in these tissues as well as measurements of plasma aluminum levels are briefly described below.

Plasma

In a review of blood aluminum concentrations for healthy individuals, plasma or serum measurements varying between 0.19 and 1.02 μg/dL in 11 studies were reported (Nieboer et al. 1995). However, according to the authors, potential problems of controlling contamination and analytical sensitivity influenced the estimates of earlier reports such that the true value more likely lies in the range of 0.11 to 0.32 μg/dL (0.04 to 0.12 μmol/L).Valkonen and Aitio (1997) reported a mean aluminum concentration of 0.16 μg/dL (0.06 μmol/L) in the serum of a healthy, non-exposed population (n = 44) who did not use antacid drugs. In another study, the mean level of aluminum in serum in 18 healthy subjects not using aluminum-containing medicines was 0.099 μg/dL (Razniewska and Trzcinka-Ochocka 2003). Liao et al. (2004) reported blood aluminum levels in workers from three optoelectronic companies in Taiwan, China. The median aluminum concentration measured was 0.36 μg/dL in the exposed workers (n = 103) and 0.32 μg/dL in the non-exposed office workers (n = 67). Higher levels of aluminum were found in aluminum welders, with mean plasma aluminum levels of 1.25 to 1.39 μg/dL (pre-shift) and 1.48 to 1.86 μg/dL plasma (post-shift) (Kiesswetter et al. 2007).

Some data on measured serum aluminum levels in animals exposed only through the normal laboratory diet were identified. Kohila et al. (2004), Johnson et al. (1992), Gonzalez-Munoz et al. (2008) and Kaneko et al. (2004) reported values ranging from approximately 0.15 to 0.66 μg/dL in different strains of rats and mice. Note that some variation in serum levels would be due to the high variability in aluminum concentration in different brands and lots of laboratory chow.

No studies were identified in which both animal and human serum levels were compared within a single study, using the same analytical methodology. The aluminum content of the standard laboratory animal diet is significantly higher than that of the typical human diet, however, so it would not be unexpected that serum aluminum concentrations observed in laboratory animals would be generally higher than reported levels in humans.

Bone

Bone exhibits more affinity to aluminum than does the brain; for example the aluminum concentrations in bone are about five-fold greater than those in the brain after repeated exposure in rats and rabbits (DuVal et al. 1986; Fiejka et al. 1996; Garbossa et al. 1998). However, the slower elimination of aluminum from the brain, as compared to bone, may be attributed in part to the bone-cell turnover and the lack of neuron turnover (Krewski et al. 2007).

In general, aluminum in bone is principally captured in the mineralization front and in the osteoid (Boyce et al. 1981; Cournot-Witmer et al. 1981; Ott et al. 1982; Schmidt et al. 1984). There are three probable mechanisms of aluminum deposition in bone that govern the elimination rate of aluminum in this matrix (Priest 2004). First, aluminum can be attached to the bone surface by heterionic exchange with calcium; this aluminum can be easily released to

the fluids close to the bone surface, and then bound to Tf. Second, aluminum can be incorporated into the structure of the developing hydroxyapatite crystal during the formation of the mineral lattice; this strongly binds the molecule to bone cells and there is little subsequent release of aluminum from the bone matrix. Third, aluminum can be complexed to organic components at the surface of bone; in this case, the migration of aluminum through its deposition at the mineralization front can occur, leading to a slow turnover.

Brain

The concentrations measured in the brains of exposed rats ranged from 0.0006% to 0.009% of aluminum administered dose per gram of brain, after intravenous or intraperitoneal injection (Krewski et al. 2007). It was suggested that 90% of the aluminum in brain is associated with citrate, 5% with hydroxide, 4% with Tf and 1% with phosphate (Yokel 2001). In humans, the aluminum accumulation is higher in the cerebral cortex and hippocampus than in other brain structures (Gupta et al. 2005).

There are two ways by which aluminum can reach the central nervous system, either through the blood-brain barrier or through the choroid plexus in the cerebrospinal fluid of the cerebral ventricles. Although there is some evidence that aluminum crosses the blood-brain barrier by Tf-receptor mediated endocytosis of the Al-Tf complexes (Roskams and Connor 1990), other mechanisms of uptake, independent of Tf, may be involved as well (Yokel and McNamara 1988; Allen et al. 1995; Radunovic et al. 1997), such as diffusion of the low molecular weight aluminum species or other carrier-mediated processes. In addition, aluminum may reach the brain through the nasal epithelium by axonal transport (Perl and Good 1987; Zatta et al. 1993), although the potential magnitude of this pathway has not been quantified. Axonal transport, however, would not be expected to contribute significantly to exposure in the general population due to the low concentration of aluminum in ambient air, outside of particular occupational settings (see section 2.3.2.1).

The transport of aluminum out of the brain seems to occur by its association with citrate (Yokel 2000). The ability to remove aluminum from the brain is low (Krewski et al. 2007). For instance, in a study in which ^{26}Al-Tf was administered intravenously in rats, Yokel et al. (2001b) reported that brain concentrations of aluminum did not significantly decrease 128 days after administration.

Placenta and foetus

Aluminum distributes to the placenta and foetus, as has been demonstrated by experimental studies in which aluminum was administered by different routes to rabbits, mice and guinea pigs during gestation (Yokel 1985; Cranmer et al. 1986; Golub et al. 1996b; Yumoto et al. 2000). Yumoto et al. (2000) estimated that approximately 0.2% of the subcutaneous injected dose of ^{26}Al-chloride was transferred to the foetus as well as to the placenta. In the study of Cranmer et al. (1986), fetal aluminum content was significantly increased following both intraperitoneal and oral administration, although the increase was greater with intraperitoneal dosing. No study investigating the level of aluminum in the human placenta was identified.

Milk

Aluminum is efficiently transferred from blood to milk in exposed lactating animals (Yokel and McNamara 1985; Muller et al. 1992; Yumoto et al. 2000) as well as in human lactating mothers (see section 2.3.2.5). According to the calculations of Findlow et al. (1990), almost all the aluminum in milk (human and bovine) should be associated with citrate, with approximately 88% as $Al(citrate)(OH)_2^{-2}$ and approximately 11% as $Al(citrate)(OH)^{-1}$.

2.3.3.3 Elimination

The principal organ of aluminum excretion is the kidney, accounting for more than 95% of the total excretion (Exley et al. 1996; Krewski et al. 2007). The urinary excretion is believed to occur by passive filtration through the glomerulus, instead of active secretion by the proximal tubules. This hypothesis is based on the results of animal studies demonstrating that when only the free fraction of aluminum was assumed to be removed from blood, the elimination rate of aluminum is approximately the same as the glomerular filtration rate (Henry et al. 1984; Yokel and McNamara 1985, 1988). If this hypothesis is true, then the factors influencing glomerular filtration rates (such as kidney disease, pregnancy and age) should also influence the rate of elimination of aluminum (Guyton 1991). Indeed, it has been observed that individuals with renal failure have lower capacity of elimination (Nieboer et al. 1995; Krewski et al. 2007).

A small portion of the absorbed aluminum appears to be eliminated through other excretion routes. The second most important route would likely be biliary excretion. Most of the experimental studies with animals have demonstrated that less than 1.5% of the total eliminated aluminum occurred by biliary excretion (Krewski et al. 2007). As well, sweat, saliva and seminal fluid can contribute, to a much lesser extent, to the elimination of aluminum from the body (Krewski et al. 2007).

The elimination rate of aluminum appears to be regulated by the presence of various aluminum complexes in the body's systemic circulation. aluminum citrate complexes are eliminated more easily than Al-Tf (Maitani et al. 1994), most likely because the lower molecular weight of the aluminum citrate complex would facilitate glomerular filtration. This may explain why the presence of citrate can enhance renal elimination (Van Ginkel et al. 1993; Cochran et al. 1994). Also, the concomitant presence of aluminum and silicon yields a filterable complex (probably the same observed in the gastrointestinal tract); this complex seems to favour renal excretion by limiting the renal reabsorption of aluminum (Bellia et al. 1996; Birchall et al. 1996). As well, fluoride is a natural element which contributes to the rapid elimination of aluminum (Chiba et al. 2002).

Some animal studies have shown lower clearances of aluminum from the body, and consequently higher elimination half-lives ($t_{½}$), after increasing the aluminum dosages (Höhr et al. 1989; Pai and Melethil 1989; Xu et al. 1991). This observation is probably explained by the fact that the fraction of ultrafilterable aluminum complexes decreased when the aluminum concentrations in blood increased (Xu et al. 1991; Yokel and McNamara 1988). Also, Greger and Radzanowski (1995) obtained a positive correlation between the $t_{½}$ of aluminum in tibia and kidneys and the age of exposed rats, indicating that the ability to remove aluminum may diminish with time.

Priest et al. (1995) and Talbot et al. (1995) investigated the elimination rates of aluminum in humans, on the basis of the time-profiles of aluminum in blood and urine of seven volunteers who had received intravenous injection of ^{26}Al-citrate. Blood, urine and feces were collected during the five days following injection, except for the volunteer in Priest et al. (1995) for which the follow-up was at 13 days. Around 59.1% (46.4% to 74.42% range) of the uptake was excreted in the cumulative urine collected during 24 hours following injection whereas after five days, around 71.8% of the dose was recovered in urine (62.3% to 82.9% range). These results are considerably different than those reported in a study by Steinhausen et al. (2004), in which two volunteers received an IV injection of ^{26}Al-chloride, where the five-day urinary excretion accounted only for 25% of the dose.

Priest et al. (1995) and Talbot et al. (1995) described the whole-body retention of aluminum, blood concentration and urinary excretion, after the first day of injection, by a power function (e.g., $C_b(t) = 0.37t^{-0.9}$, expressed as a percent of injection/L). However, in a study with a follow-up period of 11 years, Priest (2004) demonstrated that the pattern of the whole-body retention of aluminum must be represented by a multiple-exponential equation.[13] Numerous studies have actually shown that the rate of aluminum clearance in blood diminishes with time following aluminum administration, and thus a single elimination half-life ($t_{½}$) cannot describe the whole-body elimination of aluminum (Priest 2004). Some authors have attempted to calculate specific $t_{½}$ of aluminum for the tissues and organs of rats (Greger et al. 1994; Greger and Radzanowski 1995; Rahnema and Jennings 1999). In general, it was shown that aluminum deposited in well-perfused tissues/organs (e.g., kidneys and lungs) is released more rapidly than aluminum in slowly-perfused tissues (e.g., bone and spleen). These $t_{½}$ values varied from 2.3 to 113 days. However, even if the brain is well-perfused, the retention of aluminum appears to be strong (see section 2.4.2.2). According to the experimental data in animals, Krewski et al. (2007) estimated that the $t_{½}$ of aluminum deposited in brain is from 13 to 1,635 days.

A multicompartmental model was developed to describe the kinetics of aluminum in humans, based on the retention of ^{26}Al in the volunteer of the Priest et al. (1995) study, who was followed over more than ten years (Priest 2004). Five compartments are used to describe aluminum accumulation in the different organs and tissues; for each compartment, specific tissues or organs are indicated with a specific elimination half-life. These compartments are fed by the compartment of blood and extracellular fluids. As well, Nolte et al. (2001) proposed an open compartmental model to describe the kinetics of aluminum in humans based on the binding of aluminum with transferrin and citrate; this model was used by Steinhausen et al. (2004).

[13] The equation of the retention is $R(t) = 29.3e^{-0.595 \cdot t} + 11.4e^{-0.172 \cdot t} + 6.5e^{-0.000401 \cdot t}$; the corresponding elimination half-lives are 1.4, 40 and 1,727 days.

2.4 Effects characterization

2.4.1 Ecotoxicology

Below, a brief summary of effects data for the most sensitive aquatic and terrestrial organisms is presented. More extensive descriptions of environmental effects are provided in several reviews (e.g., ATSDR 2006; Bélanger et al. 1999; Roy 1999a).

When aluminum salts are added to water, they hydrolyse, and monomeric aluminum can be formed in the dissolved fraction. It is the monomeric aluminum, and not the salts, that can adversely affect organisms (Driscoll et al. 1980; Parker et al. 1989; Baker et al. 1990). The following summary focuses, therefore, on the effects of the dissolved (particularly monomeric) forms of aluminum that are produced when aluminum salts dissociate.

2.4.1.1 Aquatic organisms

Most of the research on the impact of aluminum on aquatic life has been related to the impacts of acid rain. In this report, emphasis was placed on the potential toxic impacts of aluminum in waters of neutral or near-neutral pH as the available information suggests that releases associated with the three aluminum salts being assessed occur primarily into waters of circumneutral pH (Roy 1999b; Germain et al., 2000). As described below, because of this consideration, the most relevant effects data identified were for fish. This assessment report does not provide a detailed examination of potential effects from exposure to polymeric aluminum, as polymeric aluminum is most likely to form, and to cause toxicity, during the neutralization of acidic aluminum-rich waters and this is unlikely to occur in the release scenarios considered in this assessment (Roy 1999b).

The gills are the primary target organ for aluminum in fish (Dussault et al. 2001). Aluminum binds to the gill surface, causing swelling and fusion of the lamellae and increased diffusion distance for gas exchange (Karlsson-Norrgren et al. 1986; Tietge et al. 1988). The resulting damage leads to loss of membrane permeability, reduced ion uptake, loss of plasma ions, and changes in blood parameters relating to respiration. Fish death may result from ionoregulatory or respiratory failure, or a combination of both, depending upon the pH of the water and concentration of waterborne aluminum (Neville 1985; Booth et al. 1988; Gensemer and Playle 1999). Ionoregulatory disturbances prevail at lower pH (e.g., below 4.5) and relate to decreased levels of plasma Na^+ and Cl^- ions (Neville 1985; Gensemer and Playle 1999). At pH levels above 5.5, binding of the positively charged aluminum species to negatively charged sites on the gill surface, with subsequent aluminum polymerization, leads to mucous secretion, clogging of the interlamellar spaces and hypoxia (Neville 1985; Poléo 1995; Poléo et al. 1995; Gensemer and Playle 1999).

Aluminum exposure may also disrupt ionic balance and osmoregulation in aquatic invertebrates (Otto and Svensson 1983). Reduced Na^+ and/or Ca^{2+} uptake in response to aluminum exposure have been documented in crayfish (Appleberg 1985; Malley and Chang 1985), mayfly nymphs (Herrmann 1987) and the water boatman, *Corixa* sp. (Witters et al. 1984). Aluminum reduced Na^+ influx and, to a lesser extent, increased outflux, in *Daphnia magna*, thereby impairing osmoregulation (Havas and Likens 1985). Aluminum may disrupt the respiratory organs of some invertebrates, such as the anal papillae of the phantom midge,

Chaoborus sp. (Havas 1986). Respiratory effects can occur when acidic waters are rapidly neutralized, such as when an acidic tributary enters a larger, neutral receiving stream, leading to the formation of mononuclear and polynuclear aluminum species from the dissolved ion (Gensemer and Playle 1999). These species may bind to or precipitate onto the bodies of invertebrates, creating a physical barrier to respiration. Aluminum has been reported to impair reproduction in *Daphnia magna* (Beisinger and Christensen 1972), although recent work with *Daphnia pulex* suggests that adaptive strategies which heighten survivorship and fecundity may occur following long-term exposure to sublethal levels (Wold et al. 2005). Hall et al. (1985) reported that aluminum may reduce the surface tension of water, affecting egg deposition, emergence, feeding and mating behaviour of some stream invertebrates.

2.4.1.1.1 Pelagic

Water pH is known to have a significant effect on the toxicity of dissolved aluminum. Under acidic conditions, aluminum is most toxic in the pH range 5.0–5.5. At more acidic pH, its toxicity decreases, while at still lower pH, aluminum can offer transitory protection against the toxicity of H^+ (Muniz and Leivestad 1980; Baker 1982; van Coillie et al. 1983; Roy and Campbell 1995). Elevated concentrations of the cations Ca^{2+} and Mg^{2+} reduce the toxicity of metals (Pagenkopf 1983; Campbell 1995), yet there are relatively few results examining the effects of elevated calcium on aluminum toxicity. In fish exposed to aluminum at low pH, elevated calcium has been shown to improve survival (Booth et al. 1988; Mount et al. 1988; Sadler and Lynam 1988), reduce losses of plasma ions (Brown 1981; Sadler and Lynam 1988; McDonald et al. 1989) and reduce accumulation of aluminum on gills (Wood et al. 1988a,b). However, Duis and Oberemm (2001) reported low hatching success and high embryo mortality in vendace, *Coregonus albula,* exposed to high aluminum concentrations of 2.1 and 2.4 mg/L at low pH (4.75, 5.00) and in the presence of 111 to 117 mg/L calcium. Increasing calcium concentrations to 233 to 256 mg/L had no influence on hatching and survival percentages, suggesting that the toxic effect of high aluminum levels can exceed the protective effect of high calcium.

The toxicity of dissolved aluminum is reduced in the presence of inorganic ligands, such as fluorides, sulphates and silicates, as well as organic ligands, such as fulvic and humic acids (Roy 1999a). It is well established that DOM in particular influences the speciation and absorption of aluminum. In laboratory studies with fish, the toxicity of aluminum was reduced in the presence of organic acids, such as citric acid (Driscoll et al. 1980; Baker 1982), salicylic or oxalic acid (Peterson et al. 1989), humic acid (van Coillie et al. 1983; Parkhurst et al. 1990; Peuranen et al. 2002) and fulvic acid (Neville 1985; Lydersen et al. 1990a; Witters et al. 1990; Roy and Campbell 1997). In laboratory studies with amphibians (frog eggs and tadpoles), LC_{50}s for aluminum increased (i.e., toxicity was reduced) in the presence of DOM. However, in the field, the effects of DOM in attenuating aluminum toxicity are difficult to separate from the influences of pH and aluminum concentration (Clark and Hall 1985; Freda 1991).

Most aquatic toxicity studies involving aluminum have been conducted under conditions of low pH, and a number of these accounted for the solubility of the metal in the experimental design. The general conclusion of these studies is that aluminum toxicity is related to the concentration of dissolved inorganic monomeric aluminum (Roy 1999a).

At pH < 6.0, fish, the salmonids in particular, are among the most sensitive organisms to dissolved aluminum. In soft acidic waters, the LC_{50} can be as low as 54 µg/L (for Atlantic salmon at pH 5.2), while in chronic studies, a Lowest-Observed-Effect Concentration (LOEC) of 27 µg/L was determined for growth (for brown trout [*Salmo trutta*] at pH 5.0). Some species of algae show a comparable sensitivity. Parent and Campbell (1994) determined a LOEC of 150 µg/L (as inorganic monomeric aluminum) at pH 5.0 with the alga *Chlorella pyrenoidosa*. While many invertebrates tolerate elevated levels of aluminum, Havens (1990) found that exposures to 200 µg Al/L at pH 5.0 were extremely toxic to *Daphnia galeata mendotae* and *Daphnia retrocurva*. France and Stokes (1987) concluded that stress from aluminum exposure was secondary to the stress of low-pH exposure for survival of *Hyalella azteca*. Results of other studies also suggest that invertebrates are more sensitive to low pH than to aluminum. Amphibians show a similar sensitivity. Freda (1991) summarized her work by concluding that aluminum can be lethal to amphibians that inhabit soft acidic (pH 4 to 5) waters if concentrations exceed 200 µg inorganic Al/L.

At pH 6.0 to 6.5, there are few studies that provide effects estimates in terms of inorganic monomeric aluminum. At pH 6.0, a LOEC of 8 µg/L (inorganic monomeric aluminum) for growth of the alga *C. pyrenoidosa* can be estimated from the data of Parent and Campbell (1994). Growth of the alga was reduced at this single exposure concentration in media without phosphate. This LOEC is, however, well within the likely range of natural concentrations of inorganic monomeric aluminum in surface water. In comparison, Neville (1985) observed that 75 µg Al/L (as inorganic monomeric aluminum) caused physiological distress to rainbow trout (*Oncorhynchus mykiss*) at pH 6.1 but not at pH 6.5.

At pH 6.5 to 8.0, there are few effects data available. At neutral or near-neutral pH, aluminum has a tendency to precipitate, and the chemistry of these solutions is difficult to control. While the toxicity of alum in neutral-pH waters has been the subject of many studies, the results are unreliable, due to extreme variation between replicates of the same exposure concentration and between duplicate experiments (Lamb and Bailey 1981; Dave 1985; George et al. 1995; Mackie and Kilgour 1995). However, a No-Observed-Effect Concentration (NOEC) for respiratory activity at pH 6.5 is provided by the results of the study by Neville (1985), who found that rainbow trout tolerated 75 µg Al/L (as inorganic monomeric aluminum) during exposures at this pH. Wold et al. (2005) reported a LOEC of 0.05 mg/L Al for reduced survival and reproduction in *Daphnia pulex* exposed for 21 days to concentrations ranging from 0.05 to 0.50 mg Al/L (nominal) as aluminum sulphate. The test water was maintained at a pH of 7 ± 1, suggesting that the observed effects were due to the presence of aluminum hydroxide rather than the dissolved inorganic monomeric aluminum that is usually associated with toxicity. In addition, the study reported that clonal populations of *D. pulex* derived from a lake with ongoing alum treatment showed higher age-specific survivorship, higher fecundity and faster growth rates than those collected from waters having less recent or no prior alum exposure. The researchers hypothesized that *Daphnia* may be capable of exhibiting adaptive strategies that heighten survivorship and fecundity when exposed to sublethal chemical stresses.

Gopalakrishnan et al. (2007) reported a lowest 24-hour EC_{50} value of 0.210 mg/L for development of the trochophore larva in the marine polychaete, *Hydroides elegans*. The study was conducted at a pH of 8.1 and aluminum concentrations (measured using atomic absorption

spectrophotometry) were well maintained within 2% to 15% of nominal values. Differential sensitivities were observed during embryogenesis and larval development, with lowest toxicity evident at the stage of the fertilization membrane and successively higher toxicity at the blastula and trochophore stages, respectively.

