than in animals fed any other diet. Femur concentrations of aluminium were higher in the animals fed orange juice and sponge cake than in those fed cake without orange juice, although the amount of aluminium ingested was similar in the two groups. In the kidney, concentrations of aluminium were below the detection limit in animals fed guinea-pig chow, bread, tea and jam, but aluminium was detectable in animals fed the diets containing sponge cake. Concentrations were significantly higher in the animals fed sponge cake and orange juice compared with controls, but not in animals fed sponge cake and water. None of the diets produced elevated concentrations of aluminium in the brain. Less than 1% of aluminium in the upper intestinal contents was found in the soluble fraction, and characterization by SEC-ICP-MS indicated that this aluminium was not present as citrate (Owen et al., 1994).

An 8-week feeding study in rats examined the absorption of aluminium (1.5– 2 g/kg diet) either as hydroxide or complexed with organic anions—citrate, malate, lactate or tartrate. All the organic anions significantly increased plasma aluminium concentrations compared with those in the group treated with aluminium

hydroxide. There was no significant difference in plasma aluminium concentrations between the organic anion treated groups (Testolin et al., 1996).

Rats were given ^{26}Al (3.8 ng in ^{27}Al, 63 ng) by oral gavage (300 µl), in water with a low, medium or high concentration of silicon (< 0.1, 6 or 14 mg Si/l respectively) in the presence or absence of citrate (26 g/l). While citrate significantly increased fractional intestinal absorption of ^{26}Al by a factor of 6–7, silicon had no significant effect, either in the presence or absence of citrate. The same study also found a significant 15-fold increase in ^{26}Al absorption in animals subjected to a 24 h fast compared with non-fasted animals (Drueke et al., 1997).

A small study examining aluminium concentrations in the blood 60 min after administration of a drink containing aluminium citrate (Al, 0.3 g; citrate, 4.8 g) found larger increases in blood concentrations in elderly people (aged > 77 years) compared with people aged 20–70 years. This study also found that increases in blood aluminium concentrations in younger patients with Alzheimer disease were similar to those in elderly sufferers and in controls. The authors suggested that aluminium exposure in these groups may be increased twofold (Taylor et al., 1992).

In a two-part study, the authors initially assessed the speciation of aluminium in vitro in infusions of black tea. Tea samples were incubated alone (pH 4.5) or with an equal volume of human gastric juice (pH 2.2) for 1 h at 37 °C, then centrifuged through micro-concentrators with relative molecular mass cut-offs of 3, 10 and 30 kDa. Further acid-digested samples were adjusted to pH 6.5 and then centrifuged through 3000 Da filters. Of the aluminium in the tea, 78% passed through the 3 kDa filter, and this percentage rose to over 90% with the addition of gastric juice. However, when the gastric juice-digested infusion was adjusted to pH 6.5, just 5% of the aluminium passed through the 3 kDa ultrafilter. These findings suggested that when digested tea passes from the stomach into the duodenum, the pH change would be expected to cause a rapid re-association of aluminium with species with a high relative molecular mass, such as polyphenols.

In the second part of the study, one healthy volunteer drank 2 l of tea over 4 h while commencing a 24 h urine collection. Urine collection continued for a further 24 h in which no tea was consumed, but deionized water was allowed ad libitum. There was little difference in the concentration of aluminium in urine during the two 24 h collection periods. However, urinary volume and total aluminium excretion were greater after drinking tea than during the second 24 h collection period. The authors suggested that only a small proportion of the aluminium in tea is potentially available for absorption throughout the small bowel (Powell et al., 1993).

Aluminium uptake was determined in a single human volunteer given a single oral dose of ^{26}Al (1.1 µg) in sodium citrate. Plasma concentrations of the isotope were measured 6 h after administration and uptake was determined by extrapolation. Uptake was estimated to be 1% of the administered dose (Priest et al., 1995; Priest, 2004).

In a later study, patients with Down syndrome and controls were given orange juice containing ^{26}Al and the effect of added silicate was assessed. Gastrointestinal absorption factors were calculated (aluminium absorbed :

aluminium administered). Control values ranged from 0.04 to 1.5×10^{-4}. The addition of silicate reduced absorption by a factor of approximately 7, while uptake was five times higher in patients with Down syndrome (Priest, 2004).

The uptake of various forms of aluminium was assessed in human volunteers dosed with 100 mg of aluminium via a paediatric feeding tube. Absorption fractions were calculated for aluminium citrate, aluminium hydroxide and aluminium hydroxide with sodium citrate (5×10^{-3}, 1.04×10^{-4} and 1.36×10^{-3} respectively). These results demonstrated the greater bioavailability of the citrate complex and the ability of citrate to enhance the bioavailability of aluminium in another chemical form. This study also noted that variability between the two subjects appeared to be caused by longer retention of the ^{26}Al in the intestine, before defaecation. The ^{26}Al remained in the intestine for approximately 1 day longer in one subject and this was associated with higher blood concentrations and protracted excretion (Priest et al., 1998).

In another study, a fruit drink containing ^{26}Al (27 ng) was given to 13 patients with Alzheimer disease (aged 63–76 years) and 13 age-matched controls after an overnight fast. This study found that gut absorption ranged from 0.06–0.1% of the administered dose with a 1.6-fold increase in absorption by Alzheimer patients (Moore et al., 2000).

A fractional aluminium absorption of 0.22% was determined by comparing ^{26}Al urinary concentrations in human male volunteers given ^{26}Al either by intravenous injection or in drinking-water (Priest et al., 1995b; Priest et al., 1998a cited in COT, 2005).

Three human male volunteers were given aluminium (280 mg, 104 mmol) as aluminium hydroxide with citrate (3.2 g, 1.67 mmol) in 100 ml of fruit juice, after an overnight fast. The authors suggested that it is unlikely that the aluminium was absorbed as aluminium citrate, since the blood citrate peak preceded the aluminium peak by 45–60 min. Therefore, they favoured the hypothesis proposed by Froment et al. (1989b) whereby citrate facilitates aluminium absorption by opening tight junctions in the gut epithelium (Taylor, 1998).

(b) Distribution

Groups of 10 male Wistar rats received either deionized water or aluminium chloride (5 or 20 mg Al/kg bw per day) for 6 months. Aluminium was measured in plasma, brain, liver, bone and kidney and showed dose-related significant increases in concentration when compared with the control animals (Somova & Khan, 1996).

Groups of 20 male Wistar rats were given aluminium chloride at a dose of 5 mg Al/kg bw per day by intravenous injection for 3 consecutive days. Half of the animals were sacrificed on day 4 and the other half on day 22. Haematological parameters and aluminium and iron concentrations in brain, liver, kidney and bone were studied. It was noted that aluminium had accumulated in the brain, bone and kidney of the animals sacrificed after 4 days. These levels had returned to normal after 22 days, when increased concentrations of aluminium in the liver were noted (Somova et al., 1995).

Groups of six rats were given aluminium hydroxide ($Al(OH)_3$) or aluminium chloride at a dose of 0.1, 2.0 or 100 mg Al/l (equivalent to 0, 0.01, 0.2 and 5.5 mg Al/kg bw per day) with either water, citrate or acetate for 10 weeks. Aluminium concentrations were determined in tibia, brain, liver, intestine, blood and kidney by flameless atomic absorption. These did not differ between the treatment groups, with the exception of the intestine, where intestinal cell aluminium concentrations increased in a dose-dependent manner in the presence of citrate (Fulton, 1989).

Rats were given a single oral dose of aluminium at 0, 0.2, 0.4, or 0.8 mmol as aluminium lactate by gavage in 1 ml of 16% citrate (equivalent to 0, 0.04, 0.08 and 0.16 mg Al/kg bw per day). The diet used in this study contained 7.79 mg Al/kg. Tissue aluminium concentrations were determined after 7 h by atomic absorption spectrophotometry. Significant increases above values for controls were observed in all tibia samples. Serum and kidney concentrations of aluminium in the groups at 0.2 and 0.4 mmol were significantly increased above those of the controls, with a significantly greater increase at 0.8 mmol. Significant increases in the liver and spleen were only observed at 0.8 mmol. Rats at 0.8 mmol retained significantly greater amounts of aluminium in soft tissues than those at 0.2 or 0.4 mmol. The authors suggested this might indicate that physiological mechanisms were unable to prevent the tissue accumulation of aluminium in the rats given the highest dose (Sutherland et al., 1996).

In rats dosed orally with ^{26}Al (3.8 ng with 63 ng ^{27}Al) uptake of aluminium into bone was found to be rapid (approximately 1 h) and it remained in the skeleton for the duration of a 30 day study. These authors suggested a minimum residence time of approximately 500 days (Jouhanneau et al., 1997).

Groups of growing (age 2 months), mature (age 8 months) and ageing (age 19 months) male Sprague-Dawley rats were given aluminium lactate at a concentration of 0.8 mmol by oral gavage. Rats were sacrificed on days 1, 9, 15, 21, 27, 36 and 44 (minimum of seven per age group). One day after dosing, growing rats had higher concentrations of aluminium in bone (tibia) than did mature and ageing rats, which had similar concentrations. Ageing rats had higher concentrations in the kidneys on day 1, and lower concentrations on day 9 than growing and mature rats. The half-life (time taken for tissue concentration to halve) of aluminium in the kidneys and tibias increased with age. Multiple stepwise regression analysis indicated that several factors that change with age (including animal size, kidney function, bone turnover and metabolism of other minerals), but not age itself, were predictive of tibia aluminium concentration. Age was also a predictor of liver and spleen aluminium concentrations. However, the measured changes in gut, kidney, bone and mineral metabolism were less predictive of soft tissue aluminium concentration than of bone aluminium concentration (Greger & Radzanowski, 1995).

Microdialysis was used to measure aluminium in extracellular fluid of frontal cortex, lateral ventricle and blood in rats (species not reported). The concentration of aluminium in the dialysate from the frontal cortex reached a maximal steady value within 5 min after the administration of aluminium citrate (0.5 mmol/kg bw) as an intravenous bolus. Also, there was a higher concentration of aluminium, and

higher brain : blood ratio, in the frontal cortex than in cerebrospinal fluid. The authors stated that this supports the suggestion that aluminium enters the brain from blood, through the blood–brain barrier, rather than through the choroid plexus. The concentration ratio of aluminium in extracellular fluid in the brain : blood was 0.15 at constant blood and brain extracellular-fluid aluminium concentrations, suggesting that the transfer of aluminium citrate across the blood–brain barrier is mediated by carriers, rather than by diffusion. Various substrates were included in the dialysate of the microdialysis probe implanted in the frontal cortex of rats. Addition of CN^- or 2,4-dinitrophenol as metabolic inhibitors, pyruvate as a substrate for the carrier monocarboxylate transporter (MCT), or other factors to reduce proton availability and proton gradients significantly increased the brain : blood ratio to approximately 1. These results are consistent with the hypothesis for MCT-mediated transport across the blood–brain barrier. However, lack of aluminium citrate uptake in rat erythrocytes expressing MCT1 and the band 3 anion exchange transporter suggests it is not an effective substrate for either of these transporters. Uptake of aluminium citrate into murine-derived endothelial cells appeared to be independent of sodium and pH, and dependent on energy. Uptake was inhibited by substrates and/or inhibitors of the MCT and organic anion transporter families. Determination of ^{26}Al concentrations in rat brain indicated a prolonged brain half-life (approximately 150 days). The authors noted that this is difficult to extrapolate to humans owing to insufficient insight into allometric scaling for metals between rats and humans (Yokel, 2005).

Rats aged 2 months received intraperitoneal injections of aluminium gluconate (0.667 mg Al/250 µl) three times per week for 2 months. The concentration of aluminium was estimated in brain regions and liver. Liver concentration was reported to be 44-times higher in treated rats than in controls, while a 3.5-fold increase was observed in the brain, with some regions appearing to be more vulnerable to aluminium accumulation. The highest concentrations were reported in the temporal cortex, hippocampus and anterior olfactory nuclei. The impact of aluminium exposure on distribution of glutamate, aspartate and glutamine was also studied. Of the three amino acids assayed, the distribution pattern of glutamine in the brain was distinctly different to that in controls (Struys-Ponsar et al., 1997).

Lactating rats with a litter size of 11 were injected subcutaneously with a solution containing 20 dpm ^{26}Al ($^{26}AlCl_3$) and 0.009 mg ^{27}Al ($^{27}AlCl_3$) daily from postnatal days 1 to 20. Incorporation of ^{26}Al into the brain, liver, kidneys and bone of suckling rats was measured by mass spectrometry and shown to increase significantly from days 5 to 20. After weaning, the amount of aluminium in the liver and kidneys decreased remarkably. However, in the brain the amount of ^{26}Al had only diminished slightly up to 140 days after weaning (Yumoto et al., 2003).

In a review of published studies, papers were identified in which aluminium was administered to pregnant rats, mice or rabbits and accumulation of aluminium was measured in dams, fetus or offspring. Seven studies were identified in which aluminium was administered during gestation and fetal accumulation was determined. In another seven studies, aluminium was administered at least until birth and evaluated the accumulation in the dams and/or pups. These fourteen

studies included four different aluminium compounds (hydroxide, chloride, lactate and citrate) administered by four routes (gavage, feed, intraperitoneal injection and subcutaneous injection) with total doses ranging from 14 to 8400 mg/kg bw. Fetal aluminium concentrations were not increased in six of the seven studies and pup aluminium concentrations were not increased in four of the five studies in which they were measured. Maternal aluminium concentrations were increased in some studies, but there was no consistent pattern of organ-specific accumulation and it was reported that the positive results of several of the studies were contradicted by subsequent reports from the same laboratory. Placental concentrations were increased in six out of nine studies and were greater than corresponding fetal concentrations (Borak & Pierce, 1998).

In a human volunteer, blood samples were taken at 6, 12 and 24 h after ingestion of ^{26}Al (100 ng, 70 Bq) with ^{27}Al (1 µg) in sodium citrate. The highest plasma ^{26}Al concentration (0.3 ng/l) was found in the sample collected at 6 h. Assuming a plasma volume of 3 l, 1% of the administered dose (1 ng) would have been in the circulation. Of this, 5% appeared in a fraction with low relative molecular mass. The remainder was associated with the fraction with high relative molecular mass, specifically, 80% with transferrin, 10% with albumin and 5% in other species with high relative molecular mass (Day et al., 1991). Consistent with this, studies of aluminium binding indicate that that 90% of the aluminium in blood is associated with transferrin, with the remaining 10% existing as aluminium citrate (Ohman & Martin, 1994). While binding studies have shown that transferrin is the strongest aluminium-binding protein in blood, a difference of nearly 10 log units in transferrin binding between aluminium and iron (Martin et al., 1987), indicates that aluminium is unlikely to compete with iron for transferrin binding.

(c) Excretion

Rats dosed orally with ^{26}Al (3.8 ng with 63 ng ^{27}Al) were found to have excreted approximately 90% of the aluminium in the urine within 48 h after dosing (Jouhanneau et al., 1997).

The importance of bile as an excretory route for ingested aluminium has been explored. Bile ducts of 30 male Sprague-Dawley rats were cannulated to allow both bile collection and re-infusion of bile acids. Five days after surgery, rats (average body weight, 191 ± 4 g) were given a single oral dose of aluminium (0, 0.2, 0.4, or 0.8 mmol, equivalent to 0, 0.04, 0.08 and 0.16 mg Al/kg bw per day) as aluminium lactate given by gavage in 1 ml of 16% citrate. Bile was collected from unanaesthetized rats 1–7 h after dosing. Biliary aluminium secretion was highest during the first hour of bile collection. All rats dosed with aluminium secreted significantly greater amounts of aluminium in bile than did rats in the control group. However, biliary aluminium secretion did not vary among animals given aluminium at different doses, suggesting that biliary secretion of aluminium was saturated at these doses (Sutherland et al., 1996).

A human male volunteer was given a solution of ^{26}Al (0.7 µg, 574 Bq) in trisodium citrate (35 mg) intravenously. Urinary and faecal excretion were 83% and 1.8%, respectively, of the initial dose over 13 days after administration. Whole body

retention of ^{26}Al was 15% 13 days after administration, declining to 4% at 1178 days, corresponding to a biological half-life of 7 years (Priest et al., 1995). In a second study, six human male volunteers were each given a solution of ^{26}Al (84 ng, 60 Bq) with citrate (25 mg) intravenously. On average, 59 ± 10 (SD) % was excreted in the first 24 h and by 5 days 72 ± 7 (SD) % had been excreted in the urine. The urinary excretion of ^{26}Al did not correlate with either the mass of voided urine, or excretion of sodium, potassium, calcium, magnesium or phosphorus. Faecal excretion was 1.2% ± 0.3 (SD) over the 5 days after administration. On the fifth day, whole-body retention ranged from 16% to 36%, with a mean of 27 ± 7 (SD) %. The authors suggested that the substantial interindividual variation probably reflected genuine differences in the clearance patterns, which may have implications for whole-body concentrations in the long term (Talbot et al., 1995).

2.1.2 Effects on enzymes and other parameters

Aluminium has been reported to modify the absorption of essential minerals (WHO, 1997).

In a study conducted in vitro, isolated chick duodenum enterocytes were incubated for 1 h with aluminium lactate at 100 µmol/l. In the presence of aluminium, the maximum uptake of calcium and the affinity constant (k_m) were significantly decreased. This reduction was not reversed in cells in which the aluminium-containing media was replaced by aluminium-free media before the measurement of uptake of aluminium. The effect of aluminium on calcium uptake was concentration-dependent (measured range of concentrations of aluminium: 10, 20, 50, 100, 125 or 150 µmol/l) exhibiting an inhibitory saturation-type phenomenon. Calcium uptake was lower at pH 6.5 than at pH 7.4, differences being statistically significant in the range of 20 to 50 µmol/l. Calcium channel activators A23187 and capsaicin did not modify the effects of aluminium (Orihuela et al., 2005a).

The influence of intestinal glutathione (GSH) concentrations on the effects of aluminium on calbindin-D9k-related calcium transport was assessed in adult male Wistar rats given aluminium chloride by oral gavage daily for 7 days (50 mg Al/kg bw). This treatment significantly increased the tissue aluminium content in the small intestine (as measured at the end of the experimental period) compared with control animals. At 24 h, intestinal calcium absorption was significantly decreased in rats given aluminium, or aluminium plus GSH (5 and 10 mmol/kg bw). After 7 days, the inhibitory effect of aluminium on calcium absorption was prevented by simultaneous administration of aluminium with GSH (10 mmol/kg bw). Depletion of GSH by intraperitoneal injection of buthionine sulfoximine (2 mmol/kg bw,) decreased calcium absorption in control animals, and further enhanced the inhibition of calcium absorption by aluminium. Aluminium decreased the duodenal expression of calbindin-D9k, this was prevented by co-administration of GSH at 7 days, but not at 24 h (Orihuela et al., 2005a).

The same authors carried out further studies on the effect of aluminium on GSH metabolism in the small intestine. Adult male Wistar rats were given aluminium chloride at a dose of 30, 60, 120 and 200 mg/kg bw per day by oral gavage for 7 days. The authors commented that exposure to aluminium from the diet and

drinking-water was negligible, although data were not provided. It is unclear whether the doses were expressed as aluminium or as aluminium chloride. There was a dose-related decrease of GSH in the small intestine that was statistically significant at doses of 60 mg/kg bw and above. A 71% increase in the GSH concentration was measured at the highest aluminium dose assayed. The ratio of oxidized : reduced glutathione (GSSG : GSH) increased as the aluminium dose increased becoming statistically significant at 200 mg/kg bw. Specific activities of glutathione-synthase (from 60 mg /kg bw per day) and glutathione-reductase (from 120 mg /kg bw per day) were significantly reduced (26 and 31% respectively) compared with the controls, while glutathione *S*-transferase activity was shown to only be slightly modified by treatment with aluminium. A positive linear correlation between the intestine GSH depletion and a reduction of in-situ calcium absorption, both produced by aluminium, was reported. The authors commented that the results taken as a whole indicate that aluminium alters GSH metabolism in the small intestine by decreasing its turnover, leading to an unbalanced redox state in the epithelial cells, thus contributing to deterioration in GSH-dependent absorptive functions (Orihuela et al., 2005b).

Intragastric administration of aluminium lactate at 0 or 10 mg Al/kg bw per day to six male Wistar rats for 12 weeks resulted in significant increases in intrasynaptosomal calcium concentrations, decreased Ca^{2+} ATPase, increased calcium uptake and increased calpain activity in the brain, indicating alterations in calcium homeostasis. No information on the aluminium content of the diet was provided (Kaur & Gill, 2005).

Aluminium chloride has been investigated for effects on enzymes and other parameters associated with oxidative damage. Groups of seven male Sprague-Dawley rats were treated orally at a dose of 34 mg/kg bw every other day for 30 days, equivalent to 17 mg/kg bw per day. This dose was stated to be 1/25 of the rat oral median lethal dose (LD_{50}) for aluminium and the comparison indicates that the dose is expressed as aluminium rather than aluminium chloride, although this is not clear from the paper. No other details on dosing and no information on aluminium content of the diet are provided. Treatment with aluminium chloride resulted in changes in a large number of parameters, including significantly increased thiobarbituric acid reactive substances and decreased glutathione *S*-transferase activity and levels of sulfydryl groups in plasma, liver, brain, testes and kidney. A range of aminotransferase and similar enzymes were decreased in liver and testes and increased in plasma, acetylcholinesterase decreased in brain and plasma. Concomitant administration of vitamin E (100 mg/kg bw) or selenium (200 µg/kg bw) partially or totally alleviated the effects of aluminium chloride on these parameters (El-Demerdash, 2004).

A similar study was conducted in rabbits at the same laboratory. Groups of six male New Zealand White rabbits were treated orally with aluminium chloride at a dose of 34 mg/kg bw every other day for 16 weeks, equivalent to 17 mg/kg bw per day. This dose was stated to be 1/25 of the rabbit oral LD_{50} for aluminium and the comparison indicates that the dose is expressed as aluminium rather than aluminium chloride, although this is not clear from the paper. No other details on dosing and no information on aluminium content of the diet were provided. The

effects of aluminium chloride were similar to those in the study of El-Demerdash (2004). Vitamin E and selenium were not investigated but amelioration by ascorbic acid (40 mg/kg bw) was reported (Yousef, 2004).

