Annex of the opinion on Safety of aluminium from dietary intake[1]

Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials (AFC)

(Question Nos EFSA-Q-2006-168, EFSA-Q-2008-254)

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1.1. Oral exposure

Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact materials and food additives. Aluminium is naturally present in varying amounts in most foodstuffs and levels in food crops are influenced by geographical region. A low pH value of the soil (acid rain) increases the solubility of aluminium in the soil leading to a higher aluminium content of the plants. The use of aluminium and its compounds in processing, packaging and storage of food products and as flocculants in the treatment of drinking-water may contribute to its presence in drinking water and foodstuffs, and a number of aluminium salts are used as food additives (Table 1).

Table 1. **Aluminium-containing food additives (other than colours and sweeteners) authorised for use in the European Union (Directive 95/2/EC modified)**

E No	Name	Foodstuff	Maximum level
E 520	Aluminium sulphate	Egg white	30 mg/kg
E 521	Aluminium sodium sulphate		
E 522	Aluminium potassium sulphate	Candied, crystallized and glacé fruit and vegetables	200 mg/kg, Individually or in combination, expressed as aluminium
E 523	Aluminium ammonium sulphate		
E 541	Sodium aluminium phosphate, acidic	Fine bakery wares (scones and sponge wares only)	1 g/kg expressed as aluminium
E 554	Sodium aluminium silicate	Dietary food supplements	quantum satis
E 555	Potassium aluminium silicate	Foodstuffs in tablet and coated tablet form	quantum satis
E 556	Calcium aluminium silicate		
E 559	Aluminium silicate (Kaolin)	Rice	quantum satis
		Sausages (surface treatment only)	quantum satis
		Confectionery excluding chocolate (surface treatment only)	quantum satis
		Seasonings	30 g/kg
		Tin-greasing products	30 g/kg
		Dried powdered foodstuffs (including sugars)	10 g/kg
		Salt and its substitutes	10 g/kg
		Sliced or grated hard, semi-hard and processed cheese	10 g/kg
		Sliced or grated cheese analogues and processed cheese analogues	10 g/kg
		Chewing gums	
E 555	Potassium aluminium silicate		In E 171 titanium dioxide and E 172 iron oxides and hydroxides (max 90 % relative to the pigment)
E 558	Bentonite	As carriers:	Colours, max. 5 %
E 559	Aluminium silicate (Kaolin)		Colours, max. 5 %

E No	Name	Foodstuff	Maximum level
E 1452	Starch aluminium octenyl succinate	Encapsulated vitamin prepartions in food supplements as defined in Directive 2002/46/EC	35 g/kg in food supplements

1.2. Aluminium content of foodstuffs

During the last three decades there have been many reports of the aluminium concentrations in foods and beverages. In the following text results of several studies in European countries are summarised.

In 1988 and 1991, in Germany the aluminium content of 128 items out of 12 food categories was determined (Müller *et al*., 1998). Many unprocessed foods (with the exception of some herbals and tea leaves) typically contain < 5 mg/kg aluminium. Mean concentrations (in mg/kg fresh weight) for individual products from the group 'Bread, cakes and pastries', some vegetables, sausage, offal, sugar-rich foods and a majority of farinaceous products and flours ranged from 5 to 10 mg/kg. Items with a higher mean concentration include herbs, cocoa and cocoa products, and spices. Within the group 'Bread, cakes and pastries', biscuits proved to be the item highest in aluminium (22 mg/kg), as was soft cheese in the group 'Dairy products' (8.3-16 mg/kg) and mixed mushrooms and lettuce in the group 'Vegetables' (17 ad 33 mg/kg, respectively). The authors suggest that the relatively high aluminium concentration in biscuits may result from the use of aluminium-containing food additives. The Panel noted that since the analysis of aluminium partly refers to foodstuffs from East Germany before the re-unification, the use of aluminium as food additives might have deviated from the EU permitted uses. For soft cheese the highest aluminium concentration is probably related to the use of aluminium-containing food additives. Tea leaves, especially black tea may contain high levels of aluminium. Black tea infusion (normally prepared) had an aluminium concentration of 4.2 mg/l. Results are summarised in Table 2.

Table 2. **Aluminium concentrations of different food categories in Germany (mg/kg fresh weight)*** [2]

Food category	Mean	Range
Beverages	1.5	0.4-2.6
Fruit	2.7	0.7-7.9
Fish, tinned fish	3.2	1.2-5.5
Milk, dairy products	4.5	1.2-16
Meat, sausage, offal	5.4	2.5-10
Table salt	5.6	0.5-15
Vegetables	5.7	0.7-33
Sugar, sugar-rich products	6.7	3.4-12
Bread, cake and pastries	7.4	3.4-22
Pulses	9.3	3.2-16
Farinaceous products and flours	9.5	3.8-34
Herbs	19	8.2-26
Cocoa, cocoa products	33	9.4-103
Spices**	145	6.5-695

2 The Panel noted that since the analysis of aluminium partly refer to foodstuffs from East Germany before re-unification, the use of aluminium as food additives might deviate from the EU permitted uses.

*Source: Müller *et al*., 1998.
** dry weight

Generally, in most studies conducted in other European countries all foodstuffs analysed had a relatively large variation in their aluminium concentration within or lower than the ranges reported in Table 2.

Compared with other studies a lower variation was observed in Sweden, where the concentrations of aluminium in a number of individual unprocessed foodstuffs present on the Swedish market (fruit, vegetables, cereals, milk, beverages, meat and fish) have been determined (Jorhem and Haegglund, 1992). In the Swedish study the highest aluminium concentrations were found in cereals, tea and prawns, all around 1 mg/kg. In general, the concentrations in most of the staple foodstuffs were much lower than reported in other studies.

Extended data on the aluminium content of food items in France are available from the 1st French Total Diet Study, conducted in 2000. The results showed that an aluminium level > 3 mg/kg fresh weight was only observed in the following food groups: 'Bread & rusk' (4.1 mg/kg), 'Biscuits' (5.3 mg/kg), 'Vegetables' (3.2 mg/kg), 'Nuts and oilseed' (4.1 mg/kg), 'Ice-cream' (3.9 mg/kg), 'Chocolate' (3.7 mg/kg), 'Salads' (4.9 mg/kg) and 'Shellfish' (17.1 mg/kg) (Leblanc *et al*., 2005). Mushrooms, spinach, radish, swiss card, lettuce and corn salad having the highest levels in the vegetables group ranged from 5 to 150 mg/kg.

In the UK, the most recently reported aluminium concentrations in foodstuffs come from the 2000 UK Total Diet Study (FSA, 2004). Most food categories had aluminium concentrations lower than 3 mg/kg (fresh weight) and the results were lower or similar than those reported in the 1997 Total Diet Study (Ysart *et al*., 2000). Higher levels were found in the food groups 'Sugar and Preserves' (4.1 mg/kg), 'Nuts' (5.7 mg/kg) and particularly in the group 'Miscellaneous Cereals' (19 mg/kg). The mean aluminium concentration in the 'Miscellaneous Cereals' food category was considerably higher than in the 1988 (4.8 mg/kg) and 1997 UK Total Diet Study (5.2 mg/kg) (MAFF 1993 and Ysart *et al*., 2000) but lower than in the 1994 (78 mg/kg) and 1991 Total Diet Study (64 mg/kg) (MAFF 1998, Ysart *et al*., 1999; Ysart *et al*., 2000). In 2000 this food category was made up of flour (8%), buns, cakes & pastries (17.9%), chocolate biscuits (6.9%), other biscuits (11.5%), breakfast cereals (18.4%), rice (9.7%) and other cereal products e.g. frozen cakes, pizza, pasta dried and canned, custard powder, instant puddings, cake mixes (27.6%) (personal communication FSA, 2007). The Panel noted that cereal plants show a high capacity to accumulate aluminium due to the depth and extent which their roots reach into the subsoil. Moreover, the food additives used in some bakery products might alsomake an important contribution to the aluminium content of this food category.

In Ireland, preliminary occurrence data including data from the first Irish total diet study showed a large variation in the aluminium content of 'Bread' (<5-76 mg/kg), 'Cakes and pastry' <5-179 mg/kg) and 'Glacé fruits' (<5-145 mg/kg). In 'Baking mixes' the concentration was about 13 mg/kg, whereas in 'Cheese' and 'Salt' aluminium levels < 5 mg/kg were found (Personal communication I. Pratt, 2007).

In Spain, high aluminium concentrations in 'Spices' and 'Aromatic herbs' were reported by López *et al*. (2000). Aluminium levels ranged from 3.7 to 56.5 mg/kg (dry weight), with most elevated levels found in some samples of cinnamon, mustard, oregano and paprika. In this country the aluminium content in convenience and fast foods was also studied (López *et al*., 2002). The results showed considerable amounts in many convenience foods ranging from 0.85 to 38.1 mg/kg food (fresh weight). The more elevated aluminium concentrations were detected in food with a greater content of spices and aromatic herbs, pasta, certain vegetables and

mushrooms. The highest mean aluminium concentrations were encountered in pork-based foods (mean 8.45 mg/kg, range 2.85-16.85 mg/kg) and in chicken-based foods (mean 13.9 mg/kg, range 4.0-38.1 mg/kg). A moderate increase in aluminium content was observed in convenience and fast food in aluminium vessels; this content increased slightly during heating, especially in acidic foods (containing tomato, different types of pickles and vinegars). The authors estimated that in Spain convenience and fast food contributed approximately 0.47 mg/day per capita to the exposure to aluminium.

1.3. Dietary exposure to aluminium

Total dietary exposure to aluminium from all sources has been assessed with duplicate diet studies in some European countries. In this approach, the individual diets as consumed are analysed and the methods used do not distinguish between the different compounds and sources. The aluminium concentrations reported in duplicate diet studies are the sum of the aluminium content of all the aluminium compounds. These aluminium compounds derive from different sources: natural presence, contribution by food additives and introduction by processing and storage. Therefore these studies do not allow the identification of the sources of aluminium within the diet. As shown in Table 3 mean dietary exposure to aluminium ranged from 2.5 to 13 mg/day.

Table 3. **Dietary exposure to aluminium (mg/day) determined in duplicate diet studies in several European countries**

Country	Year of Investigation	Mean (range)	Reference	Remarks
Netherlands	1978	4.6(1.4 – 33.3)	Ellen *et al*., 1990	101 adults (26 females and 75 males), one 24 h sample each
Netherlands	1984 – 1985	3.1 (0.6 – 12.9)	Ellen *et al*., 1990	110 adults (53 females and 57 males) one week sample each
Hungary	1989 – 1990	3.3 (0.3 – 19.4)	Gergely *et al*., 1991	84 samples
Germany	1988 1991/ 2 1996	5.4/6.5 4.6/4.9 3.1/3.2	Anke *et al*., 2001	Females/Males mixed diet
Germany	1996	4.1/4.1	Anke *et al*., 2001	Females/Males; ovo-lacto vegetarian diet
Italy	1989-1990	2.5 / 3.1 / 4.3 / 6.3	Gramiccioni et al., 1996	4 different regions (overall 19 24 h samples)
Sweden	Not reported	13.0 (1.2-100)	Jorhem and Haegglund, 1992	105 duplicate diets in 15 non smoking females
France	1999	2.03	Noël *et al*., 2003	calculated from 100 duplicate meals from catering establishments

Data reported in Germany suggest that the amount of aluminium in the diet of adults decreased by about half from 1988 to 1996. This decrease may in part be due to the reductions in sulphur emission, which lowered the soil pH values as well as to the more thorough cleaning of vegetables, fruits and spices (Anke *et al*., 2001). The Panel noted that the 1988 survey had been conducted in the former East Germany, whereas the other studies had been conducted after re-unification. Before that time the use of aluminium as food additive in East Germany might have

deviated from the EU permitted uses. Changes in food processing, pollution and differences in geographical regions in the 1996 studies might have influenced the dietary exposure figures as well. For children aged 5-8 years in the former West Germany, a relatively low dietary exposure to aluminium was already observed in a duplicate study conducted in 1988. Potential mean and high level exposure (90th percentile) in this study was 0.78 and 1.32 mg/day, respectively (Wilhelm *et a.l*, 1995).

Although in Sweden the aluminium levels of unprocessed foodstuffs seemed to be relatively low (see above), Table 3 shows that the highest mean exposure assessed with the duplicate portion technique, was observed in this country. The exposure was based on 105 duplicate diets, collected by 15 women working at the same institute (Jorhem and Haegglund, 1992). The major contributor to aluminium exposure was found to be a chocolate-mint cake, present in six of the diets. This cake was made from a mix with acidic sodium aluminium phosphate in the baking soda. If these six diets were excluded, the mean dietary exposure was reduced from 13.0 to 9.7 mg/day.

In addition market basket and total diet studies have been performed, allowing the assessment of mean dietary exposure in the general population and in different population groups based on a mean concentration of aluminium in each food category. In this approach, all food items which are part of the average diet are purchased, prepared according to standard household procedures, eventually aggregated into a number of food categories, and analysed. As is the case with duplicate diet studies, these studies do not allow the identification of the sources (natural or added) of aluminium within the diet. The UK Total Diet Study basically involves 199 food categories combined into 20 groups of similar foods for analysis. The relative proportion of each food category within a group reflects its importance in the average diet and is based on food consumption surveys (Ysart *et al*., 2000). In France, 998 composite samples of 300 individual food items, representative for the French diet, were analysed 'as consumed' and then combined with data from the national individual dietary survey to calculate exposures to aluminium (Leblanc *et al*., 2005).

Dietary exposure estimated through market basket studies in Europe ranged from 1.3 mg/day in French children to 11 mg/day in UK (Table 4). In 1991 and 1994, the estimated UK population exposure to aluminium was much higher than in the other years. As mentioned before, this may be partly due to differences in the use of aluminium-containing additives in bakery products, and/or in the individual products included in this food group in different years (Ysart *et al*., 2000).