At pH > 8.0, LOECs for survival of rainbow trout are ≥ 1.5 mg/L as total aluminum (Freeman and Everhart 1971). In a more recent study, Gundersen et al. (1994) reported LC_{50}s for exposures of rainbow trout in the pH range 8.0–8.6. The LC_{50}s at all pHs were approximately the same value, ~ 0.6 mg/L (range: 0.36–0.79 mg/L) as dissolved aluminum (i.e., filterable through a 0.4-µm filter), and were similar in both acute (96-hour) and longer-term (16-day) exposures at hardness levels ranging from 20 to 100 mg/L (as calcium carbonate). A NOEC for mortality of 0.06 mg dissolved Al/L can be derived from data given for one of the 16-day exposures conducted at 20 mg/L hardness and pH 8.0. Although these concentrations were measured as dissolved aluminum, it is probable that the monomeric aluminate ion, $AlOH_4^-$, predominated at this pH.

In contrast, Poléo and Hytterød (2003) reported that juvenile Atlantic salmon, *Salmo salar*, exposed under alkaline (pH 9.5) conditions to concentrations of around 0.35 mg/L (predominantly aluminate ion) showed no acute toxicity effects. The researchers noted that the aluminum concentrations used in their study were lower than those of Freeman and Everhart (1971) and Gundersen et al. (1994), and hypothesized that more environmentally relevant concentrations of aluminum do not have any acute effect on salmonids under alkaline conditions, while very high concentrations of aluminum might have. While no acute effects were observed, physiological responses in the form of elevated blood glucose and hematocrit levels and a decrease in plasma Cl^-, were evident after a three-week exposure period and were considered indicative of a stress response in the fish. The authors concluded that the combination of high pH and aluminum may impose some stress but this is unlikely to represent a serious problem unless the exposure continues for a long period of time. High alkalinity conditions such as those used in the study can occur in water bodies during periods of intense photosynthetic activity in the summer months. At these times, concentrations of aluminum present in the water would also be expected to rise as the solubility of the substance increases over that at lower pH.

While toxicity is most commonly associated with inorganic monomeric aluminum species, there is evidence that aluminum undergoing transition from one species to another is also bioavailable and can exert adverse effects on organisms. Such transition conditions can occur in mixing zones, for example, when acidic waters enter a larger, more neutral receiving system or during the liming of acidic waters. Berkowitz et al. (2005) found that the addition of alum to lake water samples (pH 8.22 to 9.08) resulted in a rapid initial decrease in pH and alkalinity followed by a gradual recovery in pH over several weeks. Dissolved Al concentrations increased following treatment, and then decreased after 150 days. Soucek (2006) determined that freshly neutralized aluminum (i.e., aluminum in transition from ionic species in acidic waters to polymers or precipitating hydroxides after a rapid pH increase) impaired oxygen consumption in *Daphnia magna* and the perlid stoneflies, *Perlesta lagoi* and *Acroneuria abnormis* (lowest LOEC for the study 0.5 mg/L, which was also the lowest concentration tested). Alexopoulos et al. (2003) reported that freshly neutralized aluminum at a concentration of 0.5 mg/L associated specifically with the gills of the freshwater crayfish,

Pacifastacus leniusculus, creating a physical barrier during precipitation that resulted in impaired respiration and asphyxiation. Particulate aluminum has been shown to decrease filter feeding in the freshwater bivalve, *Anodonta cygnea*, presumably as an avoidance response to the toxicant (Kádár et al. 2002). Poléo and Hytterød (2003) examined toxicity under steady-state (pH retained at 9.5) and non-steady state (pH lowered from 9.5 to 7.5) conditions in order to evaluate the possible impact of transient aluminum chemistry on Atlantic salmon, *Salmo salar*. No increase in toxicity occurred under the non-steady state conditions (i.e., where aluminum solubility was lowered as the pH decreased) and the physiological disturbances observed at high pH were mitigated. The results contrasted with those obtained in studies where aluminum solubility was lowered by raising the pH of aluminum-rich water. In these cases, toxicity to fish increased as the solubility of aluminum was decreased and aluminum precipitated onto the gills (e.g., Poléo et al. 1994; Poléo and Bjerkely 2000).

Verbost et al. (1995) reported enhanced toxicity in a mixing zone of acid river water containing aluminum (pH 5.1, aluminum 345 µg/L) with neutral lake water (pH 7.0, aluminum 73 µg/L). The resulting water (pH of 6.4, aluminum 235 µg/L) was expected to have low toxicity; however, the freshly mixed water was highly toxic to brown trout, *Salmo trutta*, with necrosis and apoptosis of the gills evident in exposed fish. A clear gradient in the deleterious effects occurred with increasing distance from the mixing area, with fish furthest from the mixing zone exhibiting only mild effects. The researchers concluded that freshly mixed acid and neutral water contains toxic components during the first seconds to minutes after mixing, and that even short exposure to this toxic mixing zone is detrimental to migrating trout. Farag et al. (2007) hypothesized that colloids formed in mixing zones may contribute to aluminum toxicity in fish by providing a direct route of the metal to the gills.

Finally, in a study done with DWTP sludge from Calgary and Edmonton, Alberta, AEC (1987) concluded that all sludges tested were non-toxic using a microbial test and acutely and subacutely non-toxic to rainbow trout. However, delayed release of first broods and significantly reduced reproduction were reported in the freshwater cladoceran, *Ceriodaphnia dubia*, exposed for 7 days to 100% aluminum sludge effluent collected from a DWTP in the U.S. (Hall and Hall 1989). The researchers considered that the effects were likely due to the combined effects of reductions in pH and dissolved oxygen concentrations, physical stress due to high levels of suspended solids, and possibly the presence of aqueous aluminum. Aqueous aluminum alone was probably not the factor exerting sub-lethal toxicity in 100% effluent since similar aqueous aluminum concentrations were observed in the 50% effluent where delays and significant reductions in reproduction were not observed. The same study observed significant mortality in fathead minnow, *Pimephales promelas*, exposed to the 100% effluent, as well as a lowest test concentration of 6.3%. Mortality in the intervening concentrations of 12.5, 25 and 50% were not statistically different from that in the controls. Mortality at 100% effluent was attributed to physical stress resulting from high levels of suspended solids. While a causative agent for the observed mortality at 6.3% could not be identified, the researchers noted that this test concentration had the highest concentration of aqueous aluminum, with measured levels up to 0.43 mg/L as compared with 0.05 to 0.31 mg/L at the other test concentrations. No sublethal impacts were evident in the fish testing.

2.4.1.1.2 Benthic

Alum can be used to treat eutrophic lakes to reduce the amount of phosphorus present in water or prevent its release from sediment. Lamb and Bailey (1981) concluded that a well-planned and controlled alum treatment would not result in significant mortality in benthic insect populations. Connor and Martin (1989) measured no detrimental effects on midge or alderly larvae following treatment of Kezar Lake, New Hampshire, sediment, and long-term effects on benthic invertebrates were minimal. Narf (1990) reported that benthic population diversities and numbers increased or remained the same following lake treatment with alum. Smeltzer (1990) observed a temporary impact on benthos after treatment of Lake Morey, Vermont, with an alum/sodium aluminate mixture. Benthos density, already low in the year prior to treatment, and richness were lower following treatment. However, changes were not significant, the benthic community recovered, and two new chironomids appeared the following year.

The Sludge Disposal Committee examined the impact of alum sludge discharge in aquatic environments and concluded that residue will tend to deposit near the point of discharge if the water velocity is low (Cornwell et al. 1987) and that it could have adverse effects, including development of anaerobic conditions. Roberts and Diaz (1985) related the reduction in phytoplanktonic productivity observed during alum discharge in a tidal stream in Newport News, Virginia, to the reduction in light intensity. Lin et al. (1984) and Lin (1989) found no buildup of sludge in pooled waters in the Vermillion and Mississippi rivers following sedimentation basin cleaning of DWTPs in St. Louis, Missouri. There were no significant differences in types and densities of macroinvertebrates in bottom sediments, and even higher density and diversity were found in some sites.

George et al. (1991; 1995) reported that macroinvertebrates located downstream of four DWTPs appeared to be stressed by alum discharges. In the Ohio River, effects seemed temporary and were limited in space. In addition, organisms collected from upstream locations indicated that environmental factors other than the aluminum sludge discharge may also have been affecting the system. A water–sediment microcosm study done with bottom sediment from the receiving rivers over a 72-day period showed significantly lower oligochaete content in bottom sediment treated with alum sludge. Testing with bentonite gave the same results, and the authors concluded that aluminum sludge deposits on sediment may have the potential to detrimentally affect benthic macroinvertebrate populations by limiting their access to oxygen or food and, therefore, the smothering effect from sludge may prove to be more important to aquatic organisms than aluminum content. However, in laboratory testing, filtrates obtained from aluminum sludge were toxic to the freshwater alga, *Selenastrum capricornutum*, in waters with low pH or a hardness of less than 35 mg/L $CaCO_3$, suggesting that water-soluble constituents from the aluminum sludge may be capable of affecting algal growth. The study recommended that further toxicity testing be conducted to more fully ascertain potential toxic effects, and that aluminum sludge not be discharged into soft surface waters (i.e., hardness < 50 mg $CaCO_3$/L) or those with a pH of less than 6.

A study has been undertaken to examine the environmental impact of filter backwash and basin cleaning effluents to the Ottawa River from the Britannia and Lemieux Island DWTPs in Ottawa (RMOC 2000; City of Ottawa 2002). In this study, riverine characteristics

downstream of the Britannia site were reported to be beneficial for the sampling of benthic invertebrates due to the slow water velocities of a bay environment. Unlike the Britannia site, the Ottawa River in the vicinity of the Lemieux Island DWTP was characterized by strong currents and an absence of natual benthic habitat. To examine the impact of effluents from the Lemieux Island facility, artificial habitat was installed for benthic organisms at both upstream and downstream locations from the discharge site. The results of the sampling showed that species abundance and diversity was depressed at both sites downstream from the effluent discharges in comparison to sampling sites located upstream. At sites located 150 and 6,000 m upstream from the Britannia DWTP outfall, approximately 160 and 250 organisms were counted, whereas downstream sites located at 0, 300, 500 and 1,500 m (furthest sampling location) had between 3 (at 0 m) and approximately 100 organisms (at 1,500 m) (diversity of organisms not provided for Britannia site). At the artificial sampling sites 30 and 110 m downsteam of the Lemieux Island DWTP, approximately 250 and 1,000 organisms were counted representing 17 and 21 taxa, respectively. The site located 90 m upstream from the Lemiux discharge had approximately 1,800 organisms representing 24 taxa.

Toxicity of basin sediment from each of the Britannia and Lemieux Island DWTPs was also examined. The studies showed complete mortality of midge larvae (*Chironomus riparius*) within the 10 day test exposure, while survival of *Hyalella azteca* (14 day exposure) was not significantly different from that of the control animals. The study could not determine whether the mortality was attributable to the physical characteristics of the sludge (e.g., particle size) or the presence of chemical contaninants. The sludge from the Lemieux Island DWTP was shown to inhibit growth of *Hyalella azteca* over the 14 day exposure period, but the Britannia DWTP sludge resulted in no observed effect. The study did not suggest why one sludge demonstrated growth effects, but not the other (methodology and experimental conditions were not provided).

Ultimately, the cause of the the depressed levels of organisms downstream in the Ottawa River from Britannia and Lemieux DWTPs was not due to one causal factor, rather may have resulted from a number of attributes including: physical composition of the sediment and its ability to support life; ongoing blanketing of the area due to new discharges; and toxicity of dissolved aluminum leaching out of the sediment into the water column (City of Ottawa 2002).

In studies related to wastewater releases by DWTPs, AEC (1984) reported there is potential for smothering effects on benthic organisms related to settled sludge on sediments following their release to rivers in Alberta. A number of other possible adverse impacts resulting from the discharge of aluminum sludge to receiving waters were identified, including: formation of sludge deposits in quiescent areas of streams; toxic effects on aquatic organisms from other contaminants present in the sludge; periodic high oxygen demand if water treatment plant sludge is discharged in large slugs or if previously deposited sludge is periodically re-suspended due to increased stream velocity; increased aluminum concentrations of downstream water supplies; and aesthetic problems where stream flow, stream turbidity, and/or sludge dilution are low. The researchers concluded that aluminum sludge exhibits a wide range of characteristics which depend on the raw water characteristics (turbidity, etc.) and other factors and, therefore, while numerous suspicions have been expressed regarding the potential for adverse effects resulting from the discharge of alum

sludges to receiving waters, there appeared to be a lack of good scientific evidence to substantiate these concerns. Recommendations of the report included the acquisition of baseline data through bioassay testing and other studies, as well as consideration of alternatives to direct stream disposal practices such as reduction of the quantities of alum sludge produced through substitution with other coagulants, discharge at controlled rates to a sanitary sewer, lagooning with natural freeze-thaw dewatering, thickening and dewatering followed by landfilling, and land application.

A subsequent study examining the binding, uptake and toxicity of aluminum sludges from three water treatment systems in Edmonton and Calgary determined that aluminum was effectively bound to sludges within the pH range 4.5 to 10.0, with more than 99.98% of the total aluminum being in the form of sludge (AEC 1987). Sludge collected from the three plants was found to be non-toxic to rainbow trout, Long Evans rats, and the microbial toxicity test system, Microtox.

2.4.1.2 Terrestrial organisms

Research on the effects of aluminum to soil organisms has concentrated largely on screening for aluminum-tolerant strains of root nodulating bacteria and mycorrhizal fungi, due to the importance of these species in improving crop production (Bélanger et al. 1999). In general, toxicity threshold values for bacterial species fall in the range of 0.01 to 0.05 mM (pH 4.5 to 5.5), while those of mycorrhizal fungi range from 0.1 to 20 mM (pH 3.4 to 4.5) when based on hyphal growth inhibition and 30 to 157 mg/kg soil (pH 4.5 to 5.0) when based on reduced spore germination. For soil macroinvertebrates, growth of newly hatched earthworm, *Dendrodrilus rubidus*, was significantly reduced at 10 mg Al/kg soil (soil pH 4.2 to 4.9; Rundgren and Nilsson 1997), while significantly inhibited growth and cocoon production were reported for the earthworm, *Eisenia andrei*, at concentrations ranging from 320 to 1000 mg/kg dry soil, with toxicity decreasing as soil pH increased from 3.4 to 7.3 (van Gestel and Hoogerwerf 2001). A more complete examination of potential impacts to soil-dwelling microorganisms, fungi and invertebrates can be found in Bélanger et al. (1999).

The remainder of this section focuses on the effects of aluminum on sensitive plant species. It should be noted, however, that the problem with alum sludge may be associated not only with the direct toxic effects of aluminum on plants, but also with indirect effects related to phosphorus deficiencies (Jonasson 1996; Cox et al. 1997; Quartin et al. 2001). Aluminum's capacity to fix labile phosphorus by forming stable aluminum-phosphorus complexes and hence make it unavailable to plants can be responsible for the observed effects. In addition, toxic substances captured by the floc during water treatment may be available for uptake by soil species and exert adverse effects.

The presence of aluminum in solution, soil solution or soil resulted in a decrease in seedling growth, elongation or branching of roots of hardwood and coniferous species at varying levels (Horst et al. 1990; Bertrand et al. 1995; McCanny et al. 1995; Schier 1996). The most sensitive species was honeylocust (*Gleditsia triacanthos*) (Thornton et al. 1986a, 1986b). All measures of growth, except root elongation, consistently declined as solution aluminum increased, 0.05 mM or 1.35 mg/L being the critical value for a 50% general decrease (pH = 4.0). Since honeylocust is not an important species in Canadian forests and since the

results obtained by Thornton et al. (1986b) contradict the results obtained for this species by other researchers, it was decided that the two next Lowest-Observed-Adverse-Effect Concentrations (LOAECs) are more relevant. Hybrid poplar (*Populus* hybrid) (Steiner et al. 1984) and red oak (*Quercus rubra*) (DeWald et al. 1990) showed a 50% decline in root elongation at an aluminum solution level of 0.11 mM (2.97 mg/L). The most sensitive coniferous species is pitch pine (*Pinus rigida*) (Cumming and Weinstein 1990). Seedlings inoculated with mycorrhizal fungus, *Pisolithus tinctorius*, showed increased tolerance to aluminum, whereas non-mycorrhizal seedlings exposed to 0.1 mM (2.7 mg/L) (pH 4.0) aluminum exhibited decreased root and shoot growth.

In an experiment done with scots pine (*Pinus sylvestris*), Ilvesniemi (1992) found that when nutrition was optimal, pines tolerated high levels of aluminum, but in nutrient-poor solution, their tolerance to aluminum was reduced tenfold. Hutchinson et al. (1986) and McCormick and Steiner (1978) also observed that pines were tolerant of high levels of aluminum in optimal nutrient solution.

Grain crop and forage crop species were also affected by different levels of aluminum (Bélanger et al. 1999). Wheeler et al. (1992) found that two barley (*Hordeum vulgare*) cultivars and eight common wheat (*Triticum aestivum*) cultivars were particularly sensitive, growth being decreased by more than 50% at aluminum levels as low as 0.005 mM (0.135 mg/L) (pH 4.5). Wheeler and Dodd (1995) also showed a 50% decline in growth of clover species, *Trifolium repens*, *Trifolium subterraneum* and *Trifolium pratense*, at 0.005 mM (0.135 mg/L) aluminum (pH 4.7). In a solution culture study, Pintro et al. (1996) found that the root elongation rate of maize (*Zea maize* HS777 genotype) was also negatively affected at an aluminum level of 0.005 mM (0.135 mg/L) (pH 4.4). In a study done on barley, Hammond et al. (1995) found significant amelioration of the toxic effects of aluminum on root and shoot growth when silicon was added to the solution medium. Silicon amelioration of aluminum toxicity in maize has also been reported (Barcelo et al. 1993; Corrales et al. 1997). In the presence of silicon, aluminum uptake seems to be decreased because of the formation of aluminum-silicon complexes, thus leading to a decrease in absorption of aluminum. In addition, complexes formed with organic anions, sulphate and phosphate appear to be non-toxic to plants (Kinraide 1997; Takita et al. 1999; Matsumoto 2000), while the aluminum-hydroxy species was reported to be phytotoxic in early studies (Alva et al. 1986; Wright et al. 1987; Noble et al. 1988a) but not in more recent ones (Kinraide 1997). Complexation with fluoride has been shown to ameliorate the phytoxic effects of aluminum in nutrient solutions (Cameron et al. 1986; Tanaka et al. 1987; MacLean et al. 1992); however, the aluminum-fluoride complex may also become toxic at high concentrations, with toxicity linked to the proportion and concentration of the different types of aluminum-fluoride species present in solution (Kinraide 1997; Stevens et al. 1997). Manoharan et al. (2007) reported severely restricted root growth in barley exposed to fluoride and aluminum in acidic soils (pH 4.25 to 5.48). Toxicity was attributed the activities of AlF_2^+ and AlF^{2+} complexes formed in the soil. Fluoride may enter soil through the application of phosphate fertilizers, which usually contain 1% to 4% fluoride as an impurity (Loganathan et al. 2003). Calcium supplementation has also been reported to alleviate aluminum toxicity in barley, possibly by reducing cellular absorption of the metal and enhancing protection through increased activity of antioxidant enzymes (Guo et al. 2006).

Wheeler and Dodd (1995) investigated the effect of aluminum on yield and nutrient uptake of some temperate legumes and forage crops using a low ionic strength solution. The solution aluminum levels at which top yield and root yield of 58 white clover cultivars were reduced by 50% ranged from approximately 0.005 to 0.02 mM (0.135 to 0.540 mg/L) (pH 4.5 to 4.7).

Although inorganic monomeric forms of dissolved aluminum (Al^{3+}, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$) are believed to be the most bioavailable and responsible for most toxic effects (Alva et al. 1986; Noble et al. 1988b), information on the concentrations of different dissolved aluminum complexes was not reported in many of the effects studies reviewed. For studies indicating particular sensitivity that were carried out in the laboratory in artificial solutions, it is likely that the majority of the aluminum present in these key studies was in inorganic monomeric forms. Considering that solution culture experiments gave lower LOEC values than did sand culture experiments in forest species studies, the effects data reviewed are considered to be conservative estimates of the effects levels for vegetation grown in natural soils.

2.4.2 Experimental mammal studies

The scientific literature concerning the effects of aluminum exposure in experimental mammals is large, including studies with a variety of administration routes (ingestion, inhalation, dermal, intraperitoneal, intravenous, intracisternal). The characterization of effects presented below includes studies of oral, inhalation and dermal administration, with emphasis on the oral exposure studies. This reflects the importance of the oral route in environmental exposures within the general Canadian population, as compared to dermal and inhalation as well as the research emphasis on oral studies within the scientific community. For more detailed discussion of other routes of exposure, the reader may consult the comprehensive reviews cited, in particular Krewski et al. (2007).

Health Canada considers neurotoxicity and reproductive/developmental toxicity as the categories of effects of greatest potential concern for the general population, in light of the evidence from case studies and epidemiological investigations, discussed in section 2.4.3. Recent comprehensive reviews also collectively support this conclusion (InVS-Afssa-Afssaps 2003; ATSDR 2006; JECFA 2006; Krewski et al. 2007; EFSA 2008). Thus, most of the studies presented in this section focus on neurotoxicity or reproductive/developmental toxicity in which aluminum is administered to the experimental animals through diet, drinking water or gavage.

Various aluminum salts, including chloride, nitrate, sulphate, lactate, citrate, maltolate, fluoride and hydroxide have been used in experimental animal studies to investigate the effects of Al^{3+} absorbed in the bloodstream and distributed to target organs. Aluminum speciation (i.e., the ligands associated with aluminum) and the overall composition of the diet may influence toxicokinetics and consequently the subsequent toxicity of Al^{3+} (see section 2.3.3.1.1). With respect to absorption, however, no one aluminum salt is representative of the mix of aluminum compounds in the human diet that contribute to the Al^{3+} reaching the bloodstream. Therefore, for the purpose of characterizing effects of total aluminum, all oral studies were examined, regardless of the aluminum salt administered. Relative bioavailability of particular salts is then considered in the exposure-response analysis of section 3.2.3.

A number of the experimental animal studies are designed to explore the influence of factors that may potentially exacerbate the toxic effects of aluminum (e.g., restraint) or provide protection (e.g., therapeutic substances such as Gingko). The results reported in this section, however, focus on the differences between aluminum-treated animals and controls, rather than the influence of these other factors.