In a study to investigate the possible effects of aluminium exposure on various aspects of calcium homeostasis, three male rhesus monkeys (body weight, 3–4 kg) received aluminium lactate at at dose of 25 mg Al/kg bw by gastric intubation on alternate days for 52 weeks, equivalent to 13 mg Al/kg bw per day. There was no information on the aluminium content of the diet. Aluminium exposure caused a decline in the activity of Ca^{2+} ATPase in the brain. The total calcium content was also significantly raised. Concomitant to this, the levels of lipid peroxidation were increased in the treated animals, suggesting aluminium-induced neuronal change. The authors suggest that the results indicate that the toxic effects of aluminium could be mediated through modifications in the intracellular calcium homeostasis with resultant altered neuronal function (Sarin et al., 1997a).

Three male rhesus monkeys (body weight, 3–4 kg) received aluminium lactate at a dose of 25 mg Al/kg bw on alternate days for 52 weeks, equivalent to 13 mg Al/kg bw per day. There was no information on the aluminium content of the diet. Aluminium administration caused a significant decrease in the total lipid, glycolipid and phospholipid in the brains of these primates. Phospholipid to cholesterol ratios were markedly increased, indicating a loss of membrane integrity, supported by the observation that aluminium had a significant effect on the various membrane-bound enzymes in terms of decreased activities of Na+K+ ATPase and acetylcholinesterase, along with a decrease in the myelin-specific enzyme, 2'3'-cyclic nucleotide phosphohydrolase. The authors considered the latter decrease was suggestive of possible demyelination, which in turn can be attributed to aluminium-induced lipid peroxidation and resultant loss of lipids (Sarin et al., 1997b).

2.2 Toxicological studies

2.2.1 Acute toxicity

The oral LD_{50} of aluminium chloride was 3630 ± 400 mg/kg bw, equal to 737 ± 81 mg Al/kg bw in male Wistar rats. Effects observed in rats after dosing at and above 520 mg Al/kg bw included lethargy, reduced spontaneous movement, lacrimation and breathing difficulties. No effects were observed in the animals receiving the lowest dose (325 mg Al/kg bw) (Kumar, 2001).

2.2.2 Short-term studies of toxicity

(a) Rats

Groups of 15 male albino rats (strain not reported) were given aluminium sulfate at 0, 17, 22, 29, 43, 86 or 170 mg Al/kg bw or potassium aluminium sulfate at 29 or 43 mg Al/kg bw by oral gavage for 21 days. No information was provided on the aluminium content of the diet. The effects of both compounds were similar at comparable doses of aluminium. The end-points were histopathological examination of heart, liver, kidney, brain, testes, stomach and femur. Mild

histopathological effects were reported in the kidney and liver at the lowest dose 17 mg Al/kg bw per day (as aluminium sulfate). Severity of effects increased with dose and effects on nerve cells, testes, bone and stomach were also reported at higher doses. WHO (1997) stated that the data presented were inadequate to verify the reported effects (Roy et al., 1991; WHO, 1997).

Groups of 10 male Wistar rats received aluminium chloride in deionized water as drinking-water for 6 months at doses stated to be 5 and 20 mg Al/kg bw, although it is unclear how these doses were achieved. Control animals consumed deionized water. All animals consumed 'standard pellet food' ad libitum. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. After 6 months, the body weights of animals at the lowest dose (5 mg Al/kg bw per day) and at the highest dose (20 mg Al/kg bw per day) were 80% and 84% of control, respectively. Interim body weights were not reported. Erythrocyte count in the groups at the lowest and highest dose was reduced by 31% and 23% respectively; haemoglobin by 27% and 28% respectively; erythrocyte glucose-6-phosphate dehydrogenase by 14 % and 11% respectively; erythrocyte acetylcholinesterase was reduced by 29% and 20% respectively; erythrocyte volume fraction was reduced by 10% in both groups. No significant changes in leukocyte count were found in either group (Somova & Khan, 1996).

Results of histopathological observations in the above study were reported separately. At 20 mg Al/kg bw, there were spongiform changes and neurofibrillary degeneration in the hippocampus of the brain and atrophy and fibrosis in the kidney (Somova et al., 1997).

Groups of 16 male Sprague-Dawley rats were fed diets containing aluminium hydroxide for 29 days. Groups received 1079 mg Al/kg diet, 1012 mg Al/kg diet plus 4% citrate, or 2688 mg Al/kg diet plus 4% citrate, equivalent to approximately 100, 100 and 270 mg Al/kg bw per day respectively. Concentrations of aluminium in tibia, liver and serum and urinary excretion of aluminium were highly correlated with oral exposure. Ingestion of citrate had small but significant effects on aluminium retention. Erythrocyte volume fractions were inversely correlated with tissue concentrations of aluminium (Greger & Powers, 1992; cited in WHO, 1997).

Aluminium tissue concentrations and body and organ weight changes were measured in a $2 \times 2 \times 2 \times 2$ factorial design study exposing groups of rats to diets containing 13 or 1100 mg Al/kg as hydroxide or citrate and calcium (2.7 or 10 g/kg diet) for 30 days. Further groups of six animals in a 4×2 factorial design experiment were exposed to 14 or 900 mg Al/kg diet per day and one of four levels of citrate for 28 days. Ingestion of citrate was reported to increase the retention of aluminium in bone of rats fed the highest dose (100 mg Al/kg diet per day) and on the high-calcium diet, aluminium concentrations were reported to decrease without a change in growth of the animals. In a third experiment, of $2 \times 2 \times 2$ factorial design, groups of seven rats were exposed to 9 or 1000 mg Al/kg diet per day and citrate. 'Sham' operations were carried out on these animals, or they had one kidney removed. Reduction in kidney function was insufficient to alter growth, but aluminium retention was increased in bone by 13% (Ecelbarger & Greger, 1991; cited in WHO, 1997).

Dogs

Groups of four male and four female beagle dogs were fed SALP basic at dietary concentrations of 0, 3000, 10 000 or 30 000 mg/kg for 26 weeks. The measured aluminium concentrations averaged 94, 284, 702 and 1922 mg Al/kg diet, providing average doses equal to 4, 10, 27 or 75 and 3, 10, 22 or 80 mg Al/kg bw per day for male and female dogs, respectively. Toxicity was reported to be limited to a sharp transient decrease in food consumption and a concomitant decrease in body weight in males at the highest dose, but these data are not shown in the publication. No treatment-related effects on serum chemistry, haematology or urine analysis were observed. A decrease in testes weight was seen in males at the highest dose and two animals had moderate seminiferous tubule germination epithelial cell degeneration and atrophy. Mild to moderate hepatocyte vacuolation accompanied by hypertrophy and mild bile stasis was also seen in the animals at this dose. The authors considered that the effects on the testes and liver were likely to be caused by the decreased food consumption. Very mild to mild tubular-glomerular nephritis was also reported in the males at the highest dose. There were no significant changes in bone aluminium content. A 60% increase in concentration of aluminium in the brain was recorded in the female, but not male, dogs at the highest dose (Pettersen et al., 1990). WHO (1997) concluded that the lowest-observed effect level (LOEL) from this study was 75–80 mg Al/kg bw per day.

A similar study with SALP acidic, as described in an unpublished report, was used by the Committee at its thirty-third meeting in deriving the PTWI of 7.0 mg /kg bw for aluminium (Annex 1, references *83*, *84*). The published paper describing this study was not cited in the monograph, but is available and is described here for comparison. Groups of six male and six female dogs were fed diets containing SALP acidic at concentrations at 0, 0.3, 1.0 or 3.0% for 6 months. The authors stated that the basal diet was reanalysed for contaminants and the concentration and homogeneity of SALP in the blended diets was verified analytically; however, data were not provided on aluminium content of the control diet. Groups of males and females given test diet consumed less food most weeks than controls; intake was noted sporadically to be significantly decreased in all the treated groups of females. The authors noted that the differences in food consumption were not considered to be toxicologically significant, in the absence of any correlating loss in body weight. The average daily food intake of SALP was calculated on the basis of food consumption data and body weight. For males given 0.3, 1.0 or 3.0%, the mean intakes were reported to be 120, 320 and 1030 mg/kg bw per day, and for females they were 110, 360 and 1090 mg/kg bw per day, respectively, corresponding to 10, 27 and 88 mg Al/kg bw per day and 9, 31 and 93 mg Al/kg bw per day. The Committee at its thirty-third meeting (Annex 1, reference *84*) concluded that 3% in the diet was equivalent to a dose of SALP of 1250 mg/kg bw, which was equivalent to approximately 110 mg Al/kg bw.

The nature and frequency of the adverse signs observed were such that none were considered to be related to treatment. No effect on haematological or clinical chemistry parameters was observed in treated dogs. All the measured parameters were considered to be within the normal range for dogs of this age and strain. Ophthalmological examinations, conducted before the start of the test and

at terminations, revealed no adverse ocular changes. No treatment-related changes were evident in the results of urine analysis or faecal occult blood tests. None of the organ weights (absolute or relative values) of treated animals differed significantly from those of control animals. At autopsy and upon histopathological examination the variations observed were within the normal range for dogs of this age and strain (Katz et al., 1984).

Comparison of the above two studies indicates that the basic form of SALP may be more toxic than the acidic form. The FAO specification monograph for SALP acidic (Annex 1, reference *178*) specifies not less than 95% of $NaAl_3H_{14}(PO_4)_8.4H_2O$, and 'insoluble in water', while SALP basic is a mixture of 70% of a complex of SALP (sparingly soluble) and 30% of disodium phosphate (very soluble). Both are cited as soluble in hydrochloric acid, but it is possible that differences in bioavailability resulting from differing solubilities could offer an explanation for these results.

2.2.3 Long-term studies of toxicity and carcinogenicity

No new long-term studies of toxicity or carcinogenicity were identified.

2.2.4 Genotoxicity

No new studies of genotoxicity conducted according to standard protocols were identified. Studies reported in WHO (1997) suggest that aluminium is able to form complexes with DNA and can cross-link chromosomal proteins and DNA. A number of mechanistic studies have investigated DNA damage and cell cycling.

Human peripheral blood lymphocytes were treated with aluminium chloride at 1, 2, 5, 10 and 25 µg/ml at different stages of the cell cycle, and micronucleus formation and apoptosis were assessed. The frequency of micronucleus formation increased initially, but decreased at high concentrations (10 and/or 25 µg/ml), correlating with an increase in apoptosis. The G0/G1 phase of cell cycle was found to be more sensitive than the S/G2 phases. The authors concluded that this indicates oxidative stress or liberation of DNase as a major source of DNA damage induced by aluminium (Banasik et al., 2005).

Human peripheral blood lymphocytes were treated with aluminium chloride at concentrations of 1, 2, 5, 10 and 25 µg/ml (corresponding to 4, 8, 21, 40 and 104 µmol/l) for 72 h. The level of DNA damage and apoptosis was assessed by comet assay, and apoptosis was confirmed by flow cytometry. Aluminium induced DNA damage in a concentration-dependent manner at concentrations of up to 10 µg/ml. At 25 µg/ml, DNA damage declined, accompanied by a high level of apoptosis, indicating selective elimination of damaged cells. In addition, cells were pre-treated with aluminium chloride (10 µg/ml for 72 h) and then irradiated with 2 Gy to examine effect of aluminium on DNA repair. Cells pre-treated with aluminium chloride showed a decreased DNA repair capacity (Lankoff et al., 2006).

Lymphocytes or skin fibroblasts of patients with sporadic (n = 14) or familial (n = 8) Alzheimer disease were assessed. The frequency of spontaneous micronucleus formation in cells from patients with sporadic or familial Alzheimer

disease was significantly higher than in controls. Treatment with aluminium sulfate [$Al_2(SO_4)_3$] did not increase the frequency of micronucleus formation in lymphocytes or fibroblasts of Alzheimer patients, but did induce micronucleus formation at a concentration of 1 mmol/l in cells from control subjects (Trippi et al., 2001).

The effect of aluminium ions on DNA synthesis, assessed by ^3H thymidine incorporation, was studied in normal human dermal fibroblasts in vitro using concentrations of 1.85–74 µmol Al/l (aluminium nitrate) and incubation periods of 1, 2, 3, 4 and 5 days. At 1.85 µmol/l, aluminium salts exerted a slight positive, but not significant, effect on DNA synthesis after day 3 or 5 of incubation. This effect was seen to be statistically significant at concentrations of 3.7 µmol/l and 2 days exposure onward. At 74 µmol/l and 5 days exposure, synthesis increased by 322% over control. Human dermal fibroblast proliferation was also studied. Aluminium salts moderately increased fibroblast division in a continuous manner from 7.4–74 µmol/l after 3 days incubation (Dominguez et al., 2002).

2.2.5 Reproductive toxicity

(a) Multigeneration studies

Groups of 40 Swiss Webster mice were fed diets containing 7 (control), 500 or 1000 mg Al/kg diet as aluminium lactate either from conception until weaning or from conception to age 150–170 days. According to later studies by these authors, these dietary concentrations were expected to be equivalent to < 1, 50 or 100 mg Al/kg bw per day in adult mice. A battery of six neurobehavioural tests was applied at 150–170 days. There were no treatment-related effects on the body weight of the dams of offspring or on litter size. A higher incidence of cagemate aggression was reported in the offspring at the highest dose as adults. At the conclusion of the study, grip strength was reduced in mice of both treatment groups, but this was not dose-dependent or increased by post-weaning exposure. Brain, spinal cord and liver aluminium concentrations were elevated in adults with continuous exposure after weaning, again with no clear dose–response relationship (Golub et al., 1995).

Swiss Webster mice received diets containing 7 (control), 100, 500 or 1000 mg Al/kg diet as aluminium lactate throughout development (conception to age 35 days). The authors stated that these dietary concentrations provided doses of < 1, 10, 50 or 100 mg Al/kg bw per day in adult mice. The basal diet used in this study was 'sub-optimal', intended to mimic the daily intake of nutrients by young women, which while not necessarily deficient, represents a normal deviation from recommended intakes. Data were drawn from a pool of 30 to 40 pregnancies per treatment group. There were no differences in number of dams completing pregnancy, duration of gestation, pregnancy body-weight gain, litter size at birth or birth weight. By weaning, both males and females in the groups at 500 or 1000 mg Al/kg (50 and 100 mg/kg bw per day) weighed significantly less than did the controls (Golub & Germann, 2001).

Male CD-1 mice aged 8–9 weeks were given aluminium chloride by subcutaneous injection at doses of 0, 7 or 13 mg Al/kg bw per day for 14 days before

mating. Females were not dosed at any time during this study. The doses determined for use in this study were designed to reach serum concentrations comparable to those reported in haemodialysis patients. Male mice were mated with three randomly assigned female mice daily for 9 weeks. Mean mating frequencies for the aluminium-treated groups reduced significantly from weeks 4 to 6 and a marked reduction in male fertility was also observed. Mating was reported to have returned to near normal control levels as the experiment terminated. Significantly higher numbers of postimplantion losses, fetal mortality and induced petechial haemorrhage, but no significant fetal abnormalities were observed in the groups treated with aluminium. The dominant lethal assay showed no difference in the number of implantations between aluminium-treated males and controls. Similar implantation losses were observed in all the groups except the group at the highest dose of aluminium at weeks 3 and 5. Further groups of 25 male mice were treated as before, at weeks 3, 5 and 11, and 8 animals of each group were examined for serum and testicular aluminium. The weights of the reproductive organs of the aluminium-treated animals decreased significantly as aluminium accumulated in the testes. Spermatogenic impairment in the testes within the seminiferous tubules was also apparent, but these disturbances disappeared at the end of the experiment. The authors concluded that aluminium exerted substantial negative effects on male reproductive function and produced genetic toxicity. However, these effects were found to be reversible (Guo et al., 2005).

Female Sprague-Dawley rats were given drinking-water containing aluminium (as aluminium nitrate nonahydrate) at doses of 0, 50 and 100 mg Al/kg bw per day for 15 days before mating and then throughout gestation, lactation and post-weaning. The aluminium content of the feed was 42 mg/kg. In order to enhance the gastrointestinal absorption, doses of 355 and 710 mg/kg per day of citric acid were added to the drinking-water of the groups exposed to 50 and 100 mg Al/kg bw per day, respectively. Controls received water supplemented with 710 mg/kg per day of citric acid. It is noted that doses were adjusted to maintain a constant uptake of aluminium. Body weight was decreased relative to controls on postnatal days 12–21 in pups treated with 100 mg Al/kg bw per day. Sexual maturation was delayed in all aluminium-treated females and in aluminium-treated males at 100 mg/kg bw per day. Forelimb grip strength was reduced in males at 100 mg Al/kg bw per day (Colomina et al., 2005).

(b) Developmental toxicity

Oral administration of aluminium has been reported to result in developmental effects, including growth retardation and skeletal anomalies, with the severity of effects being highly dependent on the form of aluminium and the presence of organic chelators that influence bioavailability (WHO, 1997). These data are summarized in Table 1, and indicate that the lowest-observed-adverse-effect level (LOAEL) for developmental effects was 13 mg Al/kg bw per day after treatment with aluminium nitrate, a soluble form of aluminium, administered by gavage (derived from the study of Paternain et al., 1988). Dose-related maternal toxicity (reduced body-weight gain) was also reported.

Groups of 10 pregnant Sprague-Dawley rats were given aluminium nitrate as a daily dose at 180, 360 or 720 mg/kg by oral gavage on days 6 to 14 of gestation, equivalent to 13, 26 or 52 mg Al/kg bw per day. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. Number of corpus lutea, total implantions, number of dead and live fetuses and number of resorptions were recorded and there were no significant adverse effects on these parameters. However, there was a dose-dependent increase in the number of stunted fetuses and the number of litters with runt fetuses in the groups treated with aluminium. Dams given aluminium gained significantly less body weight throughout gestation (non-dose-related) and their placentas weighed significantly less. Fetal weight, body length and tail length from the treated groups showed significant decreases, with fetal body weights being significantly lower in all treated groups in a dose-related manner. Treatment with aluminium resulted in a significantly increased incidence in skeletal variations in all the treated groups (rib and sternebral variations and reduced ossification and a significant increase in haematomas at the highest dose) (Paternain et al., 1988).

Groups of 10 pregnant Sprague-Dawley rats were given aluminium nitrate at a dose of 180, 360 or 720 mg/kg, equivalent to 13, 26 or 52 mg Al/kg bw per day. by oral gavage from day 14 of gestation until day 21 of lactation The diet was reported to contain 60 mg Al/kg, which would have provided a dose equivalent to 6 mg Al/kg bw per day. No mention was made of maternal toxicity. The dosing regime did not produce overt fetotoxic effects, other than a decrease in birth weight at the highest dose. However, the number of litters was significantly lower in the treated groups than in the controls. The growth of the offspring was significantly less from birth and throughout lactation for the group at the highest dose (52 mg Al/kg bw per day). The animals in all the aluminium-treated groups weighed significantly less than controls at day 21. Relative organ weights (heart, lungs, spleen, liver, kidneys, brain) were reported and in many cases were significantly increased in treated animals relative to controls. The effects were not dose-dependent and in some cases (especially for the brains) would simply be caused by the growth retardation of the animals. The authors concluded that very few toxic effects were observed in the group at the lowest dose (Domingo et al., 1987).

A total of 31 time-mated Charles River CD dams were fed a solution of aluminium lactate at a dose of 0, 5, 25, 50, 250, 500 or 1000 mg Al/kg bw per day by daily gastric gavage from days 5 to 15 of gestation. No information was provided on the aluminium content of the diet and therefore the total dose of aluminium is uncertain. The 390 offspring were evaluated for morphological and physiological parameters of reproductive functioning, including birth weight, anogenital distance, timing of vaginal opening, regularity of estrous cycles, duration of pseudopregnancy, number of superovulated oocytes and gonadal weight. No consistent or reproducible findings were reported in these parameters, with the exception of the regularity of estrous cycles. A temporary increase in the proportion of aberrant estrous cycles was detected in the first four cycles after vaginal opening in the group at 250 mg/kg bw per day, with none by the fifth consecutive cycle. The authors suggested that aluminium does not have a developmental reproductive toxic effect (Agarwal et al., 1996).

