2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ALUMINUM IN THE UNITED STATES

Aluminum is ubiquitous; the third most common element of the earth's crust. It is naturally released to the environment from the weathering of rocks and volcanic activity. Human activities such as mining also result in the release of aluminum to the environment. Aluminum levels in environmental media vary widely depending upon the location and sampling site. In general, background levels of aluminum in the atmosphere are low, typically ranging from about 0.005 to 0.18 μg/m^3. Much higher levels are routinely observed in urban and industrial locations. Aluminum levels in surface water is usually very low (<0.1 mg/L); however, in acidic waters or water high in humic or fulvic acid content, the concentration of soluble aluminum increases due to the increased solubility of aluminum oxide and aluminum salts. Its concentration in soils varies widely, ranging from about 7 to over 100 g/kg.

In the environment, aluminum exists in only one oxidation state (+3), and does not undergo oxidation-reduction reactions. It can react with other matter in the environment to form various complexes. The fate and transport of aluminum is largely controlled by environmental factors such as pH, salinity, and the presence of various species with which it may form complexes. In general, the solubility and mobility of aluminum in soil is greatest when the soil is rich in organic matter capable of forming aluminum-organic complexes and when the pH is low, such as in areas prone to acid rain or in acidic mine tailings.

The general population is primarily exposed to aluminum through the consumption of food items, although minor exposures may occur through ingestion of aluminum in drinking water and inhalation of ambient air. Aluminum found in over-the-counter medicinals, such as antacids and buffered aspirin, is used as a food additive, and is found in a number of topically applied consumer products such as antiperspirants, and first aid antibiotic and antiseptics, diaper rash and prickly heat, insect sting and bite, sunscreen and suntan, and dry skin products. The concentration of aluminum in foods and beverages varies widely, depending upon the food product, the type of processing used, and the geographical areas in which food crops are grown (see Section 6.4). Based on the FDA's 1993 Total Diet Study dietary exposure model and the 1987–1988 U.S. Department of Agriculture (USDA) Nationwide Food Consumption Survey, the authors estimated daily aluminum intakes of 0.10 mg Al/kg/day for 6–11-month-old infants; 0.30–0.35 mg Al/kg/day for 2–6-year-old children; 0.11 mg Al/kg/day for 10-year-old children; 0.15–0.18 mg Al/kg/day for 14–16-year-old males and females; and 0.10–0.12 mg Al/kg/day for adult (25–30- and 70+-year-old) males and females. Users of aluminum-

containing medications who are healthy (i.e., have normal renal function) can ingest much larger amounts of aluminum than in the diet, possibly as high as 12–71 mg Al/kg/day from antacid/anti-ulcer products and 2–10 mg Al/kg/day from buffered analgesics when taken at recommended dosages.

Gastrointestinal absorption of aluminum is low, generally in the range of 0.1–0.4% in humans, although absorption of particularly bioavailable forms such as aluminum citrate may be on the order of 0.5–5%. Although large bolus doses of as much as half a gram of aluminum as aluminum hydroxide throughout the day can be ingested during antacid therapy, absorption of aluminum hydroxide is usually $\leq$0.01% of the intake amount. Bioavailability of aluminum varies depending mainly on the chemical form of the ingested compound (i.e., type of anion) and the concurrent exposure to dietary chelators such as citric acid, ascorbic acid, or lactic acid. The total body burden of aluminum in healthy human subjects is approximately 30–50 mg. Normal levels of aluminum in serum are approximately 1–3 μg/L. Of the total body burden of aluminum, about one-half is in the skeleton, and about one-fourth is in the lungs.

2.2 SUMMARY OF HEALTH EFFECTS

There are numerous studies that have examined aluminum's potential to induce toxic effects in humans exposed via inhalation, oral, or dermal exposure. Most of these findings are supported by a large number of studies in laboratory animals. Occupational exposure studies and animal studies suggest that the lungs and nervous system may be the most sensitive targets of toxicity following inhalation exposure. Respiratory effects, in particular impaired lung function and fibrosis, have been observed in workers exposed to aluminum dust or fumes; however, this has not been consistently observed across studies and it is possible that co-exposure to other compounds contributed to observed effects. Respiratory effects (granulomatous lesions) have also been observed in rats, hamsters, and guinea pigs. There is concern that these effects are due to dust overload rather than a direct effect of aluminum in lung tissue. Occupational studies in workers exposed to aluminum dust in the form of McIntyre powder, aluminum dust and fumes in potrooms, and aluminum fumes during welding provide suggestive evidence that there may be a relationship between chronic aluminum exposure and subclinical neurological effects such as impairment on neurobehavioral tests for psychomotor and cognitive performance and an increased incidence of subjective neurological symptoms. With the exception of some isolated cases, inhalation exposure has not been associated with overt symptoms of neurotoxicity. A common limitation of these occupational exposure studies is that aluminum exposure has not been well characterized. The available animal inhalation studies are inadequate for assessing the potential for aluminum-induced neurotoxicity because

the only neurological end points examined were brain weight and histology of the brain; no function tests were performed.