Table 4. **Exposure to Al (mg/day) calculated with the market basket method or a model diet in several European countries**

Country	Year of Investigation	Mean	References	Remarks
UK	1988	3.9	Ysart *et al*., 1999	
	1991	10	Ysart *et al*., 1999	
	1994	11	Ysart *et al*., 1999	
	1997	3.4	Ysart *et al*, 2000	
	2000	4.7	FSA 2004	
Finland	1975 - 1978	6.7	Varo and Koivistoinen 1980	
France	2000	1.3	Leblanc *et al*., 2005	3-15 years
		1.6		15 years and above

In the most recent UK Total Diet Study 'Miscellaneous cereals' contributed 45% to the mean daily exposure and 30% came from 'Beverages' (excluding mineral water and tap water). 'Bread' contributed 7%, 'Sugar & preserves' 5%, the other food categories contributed 3% or less (FSA, 2004). According to the French total diet study for adults 'Bread' and 'Vegetables' were the most important aluminium sources (25 and 20%, respectively), 'Buns, Cakes & Biscuits' contributed 8.5%, 'Hot beverages' and 'Mixed dishes' each 5% and the other food categories 3% or less (Leblanc *et al.*, 2005). In French children aged 3 to 14 years 'Vegetables' and 'Buns, 'Cakes & Biscuits' were principal sources (each contributed 18%), 'Bread' contributed about 15%, 'Mixed Dishes' 6%, and the food categories 'Breakfast Cereals', 'Sugar & Confectionery', 'Desserts' and 'Non-alcoholic Beverages' 4% each.

In France and UK data from Total Diet Studies were also used to assess the exposure to aluminium for average and high consumers (97.5th percentile) in different age groups. The distribution of estimated dietary exposure to aluminium in the population was assessed using individual food consumption data collected within national food consumption surveys conducted in France and UK, respectively. These estimates allow to capture the variability of dietary exposure in the population. As shown in Table 5, in France the 97.5th percentile exposure for children (0.7 mg/kg bw/week) was higher than for adults (0.4 mg/kg bw/week). This was also the case in the UK. Based on the UK 2000 Total Diet Study these estimates ranged from approximately 0.9 mg/kg bw/week for adults, non-institutionalised elderly and vegetarians to 2.3 mg kg bw/week for toddlers (FSA, 2004).

Table 5. **Estimated dietary exposure to aluminium (mg/kg bw/week) from Total Diet Studies in France and UK**

Country	Population group	Estimated dietary exposure mg/kg bw per week	
		Mean	High level 97.5th percentile
France[a]	Children (3-15 years)	0.3	0.7
	Adults (15+ years)	0.15	0.4
UK[b]	Toddlers (1.5-4.5 years)	1.16	2.29
	Young people (4-18 years)	0.84	1.71
	Adults	0.47	0.94
	Elderly (non-institutionalised)	0.41	0.88
	Elderly (living in care)	0.57	1.14
	Vegetarians	0.50	0.93

[a]Leblanc *et al.* 2005

[b] FSA, 2004

FSA (2006) estimated dietary exposure to aluminium from all foods for infants of different ages using manufacturers' feeding instructions and recommendations for infant formulae consumption and maximum recommended amounts of solid foods from three manufacturers' example menus. Dietary exposure to aluminium was calculated using the mean analysed concentrations in infant formulae (altogether 48 samples of starter infant formulae, and follow-up infant formulae) and in solid foods (153 samples of solid foods including processed savoury baby food products, breakfast, rusks and breadsticks, biscuits, cereal bars and rice cakes, desserts, baby rice and fruit purées). Mean daily exposure to aluminium was 0.015 mg/kg bw, 0.029 mg/kg bw, 0.061 mg/kg bw and 0.112 mg/kg bw for infants aged 0-3, 4-6, 7-9 and 10-12 months, respectively. In this assessment the alumium content of water used to reconstitute dried or concentrated infant food and formulae was not taken into account (FSA, 2006).

According to the literature the mean or median aluminium concentration in human breast milk ranges from 0.009 to 0.380 mg/l (Baxter *et al*., 1991; Fernandez-Lorenzo *et al*., 1999; Hawkins *et al*., 1994; Koo *et al*., 1988; Simmer *et al*., 1990; Weintraub *et al*., 1986, Mandíc *et al*., 1995). Recently, JECFA estimated dietary exposure to aluminium for infants aged 3 months and fed human milk; using a concentration of < 0.05 mg/l and consumption levels of 0.7 l at the mean and 1 l at the 95th percentile. These consumption values are derived from the German DONALD study (Kersting, 1998): in a 3 month infant weighing on average 6.1 kg, the average and 95th percentile consumption of dry infant formula are respectively 105 and 144 g/day. Considering a 1:7 dilution factor, these data correspond to respectively 0.7 l/day and 1 l/day of reconstituted formulae. Consumption at the 95th percentile is therefore around 1.4 time average consumption.

Based on an average daily consumption of 0.7 l, breastfed infants would therefore be exposed to less than 0.03 mg/day, i.e. less than 0.005 mg/kg bw/day, assuming a body weight of 6.1 kg. High consumption (1 l/day) would lead to an exposure of less than 0.01 mg/kg bw/day i.e. less than 0.07 mg/kg bw/week. (JECFA, 2007).

In general, the concentration of aluminium is higher in infant formulae than in human milk. In several studies high concentrations of aluminium were found in soy-based powder for preparing infant formula (Jorhem and Haegglund, 1992; Fernandez-Lorenzo *et al*., 1999; FSA, 2003; FSA, 2006). In these studies, the alumium content of water used to reconstitute dried or concentrated infant formulae was not taken into account. Recently, Navarro-Blasco and Alvarez-Galindo (2003) determined the aluminium content of 8 different types of infant formula from the Spanish market (a total of 82 samples). In milk-based adapted starter and follow-up formulae mean aluminium values were respectively 0.252 mg/l and 0.292 mg/l. In soy-based infant formula, mean aluminium concentrations was 0.930 mg/l (7 samples). Mean aluminium concentrations in other types of infant formula were 0.449, 0.574, 0.687, 0.237 and 0.453 mg/l for preterm formula, lactose-free formula, hypoallergenic formula, non adapted starter formula and inborn metabolic errors diet formula.

Potential exposure to aluminium for infants from a variety of formulae (including water used in the reconstitution) was estimated by the Panel based on those reported analytical determination performed by Navarro-Blasco & Alvarez-Galindo (2003).

Estimated average dietary exposure based on the consumption of 0.7 l per day in a 3 month infant weighting 6.1 kg ranged from 0.2 to 0.6 mg/kg bw/week in milk-based formulae and was 0.75 mg /kg bw/week for soya-based infant formulae. Estimated dietary exposure based on the high consumption of 1 l per day ranged from 0.3 to 0.9 mg/kg bw/week in milk-based formulae and was 1.1 mg /kg bw/week for soya-based infant formulae. (Table 6).

Table 6. **Average estimated exposure to aluminium for infants aged 3 months fed on different types of infant formulae**

Formula	Daily estimated exposure (mg/kg bw)**	Weekly estimated exposure (mg/kg bw)**
Non-adapted starter formula	0.03	0.20
Adapted starter formula	0.03	0.23
Lactose free formula	0.08	0.54
Inborn metabolic errors formula	0.08	0.56
Hypoallergenic formula	0.09	0.64
Soy formula	0.11	0,75

* based on meian concentrations in infant formula considering the contribution from water used in the reconstitution (Navarro Blasco and Alvarez-Galindo (2003) .

** assumed body weight 6.1 kg

The Panel noted that in the study of Navarro-Blasco and Alvarez-Galindo (2003) the highest reported aluminium concentration for both soya-based formulae and milk-based formulae was around 4 times higher that the mean concentration estimated above, leading to a 4 times higher potential exposure in brand-loyal infants.

.The dietary exposure estimates presented above include foods that have been in contact with aluminium foil or aluminium containers or that have been cooked in aluminium utensils. It is clear that these storage and preparation steps contribute to the daily intake of aluminium especially for acidic foods (Fairweather-Tait *et al.*, 1987; Gramiccioni *et al.*, 1996; ATSDR, 1999; IPCS, 1997; Neelam *et al.*, 2000, López *et al.*, 2002). Ranau *et al*. (2001) showed that aluminium concentrations of both baked and grilled fillets wrapped in aluminium foil increase during heating, with the higher increase in grilled filets. These authors concluded that aluminium migration seems to depend on several factors such as the duration and temperature of heating, the composition and the pH-value of food, and the presence of other substances (e.g. organic acids, salt and other ions). From the study of Gramiccioni *et al.* (1996), designed to enhance aluminum migration, it appears that in extreme and occasional worst case situations, assuming that a particularly acidic meal is completely prepared or stored in aluminium containers, in the presence of unusual and border line conditions for cooking and storage, the migration would contribute to about 2 mg/ meal. The major contribution to the total amount of migrated aluminium was given by pickles simulated to be into contact with aluminium trays for 24 hours in summertime conditions (40°C) and by water boiled in aluminium pot before drinking. A ten fold lower total amount of aluminium (about 0.2 mg/meal) migrated in the other foods of the simulated meal. The highest increment of aluminium due to migration during cooking was found in tomato sauce. This is in agreement with other studies. According to Greger *et al*. (1985) a 100 g serving of tomato sauce cooked in an aluminium pan for 180 minutes might contain as much as an additional 5.7 mg of aluminium. A serving of 100 g of applesauce would accumulate 0.7 mg aluminium. However, for most foods the amount of aluminium added by cooking in aluminium utensils would amount to 0.1 mg aluminium per 100 g serving (Greger *et al*., 1985).

It is widely known that aluminium cans and multilayer carton packagings have interior protective polymeric coatings or layers that prevent the contact between the foods and the aluminium surface. This is in line with the outcome of some studies (Sepe *et al*., 2001, Abercrombie *et al*, 1997) where it was found that in some liquid foods, packed in cans or in cartons (e.g. soft drinks, tea, fruit juices) increments of the aluminium already present did not occur.

Therefore, it appears that under normal and typical conditions the contribution of migration from food contact materials would represent only a small fraction of the total dietary exposure, particularly in countries where most pans nowadays are made of stainless steel or polytetrafluoroethylene-coated aluminium (Greger *et al*., 1985; Müller *et al*., 1993; WHO, 1997).

As mentioned before, presently it is not possibly to conclude on the specific sources contributing to the aluminium content of a particular food or food group. To get an indication on the importance of some additives the Panel was provided with some additional information. A per capita exposure estimate to acidic sodium aluminium phosphate (SALP) in Europe was provided by EFPA (2005). Currently the EC Directive 95/2/EC for food additives other than colors and sweeteners permits the use of E 541, SALP, acidic, in the EU in fine bakery wares (scones and sponge wares only) with a maximum level of 1g/kg expressed as aluminium. SALP

acidic is also permitted in Norway (with the same provisions as in the EU) and in Switzerland (various authorizations at different levels in baking and other applications). The average exposure per capita was estimated by dividing the total annual sales of SALP in the European Region (about 852 tons) by the estimated number of residents (380 million). This estimate results in an average per capita intake 2.24 g SALP per year, corresponding to a daily intake of 6.1 mg SALP per resident. Based on a standard body weight of 60 kg, the average per capita exposure was estimated to be about 0.1 mg SALP/kg bw/day. The amount of aluminium in SALP is approximately 11%, the potential daily per capita exposure to aluminium caused by SALP is therefore approximately 0.01 mg/kg bw (EFPA, 2005).

The Panel noted that it should be kept in mind that the uses of sales data have several limitations. These per capita estimates are prone to underreporting. Moreover, the consumption of SALP is not homogeneously distributed among European countries and consumers and is likely to be significantly higher in those areas where scones and spongy bakery wares are most frequently consumed. According to FSA, based on UK national food consumption surveys (Gregory et al., 1995; Gregory et al., 2000; Henderson et al., 2002; Finch et al., 1998) the mean consumption of scones and spongy bakery wares range from 6 g/day (toddlers) to 33 g/day (elderly living in care), with an overall mean consumption of 14 g/day per capita.

The Panel was also provided (NATCOL, 2007) with some information on current usage levels for aluminium lakes of natural colours, aluminium concentrations resulting from use of colour lakes and an estimate of potential exposure to aluminium from use in colour lakes in UK pre-school children (1.5-4.5 years) (Gregory et al., 1995). This age group was selected because they are likely to have the highest consumption on a body weigh basis of the major coloured food items – confectionery and edible ices. Estimates of average dietary exposure to aluminium varied from 0.01 mg/kg bw/week for Cu-chlorophyllin (E141) to 0.44 mg/kg bw/week for anthocyanin (E163). For high consumers the exposure, based on a single food category, ranged from 0.01 mg/kg bw/week for carmine (E120) in for instance the food category edible cheese rind and edible casings to 2.40 mg/kg bw/week for anthocyanin (E163) in edible ices and 3.84 mg/kg bw/week for curcumin (E100) in compressed confectionary. According to the data provided, curcumin lakes are known to be used only in one brand of compressed confectionary products in Europe whereas anthocyanin lakes would be very rarely used in edible ices because they produce a purple/blue colour that does not correspond to many flavours. The Panel noted that the provided estimates are conservative, assuming that consumers always select items containing these lakes. However, data on aluminium lakes of synthetic colours are missing and estimates based on aluminium lakes in natural colours show that in specific cases the contribution to aluminium exposure could be substantial.

1.4. Drinking water

The concentration of dissolved aluminium in untreated water at near pH 7 is typically 1-50 µg/l, but this can increase to 1000 µg/l in acidic water (Yokel, 2004). Exposure through this source is therefore up to 2 mg/day, corresponding to 0.03 mg/kg bw/day based on the consumption of 2 l of water per day. Aluminium may also be present in drinking-water owing to the use of salts of aluminium as flocculants in the treatment of surface waters (e.g. aluminium sulphate, aluminium polychloride). The concentration of aluminium in water after completion of treatment is usually less than 0.2 mg/l. Based on a daily consumption of 2 l per day, dietary exposure to aluminium from treated drinking-water may be up to 0.4 mg/day, corresponding to 0.007 mg/kg bw/day (JECFA, 2007).