In most of the studies consulted, there is a lack of data on the aluminum concentration in the base diet. Studies on different brands of commercial laboratory animal chow show that aluminum levels in the chow can be significant relative to the administered doses, and also highly variable between brands and even between different lots of the same brand (ATSDR 2006). Typical levels of 250 to 350 ppm of aluminum in rodent chow (ATSDR 2006) would contribute approximately 13 to 18 mg Al/kg/d in rats and 33 to 46 mg Al/kg/d in mice, on the basis of default reference values for animal intake and body weight proposed in Health Canada (1994). While it may be hypothesized that the absorption of the base diet aluminum may differ from (and be significantly less) than the absorption of the administered aluminum, there are little relevant experimental data on this question (see section 2.3.3). Therefore the lack of data on base diet aluminum in many of the toxicity studies must be considered as a major uncertainty in the overall database, when considering these studies in the exposure-response analysis and risk characterization.

Notwithstanding the importance of quantifying total aluminum exposure in animal studies, in order to provide a qualitative summary of the literature for the purpose of hazard identification, all studies have been evaluated, regardless of whether the base diet aluminum concentration is reported. In the exposure-response analysis (section 3.2.3), however, administered and combined doses are distinguished and the influence of this factor is considered.

The description of the studies in this section is focused on the nature of the effects investigated and observed, rather than the exposure-response relationship. The database is large (138 studies) and the experimental conditions (e.g., administered salts and dosing regimen) vary, and in the majority of the studies only one dose was tested. Thus direct comparisons of the dose-effect data may be misleading. While some information on the lowest observed dose at which effects occurred is provided[14] as well as the highest dose at which no effects were observed, a more detailed discussion of the exposure-response analysis is presented in section 3.2.3. The details of the studies considered in that analysis are summarized in Tables C1 and C2 (Appendix C). Tables summarizing the full dataset are available in the Health Canada Supporting Document, prepared for this draft assessment (Health Canada 2008a).

[14] The LOELs and NOELs reported in this section may correspond to the doses reported by the researchers, or may be calculated based on reported concentrations in food or drinking water, assuming default values for animal body weight, and food and drinking water consumption rates drawn from Health Canada (1994).

2.4.2.1 Acute toxicity

Oral exposure

The oral LD_{50} (lethal dose, 50% kill, single administration) for different aluminum salts, as measured in different strains of mice, rats, guinea pigs and rabbits, varies according to the aluminum salt administered as well as according to the experimental animal species. In an early review an LD_{50} of apparently 6,200 mg Al/kg bw was reported for $Al_2(SO_4)_3$ and of 3,850 mg Al/kg bw for $Al(Cl)_3$ administered to mice (Sorenson et al. 1974), although it is unclear from the review article if these values refer to the dose in terms of aluminum or the dose in terms of the salts. Sorenson et al. (1974) also reported LD_{50} values from 260 to 4,280 mg/kg bw for $Al(NO_3)_3 \bullet 9H_2O$ in two separate studies on rats. The lower value of 260 mg/kg $Al(NO_3)_3 \bullet 9H_2O$ clearly underestimates the LD_{50} (i.e., overestimates the toxicity), as Colomina et al. (2002), Colomina et al. (2005) and Domingo et al. (1996) have shown. These research groups tested administered doses of 50 to 100 mg Al/kg bw/d, equivalent to approximately 700 to 1,400 mg $Al(NO_3)_3 \bullet 9H_2O$/kg bw/d, and the effects were limited to alterations in weight gain and subtle neurological effects (see sections 2.4.2.2 to 2.4.2.4 and section 3.3 for more detailed discussion of these studies).

In a study of oral and intraperitoneal administration during 14 days, Llobet et al. (1987) estimated the acute oral toxicity of aluminum chloride, nitrate and sulphate in Sprague-Dawley rats and Swiss mice. Aluminum chloride and nitrate produced acute toxicities of similar magnitude (LD_{50} of 222 to 370 mg Al/kg) in the mice and rats, whereas the toxicity of aluminum sulphate was considerably lower (LD_{50} > 730 mg Al/kg in both species).

Inhalation exposure

In Golden Syrian hamsters and New Zealand rabbits exposed over a short duration (four to six hours per day for three to five days at levels of 7 to 200 mg/m^3) to aluminum chlorohydrate through inhalation, the effects observed are those typically associated with inhalation of particulate matter, including alveolar wall thickening, increased number of macrophages and increased lung weight (ATSDR 2006). A more detailed discussion of the pulmonary effects in experimental animals of inhalation exposure to aluminum oxide dust and refractory alumina fibres, and aluminum hydroxide is provided by Krewski et al. (2007). The observed responses to various species of aluminum are described as "typical of foreign body reaction", including alveolar proteinosis and wall thickening, and some nodule formation.

Dermal exposure

Dermal effects of aluminum compounds (10% w/v chloride, nitrate, chlorohydrate, sulphate, hydroxide) applied to skin of mice, rabbits and pigs over five-day periods (once per day) include epidermal damage, hyperkeratosis, acanthosis and microabscesses (ATSDR 2006; Krewski et al. 2007).

2.4.2.2 Short-term toxicity (duration of exposure less than 90 days)

Oral exposure

The results of 40 short-term studies in adult mice, rats and rabbits (exposure duration between 3 and 13 weeks) are summarized below. In all the studies considered, aluminum was administered orally in drinking water, in the diet or by gavage. The aluminum salts include lactate, chloride, sulphate, nitrate and hydroxide. In some studies citrate was administered with the aluminum salt in order to enhance absorption.

As discussed in section 2.4.2, many of the short-term studies did not quantify the concentration of aluminum in the base diet. In these cases the value of the actual combined dose is highly uncertain, particularly in the studies where the administered dose was significantly less than the possible baseline dose in the diet (e.g., Basu et al. 2000; El-Demerdash 2004; Kaizer et al. 2005; Kaur and Gill 2005, 2006; Jyoti and Sharma 2006; Sparks et al. 2006; Kaur et al. 2006). In three studies (Thorne et al. 1986; Shakoor et al. 2003; Campbell et al. 2004), ambiguities in the reporting of the doses precluded consideration of the dose-response relationship; however the qualitative observations from these studies are included in the following summary of effects.

Neurobehavioural effects in adult rats and mice following oral administration from 21 to 90 days included decreased performance in the rotarod test (Bowdler et al. 1979; Shakoor et al. 2003; Kaur et al. 2006), decreased performance in passive and active avoidance tests (Commissaris et al. 1982; Connor et al. 1988; Connor et al. 1989; Kaur et al. 2006), reduced motor activity (Commissaris et al. 1982; Golub et al. 1989; Shakoor et al. 2003), decreased forelimb and hindlimb grip strength (Oteiza et al. 1993), increased sensitivity to flicker (Bowdler et al. 1979) and air puff startle response (Oteiza et al. 1993), and reduced recovery in neurological function following spinal cord injury (Al Moutaery et al. 2000).

Of the above studies, the lowest administered dose at which effects occurred was observed by Kaur et al. (2006), in which male Wistar rats were administered 10 mg Al/kg bw/d as aluminum lactate for up to 12 weeks, with testing at 0, 4, 8 and 12 weeks. A significant decrease in performance between exposed and control groups was observed at four weeks and became more pronounced following eight weeks of exposure. Decreased performance in memory function tests (passive and active avoidance responses) was also observed in the exposed animals tested at 12 weeks.

In contrast, no alterations in passive or active avoidance test results were reported in aluminum-exposed animals, at doses of 67 mg Al/kg bw/d of aluminum chloride administered by gavage to male Sprague-Dawley rats for 28 days (Bowdler et al. 1979) and 600 mg Al/kg bw/d of aluminum nitrate administered in drinking water for 14 days to male CD mice (Colomina 1999).

Reduced body weight among aluminum-exposed animals was observed by Bataineh et al. (1998), at a dose of 15 mg Al/kg bw/d of aluminum chloride administered to male Sprague-Dawley rats in drinking water for 12 weeks. On the other hand, Colomina et al. (1999) observed a reduction in body weight only at 600 mg Al/kg bw/d of aluminum nitrate, and no effect at 300 mg Al/kg bw/d, in mice administered aluminum via drinking water for 14 days. In other short-term studies, the authors either did not observe this effect, at a dose of100 mg Al/kg bw/d administered in the diet of Swiss Webster mice (Donald et al. 1989;

Golub and Germann 1998), or did not report differences in body weight between exposed and control groups.

The most extensive histopathological changes in the short-term studies were reported by Roy et al. (1991a) in which male rats were given doses of 17 to 172 mg Al/kg bw/d as aluminum sulphate via gavage. The concentration of aluminum in the base diet was not quantified. Multifocal neuronal degeneration, abnormal and damaged neurons, and reduced neuronal density were identified in specific brain regions (e.g., cerebral cortex, subcortical region and base of brain) at 29 mg Al/kg bw/d. In the liver, Roy et al. (1991a) observed cytoplasmic degeneration in the periphery of the hepatic lobule at all doses. With increasing doses, multifocal degeneration of the entire liver tissue was observed, followed by fibrous tissue proliferation. Kidney effects observed in this study at 22 mg Al/kg bw/d included increased swelling and degeneration of the cortical tubules.

Other histopathological effects reported in different strains of rats include necrosis-like changes in hippocampal CA1 cells and accumulation of synaptic vesicles in presynaptic terminals (Jyoti and Sharma 2006), congestion of cerebral and meningeal blood vessels, multifocal neuronal degeneration, neurofibrillary degeneration and foci of demyelination (El-Rahman 2003), increased membrane fluidity and decreased cholesterol/phospholipid ratio in synaptosomes (Silva et al. 2002), increased number of vacuolated spaces in the matrix of the cerebral cortex (Basu et al. 2000), decreased NADPH-diaphorase positive neurons in the cerebral cortex (Rodella et al. 2001) and increased hippocampal muscarinic receptors (Connor et al. 1988). The lowest administered doses at which such changes occurred were in the studies of Jyoti and Sharma (2006) in which exposed male Wistar rats received a dose of 10 mg Al/kg bw/d of aluminum chloride in drinking water for five weeks, and of Basu et al. (2000), in which male Sprague-Dawley rats received 10 mg Al/kg bw/d of aluminum chloride via gavage for 40 days.

The biochemical changes to the brains of adult rodents resulting from oral administration of aluminum salts for periods of less than 90 days included effects on cholinergic neurotransmission (Kumar 1998; Shakoor et al. 2003; El-Demerdash 2004; Kaizer et al. 2005; Kaur and Gill 2006) as well as changes in the levels of other neurotransmitters and signalling proteins (Flora et al. 1991; Tsunoda and Sharma 1999b; Kumar 2002; El-Rahman 2003; Becaria et al. 2006), alterations in calcium transfer, binding and signalling in the brain (Kaur et al. 2006; Kaur and Gill 2005), evidence of oxidative stress in different regions of the brain (Fraga et al. 1990; Katyal et al. 1997; Abd el-Fattah et al. 1998; El-Demerdash 2004; Nehru and Anand 2005; Becaria et al. 2006; Jyoti and Sharma 2006), changes in ATPase activity (Katyal et al. 1997), alterations to cyclic AMP second messenger systems (Johnson and Jope 1987), increased levels of amyloid precursor protein (Becaria et al. 2006) and increased TNF-∝ (alpha tumour necrosis factor) mRNA expression in the brain (Tsunoda and Sharma 1999a; Campbell et al. 2004). The lowest administered dose at which such effects were observed was 10 mg Al/kg bw/d administered to rats as aluminum lactate via gavage or as aluminum chloride via drinking water in Kaur and Gill (2006) and Basu et al. (2000).

Inhalation exposure

The toxicological literature for short-term inhalation exposure studies is limited compared to that for oral exposure. The most recent comprehensive reviews of this literature can be found in ATSDR (2006) and Krewski et al. (2007). The most sensitive and best documented endpoints concern the respiratory system. The observed effects were those commonly associated with particle inhalation exposure (> 7 mg/m^3), including a thickening of the alveolar walls, an increase in alveolar macrophages and heterophils, granulomatous nodules and lesions, and increased lung weight (ATSDR 2006).

2.4.2.3 Subchronic and chronic toxicity (exposure duration greater than 90 days, non-cancer endpoints)

Oral exposure

The results of 49 subchronic and chronic toxicity studies (exposure greater than 90 days) in adult mice, rats, rabbits, monkeys and dogs are summarized below. In all the studies considered, aluminum was administered orally in drinking water, in the diet or by gavage. The aluminum salts include lactate, chloride, sulphate, nitrate, hydroxide, citrate, maltolate, fluoride and KASAL (basic sodium aluminum phosphate).

As in the case of the short-term studies, many of the subchronic and chronic toxicity studies did not quantify the concentration of aluminum in the base diet. In those studies where the administered dose was substantially less than the possible baseline dose in the diet, the uncertainty associated with the actual combined dose was increased (see, for example, Krasovskii et al. (1979); Fleming and Joshi (1987); Bilkei-Gorzo (1993); Varner et al. (1993); Varner et al. (1994); Varner et al. (1998); Sahin et al. (1995); Somova et al. (1997); Jia et al. (2001a); Pratico et al. (2002); Abd-Elghaffar et al. (2005); Hu et al. (2005); Becaria et al. (2006); and Li et al. (2006)).

Neurobehavioural effects in adult mice and rats, following oral exposure for 90 days or more, included decreased spontaneous motor activity (Commissaris et al. 1982; Lal et al. 1993; Jia et al. 2001a; Jia et al. 2001b; Hu et al. 2005). The lowest administered dose associated with this effect was 1 mg Al/kg bw/d as observed by Huh et al. (2005) in male Sprague-Dawley rats who received aluminum maltolate at this dose in drinking water over a period of one year[15]. In contrast Domingo et al. (1996) and Colomina et al. (2002) found no differences in field activity of Sprague-Dawley rats, where animals received an administered dose of 100 mg Al/kg bw/d of aluminum nitrate (with citrate) in drinking water for periods of four to six months. Decreased motor coordination as measured by performance in the rotarod test (Sahin et al. 1995), decreased grip strength, and effects on temperature sensitivity and negative geotaxis (Golub et al. 1992a) were also observed.

Other observed neurobehavioural effects included learning and memory deficits (maze performance, passive avoidance tests) reported by Bilkei-Gorzo (1993), Lal et al. (1993),

[15] The methodological limitations and uncertainties associated with this study are discussed in section 3.2.3.

Gong et al. (2005), Gong et al. (2006) and Li et al.(2006). The lowest administered dose associated with such effects was 6 mg Al/kg bw/d, observed by Bilkei-Gorzo (1993) in Long Evans rats exposed for 90 days to aluminum chloride (plus citrate) via gavage, although there was some ambiguity in the reporting of doses in this study. In contrast, no effects on similar learning or memory tests were observed by Varner et al. (1994), Domingo et al. (1996), Colomina et al. (2002) and von Linstow Roloff et al. (2002). In the study of von Linstow Rolloff et al. (2002) an administered dose of 140 mg Al/kg bw/d was administered to male Lister hooded rats as aluminum sulphate in drinking water.

With respect to body weight, Pettersen et al. (1990), Gupta and Shukla (1995), Colomina et al. (2002) and Kaneko et al. (2004) observed reductions in body weight in aluminum-exposed animals (rodents and dogs) at doses ranging from 25 mg Al/kg bw/d of aluminum maltolate administered in drinking water to mice for up to 120 days (Kaneko et al. 2004) to 94 mg Al/kg bw/d of aluminum nitrate administered in drinking water to rats for 114 days (Colomina et al. 2002). In the Kaneko et al (2004) study, aluminum chloride was administered to another exposure group at the same dose as aluminum maltolate, and no difference in body weight between aluminum-exposed animals and controls was observed. The authors attributed the contrasting observations to the greater bioavailability of aluminum maltolate as compared to chloride, documented as well by the greater accumulation of aluminum in the brain, liver, kidney and spleen in mice exposed to aluminum maltolate.

Histopathological effects reported in rats and mice included increased damaged or abnormal neurons in specific brain regions (e.g., cerebral cortex and hippocampus) (Varner et al. 1993; Varner et al. 1998; Abd-Elghaffar et al. 2005), neurofibrillary degeneration and vacuolization of nuclei (Somova et al. 1997), and vacuolated astrocytes and vacuolization of neuronal cytoplasm (Florence et al. 1994). The lowest administered dose in which these effects were observed was less than 1 mg Al/kg bw/d in the Varner et al. (1998) and Varner et al. (1993) studies in which aluminum nitrate and sodium fluoride (to form aluminum fluoride) was administered in drinking water to male Long Evans rats for periods of 45 to 52 weeks.[16]

Petterson et al. (1990) observed mild to moderate histopathological effects in testes, liver and kidney, including hepatocyte vacuolization, seminiferous tubule germinal epithelial cell degeneration and tubular-glomerularnephritis in beagle dogs receiving a dose of 75 mg Al/kg bw/d of sodium aluminum phosphate. In this same study, no significant differences between exposure groups and controls were observed at the lower doses of 4 to 27 mg Al/kg bw/d.

The biochemical endpoints examined in subchronic and chronic experimental studies are considerably varied, as are the methodologies used to investigate these endpoints. The observed effects included a decrease in nitrergic neurons in the somatosensory cortex (Rodella

[16] The methodological limitations and uncertainties of the Varner et al. (1993) and Varner et al. (1998) studies are discussed in section 3.2.3.

et al. 2006), perturbations in ATPase activity in the brain (Lal et al. 1993; Sarin et al. 1997; Swegert et al. 1999; Silva and Goncalves 2003; Kohila et al. 2004; Silva et al. 2005), induced apoptosis in the brain (Huh et al. 2005), effects on cholinergic enzyme activities (Bilkei-Gorzo 1993; Zheng and Liang 1998; Dave et al. 2002; Zatta et al. 2002; Kohila et al. 2004), increased cytokine levels (Becaria et al. 2006), increased catalytic efficiency of monoamine oxidases A and B (Huh et al. 2005), increased caspase 3 and 12 (Gong et al. 2005; Huh et al. 2005), increased staining for amyloid precursor protein levels (Gong et al. 2005) and amyloid beta (Aβ) levels (Pratico et al. 2002), decrease in long-term potentiation in hippocampal slices (Shi-Lei et al. 2005), and alterations in phospholipid and cholesterol levels in the myelin membrane, synaptosomes or the brain (Sarin et al. 1997; Swegert et al. 1999; Pandya et al. 2001; Silva et al. 2002; Pandya et al. 2004). The lowest administered dose associated with significant effects on biochemical endpoints was 1 mg Al/kg bw/d as administered as aluminum maltolate in drinking water for one year (Huh et al. 2005)[17].

Other biochemical and biophysical effects observed in the brains of aluminum-exposed rodents included alterations in trace metal (Cu, Zn and Mn) metabolism in the brain (Sanchez et al. 1997; Yang and Wong 2001; Jia et al. 2001a; Fattoretti et al. 2003; Fattoretti et al. 2004), altered synapses in the hippocampus and frontal cortex (Jing et al. 2004), increase in area occupied by mossy fibres in the hippocampal CA3 subfield (Fattoretti et al. 2003; Fattoretti et al. 2004), increase (Flora et al. 2003) and decrease (Jia et al. 2001a) in glutathione peroxidase activity, and increase in catalase activity (Flora et al. 2003). Increased lipid peroxidation was reported by Lal et al. (1993), Gupta and Shukla (1995), Sarin et al. (1997), Pratico et al. (2002), Flora et al. (2003) and Kaneko et al. (2004). Jia (2001a), Gupta and Shukla (1995) and Abd-Elghaffar (2005) reported decreased levels of superoxide dismutase, and Jia et al. (2001a) observed increased levels in malondialdehyde. Johnson et al. (1992) observed decreased levels of cytoskeletal proteins (microtubule associated protein-2, spectrin) in the hippocampus and brain stem.

Inhalation exposure

The toxicological literature for subchronic and chronic inhalation exposure studies is limited. ATSDR (2006) and Krewski et al. (2007) report on several studies of durations of six months (six hours a day, five days a week). The most sensitive and best documented endpoints concerned the respiratory system. The observed effects are those commonly associated with particle inhalation exposure (> 600 $\mu g/m^3$), including a thickening of the alveolar walls, and an increase in alveolar macrophages, granulomatous lesions and relative lung weight (ATSDR 2006).

[17] The methodological limitations and uncertainties associated with the study by Huh et al. (2005) are discussed in section 3.2.3.

2.4.2.4 Reproductive and developmental toxicity

Oral exposure

The results of 49 studies investigating gestational, lactational and/or post-weaning exposure of rats, mice and guinea pigs to aluminum salts through diet, through drinking water or by gavage are summarized below. The aluminum salts administered in these studies included chloride, nitrate, sulphate, lactate and hydroxide. In a few studies citrate or ascorbic acid was added to enhance absorption of aluminum.

As discussed in sections 2.4.2.2 and 2.4.2.3, the lack of information on base diet for some studies is a major source of uncertainty with respect to the potential combined dose, particularly when the administered dose was low in comparison to the possible base diet dose (e.g., Clayton et al. 1992; Ravi et al. 2000). There is also uncertainty associated with reported LOELs that are of the same magnitude as the reported LD_{50} for the administered salt (Johnson et al. 1992; Misawa and Shigeta 1993; Poulos et al. 1996; Llansola et al. 1999).

The most commonly observed neurobehavioural effects in developmental studies included decreased grip strength (Golub et al. 1992b; Golub et al. 1995; Colomina et al. 2005), reduced temperature sensitivity (Donald et al. 1989; Golub et al. 1992b), reduced or delayed auditory startle responsiveness (Misawa and Shigeta 1993; Golub et al. 1994), and impaired negative geotaxis response (Bernuzzi et al. 1986; Bernuzzi et al. 1989a; Muller et al. 1990; Golub et al. 1992b). Decreased activity levels (Cherroret et al. 1992; Misawa and Shigeta 1993), locomotor coordination (Golub et al. 1987; Bernuzzi et al. 1989a; Bernuzzi et al. 1989b; Muller et al. 1990; Golub and Germann 2001b) as well as impaired righting reflex (Bernuzzi et al. 1986; Bernuzzi et al. 1989b) were also observed, although not consistently—refer to Thorne et al. (1987), Golub et al. (1992b), and Misawa and Shigeta (1993). The lowest administered dose at which effects on these endpoints were observed was 100 mg Al/kg bw/d, observed in Wistar rats administered aluminum lactate in the maternal diet during gestation (Bernuzzi et al. 1989b) as well as in Swiss Webster mice administered aluminum lactate in the maternal diet during gestation, lactation and then in the diet of offspring throughout the lifespan (Golub et al. 2000).