There is limited information on aluminum toxicity following dermal exposure. Application of aluminum compounds to the skin, such as aluminum chloride in ethanol or alum, may cause rashes in some people. Skin damage has been observed in mice, rabbits, and pigs exposed to aluminum chloride or aluminum nitrate, but not following exposure to aluminum sulfate, aluminum hydroxide, aluminum acetate, or aluminum chlorhydrate.

There is a fair amount of human data on the toxicity of aluminum following oral exposure. However, the preponderance of human studies are in patients with reduced renal function who accumulated aluminum as a result of long-term intravenous hemodialysis therapy with aluminum-contaminated dialysis fluid and, in many cases, concurrent administration of high oral doses of aluminum to regulate phosphate levels (i.e., reduce uptake of phosphate by binding it in the gut) and have limited usefulness in predicting toxicity in the general population because the very large aluminum exposure levels and impaired renal function results in aluminum accumulation. Dialysis encephalopathy syndrome (also referred to as dialysis dementia) can result from this accumulation of aluminum in the brain. Dialysis encephalopathy is a degenerative neurological syndrome, characterized by the gradual loss of motor, speech, and cognitive functions. Another neurological effect that has been proposed to be associated with aluminum exposure is Alzheimer's disease. Although a possible association was proposed over 40 years ago, this association is still highly controversial and there is little consensus regarding current evidence. A number of studies have found weak associations between living in areas with elevated aluminum levels in drinking water and an increased risk (or prevalence) of Alzheimer's disease; other studies have not found significant associations. In contrast, no significant associations have been found between tea consumption or antacid use and the risk of Alzheimer's disease; although the levels of aluminum in tea and antacids are very high compared to drinking water, aluminum from these sources is poorly absorbed. The available data do not suggest that aluminum is a causative agent of Alzheimer's disease; however, it is possible that it may play a role in the disease development.

Aluminum is found in several ingested over-the-counter products such as antacids and buffered aspirin; clinical studies on health effects of aluminum medicinals in people with normal renal function have been identified. These aluminum-containing products are assumed to be safe in healthy individuals at recommended doses based on historical use. The assumed safety of aluminum is also partly due to the generally regarded as safe (GRAS) status of aluminum-containing food additives. However, there is

some indication that adverse effects can result from long-term use of aluminum-containing medications in some healthy individuals. There are a number of case reports of skeletal changes (e.g., osteomalacia) in adults and children with normal kidney function due to long-term antacid use for the treatment of gastrointestinal disorders. These skeletal effects are secondary to hypophosphatemia and phosphate depletion caused by aluminum impairing phosphorus absorption by binding with dietary phosphorus.

There is a rather extensive database on the oral toxicity of aluminum in animals. These studies clearly identify the nervous system as the most sensitive target of aluminum toxicity and most of the animal studies have focused on neurotoxicity and neurodevelopmental toxicity. Other adverse effects that have been observed in animals orally exposed to aluminum include impaired erythropoiesis in rats exposed to 230 mg Al/kg/day and higher, erythrocyte damage (as evidenced by decreases in hemoglobin, hematocrit, and erythrocyte osmotic fragility, and altered erythrocyte morphology) in rats exposed to 230 mg Al/kg/day and higher, increased susceptibility to infection in mouse dams exposed to 155 mg Al/kg/day, delays in pup maturation following exposure of rats to 53 mg Al/kg/day, and decreases in pup body weight gain in rats and mice exposed to 103 mg Al/kg/day and higher.

Neurodegenerative changes in the brain, manifested as intraneuronal hyperphosphorylated neuro-filamentous aggregates, is a characteristic response to aluminum in certain species and nonnatural exposure situations generally involving direct application to brain tissue, particularly intracerebral and intracisternal administration and *in vitro* incubation in rabbits, cats, ferrets, and nonhuman primates. Oral studies in rats and mice have not found significant histopathological changes in the brain under typical exposure conditions; however, altered myelination was found in the spinal cord of mouse pups exposed to 330 mg Al/kg/day on gestation day 1 through postnatal day 35. Overt signs of neurotoxicity are rarely reported at the doses tested in the available animal studies ($\leq$330 mg Al/kg/day for bioavailable aluminum compounds); rather, exposure to these doses is associated with subtle neurological effects detected with neurobehavioral performance tests. Significant alterations in motor function, sensory function, and cognitive function have been detected following exposure to adult or weanling rats and mice or following gestation and/or lactation exposure of rats and mice to aluminum lactate, aluminum nitrate, and aluminum chloride. The most consistently affected performance tests were forelimb and/or hindlimb grip strength, spontaneous motor activity, thermal sensitivity, and startle responsiveness. Significant impairments in cognitive function have been observed in some studies, although this has not been found in other studies even at higher doses. Adverse neurological effects have been observed in rats and mice at doses of 100–200 mg Al/kg/day and neurodevelopmental effects have been observed in rats and mice at doses of 103–330 mg Al/kg/day.