1.5. Air

Pulmonary exposure to aluminium is determined by air concentration, particulate size and ventilatory volume. Air concentrations vary between rural and urban settings, with higher levels in industrial areas. Pulmonary exposure may contribute up to 0.04 mg/day (WHO, 1997).

1.6. Miscellaneous

Aluminium compounds are found in over-the-counter medicinal products such as aluminium hydroxide in antacids to control gastric hyperacidity, aluminium oxide in dental ceramic implants and a number of aluminium compounds in buffered aspirin (Brusewitz 1984; FDA 2002; NIH 2004; NRC 1982). Antacids may contain between 104 and 208 mg of aluminium per tablet/capsule/5mL dose (Zhou and Yokel 2005). It has been estimated that daily doses of aluminium in antacids and buffered analgesics range from 840 to 5000 mg and from 130 to 730 mg per day, respectively (Lione 1983).

Aluminium compounds are also used in the manufacture of topically applied products such as antiperspirants, first aid antibiotics and antiseptics, products for diaper rash and prickly heat, insect sting and bite and dry skin and in sunscreen and suntan products. Compared to oral sources these may be minor contributers to exposure to aluminium.

As published in the medical literature, in people of all ages with impaired renal function, aluminium accumulations may be found in the tissues and fluids of individuals undergoing treatments such as hemodialysis or receiving aluminium-containing medications (e.g. phosphate binders). Also premature infants have higher body burdens of aluminium than other infants. These risk groups can be considered as groups with special medical care.

1.7. Occupational Exposure

Occupationally exposed populations have background exposure levels similar to those of non-occupationally exposed populations, but depending on specific work tasks performed, the type and form of aluminium compound encountered and the adequacy of work place hygiene practices, their work can lead to significantly increased exposure to aluminium. Inhalation is the most important route but the extent of pulmonary uptake and retention has not been determined in most occupational settings. Based on limited data, daily occupational aluminium exposure can range from <1 mg to 40 mg per 8 h shift (WHO, 1997).

2. Absorption, distribution and excretion

This section has made substantial use of two recent, major reviews on aluminium, one from the US Agency for Toxic Substances and Disease Registry (ATSDR, 2006) and the other submitted by the International Aluminium Institute (IAI, 2007).

2.1. Introductory remarks on aluminium complexing

Metallic aluminium dissolves in diluted non oxidant acids such as hydrochloric acid in aqueous media, to form solutions containing the aluminium ion. Under similar conditions aluminium-containing compounds dissociates into the aluminium ion. In acidic solutions with pH <5, such as in the stomach, the aluminium ion exists mainly as $[Al(H_2O)_6]^{3+}$, usually abbreviated as

Al^{3+}. The progressive physiological increase in pH when passing from the stomach to the intestine induces the formation of complexes with hydroxide and finally results in the formation of insoluble $Al(OH)_3$ in neutral solutions (Martin, 1991; Harris *et al.*, 1996).

However, in most biological systems only a minimal fraction of the total aluminium is in the state of Al^{3+}. The solubility of aluminium compounds above pH 4 is strongly dependent on the presence of ligand species: the more Al^{3+} that is hidden in stable complexes, the less is able to dissociate water to precipitate in the formation of insoluble aluminium hydroxide. Therefore the toxicokinetic properties of aluminium will depend on the properties of the complexes formed between Al^{3+} and dietary or biological ligands. Biological systems always contain many potential ligands, where the formation of ternary complexes such as aluminium-ligand-hydroxide occur (Harris *et al*., 1996). Both the free Al^{3+} and the aluminium complexes tend to hydrolyse at or below neutral pH. As a consequence, mixed aluminium-ligand-hydroxide complexes are usually present at physiological pH where hydroxo-bridged polynuclear complexes are also common (Harris *et al.*, 1996).

In biological systems, the Al^{3+} mainly binds to oxygen atoms, especially if they are negatively charged. Hydroxide, citrate, phosphate and nucleoside phosphates are reported to be the most important low molecular mass ligands for Al^{3+}. On the basis of a rough comparison of the ability of various ligands to complex Al^{3+}, by calculating the amount of free Al^{3+} at pH 7.4, a decreasing binding capacity was suggested along the series citrate, catecholamines, ATP, inorganic phosphate and a lower comparable capacity for hydroxide, lactic acid and oxalic acid (Harris *et al.*, 1996). Amines are reported not to be strong Al^{3+} binders, except as part of multidentate ligand systems such as EDTA (Martin, 1991). The nitrogenous bases of DNA and RNA do not strongly bind Al^{3+} and sulfydryl groups do not bind Al^{3+} (Martin, 1991). It is recognised that the most important high molecular mass bioligand for Al^{3+} is transferrin (Ohman *et al.*, 1994; Martin, 1991; Harris *et al.*, 1996).

The final amount of aluminium present in a compartment of a biological system is therefore the result of the multiple complexation and competition equilibria involving aluminium, water molecules and ligands, and also being influenced by pH, relative solubility, transport mechanisms, presence of other metals etc. (Martin, 1991; Harris *et al.*, 1996; Ohman, 1988).

2.2. Absorption

2.2.1. Introduction

The amount of a substance that is absorbed compared to the amount administered is termed its bioavailability. The extent to which aluminium is absorbed following oral administration depends upon the chemical species present in the gut lumen and the modification of the aluminium species in the gut prior to absorption. Absorption will also be influenced by complexing ligands (e.g. citrate, lactate, silicate) and competing ions (e.g. iron, magnesium, calcium). Some authors have suggested that acid digestion in the stomach would solubilise a substantial amount of the ingested aluminium compounds to the monomolecular species Al^{+3} (e.g. hydrated $Al(H_2O)_6)^{3+}$), which would then be converted to aluminium hydroxide as the pH is neutralised in the duodenum. The majority is then expected to precipitate in the intestine with subsequent faecal excretion, leaving only a minor fraction available for absorption. Because ionized molecules are usually unable to penetrate the lipid bilayer of cell membranes due to their low lipid solubility and as aluminium would be expected to be present primarily as the free ion, with associated waters of hydration, at the low pH of the stomach, non-carrier-mediated absorption would not be predicted from the stomach. Aluminium would therefore be expected to be better absorbed from the duodenum than from the stomach and because the

stomach is not an important site of aluminium absorption, this would imply that oral aluminium bioavailability is independent of the aluminium species ingested (Reiber *et al.*, 1995).

However, citrate and many other ligands have been shown to influence aluminium absorption, suggesting that the above hypothesis is an oversimplification. Aluminium complexes, particularly the presence of carboxylic acids such as citrate, are thought to improve the solubility in the intestine and hence increase the amount of aluminium available for intestinal uptake. On the other hand the presence of ligands, such as phosphate, phytate, and polyphenols, that can form insoluble salts or complexes with aluminium may inhibit its oral absorption (Yokel and McNamara, 2001; JECFA 2007). The mechanisms mediating aluminium absorption in the gastro-intestinal tract have been suggested to include passive (diffusion) and active (carrier- and vesicular-mediated) transport across intestinal cells as well as paracellular diffusion between the cells. An energy-dependent uptake process has also been suggested (IAI 2007).

Because the oral bioavailability of aluminium is low, and also reflecting the limited sensitivity of the analytical methods, in early studies it was necessary to administer large doses of aluminium in order to determine a significant increase of aluminium in blood, urine or tissues above the endogenous aluminium concentration. As the toxicokinetics of aluminium may be dependent on the dose it can be questioned whether these high doses adequately reflect the normal situation in humans at much lower doses. More recently (since 1991), studies have been conducted to estimate oral aluminium bioavailability using the aluminium-26 radioisotope (^{26}Al) coupled with measurements by high-energy accelerator mass spectrometry (AMS). The lower limit of detection (10^{-18} g) allows physiological levels of aluminium to be quantified. This method offers a more accurate assessment of the bioavailability of aluminium and therefore most attention is paid to such studies in this assessment (JECFA, 2007).

The oral bioavailability of aluminium has been determined using several methods. Measurement of blood levels appears to be a poor indicator of the extent of aluminium absorption and, whilst urinary excretion appears to provide a better estimation of aluminium absorption, it offers no information about retention in tissues such as bone. Balance studies in which absorption was estimated based on the difference between intake and faecal excretion, or studies where the retention was estimated based on the difference between intake and urinary plus faecal excretion have also been used. Some other studies estimated oral bioavailability from the sum of urinary aluminium excretion and levels of aluminium in bone (and liver and brain) tissue (ATSDR, 2006; IAI, 2007). Comparison of the "plasma aluminium concentration × time curve" or Area under Curve (AUC) after oral vs. i.v. dosing is the generally accepted method for determining the oral bioavailability of most substances (Rowland & Tozer, 1995).

Numerous studies have shown increased serum aluminium and/or urinary aluminium excretion after oral administration of various aluminium-containing products. However, most of these studies do not permit estimation of the oral aluminium bioavailability. In addition to elevations of serum or urine aluminium levels, many studies have also shown increased levels of aluminium in the kidneys, liver, bone and brain of experimental animals after oral aluminium exposure (IAI 2007).

2.2.2. Absorption of aluminium from drinking water

Humans

Several small-scale human studies have estimated the absorption of aluminium following administration of a single dose of ^{26}Al (added as the chloride) in drinking water (Priest *et al.*, 1998; Stauber *et al.*, 1999; Steinhausen *et al.*, 2004). From a study of three healthy male volunteers who consumed 100 ng ^{26}Al together with 100 µg ^{27}Al, as aluminium chloride, Steinhausen *et al.* (2004) estimated the oral bioavailability of aluminium to be 0.1%. In another study, that modelled aluminium consumption from drinking water, two subjects consumed ^{26}Al added to water from a public supply. The results suggested that the oral bioavailability of aluminium was 0.20 and 0.14% for these two subjects (Priest *et al.*, 1998).

Twenty one subjects, who consumed a diet that provided a total intake of about 3 mg aluminium per day, drank daily either 1.6 litre of alum-treated water that contained 140 µg aluminium per litre or reconstituted soft water that had < 1 µg aluminium per litre, with and without sodium citrate. The oral bioavailability of aluminium from the water was 0.39% in the absence, and 0.36% in the presence of citrate (Stauber *et al.*, 1999). As the absorption was estimated by measuring aluminium levels in urine the gastrointestinal absorption might have been underestimated because the amount of aluminium retained in tissues or excreted by non-renal routes was not included in the calculations.

Overall, these results suggest that the oral aluminium bioavailability in humans from drinking water is in the range of 0.1 to 0.4%.

Animals

Estimates of the oral bioavailability of aluminium from drinking water in the rat, based on administration of aluminium chloride, were 0.06 to 0.2% (Ittel *et al.*, 1987) and 0.04% (Froment *et al.*, 1989a).

Several newer animal studies have utilized ^{26}Al (as aluminium chloride) to estimate the bioavailability of aluminium from drinking water in rats. When the aluminium levels in urine and bone were considered, absorption rates of 0.04–0.06% were estimated (Drueke *et al.*, 1997 and Jouhanneau *et al.*, 1993 as cited by IAI, 2007), and when liver and brain aluminium levels were also considered, an absorption rate of 0.1% was estimated (Jouhanneau *et al.*, 1997 as cited by IAI, 2007). However, newer studies that utilized a comparison of the area under the plasma aluminium concentration x time curve after oral and intravenous administration of ^{26}Al (as aluminium chloride) estimated an average oral bioavailability of about 0.3%. Thus, when the ^{26}Al (as aluminium chloride) solution was given by gavage with and without added calcium and magnesium to model hard drinking water the bioavailability was 0.28% when the rats had food in their stomach (Yokel *et al.*, 2001a) and 0.29% when the rats had empty stomachs following a special protein-rich diet and fasted for 14 hours (Zhou and Yokel, 2006).

Groups of 10 male Wistar rats received either deionised water or drinking water supplemented with aluminium chloride (5 or 20 mg aluminium/kg bw per day) for a six month period. The animals were placed in metabolism cages 6 days prior to, during (3rd month), and at the end of the study, for measurement of water consumption and diuresis (balance study). The absorption was reported to be 6.1 and 5.8 µg/kg bw per day (0.12% and 0.034%) in the 5 and 20 mg/kg bw per day dose groups, respectively (Somova and Khan, 1996) as cited by IAI, 2007).

Overall, the results from these studies suggest that the oral bioavailability of aluminium from water in the rat is in the range of 0.05 to 0.4%. The result of the study by Yokel *et al.* (2001a) was approximately 0.3% and is considered to be the most accurate measurement of the bioavailability (ATSDR, 2006).

2.2.3. Absorption of aluminium from food and beverages

Humans

Although food comprises the primary source (> 90%) of aluminium for the general population, there are only few data on the oral bioavailability of aluminium from foods or beverages other than water.

Two human studies have examined the bioavailability of aluminium in the diet. An absorption efficiency for aluminium from normal diets of 0.28% was estimated by Stauber *et al.* (1999) in 21 subjects ingesting 3 mg aluminium per day (0.04 mg aluminium/kg bw per day) or 0.76% in 8 subjects ingesting 4.6 mg aluminium per day (0.07 mg aluminium/kg bw per day) (Greger and Baier, 1983). When 125 mg aluminium per day (1.8 mg aluminium/kg bw/day) was added to the diet as aluminium lactate in fruit juice, the aluminium absorption decreased to 0.094% (Greger and Baier, 1983).

The oral bioavailability of aluminium from the diet was estimated to be 0.1 to 0.3% in humans based on a normal urinary excretion of 20 to 50 µg aluminium/person per day and a daily intake of 20 mg aluminium /person per day (Ganrot, 1986 as cited by IAI, 2007). Priest (1993) estimated the oral bioavailability of aluminium from food to be 0.1% on average (range 0.01 – 1%) assuming a daily intake of 15 mg aluminium, a daily urinary excretion of 0.025 mg aluminium and 5% retention of aluminium in the body. A similar estimate was obtained by Nieboer *et al*. (1995) by comparing an average daily urinary aluminium excretion of 0.004 to 0.012 mg to an estimated average daily intake of aluminium from food of 5 to 10 mg (Nieboer *et al*., 1995 as cited by IAI, 2007). Yokel and McNamara (2001) suggested that the bioavailability of aluminium from the diet is 0.1% based on a daily urinary excretion level of 4–12 µg aluminium and average intakes of aluminium by adults in the United States of 5–10 mg/day.