The observations on the effects on learning and memory of developmental exposure to aluminum salts also varied considerably. For example, in some studies improved performance in the maze tasks was observed (Golub et al. 2000; Golub and Germann 2001a; Colomina et al. 2005) while in others impaired performance (Golub and Germann 2001b; Jing et al. 2004) or no change (Thorne et al. 1987) was found. Golub and Germann (2001b) observed diminished maze learning in Swiss Webster mice pups when dams were exposed to aluminum lactate in the diet at a combined dose of 50 mg Al/kg bw/d, but not at 10 mg Al/kg bw/d, during gestation and lactation, and pups were exposed via diet for two weeks following weaning. In this experiment, animals (controls and aluminum-exposed) were fed a sub-optimal diet, designed to simulate the usual diet of U.S. women with regard to recommended dietary amounts of trace elements.

The observations of Roig et al. (2006) suggested a biphasic effect on learning in rats exposed to aluminum nitrate during gestation, lactation and post-weaning; in a two-dose study,

the low-dose group (50 mg Al/kg bw/d of aluminum nitrate plus citrate in drinking water) performed significantly better in the water maze test than the high-dose group (100 mg Al/kg bw/d), but there was no significant difference between the high-dose group and the controls. With respect to passive avoidance tests, the same group of researchers also reported improved performance in aluminum exposed animals at an administered dose of 100 mg Al/kg bw/d (Colomina et al. 2005).

Developmental exposure of mice and rats to aluminum salts also produced some evidence of disturbances in brain biochemistry, such as alterations in brain lipid contents and increased lipid peroxidation (Verstraeten et al. 1998; Verstraeten et al. 2002; Nehru and Anand 2005; Sharma and Mishra 2006) or decreased lipid peroxidation (Golub and Germann 2000), decreased levels in superoxide dismutase (Nehru and Anand 2005), delayed expression of a phosphorylated neurofilament protein (Poulos et al. 1996), differential effects on choline acetyltransferase activity in various brain regions (Clayton et al. 1992; Rajasekaran 2000; Ravi et al. 2000), decreased serotonin and noradrenaline levels in specific brain regions (Ravi et al. 2000), decreased concentrations of manganese in brain (Golub et al. 1992b; Golub et al. 1993), alterations to signal transduction pathways associated with glutamate receptors and decreased expression of proteins of the neuronal glutamate-nitric oxide-cGMP pathway (Llansola et al. 1999; Kim 2003), and alterations in secondary messenger systems (Johnson et al. 1992). With respect to biochemical endpoints, the lowest administered dose at which effects were measured was approximately 20 mg Al/kg bw/d, observed by Kim (2003) in which male and female Fisher rats received this dose of aluminum chloride in drinking water for 12 weeks prior to mating, after which treatment at this dose continued in dams during gestation and lactation.

Chen et al. (2002), Wang et al. (2002a) and Wang et al. (2002b) reported impairment of synaptic plasticity, as measured by field potentials in the dentate gyrus of the hippocampus. Johnson et al. (1992) reported decreased levels of microtubule associated protein-2 in the brains of rat pups exposed eight weeks following weaning, although no changes in other cytoskeletal proteins were observed. A significant decrease in myelin sheath width was observed in mice pups exposed during gestation, lactation and then through the diet following weaning (Golub and Tarara 1999), and in guinea pig pups exposed prenatally from GD30 to birth (Golub et al. 2002). These effects were observed at administered doses above 85 mg Al/kg bw/d as aluminum chloride in drinking water of Wistar rat dams (Wang et al. 2002a; Wang et al. 2002b; Chen et al. 2002) and 100 mg Al/kg bw/d in the diet of Swiss Webster mice dams (Golub and Tarara 1999).

Although the focus of the majority of the investigations of prenatal exposure was neurodevelopmental toxicity, effects on some reproductive endpoints were reported as well. Golub et al. (1987), Bernuzzi et al. (1989b), Gomez et al. (1991), Colomina et al. (1992), Belles et al. (1999), Sharma and Mishra (2006) and Paternain et al. (1988) reported reduced maternal weight gain, although no change in this parameter was observed by Donald et al. (1989), Golub et al. (1993), Golub et al. (1995) and Golub et al. (1996a), nor was it reported in the other studies. In regard to pup body weight, Sharma and Mishra (2006), Wang et al. (2002a), Llansola et al. (1999), Cherroret et al. (1995), Misawa and Shigeta (1993), Gomez et al. (1991), Paternain et al. (1988), Domingo et al. (1987), Thorne et al. (1987), Golub and Germann (2001a), Colomina et al. (1992), and Bernuzzi et al. (1989a), Bernuzzi et al. (1989b)

reported decreases in aluminum-exposed groups, while other studies reported no effects (Donald et al. 1989; Clayton et al. 1992; Golub et al. 1992b; Golub et al. 1993; Golub et al. 1995; Golub et al. 1996a; Colomina et al. 1994; Verstraeten et al. 1998). The lowest administered dose at which effects on reproductive parameters, including fetal growth, were observed was 13 mg Al/kg bw/d (Paternain et al. 1988; Domingo et al. 1987a), in which Sprague-Dawley rat dams received this dose via gavage as aluminum nitrate.

Cherroret et al. (1995) reported decreased plasma concentrations of total proteins and albumin and increased plasma $\alpha 1$ globulins, which the authors attributed to an inflammation process in young rats exposed postnatally by gavage at doses of 100 to 200 mg Al/kg bw/d. The same research group also observed effects on duodenal enterocytes, with a decrease in microvilli width and significant variation in K, Ca, S and Fe concentrations (Durand et al. 1993).

Other observed reproductive/developmental effects included a decrease in the number of corpora lutea and number of implantation sites (Sharma and Mishra 2006) as well as skeletal malformations (Paternain et al. 1988; Colomina et al. 1992; Sharma and Mishra 2006). Colomina et al. (2005) reported a delay in sexual maturation in both males and females, although this effect was produced at different dose levels in the two sexes (at 50 mg Al/kg bw/d in females and at 100 mg Al/kg bw/d in males). Misawa and Shigeta (1993) observed delayed pinna detachment and eye opening in female pups.

No significant maternal or developmental toxicity, as measured by fetal weight gain, reproductive parameters or fetal malformations, was observed by McCormack et al. (1979) at a combined dietary dose of aluminum chloride of 50 mg Al/kg bw/d, nor by Gomez et al. (1990) where 265 mg Al/kg bw/d of aluminum hydroxide was administered to dams via gavage during gestation.

Inhalation and dermal exposure

No studies were identified concerning the reproductive effects of inhalation or dermal exposure to aluminum salts.

2.4.2.5 Carcinogenicity

The literature concerning oral exposure bioassays is very limited. An increase in gross tumours was reported in male rats and female mice in a one-dose study but few study details were reported (Schroeder and Mitchener 1975a, 1975b, as reported in ATSDR 2006). Two other studies reported no increased incidence of tumours in rats and mice exposed orally to aluminum compounds (Hackenberg 1972; Oneda et al. 1994).

No increased tumour incidence was observed in rats following inhalation of alumina fibres at concentrations of up to 2.45 mg/m^3 (Krewski et al. 2007).

The International Agency for Research on Cancer did not classify specific aluminum compounds for carcinogenicity, but classified the exposure circumstances of aluminum production as carcinogenic to humans (Group 1) (IARC 1987).

2.4.2.6 Genotoxicity

The genotoxicity of various aluminum compounds is described in detail by Krewski et al. (2007) and ATSDR (2006). Briefly, aluminum compounds have produced negative results in most short-term in vitro mutagenic assays, including the Rec-assay using *Bacillus subtilis*, in *Salmonella typhimurium* TA92, TA 98, TA102, TA104 and TA1000 strains (with and without S9 metabolic activation), and in *Escherichia coli* (see Krewski et al. 2007).

In vitro studies of rat ascites hepatoma cells reported that aluminum chloride could serve as a stimulator for the crosslinking of chromosomal proteins (Wedrychowski et al 1986a, 1986b, as reported in Krewski et al. 2007, ATSDR 2006). Studies on human blood lymphocytes showed that aluminum chloride could induce positive responses for both micronuclei formation and sister chromatid exchange (see Krewski et al. 2007).

More recently Lima et al. (2007) investigated the genotoxic effects of aluminum chloride in cultured human lymphocytes. Comet assay and chromosome aberrations analysis were used to evaluate DNA-damaging and clastogenic effects of aluminum chloride at different phases of the cell cycle. All tested concentrations (5 to 25 μM aluminum chloride) were cytotoxic, reduced the mitotic index, induced DNA damage and were clastogenic in all phases.

Roy et al. (1991) administered doses of aluminum sulphate and potassium aluminum sulphate in drinking water to male rats at doses ranging from 17 to 171 mg Al/kg bw/d for up to 21 days. The frequency of abnormal cells increased in direct proportion to both the dose and the duration of exposure to the aluminum salts. Most aberrations were chromatid breaks, with translocations recorded at higher doses.

In a recent review of the safety of aluminum from dietary intake, EFSA (2008) summarized indirect mechanisms that might explain the genotoxic effects observed in experimental systems. The proposed mechanisms included cross-linking of DNA with chromosomal proteins, interaction with microtubule assembly and mitotic spindle functioning, induction of oxidative damage, and damage of lysosomal membranes with liberation of DNAase to explain the induction of structural chromosomal aberrations, sister chromatid exchanges, chromosome loss and formation of oxidized bases in experimental systems. EFSA (2008) suggested that these indirect mechanisms of genotoxicity, occurring at relatively high levels of exposure, would not likely be of relevance for humans exposed to aluminum via the diet.

2.4.3 Human studies

In this section, information on the potential human health effects associated with aluminum exposure is briefly summarized with the goal of describing the range of potential effects. As such, various exposure routes are considered in order to identify the possible target organs. This information includes data from case studies, epidemiological investigations into the potential health effects of exposure to aluminum in drinking water, occupational investigations of exposure to aluminum dust and welding fumes, and exposure to aluminum via vaccines and of dermal application of aluminum-containing antiperspirants.

In section 3.2.2 an evaluation of these health effects is presented, in order to: (a) identify critical effects; and (b) determine which, if any, of the human studies may be used to estimate the dose-response relationship. The latter determination is based on the strength of the available evidence and the relevance of the studies to environmental exposure in the general Canadian population.

2.4.3.1 Human case studies of exposure to aluminum

Human cases studies of aluminum toxicity have been well documented for specific medical conditions, most frequently in patients with renal impairment undergoing dialysis with aluminum-contaminated dialysate or receiving medications with elevated aluminum concentration. A small number of case studies or investigations have focused on children and pre-term infants receiving parenteral nutrition. Although the effects in particular sub-groups of susceptible individuals are not representative of exposure conditions for the general population, they are presented in order to identify the target organs of aluminum exposure. A more detailed discussion of these human case studies is presented in the comprehensive reviews InVS-Afssa-Afssaps (2003) and Krewski et al. (2007). As well, a case study is described below in which exposure to aluminum was associated with the accidental discharge of aluminum into the municipal water supply.

Aluminum toxicity in patients with renal impairment

Historically, patients undergoing dialysis treatment were exposed to aluminum through the water used to prepare dialysis solutions and from aluminum compounds prescribed as phosphate binders (Krewski et al. 2007). Today, this exposure is strictly controlled.[18] However, in the past, many cases of aluminum-induced encephalopathy, resulting in alterations in behaviour and memory, speech disorders, convulsions and muscle-twitching occurred in dialysis patients (Foley et al. 1981; Alfrey 1993). In cases of intoxication, the aluminum was introduced into the systemic circulation through the dialyzing membrane (in hemodialysis) or abdomen (in peritoneal dialysis) thus bypassing the gastrointestinal barrier, and was therefore completely available at the cellular level. The effects of elevated aluminum exposure in dialysis patients has provided clear evidence for the neurotoxicity of aluminum in humans.

Researchers have also identified cases of individuals with impaired renal function who, because of their reduced capacity to eliminate aluminum and chronic high exposure to aluminum-containing medications, also developed encephalopathy, even though they were not undergoing dialysis (Foley et al. 1981; Sedman et al. 1984; Sherrard et al. 1988; Moreno et al. 1991). A fatal case of aluminum-induced encephalopathy occurred in a patient with chronic renal failure who did not have dialysis treatment, but who consumed large doses of aluminum-containing antacids (Zatta et al. 2004).

[18] Cases of elevated aluminum exposure in dyalisis patients are rare, but are still occasionally reported. See www.cdc.gov/mmwr/preview/mmwrhtml/mm5725a4.htm for a recent example.

Other toxic effects of aluminum observed in dialysis-exposed patients include haematological effects such as anaemia (Bia et al. 1989; Yuan et al. 1989; Shah et al. 1990; Caramelo et al. 1995) and skeletal toxicity (osteomalacia and osteitis fibrosis) (Mathias et al. 1993; Jeffery et al. 1996; Ng et al. 2004).

Aluminum exposure via intravenous nutritional support

Klein (2005) reviewed the human evidence regarding the effects of aluminum exposure via solutions used for intravenous nutritional support with regard to effects on bone (osteomalacia) and the central nervous system. With respect to parenteral nutrition, infants may be a particularly sensitive sub-group because of the immaturity of the blood-brain barrier and renal excretory mechanisms. Bishop et al. (1997) investigated cognitive impairment in pre-term infants in relation to parenteral nutrition. In a randomized trial the researchers found that performance in neurodevelopmental testing conducted at 18 months was significantly better in 92 pre-term infants who had received a low-aluminum nutritional solution as compared to 90 pre-term infants receiving a standard solution with higher aluminum content. No follow-up testing that evaluated cognitive performance in the children of this cohort as they aged was identified.

Investigation of aluminum exposure associated with contamination event in Camelford, UK

Exley and Esiri (2006) reported an unusual case of fatal dementing illness in a 58-year-old woman, resident of Camelford, Cornwall, in the United Kingdom. Fifteen years earlier, at the age of 44 years, this person was exposed to high concentrations of aluminum sulphate in drinking water, which had been accidentally discharged in the drinking water supply of the region. During this event, up to 20,000 people were exposed to aluminum concentrations in drinking water varying from 100 to 600 mg/L. At the autopsy of the woman, a rare form of sporadic early-onset b-amyloid angiopathy in the cerebral cortical and leptomeningeal vessels, and in leptomeningeal vessels over the cerebellum was identified. Coincident high concentrations of aluminum were also found in the severely affected regions of the cortex. To date, this remains the only documented case. Exley and Esiri (2006), who reported this case, state that the role of aluminum is uncertain but may be clarified through future research in similarly exposed and unexposed populations (controls).

2.4.3.2 Epidemiological studies of aluminum exposure via drinking water

By the end of the 1980s, four epidemiological studies with an ecological design (i.e., using group rates of exposure and disease) had reported positive associations between the concentration of aluminum in drinking water and the occurrence of Alzheimer's disease (AD) or of dementia (Vogt 1986; Martyn et al. 1989; Flaten 1990; Frecker 1991). These observations resulted in further research into the relationship of aluminum in drinking water and various dementia syndromes, particularly AD.

Epidemiological studies based on observations of individuals were conducted in the 1990s with the aim of investigating the association between AD or other cognitive dysfunctions and exposure to aluminum in drinking water. Health Canada published a comprehensive review of epidemiological studies in Guidelines for Canadian Drinking Water

Quality - Technical Documents: Aluminum (Health Canada 1998b) and in the SOS report of 2000. The discussion presented below summarizes the information presented in the previous reviews and presents more recently published findings of the eight-year follow-up analysis of a large cohort in southwestern France (Rondeau et al. 2000; Rondeau et al. 2001). The study designs and findings of the relevant epidemiological studies are presented in Table B1. (Appendix B). These data have also been described in detail in Krewski et al. (2007) and InVS-Afssa-Afssaps (2003). Analysis of the epidemiological database and its applicability in a quantitative risk assessment is presented in the Hazard Characterization of this assessment (section 3.2.2.1).

Twelve studies are presented in Table B1, based on case-control, cross-sectional, or longitudinal designs. The observations from two Ontario case-control studies are drawn from the same study population—the Ontario Longitudinal study of Aging (LSA)—and all the French studies were based on observations from the "Principal lifetime occupation and cognitive impairment in a French elderly cohort" or PAQUID cohort. However, the LSA and PAQUID study populations differ with respect to the case definition and the manner of diagnosis of disease. In the PAQUID investigations, the earlier studies used a case-control design whereas the more recent studies by Rondeau et al. (2000) and Rondeau et al. (2001) used a cohort incidence analysis.

Positive findings for an association between aluminum exposure and AD or other neurological dysfunctions were found to be statistically significant ($p < 0.05$) in seven of the twelve studies, although the strength and significance of these associations depended on how the data were analysed (see Appendix B) These seven studies were carried out in Ontario (Neri and Hewitt 1991; Forbes et al. 1992; Forbes et al. 1994; Forbes et al. 1995a; Forbes et al. 1995b; Neri et al. 1992; Forbes and Agwani 1994; Forbes and McLachlan 1996; McLachlan et al. 1996), in Quebec (Gauthier et al. 2000) and in France (Michel et al. 1991; Jacqmin et al. 1994; Jacqmin-Gadda et al. 1996; Rondeau et al. 2000; Rondeau et al. 2001).

In Ontario, a series of analyses was conducted on the LSA cohort to investigate the relationship between the concentration of aluminum in drinking water and cognitive impairment, as established by interviews and questionnaires (Forbes et al. 1992; Forbes et al. 1994; Forbes et al. 1995a; Forbes and Agwani 1994). These authors observed statistically significant associations only when they controlled their analyses according to certain physical-chemical parameters of water, such as fluoride, pH, and silica. Since the methods of interviews and questionnaires for characterizing cognitive functions were deemed to be insufficiently specific for accurately detecting neurological impairments, Forbes et al. (1995b) and Forbes and McLachlan (1996) consulted death certificates from individuals on the LSA cohort and examined the association between aluminum in drinking water and AD or presenile dementia as categorized by the corresponding ICD[19] codes. Positive relationships between aluminum and AD and presenile dementia were reported with and without adjustments with different

[19] International Classification of Disease (World Health Organization).

water quality parameters. For instance, some of the highest risks for AD were observed when high concentrations of aluminum (≥ 336 µg Al/L) were combined with high pH (≥ 7.95), low levels of fluoride (< 300 µg/L) or low levels of silica (< 1.5 mg/L).

Neri and Hewitt (1991) and Neri et al. (1992) reported a significant dose-response relationship between AD or presenile dementia and aluminum using hospital discharge records from Ontario, and by matching cases and controls according to age and sex. Another study from Ontario was a case-control analysis from the Canadian Brain Tissue Bank cohort in which AD was confirmed by histopathological criteria (McLachlan et al. 1996).

Although all studies from Ontario assessed the exposure of aluminum based on the data of the water quality surveillance program of the Ontario Ministry of the Environment, only McLachlan et al. (1996) evaluated the past exposure to aluminum.[20] However, in the McLachlan et al. (1996) study, the analysis was not controlled for potential confounders and modifying factors (e.g., age, sex, education and occupation), and the significant positive associations were not adjusted for other chemical or physical parameters in water.

The single study from Quebec was a case-control analysis of AD and exposure to various aluminum species in residential drinking water (Gauthier et al. 2000). The diagnosis of AD was based on a three-step procedure to discriminate between AD and other neurological disorders. In addition to controlling for a number of confounding factors as well as the aluminum speciation, these authors took into account historical exposure to aluminum in drinking water. Gauthier et al. (2000) reported 16 odds ratios (OR) but observed only one significant positive association (i.e., OR > 1), which was related to the concentration of monomeric organic aluminum in drinking water. This significant association was found, however, when only current exposure was considered, and not for long-term exposure, which would be expected to be more biologically-relevant.

The three studies conducted on populations from the United Kingdom showed no significant association between aluminum concentration in drinking water and neurological dysfunction, following adjustment for sex and age (Wood et al. 1988; Forster et al. 1995; Martyn et al. 1997), but none of these authors adjusted their statistical tests according to the physical-chemical properties of the drinking water. The health outcome in the two case-control studies was AD, diagnosed by a three-step procedure for including cases of presenile dementia (Forster et al. 1995) or by a clinical diagnosis using unspecified criteria (Martyn et al. 1997). This latter study, which took into account past exposure, also did not observe differences between cases and controls when the analyses were restricted to subjects exposed to low levels of silica in drinking water (< 6 mg/L). The cross-sectional study of Wood et al.

[20] Present exposure (i.e., exposure based on residence at the time of the study or at the time of diagnosis) may poorly characterize the exposure relevant to development of the disease, if the subject has moved frequently in the past, or in the case of a historical change in the water supply (i.e., change in water supply or treatment process).

(1988) was based on data collected from patients from northern England with hip fractures, for whom dementia was evaluated (no information about the diagnostic tests).

The study from Switzerland (Wettstein et al. 1991), which was a cross-sectional examination of mnestic skills in octogenarians from Zurich and aluminum in drinking water, also reported no significant associations when controlling for socio-economic status, age, and education. It should be noted that the high-exposure district in this study had drinking water with a mean aluminum concentration of 98 μg/L. Thus the analysis was carried out for a drinking water supply that was generally lower in aluminum than the drinking water supplies considered in the other investigations.

All the studies from France were based on the PAQUID cohort. The studies of Michel et al. (1991) and Rondeau et al. (2001), reported significant positive associations between the exposure to aluminum in drinking water and the occurrence of AD or dementia diagnosed by a two-step procedure, whereas the positive associations reported by Jacqmin et al. (1994) and by Jacqmin-Gadda et al. (1996) were based on the scores of the Mini-Mental State Examination (MMSE). The results of Michel et al. (1991) have been discounted, however, because of a reliance on potentially unreliable historical information on drinking water concentrations (Jacqmin et al. 1994; Smith 1995; WHO 1997).