A number of human studies have examined the occurrence of cancer among aluminum industry workers and found a higher-than-expected cancer mortality rate, but this is probably due to the other potent carcinogens to which they are exposed, such as polycyclic aromatic hydrocarbons (PAHs) and tobacco smoke. Available cancer studies in animals have not found biologically relevant increases in malignant tumors. The International Agency for Research on Cancer (IARC) concluded that aluminum production was carcinogenic to humans and that pitch volatiles have fairly consistently been suggested in epidemiological studies as being possible causative agents. The Department of Health and Human Services and EPA have not evaluated the human carcinogenic potential of aluminum.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for aluminum. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

No acute-, intermediate-, or chronic-duration inhalation MRLs were derived for aluminum. Results from human and animal studies suggest that the respiratory tract, particularly the lung, is a sensitive target of airborne aluminum toxicity; human studies also suggest that the nervous system may also be a target of

inhaled aluminum. Interpretation of the human data is complicated by the lack of exposure assessment and the potential for concomitant exposure to other toxic compounds. Numerous studies have found impaired lung function in a variety of aluminum workers (Abbate et al. 2003; Al-Masalkhi and Walton 1994; Bast-Pettersen et al. 1994; Bost and Newman 1993; Burge et al. 2000; Chan-Yeung et al. 1983; Herbert et al. 1982; Hull and Abraham 2002; Jederlinic et al. 1990; Korogiannos et al. 1998; Miller et al. 1984b; Radon et al. 1999; Simonsson et al. 1985; Vandenplas et al. 1998). Other effects that have been observed include occupational asthma (Abramson et al. 1989; Burge et al. 2000; Kilburn 1998; Vandenplas et al. 1998) and pulmonary fibrosis (Al-Masalkhi and Walton 1994; De Vuyst et al. 1986; Edling 1961; Gaffuri et al. 1985; Gilks and Churg 1987; Jederlinic et al. 1990; Jephcott 1948; McLaughlin et al. 1962; Mitchell et al. 1961; Musk et al. 1980; Riddell 1948; Shaver 1948; Shaver and Riddell 1947; Ueda et al. 1958; Vallyathan et al. 1982).

Acute-, intermediate-, and chronic-duration animal studies have also reported respiratory effects. These respiratory effects include increases in alveolar macrophages, granulomatous lesions in the lungs and peribronchial lymph nodes, and increases in lung weight (Drew et al. 1974; Klosterkotter 1960; Pigott et al. 1981; Steinhagen et al. 1978; Stone et al. 1979). The lung effects observed in humans and animals are suggestive of dust overload. Dust overload occurs when the volume of dust in the lungs markedly impairs pulmonary clearance mechanisms. Lung overload is not dependent on the inherent toxicity of the compound, and dust overloading has been shown to modify both the dosimetry and toxicological effects of the compound (Morrow 1988). When excessive amounts of widely considered benign dusts are persistently retained in the lungs, the resultant lung effects are similar to those observed following exposure to dusts that are highly toxic to the lungs. Because it is unclear whether the observed respiratory effects are related to aluminum toxicity or to dust overload, inhalation MRLs based on respiratory effects were not derived.

Subtle neurological effects have also been observed in workers chronically exposed to aluminum dust or fumes. These effects include impaired performance on neurobehavioral tests (Akila et al. 1999; Bast-Pettersen et al. 2000; Buchta et al. 2003, 2005; Hänninen et al. 1994; Hosovski et al. 1990; Polizzi et al. 2001; Rifat et al. 1990; Riihimäki et al. 2000; Sjögren et al. 1990) and increased reporting of subjective neurological symptoms (Bast-Pettersen et al. 1994, 2000; Hänninen et al. 1994; Hosovski et al. 1990; Iregren et al. 2001; Rifat et al. 1990; Riihimäki et al. 2000; Sim et al. 1997; Sjögren et al. 1990, 1996; White et al. 1992). Neurological exams in the available animal studies (Steinhagen et al. 1978; Stone et al. 1979) have been limited to measurement of brain weight and/or histopathology of the brain; no function tests were performed. The identification of neurotoxicity as a sensitive end point in workers

exposed to aluminum dust and fumes is well supported by a large number of animal studies reporting a variety of neurobehavioral alterations following oral exposure. However, the poor characterization of aluminum exposure in the occupational exposure studies precludes using these studies to develop an inhalation MRL for aluminum.

Oral MRLs

Data on health effects of ingested aluminum in humans are unsuitable for MRL consideration because studies have centered on specific patient populations (i.e., dialysis, neurodegenerative disease) and are not the types typically used in risk evaluation. Studies in patients with reduced renal function who accumulated aluminum as a result of long-term intravenous hemodialysis therapy with aluminum-contaminated dialysate and the use of aluminum-containing phosphate binding agents provide evidence that aluminum is an important etiologic factor in dialysis-related health disorders, particularly the neurological syndrome dialysis encephalopathy. The effects are manifested under unnatural exposure conditions in which the gastrointestinal barrier is bypassed (exposure to aluminum in dialysate fluid) and aluminum excretion is impaired by the poor renal function. There are case reports of skeletal changes (e.g., osteomalacia) consequent to long-term ingestion of antacids in healthy adults and children with normal kidney function (Carmichael et al. 1984; Chines and Pacifici 1990; Pivnick et al. 1995; Woodson 1998), but these effects are attributable to an interaction between aluminum and phosphate in the gut (aluminum binds with phosphate in the gut resulting in decreased phosphate absorption and hypophosphatemia). Although the use of aluminum medicinals in people is widespread, there are a limited number of experimental studies that examined the potential toxicity of the aluminum in these medicinals in individuals with normal renal function.