Some studies have found increased aluminium in the urine of humans after tea consumption. Equal volumes of coffee or water did not increase the urinary aluminium concentration (Koch *et al*., 1988 as cited by IAI, 2007). After consumption of 2 litres of tea, by one subject, the urinary aluminium output suggested the bioavailability was 0.3% for aluminium (Powell *et al*., 1993). However, no elevation in serum aluminium was seen after consumption of tea that delivered 2.3 to 2.8 mg aluminium per day and comprised about 31% of the total daily dietary aluminium intake (Drewitt *et al*., 1993).

Stauber *et al*. (1999) estimated the oral aluminium bioavailability from food-plus-tea to be about 0.53%, assuming that 10% of the aluminium in the tea was available for absorption.

Animals

No increases in blood or liver aluminium concentrations were seen in rats that consumed tea as the only source of fluid for 28 days (Fairweather-Tait *et al*., 1991 as cited by IAI, 2007). In one study it was reported that increased tissue aluminium concentrations were attributed to the intake of aluminium in food. Guinea pigs that ate a test diet of sponge cake three times weekly for 3 weeks, providing a total of 40 mg of aluminium, as acidic sodium aluminium phosphate (SALP, acidic), showed a significant elevation in bone aluminium concentrations compared with those that ate only guinea pig chow, which provided a total of 3 mg aluminium (Owen *et al*., 1994).

Walton *et al*. (1994) administered aluminium sulphate and various beverages and foods to anaesthetized rats and found increased serum aluminium levels only after 8 mg of aluminium,

as aluminium sulphate, and after margarine. The aluminium content of the margarine was not determined. The other beverages and foods tested, beer, cola, coffee, orange juice, tea, wine, apple, broccoli, butter, meat and biscuits, did not increase serum aluminium levels when administered alone. However, this is not surprising as the aluminium dose provided by these beverages and foods ranged from 0.005 to 8.6 µg. Weanling rats fed a diet containing 1 to 2.7 gm of aluminium/kg diet, added as aluminium hydroxide in the absence and presence of added sodium citrate dehydrate, were estimated to absorb 0.01 to 0.04% of the aluminium (Greger & Powers, 1992).

The bioavailability of aluminium from acidic and basic SALP incorporated into foods has been determined in the rat. ^{26}Al was incorporated into the synthesis of acidic and basic sodium aluminium phosphate (SALP). Acidic SALP which is permitted as a leavening agent in scones and sponge wares only in the EU (see Table 3) was incorporated into biscuits. Basic SALP, which is not authorised in the EU, can be used in the USA as an emulsifier in cheese and was incorporated into a processed cheese (Yokel *et al.*, 2005). When rats, that had empty stomachs after being offered a special high-protein diet and then fasted for 14 hours, ate the biscuit containing either 1% or 2% acidic SALP, it was estimated that the oral aluminium bioavailability was about 0.11% and 0.13%, respectively (Yokel and Florence 2006) compared with the 0.28% from water found by Yokel *et al*. (2001a). The oral bioavailability of aluminium from the processed cheese containing either 1.5% or 3% of basic SALP was about 0.1 to 0.3% (Yokel *et al.*, 2008).

Except for SALP, acidic, no studies on bioavailability were available for other aluminium compounds authorised as food additives in the EU. However, the Panel noted the following statement in the FEEDAP opinion on Zeolite (EFSA, 2007), a form of sodium aluminium silicate used in animal feed: "Sodium aluminium silicate was considered for many years not to be absorbed in the gut (of cows). However, more recent findings indicate that absorption does occur, albeit in very small amounts. Sodium aluminium silicate may be partly hydrolysed in the digestive tract, mainly in the abomasum (because of the low pH value) resulting in release of aluminium and silicate ions. Absorption of aluminium from the intestinal tract is very low (about 0.1 %) and many organic dietary components influence this process. However, Thilsing (ref) reported, in an unpublished study, an increase of the aluminium serum level from 13 µg/l before treatment to 85 µg/l during a three-week administration of 600 g Zeolite per day. Aluminum is efficiently excreted via the kidneys. "

2.2.4. Modulation of aluminium absorption

The bioavailability of aluminium is dependent on the form in which it is ingested and the presence of dietary constituents with which the metal cation can complex. Ligands in food can have a marked effect on the absorption of aluminium, as they can either enhance the uptake by forming absorbable (usually water soluble) complexes (e.g., with carboxylic acids such as citric and lactic acids), or reduce it by forming insoluble compounds (e.g., with phosphate, dissolved silicate, phytate, or polyphenols). There is evidence that citric acid (or citrates) is the low molecular weight complexing agent of most importance for uptake of aluminium in humans. Citric acid is a constituent of many foods and beverages and can be present in the gut in high concentrations. It is well-documented in both human and animal studies that blood and tissue levels of aluminium can be increased by simply increasing the consumption of citric acid (i.e., with no concurrent increase in aluminium ingestion), or other dietary chelators such as ascorbic acid and lactic acid (IAI, 2007).

Short chain carboxylic acids

Simple short chain carboxylates, such as propionate, lactate or acetate, form only weak complexes with Al^{3+}. The type of complex is a dinuclear mixed hydroxocomplex [$(RCOO^-)Al_2(OH)_2^{3+}$] with the single carboxylate group that bridges the two Al^{3+} (Marklund *et al.*, 1989; Martin, 1991). The same scheme has been confirmed for cyclohexanecarboxylic acid, benzoic acid and 3-hydroxybenzoic acid. (Sjoberg *et al.*, 1991). With dicarboxylic acids, such as - oxalate and malate, the presence of two oxygen donor groups allow the chelate effect to form stronger complexes with Al^{3+} (Sjoberg *et al.*, 1991; Martin, 1991).

Citrate exists mainly in the form of the tricarboxylate anion at pH >6 (Martin, 1991) and is the predominant small molecule in plasma that binds Al^{3+} (Ohman *et al*., 1994, Martin, 1991). An aluminium-citrate (ligand) species [AlL] with overall zero net charge exists in appreciable amounts at pH ranging from 2-3 to 5. As the [AlL] species carries no electrical charge it provides favourable conditions for absorption of aluminium (Martin, 1991). Its presence is consistent up to pH 4, which is the pH range in the upper gastrointestinal tract (Venturini *et al.*, 1989).

As the pH increases, the [AlL] deprotonates to $[Al(LH_{-1})]^{1-}$.In neutral solution this species undergoes a further hydrolysis step and it has been demonstrated that the main species under physiological plasma conditions is $[HOAlLH_{-1}]^{2-}$ (Ohman, 1988). Even though much of the citrate in plasma occurs as a Ca^{2+} or Mg^{2+} complex, Al^{3+} easily displaces these ions from citrate (Martin, 1991).

Numerous studies in humans have shown enhanced aluminium absorption from aluminium hydroxide in the presence of citrate, other carboxylic acids, and orange juice (Coburn *et al*., 1991; Fairweather-Tait *et al*., 1994; Lindberg *et al*., 1993; Mauro *et al*., 2001; Nestel *et al*., 1994; Nolan *et al*., 1990; Nordal *et al*., 1988a; Priest *et al*., 1996; Rudy *et al*., 1991; Slanina *et al*., 1986; Walker *et al*., 1990; Weberg and Berstad, 1986 as cited by IAI, 2007). For example, Weberg and Berstad (1986, cited by IAI, 2007) reported that 0.004% of 1 g of aluminium, given as an antacid, was absorbed in the absence of citrate, compared with 0.03% when consumed with orange juice and 0.2% when consumed with a citric acid solution that delivered 0.7:1 citrate:aluminium. A more recent study using ^{26}Al estimated aluminium absorption rates of 0.523, 0.0104, and 0.136% in two subjects receiving a single dose of aluminium citrate, aluminium hydroxide, or aluminium hydroxide dissolved in a citrate solution, respectively (Priest *et al*. 1996). This is consistent with another study reporting absorption levels of 0.37–0.57% in humans ingesting 280 mg aluminium as aluminium hydroxide in sodium citrate and citric acid (Taylor *et al*. 1998 cited by IAI, 2007). A fourth study reported a higher absorption level (1%) in one subject administered ^{26}Al in a sodium citrate solution (Day *et al*., 1991).

Similarly, numerous animal studies have also shown that aluminium is more bioavailable when administered as the citrate than as other chemical species (Cunat *et al*., 2000; Deng *et al*., 1998; 2000; Drüeke *et al*., 1997; Froment *et al*., 1989b; Partridge *et al*., 1992; Schönholzer *et al*., 1997; Sutherland & Greger, 1998; Van der Voet *et al*., 1989; Yokel & McNamara, 1988). It was estimated that 3.4 to 4.2% of aluminium was absorbed by rats, from aluminium citrate; however, no direct comparison was made with aluminium dosing in the absence of citrate (Sutherland and Greger, 1998). A contemporary study also using the ^{26}Al radioisotope administered to fasted rats as either aluminium hydroxide or citrate found the absorption to be 0.1 and 5%, respectively (Schönholzer *et al*., 1997).

Although it is frequently found that citric acid enhances aluminium absorption, the mechanism is still unclear. One hypothesis is that citrate may open the junctions between the gastro-intestinal mucosal cells which functions as a barrier to non specific absorption (Froment *et al.*,

1989b). It has also been suggested that under acidic conditions (pH 1-5) the existing neutral aluminium-citrate complex can diffuse freely across the intestinal mucosa, but the lipid bilayer permeation by neutral aluminium-citrate seems to be too slow to support this hypothesis (Martin, 1991). Venturini *et al.* (1989) demonstrated that citrate is able to dissolve the $Al(OH)_3$ precipitates at pH ranges larger than those of the upper gastrointestinal tract; this may combine with the capacity of other ligands to complex aluminium in neutral forms.

Other short chain carboxylic acids, including acetate, propionate, oxalate, lactate, malate, tartrate, gluconate, ascorbate, and carbonate have also been shown to increase aluminium absorption or tissue aluminium accumulation in some, but not all, animal studies. These substances were generally less effective than citrate (Colomina *et al*., 1994; Domingo *et al*., 1991a; 1993; 1994, IAI, 2007). An 8-week feeding study in rats which examined the absorption of aluminium (1.5-2 g/kg diet) either as hydroxide or complexed with the organic anions citrate, malate, lactate, or tartrate, found that all of these significantly increased plasma aluminium concentrations compared with the aluminium hydroxide treated group. There were no significant differences in the plasma aluminium concentrations between the organic anion treated groups (Testolin *et al*., 1996).

Solubility of the aluminium species

There is evidence of greater aluminium absorption from more soluble aluminium species. A comparison of the bioavailability of different aluminium compounds was conducted by Yokel and McNamara (1988). The bioavailability of aluminium in rabbits following a single maximum dose was estimated by comparing areas under the plasma concentration x time curves after oral and intravenous dosing. The estimated bioavailability of the water-soluble compounds aluminium chloride (333 mg aluminium/kg bw), aluminium nitrate (934 mg aluminium/kg bw), aluminium citrate (1,081 mg aluminium/kg bw), and aluminium lactate (2,942 mg aluminium/kg bw) in rabbits was 0.57, 1.16, 2.18, and 0.63%, respectively. Aluminium absorption in rabbits similarly treated with the water-insoluble compounds aluminium hydroxide (780 mg aluminium/kg bw), aluminium borate (2,736 mg aluminium/kg bw), aluminium glycinate (1,351 mg aluminium/kg bw), and aluminium sucrose sulphate (20,867 mg aluminium/kg ww) was 0.45, 0.27, 0.39, and 0.60%, respectively (Yokel and McNamara, 1988). In the rat, the oral bioavailability of aluminium was 0.015% from aluminium hydroxide and aluminium sucrose sulphate, 0.037% from aluminium lactate and aluminium chloride, and 1.49% from aluminium citrate (Froment *et al.*, 1989a). Similarly, Schönholzer *et al.* (1997) examined aluminium absorption following oral exposure to ^{26}Al in rats. The bioavailability of aluminium hydroxide, aluminium citrate, aluminium citrate with added sodium citrate, or aluminium maltolate following a single gavage dose was 0.1, 0.7, 5.1, and 0.1%, respectively.

Fluoride

Fluoride, which may be present in drinking water at concentrations up to 1.5 mg/l, appears to be able to increase the absorption of aluminium. Mixed fluoro-hydroxo complexes with aluminium have been described to occur in neutral solutions (Martin, 1991). Theoretical speciation calculations for water containing 10-500 mg aluminium/l indicated that more than 99% of the aluminium was present as Al^{3+} at pH 2.5 - 3. When citrate (6000 mg/l), fluoride (50 mg F/l), or silicate (200 mg Si/l) were present in the water the percentage of Al^{3+} declined to less than 3, 36, or 99%, respectively. Thus the calculations indicated that fluoride would

solubilize more than 60% of aluminium in the stomach (Glynn *et al*., 2001). Administration of aluminium fluoride to rats increased the plasma aluminium concentration more than the administration of aluminium chloride or aluminium phosphate. The increase was similar to that seen with aluminium citrate (Allain *et al.*, 1996).

Silicon

The silicon species $Si(OH)_4$ interacts with the aluminium ion to form mono- as well as polynuclear species. In the pH range below 5, the formation of a simple mononuclear hydroxyaluminiumsilicate complex $[AlOSi(OH)_3]^{2+}$ at relatively high silicic acid concentrations is well established. At higher pH values, the slow formation of polynuclear species as well as colloidal particles has been observed. However, composition and stability of these species/particles is still under debate. In pure solutions, from pH < 5 and upwards the hydroxyaluminium silicate species are formed; in the presence of aluminium chelators, such as citrate, hydroxyaluminium silicate complexes start to form at pH 7. The normal plasma levels of silicic acid (in the range 5-20 µM/l) are far from the 100 µM/l required for the formation of hydroxyaluminum silicate species, that have been suggested to reduce the bioavailability of Al^{3+} (Birchall, 1991).