Jacqmin et al. (1994) and Jacqmin-Gadda et al. (1996) analysed the same database collected from the PAQUID cohort in different ways, with inconclusive results. The first study included an initial report of the effect of pH on the association between aluminum and cognitive impairment (Jacqmin et al. 1994). Without considering the effect of the pH-aluminum interaction, these authors reported a positive association between aluminum and cognitive impairment, whereas consideration of this interaction resulted in a negative association. These results remained statistically significant only if occupation was included in the logistic regressions. Jacqmin-Gadda et al. (1996) expanded their analyses to include the levels of silica in drinking water. While their results indicate a protective effect of aluminum against cognitive impairment with high level of silica (≥ 10.4 mg/L) and high pH (≥ 7.5), the consideration of the interaction of aluminum and silica in their logistic regression suggests an adverse effect of aluminum on neurological functions.

Rondeau et al. (2000) retained the unimpaired subjects in the studies of Jacqmin et al. (1994) and Jacqmin-Gadda et al. (1996), and evaluated the incidence of dementia and AD one, three, five and eight years after the initial MMSE. This follow-up analysis reported a positive association between aluminum and AD or dementia, after adjustment for age, sex, education and place of residence as well as for consumption of wine and bottled mineral water. This study addressed some of the limitations of previous epidemiological investigations by adjusting for the potential confounders, and while exposure levels were not weighted according to residential history, residential history was considered. At baseline, 91% of the subjects had lived more than ten years in the same parish, with a mean length of residence of 41 years. A total of 3,401 participants were included in the study at baseline, although only 2.6% of the subjects were exposed to an aluminum concentration greater than 100 μg/L. Nonetheless, the associations between aluminum in drinking water and dementia, and aluminum in drinking water and AD, were highly significant. Only two exposure groups

(< 100 μg/L or > 100 μg/L) were defined in the principal analysis and no dose-response relationship was found when exposure categories were more finely divided.

Many of the epidemiological studies investigating the association between aluminum in drinking water and the development of cognitive impairment or AD did not control for important potential confounders or modifying factors, or did not adequately characterize past exposure. The Rondeau (2000) study addressed some of these limitations. However, the subjects in the cohort were not generally exposed to high levels of aluminum (97% of subjects exposed to less than 100 μg/L), and within the limited exposure range, no dose-response relationship was observed.

2.4.3.3 Epidemiological investigations of exposure to aluminum in antacids, antiperspirants or food

Only very weak or no associations have been found between repeated exposures to aluminum in antacids and AD in a number of analytical epidemiological studies (Heyman et al. 1984; Graves et al. 1990; Flaten et al. 1991; CSHA 1994; Forster et al. 1995; Lindsay et al. 2002). Positive associations between AD and the use of aluminum containing antiperspirants were reported in two case-control studies, but the interpretation of the results is difficult due to methodological limitations of the studies (e.g., missing data, and misclassification due to varying brands and subtypes of antiperspirant with varying aluminum contents) (Graves et al. 1990; CSHA 1994). This positive observation, however, was not supported by a follow-up study on the CSHA[21] cohort (Lindsay et al. 2002); the results show that regular use of antiperspirant did not increase the risk of AD.

Rogers and Simon (1999) conducted a pilot study to examine dietary differences in individuals with AD and matched controls (n = 46: 23 subjects, 23 controls). The exposure assessment was based on questionnaires to determine past dietary habits. According to the authors, there may be an association between AD and the consumption of foods containing high levels of aluminum food additives. However, the sample size was very small and the association was statistically significant only for one category of food (pancake, waffle and biscuit).

2.4.3.4 Epidemiological investigations of exposure to aluminum in vaccines

Aluminum adjuvants are included in some vaccines to enhance and extend the immune response of some antigens. Aluminum hydroxide and phosphate salts as well as aluminum sulphate can be used as an adjuvant (Eickhoff and Myers 2002).

Possible associations between AD and historical exposure to vaccines have been investigated in the CSHA cohort (Verreault et al. 2001). Exposure to conventional vaccines appears to lower the risk of developing AD. After adjustments for age, sex and education, the ORs were 0.41 (95% CI 0.27–0.62) for the diphtheria or tetanus vaccines, 0.60 (95% CI 0.37–

[21] Canadian Study of Health and Aging.

0.99) for the poliomyelitis vaccines and 0.75 (95% CI 0.54–1.04) for the influenza vaccine. Except for the influenza vaccine, all others contain aluminum-adjuvants (Eickhoff and Myers 2002).

The possible links between the hepatitis B vaccine, which contains aluminum-adjuvants, and the risk of demyelinating diseases such as multiple sclerosis (MS) have been investigated in France (Touze et al. 2000; Touze et al. 2002), England (Sturkenboom et al. 2000; Hernan et al. 2004), the U.S. (Zipp et al. 1999; Ascherio et al. 2001), Canada (Sadovnick and Scheifele 2000) and Europe (Confavreux et al. 2001). Only the study of Hernan et al. (2004) observed a significant positive association between MS and the hepatitis B vaccine, but no association between MS and the tetanus or influenza vaccines, which also contain aluminum adjuvants.

2.4.3.5 Epidemiological investigations of occupational exposure to aluminum

Subclinical neurological effects have been observed in a number of studies of workers chronically exposed to aluminum (aluminum potroom and foundry workers, welders, and miners). Many of these studies involved small numbers of workers and involved the assessment of exposure based on occupation rather than measured airborne aluminum concentrations, and most involved mixed exposures to various dusts and chemicals. Endpoints examined in different studies varied and for those that were similar, results were not always consistent. The types of neurological effects observed included impaired motor function (Hosovski et al. 1990; Sjogren et al. 1996; Kilburn 1998), decreased performance on cognitive tests (attention, memory, visuospatial function) (Hosovski et al. 1990; Rifat et al. 1990; Bast-Pettersen et al. 1994; Kilburn 1998; Akila et al. 1999), subjective neuropsychiatric symptoms (Sjogren et al. 1990; White et al. 1992; Sim et al. 1997) and quantitative electroencephalographic changes (Hanninen et al. 1994).

In one case-control study from England (Salib and Hillier 1996) and two from the U.S. (Gun et al. 1997; Graves et al. 1998), the relationship between the occurrence of AD and occupational exposure to aluminum was investigated. In each study, disease status was defined by standard criteria (e.g., NINCDS-ADRDA and/or DSM),[22] and exposure to airborne aluminum (e.g., welding fumes, dusts and flakes) was assessed through occupational history questionnaires administered to informants. In none of these studies was there a significant association between occupational exposure to airborne aluminum and AD.

A four-year longitudinal study investigated neurobehavioural performance in 47 aluminum welders in the train and truck construction industry, with a control group drawn from assembly workers in the same industry (Kieswetter et al. 2007). Exposure to aluminum in dust was assessed through total dust collected on filter samples attached to the welders'

[22] NINCDS is the National Institute of Neurological and Communicative Disorders and Stroke; ADRDA is the Alzheimer's Disease and Related Disorders Association; DSM is the Diagnostic and Statistical Manual of Mental Disorders (published by American Psychiatric Association).

helmets as well as through biomonitoring (aluminum in plasma and urine) at the time of neurobehavioural testing (start of investigation, after two years, after four years). The battery of neurobehavioural tests included an evaluation of cognitive abilities, psychomotor performance, attention and memory. This study used a small number of participants to explore the potential use of different biomonitoring measures, dust levels and exposure duration to predict performance in neurobehavioural tests. The study was not designed to find a relationship if one existed, but rather to explore the use of different exposure measures. Although exposure to aluminum among the welders was considered to be high in comparison to other occupational studies of aluminum (88 to 140 μg Al/g creatinine in urine, or approximately 103 to 164 μg Al/L),[23] no association between exposure and neurobehavioural performances was found.

A meta-analysis was conducted for nine investigations of occupational aluminum exposure and neurobehavioural performance, with a total of 449 exposed subjects with mean urinary aluminum concentrations of 13 to 133 μg Al/L (Meyer-Baron et al. 2007). Even if almost all effect sizes indicated an inferior neurobehavioural performance of the exposed group to aluminum, only one out of ten performance variables (the digit symbol test) was statistically significant. However, the statistical significance of the digit symbol results relationship to aluminum exposure was reduced when one study, in which the biomonitoring measure was estimated on the basis of an uncertain conversion factor, was excluded from the analysis. The authors concluded that with respect to occupational exposure, as indicated by urinary concentrations of less than 135 μg Al/L, there is concurring evidence of an impact on cognitive performance and acknowledge that international standardization for exposure is needed.

2.4.4 Mode of action of toxic effects of aluminum

Information related to possible modes of action by which aluminum affects the nervous system, as explored in animal and human studies, has been discussed in a number of recent reviews (Strong et al. 1996; Savory 2000; Kawahara 2005; ATSDR 2006; Savory et al. 2006; Krewski et al. 2007; Shcherbatykh and Carpenter 2007; Goncalves and Silva 2007). In addition, Jeffery et al. (1996) and Krewski et al. (2007) consider the mode of action in relation to bone and hematopoietic tissue.

The mechanism of aluminum neurotoxicity is an area of active research, with multiple lines of investigation. The purpose of the present discussion is to briefly summarize the areas of investigation relating to mode of action of aluminum toxicity, as mostly tested in laboratory rodents or in vitro studies, and present the range of views regarding the relevance of these data to human neurodegeneration, and particularly the development of AD.

---

[23] Meyer-Baron et al. (2007) propose a conversion factor of 1.17 to obtain μg Al/L from μg Al/g creatinine, determined as the mean of reported conversion factors between 0.71 and 1.61.

Neurotoxic effects

There is evidence from studies in both laboratory animals and humans that absorbed aluminum is distributed to the brain, particularly the cerebral cortex and hippocampus. For example, the accumulation of aluminum in the brains of adult mice, rats and monkeys from the exposed groups was reported in 23 studies of neurological effects of orally administered aluminum (described in section 2.4.2).[24] Increased aluminum in the brains of pups exposed only during pregnancy was observed by Sharma and Mishra (2006), but not by others (Colomina et al. 2005; Golub et al. 1992b). Other studies of prenatal exposure in which exposure continued through lactation also reported increased aluminum in the brain (Wang et al. 2002a; Chen et al 2002; Golub et al. 1993). In contrast, Golub et al. (2000) observed decreased aluminum levels in the brains of mice exposed during gestation, lactation and through their lifespan.

Other research documenting the distribution of aluminum in the brain is described in section 2.3.3.2.

The research on aluminum neurotoxicity in laboratory animals has generally focused on the following interrelated categories of biochemical and cellular effects:

- peroxidation of membrane lipids and other sources of oxidative stress;
- increased inflammatory response;
- alterations in the lipid/phospholipid composition of myelin, with consequent effects on neurotransmission and synaptic function;
- impaired glucose metabolism;
- effects on neurotransmission, including cholinergic and glutamatergic systems;
- alterations to second messenger systems (e.g., inositol triphosphate, cAMP and Ca^{2+});
- accumulation of intracellular calcium;
- accumulation of mitochondrial Ca^{2+}, resulting in release of cytochrome c and subsequent apoptosis;
- perturbation in the distribution and homeostasis of essential metals with potential adverse metabolic effects;
- alteration of phosphorylation level of neurofilaments, including phosphorylation of tau-protein, and resulting neurofibrillary tangle formation;
- inhibition of axonal transport;
- accumulation of amyloidβ peptide;

[24] Studies showing accumulation of aluminum in brain regions include Flora et al. (1991, 2003), Golub et al. (1992a), Lal et al. (1993), Varner et al. (1993, 1994, 1998), Florence et al. (1994), Gupta and Shukla (1995), Domingo et al. (1996), Sarin et al. (1997), Somova et al. (1997), Zheng and Liang (1998), Colomina et al. (1999), Kumar (1999), Swegert et al. (1999), Jia et al. (2001a), Baydar et al. (2003), Fattoretti et al. (2004), Jing et al. (2004), Abd-Elghaffar et al. (2005), Huh et al. (2005), Kaur et al. (2006) and Roig et al. (2006).

- alterations in gene expression and binding to DNA;
- alterations to the permeability of the blood-brain barrier.

There has been some effort to integrate the evidence for the above biochemical effects into a common mechanism, or at least a group of mechanisms of action for the neurotoxicity of aluminum (for example, see Kawahara (2005) and Shcherbatykh and Carpenter (2007)). Strong et al. (1996) argued that "a single unifying mechanism of aluminum neurotoxicity that will encompass all the potential means by which aluminum acts at the cellular level probably does not exist." These authors did, however, propose the following general categories by which aluminum neurotoxicity may be characterized, as a means for focusing future research on mechanisms of action:

- the induction of cytoskeletal pathology in the form of neurofilamentous aggregates: the mechanisms of this induction include those at the level of gene expression to altered post-translational processing (phosphorylation or proteolysis) of neurofilaments;

- alterations in cognition and behaviour in the absence of cytoskeletal pathology but with significant neurochemical and neurophysiological modifications: these include effects on cholinergic activity, signal transduction pathways and glucose metabolism;

- developmental neurotoxicity: research into this lifestage could focus on whether the mechanisms of action of aluminum that have led to neurobehavioural alterations in the developing fetus are similar to those responsible for toxicity in the adult as well as the nature of these alterations (permanent versus transient).

The relationship between the mechanism of aluminum neurotoxicity in animals and to the potential mechanism in AD remains an important topic of discussion. This is a complex debate as the basic cellular mechanism for AD is not clear. The presence of senile plaques composed of Aβ peptides in the brains of individuals with AD is well-documented, but the means by which these peptides produce neurotoxicity is not known (Marchesi 2005). Superimposed on the debate on the mechanisms for AD is the controversy as to whether environmental exposure to aluminum could contribute to the development of AD. The recent literature includes arguments across the spectrum, from the view that no compelling evidence for the "aluminum hypothesis" exists today (Becking and Priest 1997; Wisniewski and Lidsky 1997) to the view that the different animal and epidemiological evidence suggest that environmental aluminum may indeed be an important contributing factor for AD and that it is important not to prematurely reject this hypothesis (Yokel 2000; Gupta et al. 2005; Kawahara 2005; Exley 2006; Miu and Benga 2006; Savory et al. 2006). The proponents of further investigation into the role of aluminum in the development of AD cite, among others, the following lines of evidence, in addition to the epidemiological evidence (described in section 2.4.3.2), for which counter arguments have also been put forward:

- Increased aluminum in the whole brains of AD individuals at autopsy, as compared to age-matched non-AD brains, has been observed in some studies,

although not in others. Investigations focusing on the measurement of aluminum in the senile plaques and neurofibrillary tangles of AD brains have also produced variable results, possibly as a result of difficulties and differences of the analytical methods used (Environment Canada and Health Canada 2000; Yokel 2000).

- Aluminum injected into the brain or spinal cord of certain species (e.g., rabbit, cat, guinea pig and ferret) produce effects that have some similarities to AD pathology, although there are significant differences as well.

  For example, abnormally phosphorylated tau is the principal protein of the paired helical filaments that make up the neurofibrillary tangles that are diagnostic of AD. Aluminum-induced phosphorylation of tau protein has been demonstrated in some in vitro and in vivo studies (Yokel 2000; Savory et al. 2006). Yet, although aluminum induces neurofilament aggregates in model species, these differ structurally from neurofibrillary tangles that are diagnostic of AD in humans.

  The deposition of senile plaques, also a hallmark of AD, is not observed in animal models, but increased immunoreactivity to Aβ and its parent molecule, amyloid precursor protein, via aluminum has been demonstrated in both in vitro and in vivo studies (Environment Canada and Health Canada 2000).

- Dialysis encephalopathy (see section 2.4.3.1 for discussion) is clearly recognized as resulting from aluminum intoxication. This condition has provided clear evidence for the neurotoxicity of aluminum in humans. Nonetheless, the very different clinical symptoms and progression of the two diseases as well as their differing pathologies have been cited as evidence of a lack of causal relationship between aluminum and AD (Wisniewski and Lidsky 1997).

Bone toxicity

In the case of osteomalacia associated with aluminum exposure, two distinct mechanisms of actions are recognized (ATSDR 2006). Firstly, the oral exposure to high levels of aluminum can produce a complex with dietary phosphorus, impairing gastrointestinal absorption of this element necessary for bone mineralization. Secondly, the osteomalacia associated with increased bone concentrations of aluminum, principally located at the mineralization front, is associated with increased mineralization lag time, increased osteoid surface area, low parathyroid hormone levels, and elevated serum calcium levels (ATSDR 2006).

Hematopoetic tissue

Among patients with chronic renal failure who receive dialysis treatment, some individuals will develop a hypochromic microcytic anemia, the severity of which correlates with the plasma and red blood cell aluminum levels and can be reversed by terminating exposure to aluminum or by aluminum chelation with desferrioxamine (Jeffery et al. 1996). While the mechanism for this effect in dialysis patients is not known, Jeffery et al. (1996)

suggest that it may be aluminum interference with iron metabolism, possibly through disruption in cellular transfer of iron to ferritin to heme.

3 ASSESSMENT OF "TOXIC" UNDER CEPA 1999

3.1 CEPA 1999 64(a) and 64(b) Environment

3.1.1 Environmental risk characterization

The approach taken in the ecological component of this risk assessment was to review new information relevant to the three aluminum salts recommended for assessment by the Ministers' Expert Advisory Panel (i.e., aluminum chloride, aluminum nitrate, aluminum sulphate), and to evaluate this information with reference to the original characterization of potential risk presented in Environment Canada and Health Canada (2000).

Environment Canada and Health Canada (2000) identified the pelagic, benthic and soil compartments as primary media of potential exposure for aluminum derived from the three salts subject to assessment, and conducted an analysis of potential risk for each compartment. This analysis is provided in the sections below, along with additional information collected subsequent to the publication of this assessment and deemed relevant to the evaluation of potential risk.

3.1.1.1 Aquatic organisms

3.1.1.1.1 Pelagic

Environmental exposure in water to aluminum from the three aluminum salts is expected to be greatest in areas near direct releases of process wastewater to the aquatic environment. Unfortunately, few measured data are available for receiving environments following direct releases from water treatment facilities or pulp and paper mills. In addition, measurements of total concentrations of a metal can rarely be correlated directly with their biological effects. Metal in particulate form is generally considered to be less available for uptake by organisms, and the formation of complexes with inorganic (e.g., OH^-, SO_4^{2-}) or organic (e.g., fulvic acid) ligands can reduce the available fraction of the dissolved form of a metal. Speciation modelling using the estimation models MINEQL+ and WHAM was conducted in order to estimate the level of dissolved inorganic monomeric aluminum present in rivers following release of wastewater from eight DWTPs and two pulp and paper plants (Germain et al. 2000). The modelling provided results in the pH range of 6.56 to 8.38 and therefore the dissolved monomeric aluminate ion, $Al(OH)_4^-$, would be the predominant aluminum species present (see Figure 2.1). As indicated in Section 2.4.1, dissolved inorganic monomeric aluminum is considered to have the highest bioavailability to aquatic species and to present the greatest risk of adverse effects to pelagic organisms. The level of dissolved inorganic monomeric form of aluminum was calculated, using aluminum levels estimated in effluents (Fortin and Campbell 1999) and assuming a 1:10 dilution. For the DWTPs considered, average concentrations of dissolved inorganic monomeric forms of aluminum (which are assumed to be the bioavailable forms) at saturation varied from 0.027 to 0.348 mg/L during backwash events, assuming that microcrystalline gibbsite is controlling the aluminum solubility. According to Hem and Robertson (1967), microcrystalline gibbsite controls aluminum solubility at pH values of less than 7, while the precipitate formed when the pH of water is in the 7.5–9.5 range has a solubility similar to that of boehmite. This precipitate will evolve to bayerite, a more stable and insoluble form of aluminum hydroxide, within a week. If it

is assumed that boehmite is controlling the solubility, dissolved aluminum levels would be lower, ranging from 0.005 to 0.059 mg/L (Fortin and Campbell 1999). For the two pulp and paper mills considered, the dissolved aluminum values were among the lowest, whatever form is controlling the aluminum solubility.

The calculated dissolved aluminum concentration of 0.348 mg/L represents the saturation concentration, assuming that microcrystalline gibbsite controls solubility when aluminum salts are used to treat drinking water. This value was calculated for a location in the Canadian Prairies, where the pH of receiving waters (8.38) and solubility were the highest of all sites examined (Fortin and Campbell 1999). Backwash events can be considered to last for about 30 minutes and occur every 48 to 72 hours for each filter at a DWTP (Environment Canada and Health Canada 2000). If it is assumed that most DWTPs have about 20 filters (small DWTPs have fewer filters), it is estimated that concentrations in receiving waters near the point of discharge could be as high as 0.348 mg/L as much as 10% of the time. The rest of the time, aluminum concentrations would approach background values, which, for locations on the Prairies, are likely on average to be about 0.022 mg/L as monomeric inorganic aluminum (Environment Canada and Health Canada 2000). The temporally weighted concentration of dissolved monomeric aluminum at this location averaged over a period of several days would therefore be about 0.055 mg/L. This concentration was taken as a conservative (reasonable worst-case) Predicted Environmental Concentration (PEC) for waters close to discharge points.

Because aluminum releases reported by DWTPs occur in circumneutral to neutral waters, two Critical Toxicity Values (CTVs) corresponding to the pH of waters where releases occur could be chosen. The work of Neville (1985) provides a NOEC of 0.075 mg/L as inorganic monomeric aluminum, based on the absence of deleterious effects on ventilation and respiratory activity of rainbow trout at pH 6.5. This CTV is considered valid for the pH range 6.5–8.0. A second CTV for alkaline conditions (pH > 8.0) is based on the work of Gundersen et al. (1994), who determined similar LC_{50}s (~ 0.6 mg dissolved Al/L) during several experiments in the pH range 8.0–8.6 and water hardness range 20 to 100 mg/L (as calcium carbonate). A NOEC for mortality of 0.06 mg dissolved Al/L can be derived for rainbow trout from data given for one of the 16-day exposures at 20 mg/L hardness and pH 8.0. The chemical concentrations in Gundersen et al. (1994) are expressed as “total” and “dissolved” aluminum; there was, unfortunately, no attempt to identify the forms of dissolved aluminum present. At the experimental pH, it is probable that a good proportion of the dissolved aluminum was the monomeric aluminate ion as the predominant species.Since the pH in waters for which the PEC was estimated is 8.38, the corresponding CTV is 0.06 mg/L as dissolved inorganic monomeric aluminum.