Derivation of an MRL(s) for aluminum based on animal studies is complicated by limitations in the database, particularly the lack of information on aluminum content in the base diet. As discussed in the introduction to Section 3.2.2, commercial laboratory animal feeds contain high levels of aluminum that can significantly contribute to total experimental exposure. Due to the likelihood of significant base dietary exposure to aluminum, studies with insufficient information on aluminum content in the base diet must be assumed to underestimate the actual aluminum intake. The magnitude of the underestimate can be considerable; for example, approximate feed concentrations of 250 and 350 ppm aluminum reported in some rat and mouse studies, respectively (Colomina et al. 1998; Domingo et al. 1993; Oteiza et al. 1993), are roughly equivalent to daily doses of 25 mg Al/kg/day (rats) and 68 mg Al/kg/day (mice), which represents a significant portion of the lethal dose for these species. Consequently, although studies with

inadequate data on base dietary levels of aluminum provide useful information on health effects of aluminum, no-observed-adverse-effect levels (NOAELs) and lowest-observed-adverse-effect levels (LOAELs) from these studies cannot be assumed to be accurate, are not suitable for comparing with effect levels from studies that used diets with known amounts of aluminum, and are inappropriate for MRL consideration.

The available data were considered inadequate for derivation of an acute-duration oral MRL for aluminum. Two studies were identified that provided sufficient information on the levels of aluminum in the basal diet. McCormack et al. (1979) and Domingo et al. (1989) did not find any significant alterations in pup viability/lethality, pup body weight, or the incidence of malformation in rats exposed to 110 mg Al/kg/day as aluminum chloride in the diet on gestation days 6–19 (McCormack et al. 1979) or 141 mg Al/kg/day as aluminum nitrate administered via gavage on gestation days 6–15 (Domingo et al. 1989). Neither study evaluated the potential neurotoxicity of aluminum following acute-duration exposure; intermediate-duration studies provide strong evidence that the nervous system (in adults and developing organisms) is the most sensitive target of aluminum toxicity.

- An MRL of 1 mg Al/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to aluminum.

A fair number of animal studies have examined the oral toxicity of aluminum following intermediate-duration exposure. A subset of these studies that provide information on the aluminum content of the basal diet and involved exposure to aluminum via the diet or drinking water will be the focus of this discussion. With the possible exception of reproductive function, these studies have examined most potential end points of aluminum toxicity. Systemic toxicity studies have not consistently reported adverse effects in rats exposed to up to 284 mg Al/kg/day (Domingo et al. 1987b; Gomez et al. 1986; Konishi et al. 1996), mice exposed to doses as high as 195 mg Al/kg/day (Oteiza et al. 1989), or dogs exposed to doses as high as 88 mg Al/kg/day (Katz et al. 1984; Pettersen et al. 1990). An increased susceptibility to bacterial infections was observed in mouse dams exposed to 155 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through lactation day 21 (Yoshida et al. 1989). However, a similar aluminum dose did not result in a change in susceptibility in virgin female mice exposed to 107 mg Al/kg/day as aluminum lactate in the diet for 6 weeks (Yoshida et al. 1989). Immunological alterations (decreased spleen concentrations of interleukin-2, interferon g, and tumor necrosis factor and a decrease in CD^{4+} cells) were observed in mice exposed to 200 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through postnatal day 180 (Golub et al. 1993). There is limited information on the potential for aluminum to induce reproductive effects. Although a number of studies have reported no

alterations in the occurrence of resorption, litter size, sex ratio, or pup body weight, no studies have examined fertility or potential effects on sperm morphology or motility. A significant alteration in gestation length was observed in mice exposed to 155 or 330 mg Al/kg/day as aluminum lactate in the diet on gestation day 1 through lactation 21 (Donald et al. 1989); in the aluminum exposed mice, 4 of the 17 litters were born earlier or later (days 17, 19, or 20 versus day 18 in controls) than control litters. However, this has not been reported in other studies in mice or rats (Colomina et al. 2005; Golub and Germann 2001; Golub et al. 1992a, 1995).