Silicon is present in the animal gastrointestinal tract as monomeric silicic acid, which can react with aluminium to form hydroxyaluminosilicate species and slowly, but eventually, amorphous solids (Birchall *et al*., 1996). Taylor *et al*. (1995) have cited previous studies showing an inverse relationship between aluminium absorption and silicon concentrations in drinking water. These results suggest that increasing dietary silicon may reduce aluminium absorption and facilitate its excretion.

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

Iron

Iron (Fe) status impacts on the absorption of aluminium and its accumulation in the brain. Rats maintained on an iron-deficient diet had greater (0.0065%), and rats maintained on an iron-supplemented diet had lower (0.0028%) oral aluminium bioavailability than controls (0.0040%) (Winklhofer *et al*., 2000). Oral exposure to aluminium hydroxide produced a greater increase in aluminium excretion and brain aluminium levels in iron-deficient than in normal and iron-overloaded rats, whereas serum aluminium did not show consistent changes (Cannata *et al*., 1991; Fernández *et al*., 1989). This is perhaps due to a similar mechanism for uptake of aluminium and iron from the gut, which was suggested to involve an active process mediated by transferrin (Tf). (Cannata *et al*., 1991; Fernandez Menendez *et al*., 1991; Van der Voet and de Wolff, 1987b).

Calcium

Like iron, calcium (Ca) status impacts on aluminium absorption and accumulation. Dietary calcium deficiency increased the absorption of aluminium from aluminium chloride, and the extent of tissue aluminium accumulation, and aluminium-induced neuropathology in rats (Provan & Yokel, 1990; Taneda, 1984). Increased calcium concentrations decreased aluminium uptake from the chloride and its appearance in plasma in studies that used the rat everted gut sac and *in situ* rat gut technique, suggesting a common uptake mechanism for aluminium, introduced as the chloride, and calcium (Cunat *et al*., 2000; Feinroth *et al*., 1982). Calcium channel blockers decreased, and calcium channel activators increased, aluminium uptake into rat jejunal slices (Provan & Yokel, 1988b) and duodenum (Cochran *et al*., 1990) when introduced as aluminium chloride; however, Provan & Yokel (1988a) did not find an effect of calcium channel blockers or a facilitator in the in situ rat gut preparation. There were no differences in absorption of aluminium when ^{26}Al chloride was added to hard water (300 mg calcium carbonate/l added) or soft water.

There were no differences in absorption when the ^{26}Al was added to hard water (added 300 mg calcium carbonate/l) or soft water (Yokel *et al*. 2001a).

Uraemia

The primary documented problems with aluminium as a toxicant to bone and the brain have occurred in persons with uraemia, who accumulate aluminium due to their inability to excrete it. Aluminium levels were higher in the liver, but not in other organs, of chronically uraemic rats given oral aluminium hydroxide than in pair-fed controls (Drüeke *et al*., 1985). The oral bioavailability of aluminium introduced as ^{26}Al plus aluminium chloride was estimated to be 0.133% in controls and 0.175% in uraemic (5/6 nephrectomized) rats (Ittel *et al*., 1997). Reduction of renal function in rabbits, to ~ 23% of controls, increased the percentage of aluminium absorbed from aluminium chloride, citrate and lactate by ~ 50 to 100% (Yokel & McNamara, 1988).

Presence of food in the stomach

It has been assumed that the presence of food in the stomach inhibits aluminium absorption, due to aluminium association with organic ligands such as phytate and polyphenols in food. However, only a few studies have directly addressed this hypothesis. Overall, results suggesting that the presence of food in the stomach significantly affects oral aluminium bioavailability have only been obtained in a few studies (Walton *et al*., 1994; Drüeke *et al*., 1997; Yokel & Florence, 2006) whereas another study did not find a significant effect of the presence of food in the stomach on oral aluminium bioavailability (Yokel *et al*., 2001a). Thus, the presence of food in the stomach appeared to delay the absorption of ^{26}Al, but did not significantly alter the amount of aluminium absorbed in rats (Yokel *et al*. 2001a). Aluminium bioavailability was 0.23% with no food in the stomach and 0.21% when food was present.

Aluminium absorption in subjects with dementia

In a study of 20 subjects with Alzheimer's disease (AD), aged 65 to 76 (n=10) and 77 to 89 (n=10) blood aluminium levels were compared to those of 20 controls. Subjects and controls consumed ~ 4.5 mg/kg aluminium hydroxide and 3.3 to 6.5 g citrate (citrate: aluminium, 1.6:1

to 3.2:1) after an overnight fast (Taylor *et al.*, 1992). In the younger AD subjects, the blood aluminium was significantly greater at 60 minutes than in control subjects (104 vs. 38 µg/l). This association was not seen in the older subjects group.

Aluminium absorption was studied in AD subjects and compared to age-matched controls after consumption of a fruit drink containing ^{26}Al (Moore *et al.,* 2000). The percent of aluminium absorbed in AD subjects, estimated from single plasma samples obtained 1 h after the oral aluminium consumption, was 164% of that seen in controls. The authors attributed these differences to absorption, although reduced renal aluminium clearance in the aged could also contribute to this difference. The lack of consistent overall differences as a function of age or dementia status makes it difficult to draw any general conclusion from this study.

Zapatero *et al.* (1995) found significantly higher serum aluminium concentrations in 17 AD subjects, compared to age-matched controls, but no difference between 15 subjects with senile dementias and controls.

Based on higher serum and urine aluminium levels in 8 patients (65 to 86 years old) with dementia including AD compared to 144 controls (30 to 65 years old) (18 and 6 µg/l and 77 and 26 µg/l, respectively), Roberts *et al.* (1998) claimed to have confirmed earlier findings that patients with dementia appear to absorb more aluminium from the diet than healthy subjects. However, the difference in the serum and urine aluminium levels could be due to factors other than dementia, such as the significant age differences between the subjects (ATSDR 2006).

Utilizing both ^{26}Al and ^{27}Al in separate studies, greater aluminium absorption was seen in subjects with Down's syndrome than in controls, 0.14 vs. 0.03% for ^{27}Al administered with citrate and 0.14 vs. 0.022% for ^{26}Al administered with orange juice (Moore *et al.,* 1997). However, these results are based on a single blood sample drawn 60 minutes after aluminium administration, a method that may not very reliably estimate oral bioavailability.

2.3. Distribution

2.3.1. Binding and complexation of aluminium in the body

Aluminium is believed to exist in four different forms in the body: as the free ion, as low-molecular-weight complexes, as physically bound macromolecular complexes, and as covalently bound macromolecular complexes (Ganrot 1986).

The main carrier of Al^{3+} in plasma is the iron binding protein transferrin (Tf). Studies have demonstrated that about 89% of the Al^{3+} in plasma is bound to transferrin and about 11% to citrate (Ohman *et al.*, 1994). Displacement of bound Fe^{3+} on transferrin in plasma is not necessary because the capacity in terms of unoccupied sites is large enough to also accommodate Al^{3+} (Martin, 1991).

There is evidence of a weak binding between Al^{3+} and albumin. The Al^{3+} was shown to be linked to six oxygen ligands in an octahedric site, which led to the suggestion that the Al^{3+} may be binding at the Ca^{2+} binding site of albumin (Fatemi *et al.*, 1992). However, it was considered unlikely that albumin can compete with transferrin and other ligands in serum and consequently albumin is not a major carrier of Al^{3+} in serum (Martin *et al.*, 1987; Martin, 1986a; Martin, 1988).

Aluminium may also form low-molecular-weight complexes with organic acids, amino acids, nucleotides, and phosphates. These low-molecular-weight complexes are often chelates and

may be very stable. The importance of complexation with organic acids, in particular with citrate, has already been mentioned in the section on absorption.

Inorganic phosphates are widely distributed in the body and may complex Al^{3+}. Species such as $[Al(H_2PO_4)]^{2+}$, $[Al(HPO_4)]^{+}$ and $[Al(PO_4)]$ have been directly studied only at acidic pH (up to pH 4) (Harris *et al.*, 1996). At neutral pH the Al^{3+} ion forms poorly soluble compounds with phosphate; although usually indicated as $AlPO_4$, these species are a mixed phosphate-hydroxo complexes (Ohman *et al.*, 1994). Harris *et al.* (1996) reported that because of precipitation of these complexes, the concentration of soluble Al-phosphate hydroxo species is limited to 20 μM at neutral pH. The complexation between aluminium and inorganic phosphates at neutral pH is still under debate (Harris *et al.*, 1996; Martin, 1991; Ohman *et al.*, 1994).

The alpha carboxylate group of amino acids is so weakly basic that meaningful interaction with Al^{3+} does not occur. However, there is some evidence that chelation of Al^{3+} by simple alpha aminoacids begins to be distinguishable by hydrolysis at ligand concentrations greater than 20 mM (Martin, 1991).

According to Martin (1991), catecholamines can chelate Al^{3+} between two negatively charged oxygens, but in neutral solutions the existing competition with protons reduces the stability of the complex with catecholamines. On the basis of the results of studies on fluids low in citrate and transferrin it was suggested that in the absence of other stronger ligands, catecholamines may be important binders for Al^{3+} (Kiss *et al.*, 1989; Wenk *et al.*, 1981).

Nucleotides that contain basic terminal phosphate groups (pKa >6) are strong Al^{3+} binders and readily form mono and bis complexes (Martin, 1991; Harris *et al.*, 1996). Nucleic acids contain only weakly basic phosphate functionalities and can bind Al^{3+} at least 10^5 times less strongly than nucleotides (Martin, 1991). Strong competition from hydroxo complex formation implies only weak association with DNA in neutral solutions (Karlik *et al.*, 1980). Several authors (Martin, 1991; Harris *et al.*, 1996) have reported that DNA cannot compete with ATP or other nucleotides or biophosphates for Al^{3+}. It was reported that Al^{3+} binding to DNA is very weak under physiological intracellular conditions (Martin, 1991).

2.3.2. Human studies

After absorption, aluminium distributes unequally to all tissues in humans. The total body burden of aluminium in healthy human subjects has been reported to be approximately 30–50 mg (Alfrey et al. 1980; Cournot-Witmer et al. 1981; Ganrot 1986; Hamilton et al. 1973 as cited by IAI, 2007). Normal levels of aluminium in serum are approximately 1–3 μg/l (House 1992; Liao et al. 2004) and may increase with aging (Zapatero et al. 1995). As detection methods have improved, the suggested normal values for aluminium levels in plasma have been revised downwards, and Nieboer et al. (1995) suggested that the value in normal subjects lies in the range of 1.1 to 1.9 μg/l.

About one-half of the total body burden of aluminium is in the skeleton, and about one-fourth is in the lungs (Ganrot 1986). The levels of aluminium in the lung of adult humans probably result from an accumulation of insoluble aluminium compounds that have entered the body via inhalation (Ganrot 1986). Most of the aluminium in other parts of the body probably originates from food intake. Reported normal levels in human bone tissue range from 5 to 10 mg/kg (Alfrey 1980; Alfrey *et al*., 1980; Cournot-Witmer *et al*., 1981; Hamilton *et al*., 1973 as cited by IAI, 2007). Aluminium is also found in human skin , lower gastrointestinal tract, lymph nodes, adrenals, and parathyroid glands. Low aluminium levels (0.3–0.8 mg/kg w/w) are found in most soft tissue organs, other than the lungs (IAI, 2007; ATSDR, 2006)).

One hour after ingestion of ^{26}Al citrate by one volunteer, 99% of the aluminium in the blood was present in the plasma and the remaining 1% was in erythrocytes. In blood taken after 880 days, 86% of the ^{26}Al was in plasma and 14% associated with erythrocytes (Day *et al.*, 1994). However, the aluminium concentration in plasma from haemodialysis patients showed little difference from that in erythrocytes (Van der Voet & de Wolff, 1985). These results are similar to those from animal studies showing similar aluminium concentrations in plasma and erythrocytes at equilibrium.

In serum from 10 uraemic patients, ~ 90% of the aluminium in plasma was found bound to Tf (Ohman & Martin, 1994; Wrobel *et al*., 1995). This percentage did not change as a function of the aluminium concentration or the presence of uraemia. The remaining aluminium was associated with a low molecular mass entity, suggested to be citrate. Steinhausen *et al.* (2004) suggested that the ultrafilterable fraction of aluminium in healthy humans and patients with chronic renal failure was 6 and 11%, respectively and analytical speciation studies suggest that the ultrafilterable species are citrate, phosphate, and citrate-phosphate aluminium complexes (Sanz-Medel *et al.*, 2002).

Clearance of ^{26}Al from the blood was assessed in two male volunteers orally exposed to 100 mg aluminium as aluminium chloride (Hohl *et al*. 1994). Plots of the serum and urine concentrations showed several changes in slopes, indicating that the clearance from blood involves one central and three peripheral compartments.

In the human brain normal levels of aluminium range from 0.25 to 0.75 mg/kg wet weight, with the grey matter containing about twice the concentration found in the white matter. There is evidence that with increasing age, aluminium concentrations may increase in the human brain tissue (IAI, 2007; ATSDR, 2006).

Aluminium concentrations (dry weight basis) in the bone of normal humans were a few-fold higher than those in the brain (Alfrey *et al*., 1980), ~ 1 to 3 mg/kg (Nieboer *et al*., 1995). Human bone aluminium concentration significantly increased with age in a study of one hundred seventy-two 16-98 year old subjects. It was less than 0.4 µg/gm dry bone weight in quartile 1 compared to >1.7 in quartile 4 (Hellström *et al*., 2005).

As regards occurrence of aluminium in human milk, a literature review of studies published in the previous 30 years showed values between 39 and 250 µg/l (Caroli *et al*., 1994). Another review concluded that the normal values were 4 to 65 µg Al/l (American Academy of Pediatrics, 1996). Other studies have reported mean values between 9 and 86 µg/l (IAI, 2007, ATSDR, 2006; Weintraub *et. al.*, 1986; Koo et. al., 1988; Simmer *et al*., 1990; Baxter *et al*., 1991; Coni *et al*., 1990; Bougle *et al*., 1992; Hawkins *et al*., 1994; Vinas *et al*., 1997; Krachler *et al*., 2000).