It is possible that effects may be elicited at concentrations below that of the selected CTV of 0.06 mg/L. Wold et al. (2005) reported a 21-day LOEC for reduced survival and reproduction in *Daphnia pulex* at a lowest test concentration of 0.05 mg/L. Testing was conducted at a pH of 7 ± 1, suggesting that the observed effects were due to the presence of aluminum hydroxide rather than the dissolved inorganic monomeric aluminum that is usually associated with toxicity. Recent studies (e.g., Verbost et al. 1995; Kádár et al. 2002; Alexopoulos et al. 2003) provide evidence that the particulate and/or colloidal forms of aluminum, such as may be present under the transition conditions of mixing zones, are

bioavailable and can exert adverse effects on organisms. Impaired oxygen consumption, gill damage, and reduced feeding behaviour have been reported in aquatic invertebrates and fish present in waters containing freshly neutralized aluminum (i.e., aluminum in transition from ionic species to polymers or precipitating hydroxides), although it is not clear whether these effects result from physical damage to structures such as the gills, or from direct chemical toxicity. Therefore, while there may be circumstances or conditions under which particulate and colloidal forms of aluminum can exert adverse effects on aquatic organisms, these conditions are likely to be localized and/or transitory in nature, and the selected CTV of 0.06 mg/L, based on the inorganic monomeric form, is considered sufficiently representative of the overall potential for adverse impacts in aquatic species.

In determining Predicted No-Effect Concentrations (PNECs) for aluminum, the nature of the biological response was considered, since some organisms respond to a narrow aluminum concentration range. This results in an abrupt "threshold" where an evident biological response occurs, with no observable effects at slightly lower concentrations (Hutchinson et al. 1987; Roy and Campbell 1995). Consequently, since the CTV chosen is a NOEC, the application factor used to derive a PNEC from the CTV was 1. Aluminum being a natural element, it is also useful to consider whether the PNEC is within the range of natural background concentrations. Although based on limited data, on an overall basis, the 90th-percentile value for dissolved aluminum at sampling stations located upstream of points of discharge of aluminum salts is 0.06 mg/L (Germain et al. 2000). It should be noted that only a portion of this dissolved aluminum is in inorganic monomeric forms (corresponding to the PNEC). Thus, the 90th-percentile value for inorganic monomeric aluminum in uncontaminated water is expected to be less than 0.06 mg/L.

The reasonable worst-case quotient for receiving water can therefore be calculated as follows:

$$\text{Quotient} = \frac{\text{PEC}}{\text{PNEC}}$$

$$= \frac{0.055 \text{ mg/L}}{0.06 \text{ mg/L}}$$

$$= 0.92$$

Since this conservative quotient is relatively close to 1, it is helpful to consider further the likelihood of biota being exposed to such concentrations in Canada.

It is likely that chemical equilibrium modelling overestimates inorganic forms of aluminum in solution, since it appears to overestimate dissolved aluminum. One reason for the overestimate is that a very large fraction of the aluminum released from DWTPs during backwash events is most probably in solid form, while calculations used to estimate the PEC assumed that all of the aluminum was in dissolved form (Germain et al. 2000). Although the modelling assumed that saturation was achieved instantly, this "solid" aluminum may take a relatively long time to dissolve such that aluminum levels in receiving waters do achieve saturation. In fact most of the aluminum solids released are expected to settle relatively

quickly to bottom sediment. Dissolved concentrations may also be overestimated because of the assumption that the solubility of aluminum is controlled by microcrystalline gibbsite. Based on limited data on concentrations of dissolved aluminum at different treatment steps at one Canadian DWTP, solubility may be controlled by less soluble forms of aluminum hydroxide, such as boehmite (Fortin and Campbell 1999).

The possibility that modelled concentrations overestimate actual values is further supported by data for two sites on the North Saskatchewan River, where the dissolved inorganic aluminum concentrations predicted by modelling are 0.110 and 0.099 mg/L, while the measured concentrations at these sites are 0.005 and 0.010 mg/L (Roy 1999b).

Srinivasan et al. (1998) studied the speciation of aluminum at six different stages of water treatment at Calgary's DWTP. The total aluminum concentration ranged from 0.038 to 5.760 mg/L, and the dissolved inorganic aluminum concentration varied from 0.002 to 0.013 mg/L. George et al. (1991) measured < 0.06 mg monomeric Al/L in alum sludge from ten different DWTPs containing up to 2,900 mg total Al/L. These results show that the concentration of dissolved aluminum in process wastewaters is less than the PNEC.

Finally, while the potential for aluminum to influence the cycling and availability of phosphorus and other trace elements in aquatic systems is recognized (see Section 2.3.1; Environmental Fate), no empirical data were found to suggest the occurrence of this process in Canadian surface waters and, in particular, as a result of aluminum released from the three aluminum salts that are the subject of this assessment. For this reason, the potential for risk from this source will not be evaluated further here.

3.1.1.1.2 Benthic

Acute toxicity to benthic and pelagic organisms resulting from exposure to potentially high concentrations of aluminum in aluminum-based sludge is unlikely, because of the solubility constraints in receiving waters discussed above. Filtrates obtained from alum sludge were toxic to freshwater algae in waters with low pH (less than 6) or low hardness (less than 35 mg/L $CaCO_3$/L); however, the available information indicates these conditions are not prevalent in Canadian waters that receive large inputs of aluminum from the three aluminum salts being assessed. AEC (1987) determined that aluminum was effectively bound to sludge within the pH range of 4.5 to 10.0, with less than 0.02% of the total aluminum released in waterwaters dissolved in the liquid phase associated with the sludge.

Hall and Hall (1989) reported delayed and reduced reproduction in *Ceriodaphnia dubia* following exposure to undiluted alum sludge effluent, suggesting that sublethal effects may be possible in the environment. However, effluent dilution occurs immediately upon release into a receiving water body. In addition, any observed ecosystem impacts would be difficult to link directly to the presence of aluminum given the potentially large number of contaminants that may also be present in the sludge.

There is evidence that aluminum sludge released from DWTPs can deposit and form a blanket over sediments in rivers with slow water velocity, and macroinvertebrate populations may be stressed due to a lack of oxygen and carbon sources on which to feed. For this reason,

George et al. (1991) recommended that sludge be discharged during periods of fast water movement as this may be less detrimental to primary producers and benthic communities. AEC (1984) reported smothering effects related to settled sludge on sediments following disposal to rivers in Alberta may occur but concluded that while there is potential for adverse impacts resulting from the deposition of alum sludge in receiving waters, further research is needed. The study recommended alternative treatment and disposal methods for alum sludge be considered, including reduction in the quantities produced through substitution with alternative coagulants, routing of the sludge through sanitary sewer, lagooning, and landfilling or land application.

The City of Ottawa (2002) found depressed abundance of benthic organisms downstream from the Britannia DWTP up to 1,500 m from the discharge site compared to upstream sampling sites. Areas of sediment with an appearance similar to depositons at the outfall from the Britannia DWTP and with higher levels of aluminum were found 1,500 m downstream from the outfall while sampling sites closer to the discharge did not exhibit such strong similarities, and had lower concentrations of aluminum which approached aluminum concentrations found in the sediment 150 m upstream from the discharge. This study thus showed that sludge sediment from the the Britannia DWTP can travel to distant locations from the point of discharge where deposition may occur due to site specific hydrological characteristics. In this study, it was also unclear whether the identified impacts were a result of the physical composition of the sediments (e.g., grain size), on-going blanketing of the area, and/or toxicity of dissolved aluminum leaching out of sediment and into the water column.

In their environmental risk assessment guidance document for metals, ICMM (2007) indicate that trace metals discharged into aquatic ecosystems are most likely to be scavenged by particles and removed to sediments. Once associated with surface sediments, the metals are subjected to many types of transformation reactions, including formation of secondary minerals, and binding to various sediment fractions (e.g., sulphides, organic carbon, iron hydroxides). For this reason, it may be difficult to establish clear relationships between measured concentrations of a metal in sediment and the potential for impacts to benthic organisms.

Overall, the greatest potential for risk to the benthic environment resulting from the release of aluminum-based effluents and sludges likely relates to the physical effects of blanketing and smothering of benthic communities in the vicinity of the outfall. While this impact does not constitute direct aluminum toxicity, the presence of aluminum coagulants and flocculants in water treatment processes results in the formation of substantial quantities of sludge, which may then be released into the environment. It is reasonable to expect that physical impairment of bethic populations would not be limited to alumimum coagulants sludge, but could also result from any other chemical coagulant used for the treatment of drinking water. However although the potential for local impacts to benthic organisms exists, there are relatively few reports of such damage.

In recognition of the potential for adverse ecosystem effects, many Provinces have implemented strategies designed to reduce or eliminate the release of water treatment plant effluents and sludges to receiving water bodies (see Section 2.2.2). It is expected that addressing issues relating to overall effluent and sludge concerns, most notably the extremely

high levels of total suspended solids (TSS) should also effectively deal with physical and chemical aspects of aluminum sludge toxicity in the aquatic receiving environment.

3.1.1.2 Terrestrial organisms

Terrestrial organisms are exposed to added aluminum when alum sludge from water treatment facilities, primarily MWWTPs, is applied to agricultural soils.

The lowest level of dissolved aluminum reported to adversely affect terrestrial organisms is 0.135 mg/L, which can reduce root and seedling growth in sensitive grain and forage crops. This concentration was therefore selected as the CTV, assuming that most of the dissolved aluminum was in inorganic monomeric forms. Considering that this CTV was derived from experiments using solution cultures, the effects data on which the CTV is based could overestimate the sensitivity of crops grown in soils in the field. Because of that, the fact that many species were affected at the same low level and the fact that aluminum is naturally present in soil, an application factor of 1 was applied to the CTV to derive the PNEC. The conservative PNEC for soil-dwelling organisms is therefore 0.135 mg dissolved monomeric Al/L.

No data were identified on concentrations of dissolved aluminum in soils that have received applications of alum sludge. However, as was noted in section 2.2.2.2, spreading on agricultural land is permitted in Canada only when the pH is greater than 6.0 or when liming and fertilization (if necessary) are done. Thus, the pH of receiving soils will likely be in the circumneutral range, where the solubility of aluminum is at a minimum. Based on results of equilibrium modelling, with the total dissolved aluminum concentrations being controlled by the precipitation of microcrystalline gibbsite, total dissolved aluminum concentrations would not exceed the PNEC unless soil pHs were less than about 5.1 (Bélanger et al. 1999). Because it is very unlikely that the pH of soils receiving alum sludge applications will be this low, it is very unlikely that the PNEC of 0.135 mg/L is exceeded in Canadian soils receiving such applications. In addition, while a shift in soil pH at the site of sludge application could mobilize the aluminum present in the sludge, the events causing such a shift (e.g., storm events) and the resulting impacts are likely to be local and transitory in nature.

The expectation that the solubility and hence bioavailability of aluminum in sludges applied to agricultural soils will be extremely limited is supported by data on aluminum levels in plants growing on such soils. For example, aluminum in yellow mustard seed (*Sinapsis alba*) and Durum wheat seed (*Triticum turgidum* var. *durum*) collected from plants grown in soil amended with alum sludge from Regina's DWTP were found to be not statistically different from those of seeds collected in control plots (Bergman and Boots 1997).

Finally, although it has been noted that aluminum in the sludge can fix labile phosphorus by forming stable aluminum-phosphorus complexes and hence make it unavailable to plants, causing deficiencies (Jonasson 1996; Cox et al. 1997), this is unlikely to occur when soil receiving sludge is also fertilized as required in Canada.

3.1.2 Other lines of evidence relating to aluminum salts

Trends in production and use

An apparent increase in production and use of aluminum salts occurred over the period 1995 to 2000; however, from 2000 to 2006, user demand remained relatively constant and the total amount of aluminum contained in the salts (i.e., aluminum chloride, aluminum nitrate, aluminum sulphate, PAC, PASS, ACH and sodium aluminate), and therefore available for release to the Canadian environment, appeared stable at around 16,000 tonnes per year (Cheminfo Services Inc. 2008). Water treatment applications continued to be the primary consumer of sulphate and chloride salts in the years following publication of the original State of the Science report (Environment Canada and Health Canada 2000), with lesser quantities used in the pulp and paper sector.

Despite the proportionally higher demand for aluminum sulphate in comparison with the other aluminum salts (86% of the total demand in 2006), aluminum producers reported declining use of alum (and sodium aluminate) over the period 2000 to 2006, with increased use of other aluminum-based products, such as polyaluminum chloride (PAC), aluminum chlorohydrate (ACH) and polyaluminum silicate sulphate (PASS), as well as non-aluminum products such as iron salts. PAC and iron chlorides were increasingly used as substitute coagulants/flocculants for alum in drinking water treatment, the former substance for its superior settling properties in colder water temperatures and the latter due to awareness of residual aluminum issues and superior performance in floc settling and dewatering of sludge (Cheminfo Services Inc. 1008). PAC is also particularly effective at water treatment facilities experiencing large fluctuations in water temperature, turbidity, pH and alkalinity. ACH, which is a highly concentrated and highly charged type of PAC, is sometimes used preferentially over alum because of its better buffering capacity, and PASS is very effective at removing phosphorus in cold waters with lower dosing rates and less sensitivity to variable conditions of alkalinity, pH, temperature and suspended solids (Cheminfo Services Inc. 2008). Physical process changes, such as conversion from acid to alkaline paper-making, have also contributed to reduced demand for alum.

Trends in sources and releases to the environment

No evidence of significant new sources of aluminum derived from the three salts that are the subject of this assessment has been identified.

Data provided in the study by Cheminfo Services Inc. (2008) indicated that while a slight decrease in Canadian consumption of aluminum salts occurred over the period from 2000 to 2006, the total amount of aluminum contained in these salts remained virtually unchanged, and this suggests that overall concentrations and total entry of aluminum into the environment have remained relatively constant.

Information collected since the publication of Environment Canada and Health Canada (2000) indicates that primary exposure routes for aluminum derived from the three salts have also remained unchanged. For drinking water treatment, releases are primarily to surface

waters, with lesser proportions of aluminum released to sewer for subsequent wastewater treatment or present in sludge that is directed to landfill. While low levels of aluminum have been measured in final effluents leaving municipal wastewater treatment plants, the majority of the metal appears to remain within sludge which is then transferred to landfill or processed for landfarming. Releases related to industrial applications have decreased in recent years, largely due to lower aluminum use in the pulp and paper sector and therefore lower quantities entering receiving waters from industrial treatment plants and reduced quantities sent to landfill in paper products (Cheminfo Services Inc. 2008).

3.1.3 Sources of uncertainty

There are a number of uncertainties in this risk characterization. Regarding effects of aluminum on pelagic organisms, there are only a few acceptable studies conducted at circumneutral pH (6.5–8.0), conditions similar to those of aquatic environments receiving releases from DWTPs. There are also uncertainties associated with the decision to use an application factor of 1 to derive a PNEC for pelagic organisms, a choice that was made considering concentrations of aluminum in uncontaminated waters and the biological response of organisms to a narrow concentration range, resulting in an abrupt "threshold" where biological response occurs.

There are uncertainties associated with levels of aluminum released by DWTPs and with the levels and form of aluminum present in the aquatic environment. The use of the MINEQL+ and WHAM models provided aluminum results higher than those measured in the receiving environments when calculations were done assuming that aluminum solubility is controlled by microcrystalline gibbsite. When calculations were done with the boehmite form of aluminum hydroxide, levels were much lower than what was calculated with the microcrystalline gibbsite form (Fortin and Campbell 1999). Direct measurement and determination of aluminum speciation in final effluents from water treatment plants would confirm the estimated levels and forms provided by MINEQL+ and WHAM models.

Other uncertainties exist relating to the impact of aluminum sludge releases on benthic organisms. There are some indications that sludge releases, whatever the coagulant or flocculant used, may have a smothering effect on benthos. In recognition of the potential for adverse ecosystem effects, many Provinces have implemented strategies designed to reduce or eliminate the release of water treatment plant effluents and sludges to receiving water bodies (see Section 2.2.2). It is expected that addressing issues relating to overall effluent and sludge concerns, most notably the extremely high levels of total suspended solids (TSS) should also effectively deal with physical and chemical aspects of aluminum sludge toxicity in the aquatic receiving environment.

In relation to terrestrial organisms, there are uncertainties associated with the limited data available for effects on soil-dwelling organisms other than plants. The lack of information on aluminum levels in pore waters of soils receiving applications of alum sludge is not considered critical, since these levels are constrained by theoretical limits on solubility that are below the PNEC for sensitive vegetation.

3.2 CEPA 1999 64(c): Human health

3.2.1 Estimated population exposure

The average daily intake of aluminum in six age groups in Canada is estimated on the basis of concentrations measured in: (a) indoor and outdoor air (section 2.3.2.1); (b) drinking water (section 2.3.2.2.2); (c) soil (section 2.3.2.4); and (d) food (section 2.3.2.6). Table 3.1 shows the overall estimate of average daily intakes by age group and different environmental media (water, indoor air, outdoor (ambient) air, soil, and food and beverages) for total aluminum. Total aluminum was considered, instead of the three specified salts, as concentrations of aluminum in foods, soil, drinking water, and air are generally reported as total aluminum, and not in terms of specific salts.

The average daily intake values were derived using a deterministic exposure assessment, which provides a single point estimate of intake (in this case and estimate of the mean). Probabilistic exposure assessments, on the other hand, provide information on the full range of possible intakes in the study population, and may, as well, give a more accurate estimate of mean exposure. The potential influence of a probabilistic analysis on the current assessment, with regard to the daily total aluminum intake in food, is discussed in more detail in section 3.2.1.4.

Consideration of the environmental media—drinking water, air, soil and food—in the derivation of the average daily intake is consistent with other assessments of priority substances. Daily intake of other sources of aluminum (e.g., antacids, vaccines and cosmetics) is difficult to quantify for the general Canadian population, both because of the limited data on exposure and absorption, and the variability in usage within the population. Therefore, these sources were not included in the estimation of the average daily intake. All of these additional sources may however, constitute non-negligible exposures to aluminum, and should be considered in the qualitative evaluation of uncertainty associated with the estimate of the average daily intake.

3.2.1.1 Air

3.2.1.1.1 Estimated average daily intake of total aluminum in outdoor air

The estimated average daily intake of total aluminum in airborne particles in outdoor air was determined using more than 10,000 measurements taken over the past ten years at some 50 sites in Canada. The average provincial/territorital total aluminum concentration of 0.17 $\mu g/m^3$ in PM_{10} in Canada was used in the daily intake estimate (section 2.3.2.1.1). By age group, average daily intakes for PM_{10} were very low, ranging from 0.03 µg/kg bw/d for seniors to 0.1 µg/kg bw/d for young children aged six months to four years old.

3.2.1.1.2 Estimated average daily intake of total aluminum in indoor air

In the case of indoor air, only measurements conducted on PM_{10} samples were evaluated to estimate intake since the concentration of aluminum in $PM_{2.5}$ was often below the detection limit. The concentration based on the average daytime and nighttime concentrations of total aluminum is estimated to be 1.49 $\mu g/m^3$ (section 2.3.2.1.2). The estimated average daily intake from indoor air is therefore higher than that from outdoor air, ranging from

0.3 μg/kg bw/d in adults and seniors to 0.8 μg/kg bw/d in young children aged six months to four years old.

3.2.1.2 Water

On the basis of data provided by municipal drinking water treatment plants from across Canada (section 2.3.2.2.2), the mean total aluminum concentration was estimated to be 101 μg/L. This estimate applies to plants that use coagulant/flocculents containing aluminum salts and secure their water supply from surface water sources. The average daily intake for each age group ranged from 2.0 μg/kg bw/d for adolescents and adults to 10.8 μg/kg bw/d for non-breastfed infants.

3.2.1.3 Soil

The mean total aluminum concentration in soil of approximately 41,000 mg/kg (section 2.3.2.4) was used to estimate the exposure of the Canadian population via soil. The average daily intake of aluminum from soil among infants was 166 μg/kg bw/d, and significantly higher in young children aged six months to four years old, who were found to have an estimated average daily intake of 268 μg/kg bw/d. For the other groups, the average daily intakes of total aluminum are progressively lower from 87 μg/kg bw/d for children aged 5 to 11 years old to 17 μg/kg bw/d for seniors.

3.2.1.4 Foods

For each age group defined in the Canadian population, the estimated mean dietary intake of total aluminum was derived using the fifth Total Diet Study completed in 2000–2002 (Dabeka 2007). Daily intakes of aluminum from food and beverages are presented in Table 3.1. For breastfed infants aged zero to six months old, the exposure to aluminum from human milk was approximately 12 μg/kg bw/d, whereas an intake of 85 μg/kg bw/d was calculated in non-breastfed infants. Among young children aged six months to four years old, the estimated mean daily intake from food was approximately 268 μg/kg bw/d. In the other groups, the mean daily intake of total aluminum ranged from 341 μg/kg bw/d in children aged 5 to 11 years old to 113 μg/kg bw/d in adults over 60 years old.

The above mean intake values of total aluminum in food were derived using a deterministic exposure assessment, which provides a single point estimate of intake but does not provide information about the full range of possible exposures within a population. The deterministic approach in this case is expected to overestimate mean estimates of exposure, in part because the aggregation of food categories inflates the contribution of less frequently consumed foods having higher levels of contamination. Further, the deterministic assessment does not take into account the day-to-day variability in the types of foods consumed by individuals.

Probabilistic exposure assessments estimate the probability of a given exposure in a population. The distribution of intakes that is generated provides more information about the full range of possible intakes in that population. Such statistical modelling can also account for intra- and interindividual variability in eating behaviours. As such, probabilistic exposure assessments, when the datasets are available to allow such assessments, are considered to provide a more accurate picture of exposure than deterministic exposure assessments.

3.2.1.5 Overall estimate of exposure in the Canadian population

The estimated mean daily intake of total aluminum was lower in breastfed than in non - breastfed infants, with levels of 179 and 262 μg/kg bw/d, respectively. The highest EDI of total aluminum was found in young children aged six months to four years old with 541 μg/kg bw/d, whereas for other age groups this intake decreased significantly to 432 μg/kg bw/d in children aged 5 to 11 years old, 293 μg/kg bw/d in adolescents, 163 μg/kg bw/d in adults aged 20 to 59 years old and finally 133 μg/kg bw/d in adults over 60 years old.