The preponderance of available intermediate-duration studies has focused on the potential for aluminum to induce neurological and neurodevelopmental effects. Although neurotoxicity of aluminum has not been established in people with normal renal function, the data for dialysis encephalopathy (as well as some occupational studies) establish that the human nervous system is susceptible to aluminum and neurotoxicity is a well-documented effect of aluminum in orally-exposed in mice and rats. A wide variety of behavioral tests were conducted in rats and mice, in which the most consistently affected behaviors involve motor function. Alterations in forelimb and hindlimb grip strength have been observed in adult mice exposed to 195 mg Al/kg/day as aluminum lactate in the diet for 90 days (Golub et al. 1992b), mice (6 weeks of age at study beginning) exposed to 195 mg Al/kg/day as aluminum lactate in the diet for 5–7 weeks (Oteiza et al. 1993), the offspring of mice exposed on gestation day 1 through lactation day 21 to 155 mg Al/kg/day (Donald et al. 1989; Golub et al. 1995) or 250 mg Al/kg/day (Golub et al. 1995) as aluminum lactate, and the offspring of rats exposed to 103 mg Al/kg/day as aluminum nitrate in drinking water (with added citric acid) for 15 days prior to mating and on gestation day 1 through lactation day 21 (Colomina et al. 2005). Decreases in spontaneous motor activity were observed in mice exposed to 130 mg Al/kg/day for 6 weeks (Golub et al. 1989) or 195 mg Al/kg/day for 90 days (Golub et al. 1992b). Motor impairments have also been detected in mice in the wire suspension test in which offspring exposed to 130 mg Al/kg/day had a shorter latency to fall from the wire and in the rotorod test in which offspring exposed to 260 mg Al/kg/day had a higher number of rotations (which occur when the animals lost its footing, clung to the rod, and rotated with it for a full turn) (Golub and Germann 2001). Neurobehavioral alterations that have occurred at similar dose levels include decreased responsiveness to auditory or air-puff startle (Golub et al. 1992b, 1995), decreased thermal sensitivity (Golub et al. 1992a), increased negative geotaxis latency (Golub et al. 1992a), and increased foot splay (Donald et al. 1989). Additionally, one study found significant impairment in performance of the water maze test in offspring of mice exposed to 130 mg Al/kg/day on gestation day 1 through lactation day 21 (Golub and Germann 2001). Colomina et al. (2005) did not find alterations in this test in rats exposed to 53 mg Al/kg/day; however, this study did not run probe tests, which showed significant

alterations in the Golub and Germann (2001) study. Other studies have utilized passive avoidance tests or operant training tests to evaluate potential impairment of cognitive function. However, the interpretation of the results of these tests is complicated by an increase in food motivation in aluminum exposed mice (Golub and Germann 1998).

There is also strong evidence that gestational and/or lactational exposure can cause other developmental effects. Gestation and/or lactation exposure can result in significant decreases in pup body weight gain in rats and mice (Colomina et al. 2005; Golub and Germann 2001; Golub et al. 1992a). The decreases in pup body weight are often associated with decreases in maternal body weight during the lactation phase of the study; however, decreases in body weight have also been observed in a cross-fostering study when gestation-exposed pups were nursed by control mice (Golub et al. 1992a). Other studies involving gestation and lactation exposure to aluminum did not find changes in pup growth in mice (Donald et al. 1989; Golub and Germann 1998; Golub et al. 1995). In rats, a delay in physical maturation, particularly delays in vagina opening, testes descent, and incisor eruption, has been reported at 53 mg Al/kg/day (Colomina et al. 2005). In the Colomina et al. (2005) study, a delay in vagina opening was observed in rat offspring exposed to 53 mg Al/kg/day. The number of days to vagina opening was 31.1, 40.9, and 45.9 days in the control, 53, and 103 mg Al/kg/day groups, respectively. Delays in maturations were also observed for testes descent (23.9, 22.8, and 27.1 days in the control, 53, and 103 mg Al/kg/day groups, significant at 103 mg Al/kg/day) and incisor eruption in males (5.5, 6.1, and 5.3 days, significant at 53 mg Al/kg/day, but not at 103 mg Al/kg/day). Significant delays in vagina opening and testes descent were also observed at 103 mg Al/kg/day in the offspring of rats similarly exposed but with the addition of restraint stress on gestation days 6–20. The mean number of days to maturation in the control, 53, and 103 mg Al/kg/day groups were 32.5, 40.4, and 44.9 days for vagina opening and 24.9, 23.2, and 27.7 days for testes descent. However, another study by Colomina et al. (1999) did not find significant delays in vagina opening or testes descent, but did find significant delays in pinna attachment and eye opening following administration of 75 mg/kg/day (15 mg Al/kg/day) aluminum chloride via intraperitoneal injection to mice on gestation days 6–15. Another study did not find delays in pinna attachment, eye opening, or incisor eruption in the offspring of rats administered via gavage 73 mg Al/kg/day as aluminum chloride (aluminum content of the diet was not reported) on gestation days 8–20 (Misawa and Shigeta 1992). Collectively, these studies provide equivocal evidence that aluminum induces delays in maturation.

The Golub et al. (1989), Golub and Germann (2001), and Colomina et al. (2005) studies identified the lowest LOAELs for the critical effects (neurotoxicity, neurodevelopmental toxicity, and delays in