2.3.3. Animal studies

The distribution of aluminium in animals after oral exposure has been determined in a number of studies (Cranmer *et al*., 1986; Deng *et al*., 2000; Dlugaszek *et al.*, 2000; Domingo *et al*,. 1993; Greger and Donnaubauer 1986; Julka *et al*., 1996; Ogasawara *et al*., 2002; Sutherland and Greger 1998; Walton *et al*., 1995; Yokel and McNamara 1985; Zafar *et al*., 1997 as cited by IAI, 2007). Aluminium is not equally distributed throughout the body following dietary exposure to rats. Aluminium accumulation was higher in the spleen, liver, bone, and kidneys than in the brain, muscle, heart, or lung. Aluminium concentrations in these tissues decreased significantly 3 days after withdrawal of aluminium hydroxide from the diet. Tissue concentrations of aluminium were similar in treated and control rats 7 days after withdrawal

(Greger and Sutherland 1997). Eight days after a single gavage dose of 2.6 mg of ^{26}Al as aluminium chloride, the descending order of aluminium levels was bone>spleen>liver>kidney (Zafar *et al*., 1997).

Aluminium levels have been found to increase with ageing in a number of tissues and organs (bone, muscle, lung, liver, and kidney) of experimental animals (Golub *et al*., 1996a, Kukhtina, 1972, Stone *et al*., 1979, Massie *et al*., 1988, Greger & Radzanowski, 1995). The limited data available suggest that brain and blood aluminium concentrations also increase with age.

Most of the results of animal studies (rats, rabbits) suggest an initial volume of distribution (Vd) of aluminium consistent with the blood volume. However, when samples were collected over longer periods, greater Vds became evident. In rats, the Vd 10 hours after i.v. and oral administration of 8.1 mg aluminium/kg bw was 38 and 46 ml/kg bw, respectively (Gupta *et al*., 1986). After i.v. injection of aluminium lactate in rabbits, the initial Vd was reported to be 54 mL/kg after 1.1 mg/kg bw and 44 ml/kg bw after 2.2 mg/kg (Yokel & McNamara, 1985). When sampling time was increased to 48 hours, the steady-state Vd in rabbits after i.v. injection of 2.7 mg Al lactate/kg bw was reported to be 1175 ml/kg (Yokel & McNamara, 1988).

Studies in rats and rabbits have shown the distribution of aluminium in blood to be equal between plasma and erythrocytes (Mayor *et al*., 1977; Yokel & McNamara, 1985; Pai and Melethil, 1989). However, according to Priest (2004), only about 10% of the aluminium in blood is found in the erythrocytes. In plasma, aluminium is believed to be present bound mainly to transferrin (Tf) (Ganrot 1986; Harris and Messori 2002; Martin 1986). There is *in vitro* evidence indicating that aluminium can bind to the iron-binding sites of Tf , and that Al^{+3} may compete with similar ions in binding to Tf (Ganrot 1986). The percentage of aluminium bound to plasma proteins was reported to be 92 to 98% in the rat, at a serum aluminium concentration of 2,000 to 10,000 µg Al/l following aluminium chloride injection (Burnatowska-Hledin *et al*., 1985 as cited by IAI, 2007). Similarly, 98% of aluminium was found to be protein bound when rat serum aluminium concentrations were 110,000 to 440,000 µg Al/l (Gupta *et al*., 1986). These aluminium concentrations greatly exceed those seen in humans.

Cellular uptake of aluminium in organs and tissues is believed to be relatively slow and most likely occurs from the aluminium bound to transferrin by transferrin-receptor mediated endocytosis (TfR-ME) (Ganrot 1986). Studies of the sub-cellular localization of the aluminium ion in rat liver cells (at pH 7) showed considerably more aluminium in the nuclear fraction than in the mitochondrial or other sub-fractions, suggesting selective nuclear uptake of aluminium (Kushelevsky *et al*., 1976). In Caco-2 cells the nucleus also appeared to selectively take up ^{26}Al , irrespective of the chemical species of ^{26}Al to which the cells were exposed (Zhou & Yokel, 2005). In contrast, Muller & Wilhelm (1987) found aluminium to be present mainly in the mitochondrial (~ 45 to 50%) and post-mitochondrial fractions (~ 40 to 45%) of rat hepatocytes with only 6-7% in the nucleus. In another study using piglet hepatocytes, aluminium was shown to be present in the lysosomes (Klein *et al*., 1987). Aluminium was also seen in the lysosomes of kidney cells of rats given i.p. aluminium chloride (Linss *et al*., 1991; 1992).

The aluminium concentration in the brains of mice fed a commercial diet (aluminium content not described) increased several fold from 1 week to 4 weeks of age, then remained constant until declining several fold from 52 to 104 weeks of age. In contrast, rat brain aluminium showed no consistent changes over the same time period (Takahashi *et al*., 2001). However, oral administration of ^{26}Al (as the chloride) resulted in higher brain ^{26}Al concentrations than seen in control rats (Drüeke, 2002; Fink *et al*., 1994; Jouhanneau *et al*., 1997b; Walton *et al*.,

1995 as cited by IAI, 2007), demonstrating the ability of aluminium to be orally absorbed and to distribute into the brain.

There are two routes by which aluminium might enter the brain from the blood: 1) through the blood brain barrier (BBB) and 2) through the choroid plexuses into the cerebrospinal fluid (CSF) of the ventricles within the brain and then into the brain. Aluminium has been shown to rapidly enter the brain extracellular fluid (ECF) and the CSF, with smaller concentrations in these two fluids than in the blood (Allen & Yokel, 1992; Allen *et al*., 1995; Xu *et al*., 1992b; Yokel *et al*., 1991b as cited by IAI, 2007).

The aluminium Tf complex is the predominant aluminium species in plasma and evidence has been provided that aluminium transport across the BBB can be mediated by transferrin-receptor mediated endocytosis (TfR-ME) of the aluminium Tf complex (Roskams & Connor, 1990). This process would presumably release free aluminium in the brain ECF. However, there appears to be more than one mechanism for distribution of aluminium across the BBB into the brain. When aluminium citrate was given i.v. at a rate that produced plasma concentrations in excess of the ability of Tf to bind the aluminium the appearance of aluminium in brain ECF was too rapid to be mediated by TfR-ME (Allen *et al*., 1995). This suggests a second mechanism, independent of Tf, which can transport aluminium citrate into the brain.

Morris *et al*. (1989) reported a positive correlation between aluminium concentration in neurons in the cortex and hippocampus and the density of TfR. From *in vitro* studies it also appears that Tf enhances aluminium uptake into neurons and that many different chemical species of aluminium can enter neurons and glial cells. However, it has been suggested by Bradbury (1997) that there are no available binding sites for aluminium on Tf in brain ECF. If this is the case, this mechanism may not be very important *in vivo*. The mechanisms of aluminium uptake by brain cells therefore appear to include a combination of diffusion, TfR-ME and other, as yet un-resolved, carrier-mediated processes. The monocarboxylate transporter (MCT) and organic anion transporter families have been suggested to act as carriers (Ackley and Yokel, 1997; 1998).

After repeated s.c. or i.v. injections of aluminium lactate to rabbits, aluminium in neurons was primarily seen in the nucleus (nucleolus, interchromatin granules, euchromatin, and the heterochromatin) and to a lesser extent in the cytoplasm (rough endoplasmic reticulum and free ribosomes) (Uemura, 1984; Wen & Wisniewski, 1985). Variable results have been obtained *in vitro* using murine neuroblastoma cells, rat cerebral organotypic cultures, human neuroblastoma cells, and neuron- and astrocyte-like cells. Between 20 and 55% of aluminium was seen in the nuclear fraction and the remainder in subfractions of the cytoplasm, such as lysosomes and mitochondria (Shi & Haug, 1990; Schuurmans Stekhoven *et al*., 1990; King *et al*., 1994; Lévesque *et al*., 2000; Yumoto *et al*., 2003). Yumoto *et al*. (1997) showed that ~ 89% of the nuclear ^{26}Al was in the chromatin fraction.

Repeated treatment of rats and rabbits with aluminium resulted in ~ 5-fold greater elevation of aluminium levels in bone than in brain (DuVal *et al*., 1986; Fiejka *et al*., 1996; Garbossa *et al*., 1998b; Henry *et al*., 1984; Yokel, 1983 as cited by IAI, 2007). In rats given an oral dose of ^{26}Al, the ^{26}Al rapidly entered the bone, peaked within hours, with no significant decrease over the subsequent 720 hours (Jouhanneau *et al*., 1997b). After oral ingestion of ^{26}Al, ~ 0.25 to 0.3% of the dose was found in the skeleton after 2 (Jouhanneau *et al*., 1997b) and 48 hours (Drüeke *et al*., 1997). Consistently more ^{26}Al was found in the skeleton and urine when it was administered together with citrate, than without (Jouhanneau *et al*., 1997b). Forty-eight hours after an oral dose of ^{26}Al to rats, 1 x 10^{-3} and 1 x 10^{-6} % of the dose was found in each gram of bone and brain, respectively (Drüeke *et al*., 1997). This would suggest that about 100-fold more aluminium enters bone than brain after a single exposure. However, as noted above, the

steady state concentration of aluminium in the bone is not 100-fold greater than in the brain. This suggests that the clearance of aluminium from the bone is more rapid than from the brain. Potential mechanisms of bone aluminium deposition have been suggested to be heterionic exchange with calcium, co-precipitation with calcium and complexation with organic components of the bone matrix (Priest, 2004).

Repeated administration results in greater aluminium accumulation in bone of uraemic animals than in bone of controls (Alfrey *et al*., 1985; Chan *et al*., 1983; Ecelbarger & Greger, 1991; Hirschberg *et al*., 1985; Walker *et al*., 1994; Yokel & McNamara, 1988). In the brain, the aluminium concentration was significantly elevated in only one of these studies (Alfrey *et al*., 1985). Also, after a single oral administration, bone of uraemic rats contained significantly more ^{26}Al than did bone of control rats (Ittel *et al*., 1997).

It has been reported that aluminium can reach the placenta and fetus and to some extent distribute to the milk of lactating mothers (Cranmer *et al*. 1986; Golub *et al*. 1996; Yokel 1985; Yokel and McNamara 1985). The studies included four different aluminium compounds (hydroxide, chloride, lactate and citrate) administered by four routes (gavage, feed, intraperitoneal injection and subcutaneous injection) with total doses ranging from 14 to 8,400 mg/kg bw per day.

The concentration of aluminium in milk of rats that ingested 420 mg aluminium/kg bw per day as aluminium lactate in the diet during gestation and lactation increased at least 4-fold beginning on postnatal day 12 (Golub *et al*., 1996). Peak concentrations of aluminium were detected in the milk of lactating rabbits 12–24 hours after a single large gavage dose of aluminium lactate (Yokel and McNamara 1985). Aluminium levels in rabbit pups exposed during lactation were not significantly different from levels in control pups, suggesting that only a small amount of the aluminium in breast milk was absorbed by the offspring (Yokel 1985).

2.3.4. Modulation of the distribution of aluminium

It has been suggested that citrate can promote the distribution of aluminium from plasma to organs as well as the renal elimination of aluminium. In contrast, administration of aluminium as the lactate or chloride may result in sequestration in tissues and reduced renal clearance resulting in increased body burden. Thus, tissue aluminium concentrations in rabbits 1 week after completion of a series of daily i.v. injections for 20 days of aluminium citrate were considerably lower than after the same molar dose of aluminium lactate (Yokel *et al*., 1996a). However, citrate promotion of aluminium distribution and excretion would only be favoured when the aluminium concentration exceed the Tf metal binding capacity. This will seldom occur in humans, but has occurred in many of the experimental animal studies.

The iron status is negatively correlated with aluminium accumulation in tissues. It has been suggested that this may be due to competition between these two chemically similar trivalent cations, enabling greater transferrin-mediated extravascular distribution and storage of aluminium on ferrin when the iron concentrations are low (Greger & Sutherland, 1997).

Some studies have suggested that formation of aluminium fluoride in plasma may influence aluminium distribution out of plasma. Thus, studies using s.c. injections of aluminium to rats together with fluoride resulted in significantly higher aluminium levels in liver, spleen and adrenals than those from aluminium-alone injections and increased aluminium-induced behavioural toxicity (Stevens *et al*., 1987). Addition of fluoride to the aluminium in the drinking water of rats reduced bone aluminium levels but appeared to exacerbate the

osteomalacic lesion of aluminium-associated bone disease (Ittel *et al*., 1992b). However, calculations have shown that only insignificant amounts of aluminium fluoride will be formed in the plasma of humans in the presence of normal plasma fluoride concentrations and normal or elevated plasma aluminium concentrations. Also, aluminium fluoride constitutes < 1% of intracellular aluminium, making any effect of fluoride on aluminium distribution unlikely (Yokel and McNamara, 2001).

Co-administration of folic acid with aluminium to rats, 5 days weekly for 8 weeks, resulted in significantly less aluminium in femur, brain and kidney, but not serum, than in the control rats (Bayder *et al*., 2005), probably by either reducing the absorption or enhancing the elimination of aluminium.

There is evidence suggesting that vitamin D enhances oral aluminium absorption in the rat and rabbit, as increased serum and urinary aluminium were seen in vitamin D treated animals after oral but not i.v. administration of aluminium (Adler & Berlyne, 1985; Ittel *et al*., 1988; Long *et al*., 1991; 1994).