Table 1. Studies of developmental toxicity with aluminium salts administered orally, published since the previous evaluation performed by the Committee

Species	Route	Compound	Dose	Duration (days of gestation)	NOAEL/LOAEL	Reference
Mouse (Swiss, 20 per group)	Gavage	$Al(OH)_3$	66.5, 133, 266 mg/kg bw per day [a,b]	Days 6–15	No evidence of maternal toxicity, embryo/fetal toxicity or teratogenicity reported	Domingo et al. (1989)
Mouse (Swiss Webster)	Diet	Aluminium lactate	25 and 1000 mg Al/kg diet, equivalent to 4 and 100 mg Al/kg bw per day	Day 0 to weaning	LOAEL: 100 mg/kg bw per day (gestation and lactation exposure: growth retardation in offspring beginning on postnatal day 10)	Golub et al. (1992)
Mouse (Swiss CD1, 10–13 per group)	Gavage	$Al(OH)_3$ Aluminium lactate $Al(OH)_3$ + lactic acid	57.5 mg/kg bw per day [a,b] 166 mg/kg bw per day [a,b] 627 mg/kg bw per day [a,b]	Days 6–15	$Al(OH)_3$: No toxicity reported Aluminium lactate: LOAEL: 166 mg/kg bw per day (poor ossification, skeletal variations, cleft palate) $Al(OH)_3$ + lactic acid: no toxicity reported	Colomina et al. (1992)
Mouse (CBA)	Water	$Al_2(SO_4)_3$	750 mg/l [a,b]	Days 10–17	No toxicity reported	Clayton et al. (1992), cited in WHO (1997)

Table 1. (contd)

Species	Route	Compound	Dose	Duration (days of gestation)	NOAEL/LOAEL	Reference
Rat (Sprague-Dawley)	Gavage	$Al(NO_3)_3$	13, 26 or 52 mg Al/kg bw per day (+ equivalent of 6 mg/kg bw per day from diet)	Days 14–21	LOAEL: 13 mg Al/kg bw per day (decreased survival ratios)	Domingo et al. (1987)
Rat (Sprague-Dawley, 10 per group)	Gavage	$Al(NO_3)_3$	13, 26 or 52 mg Al/kg bw per day [a]	Days 6–14	LOAEL: 13 mg Al/kg bw per day (fetal malformations and variations)	Paternain et al. (1988)
Rat (Sprague-Dawley)	Diet	$AlCl_3$ with accompanying parathyroid hormone injection	50 mg/kg bw per day [a,b]	Days 6–19	LOAEL: 50 mg/kg bw per day (reduced skeletal ossification and increased incidence of skeletal varitions)	McCormack et al. (1979), cited in WHO (1997)
Wistar rats (13–14 per group)	Diet	$AlCl_3$	160 or 200 mg Al/kg bw per day [a]	Day 8 to parturition	LOAEL: 160 mg/kg/ per day (pre-weaning mortality)	Bernuzzi et al. (1986)
Rat (Sprague-Dawley, 15–19 per group)	Gavage	$Al(OH)_3$ Aluminium citrate $Al(OH)_3$ + citric acid	384 mg/kg bw per day [a,b] 1064 mg/kg bw per day [a,b] 384 mg/kg bw per day + 62 mg citric acid/kg bw per day [a,b]	Days 6–15	LOAELs (reduced fetal body weight; increased skeletal variations)	Gómez et al. (1991), cited in WHO (1997)

Table 1. (contd)

Species	Route	Compound	Dose	Duration (days of gestation)	NOAEL/LOAEL	Reference
Rat (Wistar, 18–19 per group)	Gavage	$Al(OH)_3$	192, 384, 768 mg/kg bw per day [a,b]	Days 6–15	NOEL: 768 mg/kg bw per day	Gómez et al. (1990), cited in WHO (1997)
Rat (CD, approx. three per group)	Gavage	Aluminium lactate	0, 5, 25, 50, 250, 500 or 1000 mg Al/kg bw per day [a]	Days 5–15	No consistent neuroendocrine or reproductive effects	Agarwal et al. (1996)

Adapted from WHO (1997)

$AlCl_3$: aluminium chloride; $Al(OH)_3$: aluminium hydroxide; $Al(NO_3)_3$: aluminium nitrate; $Al_2(SO_4)_3$: aluminium sulfate; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.

[a] No information on aluminium content of diet; [b] Unclear if dose is expressed as aluminium or as administered substance.

Groups of 20 pregnant mice were given $Al(OH)_3$ at a daily dose of 0, 66.5, 133 or 266 mg/kg by oral gavage on days 6 to 15 of gestation and killed on day 18 of gestation. Females were evaluated for body-weight gain, food consumption, appearance and behaviour, survival rates and reproduction data. No significant effects attributable to treatment were seen in any of these parameters. No treatment-related changes were recorded in the number of total implants, resorptions, number of live and dead fetuses, fetal size parameters and fetal sex distribution. Examination of the fetuses did not reveal any external, skeletal or soft tissue differences in comparison with the controls. Thus, the authors concluded that no maternal, embryo/fetal or teratogenicity was observed with the doses of aluminium administered to mice in this study (Domingo et al., 1989).

2.2.6 Special studies

(a) Studies of neurotoxicity and neurobehaviour

There is considerable evidence that aluminium is neurotoxic in experimental animals, but species variation exists. In susceptible species (rabbit, cat, guinea-pig, ferret), the toxicity is characterized by progressive encephalopathy resulting in death associated with status epilepticus. The progressive neurological impairment is associated with neurofibrillary pathology in large and medium size neurons predominantly in the spinal cord, brain stem and selected areas of the cortex. These fibrils are morphologically and biochemically different from those that occur in Alzheimer disease. In addition, aluminium has been found to induce epileptic seizures in all species studied (e.g. primates, rodents and fish). These effects have been observed after parenteral injection (e.g. intrathecal, intracerebral and subcutaneous) and there have been no reports of progressive encephalopathy or epilepsy when aluminium compounds were given orally (WHO, 1997).

Behavioural impairment has been observed in the absence of overt encephalopathy or neurohistopathology in rats and mice given diets or drinking-water containing soluble aluminium salts (e.g. lactate, chloride) generally at doses of 200 mg Al/kg bw per day or more, as summarized in Table 2. Effects involved impairment of performance on passive and conditioned avoidance responses (COT, 2005). Because these studies were designed specifically to investigate behavioural effects and other potential end-points were incompletely evaluated, a possible role of organ damage (kidney, liver, immunological) cannot be discounted (WHO, 1997).

The effects of oral exposure to aluminium on brain development have been studied in mice. Effects recorded in more than one study in immature animals included impaired performance of reflexes and simple behaviours. Postnatal mortality and growth were also affected at the higher doses in some of these studies. Adult rats and mice have also been assessed for brain function after development exposures. Reduced grip strength and startle responsiveness were found to persist up to age 150 days. There was no effect on reactions to the light avoidance task in rats after gestational or postnatal exposure (WHO, 1997).

Swiss Webster mice were fed diets containing aluminium at 25 (control), 500 or 1000 mg Al/kg (as aluminium lactate) from conception through weaning. Maternal intakes were reported to be 5, 100 and 200 mg Al/kg bw, respectively at the

beginning of pregnancy and 10, 210 and 420 mg Al/kg bw, respectively near the end of lactation. Weights, food intake and toxic signs were recorded at regular intervals and pregnancy outcome evaluated. Pups were assessed for growth, neurobehavioural development and toxic signs before weaning. They were then assessed immediately after weaning and 2 weeks after weaning during which time they were maintained on control (25 mg Al/kg) diet. No maternal or reproductive toxicity was detected and there were no group differences in pup mortality, growth, toxic signs or neurobehavioural development before weaning. In general, dietary aluminium was associated with dose-related greater foot splay, decreased sensitivity to heat and greater forelimb and hindlimb grip strength shortly after weaning and, to some extent, after a 2-week recovery period on control diet (Donald et al., 1989).

Male Swiss Webster mice were fed diets containing 7 (control, with and without citrate), 100, 500, 750 or 1000 mg Al/kg diet as aluminium lactate (with 3.2% citrate to promote aluminium absorption) from the beginning of puberty (45 days of age) for either 4 or 8 weeks. There was no effect of aluminium content on food intake in any of the treatment groups, or on liver, spleen and tibia weights. A decrease in brain weight was recorded in the animals that received 1000 mg Al/kg diet (which the authors considered provided 100 mg Al/kg bw per day), for 4 weeks but not in the same group treated for the longer duration. A dose-related effect of aluminium on forelimb grip strength was recorded in the groups exposed for 4 weeks (i.e. in pubertal mice) but this effect disappeared in young adulthood, despite continued administration of aluminium (Golub & Keen, 1999).

Groups of 18 male and female Swiss Webster mice were fed diets containing aluminium at a dose of 1000 mg Al/kg diet in the form of aluminium lactate, from conception and throughout their lifespan. The authors considered this diet to provide a dose to adult mice of 100 mg Al/kg bw per day, control diet provided less than 1 mg Al/kg bw per day. Animals in the control and treated groups had a similar mortality rate and no evidence of gross neurodegeneration was seen. There were no consistent differences in neurobehavioural tests based on grip strength, temperature sensitivity or negotiating a maze. The only toxic signs reported were red eyes, fur loss and circling (motor stereotypy) all with a low incidence (no group incidences reported) (Golub et al., 2000).

In the study described in section 2.2.5.1, Swiss Webster mice received diets containing 7 (control), 100, 500 or 1000 mg Al/kg diet as aluminium lactate, throughout development (conception to age 35 days) and were subjected to behavioural tests as adults (aged more than 90 days). The authors considered these dietary doses to be equivalent to less than 1, 10, 50 and 100 mg Al/kg bw per day in adult mice. By weaning, both males and females in the groups at 500 or 1000 mg Al/kg weighed significantly less than controls. One offspring from each litter was used for behavioural testing. Subtle deficits in several neuroparameters, including impaired learning in a maze, were observed in the animals that received diet containing 1000 mg Al/kg, but not at the lower doses. A reduction in hindgrip strength was reported in approximately 15% of animals receiving the highest dose, this was no longer significant after adjustment for body weight (Golub & Germann 2001).

Table 2. Studies of developmental neurotoxicity with aluminium salts administered orally, published since the previous evaluation by the Committee

Species	Route	Compound	Dose	Duration	NOAEL/LOAEL	Reference
Mouse (Swiss Webster; males and females, eight per group)	Diet	Aluminium lactate	7 (control), 500 or 1000 mg Al/kg diet 50 or 100 mgAl/kg bw per day	Conception through weaning, or; conception through adulthood	LOAEL: 50 mg/kg bw per day (reduced grip strength)	Golub et al. (1995)
Mouse (Swiss Webster; 20 per group)	Diet	Aluminium lactate	7 (control), 100, 500 or 1000 mg/kg diet < 1, 10, 50 or 100 mg/kg bw per day	Conception to age 35 days	LOAEL: 50 mg/kg bw per day (weighed significantly less than controls) 100 mg/kg bw per day (neuroparameters)	Golub & Germann (2001)
Mouse (Swiss Webster)	Diet	Aluminium lactate	25 (control), 500 or 1000 mg Al/kg diet, equivalent to 4, 75 or150 mg/kg bw per day	Conception through weaning	LOAEL 75 mg/kg per day (foot splay, forelimb and hind limb grip strengths, thermal sensitivity)	Donald et al. (1989)*
Mouse (Swiss Webster)	Diet	Aluminium lactate	25 (control) or 1000 mg Al/kg 100 mg/kg bw per day	Conception through lactation	LOAEL: 100 mg/kg bw per day (growth retardation, forelimb grasp strength)	Golub et al. (1992)
Mouse (Swiss CD1, 10–13 per group)	Diet	Aluminium lactate	25 (control), 500 and 1000 mg Al/kg diet equivalent to 4, 75 and 100 mg Al/kg bw per day	Day 0 of gestation to weaning	LOAEL: 100 mg/kg bw per day (increased landing foot splay, increased hindlimb grip strength, decreased temperature sensitivity in 21 day old mice)	Donald et al. (1989)

Table 2. (contd)

Species	Route	Compound	Dose	Duration	NOAEL/LOAEL	Reference
Mouse (Swiss Webster)	Diet	Aluminium lactate with and without citrate	7 (control),100, 500, 750 or 1000 mg/kg diet <1, 10, 50, 75 or 100 mg/kg bw per day	4 or 8 weeks from beginning of puberty (45 days)	NOAEL: 100 mg/kg bw per day (no consistent toxic effects recorded)	Golub & Keen (1999)
Mouse (Swiss Webster; 18 per group)	Diet	Aluminium lactate	7 (control) and 1000 mg Al/kg diet < 1 and 100 mg/kg bw per day in adults	Conception through lifespan	LOAEL: 100 mg/kg (red eyes, fur loss, circling)	Golub et al. (2000)
Mouse (Swiss Webster)	Diet	Aluminium lactate	7 (control) or 1000 mg Al/kg diet 200 mg Al/kg bw per day in pregnant mice 420 mg Al/kg bw per day in lactating mice 130 mg Al/kg bw per day in adult offspring	Conception to weaning or; conception to age 52 days	LOAEL: 1000 mg Al/kg diet (lower response amplitudes, reduced auditory startle)	Golub et al. (1994)
Rat (Wistar; (13–14 per group)	Diet	$AlCl_3$	160 or 200 mg Al/kg bw per day [a]	GD 8 to parturition	LOAEL: 160 mg/kg per day (pre-weaning mortality, delay in neuromotor development)	Bernuzzi et al. (1986)

Table 2. (contd)

Species	Route	Compound	Dose	Duration	NOAEL/LOAEL	Reference
Rat (Wistar; 6–12 per group)	Diet	Aluminium lactate or $AlCl_3$	100, 200 or 300 mg Al/kg bw per day ($AlCl_3$) [a] 100, 200 or 400 mg Al/kg bw per day (Allactate) [a]	Days 1–21 of gestation	LOAEL: 200 mg Al/kg bw per day as $AlCl_3$ (grip strength) 100 mg Al/kg bw per day as Allactate (grip strength)x	Bernuzzi et al. (1989a)
Rat (Wistar; 25–38 per group)	Oral gavage	Aluminium lactate	100, 200 or 300 mg Al/kg bw per day [a]	Postnatal days 5–14	LOAEL: 100 mg Al/kg bw per day (negative geotaxis test)	Bernuzzi et al. (1989b)
Rat (Wistar; four per group; multiple groups per dose)	Oral gavage	Aluminium lactate	100 or 200 mg Al/kg bw per day [a]	Postnatal days 5–14	LOAEL: 200 mg/kg bw per day (increased brain Al, decreased choline acetyltransferase & general activity)	Cherroret et al. (1992)
Rat (Wistar; 6–9 per group)	Diet	Aluminium lactate	400 mg Al/kg bw per day [a]	Days 1–7 of gestation; or days 1–14 of gestation; or conception to parturition	LOAEL: 400 mg Al/kg bw per day (locomotor co-ordination)	Muller et al.(1990)

$AlCl_3$: aluminium chloride; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level.

[a] No information on aluminium content of diet

Soluble (aluminium chloride, 30 and 100 mg/kg bw) and insoluble (aluminium hydroxide, 100 and 300 mg/kg bw) aluminium were administered orally once per day for 90 days to Long-Evans rats (groups of 10 males and 10 females). It is unclear if these doses related to the content of aluminium or of the substance tested. No information was provided on aluminium content of the chow. No relevant differences in body weight or general condition were observed between treatment groups. Performance in learning to negotiate a maze was significantly impaired in all of the aluminium-treated groups, with the performance of those receiving the highest dose of aluminium chloride being worst. The aluminium content of the brains was significantly elevated in each treatment group. The elevation was highest in those animals treated at 100 mg/kg bw (336% of control values). Brain acetylcholinesterase activity was significantly elevated by 65–83% in the two groups receiving the highest dose. Brain choline acetyltransferase activity was significantly lowered to 58% of control in the group treated with aluminium chloride at 100 mg/kg bw . (Bilkei-Gorzo, 1993).

Male Wistar rats (age 2 months, n = 19 test, n = 10 control) were given aluminium gluconate daily by intraperitoneal injection three times per week for 2 months or 3 months, or 2 months with 1 months rest. The test animals received 0.667 mg Al/250 µl and controls received an equal volume of sodium gluconate by intraperitoneal injection. Treatment began 2 months before behavioural testing and was maintained throughout the maze learning to avoid any decrease in tissue aluminium concentrations. No significant difference in body weight was observed at the end of the 2 months of treatment. Before the maze experiment the body weight of the rats was reduced by food deprivation and maintained at 80% of their free-feeding value. Rats were submitted to a radial maze test to determine the influence of aluminium on cognitive and non-cognitive behavioural processes. Both learning abilities (working memory and reference memory) and rapidity (time spent to respond to and master a trial) were recorded. Aluminium concentration was evaluated in the brain, serum and liver, significant increases were recorded in all tissue measurements. In the brain, aluminium accumulation was area-specific; the highest levels being observed in the temporal cortex, anterior olfactory nucleus and hippocampus. Despite the accumulation in the brain, no decrease in learning ability was observed, the only behavioural difference observed was a decrease in rapidity (Struys-Ponsar et al., 1997).

Pregnant rats received diets containing aluminium lactate at 400 mg Al/kg bw per day for either the first week (days 1–7 of gestation); first and second (days 1–14 of gestation); or from day 1 of gestation to parturition. Maternal body weight was significantly decreased on days 16 and 19 of gestation by 26% and 35%, respectively, for the group treated from day 1 of gestation to parturition, but not at the other doses. No effect of treatment on litter size, mortality rate or body-weight gain of pups was noted. Performance of the pups was impaired in a negative geotaxis test for those receiving the second two dosing regimes, and in locomotor co-ordination and operant conditioning tests for all three treatment groups. No differences were apparent in grasping and righting reflexes (Muller et al., 1990)

Pregnant Wistar rats received diets containing either aluminium chloride (100, 300 or 400 mg Al/kg bw per day) or aluminium lactate (100, 200 or 400 mg

Al/kg bw per day) from day 1 of gestation to parturition. Maternal food and water consumption was not affected by treatment. A 5–10% deficit in maternal body weight was reported at day 18 of gestation in the groups receiving the intermediate and highest dose of aluminium chloride and the highest dose of aluminium lactate, but not at earlier times. No effect of treatment on litter size was detected, but increased mortality was reported during the first week. This effect was significant in the groups receiving 300 mg Al/kg bw per day as aluminium chloride or 400 mg Al/kg bw per day as aluminium lactate. The neuromotor maturation of surviving pups treated with aluminium showed impairment during the first 2 weeks of life, with grasping reflex being significantly affected in all three groups receiving aluminium lactate and in all treated groups except that receiving the lowest dose of aluminium chloride (Bernuzzi et al., 1989a).

Astrocytes were prepared from cerebral cortex of rats aged 1–3 days. A 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test gave an IC_{50} of approximately 343 µmol Al/l. In subsequent experiments, cultures were exposed to aluminium chloride at concentrations of 200, 400 or 800 µmol/l. Lactate dehyrogenase release showed a significant increase at 800 µmol/l. Cellular toxicity measured by vacuolation of cytoplasmic compartment and swollen appearance was measured in all but the control cultures. Exposure of cultures to 200, 400 or 800 µmol/l specifically increased glutamine synthetase at all three doses. In parallel with this increase, a higher rate of disappearance of glutamate from culture media was observed, as well as an accumulation of glutamine in the cellular extract. The authors suggest that these results indicate that the astrocyte population is a potential target for toxic action of aluminium that could mediate the pathogenesis of this metal (Struys-Ponsar et al., 2000).

(b) Hormonal activity/effects

Groups of six ICR mice (CD-1 derived) were given aluminium chloride at a dose of 35 mg Al/kg bw per day by intraperitoneal injection for 12 days to study effect on nitric oxide production in serum and testis. Serum and testicular aluminium concentrations increased significantly compared with controls. Aluminium administration significantly increased the production of nitric oxide and decreased testicular adenosine 3',5'-cyclic monophosphate (cAMP). As a consequence of the decreased cAMP activity, the observed transport of cholesterol into the mitochondria of Leydig cells, and thus the secretion of testosterone, was reduced (Guo et al., 2005).

(c) Effects on bone

Excessive deposits of aluminium in the skeleton may result in a syndrome referred to as 'aluminium-induced bone disease', and a number of animal models of osteomalacia involve intraperitoneal or intravenous injection of aluminium. Osteomalacia occurs when aluminium concentrations in bone reach 100 µg/g bone ash, which is more than 10 times the normal human bone aluminium concentration. No studies of oral administration were identified (WHO, 1997).

3. OBSERVATIONS IN HUMANS

3.1 Biomarkers of exposure

Concentrations of aluminium in blood, urine and faeces can be measured in humans, but these measurements are not directly related to oral exposure to aluminium (ATSDR, 1999). One reason is that the gastrointestinal tract is a barrier to aluminium uptake (Priest, 2004), and a second reason is that the composition and acidity of the diet affect how much aluminium will be absorbed (Becaria et al., 2002). ATSDR (1999) pointed out that high levels of exposure to aluminium can be reflected by aluminium concentrations in urine, but noted that the rapid excretion of aluminium in urine affects the validity of this parameter as a measurement of bioavailability.

Priest (2004; p. 375) estimated that "…most aluminium that enters the blood is excreted in urine within a few days or weeks." Aluminium concentrations in faeces can also be measured and used to estimate a part of the oral exposure to aluminium, but not the portion that is absorbed by the body (ATSDR, 1999).

3.1.1 Biomarkers of effects

At the current time, no simple non-invasive test was available to measure the effects of oral exposure to aluminium in humans (ATSDR, 1999).

3.1.2 Clinical observations

(a) Dialysis encephalopathy and other disorders in patients with chronic renal failure

In the early to mid 1970s, reports were published describing a cluster of symptoms observed in patients from different dialysis units (Alfrey et al., 1972; Mahurkar et al., 1973; Barratt & Lawrence, 1975; Rosenbek et al., 1975). According to Alfrey et al. (1976), the majority of patients described in the reports had been on intermittent haemodialysis for several years, and the clinical findings included speech difficulty, asterixis, myoclonus, dementia, focal seizures and an abnormal electroencephalogram. The symptoms often progressed to coma and death. This cluster of symptoms became defined as dialysis encephalopathy syndrome (DES) and the cause of the syndrome was investigated. Initially, clinicians suggested a number of possible causes: viral infections, vitamins, amino acid or dopa deficiency, hypertension, drug intoxication, toxic metal deposition or aluminium accumulation (Starkey, 1987). Over the past 30 years, a substantial amount of evidence on this disorder has been collected and aluminium is now widely considered to be a primary cause of DES (Kerr et al., 1992; WHO, 1997; Flaten, 2001; Goyer & Clarkson, 2001). This evidence included findings of elevated concentrations of aluminium in blood, bone, muscle, and brain tissue in patients with DES (Starkey, 1987; Goyer & Clarkson, 2001). Kerr et al. (1992) discussed four factors involved in aluminium intoxication of patients with chronic renal failure: "1) exposure to large volumes of contaminated fluid during haemodialysis, peritoneal dialysis, haemofiltration and occasionally, intravenous therapy; 2) ingestion of grams of aluminium daily as a

phosphate binder; 3) loss of the renal excretory pathway for aluminium; and 4) increased aluminium absorption from the gut in uraemia" [p. 123]. In addition to DES, other disorders associated with aluminium have been observed in patients with chronic renal failure on dialysis, including osteomalacia, extraskeletal calcification, microcytic anaemia and cardiac arrest (Starkey, 1987; Drüeke, 2002).