maturation) and were considered as possible principal studies. Golub et al. (1989) identified the lowest LOAEL for neurotoxicity. In this study in which mice were exposed to aluminum lactate in the diet for 6 weeks, significant decreases in total activity and vertical activity (rearing) were observed at 130 mg Al/kg/day; no significant alterations were observed at 62 mg Al/kg/day. One limitation of this study is that motor activity was the only neurobehavioral test evaluated; other studies have shown that grip strength is one of the more sensitive end points. Golub and Germann (2001) examined a number of sensitive end points of neurodevelopmental toxicity in the offspring of mice exposed to aluminum lactate in the diet on gestation day 1 through lactation day 21, after which the pups were fed a diet containing the same levels of aluminum as the dams on postnatal days 21–35. The study identified a NOAEL of 26 mg Al/kg/day and a LOAEL of 130 mg Al/kg/day for alterations in tests of motor function (a shorter latency to fall off a wire) and cognitive function (impaired performance in the water maze test). This study used a suboptimal diet, which complicates the interpretation of the study results. The dietary levels of phosphorus, calcium, magnesium, iron, and zinc were lower than the National Research Council's recommendation in an attempt to mimic the intakes of these nutrients by young women. The investigators noted that even though the intakes of several nutrients were below the recommendations, the diet was not deficient. The impact of the suboptimal diet on the developmental toxicity of aluminum is not known. The observed effects are similar to those reported in other studies, as are the adverse effect levels. In the Colomina et al. (2005) study, a significant decrease in forelimb grip strength was observed in the offspring of rats exposed to 103 mg Al/kg/day as aluminum nitrate in the drinking water (with citric acid added to increase aluminum absorption) for 15 days prior to mating and during gestation and lactation; grip strength was not adversely affected at 53 mg Al/kg/day. This study also found significant delays in vagina opening at 53 mg Al/kg/day. As previously noted, there are limited data to confirm or refute the identification of delays in maturation as a critical effect of aluminum. The delays in maturation may be secondary to decreases in maternal weight or food intake or decreases in pup body weight and/or food intake; however, these data are only reported for some time periods. The Golub et al. (1989) study was not selected as the principal study because the NOAEL of 62 mg Al/kg/day identified in this study is higher than the dose associated with delayed maturation in the Colomina et al. (2005) study. The Golub and Germann (2001) and Colomina et al. (2005) studies were selected as co-principal studies. A short description of these studies follows.

In the Golub and Germann (2001) study, groups of pregnant Swiss Webster mice were exposed to 0, 100, 500, or 1,000 mg Al/kg diet on gestational days 0–21 and during lactation until day 21. On postnatal day (PND) 21, one male and one female pup from each litter were placed on the same diet as the dam. The offspring were exposed until PND 35. The composition of the diet was modified from the National

Research Council's recommendations; the investigators noted that the nutrients were reduced to correspond to the usual intake of these nutrients by young women. The average daily intakes of phosphorus, calcium, magnesium, iron, and zinc in women aged 18–24 years are 83, 56, 71, 69, and 67% of the recommended dietary allowance (RDA); these percents were used to modify the recommended dietary intake for the mice used in this study. Doses of 26, 130, and 260 mg Al/kg/day are calculated by averaging reported estimated doses of 10, 50, and 100 mg Al/kg/day for adults (i.e., at beginning of pregnancy) and 42, 210, and 420 mg Al/kg/day maximal intake during lactation. The doses at lactation were calculated using doses estimated in previous studies with similar exposure protocols performed by the same group of investigators (Golub et al. 1995). At 3 months of age, the females were tested for neurotoxicity using the Morris water maze. At 5 months of age, males were tested for motor activity and function using rotarod, grip strength, wire suspension, mesh pole descent, and beam traversal tests. No alterations in pregnancy weight gain or pup birth weights were observed. At PND 21, significant decreases in pup body weights were observed at 130 and 260 mg Al/kg/day. No information on maternal weight gain during lactation was reported; however, the investigators noted that the decrease in pup weight was not associated with reduced maternal food intake. At PND 35, the decrease in body weight was statistically significant at 260 mg Al/kg/day. On PND 90, female mice in the 260 mg Al/kg/day group weighed 15% less than controls. Decreases in heart and kidney weights were observed at 260 mg Al/kg/day in the females. Also, increases in absolute brain weight were observed in females at 26 mg Al/kg/day and relative brain weights were observed at 26 or 260 mg Al/kg/day, but not at 130 mg Al/kg/day. In the males, significant decreases in body weight were observed at 130 (10%) and 260 (18%) mg Al/kg/day at 5 months; an increase in food intake was also observed at these doses. In the Morris maze (tested at 3 months in females), fewer animals in the 260 mg Al/kg/day group had escape latencies of <60 seconds during sessions 1–3 (learning phase) and a relocation of the visible cues resulted in increased latencies at 130 and 260 mg Al/kg/day. Body weight did not correlate with latency to find the platform or with the distribution of quadrant times. The investigators concluded that controls used salient and/or nonsalient cues, 26 and 130 mg Al/kg/day animals used both cues, but had difficulty using only one cue, and 260 mg Al/kg/day animals only used the salient cues. In the males tested at 5 months, a significant decrease in hindlimb grip strength was observed at 260 mg Al/kg/day, an increase in the number of rotations on the rotorod as observed at 260 mg Al/kg/day, and a shorter latency to fall in the wire suspension test was observed at 130 and 260 mg Al/kg/day. The investigators noted that there were significant correlations between body weight and grip strength and number of rotations. When hindlimb grip strength was statistically adjusted for body weight, the aluminum-exposed mice were no longer significantly different from controls; the number of rotations was still significantly different from control after adjustment for body weight.