It has been hypothesised that calcium and magnesium deficiency may contribute to accumulation of aluminium in the brain in the amyotrophic lateral sclerosis (ALS) and parkinsonism dementia disorders. In cynomolgus monkeys, maintained for 41 to 46 months on a low calcium diet and administered 150 mg aluminium and 50 mg manganese per day, preliminary analysis of the spinal cord of one of the animals showed accumulation of aluminium, but not manganese (Garruto *et al*., 1989). In another study, mice fed a diet low in calcium and magnesium and high in aluminium had increased levels of aluminium in the brain, kidney, liver and muscle (Yasui *et al*., 1990b). Rats fed a calcium- and magnesium-deficient diet had a non-significant increase of aluminium in the CNS, whereas rabbits fed a calcium- and magnesium-deficient diet with added aluminium, as the lactate, did not show an elevation of aluminium in the CNS compared to rabbits consuming the deficient diet without aluminium (Yase, 1980). In a study in rats, low dietary calcium and magnesium significantly increased the aluminium levels in lumbar spinal and femoral bone. Addition of aluminium increased the aluminium concentration more, but the lack of a group given standard diet with aluminium for comparison does not allow determination of whether or not calcium and magnesium deficiency increased aluminium accumulation in the nervous system in the presence of elevated aluminium in the diet (Yasui *et al*., 1991a). Mice that consumed a low calcium, low magnesium diet or the same diet plus aluminium, as 15.6 g aluminium hydroxide/kg diet, for 11 to 31 months, had aluminium and calcium deposition in cortical and hippocampal neurons (Kihira *et al*., 2002).

Age-related differences in the distribution of aluminium have been observed in rats exposed to 0, 50, or 100 mg Al/kg bw per day as aluminium nitrate in the drinking water with added citrate (Gómez *et al*. 1997a). The levels of aluminium in the brain and bone were significantly higher in the older rats (16 months of age at study beginning) compared to young (21 days of age) or adult (8 months of age) rats; this was observed in the control and aluminium-treated rats. The levels of aluminium in the liver were significantly higher in adult and older rats as compared to the young rats.

2.4. Elimination and Excretion

Following ingestion in humans, absorbed aluminium from the blood is eliminated primarily by the kidneys, presumably as the citrate, and excreted in the urine. Unabsorbed aluminium is excreted in the faeces (Gorsky *et al*. 1979; Greger and Baier 1983; Kaehny *et al*. 1977; Recker

et al. 1977; Sutherland and Greger 1998 as cited in AIA, 2007). Excretion via the bile constitutes a secondary, but minor route. For example, during the first 5 days after i.v. injection of aluminium citrate in seven humans approximately 1.5% of the dose appeared in the faeces and approximately 70% in urine (Priest, 2004). Aluminium has also been detected in sweat (laboratory reference value of 11 µg/l; Omokhodion & Howard, 1994), saliva (children aged ~ 10 averaged 54 µg/l; Sighinolfi *et al*., 1989), and seminal fluid (average 0.46 - 3.3 mg/kg; Yamamoto *et al*., 1959; Hovatta *et al*., 1998; Dawson *et al*., 2000 as cited by IAI, 2007).

It has been reported that humans who do not consume any specific diet, take no medications containing aluminium, and who have normal renal function excrete 0.15 to 0.45 µmole (4 to 12 µg) (Nieboer *et al*., 1995), less than 20 µg (Wilhelm *et al*., 1990) or less than 50 µg aluminium per day in urine (Greger & Sutherland, 1997). Based on studies published over 30 years, Caroli *et al*. (1994) established a reference value of 2.3 to 110 µg/l in urine. The mean serum and urine levels of aluminium in 44 non-exposed persons who did not use antacids were 0.06 and 0.33 µM (1.6 and 8.9 µg/l) (Valkonen & Aitio, 1997) and the median aluminium concentration in urine was reported to be 3.3 µg/l in 67 office workers who had not been exposed to aluminium (Liao *et al*., 2004).

In humans orally exposed for 20 days to 1.71 mg aluminium/kg bw per day, as aluminium lactate, in addition to 0.07 mg aluminium/kg bw per day in the basal diet, 0.09 and 96% of the amount of aluminium ingested per day was eliminated through the urine and faeces, respectively (Greger and Baier 1983).

Urinary aluminium concentrations were significantly increased (3 to 34-fold) in volunteers who received 2.2 g of aluminium phosphate, carbonate, hydroxide, or dihydroxyaluminium aminoacetate as antacids. The serum aluminium concentrations only increased 1.3 to 2.8-fold (Kaehny *et al*. 1977). Similarly, after aluminium-containing antacid consumption, serum aluminium concentration increased ~ 2.4-fold and urine aluminium concentration increased ~4.5-fold (Gorsky *et al*., 1979).

Excretion of aluminium may be lower in premature infants compared to full-term infants (Bougle *et al*. 1992; 1997). Plasma levels of aluminium in premature infants were 14.6 µg/l compared to 7.8 µg/l in full-term infants, and absolute urinary excretion was reduced. The aluminium-creatinine ratio in the urine was similar in both groups, indicating that the lower excretion in the premature infants may be due to lower metabolic and glomerular filtration rates.

Numerous studies have reported marked increase in urinary, but very little increase in serum, aluminium levels following occupational, inhalatory exposure to aluminium fumes and dusts (IAI 2007).

Multiple values for the elimination half life of aluminium in humans have been reported, suggesting that there is more than one compartment of aluminium storage from which aluminium is eliminated. Typically, as the duration of sampling after exposure was increased, a longer half life was observed.

Based on an estimated human body burden of 60 mg aluminium, an assumed daily dietary intake of 20 mg and absorption of 1%, Jones *et al*. (1986) calculated a mean retention time of aluminium in the human of 300 days and a half life of 210 days. This calculation assumed steady state conditions and was based on a single compartment or one compartment that is responsible for a majority of the aluminium body burden. However, elimination half lives of hours, weeks and years were seen after termination of short-term inhalation exposure, less than 1 year exposure, and upon retirement, respectively (Ljunggren *et al*., 1991). The aluminium elimination half life positively correlated with exposure time (Ljunggren *et al*., 1991). These

results are consistent with more than one compartment of aluminium storage and might result from retention of aluminium in a depot from which it is slowly eliminated. This depot is probably bone which stores ~ 60% of the human aluminium body burden. Slow aluminium elimination coupled with continued exposure would be predicted to produce an increasing body burden with age.

Within the first day of receiving a single injection of ^{26}Al citrate, approximately 59% of the dose was excreted in the urine of six subjects; 72 and 1.2% was excreted in the urine and feces, respectively, during the first 5 days (Talbot *et al*. 1995). At the end of 5 days, it was estimated that 27% of the dose was retained in the body (Priest *et al*. 1995; Talbot *et al*. 1995). When ^{26}Al levels were monitored more than 3 years after a single subject received the injection, a half-life of approximately 7 years was calculated (Priest *et al*. 1995). However, when the subject was re-examined approximately 10 years after the injection, a half-life of about 50 years was estimated (Priest 2004). It was suggested that the aluminium body burden after long term (years) of constant aluminium intake would be ~ 400 times the daily aluminium intake (Priest, 2004).

Zapatero *et al*. (1995) found that serum aluminium concentration positively correlated with age in 356 healthy adults. This could not be attributed to the age-related decrease of renal function. It is unknown if it relates to the long half life of aluminium in one or more compartments in the human so that steady state is not reached in a lifetime, to age-related increased absorption, or to other factors.

There are no reported determinations of retention time in specific tissues in humans.

Excretion data collected in animal studies are consistent with the results from human studies. A single oral dose of 11 mg aluminium to healthy Sprague-Dawley rats resulted in a 14-fold increase in the aluminium levels in urine, as compared to baseline levels (Ittel *et al.,* 1987). The aluminium was primarily excreted during the first 24-hour period, and was comparable to the baseline levels 5 days postexposure. Similarly exposed uremic rats excreted more aluminium than the healthy rats. The study authors postulated that this increase in excretion was probably due to increased gastrointestinal absorption. Rats administered a single dose of one of eight aluminium compounds (all contained 35 mg aluminium) excreted 0.015–2.27% of the initial dose in the urine. The range most likely reflects differences in gastrointestinal absorption (Froment *et al*. 1989b). Following administration of a single dose of 6.7–27 mg aluminium/kg bw, 1.3–2.8% of the dose was excreted within the first 3 hours; the percent of the dose excreted in the urine did not differ among the three dose groups (Sutherland and Greger, 1998).

Faecal aluminium represents unabsorbed aluminium as well as aluminium excreted via bile. In rats receiving a gavage dose of 6.7–27 mg Al/kg, the levels of aluminium in bile were significantly higher than in controls within 15 minutes (Sutherland and Greger 1998). The percentage of the total dose excreted in bile during the first 3 hours after dosing ranged from 0.06 to 0.14%.

In rats, the half-life of aluminium elimination, based on studies in which samples for aluminium determination were collected for ≤ 24 hr after i.v. injection of aluminium chloride, sulphate, or citrate, were found to be between 1 and 5 hours. Similar values have been obtained in mice: 1.5 hours after i.p. injection of aluminium gluconate or lactate. Studies in the dog provided similar results: 1.5 to 4.6 hours after aluminium chloride given i.v. (IAI, 2007).

The apparent half-life increased with increased duration of sampling after acute administration of aluminium to rabbits, suggesting the presence of one or more compartments with very long half-lives. Half lives of 2.1 - 3.8 hours in lactating rabbits and 8.6 hours in 17 to 21 day old suckling offspring were found after i.p injection of aluminium lactate (Yokel & McNamara,

1985). However, when blood samples were obtained after 48 h, a half-life of 27 hours was seen in normal rabbits that received aluminium lactate (Yokel & McNamara, 1988). A similar study, in which blood was obtained to 72 h, resulted in a t½ of 43 h in normal rabbits (Yokel & McNamara, 1989). To determine the half-life of aluminium elimination from organs, adult rabbits were given a single i.v. infusion of aluminium lactate over 6 hours and then terminated up to 128 days later. The half-life of aluminium was estimated to be 113, 74, 44, 42, 4.2 and 2.3 days in spleen, liver, lung, serum, kidney cortex, and kidney medulla, respectively. A second half-life in the kidney greatly exceeded 100 days (Yokel & McNamara, 1989). In rats, the whole organism elimination half-life was estimated to be 8 to 24 days in serum, kidney, muscle, liver, tibia and spleen (Greger *et al*., 1994).

Aluminium persists for a very long time in the rat brain following systemic injection of very small doses of ^{26}Al. When ^{26}Al (as citrate) was given i.v. to rats that were euthanatized 0.17 to 256 days later, the half-life of brain aluminium was estimated to be approximately 150 days (Yokel *et al*., 2001a). This estimate is not expected to have a high degree of accuracy as brain samples were not obtained for at least 3 half-lives. In offspring of rats that were given ^{26}Al injections daily from day 1 to 20 postpartum and examined on days 40, 80, 160, 320 or 730 postpartum the aluminium concentrations decreased over the 730 days in all tissues (Yumoto *et al*., 2003). IAI (2007) calculated the elimination half-lives to be approximately 13 and 1635 days in the brain. Half-lives of 7 and 520 days in parietal bone were suggested and after 730 days, the amount of ^{26}Al remaining in the liver and kidneys was about 2% of that seen at weaning. For liver and kidney, the half-lives were 5 and 430 days and 5 and 400 days, respectively. In blood the values were 16 and 980 days.

There is little published information on allometric scaling of aluminium elimination rates that can be used to extrapolate these results from the rat to the human. For aluminium in the brain 150 days is approximately 20% of, and 1365 days exceeds, the rat's normal life span. For comparison, the whole-body half-life of aluminium in the human was estimated to be 50 years (Priest, 2004).

3. Acute toxicity

The acute oral toxicity of a number of aluminium inorganic salts has been evaluated in rats and mice, and shows a wide range of LD_{50} values for different compounds (Table 7).

Table 7. **Reported oral LD_{50} values for aluminium salts (adapted from FAO/WHO 1989 and WHO 1997)**

Salt	Species	LD_{50} (mg test substance/kg bw)	LD_{50} (mg Al/kg bw)
$AlBr_3$	mouse	-	164
	rat	-	162
$Al(NO_3)_3$	Mouse	-	286
	Rat	-	261
$AlCl_3$	mouse	3800	770
	mouse	-	220
	rat	-	370
	rat	3700	750
	rat	1600 *	737 *
$Al_2(SO_4)_3$	mouse	6200	980
	mouse	-	>730
	Rat	-	>730

* Kumar (2001)

The range of available LD_{50} data obtained with intraperitoneal administration is much narrower than that for oral administration (Table 8).

Table 8. **Reported intraperitoneal LD_{50} values for aluminium salts (adapted from FAO/WHO 1989 and WHO 1997)**

Salt	Species	LD_{50} (mg test substance/kg bw)	LD_{50} (mg Al/kg bw)
$AlBr_3$	mouse	-	108
	rat	-	82
$AlCl_3$	mouse	-	105
	rat	-	81
$Al(NO_3)_3$	mouse	-	133
	rat	-	65
$Al(OH)_3$	rat	1100	35
$Al_2(SO_4)_3$	mouse	-	40
	rat	-	25

These data indicate that within the body different aluminium compounds have similar potency, which is based upon the dose of the aluminium ion. The range of different potencies following oral administration is therefore likely to be dependent upon the bioavailability. The difference between the oral and i.p. LD_{50} values suggests that the extent of absorption for different aluminium compounds is in the following order: $AlBr_3 > Al(NO_3)_3 > AlCl_3 > Al_2(SO_4)_3$.

There is little indication that aluminium is acutely toxic by oral exposure in humans, despite its widespread use in antacids at doses up to 1200 mg/day (as aluminium glycinate and/or hydroxide) (WHO, 1997).

Overall, it can be concluded that the acute oral toxicity of those aluminium compounds for which data are available is moderate to low.

4. Sub-chronic toxicity

The available sub-chronic toxicity studies relating to drinking water administration of aluminium compounds are summarised in Table 8, those relating to gavage or dietary administration are summarised in Table 9. Most of these were not conducted in accordance with the guidelines required for regulatory submissions, and the study design and reporting does not allow NOAELs and LOAELs to be identified. In particular, most of the studies did not measure the aluminium content of the basal diet fed to the animals, and therefore the stated dose is likely to be an underestimate of total aluminium exposure. Furthermore, there is little consistency in the effects observed in different studies. The few studies indicating NOAELs or LOAELs are described below.