Among the early studies on DES and concentrations of aluminium in water used to make dialysis fluids, one study found that DES rarely affected patients in those centres in the UK that used water with aluminium concentrations of less than 50 µg/l (Parkinson et al., 1979, 1981). Another study in the Trent region in England found that the average water concentration for patients on dialysis that developed encephalopathy was 328 µg/l (average aluminium concentration in water for patients experiencing multiple bone fractures was 160 µg/l, for patients on dialysis without either disorder, 80µg/l) (Platts et al.,1977). When Parkinson et al. (1981) summarized the early clinical studies or outbreaks of DES that included measures of the concentration of aluminium in water used to make dialysis fluid, they found that the concentrations of aluminium in water associated with DES were usually reported to be greater than 200 µg/l. Treating water used for dialysis with various methods such as filtration, carbon adsorption, reverse osmosis and de-ionization, depending on the water supply, has been found to reduce the incidence of DES (Parkinson et al., 1981; Kerr et al., 1992).

(b) Osteomalacia

In addition to bone changes observed in patients on dialysis, osteomalacia has also been observed in several patients on long-term parenteral nutrition (TPN) who had a variety of gastrointestinal illnesses with malabsorption but who had not been taking large amounts of antacids (Klein et al., 1982; Ott, 1985). Klein et al. (1982) found a substantial quantity of aluminium delivered intravenously in the TPN when casein hydrolysate was used as the protein source. They found that the patients had elevation of serum aluminium content, increased urinary excretion of aluminium and a high content of aluminium in trabecular bone. While suspicious, the researchers state that the "…data do not prove a pathogenic relationship between Al and bone disease" (Klein et al., 1982; p. 1425).

There have also been a few case reports of adults, infants and a child with normal renal function who experienced skeletal changes from frequent use of aluminium-containing antacids for the treatment of gastrointestinal illness (Neumann & Jensen, 1989; Foldes et al., 1991; Pivnick et al., 1995; Shetty et al., 1998; Woodson, 1998; ATSDR, 1999). The antacids in these cases were considered to induce phosphate depletion that resulted in alteration of bone. One example of such a case was described by Woodson (1998). A woman aged 39 years, taking large doses of an antacid containing a high concentration of aluminium and magnesium hydroxide for peptic ulcer and gastritis, reported pain in the right foot. X-ray examination of the foot revealed a callous around a stress fracture of the calcaneous. Bone biopsy found that 27.6% of the bone surface had aluminium deposits. The amount of intake of elemental aluminium in the antacid was estimated to be 6.3 g/day and 18 kg over 8 years. Woodson suggested that the antacid had bound phosphate in the gut causing its malabsorption and that profound

phosphate depletion had occurred that resulted in osteomalacia. When the patient stopped intake of the antacid, she had improvement in symptoms and objective findings.

(c) A case of severe cerebral congophilic angiopathy, an Alzheimer-related disease

In July 1988, a water authority inadvertently discharged 20 tonnes of aluminium sulfate into the drinking-water supplied to Camelford, UK and its vicinity. The drinking-water was considered to be heavily polluted for 3 days, not only with the increased concentration of aluminium but also with copper, lead and zinc that had leached from pipes, owing to the increased acidity of the water (Coggon, 1991; Owen et al., 2002). The highest aluminium concentration measured in water for this accident was 620 mg/l (Owen et al., 2002). The highest concentration considered palatable for drinking was 100 mg/l (WHO, 1997 cites Clayton, 1989). Initial acute effects reported in this population were gastrointestinal problems and oral ulceration (Coggon, 1991). Since January 1989, an advisory group of independent experts has met on several occasions to evaluate possible long-term health effects from the accident. Other epidemiology studies from this setting are discussed in section 3.1.3, but a recently published case study (Exley & Esiri, 2006) will be considered here.

Exley & Esiri (2006) report postmortem findings on brain tissues from a resident of Camelford who was referred for a neurological examination in 2003 at age 58 years and died in 2004 of an unspecified neurological condition. Examination of brain tissue revealed "a rare form of sporadic early-onset ß amyloid angiopathy in cerebral cortical and leptomeningeal vessels, and in leptomeningeal vessels over the cerebellum" (Exley & Esiri, 2006, p.1). DNA testing of brain tissue detected APOE genotype ε4/4. During the analysis of brain tissue for concentration of aluminium, the examiner was masked to whose tissue sample was under study and conducted analysis on tissue from this patient along with three other patients. A range of aluminium concentrations in tissues were found for the present case, from a low in the range of 3–7 µg/g dry weight (exact measurement not presented in paper) to a high of 23.0 µg/g dry weight. The authors report these concentrations as coincident with the severely affected areas of the cortex and find them to be high in comparison to what they consider to be usual aluminium concentration in brain tissue, 0–2 µg/g dry weight. One of the three other individuals had neuropathology similar to the case but was 22 years older; and the highest aluminium concentration in the tissue of this person was 25.16 µg/g. The paper does not state that this person was exposed in the Lowermoor incident so presumably he/she was not exposed. The researchers also discuss findings from other studies regarding APOE genotype ε4/4 as a risk factor for early age onset of Alzheimer disease and for deposition of ß-amyloid angiopathy in walls of cortical and leptomeningeal blood vessels. They were not aware of examination of brain tissue for aluminium concentrations in other studies of similar cases. They conclude by indicating that it is not yet understood what role aluminium has, if any, in the initiation and progression of this rare disease.

3.1.3 Epidemiological studies

(a) Alzheimer disease, dementia or cognitive impairment

(i) Exposure to aluminium in drinking-water

There have been several reviews of the epidemiology studies of aluminium in water in relation to Alzheimer disease, dementia or cognitive impairment (e.g. Doll, 1993; WHO, 1997; Flaten, 2001; Jansson, 2001 & COT, 2005). A table summarizing the epidemiology studies reviewed in the WHO report (1997) updated by COT (2005) is shown at the end of this section (Table 3). Studies reviewed in this document include additional studies not listed in the original table and, for the purpose of the discussion below, have been inserted in the table (indicated by asterisks). The studies are listed by publishing year in the table, but they are grouped according to study design in the text for the purpose of comparison.

An epidemiology study was included in this review only if information on exposure assessment and disease definition were provided and if the study was adjusted for one or more of the possible risk factors for Alzheimer disease. The one exception to this definition was the inclusion of a brain autopsy case–control study (McLachlan et al., 1996). Potential confounders of the relationship between aluminium and Alzheimer disease were not collected in the study, but classification of cases was meticulous at the tissue level and information on exposure to aluminium in water 10 years before death was ascertained, warranting its inclusion in this review.

One comment by investigators that recurs throughout these studies is the issue regarding bioavailability of aluminium. They recognize that the concentration of aluminium is much higher in food than in water and that only a small portion of the daily intake of aluminium is from water. One investigator postulated that "aluminium in drinking-water is either dissolved or readily brought into solution and its bioavailability may therefore be much higher than aluminium from other sources" (Martyn, 1989; p. 59); however, the bioavailability of aluminium from water versus foods is not yet fully understood and the relation of different species of aluminium in human absorption requires further study. Only one of the epidemiology studies below examined any form of aluminium other than total aluminium concentrations in water.

Ecological study

Flaten (1990) conducted an exploratory ecological study to determine the association of age-adjusted death rates from dementia, Parkinson disease and amyotrophic lateral sclerosis (ALS) for 193 municipality aggregates in Norway with the weighted mean aluminium values in drinking-water for each of these aggregates (weighted by the number of persons served by the individual water works). Death certificates containing ICD-8 codes for dementia, Parkinson disease and ALS as the underlying or contributory cause of death were used to ascertain disease. Death rates were calculated for 10 year and for 5 year intervals for the years, 1969–1983. Aluminium content in finished water was obtained across four seasons in 1982–1983 from each of the Norwegian waterworks and ranged from: not

detectable (< 0.008 mg/l) to 4.10 mg/l (the value at the 90th percentile was 0.238 mg/l). For dementia, the death rates for the period 1974–1983 increased for both men and women across low, medium and high categories of aluminium concentration in water, with no overlap in the 95% confidence intervals. For Parkinson disease, there were somewhat higher death rates at higher aluminium concentration in water but the 95% confidence intervals overlapped. No significant association between ALS death rates and aluminium concentrations in water was observed.

Flaten (1990) emphasized the exploratory nature of his ecological study and noted that increased dementia rates might be explained by other factors, such as socioeconomic variables. He found, for example, that dementia rates also correlated strongly with population density although "Al concentration and the percentage living in densely populated areas were not strongly inter-correlated" [p. 165]. Furthermore, while a patient may have dementia, the disease may not be considered by a physician as an underlying or contributory cause of death and reporting of this disorder on a death certificate may vary widely; however, unless under-reporting varied systematically by area of aluminium concentration, it is difficult to perceive how this would strongly influence the findings.

Flaten (1990) mentioned that aluminium levels in Norway may be changing in water over time owing to increased acidification of rain which helps mobilize aluminium from the soil. This change should be distributed throughout the country and not in a specific region. Because death rates were ascertained over a 10-year period and aluminium concentrations in water were ascertained over four seasons in a single year, exposure levels of aluminium before onset of disease are not known on an ecological or individual level.

Prevalence (cross-sectional) studies

Martyn et al. (1989) studied prevalence of dementia and epilepsy in 88 county districts in England for the age group 40–69 years. Dementia and epilepsy were defined by results on a computer tomography (CT) scan and by clinical information supplied on a request form for the CT scan. Dementia was detected in 1203 persons and categorized as probable Alzheimer disease, possible Alzheimer disease, cerebrovascular dementia or other causes of dementia. Age-adjusted rates of disease for each county district were calculated and adjusted for differences from nearest CT scan as well as size of the population served by the CT scan units.

Exposure to aluminium was based on the residual concentration of aluminium for each water source across the 88 county districts over a 10 year period before diagnosis of patients. A mean concentration of aluminium in the water was determined for each county district. Five categories of water exposure were created: 0–0.01 mg/l, 0.02–0.04 mg/l, 0.05–0.07 mg/l, 0.08–0.11 mg/l, and > 0.11 mg/l. Risk of Alzheimer disease and epilepsy in patients in county districts with higher mean aluminium concentration were calculated relative to the lowest category of aluminium concentration (0 to 0.01 mg/l). Significant relative risks were found for the 'probable Alzheimer disease' category, but not for other dementia disease categories or epilepsy. Table 3 indicates this finding, but a clear dose–response

trend of increasing risk of probable Alzheimer disease with increasing aluminium concentration in water was not observed.

Michel et al. (1991) studied a sample of persons residing in France (they used a three-stage approach to sampling: they randomly sampled cantons (districts) in the department of Gironde, then communes (parishes) from the cantons and finally, study participants from the parishes); 4050 subjects were obtained through sampling, but 1258 (31%) persons declined to participate in the study. The final sample size was 2792 persons.

Alzheimer disease was determined in a two-stage process. For the first stage, a screening was done by psychologists (Diagnostic Statistical Manual (DSM)-III criteria for dementia and psychometric tests). Patients who tested positive by the criteria in the first stage went to a second stage. In the second stage, senior neurologists interviewed patients and performed a clinical examination (using the criteria for Alzheimer disease specified by the Joint Working Group of the US National Institute of Neurological and Communicable Disorders and Stroke and Alzheimer Disease and Related Disorders (NINCDS-ARDA)) (McKhann et al., 1984). Exposure to aluminium was based on the concentration of aluminium in the well water for a parish. Aluminium concentration in well water ranged from 0.01 to 0.16 mg/l. The researchers evaluated whether increasing levels (across four categories) of aluminium in well water across parishes correlated with increasing prevalence of Alzheimer disease across parishes. They found a statistically significant association between prevalence of probable Alzheimer disease and category of aluminium concentration. This association continued to be observed after adjusting for age, education, and urban/rural. They found a relative risk of 1.16 for an increase of 0.01 mg/l and relative risk of 4.53 for an increase in 0.1 mg/l (95% confidence interval (CI), 3.36–6.10).

Wettstein et al. (1991) conducted a prevalence study in Switzerland to test the mnestic and naming performance of 800 persons aged 81 to 84 years, who had lived for more than 15 years in either of two districts: in one of these districts the water supply contained a high concentration of aluminium and in the other the concentration of aluminium in the water supply was low (98 µg/l versus 4 µg/l). The researchers also examined the serum and urinary aluminium concentration of 40 nursing-home patients living in either of the two districts to examine differences in intake of metal from water; 20 of the nursing home patients (10 patients in each district) were diagnosed as having senile dementia.

The two city-districts selected for the study had socioeconomically similar populations. Four hundred study participants from the low-aluminium district and 405 study participants from the high-aluminium district were enrolled in the study, using the same method for selecting individuals for contact in each district. The participants were interviewed and given the mnestic and naming subtest of the Mini Mental Status test.

No significant differences in test scores for the participants in the two districts were found. Age and education affected these scores but these factors were equally distributed in the two districts and did not appear to confound the findings. Serum aluminium concentrations in the nursing-home patients with Alzheimer disease

were somewhat higher in the low-aluminium district than the high-aluminium district, the opposite of postulated results. For the 10 control patients in each district there was no significant difference in mean serum aluminium concentrations. Urinary excretion of aluminium was similar in the two groups of nursing-home patients with Alzheimer disease; however, the urinary aluminium : creatinine ratio was significantly higher in control patients from the low-aluminium district than from the high-aluminium district. Researchers concluded that they could not demonstrate a consistent effect of residence in nursing homes with high or low aluminium in drinking-water (Wettstein et al., 1991, p.100). This study is interesting because it attempted to evaluate biomarkers for exposure to aluminium while determining whether poorer mnestic and naming performance are associated with higher concentrations of aluminium in water.

Forbes et al. (1992; 1994a; 1994b; 1995a; 1997) studied participants from a cohort **o**f men enrolled in the Ontario Longitudinal Study of Aging; this cohort initially enrolled 2000men at age 45 years and has been followed for more than 30 years. Forbes et al. evaluated the association between impaired mental function and various chemicals in drinking-water. In 1990–1991, the researchers administered a questionnaire by telephone to the remaining participants. Participants responded directly to the questionnaire (n = 513) or the questionnaire was administered to proxy persons if participants were deceased (usually to a relative of a deceased cohort member, n = 224) or unable to respond for other reasons (n = 45). A total of 782 interviews were conducted (Forbes et al., 1992; 1994b). In later papers, the number of interviews increased to 870 (545 participants, 276 relatives, and 49 proxies) (Forbes et al., 1994a; 1997). The questionnaire contained about 100 questions, including a modified mental status test and nine questions which tested short-term memory. When all 100 questions were answered correctly, the study participant was characterized as having no impairment of mental function; otherwise, a study participant was considered to have some impairment of mental function (Forbes et al., 1992; 1994b).

Information on the residence of these men over the past 30 years was known. The researchers estimated exposure to aluminium for each individual based on the concentration of aluminium currently in the water supply of the city in which the individual had lived the longest. For the analysis, there was information available on both the questionnaire results and water exposure information for 485 men from the cohort (Forbes et al., 1992); in later papers, information for a somewhat higher number of men was available. The odds ratio for the association of some impairment of mental function with high concentrations of aluminium (≥0.085 mg/l) in drinking-water compared with lower concentrations was 1.14, which was not statistically significant (p > 0.05) (Forbes et al., 1992). When only the data from the study participants who were directly interviewed were analysed, the odds ratio increased to 1.53, but was not statistically significant (95% CI, 0.94–2.51) (Forbes et al., 1994b).

Forbes et al. (1992; 1994a; 1994b; 1995a; 1997) also evaluated a number of other water constituents or characteristics, including fluoride, pH, organic matter (DOC), turbidity, silica, and iron, and found the association between aluminium and impairment of mental function to be somewhat more complicated (data from all study

participants used). For example, a significant association of aluminium and fluoride concentrations with impairment of mental function was found when persons consuming water with high aluminium concentrations (≥0.085 mg/l) and low fluoride levels (< 0.13 mg/l) were compared with persons consuming water with low aluminium concentrations and high fluoride concentrations (OR = 2.72, p = .01) (Forbes et al., 1992). Low or high iron concentrations in the water did not appear to affect this association (Forbes et al., 1997). As another example, at a pH of greater than 8.05, the association between high aluminium concentrations and impairment of mental function was in a positive direction (OR = 1.30 (95% CI, 0.85–2.04), while at medium and low pH, the association was in the opposite direction (at pH range 7.85–8.05, OR = 0.68 (95% CI, 0.21–2.19); at pH < 7.85, OR = 0.76 (95% CI, 0.28–2.06), although none of the findings were statistically significant. A logistic regression model that accounted for various water constituents or characteristics and other factors (that is, fluoride, pH, turbidity, silica, iron, source of water, level of education, health at age 62 years, income at age 45 years, total number of moves, and age) found a significant association between high aluminium concentrations and impairment of mental function (OR = 2.35, 95% CI, 1.32–4.18) (Forbes et al. 1995a). In regard to their findings, Forbes et al. (1994a, 1994b) comment that the results must be considered in light of the problem of substantial drop-out of participants from Ontario Longitudinal Study of Aging over 30 years, which potentially affects whether the results from the remaining participants are representative of the study population. They also comment that the presence of any impairment of mental function on screening tests is not the same as dementia, and comment that they may not have considered other possible important confounding factors.

Jacqmin et al. (1994) studied 3777 French men and women aged 65 years and older enrolled at baseline for a population-based cohort study (the Paquid study). These participants were randomly selected from electoral rolls from one of 75 randomly selected rural or urban parishes in the administrative areas of Gironde or Dordogne in south-western France (more sampling detail described above, Michel et al., 1991, Gironde only). A study participant who scored less than 24 on the Mini-Mental State Examination (MMSE) was considered to have cognitive impairment.

Exposure to aluminium was based on information collected in two surveys in 1991 of 75 drinking-water areas that supplied the cohort participants. In addition to aluminium, these surveys measured pH and various chemicals in the water (aluminium, calcium, and fluorine). The researchers also collected historical information on chemical concentrations in water for the period 1981–1991. For each parish, the researchers calculated a weighted mean of all measures for each chemical.

The researchers analysed prevalence rates for cognitive impairment across increasing concentrations of aluminium, calcium, fluorine and increasing pH. The main finding of the study was a significant protective effect of high calcium concentrations for cognitive impairment before and after adjusting for the effects of age, sex, level of educationa and occupation of the study subjects. No significant association of aluminium concentrations with cognitive impairment was observed

(highest category of aluminium in this study was greater than or equal to 18 µg/l), except when the logistic regression model incorporated an interactive term for aluminium and pH. The researchers further evaluated this relationship. A stratified analysis of pH and aluminium demonstrated that the odds ratio for the association of aluminium with cognitive impairment mildly increased in magnitude with increasing doses of aluminium when the pH was 7.0 but decreased in magnitude with increasing doses of aluminium when the pH was 8.0. It was not clear to the researchers how to interpret this finding. The study did not demonstrate an effect of calcium on the aluminium-cognitive impairment relationship. Researchers also analysed exposure to aluminium cooking vessels, but did not find an association of this exposure with cognitive impairment.

The results of this study conflict with those of the study conducted by Michel et al. (1991), athough the participants in the Michel study are a sub-sample (from Gironde) of the participants in this study. Jacqmin et al. (1994; p. 49) consider that the difference in the two studies is related to problems with the historical assessment of aluminium in drinking-water: "A previous report on the Paquid study supported the hypothesis of an association between the risk of Alzheimer's disease and high levels of aluminium in the drinking water. However, this was based on retrospective measures of the concentrations of aluminium that were not reliable: in particular, some of these measures were old and sampling and dosage techniques have changed in recent years" There were other differences between the two studies, including a difference in the health end-point (probable Alzheimer disease versus cognitive impairment).

Forbes et al. (1995b) used Ontario death certificate data from Statistics Canada for a cross-sectional mortality study. Researchers searched for ICD-9 codes for Alzheimer disease (ICD-9 code 331.0), presenile dementia (ICD-9 code 290.1) or bronchopneumonia (ICD-9 code 485) when they examined records. A total of 3161 persons who died with Alzheimer disease or presenile dementia as the underlying cause of death were enrolled in the study. It is difficult to determine from the paper how the researchers calculated death rates for Alzheimer disease or presenile dementia, but presumably all other deaths in the same age category as those from Alzheimer disease were used as the denominator for the rates. Bronchopneumonia deaths were considered only briefly in the paper in relation to water concentrations of chemicals because bronchopneumonia may be the actual cause of death in many patients with Alzheimer disease.

Presumably, the researchers estimated exposure to aluminium in water for each individual based on the concentration of aluminium currently in the water supply for the residence where the individual lived at the time of death. They had used residence water-supply information for living individuals when evaluating exposure in their earlier studies (Forbes et al., 1992, 1994a, 1994b), but the method of assigning exposure for persons who died was not explicitly stated.

Researchers categorized aluminium concentrations in water according to three categories: 0.067 mg/l, 0.068–0.20 mg/l and ≥ 0.336 mg/l. There were no values between 0.21 and 0.335 mg/l. More than 75% of the individuals with Alzheimer disease were exposed at the intermediate level, 0.068–0.20 mg/l, while

less than 1% ($n = 14$) of individuals were exposed at the highest level, > 0.336 mg/l. It is not clear from the methods why the researchers selected these particular ranges of aluminium concentration for each category. Results of the analysis showed a significantly greater than 1.0 rate ratio for the association of death from Alzheimer disease with the highest category compared with the lowest (all ages of Alzheimer disease: rate ratio = 2.42 (95% CI, 1.42–4.11); this rate ratio increased when higher ages were used to identify deaths from Alzheimer disease (i.e. at 75 years and older, at 85 years and older). However, there was a less than 1.0 rate ratio for the association of death from Alzheimer disease with the intermediate category of values compared with the lowest (all ages of Alzheimer disease: rate ratio, 0.92, 95% CI, 0.84–1.01). From these findings, the researchers proposed that there may be a J-shaped rather than linear dose–response relationship when aluminium concentrations in water reach the levels found in this study. When only two categories were used in analysing the data, above or below 0.075 mg/l (or 0.068 or 0.085 mg/l), the rate ratios for the association of Alzheimer disease death with the higher category compared with the lower category were all below 1.0 (Forbes et al., 1995b; p. 646).