The contribution from various environmental media was evaluated for each of the age groups (Table 3.2). In young children aged six months to four years, approximately 50% of the aluminum intake was from food, 50% from ingestion of soil, and less than 1% from the ingestion of drinking water and inhaled particles. The contribution from the ingestion of food increased in the other age groups to 80% or more, whereas the contribution from soil decreased with age to 20% in children aged 5 to 11 years old and approximately 10% in the older age groups. The contribution from the ingestion of drinking water and inhaled particles is very low, at less than 2% or 0.2%, respectively for all age groups other than infants.

In infants, for the exclusively breastfed group, more than 90% of the total aluminum intake was found to be from the ingestion of soil and approximately 7% from the ingestion of human milk. For those infants who consumed infant formula and different food groups and beverages, approximately 30% of total aluminum intake was from the ingestion of food and about 63% from the ingestion of soil.[25]

With respect to the three salts—aluminum chloride, aluminum nitrate, and aluminum sulphate—the only media in which the mean concentration is significantly affected by these the use of these salts is drinking water, in which aluminum sulphate or aluminum chloride may be added during the treatment process. While aluminum sulphate is permitted as an additive in some food products, this use is infrequent and would be expected to have a very minor influence on the total aluminum intake from food. The question of the relative contribution of the three salts to overall exposure to aluminum is discussed in more detail in section 3.2.4.

For those who regularly use aluminum-containing over-the-counter oral therapeutic products (e.g., pharmaceuticals such as antacids), these products represent the major source of daily aluminum intake. Based on the manufacturers' maximum recommended daily doses, EDIs of aluminum from these products may reach approximately 31,000 μg/kg bw/d. However, these are not generally the three salts considered in this assessment.

[25] Soil would most likely be in the form of household dust for this age group.

Table 3.1 Estimated mean daily intake of total aluminum based on Canadian data

Source of exposure	Estimated mean daily intake of total aluminum (µg/kg bw/d)						
	Infants[1] (0–6 months)		Toddlers[2] (0.5–4 years)	Children[3] (5–11 years)	Teens[4] (12–19 years)	Adults[5] (20–59 years)	Seniors[6] (> 60 years)
	Breastfed (exclusively)	Non-breastfed					
Drinking water[7]	0	10.8	4.57	3.59	2.04	2.14	2.25
Food and beverages[8]	12.2	85.0	268	341	270	143	113
Ambient air[9]	0.05		0.1	0.08	0.05	0.04	0.03
Indoor air[10]	0.37		0.78	0.61	0.35	0.30	0.26
Soils[11]	166		268	87	21	18	17
TOTAL	179	262	541	432	293	163	133

[1] Assumed to weigh 7.5 kg, to breathe 2.1 m^3 of air per day, to drink 0.8 L of water per day (non breastfed) or 0 L of water per day (breastfed), and to ingest 30 mg of soil per day (Health Canada 1998a).
[2] Assumed to weigh 15.5 kg, to breathe 9.3 m^3 of air per day, to drink 0.7 L of water per day and to ingest 100 mg of soil per day (Health Canada 1998a).
[3] Assumed to weigh 31.0 kg, to breathe 14.5 m^3 of air per day, to drink 1.1 L of water per day and to ingest 65 mg of soil per day (Health Canada 1998a).
[4] Assumed to weigh 59.4 kg, to breathe 15.8 m^3 of air per day, to drink 1.2 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998a).
[5] Assumed to weigh 70.9 kg, to breathe 16.2 m^3 of air per day, to drink 1.5 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998a).
[6] Assumed to weigh 72.0 kg, to breathe 14.3 m^3 of air per day, to drink 1.6 L of water per day and to ingest 30 mg of soil per day (Health Canada 1998a).
[7] Based on the mean total aluminum concentration from all the drinking water treatment plants in Canada, estimated to be 101.16 µg/L (see section 2.3.2.2.2).
[8] Based on dietary intake data from the fifth partial Total Diet Study in Canada (Dabeka 2007; see section 2.3.2.6). Data were adjusted for age categories from Health Canada (1998a). For breastfed infants, mean breast milk aluminum concentration of 0.11 mg/kg (section 2.3.2.6) was used, with a human milk density of 1.03 kg/L and an ingestion rate of 0.8 L/d.
[9] Based on the mean concentration of total aluminum for all Canadian data in ambient air between 1986 and 2006, which is 0.17 µg/m^3 in PM_{10} (see section 2.3.2.1.1).
[10] Based on average daytime and nighttime concentrations of all Canadian data in indoor air for total aluminum, which is about 1.49 µg/m^3 (see section 2.3.2.1.2).
[11] Based on the mean concentration of total aluminum of 41,475 mg/kg measured in soils and sediments on the entire Canadian territory (see section 2.3.2.4).

Table 3.2 Contribution (%) of each source of exposure based on Canadian mean daily intake of total aluminum

Source of exposure	**Contribution (%) of each source of exposure**						
	Infants (0–6 months)		Toddlers (0.5–4 years)	Children (5–11 years)	Teens (12–19 years)	Adults (20–59 years)	Seniors (> 60 years)
	Breastfed (exclusively)	Non breastfed					
Drinking water	0.00	4.1	0.84	0.83	0.70	1.31	1.69
Food and beverages	6.80	32.4	49.5	78.9	92.2	87.7	85.0
Ambient air	0.030	0.02	0.02	0.02	0.02	0.02	0.02
Indoor air	0.21	0.14	0.14	0.14	0.12	0.18	0.20
Soils	92.7	63.4	49.5	20.1	7.17	11.0	12.8
TOTAL	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

3.2.2 Hazard characterization

The discussion in this section focuses on the broad characterization of the types of effects of concern for the human health risk assessment of aluminum, on the basis of both human and experimental animal data. The suitability of the different sources of data for the exposure-response analysis, presented in section 3.2.3, is evaluated as well.

3.2.2.1 Effects in humans

The epidemiological data on aluminum exposure in drinking water were not used in this assessment for developing the dose-response relationship (see section 3.2.3), because of the lack of evidence for a causal relationship between aluminum in drinking water and AD, and the lack of data on total exposure to aluminum, for which food is the predominant contributor. Nonetheless, the observed associations in some studies between aluminum in drinking water and the development of AD do support further consideration of neurotoxicity as an endpoint of concern in the human health risk assessment for aluminum.

Aluminum has been shown to produce neurotoxic effects in humans as well as bone and blood toxicity, during medical treatment in which the gastrointestinal barrier is bypassed (e.g., aluminum-induced encephalopathy through dialysis treatment in patients with renal failure). There is also some epidemiological evidence for long-term cognitive impairment, in pre-term infants receiving aluminum-containing nutritional solution intravenously, and associated with occupational exposures, as discussed in section 2.4.3.1. These exposure conditions are not applicable to the general population, particularly as the exposure to aluminum generally does not occur via ingestion, and therefore human studies have not been used as a basis for characterizing the dose-response relationship for environmental exposures (see section 3.2.3). However, this evidence does support the identification of neurotoxicity and developmental neurotoxicity as endpoints of concern in the human health risk assessment for aluminum.

With respect to the conditions of exposure in the general population, the most relevant available information is provided by the epidemiological investigations into the association between exposure to aluminum through drinking water and AD and other forms of dementia (see section 2.4.3.2). The use of these findings for first identifying an endpoint of concern (i.e., hazard identification), and then for evaluating the exposure-response relationship is discussed below.

The hypothesis of aluminum in drinking water as a risk factor for AD or impaired cognitive function in the elderly is controversial in the scientific community, and has important implications for public health. Hence, it is important to evaluate in detail the weight of evidence for the observed associations, in the context of traditional criteria for causality. This evaluation, for studies published prior to 1998 is presented in the Guidelines for Canadian Drinking Water Quality - Technical Documents: Aluminum (Health Canada 1998b) and in the SOS report (Environment Canada and Health Canada 2000). In the SOS report the criteria of consistency and specificity, strength, dose-response, temporality, biological plausibility, and coherence of the observed association were evaluated, and the conclusion was as follows:

"Overall ... the weight of evidence for causality for the observed associations between aluminum and Alzheimer's disease is weak, at best. There is only limited consistency in the results of the analytical epidemiological studies. While the criteria for diagnosis were generally more stringent in the studies in which there was a positive outcome, there was more consistent control of potential confounding factors in the studies in which no associations were reported. Moreover, while there is some evidence of exposure-response in the individual available studies for the reported association between aluminum and Alzheimer's disease, there is little consistency in results among the different investigations in this respect, at least based on the limited extent of comparison permitted by the available data. There are also limited data to serve as a basis of the extent to which the observed association between aluminum and Alzheimer's disease meets the criterion of temporality. Most limiting, however, in the assessment of the weight of evidence for causality of the observed association is the lack of relevant data on biological plausibility; indeed, there is no hypothesized plausible pathway from exposure to effect with measurable key events, for which sufficient investigation has been conducted to assess weight of evidence against traditional criteria of causality, such as consistency, strength, specificity, dose-response, temporal patterns, biological plausibility and coherence."

Since the publication of the SOS report, a significant positive association between AD and aluminum in drinking water has been observed in the additional analysis of the data from the PAQUID cohort in southwestern France (Rondeau et al. 2000; Rondeau et al. 2001, as described in section 2.4.3.2). While the exposure assessment in this cohort study is improved in relation to previous case-control studies, it is still limited by two factors: the quantification of the aluminum exposure of individuals from other dietary sources and the relatively narrow range of aluminum exposure in the population studied.

Recent reviews of the epidemiological literature have reiterated the limitations of the epidemiological data base, in its entirety, in regard to the causality of the occurrence of aluminum in the environment and AD, while also maintaining that the hypothesis cannot be rejected at this time (InVS-Afssa-Afssaps 2003; ATSDR 2006; JECFA 2006; Krewski et al. 2007). As a result of these limitations, JECFA (2006) and ATSDR (2006) chose not to base their regulatory values for aluminum intake on epidemiological studies.

3.2.2.2 Effects in experimental animals

The scientific community has primarily focused its investigations of aluminum toxicity on the endpoints of neurotoxicity and reproductive/developmental toxicity, principally because of the evidence from human case studies and epidemiological studies indicating that these effects may be of concern. A total of 138 toxicological studies, published from 1979 to 2007, reporting on neurotoxicity and reproductive/developmental effects of oral aluminum exposure in rodents, monkeys and dogs, have been evaluated for the present assessment.

The observations of the toxic effects of aluminum may be influenced by dose, aluminum salt, dosing regimen and exposure media as well as animal species and strain, age,

sex, and health status. Considering the database evaluated for this assessment, the different studies vary with respect to all of these factors, and with respect to the specific endpoints investigated. Moreover, the majority of studies compare animals exposed at a single dose to a control group. In these single-dose studies, the dose corresponding to a lowest observed effect level (LOEL) or to a no observed effect level (NOEL) is strongly influenced by the researcher's choice of administered dose.

In 2000, in its SOS report, Health Canada summarized the experimental database on aluminum toxicity as follows (Environment Canada and Health Canada 2000):

> *"Altered performance in a variety of neurobehavioural tests and pathological and biochemical changes to the brain have been observed in studies of the oral administration (i.e., drinking water, diet, gavage) of aluminum salts to mice, rats and monkeys for varying periods of time as adults or during gestation, weaning and/or post-weaning. Interpretation of the results of a number of these studies is limited by designs that focus on testing specific hypotheses rather than examination of a range of neurotoxicity endpoints, the administration of single doses or a lack of an observed dose–response, lack of information on concentrations of aluminum or bioavailability from basal diets, the use of specific ligands to enhance accumulation of aluminum and small group sizes. Indeed, there have been no studies in which a broad range of neurological endpoints (biochemical, behavioural and histopathological) have been investigated in a protocol including multiple dose groups."*

Since 2000 the database for neurological and reproductive/developmental endpoints has been considerably expanded. Yet the same limitations apply, most notably in regard to an emphasis on testing specific hypotheses rather than examining a range of neurotoxicity endpoints, testing of single doses or lack of an observed dose-response relationship, and small group sizes. There is no single study that has investigated multiple dose groups for a broad range of neurological endpoints.[26]

The database does, however, provide a broad range of studies carried out by researchers from many different laboratories. Considered in its entirety, it gives evidence for neurological, neurodevelopmental and reproductive toxicity in experimental animals, including motor (e.g., rotarod test and grip strength), sensory (e.g., auditory startle) and cognitive effects (e.g., maze learning and passive avoidance tests) as well as neuropathological (e.g., neuronal degeneration), and biochemical changes (e.g., alterations in energy metabolism, trace element tissue concentrations and neurotransmission systems).

While no single or limited number of studies provides an adequate basis for characterizing the dose-response relationship, consideration of the database, as a whole, does

[26] A good laboratory practice (GLP) study generally following OECD and U.S. EPA Developmental Neurotoxicity guidelines, commissioned by a consortium of aluminum salt producers, is currently underway. The results, however, will not be available before mid-2009.

provide a basis for approximately determining the lower range of doses at which researchers have repeatedly observed statistically significant changes in neurological, neurodevelopmental and/or reproductive endpoints in experimental animals orally exposed to aluminum salts.

3.2.3 Exposure-response analysis

The objective of the exposure-response analysis was to identify the lower range of doses for which oral exposure to aluminum has been shown to produce toxicologically significant effects in multiple studies.

In order to characterize the lower range of doses at which oral exposure to aluminum produces effects in experimental animals, two subsets of the studies, based primarily on exposure period, were evaluated: (a) neurotoxic effects in adults following subchronic or chronic exposure (greater than 90 days); and (b) neurodevelopmental and reproductive effects in prenatal/lactation exposure studies. The studies included in these subsets are briefly described in Tables C1 and C2 (Appendix C). These two exposure periods were considered to be of greatest relevance to the evaluation of risks from long-term exposure to aluminum. Studies pertaining to other age categories (juvenile or older animals) are discussed separately in section 3.2.3.1.

These subsets include studies with highly diverse experimental conditions, notably with respect to the animal species and strain, type of aluminum salt administered, exposure vehicle as well as other aspects of the experimental methodology.[27] There is also variability in the reporting of doses. Some researchers adjust the concentration in drinking water for a constant dose in mg Al/kg bw/d and report this value (e.g., Colomina et al. 2005; Colomina et al. 2002; Roig et al. 2006), while others estimate doses in terms of mg Al/kg bw/d based on measures of animal body weight and food and water intake, but keep the same concentration in the diet throughout the experiment (e.g., Golub and Germann 2001b; Golub et al. 2000). In other cases, the dose is reported only as a concentration administered via diet, drinking water or gavage, and the intake in mg Al/kg bw/d has been estimated using Health Canada (1994) reference values for animal body weight and intake.

[27] Further categorization of the studies, based on salt administered, animal species, exposure vehicle and a more precisely defined exposure period, was considered but found to be not feasible. Narrowly defined subgroups did not provide an adequate number of studies with common endpoints and dose ranges. On the other hand, the comparison of pooled studies (e.g., drinking water studies vs. dietary administration studies), in order to determine the relative importance of different experimental variables, is limited by the confounding between these variables. Researchers tend to chose similar sets of experimental conditions from one experiment to another. Thus differences in the LOELs observed in a series of studies might be attributed to a particular factor (e.g., drinking water vs. diet) but could also be the result of the researchers' choices to repeatedly use the same single dose of the same salt, in the same exposure vehicle (diet or drinking water). Likewise, evaluation of pools of single-dose studies can mask the influence of an experimental condition, as reported LOELs may be poor estimates of real effect levels.

In the case of the developmental studies, the LOELs are reported as the maternal dose at the beginning of gestation. In the studies where the concentration in drinking water or the diet remained constant, this dose would generally be lower than the received dose, due to increased food and water intake during gestation and lactation. For the purpose of human health risk assessment, however, the maternal dose at the beginning of pregnancy was considered, as this provided a common point of comparison between studies.

One condition that was applied to both subsets of studies was that the experimental administered dose constitutes the principal contribution to total aluminum. As previously discussed, the concentration of aluminum in standard laboratory rodent chow may be significant, contributing approximately 10 mg Al/kg bw/d in rats and 30 mg Al/kg bw/d in mice for a typical concentration of 250 ppm.[28] In the majority of studies, this base diet concentration is not measured. Base diet concentration would considerably impact the exposure-response analysis if: (a) the bioavailability of the aluminum contained in the chow was of a similar magnitude to the bioavailability of the administered aluminum; and (b) the lab chow were to contribute a large percentage of the total aluminum exposure. While it could be hypothesised that the aluminum in the lab chow, associated with ligands in the food matrix, would be less soluble and therefore less bioavailable than added aluminum, no experimental data were identified to assess the relative bioavailabilities of aluminum in lab chow and added aluminum salts. Therefore, with regard to those studies where base diet was not quantified, studies were included in the two subsets only if the administered dose (D_a) likely exceeded the base diet dose (i.e., $D_a > 10$ mg Al/kg bw/d for rats and $D_a > 30$ mg Al/kg bw/d for mice). This approach limits the influence of the unknown base diet aluminum concentration on the exposure-response analysis, but does introduce a bias against inclusion of low dose studies in the exposure-response analysis.[29] This issue is considered further in the discussion of uncertainties (section 3.2.3.2).

Other conditions applied in the compilation of these subsets were that the doses and other experimental conditions be reported unambiguously. In addition, in the subset of adult studies, studies of juvenile and older animals were not included. Studies based on these other exposure periods are discussed in section 3.2.3.1.

The LOELs of the studies meeting the conditions described above are presented graphically inFigure 3.1. In the four studies in which a LOEL for a specific endpoint is also

[28] See discussion in section 2.4.4 on typical levels of aluminum in lab chow.

[29] The low-dose studies for adult exposure, in which base diet aluminum concentration is not reported, include findings of altered levels of neurotransmitters (Silva and Goncalves 2003; Dave et al. 2002; Bilkei-Gorzo 1993), of changes in the phospholipid content of synaptic plasma membrane (Pandya et al. 2001) or of increased lipid peroxidation in the brain (Kaneko et al. 2004, Pratico et al. 2002, Abd-Elghaffar et al. 2005). Some low-dose studies also documented increased neuronal damage (Varner et al. 1998, 1993; Somova et al. 1997; Abd-Elghaffar et al. 2005) and neuromotor and coordination effects (Bilkei-Gorzo 1993; Sahin et al. 1995). The low-dose prenatal/lactation exposure studies included findings of alterations in neurotransmission (Kim 2003; Ravi et al. 2000) and effects on fetal growth (Paternain et al. 1988; Domingo et al. 1987a).

associated with a NOEL, this is so indicated. Six other studies listed in Tables C1 and C2 found no effects for any endpoints measured (von Linstow Roloff et al. 2002; Domingo et al. 1996; Roig et al. 2006; McCormack et al. 1979; Colomina et al. 1994 and Katz et al. 1984). Consideration of these studies is important in assessing the consistency of the database and are included in the evaluation presented below. However, the studies are not included in Figure 3.1 as no corresponding LOELs for the endpoints were observed.

Considering the studies of Tables C1 and C2 collectively, the following observations concerning the exposure-response relationship for aluminum may be made:

- There is a wide variation in reported LOELs (from 1 to 663 mg Al/kg bw/d). As previously discussed, this variation would be expected, considering the diverse experimental conditions (species, strains, aluminum salt, dosing regimes, dosing vehicle, statistical power and endpoints measured).

- There is a predominance of single dose studies or studies where the LOEL was observed at the lowest dose. Thus, the LOELs in Figure 3.1 may be elevated with respect to the effect levels that might be observed in multiple dose studies.

- For the 16 subchronic and chronic exposure studies for neurotoxicity in adults, the LOELs range between 1 and 500 mg Al/kg bw/d (administered and combined doses—D_a and D_c—considered together). Among these studies the neurobehavioural endpoints examined included Morris water maze performance and impaired learning in the shuttle box as well as effects on reflex and motor activity. Biochemical endpoints included alterations in neurotransmission systems, increased apoptosis in the brain, alterations in synaptosomal membrane fluidity and increased lipid peroxidation in the brain.

- For the 22 studies of exposure during gestation and lactation, the LOELs (D_a and D_c) vary between 29 and 663 mg Al/kg bw/d. Neurobehavioural endpoints included grip strength, auditory startle, negative geotaxis and other reflexes, maze learning, thermal sensitivity, and motor development. The observed reproductive/developmental effects included a decrease in the number of corpora lutea and the number of implantation sites, a decrease in placental and fetal weight or reduced pup body weight, an increase in skeletal malformations, and an increase in the number of days to sexual maturity. In addition, alterations in essential element metabolism, deficits in synaptic plasticity in the hippocampus, a decrease in myelin sheath width as well as increased lipid peroxidation and a decrease in superoxide dismutase and catalase activity in the cerebrum and cerebellum were reported in developmental studies.

In order to estimate the lower range of doses at which oral exposure to aluminum produces toxicologically significant neurological or reproductive/developmental effects, the individual studies presented in Tables C1 and C2 were critically reviewed. The limitations of the collective database previously described—including the use of a single exposure dose, examination of a limited number of endpoints, lack of information on base diet aluminum

concentration and small group sizes—often apply to these studies as well. Nonetheless, some of the studies provided stronger evidence than others for establishing the dose range at which neurological and reproductive/developmental effects may occur. The following discussion focuses particularly on studies documenting LOELs at the lowest doses, and evaluates the findings in relation to three issues: (a) use of a low administered dose; (b) toxicological significance of different endpoints; and (c) methodological strengths and limitations and consistency of study findings.

(a) Use of a low administered dose:

Of the studies included in Figure 3.1 the lowest LOEL was observed by Huh et al. (2005). This study reported apoptosis as well as the activation of the catalytic activity of monoamine oxidases A and B in the brains of Sprague-Dawley rats at a reported combined dose of 1 mg Al/kg bw/d. The aluminum-exposed group received aluminum maltolate in drinking water over a period of 12 months.

This study reported an aluminum concentration of 11.5 ppm in the base diet. Although this is a relatively low value for laboratory chow, it does constitute an aluminum dose (0.6 mg Al/kg bw/d) of nearly twice that of the administered dose (0.38 mg Al/kg bw/d). The use of an administered dose less than the base diet dose raises the question of exposure misclassification of individual animals, as the normal variability in intake between animals may create overlap between the two groups with respect to the dose received. This is considered to be a major limitation of this study.