In the Colomina et al. (2005) study, groups of female Sprague Dawley rats were exposed to 0, 50, or 100 mg Al/kg/day aluminum nitrate nonahydrate in drinking water; citric acid (710, 355, and 710 mg/kg/day in the control, 50, and 100 ppm groups, respectively) was added to the drinking water to increase aluminum absorption. The adult rats were exposed to aluminum for 15 days prior to mating and during gestation and lactation periods; after weaning, the pups were exposed to the same aluminum concentration as the mothers from PND 21 through 68. The basal diet (Panlab rodent chow) contained 41.85 µg Al/g diet. Aluminum doses were calculated by adding the basal dietary aluminum doses (calculated using reference values for mature Sprague-Dawley rats) to reported aluminum doses from water; the total aluminum doses were 3, 53, and 103 mg Al/kg/day. In addition to aluminum exposure, some animals in each group underwent restraint stress for 2 hours/day on gestation days 6–20; the restraint consisted of placing the rats in cylindrical holders. The following neurobehavioral tests were performed on the offspring: righting reflex (PNDs 4, 5, 6), negative geotaxis (PNDs 7, 8, 9), forelimb grip strength (PNDs 10–13), open field activity (PND 30), passive avoidance (PND 35), and water maze (only tested at 53 mg/kg/day on PND 60). The rats were killed on PND 68. No significant alterations in body weight, food consumption, or water consumption were observed during gestation in the dams exposed to aluminum. The investigators noted that decreases in water and food consumption were observed during the lactation period in the rats exposed to 103 mg Al/kg/day, but the data were not shown and maternal body weight during lactation was not mentioned. No significant alterations in the number of litters, number of fetuses per litter, viability index, or lactation index were observed. Additionally, no differences in days at pinna detachment or eye opening were observed. Age at incisor eruption was significantly higher in males exposed to 53 mg/kg/day, but not in males exposed to 103 mg/kg/day or in females. A significant delay in age at testes descent was observed at 103 mg/kg/day and vagina opening was delayed at 53 and 103 mg/kg/day. A decrease in forelimb grip strength was observed at 103 mg/kg/day; no alterations in other neuromotor tests were observed. Additionally, no alterations in open field behavior or passive avoidance test were observed. In the water maze test, latency to find the hidden platform was decreased in the 53 mg/kg/day group on test day 2, but not on days 1 or 3; no significant alteration in time in the target quadrant was found.

The Golub and Germann (2001) and Colomina et al. (2005) studies identify four end points that could be used as the point of departure for derivation of the intermediate-duration oral MRL:

(1) latency to fall off wire in wire suspension test; adverse effect level of 130 mg Al/kg/day, no effect level of 26 mg Al/kg/day (Golub and Germann 2001);

(2) latency to locate the platform following cue relocation in the water maze test; adverse effect level of 130 mg Al/kg/day, no effect level of 26 mg Al/kg/day (Golub and Germann 2001);

(3) decreased forelimb grip strength; adverse effect level of 103 mg Al/kg/day, no effect level of 53 mg Al/kg/day (Colomina et al. 2005); and

(4) delay in vagina opening; adverse effect level of 53 mg Al/kg/day, no effect level not identified (Colomina et al. 2005).

Benchmark dose (BMD) modeling was considered for each of these end points. As discussed in Appendix A, BMD modeling was not used to identify the point of departure due to incomplete reporting of the data or because the models did not provide adequate fit.

Using a NOAEL/LOAEL approach, the NOAEL of 26 mg Al/kg/day identified in the Golub and Germann (2001) study was selected as the point of departure for the MRL. An MRL based on this NOAEL should be protective for neurological effects, neurodevelopmental effects, and for delays in maturation. Dividing the NOAEL by an uncertainty factor of 100 (10 to account for the extrapolation from mice to humans and 10 for human variability) and a modifying factor of 0.3 to account for possible differences in the bioavailability of the aluminum lactate used in the Golub and Germann (2001) study and the bioavailability of aluminum from drinking water and a typical U.S. diet results in an MRL of 1 mg Al/kg/day. No studies were identified that estimated the bioavailability of aluminum lactate following long-term dietary exposure; however, a bioavailability of 0.63% was estimated in rabbits receiving a single dose of aluminum lactate (Yokel and McNamara 1988). Yokel and McNamara (2001) and Powell and Thompson (1993) suggest that the bioavailability of aluminum from the typical U.S. diet was 0.1%; the bioavailability of aluminum from drinking water ranges from 0.07 to 0.39% (Hohl et al. 1994; Priest et al. 1998; Stauber et al. 1999; Steinhausen et al. 2004). These data suggest that aluminum lactate has a higher bioavailability than aluminum compounds typically found in drinking water or the diet.

- An MRL of 1 mg Al/kg/day has been derived for chronic-duration oral exposure (365 days or longer) to aluminum.