The researchers also modeled the effects of fluoride, pH and silica (SiO_2) on the aluminium–Alzheimer disease association, finding possible interactive effects of some of these chemicals. For example, when high concentrations of fluoride (> 0.5 mg/l) and pH (≥7.96) are present, the risk ratio for high concentrations of aluminium associated with Alzheimer disease is reduced. In a later paper, Forbes et al. (1997) added iron concentrations in water to the multivariate analysis (poisson regression model) and continued to observe the J-shaped association between aluminium concentrations and death from Alzheimer disease for persons aged 85 years or older.

Gillette-Guyonnet et al. (2005) analysed baseline data from an ongoing cohort study, the Epidemiology of Osteoporosis (EPIDOS) study, that follows women aged more than 75 years residing in five cities in France ($n = 7598$ women) to determine risk factors for fracture of the femoral neck. Gillette-Guyonnet et al. (2005), however, used data from this study to compare exposures to concentrations of chemicals in water in women with cognitive impairment at baseline of the study to exposures in women without cognitive impairment at baseline.

Cognitive impairment at baseline was assessed by the Short Portable Mental Status Questionnaire (SPMSQ) and women with a Pfeiffer score of < 8 were considered to have cognitive impairment. Exposure to chemicals in water was assessed from questioning women about water intake, from the tap or from bottled mineral water, and from obtaining data about the concentrations of aluminium, silica and calcium concentrations in brands of bottled water or the city water supply. Aluminium in water varied from undetectable to 0.032 mg/l in bottled water and from 0.01 to 0.063 mg/l in the five cities in the study.

The researchers did not find an association between cognitive impairment and aluminium in the baseline study. They were able to adjust for a number of potential confounders in their analyses, including age, education and income. They

did find that cognitive impairment was associated with lower silica concentrations in drinking-water. No effect of calcium in water was observed.

Only one of the seven prevalence studies presented here examined daily ingestion of water at the individual level; most used average concentration of aluminium in the water supply to residences as an estimate of aluminium exposure. For three of the seven studies, an association of Alzheimer disease with aluminium was found, although the measurement of historical aluminium concentrations was called into question for one of these studies. Of the other four studies, all of which examined cognitive impairment, only one found an association between cognitive impairment and aluminium. The issue of the possible effect of other chemicals in drinking-water on the relationship between Alzheimer disease and aluminium was evaluated in two studies, but little information on potential confounding factors was available overall. A difficulty for several of these studies is determining an estimate of exposure before development of disease.

Case–control studies

Neri & Hewitt (1991) conducted a cross-sectional case–control study in Ontario, Canada in 1986. Cases were 2344 individuals aged 55 years or older who had a diagnosis of Alzheimer disease or presenile dementia by ICD-9 code (331.0 or 290.0) on their hospital summary record. Controls were 2232 individuals matched by age and sex to cases that had non-psychiatric diagnoses on their hospital record. Aluminium concentration of finished water in the municipal water supply for the area of the patient's current residence was obtained (if water information was not available for the patient's locality, the individual was not enrolled in the study). The unmatched analysis indicated that the estimated relative risk for Alzheimer disease increased with increasing concentrations of aluminium in the water supply (from 0.01 mg/l to above 0.20 mg/l), although the statistical significance of this finding was not shown.

Forster et al. (1995) conducted a case–control study of presenile dementia of the Alzheimer type (PDAT) in northern England to determine risk factors for this disease. Cases (n = 109) were ascertained through hospital admission and other medical records; they were individuals who were aged less than 65 years when they were diagnosed as having dementia by clinical algorithm criteria for Alzheimer disease in the period 1981–1989 and confirmed at the study interview. Controls (n = 109) were randomly selected from the same northern regional health authority (although not the same general practice) as the cases and pair-matched to cases on age and sex. Close relatives were used as informants for both cases and controls to respond to questions about exposure to risk factors in the period before the onset of PDAT in the cases. Historical data on aluminium concentration in drinking-water was obtained from the local water authority in the mid to late 1980s where each case lived the longest in the 10 years before onset of PDAT (the same time period and residence criteria was used for the matched control). Aluminium concentration in water was analysed and no significant association of Alzheimer disease with this exposure was seen across four categories of increasing concentration of aluminium (< 50 µg/l, > 50 µg/l, > 99 µg/l, > 149 µg/l). Taylor et al. (1995) used the same study participants and used exposure to current water supply levels of aluminium and

silicon to examine if dissolved silicon in drinking-water was related to PDAT. The reason for studying this relationship was based on the possibility that silicon might affect the bioavailability of aluminium. They found that soluble silicon concentrations for the cases did not differ significantly from those for the controls, but did find a significant inverse relationship between silicon and aluminium concentrations (Spearman's rank order correlation coefficient 0.43, $p < 0.001$).

McLachlan et al. (1996) conducted a case–control study in Canada. Definition of cases and controls was based on postmortem histopathological examination of brains of individuals who were residents of Ontario at time of death. Cases were categorized as having definitive pathological diagnosis of Alzheimer disease ($n = 296$) with an absence of any other degenerative process, or Alzheimer disease pathology with other neuropathologic changes and clinical dementia ($n = 89$). Controls were categorized as having either no histopathology on postmortem ($n = 125$) or having histopathological changes for several other diseases, none for which an association with aluminium has been found ($n = 340$).

Exposure to aluminium in water was based on water source for residence at time of death and also on a 10-year residential history obtained for a subset of the cases ($n = 119$) and controls ($n = 51$) from a telephone interview of next of kin of these individuals. The 10-year exposure was calculated using water supply data for each residence of an individual weighted by duration of residence in that location. In the analysis, the cases from the first category were compared with controls and then all cases were combined and compared with controls (all controls and a subset of controls). A significant association was found between Alzheimer disease and aluminium concentrations in water > 100 µg/l for residence at time of death or 10-year exposure history (all cases and controls, current: OR = 1.7 (95% CI, 1.2–2.5); 0-year: OR = 2.6; 95% CI, 1.2–5.3). Similar results were found for other configurations of case–control comparisons. Other potential confounders of the relationship between Alzheimer disease and aluminium were not available in this study, including age. The 10-year residential history may not be sufficient to detect exposures that cause disease.

Martyn et al. (1997) conducted a case–control study that involved men from eight regions of England and Wales who were aged between 42 and 75 years. Study participants were identified from the CT records of eight neuroradiology centres. Men with a possible diagnosis of dementia on the CT record, a normal CT scan or a CT scan which showed only cerebral atrophy without evidence of infarction, and hospital notes indicating a clinical diagnosis of Alzheimer disease were enrolled as cases ($n = 106$). Men were enrolled into one of three control groups when: (a) the CT scan indicated a diagnosis of dementia without other evidence indicating Alzheimer disease ('other dementia' control group, $n = 99$); (b) the CT scan indicated a diagnosis of primary brain cancer ('brain cancer' control group, $n = 226$); or (c) the CT scan indicated another diagnosis other than dementia, such as malignant brain tumour, epilepsy or chronic disabling disease ('other' control group, $n = 441$).

Exposure to aluminium in drinking-water was ascertained through questionnaire information given to the study participant or the next of kin (if participant had died or was unable to fill out questionnaire). The questionnaire elicited the

addresses of all places of residence of 3 years or more since the study participant was aged 25 years. The investigators collected information on concentrations of aluminium and molybdate-reactive silica in drinking-water for these addresses and estimated an average concentration of the chemicals in drinking-water over time for each individual. The exposure for aluminium in water ranged from less than 0.015 mg/l to greater than 0.109 mg/l. In a comparison of the cases with each control group, no increased risk of Alzheimer disease with aluminium in drinking-water was observed, nor was a protective effect of silicon in drinking-water found as hypothesized. The use of three control groups aided in providing evidence that the lack of association was consistently found and less likely to be a result of study bias from control selection or control response to questionnaire.

Gauthier et al. (2000) conducted a case–control study in Quebec, Canada, enrolling 68 cases of Alzheimer disease and 68 controls matched pair-wise to cases on age and sex. These study participants were a subset of a larger random sample of persons aged 70 years or older selected from the Quebec health plan files. Alzheimer disease diagnosis occurred after a three-phase assessment of sampled individuals. A screening test, the Modified Mini-Mental State Examination (3MS), determined if sampled subjects were considered cognitively impaired or not. If they were considered cognitively impaired, they underwent a second phase of assessment for dementia (standardized clinical interview with proxy respondent and a series of neuropsychological tests with subject), and if considered to have dementia, underwent a standardized medical examination with a neurologist. The cases were selected from those persons diagnosed as possible or probable Alzheimer disease without other co-morbid anomalies by the neurologist, and controls were selected from among persons without dementia. Individual exposure of the cases and controls to aluminium in water was determined in two ways using data from a water sampling campaign in 1995–1996: (a) concentrations in the water supply to the participant's current residence; and (b) estimated long-term exposure to aluminium since 1945 based on the study participant's residential history (constructed with current water data from municipalities). During the laboratory analysis of water a total of 19 physicochemical variables were assessed. Speciation of aluminium was also performed, including concentrations of total aluminum, total dissolved aluminium, total monomeric aluminium, organic monomeric aluminium, and inorganic monomeric aluminium. Other information collected about the cases and controls included occupational history, highest level of education, family history, medical history and presence or absence of ApoE ε4 allele (from the participants' blood samples).

The results of the study showed no significant association between long-term exposure to forms of aluminium found in drinking-water and Alzheimer disease. However, for current exposure, a significant association was found between organic monomeric aluminium and Alzheimer disease (OR = 2.67, 95% CI, 1.04–6.90), adjusted for education, presence of family cases of Alzheimer disease, and presence of at least one ApoE ε4 allele. The authors suggest that there is biological plausibility for this finding, citing research about how this form of aluminium complexes with organic acids of low relative molecular mass and how complexes are absorbed into the gastrointestinal tract, circulate in blood and cross the

blood–brain barrier (p. 239). They suggest that the reason for not observing this association for long-term exposure may be related to the imprecision of the long-term exposure estimates.

Gillette-Guyonnet et al. (2005) studied women in a single city (Toulouse) of a five-city cohort study who agreed 7 years after enrolling in the Epidemiology of Osteoporosis (EPIDOS) study to be enrolled in another prospective study; this other prospective study aims to determine risk factors for Alzheimer disease. The researchers compared women who enrolled in this additional study and were diagnosed with Alzheimer disease (cases, $n = 60$) to women enrolled in the study of Alzheimer disease who had normal cognitive function (controls, $n = 323$). They described the design of this study a nested case–control study (nested within the larger cohort). They retrospectively used the information on exposure to chemicals in water collected 7 years earlier at baseline of EPIDOS (1992–1994) and again in 1999–2000. Exposure to chemicals in water was determined by questioning women about water intake, from the tap or from bottled mineral water, and obtaining data about the concentrations of aluminium, silica and calcium concentrations in brands of bottled water or the city water supply. Aluminium concentration in the water supply of Toulouse was measured as 0.063 in 1992–1994 and 0.060 mg/l in 1999–2000.

To determine the status of the women with respect to Alzheimer disease, researchers began by employing the SPMSQ, the MMSE and the Grober and Buschke test to assess cognitive function. These tests, an assessment in the home of the study participant's independence in instrumental activities of daily living and computed tomography reports or scans were evaluated by a geriatrician and neurologist to determine if the woman had normal cognitive function, mild cognitive impairment, Alzheimer disease or other types of dementia. The clinicians used NINCDS-ADRDA criteria in the diagnosis of Alzheimer disease.

The researchers did not find an association between Alzheimer disease and aluminium in the study. They were able to adjust for a number of potential confounders in their analyses, including age, education and income. They did, however, find that Alzheimer disease was associated with lower silica concentrations in drinking-water. No effect of calcium in water was observed.

An additional case–control study conducted by Altmann et al. (1999) that examined association of aluminium in water from the Camelford water pollution incident with disease differs from the above case–control studies in that it aimed to measure disturbance of cerebral function, rather than Alzheimer disease, using psychological tests and visual evoked potentials (VEP). A difficulty with this study is that selection of cases ($n = 55$) was not investigator-initiated but was initiated by lawyers on behalf of persons who were considering litigation on account of alleged effects from the water pollution incident. The cases complained about short-term memory loss and impaired concentration. Therefore, the cases had an idea about both the exposure and possible health effects before the study was begun. The investigators selected as controls 15 siblings of the cases (nearest in age to the cases of his or her siblings) who had not lived in the area of water contamination since before the incident. Because of the likelihood that controls were aware that

cases were considering litigation, it is possible that this awareness had some affect on the control's performance on tests. The least subjective test in the battery of tests used in the study was VEP. A significant difference between the 15 pairs of cases and their siblings was detected on VEP, with the siblings having a better flash pattern. Potential confounders of this finding, if any, are not discussed in the paper. Due to issues with the study design and differences in end-points, this study will not be included in the comparison with the other case–control studies.

In summary, two of the six case–control studies presented here showed a statistically significant association of Alzheimer disease with aluminium. Exposure assessment varied in the six studies, from ascertaining aluminium concentration in the current residential water supply in a cross-sectional case–control study (Neri & Hewitt, 1991) to questioning individuals directly about their ingestion of tap or bottled mineral water in a nested case–control study (Gillette-Guyonnet et al., 2005). Three of the studies depended on informants to recall the past residential history for cases or controls, which was used to construct historical exposure to water. The use of informants, rather than direct questioning of study participants, are often required in retrospective studies of patients with a disease involving memory loss but may result in inaccurate exposure assessment.

Another issue to consider when examining the results of these studies is disease definition. Disease definition varied from identification of cognitive impairment in women (Gillette-Guyonnet et al., 2005) to Alzheimer disease identification using postmortem histopathological examination of brain tissue (McLachlan et al., 1996). If aluminium has a role only in the pathogenesis of Alzheimer disease and not cognitive impairment or other forms of dementia then careful attention to criteria for evaluating the form of the disease is warranted.

Of the two studies showing an association of Alzheimer disease with aluminium, the study by Gauthier et al. (2000) evaluated the speciation of aluminium in water and the association of Alzheimer disease was found for only one of these species, organic monomeric aluminium. This finding leads to questions about bioavailability of different species of aluminium and the effect, if any, on pathogenesis of Alzheimer disease.

Prospective cohort study

Rondeau et al. (2000) analysed data from a prospective cohort study (the Paquid cohort) of 3777 persons who were aged 65 years or older and lived at home at the onset of the study. These participants were randomly selected from electoral rolls from one of 75 randomly selected rural or urban parishes in the administrative areas of Gironde or Dordogne in south-western France (more sampling detail described above (Michel et al., (1991), Gironde only). Alzheimer disease was determined in a two-stage process. For the first stage, a screening was done by psychologists (DSM-III criteria for dementia and psychometric tests). Patients who tested positive by the criteria in the first stage went to a second stage. In the second stage, senior neurologists interviewed patients and performed a clinical examination (NINCDS-ADRDA criteria for Alzheimer disease and Hachinski score for vascular dementia). Study participants were re-examined at specific intervals after

the start of the study with the same criteria as above to detect new cases of dementia. An additional test for dementia, the MMSE, was added after the baseline exams.

Exposure to aluminium was based on information collected in two surveys in 1991 of 77 drinking-water areas that supplied the cohort participants. These surveys measured pH and various chemicals in the water (aluminium, calcium, and fluorine). Information on chemical analyses of drinking-water (aluminium and silicon) conducted by the sanitary administration between 1991 and 1994 was also used. The researchers additionally collected historical information on chemical concentrations in water for the period 1981–1991. For each parish, the researchers calculated a weighted mean of all measures for each chemical (for the 70 of 77 drinking-water areas that had all available information). For the analysis, aluminium was characterized in three ways: (1) ≥ 0.1 vs < 0.1; (2) as a continuous variable; and (3) grouped into four categories (< 0.0038 mg/l, ≥ 0.0038 to < 0.0110 mg/l, ≥ 0.0110 to < 0.1000 mg/l, ≥ 0.1000). Aluminium concentrations in water ranged from 0.001 to 0.459 mg/l, with a median value of 0.009 mg/l.

For analysis of the data, the researchers evaluated 2698 participants of the original cohort. The reason for the drop in sample size from the baseline number was because of the following reasons: the exclusion of persons who were demented at baseline (n = 102) in order to ascertain incident cases during 8 years of follow-up, the lack of participation of study subjects in follow-up visits (due to death or refusal (n = 703)), and the lack of water measurement and adjustment co-variates for some participants (n = 274).

Results of the analysis showed that the risk associated with dementia (incident cases, n = 253) or the risk associated with Alzheimer disease (incident cases, n = 182) was significantly elevated at aluminium concentrations in drinking-water of 0.1 mg/l compared with the lowest aluminium concentrations (respectively, relative risk, RR = 1.99, 95% CI, 1.20–3.28; RR = 2.14, 95% CI, 1.21–3.80, adjusted for age, gender, level of education, place of residence, and wine consumption). There was no linear dose–response observed across the four aluminium categories with dementia or Alzheimer disease, and the researchers suggest the possibility of a threshold effect at the highest category of aluminium concentration. Concentrations of silicon in the water of > 11.25 mg/l were associated with a reduced risk for developing dementia or Alzheimer disease, but there did not appear to be an interaction between aluminium and silicon associated with outcome of disease. No effect of pH was observed.

This study found an association of Alzheimer disease and dementia with aluminium concentrations ≥ 0.1 mg/l in water, although no dose–response was observed. The study had several strengths. Exposure was assessed before onset of disease and information about potential confounders of the relationship between Alzheimer disease and aluminium was obtained directly from study participants rather than informants; disease was diagnosed carefully with standard tests and clinical evaluation; and researchers examined whether loss to follow-up of cohort participants explained findings. The researchers also analysed water for other chemicals and could examine the effect of these chemicals on the relationship

between Alzheimer disease and aluminium. Findings from the study conflicted with findings from another study using the full cohort and the same exposure assessment (Jacqmin et al., 1994), but the latter study evaluated study participants at baseline in the cohort and used a different health end-point, cognitive impairment (scoring below 24 on the MMSE).

Conclusions about studies of exposure to aluminium in drinking-water

Some of the epidemiology studies suggest the possibility of an association of Alzheimer disease with aluminium in water, but other studies do not confirm this association. Many of the methodological issues concerning the studies are discussed above. All studies lack information on ingestion of aluminium from food and how concentrations of aluminium in food affect the association between aluminium in water and Alzheimer disease. Some of the studies have examined the effect of other chemicals in water but more information is needed in this area.

(ii) Exposure to aluminium in food

There are very few studies that incorporate information about dietary intake in epidemiological studies of Alzheimer disease, and all studies used a case–control design.

Rogers & Simon (1999) conducted a pilot case–control study to determine whether intake of food containing aluminium additives differs in individuals with and without newly diagnosed Alzheimer disease. The cases and the matched controls were selected from a nursing home in New York, USA from March to November 1993. The cases were defined as persons with newly-diagnosed Alzheimer disease from 1990 to 1993. The diagnosis of Alzheimer disease was ascertained using criteria specified by NINCDS-ADRDA. A total of 46 participants composing 23 matched pairs were enrolled in the study. Next-of-kin or spouse responded to questions on the participants' medical history, lifestyle behaviour and dietary intake before admission to the Centre.

The crude ORs for the association between categories of aluminium-containing foods and Alzheimer disease were generally low and non-significant. A statistically significant association between food intake and disease was only found for one category "pancakes, waffles, biscuits, muffins, cornbread, corn tortillas" based on five discordant matched case–control pairs (OR = undefined; $p = 0.025$).

Several of the ORs markedly increased when adjusted for other possible factors that may affect the food-disease relationship, thus indicating the instability of several of these estimates. The large difference between the crude and adjusted ORs after adjusting for up to six covariates in the conditional logistic regression model also indicates the difficulty of using complex models to analyse a small number of matched case–control pairs. The authors did not discuss or show the confidence interval around each OR, and therefore, the reader could not examine the extent of variability of the estimates. Other difficulties with the study included: (1) the long time to recall of dietary intake information—surrogates had to recall diet in a time period up to 8 years before the interview; (3) the potential for differential recall between case versus control surrogates owing to the likely differences in

intensity of care of study participants and the influence that intensity of care may have on knowledge of diet; (4) the potential for a control to change usual dietary patterns when diagnosed with heart disease or high blood pressure, especially if he or she reduces intake of fatty foods such as biscuits containing aluminium; (5) the lack of validation of dietary intake questions developed for this study; and (6) the problems with incomplete information about the amount of aluminium in different brands of foods. Although the findings in this pilot study should be interpreted cautiously and considered exploratory, they are intriguing enough to suggest that future epidemiological studies of intake of food containing aluminium and Alzheimer disease are warranted.

A few epidemiology studies have also examined the relation between tea drinking and Alzheimer disease. Pennington (1987) summarized the literature on aluminium content in individual foods and found that the concentration of aluminium in tea leaves and powder ranged from 67.0 to 14.0 mg/100 g, but that aluminium concentration in a cup of brewed tea (8 fl oz) ranged from 0.05 to 1.07 mg/100 g. Findings from the epidemiology studies on tea are presented below.

Broe et al. (1990) conducted a case–control study in Australia in 1986–1988 that enrolled 170 newly diagnosed cases of Alzheimer disease and 170 controls matched to cases on age, sex and, when possible, attendance of the same general practice clinic as the case. Cases were evaluated by neurologists and underwent a standardized battery of tests and examinations for Alzheimer disease and were classified as probable or possible Alzheimer disease by NINCDS-ADRDA criteria. Trained interviewers questioned individuals, usually in their homes, about health history, family history, lifestyle and occupational or domestic exposures. Interviewers asked about tea drinking history. No significant association between tea drinking and Alzheimer disease was detected on analysis of matched pairs, even at levels of “>4 cups of tea daily sometime in life” (odds ratio = 1.42, 95% CI, 0.93–2.17).