In spite of the extremely low administered dose, the animals receiving aluminum maltolate were found, after one year, to have approximately four times the amount of aluminum in the brain (462 ng/g) as compared to the controls (110 ng/g).[30] This finding suggested a comparable increase in both the fraction of aluminum absorbed into the bloodstream and/or the amount of aluminum distributed to the brain when the aluminum is administered as the maltolate salt. Recently, Zhou et al. (2008) found differences in aluminum oral bioavailability, which were not statistically significant, between the citrate, maltolate and fluoride salts in drinking water. The measured bioavailabilities of all the salts were low (estimated means of 0.5%, 0.61% and 0.35% for maltolate, citrate and fluoride, respectively) and approximately twice the estimated bioavailability of aluminum in food (0.1% to 0.3%, as presented in Table 2.7) as measured with the same experimental protocol.

[30] In contrast, Colomina et al. (2002) administered aluminum nitrate, enhanced with citrate, in drinking water, at an average dose of 94 mg Al/kg bw/d, to groups of male rats aged 21 days and 18 months old. The increase in whole brain aluminum concentration in the aluminum-exposed group was not statistically significant. Roig et al (2006) observed an increase of aluminum in brain regions of rats exposed to 100 mg Al/kg bw/d of aluminum nitrate with citrate in drinking water for one year. Observations were made in two-year-old rats, and increases were on the order of three- to ten-fold, depending on the brain region, and with a 22-fold increase in the striatum.

These findings suggest that while aluminum maltolate may be more bioavailable, the increase would not be sufficient to explain the results of Huh et al (2006).

In light of the uncertainty associated with the reported increased brain concentrations in the Huh et al. (2005) study, in addition to the methodological limitation of testing an administered dose that is less than the base diet dose, the study by Huh et al. (2005) was not retained for the purpose of estimating the lower range of aluminum doses at which neurological effects may be expected to occur.

Other investigations with relatively low doses over periods of 12 weeks or longer have also reported neurotoxic effects. These studies were not considered in the exposure-response analysis as the aluminum content in the laboratory chow was not reported, and thus, unlike the study by Huh et al. (2005), the relative contribution of the aluminum in the base diet could not be evaluated. However, it should be noted that LOELs ranging from 0.07 to 22 mg Al/kg bw/d (administered dose) have been associated with a significant increase in brain aluminum levels as well as significant increases in neurobehavioural or histopathological effects (refer to Kaur and Gill 2006; Kaur et al. 2006; Varner et al. 1993; Varner et al. 1994; Varner et al. 1998; Somonova et al. 1997; Fleming and Joshi 1987; Kaneko et al. 2004; and Abd-Elgahaffar et al. 2005). These results were found for different species and for different aluminum salts, administered either in drinking water or by gavage. Thus, the possibility of toxicologically significant neurological effects in this low dose range cannot be discounted. However, the difficulty of interpreting the results of these studies underlines the importance of: (a) quantifying the aluminum content in the base diet and drinking water; and (b) using a purified low-aluminum diet in studies in which the administered dose is also very low.

Among the investigations mentioned above, the study findings with respect to aluminum fluoride are of particular concern, because of the presence of both of these ions in drinking water, either naturally or through addition during the treatment process. Varner et al. (1993), Varner et al. (1994) and Varner et al. (1998), in observing increased aluminum levels in the brain associated with a low administered aluminum fluoride dose, suggested that fluoride may enhance the uptake of aluminum by the brain. At present, the scientific database is very limited with respect to the toxicokinetics and health effects specific to aluminum fluoride.

(b) Toxicological significance of different endpoints:

Considering the 16 subchronic and chronic adult exposure studies, the LOELs range between 19 and 500 mg Al/kg bw/d (administered and combined doses—D_a and D_c—considered together, and excluding the Huh et al. (2005) study). For neurobehavioural endpoints (Morris water maze performance, impaired learning in the shuttle box and motor activity), the LOELs of the seven relevant studies vary between 40 to 500 mg Al/kg bw/d (D_a and D_c), with four studies having LOELs at D_as of 40 to 70 mg Al/kg bw/d (Commissaris et al. 1982; Lal et al. 1993; Gong et al. 2005; Mameli et al. 2006). The neurobehavioural endpoints examined constitute standard elements of neurobehavioural testing and impaired performance is considered to be toxicologically significant in the experimental animal.

The biochemical effects observed in the remaining studies included alterations in neurotransmission systems, alterations in synaptosomal membrane fluidity and increased lipid peroxidation in the brain, and were associated with LOELs varying from 19 to 420 mg Al/kg bw/d. These observations provide supportive evidence for neurotoxicity observed via other endpoints as well as information on mechanisms of action, but are more difficult to evaluate with respect to toxicological significance. For this reason, studies with these endpoints were given less weight in the exposure-response evaluation, in comparison to studies that include neurobehavioural endpoints.

Considering the 22 studies of exposure during gestation and lactation, the LOELs (D_a and D_c) varied between 29 and 663 mg Al/kg bw/d. For neurobehavioural endpoints (grip strength, auditory startle, negative geotaxis and other reflexes, maze learning and thermal sensitivity, and motor development), the LOELs (administered doses) ranged from 50 to 155 mg Al/kg bw/d, with the LOELs of two studies falling in the range of 50 to 60 mg Al/kg bw/d (Colomina et al. 2005; Golub and Germann 2001b).

With respect to reproductive parameters, the lowest LOEL was reported by Belles et al. (1999), where aluminum nitrate was administered to pregnant mice via gavage at a dose of 29 mg Al/kg bw/d and observed an increase in the number of early deliveries and reduced fetal body weight. Reduced birth or fetal weight was also observed by Colomina et al. (1992) and Sharma and Mishra (2006) at LOELs ranging between 50 and 70 mg Al/kg bw/d. Morphological effects in offspring were also observed in the latter two studies.

The motor, reflex and learning endpoints examined in the developmental studies as well as the reproductive parameters of fetal growth and morphological variations are all standard endpoints included in neurodevelopmental testing procedures, and considered to be toxicologically significant.

(c) Evaluation of methodology and consistency of results in studies with LOELs of less than 70 mg Al/kg bw/d:

The methodologies and findings of the abovementioned studies with LOELs of less than 70 mg Al/kg bw/d for neurobehavioural or reproductive/developmental endpoints were compared in order to characterize the strength of evidence for the effects observed at these dose levels. With respect to the neurobehavioural effects in adults at exposures greater than 90 days, four studies were evaluated: Mameli et al. (2006), Gong et al. (2005), Lal et al. (1993) and Commissaris et al. (1982). The reproductive/developmental studies included Sharma and Mishra (2006), Belles et al. (1999), Colomina et al. (1992), Colomina et al. (2005) and Golub and Germann (2001b). In addition, investigations in which NOELs were observed for these same endpoints are discussed.

Neurobehavioural effects in adults

Of the four neurobehavioural studies in adults, all were carried out in rats using aluminum chloride, in drinking water (Gong et al. 2005; Mameli et al. 2006; Lal et al. 1993), or in the diet (Commissaris et al. 1982), for periods varying between 90 days and 11 months.

Several weaknesses were identified in the investigations of Commissaris et al. (1982) and Gong et al. (2005). First, exposure information in these two reports was expressed as concentrations in the food or drinking water, and no information was included on intake rates or body weight of the animals. Thus the administered doses (50 and 60 mg Al/kg bw/d, respectively) were calculated on the basis of default intake and body weight values (refer to Health Canada 1994), and are therefore associated with greater uncertainty than had the doses been reported by the researchers on the basis of experimental observations. Moreover, the concentration of aluminum in the base diet was not reported in the two studies, and so the combined dose could not be calculated.

The investigations of Commissaris et al. (1982) and Gong et al. (2005) were also limited by the use of a single aluminum dose and the absence of a group receiving sodium chloride. Thus, a dose-response relationship could not be examined, and the observed effects could not be definitively attributed to the aluminum ion. It should be added that these two investigations were carried out with the primary objective of examining the influence of other test substances on aluminum toxicity—parathyroid homone and Ginkgo biloba leaf extract, respectively—and not for the purpose of evaluating aluminum toxicity at different dose levels for different endpoints.

In the study of Lal et al. (1993), adult male Druckrey albino rats were exposed to an administered dose of 52 mg Al/kg bw/d for 180 days in drinking water. Although this dose was not reported directly in this form, information on daily water consumption and average body weight was provided, allowing for calculation of the dose based on experimental data. The investigation included a range of behavioural, biochemical and histopathological endpoints. The researchers observed reduced spontaneous motor activity and impaired learning in the shuttle box and maze tests, in addition to increased lipid peroxidation and decreased Mg^{2+} and $Na^{+}K^{+}$-ATPase activities in the brain. The aluminum concentration in different brain regions was significantly increased in the aluminum-exposed animals, but no pathological alterations were observed.

In the context of evaluating the exposure-response relationship, the study by Lal et al. (1993) is more informative than the Commissaris et al. (1982) and Gong et al. (2005) studies, in that the dose is more accurately reported, brain aluminum content was measured and a range of endpoints were examined, with generally consistent findings reported for the different endpoints. Its limitations include the use of a single dose, the absence of a group exposed to sodium chloride and the lack of information on the aluminum concentration in the base diet. Assuming a concentration of 250 ppm of aluminum in the laboratory chow (ATSDR 2006), the corresponding approximate aluminum dose would be 13 mg Al/kg bw/d, leading to an estimated combined dose for the Lal et al (1993) study of 65 mg Al/kg bw/d.

It should be noted that NOELs for impaired learning in the maze and shuttle box tests in aluminum-exposed adults have been observed at doses of 100 and 140 mg Al/kg bw/d,

respectively by Domingo et al. (1996) and VonLinstow Roloff et al. (2002). In the study by Domingo et al. (1996) the aluminum was administered to rats as Al nitrate, with added citrate, in drinking water for a period of 6.5 months. Von Linstow Roloff (2002) administered Al sulphate in drinking water to rats for a period of seven months.

Of these four studies, only Mameli et al. (2006) included more than one dose group, and were thereby able to establish a LOEL of 43 mg Al/kg bw/d and a NOEL of 22 mg Al/kg bw/d. At this administered dose the researchers found impairment of the vestibulo-ocular reflex in male rats of different ages (3, 10 and 24 months old) exposed to aluminum chloride in drinking water. Significant increases of aluminum were observed in brain regions (brainstem-cerebellum and cerebrum). This study, which used 20 animals per dose per age group, also included an exposure group for the salt, in this case sodium chloride, such that the observed effects could be more clearly attributed to the aluminum and not the chloride ion. It should be noted, however, that evidence from other studies supporting the effects of aluminum on the vestibulo-ocular reflex is not available, as this endpoint has not been evaluated by other researchers.

In the study by Mameli et al. (2006), the base diet aluminum concentration was measured but not clearly reported, nor was food intake measured. The LOEL of 43 mg Al/kg bw/d is thus the administered dose. The combined dose may be estimated at approximately 50 mg Al/kg bw/d, based on default values for rat dietary intake.

Considering the observations of LOELs and NOELs associated with neurobehavioural effects in adults as well as the probable combined doses, alterations in learning and reflexes may be observed at approximately 50 to 65 mg Al/kg bw/d, based on the LOELs of Mameli et al. (2006) and Lal et al. (1993) expressed as estimated combined dose.

Reproductive effects

With respect to reproductive effects, the lowest LOEL presented in Figure 3.1 is associated with the study of Belles et al. (1999). In this investigation, mice were exposed to aluminum nitrate via gavage from gestational day 6 to 15 at a dose of 29 mg Al/kg bw/d. In addition to the control group, one group received sodium nitrate at a similar nitrate dose. A high mortality (52%) in the aluminum-exposed pregnant mice was observed in this study, which was not observed in other developmental studies in which aluminum nitrate or other aluminum salts were administered at similar or greater doses. Other observations included reduced body weight gain in the dams during gestation and reduced fetal body weight. The number of early deliveries was also increased in the aluminum-exposed animals as compared to the control group, but there was no significant difference in this regard when compared to the sodium nitrate-exposed group.

This study is limited to a single dose, and the aluminum content in the base diet was not measured. The lack of information on base diet is particularly important in studies with mice because of their small body weight. A laboratory chow containing 250 ppm of aluminum would be equivalent to a dose of approximately 33 mg Al/kg bw/d, which is higher than the administered dose in this investigation.

Reduced maternal body weight gain and reduced fetal weight in aluminum-exposed animals were also observed at the LOELs associated with the Sharma and Mishra (2006) and Colomina et al. (1992) studies. A significant reduction in pup weight was also observed at the higher doses tested in the studies of Golub and Germann (2001b) and Colomina et al. (2005), at approximately 100 mg Al/kg bw/d.

In the study by Sharma and Mishra (2006), rats received 70 mg Al/kg bw/d as aluminum chloride via gavage during gestation and lactation. In addition to the effects on fetal weight, the authors observed an increase in skeletal malformations and in oxidative stress in the brains of mothers, fetuses and sucklings. The dose level in this study is based on the measured maternal weights. However, no information on base diet was included. The combined dose, based on a concentration of 250 ppm of aluminum in a typical lab chow and default values of Health Canada (1994), is estimated at approximately 83 mg Al/kg bw/d.

Colomina et al. (1992) administered aluminum lactate to mice through gavage. A LOEL of 57.5 mg Al/kg bw/d (administered dose) was observed for an increased incidence of morphological effects (cleft palate, delayed ossification of parietals), in addition to reduced fetal weight. This study did not report the aluminum content in the base diet. Considering the reported concentration in the laboratory chow used by this research group in other experiments of 42 ppm of aluminum, the estimated base diet dose would be approximately 5.5 mg Al/kg bw/d, based on Health Canada (1994) default values for body weight and food intake in mice. The combined dose would then be estimated at 63 mg Al/kg bw/d.

In contrast to the findings mentioned above, in the study of McCormack et al. (1979), rats were fed aluminum chloride in the diet at maternal dose levels of 25 and 50 mg Al/kg bw/d during gestation, and no differences in fetal growth or skeletal anomalies were observed. Colomina et al. (1994) found no differences in dam body weight, fetal growth or morphological variations in mice exposed via gavage to 104 mg Al/kg bw/d of aluminum hydroxide, during gestation. The latter finding may have resulted from the lower solubility and therefore the lower bioavailability of the hydroxide salt.

Considering the observations of LOELs and NOELs associated with reproductive effects, and the probable combined doses, reductions in fetal and pup body weight may be observed beginning at approximately 60 mg Al/kg bw/d (e.g., Colomina et al. (1992)). The study of Belles (1999), in which a LOEL of 29 mg Al/kg bw/d was observed for reduced fetal weight, is given less weight in this evaluation, in light of the uncertainty associated with the high maternal mortality rate observed in the exposed animals, and the elevated contribution of the base diet to aluminum exposure as compared to the administered dose.

Neurodevelopmental effects

With respect to neurodevelopmental effects, the lowest LOELs presented in Figure 3.1 are associated with the investigations of Colomina et al. (2005) and Golub and Germann (2001b). Both of these studies included exposure through gestation and lactation. The

experimental conditions of the two studies, however, differed in many other respects, and these are described briefly below.

Colomina, Roig et al. (2005) exposed female Sprague-Dawley rats to 0, 50, or 100 mg Al/kg bw/d as aluminum nitrate in drinking water with citric acids, in combination with a base diet dose of approximately 3 mg Al/kg bw/d. Aluminum exposure was maintained through gestation, lactation and the life of the dams.

The maternal effects of aluminum administration included decreased food intake (with reduced body weight) during gestation and lactation and decreased water intake during lactation in the 100 mg Al/kg bw/d dose group. No effects were observed with respect to the length of gestation, the number of litters or the number of fetuses per litter. With respect to the pups, there was a significant increase in the number of days until sexual maturation in males in the 100 mg Al/kg bw/d dose group and in females at both 50 and 100 mg Al/kg bw/d. A significant reduction in forelimb grip strength in males was observed in the 100 mg Al/kg bw/d dose group on PND 11 compared controls.

In the water maze task, assessing spatial learning, the performance of aluminum treated rats (50 mg Al/kg bw/d) was significantly improved in comparison to the control group. The pups in the 100 mg Al/kg bw/d dose group were not tested in the water maze test, because of altered maternal food and water intakes in this group. No differences in aluminum-exposed animals were observed with respect to surface righting, negative geotaxis or activity in an open field. The authors also measured aluminum concentration in brain regions but did not find increased levels in any regions in the aluminum-exposed animals.

The study of Golub and Germann (2001b) investigated the long-term consequences of prenatal exposures to aluminum in Swiss Webster mice, in conjunction with a suboptimal base diet. The base diet was designed to simulate the usual diet of young women in the U.S., with respect to estimated phosphate, calcium, iron, magnesium, and zinc intakes. Following breeding, dams were exposed to aluminum in the diet as aluminum lactate. The doses were equivalent to approximately < 1, 10, 50 and 100 mg Al/kg bw/d, as estimated at the beginning of gestation.

The dams were exposed throughout gestation and lactation. Following weaning at 21 days, the pups were fed the same diet as the dams for two weeks (although the per kg dose levels were higher). No effects were observed in the number of dams completing pregnancy, gestation length, weight gain of the dams (GD0 to GD15), litter size or birth weight. By weaning, both males and females in the two highest dose groups weighed significantly less than the controls, although by PND35 only the highest dose group showed this effect.

The female offspring of the highest dose group (maternal exposure of 100 mg Al/kg bw/d) were found to be slower in maze learning at three months old, as indicated by longer latencies during the first three sessions of the four-session learning series. All aluminum treated groups were similar to controls by the fourth session. Differences in aluminum exposed groups were also observed in the cue relocation trials, in which average

trial latency was significantly increased at the two highest dose levels (50 and 100 mg Al/kg bw/d) as compared to the control group.

In the motor testing of male offspring at five months old, males in the highest dose group (maternal exposure of 100 mg Al/kg bw/d) had significantly lower hindlimb grip strength and greater number of rotations in the rotarod test (animal losing footing). When body weight was taken into account, only the findings for the rotarod test remained significant.

The investigations by Colomina et al. (2005) and Golub and Germann (2001b) are methodologically superior in many respects to the majority of the studies described in Tables C1 and C2. Both include two dose levels in addition to the control group, quantify the aluminum dose associated with the base diet, and examine a range of reproductive and neurodevelopmental endpoints. The Colomina et al. (2005) study includes measurement of aluminum concentration in different brain regions. The Golub and Germann (2001b) study, however, used an experimental protocol designed to test the influence of a suboptimal diet, which limits comparisons of the findings with other investigations of aluminum toxicity, particularly as no groups were included with equivalent aluminum dose levels and a standard diet.

Interpretation of cognitive and motor test findings in the studies investigating the effects of aluminum exposure is also complicated by a possible biphasic dose-response relationship. For example, in the study by Roig et al. (2006), rats received aluminum nitrate in drinking water during gestation and lactation at administered doses of 50 and 100 mg Al/kg bw/d. No difference in the motor activity of aluminum-exposed pups and controls was found. However, the animals exposed to 50 mg Al/kg bw/d showed an improved performance in maze learning. The performance of animals exposed to 100 mg Al/kg bw/d was significantly reduced as compared to the animals exposed to 50 mg Al/kg bw/d, but not significantly different from controls. Colomina et al. (2005) also observed improved maze performance in aluminum-exposed animals, although the highest exposure group in that study was not tested for this endpoint.

Considering the neurodevelopmental studies described above, diminished performance in learning or motor tests may be observed in animals exposed prenatally or through lactation at maternal combined doses beginning at approximately 50 mg Al/kg bw/d. There is, however, considerable variability in various study results with respect to these endpoints, which also suggest a possible biphasic dose-response relationship in relation to maze learning.

3.2.3.1 Studies pertaining to other life stages

Some experimental animal studies have focused on life stages not included in the subsets discussed above. These are described below.

Golub and Keen (1999) investigated the effects of aluminum lactate administered in the diet to pubertal mice for four- or eight-week periods at doses of 17, 78, 122 and 152 mg Al/kg bw/d. A significant association between aluminum intake and reduced brain weight was observed in the four-week cohort at 152 mg Al/kg bw/d, but not in the eight-

week cohort, suggesting that effects in young animals are reversible, even as exposure continues. There were no consistent effects, however, on startle response or grip strength.

Rajasekaran (2000) administered 53 mg Al/kg bw/d of aluminum chloride via gavage to male pubertal Wistar rats for 30 days. Testing at the end of the exposure period showed a decrease in spontaneous motor activity in the exposed rats, but no effect on motor coordination. Acetyl cholinesterase activity was decreased in the cerebrum but not the cerebellum or brain stem.

Fattoretti et al. (2004) administered aluminum chloride in drinking water to 22-month-old rats, at a dose of 31 mg Al/kg bw/d for six months. They observed an increase in trace elements and aluminum in brain regions, and an increase in the area occupied by the mossy fibres in the hippocampal CA3 zone. No neurobehavioural endpoints were examined in this study.

Colomina et al. (2002) administered aluminum nitrate in drinking water (with citric acid) for 114 days to rats who were 18 months old at the start of the experiment. The weighted dose over the four months was 94 mg Al/kg bw/d. They found a decrease in mean body weight in aluminum-exposed older rats but no differences in brain aluminum concentration. No effects were observed in the passive avoidance test or in open-field activity. However, the percentage of perforated synapses in the brain increased with age and aluminum exposure.

A recent study by Walton (2007a, 2007b) of rats exposed from 12 months to the end of life investigated neurotoxicity endpoints at combined doses of 0.4 and 1.6 mg Al/kg bw/d, doses simulating current estimated low-end and high-end human exposures. Two of the six rats in the high exposure group developed significant impairment in memory tests in old age, and the brains of these rats were examined with respect to aluminum loading and inhibition of PPP2 activity (a major phosphate-removing enzyme active against tau hyperphosphorylation[31]). The study, limited by the small group size, did not report on differences between the two aluminum-exposure groups, and thus does not provide a basis for conclusions in regard to the relationship between observed biochemical and behavioural effects and aluminum exposure.

3.2.3.2 Identification of the level of concern and associated uncertainties

On the basis of the 43 studies presented in Tables C1 and C2, and considering additional studies on other age groups, it is recommended that a dose of 50 mg Al/kg bw/d, expressed as a combined dose of total aluminum, be considered as the level at which neurological and reproductive/developmental effects begin to be repeatedly observed in animal studies.

---

[31] Neurofibrillary tangles in AD brains are formed from the hyperphosphorylation of tau protein.