A small number of animal studies examined the chronic toxicity of aluminum. Schroeder and Mitchener (1975a, 1975b) examined the systemic toxicity of aluminum following lifetime exposure of rats and mice to very low doses of aluminum sulfate in the drinking water. Although the levels of aluminum in the diet were not reported, they are assumed to be low because the animals were fed a low-metal diet in metal-free environmental conditions. Studies conducted by Roig et al. (2006) and Golub et al. (2000) primarily

focused on the neurotoxicity of aluminum following lifetime exposure (gestation day 1 through 24 months of age). In the Golub et al. (2000) study, significant decreases in forelimb and hindlimb grip strength, and a decrease in thermal sensitivity were observed in mice exposed to 100 mg Al/kg/day; negative geotaxis was significantly altered at 18 months, but not at 24 months. No effect on horizontal activity was observed. A 10% increase in body weight and a 20% decrease in body weight were observed in the males and females, respectively. In a companion study by this group, no significant cognitive impairments were found in the Morris water maze test; in fact, aluminum-exposed mice performed better than controls in the learning tasks. Roig et al. (2006) also found no significant alterations in performance on the Morris water maze in rats exposed to 100 mg Al/kg/day as aluminum nitrate in the drinking water (with added citric acid). Although significant differences were found between the two aluminum groups (50 and 100 mg Al/kg/day); this was primarily due to the improved performance (as compared to controls, no significant differences) in the 50 mg Al/kg/day group. Roig et al. (2006) also found no significant alterations in open field activity.

Based on the results of these chronic-duration studies, the decreases in forelimb and hindlimb grip strength and the decrease in thermal sensitivity identified in the Golub et al. (2000) study were selected as the critical effect for derivation of a chronic-duration oral MRL for aluminum. The selection of these end points, and neurotoxicity in general, is well supported by the findings of a number of intermediate-duration studies that indicate that this is one of the most sensitive targets of aluminum toxicity (Colomina et al. 2005; Donald et al. 1989; Golub and Germann 2001; Golub et al. 1992a, 1995).

In the Golub et al. (2000) study, groups of 8 male and 10 female Swiss Webster mice were exposed to 7 or 1,000 μg Al/g diet as aluminum lactate in a purified diet. The investigators estimated adult doses of <1 and 100 mg/kg/day. The mice were exposed to aluminum from conception (via feeding the dams) through 24 months of age. Body weight, food intake, and clinical signs were determined during the last 6 months of the study. A neurobehavioral test battery (foot splay, temperature sensitivity, negative geotaxis, and grip strength), 1 hour spontaneous activity measurement, and auditory startle tests were conducted at 18 and 24 months. In a companion study, groups of 6–9 male and female Swiss Webster mice or 7 male and female C57BL/6J mice (number per sex were not reported) were exposed to 7 or 1,000 μg Al/g diet as aluminum lactate in a purified diet (<1 and 100 mg/kg/day) from conception (via feeding the dams) through 24 months of age. Body weight, food intake, and clinical signs were determined during the last 6 months of the study. A neurobehavioral test battery (foot splay, temperature sensitivity, negative geotaxis, and grip strength) and Morris maze testing were performed at 22–23 months of age. In the principal study, no significant alterations in mortality were observed. A

significant decrease in body weight was observed in the female mice (approximately 20%). In the males, there was a significant increase in body weight (approximately 10%). No significant alterations in food intake were observed in either sex. However, food intake/g body weight was significantly higher in the aluminum-exposed mice. No significant alterations in the occurrence of clinical signs or indications of neurodegenerative syndromes were found. Significant increases in relative spinal cord, heart, and kidney weights were found. Significant alterations in negative geotaxis and tail withdrawal time in the temperature sensitivity test (males only) were observed at 18 months. At 24 months, significant alterations in forelimb and hindlimb grip strength and temperature sensitivity were found in male and female mice. Forelimb and hindlimb grip strengths were decreased and thermal sensitivity was decreased, as evidenced by an increase in tail withdrawal times. Auditory startle response tests could not be completed in the older mice. Similarly, vertical spontaneous movement could not be measured; no effect on horizontal movement was found. In the companion study, no alterations in neurobehavioral battery test performance were observed; the investigators note that this may be due to the small number of animals per group. In general, aluminum-exposed mice performed better on the water maze test than controls.

A chronic-duration oral MRL was derived using the LOAEL of 100 mg Al/kg/day for decreased forelimb and hindlimb grip strength and decreased thermal sensitivity identified in the Golub et al. (2000) study. A BMD approach for deriving an MRL was not utilized because the Golub et al. (2000) study only tested one aluminum group. The MRL of 1 mg Al/kg/day was calculated by dividing the LOAEL of 100 mg Al/kg/day by an uncertainty factor of 300 (3 for use of a minimal LOAEL, 10 for extrapolation from animals to humans, and 10 for human variability) and a modifying factor of 0.3 to account for possible differences in the bioavailability of the aluminum lactate used in the Golub and Germann (2001) study and the bioavailability of aluminum from drinking water and a typical U.S. diet. No studies were identified that estimated the bioavailability of aluminum lactate following long-term dietary exposure; however, a bioavailability of 0.63% was estimated in rabbits receiving a single dose of aluminum lactate (Yokel and McNamara 1988). Yokel and McNamara (2001) and Powell and Thompson (1993) suggest that the bioavailability of aluminum from the typical U.S. diet was 0.1%; the bioavailability of aluminum from drinking water ranges from 0.07 to 0.39% (Hohl et al. 1994; Priest et al. 1998; Stauber et al. 1999; Steinhausen et al. 2004). These data suggest that aluminum lactate has a higher bioavailability than aluminum compounds typically found in drinking water or the diet.