In another case–control study in northern England (Forster et al., 1995, see description of study above), the measure “>4 cups of tea daily” was also used, but no significant association between tea drinking and PDAT was detected although, as in the Australian study, the odds ratio was above 1.0 (OR = 1.4; 95% CI, 0.81–2.63).

Table 3. Epidemiological studies of aluminium in drinking-water and dementia or Alzheimer disease

Type of study	Measure of exposure to aluminium in water	Outcome measure/data source	Results	Reference
Ecological	Aluminium in drinking-water (concurrent) Four seasonal samples	Mention of dementia ICD9 290, 290.1 (dementia), 342.0 (Parkinson), 348.0 (ALS); sex-adjusted death certificate	AD only: Al (mg/l) Males Females RR < 0.05 1.00 1.00 0.05-0.2 1.15 1.19 > 0.2 1.32 1.42 PD and ALS—no gradient	Flaten (1990)
Morbidity prevalence	Aluminium in finished drinking-water; historical	Dementia by diagnostic category (not standard) CT scan centre records age-sex-adjusted	All males and females RR = 1.3–1.5, no dose-response relationship Males and females aged < 65 years RR = 1.4–1.7, dose response	Martyn et al. (1989)
Morbidity prevalence case–control	Finished drinking-water aluminium; historical	'Cases' were hospital discharges of AD (ICD 9 331.0), Presenile dementia (ICD 9 290) Age/sex/residence-matched controls with other diagnoses Hospital Medical Records Institute database-Ontario	RR from OR, gradient for AD Al (mg/l) RR from OR < 0.01 1.00 0.01–0.099 1.13 0.010–0.199 1.26 > 0.2 1.46	Neri & Hewitt (1991)

Table 3. (contd)

Type of study	Measure of exposure to aluminium in water	Outcome measure/data source	Results	Reference
Morbidity prevalence	Aluminium in finished drinking-water; residence > 15 years; urinary aluminium and serum aluminium; historical and concurrent	Mnemic skills in octogenerarians; sample of 800 residents in high & low aluminium areas; urinary and serum aluminium from 10 AD patients and controls in each area age, sex, education population-based	No difference in mean scores of tests for cognitive function Slightly higher serum aluminium in AD in low aluminium areas; similar urinary excretion in AD and controls; hypothesis of association not supported	Wettstein et al. (1991)
Morbidity prevalence	Aluminium in drinking-water, historical	Cognitive function in sample of > 65 year olds by test battery (DSM III), population-based (2792); age, sex, education, SES, aluminium in water-many sources for the data	Probable AD, gradient-adjusted for age, education, residence, RR = 4.53/100 µg/l aluminium; RR corrected to NS with current aluminium measurement [Jacqmin et al. 1994]	Michel et al. (1991)
Morbidity prevalence*	Aluminium in drinking-water, historical	Cognitive function; 100 questions including a modified mental test and 9 questions short-term memory	NS/OR = 1.14 (Al ≥ 0.085 mg/l) S/OR = 2.35 (Al ≥ 3.14 µmol/l) adjusted for fluoride, pH, turbidity, silica, iron, source of water, education, health, income, moves, and age	Forbes et al. (1992), (1995a)
Morbidity prevalence	Aluminium in water, pH, calcium	Cognitive function	Calcium protective RR = 1.2 with pH < 7.3 NS/all other pH values	Jacqmin et al. (1994)

Table 3. (contd)

Type of study	Measure of exposure to aluminium in water	Outcome measure/data source	Results	Reference
Case-control*	Aluminium in drinking-water, historical	PDAT Persons aged < 65 years; 109 cases, 109 controls (matched to cases, age, sex)	OR/NS for aluminium measures at all levels (highest > 149 µg/l)	Forster et al. (1995)
Morbidity prevalence	Aluminium in drinking-water	Males, mention of AD or presenile dementia (ICD-9), death certificate No age-education adjustments. Controlled for pH, SiO2, F in water	RR of AD at all ages = 2.42 for aluminium >336 µg/l RR of AD at age 75 + = 3.15 for aluminium > 336 µg/l *sample size = 14 at aluminium > 336 µg/l No linear dose-response (J-shaped response curve)	Forbes et al. (1995b)
Case-control	Aluminium in drinking-water; residence-weighted; historical	Pathological confirmation of diagnosis in all cases and controls; no age-sex-education adjustment	RR = 1.7 for aluminium > 100 µg/l RR = 2.5 for aluminium > 100 µg/l when based adjustment for 10-year weighted exposure history	McLachlan et al. (1996)
Morbidity incidence (Case-control)	Aluminium in drinking-water, exposure as years before diagnosis	106 males clinical diagnosis AD, Controls = other dementia, brain cancer, other neurodegeneration, *no disease, all aged 45–75. Adjusted for age, SiO2	No increased risk, highest aluminium > 109 µg/l vs lowest aluminium< 16 µg/l	Martyn et al. (1997)

Table 3. (contd)

Type of study	Measure of exposure to aluminium in water	Outcome measure/data source	Results	Reference
Case-control	Aluminium in drinking-water, estimates (historical) of long term exposure, variety of aluminium species	68 elderly clinically diagnosed age and sex-matched controls adjusted for education, ApoE ε4 allele, AD or dementia family history	NS for all aluminium measures except monomeric organic aluminium at time of diagnosis, OR = 2.67	Gauthier et al. (2000)
Prospective cohort	Aluminium in drinking-water, time-weighted historical	2698 males and females aged > 65 at baseline, 8 year follow-up dementia (DSM-IIIR, MMSE), AD (NINCDS-ARDRA) population-based (3401), adjusted for age, sex, education, place of residence, wine consumption	RR of dementia = 1.99 for aluminium > 0.1 mg/l RR of AD = 2.20 for aluminium > 0.1 mg/l High silica levels may be protective	Rondeau et al. (2000)
Morbidity prevalence*	Aluminium in drinking-water and questionnaire response about bottled and tap water	5691 women aged > 75 years at baseline, 5 cities, Epidemiology of Osteoporosis study, cognitive impairment, Pfeiffer score < 8	NS for aluminium Tap water range: 0.01-0.063 mg/l, five cities Bottled mineral water range: non-detectable to 0.032 mg/l	Gilette-Guyonnet et al. (2005)

Table 3. (contd)

Type of study	Measure of exposure to aluminium in water	Outcome measure/data source	Results	Reference
Nested case–control*	Aluminium in drinking-water and questionnaire response about bottled and tap water	60 cases diagnosed with SPMSQ, MMSE, Grober and Buschke test, assessment of daily living, NINCDS-ADRA (AD); 323 controls, normal cognitive function adjusted for age, education, income	NS for aluminium Tap water range: 0.060-0.063 mg/l, one city Bottled mineral water range: non-detectable to 0.032 mg/l	Gilette-Guyonnet et al. (2005)

From ICPS (1997), updated from COT (2005)
*Updated or inserted in this document)
MMSE: Mini-Mental State Examination; AD: Alzheimer disease; ALS: amyotrophic lateral sclerosis; NINCDS-ADRA: US National Institute of Neurological and Communicable Disorders and Stroke and Alzheimer Disease and Related Disorders; NS: non-significant; OR: odds ratio; PD: Parkinson disease; PDAT: presenile dementia of the Alzheimer type; RR: relative risk; SES, socioeconomic status; SPMSQ: Short Portable Mental Status Questionnaire.

A third case–control was conducted in Canada (The Canadian Study of Health and Aging, 1994). This study enrolled study participants aged 65 years or older from communities and institutions across Canada (except Ontario), using health insurance plan information. Sampled individuals from the community were screened for cognitive impairment with the 3MS. If the 3MS suggested impairment, individuals underwent clinical examination. Diagnosis of dementia was based on DSM-III-R, while possible or probable Alzheimer disease was based on the findings of dementia plus NINCDS-ADRDA criteria. Persons in institutions were examined clinically without first undergoing screening. Cases were selected from the possible and probable Alzheimer disease groups of patients ($n = 258$) and controls ($n = 535$) were selected from individuals assessed to be cognitively normal. Controls were frequency-matched to cases on age group, study centre, and residence in a community or institution. Proxy respondents for cases and controls answered a risk-factor questionnaire that included a limited dietary history, including a question about tea intake. The researchers did not find an association between Alzheimer disease and tea (OR = 1.40; 95% CI, 0.86–2.28, adjusting for age, sex, education and residence.

A fourth pilot case–control study conducted by Rogers & Simon (1999) in the USA and described immediately above also found no association between Alzheimer disease and tea (OR = O.6, $p = 0.69$). The OR was calculated from a very small number of discordant pairs ($n = 11$ pairs).

(iii) Exposure to aluminium in antacids

Aluminium concentrations in antacids, when present, are at a much higher concentration than are found in water. Daily intake of aluminium in antacids has been estimated as 1 g or more by Anke et al. (2001) and up to 5 g by Lione (1985). Case-reports of the effects of aluminium-containing antacids related to skeletal changes are described earlier in this document. Flaten (2001) reviewed 13 epidemiological studies that were germane to evaluating antacid use and Alzheimer disease, including three studies that indirectly evaluated this relationship by studying groups of patients with peptic ulcer or who were regular users of the H_2 blocker cimetidine. The 13 studies were published from 1984 to 1999, and the majority of the studies had a case–control design (9 of the 13). There was no prospective study design among these studies that could have allowed for ascertainment of antacid use directly from the patient before onset of disease. None of the estimates of risk (OR, RR or standardized mortality ratio, SMR) achieved statistical significance, and in 11 of the 13 studies, the risk estimate was below 1.0, tending in the direction of a protective association of the use of antacids with Alzheimer disease. However, sample size in most of these studies is very small, dose and frequency of use information for the antacid was not obtained, and information about whether antacids contain aluminium is sometimes not presented.

Case-reports of frequent use of antacids and its effect on bone are discussed above in the section on osteomalacia.

(b) Other neurological conditions: ALS, parkinsonism–dementia

Studies of patients and sampling of drinking-water and garden soil have been conducted in three areas of the western Pacific with high incidence rates of amyotrophic lateral sclerosis (ALS) and parkinsonism–dementia (Gajdusek & Salazar, 1982; Perl et al., 1982). These three areas include Guam, the Kii Peninsula of Japan and southern West New Guinea. Unusually low concentrations of calcium and magnesium were found in the drinking-water and soil from these areas, while relatively high concentrations of other elements were found, including aluminium. Gajdusek & Salazar (1982) observed that villages with a high incidence of ALS or parkinsonism–dementia had different geographical terrain compared with other villages in the same general regions with low incidence of disease; however, investigators only obtained samples of water and soil from the high but not the low incidence villages. Perl et al. (1982) examined accumulation of aluminium within the brain tissue of eight Guamanian persons, three of whom had died of ALS or parkinsonism–dementia and five of whom (the controls) had died of non-neurological disorders and did not show signs of neurological disease before death. The brain tissue of all eight persons contained aluminium, but concentrations were higher in the patients with ALS and parkinsonism–dementia and in one control compared with the remaining four controls. Perl et al. (1982) found the presence of neurofibrillary tangle-bearing hippopcampal neurons in the persons with the higher concentrations of aluminium, but did not detect neurofibrillary tangle-bearing neurons in the four remaining controls. The role of aluminium, if any, in the initiation and development of the disease is not elucidated in these studies since the effect of the other factors potentially associated with the disease or their interactions are not yet fully understood.

In Italy, Bergomi et al. (2002) conducted a population-based case–control study to evaluate the association between exposure to trace elements, including aluminium, and sporadic ALS. They enrolled patients from five provinces of Italy whose first diagnosis of ALS occurred in 1998–1999. A neurologist diagnosed ALS and determined if the disease was possible, probable or definite, based on the El Escorial criteria (Brooks, 1994). A patient with possible or probable ALS was followed until a conclusive diagnosis was made and then defined as a case in the study. Controls were selected from the same population as the cases, employing random sampling from the National Health Service directory (all citizens in Italy are included in the National Health Service) and matching by same birth year and gender as the cases. The sample size for the study was 62 persons, 22 cases (10 women and 12 men) and 40 controls (18 women and 22 men). Investigators administered a questionnaire on clinical, life-style and dietary factors and sampled toenail specimens and blood. Toenails were analysed for a number of trace elements, including cadmium, lead, copper, manganese, selenium, chromium, cobalt, iron and aluminium. No association between ALS and aluminium was found. The meaning of this result is difficult to determine since toenail concentration of aluminium as a biomarker for chronic environmental exposure to aluminium has yet to be validated.

(c) All-cause mortality

As a follow-up to the water pollution incident in Camelford, UK and its vicinity (see section 3.1.2, case of severe cerebral congophilic angiopathy, for a fuller description of this incident), Owen et al. (2002) compared mortality in the area with water pollution (n = 11 114 residents) to mortality in an adjacent area free of pollution (n = 5359 residents). They collected information on deaths from July 1988 to December 1997 and corrected death rates for differences in age distribution and sex between the two populations. They calculated an SMR for the exposed population and a SMR for the unexposed population, standardized to England and Wales or standardized to Cornwall and the Isles of Scilly. The ratio of the SMR for the exposed population to the SMR for the unexposed population was 1.08 (95% CI, 0.97–1.21), standardized to England and Wales. The ratio of SMRs, standardized to Cornwall and the Isles of Scilly was closely similar. These results suggest a very small but not statistically significant excess of mortality in the exposed group. No other factors were considered in this study. The authors noted that not all deaths were accounted for if individuals moved out of either area before the recommendation in 1991 to ascertain deaths. It is not clear how much influence under-reporting of deaths had on the estimates.

(d) Other disorders

At the present time, oral exposure to aluminium in humans has not been associated with cancer, genotoxicity or reproductive toxicity.

4. ANALYTICAL METHODS

4.1 Food additives

Each specification monograph for food additives containing aluminium has methods of analysis for identity and purity (qualitative methods for identification, and quantitative methods for assessing the purity of the additive). The methods are either included in the monographs or refer to methods in common for two or more substances in Volume 4 of the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*).

4.2 Food samples.

Food samples would normally be rendered into soluble samples, e.g. by microwave-assisted acid digestion, before instrumental measurement of aluminium. Graphite-furnace atomic absorption spectrometry, inductively coupled plasma-optical/atomic emission spectrometry (ICP-OES/AES) and inductively coupled plasma mass spectrometry (ICP-MS) are commonly used methods for measuring aluminium in foods. All offer low detection limits, which are typically 0.1 mg/kg sample or lower, depending on the sample type.

Graphite-furnace atomic absorption spectrometry can be affected by high-chloride matrices and this can be a problem especially for biological samples. ICP-AES and ICP-MS are relatively free from interference, although Ti and Ca can give

high background signals in ICP-AES and ICP-MS can suffer interference from beryllium oxide, boron oxide and cyanide.

In proficiency testing schemes, errant results that deviate from the mode cannot be ascribed to any particular analytical method. All the methods described above are capable of returning reliable results for aluminium in food.

Aluminium is an abundant element in the environment so background levels in laboratory analysis can be a problem, particularly when measuring biological samples (blood, plasma etc) where concentrations are low (low parts per billion, µg/kg). Concentrations in food are generally higher (low parts per million, mg/kg) and so analytical background contamination is not such a problem but it always needs to be guarded against.

A large proportion of errant results in proficiency testing schemes are submitted by laboratories using non-accredited procedures. These errant results tend to be too high compared with the norm, and this may be owing to insufficient care taken to exclude high background levels of aluminium in reagents and glass/plastic ware or other contamination issues.

5. SAMPLING PROTOCOLS

There is no specific Codex method of sampling of food to be analysed for aluminium, but there are Codex Alimentarius Committee Guidelines CAC/GL 50 (2004) 'General guidelines in sampling' which are helpful.

6. EXPOSURE TO ALUMINIUM IN THE DIET AND OTHER SOURCES

Only consumer exposure to aluminium in the diet and other routes or commodities was considered by the Committee, without consideration for occupational exposure. Previous reviews by the Committee (WHO, 1989a; WHO, 1989b) IPCS (WHO, 1997) & COT (COT, 2005) and recent literature data were 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 kg body weight basis, a standard 60 kg adult was considered, unless otherwise specified.

6.1 Dietary exposure (including drinking-water)

In the last evaluation made by the Committee, dietary exposure, particularly through foods containing aluminium compounds used as food additives, was found to represent the major route of aluminium exposure by the general public excluding persons who regularly ingest aluminium-containing drugs (WHO, 1989a; 1989b). The review by IPCS in 1997 confirmed that non-occupational human exposure to aluminium in the environment is primarily through ingestion of food and water (WHO, 1997). Of these, food appeared as the principal contributor.

The three dietary sources of aluminium are natural sources (foods and beverages, drinking-water), packaging and utensils used during food preparation and storage and food additives.

The geological origins of the soil and its conditions, especially its pH, have a significant influence on the aluminium content of the food chain. The solubility of aluminium compounds may increase when acid rain decreases the pH of the soil, as a consequence aluminium content increases in surface water, plants and animals (Anke, 2001).

6.1.1 Drinking-water

In the last evaluation by the Committee (Annex 1, reference *84*), although water was not found to contribute significantly to the total aluminium exposure from all sources for most individuals, elevated aluminium concentrations were reported in water from certain areas and resultant aluminium exposure could be as high as the dietary contribution.

Aluminium in natural waters is mainly derived from weathering of rocks and minerals. Analytical data from drinking-water in the USA suggest that the aluminium content of raw surface water is higher than that of raw ground water. Thus 55% of the raw surface waters had a concentration of greater than 50 µg/l vs only 4% of the raw ground waters (Miller, 1984).

Concentration of dissolved aluminium in raw water near pH 7 is typically between 1 and 50 µg/l, but can increase to 500–1000 µg/l in acidified water (Yokel, 2004, cited in Schafer). Based on the consumption of 2 l of water per day, exposure through this source is therefore up to 2 mg/day, corresponding to 0.03 mg Al/kg bw per day

Aluminium may also be present in drinking-water owing to the use of salts of aluminium as a chemical coagulation-based treatment of surface waters, which is the most common approach for treatment of surface waters (WHO, 2004). Chemical coagulants are usually salts of aluminium or iron. Typical coagulant doses are 2–5 mg Al/l. Coagulation is used for removal of microorganisms, turbidity and colour and can also remove certain heavy metals and low-solubility organic chemicals, such as certain organochlorine pesticides. No health-based guideline value for aluminium in drinking-water has been established by WHO. However, practical levels were derived which minimize concentrations of aluminium in finished water: 0.1 mg/l or less in large water treatment facilities, and 0.2 mg/l or less in small facilities (WHO, 2004). These recommendations provide a compromise between the beneficial effects of the use of aluminium salts as coagulants in water treatment on the one hand, and discoloration and health concerns about aluminium as a potential neurotoxicant, on the other hand. The presence of aluminium at concentrations in excess of 0.1–0.2 mg/l is unlikely since it often leads to consumer complaints as a result of deposition of aluminium hydroxide floc in distribution systems and the exacerbation of discoloration of water by iron.

Based on a daily consumption of 2 l per day, dietary exposure to aluminium from treated drinking-water may be up to 0.4 mg/day, corresponding to 0.007 mg/kg bw per day.

6.1.2 Aluminium from natural dietary sources

The aluminium content of the flora also depends on the variety, part and age of the plant. The concentration of aluminium is high in leaves, medium in blossoms, ears, fruits and seeds, and low in stalks (Anke et al., 2001).

Müller et al. (1998) reported analytical data on 128 foods and drinks in Germany in 1988 and 1992, including non-processed foods. The highest concentrations of aluminium (mg/kg fresh matter) were found in spices (mean, 145 mg/kg fresh matter; range, 6.5–695), cocoa and cocoa products (mean, 33 mg/kg; range, 9–103) and herbs (mean, 19 mg/kg; range, 8–26). Intermediate concentrations were found in vegetables (mean, 5.7 mg/kg; range, 0.7–33) and in meat, sausage, offal (mean, 5.4 mg/kg; range, 2.5–10). Lowest concentrations were found in fruit (mean, 1.5 mg/kg; range, 0.4–2.6). Generally, a relatively large variation in concentration was found within all categories of foodstuffs. Most foodstuffs contained less than 5 mg/kg.

Tea leaves contain high concentrations of aluminium, but only a small proportion of it remains in the tea decoction, providing around 0.4 mg Al/cup (Neelam, 2000).

According to Greger (1992), most unprocessed foods in the USA contain aluminium at less than 5 mg/kg and most individuals consume aluminium at 1 to 10 mg/day from natural dietary sources. The average Swedish daily diet from unprocessed foods was calculated to contain about 0.6 mg aluminium with three food items providing 80%: coffee, wheat flour and tea (Jorhem & Haegglund, 1992).

6.1.3 Aluminium migrating from food-contact material (food containers, cookware, utensils and packaging)

Because of its lightness, malleability, tensile strength and corrosion resistance, aluminium is used extensively in structural materials in the packaging of foodstuffs and beverages (cans, cartons, laminated paperboard packages, tubs, foil), in kitchen utensils (knives and forks, pots and pans, baking trays, mocha-type coffee pot). The use of aluminium has increased in recent years owing to the widespread use of precooked or frozen foods sold in disposable trays or wrapped in aluminium foil. Aluminium dissolves in non-oxidizing acids and can therefore be released from aluminium-containing packaging into the foodstuff in presence of an acidic medium. A number of studies have been conducted to estimate potential exposure from this source.

In Sweden, the aluminium content of beverages packed in glass bottles was not found to be different from that of aluminiumcans, indicating that the release of aluminium from the cans to the contents is small (Jorhem & Haegglund, 1992). A number of studies show that migration of aluminium from aluminium-containing cookware and utensils into food was found to be high if acidic foods (tomato sauces,

sauerkraut) are cooked in uncoated aluminium containers. The highest rate of migration is found when aluminium utensils are used for acid foods (AFSA/AFSSPS/AVS, 2003; Scancar, 2003).

In the Netherlands, aluminium in duplicate diets of 18 subjects cooking in aluminium pans was found to be similar to that of the other subjects (Ellen et al., 1990). A study performed in Italy showed that aluminium does migrate from containers to foods and beverages in conditions representative of actual use, with the highest release into acidic and salty foods (pickles and tomatoes); the overall increase in dietary exposure through this source could reach 6 mg/day under theoretical worst-case assumptions (Gramiccioni et al., 1996). On the other hand, aluminium in duplicate diets of Swedish women using regularly aluminium utensils and foils was found to be 2 mg/day higher than that of women who did not use them (Jorhem & Haegglund, 1992).

A higher contribution from aluminium migration was reported in a study conducted in India (Neelam, 2000). Food was prepared according to traditional recipes in stainless steel, old aluminium vessels (age 10 years) and new aluminium vessels (age 1 to 15 days) and analysed for aluminium. Based on food consumption data, exposure in the urban population was estimated to be 9.6 (range, 5.6–16.2), 14.2 (range, 8.3–23.2) and 18.2 (range, 10.5–32) mg/day, respectively. These data suggest that daily use of an aluminium vessel may lead to an increased exposure of around 7 mg/day.

6.1.4 Aluminium present in food additives

Table 4 presents the provisions made for aluminium compounds in the current draft Codex GSFA.

Some aluminium-containing additives are listed in the current draft versions of Table 1 and 2 of the Codex GSFA and for these additives reference is made to the PTWI for aluminium established in 1988 by the JECFA Committee. This is the case for aluminium ammonium sulfate and SALP, acidic and basic. These aluminium compounds may be used according to good manufacturing practices (GMP) in a large number of products and at maximum levels in other products. The Committee noted that maximum levels are generally expressed as aluminium (e.g. 35 000 mg/kg expressed as Al for SALP used in processed cheese) but that in some cases the reporting basis is not specified (aluminium ammonium sulfate, up to 10 000 mg/kg in bakery products).

Some additives containing aluminium are listed in Tables 1, 2 and 3 of the current draft Codex GSFA. In Table 3, reference is made to an ADI 'not specified' for aluminium, and sodium aluminium silicate, calcium aluminium silicate and aluminium silicate are allowed at GMP in food in general. Specifications are available in the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*) for other aluminium compounds, but no provision has yet been made for them in the Codex GSFA. This is the case for aluminium lakes of dyes and colours, aluminium sulfate and potassium aluminium sulfate. Other aluminium compounds are used in a number of countries but are not reported in the Codex

GSFA or in the *Combined Compendium*. This is the case for aluminium powder, aluminium oxide, potassium aluminium silicate and SALP.

The Committee was provided with an assessment of exposure to SALP in Europe (EFPA, 2005). In this assessment, the total annual sales of SALP in the countries of the European Union (EU) in 2004 (852 tonnes) was divided by 380 million (the estimated number of residents) to calculate the average exposure per capita: 2.24 g of SALP per year. Based on a standard body weight of 60 kg, the average exposure was estimated to be about 0.1 mg/kg bw per day, expressed as SALP. This exposure corresponds to an intake of less than 0.01 mg Al/kg bw per day, based on the 8.5% aluminium content of the tetra hydrate SALP acidic.

The Committee was also provided with disappearance data for the USA, collected by the International Food Additives Council, for a number of aluminium compounds used as food additives (O'Brien Nabors, 2006). Overall, aluminium present in SALP, basic and acidic; aluminium sodium sulfate; sodium aluminium silicate and aluminium lakes intended for human consumption, sold in the USA in 2003 and 2004 amounted to respectively 5921 pounds (x 0.45 = 2664 kg) and 5961 pounds (x 0.45 = 2682 kg), equivalent to 9 mg Al/capita per year (based on populations of 290 850 005 and 293 656 842 in 2003 and 2004 respectively).

These data may provide an estimate of average exposure to aluminium through additives in two very large populations (the EU and USA). However, the consumption is not homogeneously distributed among EU countries with the UK being the largest consumer of SALP) and may not be homogeneously distributed among consumers. Thus, from the use of approximately four million pounds in weight of aluminium in food additives in the USA in 1982, it has been estimated that the average citizen of the USA theoretically consumes 21.5 mg Al/day from food additives. However, further information allows it to be estimated that about 5% of adults in the USA were exposed to more than 95 mg Al/day from additives while 50% of them were exposed to 24 mg or less. These data indicate that individual use of aluminium-containing food additives varies greatly among consumers (Greger, 1992).

Further data are available to estimate exposure in the population of interest i.e. regular consumers of products containing aluminium compounds.

According to Greger (1992), the most commonly used aluminium-containing food additives in the USA are acidic SALP (leavening agent in baked goods); the basic form of SALP (emulsifying agent in processed cheese); aluminium phosphates (acidifying agents); bentonite (materials-handlings aid), aluminium lakes of various food dyes and colours, aluminium silicates (anti-caking agents). Although aluminium-containing additives were found to be present in only a limited number of foods, some processed foods have very high contents. Greger (1992) reports concentrations of 297 mg/kg in processed cheese, 400 mg/kg in home made corn bread, 128 mg/kg in muffins, 2300 mg/kg in baking powder and 164 mg/kg in salt.

Table 4. Aluminium compounds used as food additives present in the current draft GSFA

Name	Function	Applications	Levels of use (expressed as aluminium)	INS No.	JECFA evaluationa
SALP, acidic & basic	Acidity regulator, emulsifier in processed cheeses, raising agent in bakery products, stabilizer, thickener	Baking powder, flours, bakery products, cheese, cocoa powders, desserts, bakery wares, confectionery, mixes for soups and sauces, concentrates for water-based flavoured drinks	Up to 35 000 mg/kg in processed cheese and 45 000 mg/kg in flours	541(i), 541(ii)	PTWI for aluminium powder (GSFA Tables 1 and 2)
Aluminium ammonium sulfate	Firming agent, raising agent, stabilizer	Bakery products (including ordinary bakery products), egg products, herbs and spices, soya-bean products, snacks, processed fish, processed vegetables, candied fruit	Up to 10 000 mg/kg in bakery products GMP in starch and soya-bean products	523	PTWI for aluminium powder (GSFA Tables 1 and 2)
Sodium aluminium silicate	Anti-caking agent	Salt and salt substitutes, sugar, grain Permitted for use in food in general	Up to 20 000 mg/kg in salt GMP in grain and food in general	554	ADI 'not specified'

Table 4 (contd)

Name	Function	Applications	Levels of use (expressed as aluminium)	INS No.	JECFA evaluationa
Calcium aluminium silicate	Anti-caking agent	Salt and salt substitutes, sugar, Grape wines, grain Permitted for use in food in general	Up to 20 000 mg/kg in salt GMP in grain, grape wine and food in general	556	ADI 'not specified' (GSFA Tables 1, 2 and 3)
Aluminium silicate	Anti-caking agent	Salt and salt substitutes Grain, herbs and spices Permitted for use in food in general	Up to 10 000 mg/kg in salt GMP in grain, herbs and spices and in food in general	559	ADI 'not specified' (GSFA Tables 1, 2 and 3)

ADI: acceptable daily intake; GMP: good manufacturing practice; GSFA: General Standard for Food Additives; SALP: sodium aluminium phosphate
[a]As reported in current draft Codex GSFA.

Also, the Swedish study of Jorhem & Haegglund (1992) clearly demonstrated that aluminium compounds used as food additives increase the daily intake of aluminium by one order of magnitude in consumers of the foods which contain such compounds. The major contributor of aluminium to the Swedish duplicate diets was found to be a chocolate/mint cake. The high concentration of aluminium in this cake may derive from its known ingredients cocoa, mint and/or baking soda. The six diets including this cake contained on average 72 mg Al/day while the mean content of the remaining 99 diets was 9.7 mg Al/day.

In Germany, the highest aluminium content in processed foods was found in biscuits (22 mg/kg) and in soft cheese (8 to 16 mg/kg) Müller et al., 1998).

In the 2000 UK TDS, the miscellaneous cereals group was reported with the highest mean concentration of aluminium (19 mg/kg fresh weight) (FSA, 2004).

In the second Chinese Total Diet Study (Junquan Gao, 2006) the highest content was found in cereal products (50 mg/kg), owing to the use of leavening agents containing aluminium. The maximum concentration of aluminium given in Chinese standards was 100 mg/kg in cereals and cereal products.

The potential high aluminium content of cereal products and in particular of ordinary baked goods may be of special importance in a number of countries since they constitute staple food and may therefore be consumed regularly in large quantity.

6.1.5 Assessment of total dietary exposure

Duplicate diet studies have been performed in a number of countries (Table 5). Mean values varied between 3 and 13 mg/day. The highest single reported value was 100 mg/day in a sample from Sweden. Data reported in Germany suggest that aluminium progressively decreased in the diet by about half from 1988 to 1996, probably owing to efforts to reduce the acidity of rain (Anke et al., 2001).

Moreover, duplicate diet studies were collected and analysed in nine developed and developing countries involved in a multicentre study published in 1991 (Parr et al., (1991). The 75th percentile of exposure to aluminium was estimated after normalizing for a 10 MJ daily energy intake. The lowest values were observed in Japan and Norway (2 mg/day). Increasing values were observed in Italy, Spain, Thailand, Brazil, China, Iran and Turkey reaching 18 mg in Sudan.

A number of market-basket studies have been performed, allowing estimation of exposure in different population groups by calculation (Table 6). These results are based on mean content of aluminium in food groups and mean consumption.

In the adult population, mean exposure to aluminium estimated by model diet or market basket varied from approximately 2 mg in the French survey to more than 40 mg/day in China.

Table 5. Intake of aluminium determined by the duplicate portion technique in several countries

Country	Year of investigation	Mean (range) in mg/day	Reference	Remarks
Netherlands	1978	4.6 (1.4–33.3)	Ellen et al. (1990)	101 adults (26 females and 75 males), one 24 h sample each
Netherlands	1984–1985	3.1 (0.6–12.9)	Ellen et al. (1990)	110 adults (53 females and 57 males) 1 week sample each
Hungary	1989–1990	3.3 (0.3–19.4)	Gergely et al. (1991)	84 samples
Japan	1981	4.0 (1.3–10.3)	Shiraishi et al. (1989)	31 males, 62 24 h samples
Germany	1988 1991/2 1996	5.4/6.5 4.6/4.9 3.1/3.2	Anke et al. (2001)	Females/males mixed diet
Germany	1996	4.1/4.1	Anke et al. (2001)	Females/males; ovo-lacto-vegetarian diet
Italy	Not reported	2.5/3.1/4.3/6.3	Gramiccioni et al. (1996)	Four different regions (overall 19 24 h samples)
India	2000–2001	6.4 (1.9–12.1)	Tripathi et al. (2002)	45 24 h samples
Taiwan (China)	1989, 1990	5.2/4.9	Liu & Chung (1992)	15 subjects, three 24 h samples, females/males
Sweden	Not reported	13.0 (1.2–100)	Jorhem & Haegglund (1992)	105 duplicate diets in 15 non-smoking females

Exposure to aluminium was found to be lower in the 1993 US Food and Drug Administration (FDA) Total Diet Study (TDS) when compared with that conducted in 1984 (Pennington & Schoen, 1995). The highest mean exposure per kg bw was found in small children: 6 mg/day for children aged 2 years, which corresponds to approximately 0.5 mg/kg bw per day based on a standard 12 kg bw.

Table 7. Intake of aluminium (mg/day) calculated with the market basket method or a model diet in several countries

Country	Year of investigation	Mean or range, in mg/day; Males/ females	Remarks	Reference
China	1992–1993	17.8 31.5 43.4/41.5	Young children (2–7 years) Older children (8–12 years) Adults (20–50 years)	Junquan Gao (2006)
Japan	1986	3.8/3.5 4.1/3.0 2.3/2.3	Children (3 years) Teenagers (16 years) Adult (40 years) males	Shiraishi et al. (1988)
UK	1997 2000	3.4 4.7		MAFF (1999) MAFF (2004)
Finland	1975–1978	6.7		Varo & Koivistoinen (1980)
USA	1993	0.7 11.5 7 8–9	6—11 months 14–16 years, males Adult females Adult males	Pennington & Schoen (1995)
USA	1984	1.8 6.3 8.6 12.7 8.7 13.7 8.9 11.8	6–11 months 2 years 14–16 years females 14–16 years males 25–30 years females 25–30 years males 60–65 years females 60–65 years males	Pennington & Jones (1989)
USA	1985	14.3	25–30 years males	Iyengar et al. (1987)
France	2000	1.3 1.6	3–15 years 15 years and above	Leblanc et al. (2005)

MAFF: Ministry of Agriculture, Fisheries and Food

In contrast, the 2000 UK TDS revealed that dietary exposure to aluminium has increased by about one third, reaching 4.7 mg/day (FSA, 2004) versus 3.4 mg/day in the previous UK TDS conducted in 1997 (MAFF, 1999). In the more recent study, miscellaneous cereals, which contained aluminium at a mean concentration of 19 mg /kg, were the most significant (45%) contributor to the dietary exposure of the population, probably owing to the use of aluminium-containing food additives. On the other hand, bread contained aluminium at an average concentration of 3 mg/kg and contributed only 7% of the overall exposure. Exposure expressed per kg bw varied from 0.06 mg/kg bw per day in the elderly to 0.16 mg/kg bw per day in toddlers (1.5–4.5 years), based on measured body weight. High levels of exposure, estimated on the basis of high level of consumption, was estimated to vary from 0.13 mg/kg bw per day in the elderly to 0.33 mg/kg bw per day in toddlers.

In the second Chinese Total Diet Study conducted in 1992–93, high exposure to aluminium was estimated owing to the high mean aluminium content of cereal products. In children, mean estimated dietary exposure was around 1 mg/kg bw per day in both age class 2–7 years and 8–12 years: considering as standard body weight 16.5 kg and 29.4 kg respectively. Exposure in high consumers of these products or in regular consumers of products would be higher.

Infants

Since the 1990s, there has been some concern about the aluminium content of infant formulae (Greger, 1992). The aluminium content of human milk and cows' milk was found to be negligible (< 0.05 mg/l) (Koo et al., 1989, cited by Greger, 1992) while high levels of aluminium were found in milk-based formulae and soya-based formulae leading to the presence of aluminium at of 0.01–0.36 and 0.4–6.4 mg/l respectively in the products ready for consumption (Greger, 1992). A high concentration of aluminium was also found in soya-based powder infant formula present on the Swedish market (Jorhem & Haegglund, 1992): 14 mg/kg which, based on a typical dilution factor of 1 : 7, corresponds to 2 mg/l in the reconstituted milk.

Based on the German DONALD study (Kersting, 1998), in a 3 month infant weighing on average 6.1 kg, average and 95th percentile consumption of dry infant formula are respectively 105 and 144 g/day, which, based on a 1:7 dilution factor correspond to respectively 0.7 and 1 l/day of reconstituted formulae.

Thus, infants aged 3 months consuming a soya-based formula containing aluminium at a concentration of 6 mg/l (the highest concentration reported) once reconstituted could be exposed to approximately 4 mg/day on average and 6 mg/day for high percentile consumption. In the case of milk-based formulae containing aluminium at 0.4 mg/l (highest concentration reported), once reconstituted, potential exposure to aluminium would be up to 0.3 mg/day for average consumption and 0.4 mg/day for high consumption. Infants fed the same quantities of human or cows' milk would be exposed only to less than 0.03 mg/day for average consumption and less than 0.05 mg/day for high consumption.

Expressed on a kg body weight basis these values correspond to 1 mg/kg bw and 0.06 mg/kg bw for high consumption in infants fed soya-based formulae and milk-based formulae, respectively. In the case of infants fed human or cows' milk, high consumption would lead to an aluminium exposure of less than 0.01 mg/kg bw.

6.2 Other sources of exposure

6.2.1 Inhalation

When aluminium was reviewed by the Committee in 1988, exposure to aluminium from air, even in industrial areas, was found to be minor relative to that from food (Annex 1, reference *84*).

Atmospheric concentrations of aluminium in non-industrial rural and urban areas range from 0.05 to 0.5 and 0.1 to 5 μg/m^3 and are typically 0.2 and 1 μg/m^3, respectively. In industrial areas, the concentration of aluminium may rise to 25–2500 μg/m^3. The main source in remote locations is weathering of aluminosilicate rocks and soils. Anthropogenic sources are coal combustion, iron, pumice stone, cement, kaolin and chalk works as well as waste incineration. Atmospheric particulate aluminium consists of silicates, oxides and hydroxides (Yokel & McNamara, 2001; Yokel, 2004, cited in Schafer, 2005).

Consumer exposure through air is a minor source of exposure. According to *Environmental Health Criteria 194*, pulmonary exposure may contribute up to 0.04 mg/day (WHO, 1997).

6.2.2 Dermal exposure to consumer products containing aluminium

Aluminium chlorohydrate in antiperspirants produces insoluble aluminium hydroxide on the skin to form an obstructive plug in the sweat gland duct. Many deodorant stones contain aluminium sulfate. In dental rinses and toothpastes, aluminium is used to reduce dentinal hypersensitivity. Aluminium is found in some acne cleaning preparations as an abrasive (Yokel, 2004, cited in Schafer, 2005). Results of a preliminary study on dermal absorption of aluminium chlorohydrate used as active ingredient of antiperspirant suggest that about 4 μg of aluminium is absorbed from a single use on both underarms (Flarend, 2001).

6.2.3 Consumption of medicines containing aluminium

Aluminium hydroxides administered orally are used as antacids and in phosphate binders. Aluminium is also an auxiliary in diarrhoeal remedy preparations and buffered analgesics, in anorectic preparations (as a keratolytic) and vaginal douches, in products for dermatitis (as an astringent), in first-aid antibiotics, and antiseptics. Furthermore, aluminium salts are added as adjuvant to vaccines and allergy immunotherapeutics in order to increase their antigenic properties (Yokel, 2004, cited in Schafer, 2005; Anke et al., 2001). According to Anke et al. (2001), daily intake of aluminium in e.g. antacids may be 1 g and more. According to Lione (2005), if taken as directed, the daily intake of aluminium from antacids can be as much as 5 g, while aluminium-buffered aspirin used for rheumatoid arthritis can contribute 0.7 g/day.

Bioavailability of aluminium

Available data are not sufficient to correct the exposure assessment on the basis of bioavailability. Aluminium contained in some additives such as silicates may have a low bioavailability, but the main sources of exposure are sulfates and phosphates used in cereal products. Experimental data suggest that absorption of aluminium increases in presence of citric acid through chelation (Fulton & Jeffery, 1990). Very few data are available on the content of citric acid in the diet. Citric acid is one of the main organic acids present in fruit, with amounts varying from 0.05 to 3.2 g/l in juices (Chinnici et al., 2005). Citric acid in cheese was shown to vary from 0.07 g/kg in brie to 1.5 g/kg in cheddar cheese (Mullin & Emmons, 1997). Citric acid may also be added to fruit based products (fruit juices, jam, cocoa products) and to cheese as an additive. A diet high in fruit and fruit based products could eventually lead to a higher bioavailability of aluminium.

7. EFFECTS OF PROCESSING

The Committee found no information on reduction of the aluminium content of foodstuffs by processing.

8. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF RISK OF CARCINOGENICITY/TOXICITY

8.1 Contribution of data to assessment of risk

8.1.1 Pivotal data from biochemical and toxicological studies

Assessment of the bioavailability of aluminium compounds is confounded by limitations in the analytical methodology, particularly for older studies, by concurrent exposure to modifying factors and by dose-dependency. Speciation appears to be an important factor in absorption and it is widely assumed that soluble aluminium compounds, such as the chloride and lactate salts, are more bioavailable than insoluble compounds, such as aluminium hydroxide or silicates. Studies in laboratory animals and in human volunteers generally show that absorption of aluminium is less than 1%. However, because of the differences in methodology, it is not possible to draw precise conclusions on the rate and extent of absorption of different aluminium compounds. Concurrent intake of organic anions (particularly citrate) increases the absorption of aluminium, while other food components, such as silicates and phosphate, may reduce the absorption of aluminium.

Studies reviewed by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*) showed no detectable aluminium in the urine of normal subjects given aluminium hydroxide gel (2.5 g Al/day, equivalent to 42 mg/kg bw per day assuming body weights of 60 kg) for 28 days. In contrast, faecal excretion of aluminium in patients with chronic renal disease given aluminium hydroxide (1.5–3.5 g Al/day, equivalent to 25–57 mg/kg bw per day, assuming body weights of 60 kg) for 20–32 days indicated a daily absorption of 100–568 mg of aluminium.

Slight increases in concentrations of aluminium in plasma were reported over the study period.

Oral dosing of rats with aluminium compounds has been shown to result in increased concentrations of aluminium in blood, bone, brain, liver and kidney. Studies with ^{26}Al administered intravenously to a small number of human volunteers indicate a biological half-life of about 7 years (in one individual) and interindividual variation in clearance patterns.

Aluminium compounds have been reported to interfere with the absorption of essential minerals such as calcium and phosphate, although the extent to which this occurs at dietary exposure levels is unclear.

The available toxicological studies were from the published literature and were not designed to assess the safety of food additives. Most were conducted to investigate specific effects or mechanisms of action, and many do not provide information on the dose–response relationship. Some do not make clear whether the stated dose relates to aluminium or to the aluminium compound tested. A further complication is that many studies do not appear to have taken into account the basal aluminium content of the animal feed before addition of the test material. Some studies refer to a basal aluminium content of about 7 mg/kg, which would not add significantly to the doses of aluminium under investigation. However, ATSDR (1999) reported that there are diverse concentrations ranging from 60 to 8300 mg/kg feed and that substantial variation between brands and between lots occurs. For chow containing aluminium at a concentration of 200 mg/kg, applying the default JECFA conversion factors indicates doses equivalent to 30 mg Al/kg bw for mice and 20 mg Al/kg bw for rats. The toxicological data are influenced by the solubility, and hence the bioavailability, of the tested aluminium compounds, and the dose–response relationship will be influenced by the aluminium content of the basal animal feed.

Recent studies have identified effects of aluminium compounds at doses lower than those reviewed previously by the Committee. Studies in rats, rabbits and monkeys have indicated effects on enzyme activity and other parameters associated with oxidative damage and calcium homeostasis in short-term studies with aluminium compounds administered at oral doses of 10–17 mg Al/kg bw per day. These studies involved administration at a single dose and did not take into account the aluminium content of the diet. The functional relevance of the observations is unclear and since the total exposure is unknown, they are not suitable for the dose–response analysis.

Mild histopathological changes were identified in the kidney and liver of rats given aluminium sulfate by gavage at a dose of 17 mg Al/kg bw per day for 21 days. Rats given drinking-water containing aluminium chloride at a dose of 5 and 20 mg Al/kg bw per day for 6 months showed non-dose-dependent decreases in body weight and changes in haematological parameters and acetylcholine-associated enzymes in the brain. Histopathological changes were observed in the kidney and brain at doses of 20 mg Al/kg bw per day in the latter study. These effects have not been observed in other studies and total exposure is unknown since aluminium content of the diet was not taken into account.

Beagle dogs given diets containing SALP (basic) for 6 months showed decreased food intake and body weight and histopathological changes in the testes, liver and kidneys in the males at the highest concentration tested, 1922 mg Al/kg of diet, equal to 75 mg Al/kg bw per day. No effects were seen in female dogs at this dietary concentration, equal to 80 mg Al/kg bw per day. The no-observed-effect level (NOEL) in this study was a dietary concentration of 702 mg Al/kg, equal to 27 mg Al/kg bw per day. This study is similar to that providing the basis for the previously established PTWI, which used SALP acidic. The Committee noted that there was no explanation for the observed sex difference, and limitations in the reporting made interpretation of this study difficult.

Special studies have highlighted a potential for effects on reproduction, on the nervous system and on bone. Few of these studies are adequate to serve as a basis for the determination of no-effect levels, as they were designed to address specific aspects and only a very limited range of toxicological end-points were examined.

Soluble aluminium compounds have demonstrated reproductive toxicity, with LOELs in the region of 13–200 mg Al/kg bw per day for reproductive and developmental effects with aluminium nitrate. None of these studies identified NOELs. The lowest LOELs were obtained in studies in which aluminium compounds were administered by gavage; taking into account the aluminium content of the diet, the total dose may have been in the region of 20 mg Al/kg bw per day or more.

Neurotoxicity potential has received particular attention because of a speculated association of aluminium with Alzheimer disease. Many of the studies in laboratory animals have been conducted using parenteral administration and are of uncertain relevance for dietary exposure because of the limited bioavailability of aluminium compounds likely to be present in food. In contrast to studies with other routes of administration, the available data from studies using oral administration do not demonstrate definite neuropathological effects. Some studies indicate that certain aluminium compounds, especially the more soluble forms, have the potential to cause neurobehavioural effects, at doses in the region of 50 to 200 mg Al/kg bw per day administered in the diet. The studies indicating the lowest LOELs took account of the basal diet content of aluminium and one of these studies also indicated a NOEL of 10 mg Al/kg bw per day.

The previously established PTWI of 0–7.0 mg/kg bw for aluminium was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing SALP acidic at a dietary concentration of 3% for 189 days, equivalent to approximately 110 mg Al/kg bw.

The new data reviewed at the present meeting indicated that soluble forms of aluminium may cause reproductive and developmental effects at doses lower than that used to establish the previous PTWI. Although insoluble aluminium compounds may be less bioavailable, the evidence that other dietary components, such as citrate, can increase uptake of insoluble aluminium suggests that data from studies with soluble forms of aluminium can be used as a basis for deriving the PTWI.

8.1.2 Pivotal data from human clinical/epidemiological studies

The previous evaluation of aluminium made by the Committee at its thirty-third meeting (Annex 1, references *83*, *84*) did not include epidemiology studies. Since then, a number of epidemiology studies have been conducted, with most focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some epidemiology studies suggest an association of consumption of aluminium in water with Alzheimer disease, but this was not confirmed in others. None of the studies accounted for ingestion of aluminium in food, a potentially important confounding factor. The studies relied on concentrations of aluminium in the residential water supply as a measure of exposure, with the one exception of a study that also assessed ingestion of bottled water.

There is minimal information from the epidemiology literature about the association between intake of aluminium in food and neurological conditions, and the current information from a pilot case–control study evaluating Alzheimer disease is considered to be preliminary. Epidemiology studies of the use of antacids did not capture dose information and did not demonstrate an association with neurological conditions. In the literature there have been a few case reports of adults, infants and a child with normal kidney function who experienced skeletal changes attributable to frequent use of aluminium-containing antacids considered to induce phosphate depletion.

In summary, no pivotal epidemiology studies were available for the risk assessment.

9. COMMENTS

Absorption, distribution, metabolism and excretion

Assessment of the bioavailability of aluminium compounds is confounded by limitations in the analytical methodology, particularly for older studies, by concurrent exposure to modifying factors and by dose-dependency. Speciation appears to be an important factor in absorption and it is widely assumed that soluble aluminium compounds, such as the chloride and lactate salts, are more bioavailable than insoluble compounds, such as aluminium hydroxide or silicates. Studies in laboratory animals and in human volunteers generally show that absorption of aluminium is less than 1%. However, because of the differences in methodology, it is not possible to draw precise conclusions on the rate and extent of absorption of different aluminium compounds. Concurrent intake of organic anions (particularly citrate) increases the absorption of aluminium, while other food components, such as silicates and phosphate, may reduce the absorption of aluminium.

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Oral dosing of rats with aluminium compounds has been shown to result in increased concentrations of aluminium in blood, bone, brain, liver and kidney. Studies with ^{26}Al administered intravenously to a small number of human volunteers indicate a biological half-life of about 7 years (in one individual) and interindividual variation in clearance patterns.

Aluminium compounds have been reported to interfere with the absorption of essential minerals such as calcium and phosphate, although the extent to which this occurs at dietary exposure levels is unclear.

Toxicological data

The available studies were from the published literature and were not designed to assess the safety of food additives. Most were conducted to investigate specific effects or mechanisms of action, and many do not provide information on the dose–response relationship. Some do not make clear whether the stated dose relates to elemental aluminium or to the aluminium compound tested. A further complication is that many studies do not appear to have taken into account the basal aluminium content of the animal feed before addition of the test material. Some studies refer to basal aluminium content in the region of 7 mg/kg, which would not add significantly to the doses of aluminium under investigation. However, it has been reported that there are diverse concentrations ranging from 60 to 8300 mg/kg feed and that substantial brand-to-brand and lot-to-lot variation occurs. For chow containing aluminium at a concentration of 200 mg/kg, applying the default JECFA conversion factors indicates doses equivalent to 30 mg Al/kg bw for mice and 20 mg Al/kg bw for rats.

The toxicological data are influenced by the solubility, and hence the bioavailability, of the tested aluminium compounds, and the dose–response relationship will be influenced by the aluminium content of the basal animal feed.

Recent studies have identified effects of aluminium compounds at doses lower than those reviewed previously by the Committee. Studies in rats, rabbits and monkeys have indicated effects on enzyme activity and other parameters associated with oxidative damage and calcium homeostasis in short-term studies with aluminium at oral doses in the region of 10–17 mg/kg bw per day. Those studies involved administration at a single dose and did not take into account the aluminium content of the diet. The functional relevance of the observations is unclear and since the total exposure is unknown, they are not suitable for the dose–response analysis.

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in the brain. Histopathological changes were observed in the kidney and brain at doses of 20 mg Al/kg bw per day, in the latter study. Such effects had not been observed in other studies and total exposure was unknown since the aluminium content of the diet was not taken into account.

Beagle dogs given diets containing SALP basic for 6 months showed decreased food intake and body weight and histopathological changes in the testes, liver and kidneys in the males at the highest tested concentration of aluminium, 1922 mg/kg of diet, equal to 75 mg/kg bw per day. No effects were seen in female dogs at this dietary concentration, equal to 80 mg Al/kg bw per day. The NOEL in this study was a dietary concentration of 702 mg/kg, equal to 27 mg Al/kg bw per day. This study is similar to that providing the basis for the previously established PTWI, which used SALP acidic. The Committee noted that there was no explanation for the observed sex difference, and limitations in the reporting made interpretation of this study difficult.

Special studies have highlighted a potential for effects on reproduction, on the nervous system and on bone. Few of those studies are adequate to serve as a basis for the determination of no-effect levels, as they were designed to address specific aspects and only a very limited range of toxicological end-points were examined.

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Neurotoxicity potential has received particular attention because of a speculated association of aluminium with Alzheimer disease. Many of the studies in laboratory animals have been conducted using parenteral administration and are of uncertain relevance for dietary exposure because of the limited bioavailability of aluminium compounds likely to be present in food. In contrast to studies with other routes of administration, the available data from studies using oral administration do not demonstrate definite neuropathological effects. Some studies indicate that certain aluminium compounds, especially the more soluble forms, have the potential to cause neurobehavioural effects at doses in the region of 50 to 200 mg Al/kg bw per day, administered in the diet. The studies indicating the lowest LOELs took account of the basal diet content of aluminium and one of those studies also indicated a NOEL of 10 mg Al/kg bw per day.

The previously established PTWI of 0–7.0 mg/kg bw for aluminium was based upon a study in which no treatment-related effects were seen in beagle dogs given diets containing SALP acidic at a dietary concentration of 3% for 189 days, equivalent to approximately 110 mg Al/kg bw.

The new data reviewed at the present meeting indicated that soluble forms of aluminium may cause reproductive and developmental effects at a dose lower than that used to establish the previous PTWI. Although insoluble aluminium

compounds may be less bioavailable, the evidence that other dietary components, such as citrate, can increase uptake of insoluble aluminium suggests that data from studies with soluble forms of aluminium can be used as a basis for deriving the PTWI.

Observations in humans

The previous evaluation of aluminium made by the Committee at its thirty-third meeting did not include epidemiology studies. Since then a number of epidemiology studies had been conducted, with most focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some epidemiology studies of aluminium in water suggested an association of consumption of aluminium in water with Alzheimer disease, but such an association was not confirmed in others. None of the studies accounted for ingestion of aluminium in foods, a potentially important confounding factor. The studies relied on concentrations of aluminium in the residential water supply as a measure of exposure, with the one exception of a study that also assessed ingestion of bottled water.

There was minimal information from the epidemiology literature about the association between intake of aluminium in food and neurological conditions, and the current information from a pilot case–control study evaluating Alzheimer disease was considered to be preliminary. The epidemiology studies of the use of antacids did not capture dose information and did not demonstrate an association with neurological conditions. In the literature there have been a few case reports of adults, infants and a child with normal kidney function who experienced skeletal changes attributable to frequent use of aluminium-containing antacids considered to induce phosphate depletion.

In summary, no pivotal epidemiology studies were available for the risk assessment.

Exposure to aluminium from the diet and other sources

Only consumer exposure to aluminium in the diet and via other routes or commodities were considered by the Committee; occupational exposure was not taken into account. 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 kg body weight basis, a standard body weight of 60 kg for an adult was considered by the Committee, unless otherwise specified.

Soil composition has a significant influence on the aluminium content of the food chain. The solubility of aluminium compounds may increase when acid rain decreases the pH of the soil; as a consequence, aluminium content increases in surface water, plants and animals. Most foods contain aluminium at concentrations of less than 5 mg/kg. It is estimated that quantities of about 1–10 mg/day per person generally derive from natural dietary sources of aluminium, corresponding to up to 0.16 mg Al/kg bw per day. The concentration of dissolved aluminium in untreated water at near pH 7 is typically 1–50 µg/l, but this can increase to 1000 µg/l in acidic water. Exposure through this source is therefore up to 2 mg/day, corresponding to

0.03 mg/kg bw per day based on the consumption of 2 l of water per day. Aluminium may also be present in drinking-water owing to the use of aluminium salts as flocculants in the treatment of surface waters. The concentration of aluminium in finished water is usually less than 0.2 mg/l. Based on a daily consumption of 2 l per day, dietary exposure to aluminium from treated drinking-water may be up to 0.4 mg/day, corresponding to 0.007 mg/kg bw per day.

Aluminium is used extensively in structural materials used in food-contact materials, including kitchen utensils. Aluminium can be released into the foodstuff in the presence of an acidic medium. Conservative assessments suggest that mean potential dietary exposure through this source may be up to 7 mg Al/day. Such dietary exposure corresponds to 0.1 mg Al/kg bw per day.

The current draft provisions made for aluminium compounds in the Codex GSFA are reported in Table 4. Some aluminium-containing additives are listed only in the current versions of Table 1 and 2 of the Codex GSFA, and for those additives reference is made to the PTWI for aluminium established in 1988 by JECFA. It is the case for aluminium ammonium sulfate and SALP, acidic and basic. Those aluminium compounds may be used according to GMP in a large number of products and at maximum levels in other products. The Committee noted that maximum levels are generally expressed as aluminium (e.g. 35 000 mg Al/kg, for SALP used in processed cheese) but that in some cases the reporting basis is not specified (up to 10 000 mg/kg in bakery products containing aluminium ammonium sulfate).

The Committee also noted that some food additives containing aluminium are listed in Tables 1, 2 and 3 of the current draft Codex GSFA. In Table 3, reference is made to an ADI 'not specified', and sodium aluminium silicate, calcium aluminium silicate and aluminium silicate are allowed at concentrations consistent with GMP in food in general. Specifications for other aluminium compounds are available in the *Combined Compendium of Food Additive Specifications* (Annex 1, reference *180*), but no provision had yet been made for them in Codex GSFA. This is the case for aluminium lakes of colouring matters, aluminium sulfate, aluminium powder and potassium aluminium sulfate. Other aluminium compounds are used in a number of countries but are not reported in the Codex GSFA or in the *Combined Compendium of Food Additive Specifications*. This was the case for aluminium oxide and potassium aluminium silicate.

The Committee was provided with an exposure assessment based on annual sales of SALP in Europe suggesting that the average exposure in the general population is about 0.1 mg/kg bw per day, corresponding to less than 0.01 mg Al/kg bw per day, based on the fact that tetrahydrate SALP acidic has an aluminium content of 8.5%. The Committee was also provided with disappearance data from the USA for a number of aluminium compounds used as food additives. Overall, aluminium present in SALP, basic and acidic; aluminium sodium sulfate; sodium aluminium silicate and aluminium lakes intended for human consumption sold in the USA in 2003 and 2004 would provide 9 mg of aluminium per capita per year, corresponding to 0.0004 mg/kg bw per day. Other data provided to the Committee suggest that there is a large range of exposure among consumers. A survey

conducted in 1979 suggests that 5% of adults in the USA were exposed to more than 1.5 mg Al/kg bw per day, from food additives.

Additional data were available to estimate exposure in the population of interest i.e. regular consumers of products containing food additives containing aluminium. In the USA, although aluminium-containing additives were found to be present in only a limited number of foods, some processed foods have a very high aluminium content: processed cheese, 300 mg/kg; home-made corn bread, 400 mg/kg (owing to the use of aluminium-containing leavening agents); muffins, 130 mg/kg; baking powder, 2300 mg/kg; and table salt, 164 mg/kg. In Germany, the processed foods found to have the highest aluminium content were biscuits (22 mg/kg) and soft cheese (8–16 mg/kg). In the 2000 UK Total Diet Study, the miscellaneous cereals group was reported to have the highest mean concentration of aluminium (19 mg/kg). In the 1992–1993 Chinese Total Diet Study, cereal products were also found to have the highest aluminium content (50 mg/kg) owing to the use of leavening agents containing aluminium. The potentially high aluminium content of cereal products and, in particular, of ordinary baked goods may be of special importance in a number of countries where they constitute staple food and may therefore be consumed regularly in large quantities by a significant proportion of the population.

Total dietary exposure to aluminium from all sources has been estimated through duplicate diet studies performed in adults in a number of countries. Mean values varied between 3 and 13 mg/day. The highest single reported value was 100 mg/day. In a multicentre study, exposure at the 75th percentile ranged from 3 to 26 mg/day, according to country. Data reported in Germany suggest that the amount of aluminium in the diet decreased by about half between 1988 and 1996.

A number of market-basket studies have also been performed, allowing estimation of exposure in different population groups based on mean content of aluminium in food groups, and on mean consumption. Exposure for consumers with a high consumption of cereal products or in regular consumers of products that contain higher-than-mean concentrations of aluminium will therefore be higher than estimated in those studies. In the adult population, mean exposure to aluminium estimated by model diet or market basket varied from 2 mg/day in the most recent French survey to more than 40 mg/day in China.

The highest mean exposure to aluminium per kg bw was found in young children: 0.16 mg/kg bw per day in the 1.5–4.5 years age group in the UK, based on measured body weight; approximately 0.5 mg/kg bw per day in the USA in children aged 2 years, considering a standard body weight of 12 kg; approximately 1 mg/kg bw per day in China in age groups 2–7 years and 8–12 years, considering as standard body weight 16.5 kg and 29.4 kg, respectively.

Values for high levels of exposure, estimated on the basis of high levels of consumption, were available for UK children aged 1.5–4.5 years (0.33 mg/kg bw per day).

The issue of bioavailability was considered by the Committee, but available data were not sufficient to correct the exposure assessment on the basis of

bioavailability. Aluminium contained in some food additives such as silicates may have a low bioavailability, but the main sources of exposure are sulfates and phosphates used in cereal products. A diet high in fruit and fruit-based products could lead to higher bioavailability owing to the increased absorption of aluminium in the presence of citric acid. Citric acid is one of the main organic acids present in fruit and may also be added to fruit-based products and to cheese.

The aluminium content of milk and formulae was considered when estimating exposure for infants. The aluminium content of human and cows' milk was found to be negligible (less than 0.05 mg/l), while cows' milk-based and soya-based formulae were found to contain high levels of aluminium, leading to concentrations of 0.01–0.4 and 0.4–6 mg/l, respectively, in the ready-to-drink product. The Committee estimated dietary exposure to aluminium based on the highest of those values in an infant aged 3 months weighing an average of 6 kg, considering 1 l of reconstituted formula per day as consumption at the 95th percentile. Expressed on a kg body weight basis, dietary exposure to aluminium was estimated to be up to 1 mg/kg bw per day and 0.06 mg/kg bw per day in infants fed soya-based formulae and milk-based formulae respectively. In the case of infants fed human or cows' milk, high consumption would lead to aluminium exposures of less than 0.01 mg/kg bw per day.

Sources of exposure to aluminium other than in the diet that were considered by the Committee were air, cosmetic and toiletry products and medicines. aluminium from air, in industrial areas, contributes up to 0.04 mg/day and therefore constitutes a minor source of exposure. Estimates of dermal absorption of aluminium chlorohydrate used as an active ingredient of antiperspirant suggest that only about 4 µg of aluminium is absorbed from a single use on both underarms. Some medical applications of aluminium may lead to long-term exposure: aluminium hydroxides in antacids, phosphate-binders and buffered analgesics. If taken as directed, the daily intake of aluminium from antacids could be as much as 5 g, while aluminium-buffered aspirin used for rheumatoid arthritis could contribute 0.7 g of aluminium per day.

In conclusion, the present assessment confirms 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.

10. EVALUATION

The Committee 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 and therefore the PTWI should be revised. However, the available studies have many limitations and are not adequate for defining the dose–response relationships. The Committee therefore based its evaluation on the combined evidence from several studies. The relevance of studies involving administration of aluminium compounds by gavage was unclear because the toxicokinetics after gavage were expected to differ from toxicokinetics after

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

Mean exposure	Natural dietary sources	Water (assuming a consumption of 2 l/day)	Food-contact materials	Overall diet, including additives
Expressed as mg Al/week	7–70	< 0.7 (typical untreated water) 1.4–2.8 (water treated with aluminium salts) 14 (acidic untreated water)	0–49[a]	14–280
Expressed as percentage of PTWI (assuming a body weight of 60 kg)	2–120	1–20	< 80[a]	20–500

Al: total aluminium
[a]Theoretical exposure using conservative assumptions
PTWI: provisional tolerable weekly intake

dietary administration, and the gavage studies generally did not report total aluminium exposure including basal levels in the feed. The studies conducted with dietary administration of aluminium compounds were considered most appropriate for the evaluation. The lowest LOELs for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50–75 mg Al/kg bw per day.

The Committee applied an uncertainty factor of 100 to the lower end of this range of LOELs (50 mg Al/kg bw per day) to allow for inter- and intraspecies differences. There are deficiencies in the database, notably the absence of NOELs in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The deficiencies are counterbalanced by the probable lower bioavailability of the less soluble aluminium species present in food. Overall, an additional uncertainty factor of three was considered to be appropriate. The Committee confirmed that the resulting health-based guidance value should be expressed as a PTWI, because of the potential for bioaccumulation. The Committee established a PTWI of 1 mg Al/kg bw, which applies to all aluminium compounds in food, including additives. The previously established ADIs and PTWI for aluminium compounds were withdrawn.

The potential range of exposure from dietary sources is summarized in Table 8.

The Committee noted that the PTWI is likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing additives. The Committee also noted that dietary

exposure to aluminium is expected to be very high for infants fed on soya-based formula.

Further data on the bioavailability of different aluminium-containing food additives are required.

There is a need for an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioural end-points, to be conducted on a relevant aluminium compound(s).

Studies to identify the forms of aluminium present in soya formulae, and their bioavailability, are needed before an evaluation of the potential risk for infants fed on soya formulae can be considered.

Recommendations to Codex

The Committee recommended that provisions for aluminium-containing additives included in the Codex GSFA should be compatible with the newly established PTWI for aluminium compounds of 1 mg Al/kg bw The Committee noted in particular that provisions for such additives used at levels consistent with GMP in staple foods may lead to high exposure in the general population and in particular in children.

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