4.1. Rats

$Al(NO_3)_3$ was administered to groups of 10 female Sprague-Dawley rats via the drinking water at concentrations providing doses of 1, 26, 52 or 104 mg Al/kg bw per day for 28 days (Gómez *et al*., 1986). There were no clinical signs, effects on food or water consumption, growth, haematological and serum analyses at any dose level. Mild histopathological changes were reported in the spleen and liver at 104 mg Al/kg bw per day, with a NOAEL of 52 mg Al/kg bw per day (Gómez *et al*., 1986). In contrast, the same researchers reported that administration of $Al(NO_3)_3$ to female Sprague-Dawley rats via the drinking water at a concentration resulting in 261 mg Al/kg bw per day for 100 days resulted in decreased body weight gain associated with decreased food consumption, but no histopathological changes. Again there were no clinical signs, or effects on haematological and serum analyses at any dose level. The NOAEL in this study was also 52 mg Al/kg bw per day (Domingo *et al*., 1987).

Some studies with drinking water administration of $AlCl_3$ or $Al_2(SO_4)_3$ have indicated that effects on haematological parameters and in the brain might occur at lower doses (Somova & Khan, 1996; Somova *et al*., 1997). Groups of 10 male Wistar rats received $AlCl_3$ in deionised water as drinking-water for 6 months at doses stated to be 0, 5 and 20 mg Al/kg bw. After 6 months, the body weights of animals at the lowest dose (5 mg/kg bw per day) and at the highest dose (20 mg/kg bw per day) were 80% and 84% of control, respectively. Interim body weights were not reported. Non-dose-related changes in some haematological parameters and serum enzymes were also reported. (Somova & Khan, 1996). At 20 mg Al/kg bw, there were spongiform changes and neurofibrillary degeneration in the hippocampus of the brain and atrophy and fibrosis in the kidney (Somova *et al*., 1997). These studies provided inadequate information on how the reported doses were calculated, the aluminium content of the diet was not taken into account, and the effects were not clearly related to dose, and therefore these studies could not be taken into account in the evaluation.

Groups of 15 male albino rats (strain not reported) were given $Al_2(SO_4)_3$ at 0, 17, 22, 29, 43, 86 or 172 mg Al/kg bw or $KAl(SO_4)_2$ at 29 or 43 mg Al/kg bw by oral gavage for 21 days. The effects of both compounds were similar at comparable doses of aluminium. Mild histopathological effects were reported in the kidney and liver at the lowest dose of 17 mg Al/kg bw per day (as $Al_2(SO_4)_3$). Severity of effects increased with dose and effects on nerve cells, testes, bone and stomach were also reported at higher doses (Roy *et al*., 1991a). WHO (1997) stated that the data presented were inadequate to verify the reported effects as the study provided inadequate information on how the reported doses were calculated and the aluminium content of the diet was not taken into account.

Studies involving dietary administration of $Al(OH)_3$ and SALP basic to groups of 25 male Sprague-Dawley rats for 28 days resulted in no effects at the highest tested doses, which were in the region of 140-300 mg Al/kg bw per day (Hicks *et al*., 1987).

4.2. Dogs

Two studies have involved dietary administration of SALP to beagle dogs. Administration of dietary concentrations of 0, 3,000, 10,000 or 30,000 mg/kg SALP, acidic to groups of six male and six female dogs for 26 weeks produced no toxicologically relevant effects on haematological or clinical chemistry parameters, ophthalmological examination, urine analysis, faecal occult blood tests, organ weights or histopathological observations. Based on food consumption data, these dietary concentrations were equal to doses of 10, 27 and 88 mg Al/kg

bw per day and 9, 31 and 93 mg Al/kg bw per day for males and females respectively, but these were not corrected for the basal aluminium content of the diet (Katz *et al*., 1984).

In contrast, dietary concentrations of 0, 3,000, 10,000 or 30,000 mg/kg SALP basic, administered to groups of four male and four female beagle dogs for 26 weeks resulted in decreased food consumption, decreased body and testis weight and histopathological changes in liver and kidney of male dogs at the top dose. No effects were seen in females. These dietary concentrations were equal to average doses of 4, 10, 27 or 75 and 3, 10, 22 or 80 mg Al/kg bw per day for male and female dogs, respectively suggesting a NOAEL of 27 mg Al/kg bw in male dogs (Pettersen *et al*., 1990).

Table 9. **Summary of sub-chronic studies of oral toxicity of aluminium compounds administered via drinking water (adapted from WHO, 1997)**

Al salt	Species	Dose regime	Endpoints	NOAEL/LOAEL	Ref
$AlCl_3$	Male Weizman rats (n=5-10)	250 mg Al/kg bw per day via drinking water (period not specified) *	Clinical signs Tissue concentration	Not possible to establish NOAEL/LOAEL	Berlyne *et al.* 1972
	Male Wistar rats (n=10)	5 and 20 mg Al/kg bw per day via drinking water for 6 months *	Body weight, haematological status, histopathology	Decrease in body weight and haematological parameters at both doses, with effect greater at 5 mgAl/kg bw per day. Spongioform changes and neurofibrillary degeneration of the hippocampus and change in the kidney at 20 mgAl/kg bw per day Not possible to establish NOAEL/LOAEL	Somova & Khan, 1996; Somova *et al.*, 1997
$Al(NO_3)_3$	Female Sprague-Dawley rats (n=10)	1, 26, 52 or 104 mg Al/kg bw via drinking water for 28 days *	Clinical signs, food and water consumption. Tissue concentrations, bw and haematological status. Histopathology	Mild histopathological changes in spleen and liver NOAEL = 52 mg Al/kg bw per day LOAEL = 104 mg Al/kg bw per day	Gómez *et al.*, 1986
	Female Sprague-Dawley rats (n=10)	0, 26, 52 or 261 mg Al/kg bw via drinking water for 100 days *	Clinical signs, food and water consumption. Tissue concentrations, bw and haematological status. Histopathology	Decreased bw gain, no dose-dependent accumulation of aluminium in tissues NOAEL = 52 mg Al/kg bw per day LOAEL = 261 mg Al/kg bw per day	Domingo *et al.*, 1987
$Al_2(SO_4)_3$	Male Weizman rats (n=5-10)	1 or 2% in drinking water, equiv. to 200 or 350 mg Al/kg bw per day *	Clinical signs Tissue concentration	Periorbital bleeding in 3/5 animals at 350 mg Al/kg bw per day Not possible to establish NOAEL/LOAEL	Berlyne *et al.* 1972

* Not clear that aluminium content of feed has been taken into account

Table 10: **Summary of sub-chronic studies of oral toxicity of aluminium compounds administered via gavage or diet (adapted from WHO, 1997)**

Al salt	Species	Dose regime	Endpoints	NOAEL/LOAEL	Ref
$Al(OH)_3$	Male Weizman rats (n=5-10)	150 mg Al/kg bw per day by gavage (period not specified) *	Clinical signs Tissue concentration	Not possible to establish NOAEL/LOAEL	Berlyne *et al.* 1972
	Male Sprague-Dawley rats (n=16)	1079, 1012 or 2688 mg Al/kg diet, equiv. to 100, 100 or 270 mg Al/kg bw per day for 12 or 29 days with and without 4% citrate *	Body and organ weights, food and water consumption, haematological status Tissue and urinary concentrations	Haematocrits inversely correlated to tissue concentrations of aluminium. Not possible to establish NOAEL/LOAEL	Greger & Powers, 1992
	Male Sprague-Dawley rats (n=25)	Control or 14,470 mg/kg $Al(OH)_3$ in diet, equiv. to 5 or 302 mg Al/kg bw per day for 28 days	Clinical signs, food and water consumption, organ and body weights, haematological and serum analyses, urinalysis, ophthalmological examination, bone Al concentration, histopathology	No treatment-related effects or Al deposition in bone NOAEL = 302 mg Al/kg bw per day	Hicks *et al.*, 1987
$Al_2(SO_4)_3$	Male rats (n=5 per group)	0, 17, 22, 29, 43, 86 and 172 mg Al/kg bw per day by gavage for 7, 14 or 21 days *	Histopathological examination of heart, liver, kidney, brain, testes, stomach and femur	Dose-related histopathological effects in liver and kidney at all doses. Effects on other organs at higher doses. WHO considered the reported detail inadequate to evaluate (WHO 1997)	Roy *et al.*, 1991a
KASAL I [a] 6% SALP basic	Male Sprague-Dawley rats (n=25)	Control or 30,000 mg/kg KASAL I in diet, equiv. to 5 or 141 mg Al/kg bw per day for 28 days	Clinical signs, food and water consumption, organ and body weights, haematological and serum analyses, urinalysis, ophthalmological examination, bone Al concentration, histopathology	No treatment-related effects or Al deposition in bone NOAEL = 141 mg Al/kg bw per day	Hicks *et al.*, 1987
KASAL II 13% SALP basic	Male Sprague-Dawley rats (n=25)	Control, 7000 or 30,000 mg/kg KASAL II in diet, equiv. to 5 (control), 67 and 288	Clinical signs, food and water consumption, organ and body weights, haematological and serum analyses, urinalysis, ophthalmological examination, bone Al	No treatment-related effects or Al deposition in bone NOAEL = 288 mg Al/kg bw per day	Hicks *et al.*, 1987

		mg Al/kg bw per day for 28 days	concentration, histopathology		
$KAl(SO_4)_2$	Male rats (n=5 per group)	0, 29 and 43 mg Al/kg bw per day by gavage for 7, 14 or 21 days *	Histopathological examination of heart, liver, kidney, brain, testes, stomach and femur	Reported detail inadequate to evaluate.	Roy *et al.*, 1991a
SALP, acidic	Male and female beagle dogs (n=6)	0.3, 1.0 and 3.0% SALP, acidic in diet, equiv to 10, 27 and 88 mg Al/kg bw per day (males) or 9, 31 and 93 mg Al/kg bw per day (females) for 26 weeks *	Clinical signs, food and water consumption, organ and body weights, haematological and serum analyses, urinalysis, ophthalmological examination, tissue concentrations, histopathology	Decreased food consumption in females, but no associated decrease in body weight. Changes not considered to be toxicological relevant NOAEL = c. 90 mg Al/kg bw per day	Katz *et al.*, 1984
SALP, basic	Male and female beagle dogs (n=4)	0, 0.3, 1.0 and 3.0% SALP, basic in diet, equal to 4 (control), 10, 27 and 75 mg Al/kg bw per day (males) or 3 (control), 10, 22 and 80 mg Al/kg bw per day (females) via diet for 26 weeks	Clinical signs, food and water consumption, organ and body weights, haematological and serum analyses, urinalysis, ophthalmological examination, tissue concentrations, histopathology	Sharp, transient decrease in food consumption in males with concomitant decrease in body weight, testes weight and histopathological changes in liver and kidney. Males: LOAEL = 75 mg Al/kg bw per day NOAEL = 27 mg Al/kg bw per day Females: NOAEL = 80 mg Al/kg bw per day	Pettersen *et al.*, 1990

* Not clear that aluminium content of feed has been taken into account

[a] KASAL is a synonym for SALP basic, which is a mixture of 70% of a complex of SALP and 30% of disodium phosphate.

5. Genotoxicity

Aluminium ion (Al^{3+}) is known to interact with DNA *in vitro* (WHO, 1997, ATSDR, 2006, IAI 2007). Effects include cross-linking of chromosomal protein and DNA (WHO, 1997). Studies using NMR spectroscopy and circular dichroism of DNA: aluminium complexes have indicated that Al^{3+} binds to the phosphate oxygen while hydroxylated aluminium species may preferentially bind to other sites such as DNA bases (Rao & Divakar, 1993, as quoted in WHO, 1997). Ahmad *et al*. (1996) carried out DNA binding studies with aluminium chloride (0.6-25 mM) in calf-thymus DNA and reported that aluminium was bound to the backbone phosphate group and the guanine N-7 site of the G-C base pairs by the process of chelation.

A number of aluminium compounds have given negative results in the majority of short-term bacterial mutagenicity assays (IAI 2007). No evidence of a mutagenic response was seen in *Salmonella typhimurium* strains TA104, TA92, TA98, TA100 exposed to aluminium acetylacetonate (1.9-48 micromol/plate), aluminium lactate (1.8 - 5.5 micromol/plate), or aluminium maltolate (0.5-3.7 micromol/plate) (Gava *et al*., 1989), while aluminium fluoride (0.02-119 micromol/plate) did not produce mutations in TA98, TA100, TA1535, TA1537, and TA1538 strains (Shimizu *et al*., 1985). Zeiger *et al*. (1987) obtained negative results in the TA98, TA100, TA1535, and TA1537 strains following exposure to sodium aluminium silicate (0.96 - 38.5 micromol/plate). No evidence of mutagenicity was seen in *Salmonella typhimurium* TA 102 strain exposed to aluminium chloride hexahydrate at concentrations of 10-100 nanomol/plate (Marzin & Phi, 1985), while aluminium chloride (at concentrations of 0.3 and 3.0 mg/l) also gave negative results in the TA98 strain in an assay carried out in suspension culture (Ahn & Jeffrey, 1994). Similarly, Prival *et al*. (1991) showed no positive responses to sodium aluminium silicate (0.36 - 108.1 micromol/plate) or calcium aluminosilicate (0.033 - 10 mg/plate) in TA98, TA100, TA1535, TA1537, and TA1538 strains. While positive results have been obtained in studies using dye-alumina complexes, these results have been attributed to impurities present in the dye complexes rather than an effect of aluminium (Brown *et al*., 1979).

Studies with a range of aluminium compounds (aluminium chloride, aluminium fluoride, calcium aluminosilicate and sodium aluminium silicate) using *Escherichia coli,* WP2 strain, have also shown negative responses (Seo & Lee, 1993; Shimizu *et al*., 1985; Prival *et al*., 1991). No mutagenic activity was demonstrated for aluminium oxide, aluminium chloride or aluminium sulphate at concentrations of 1-10 mM in the rec-assay using *Bacillus subtilis* strains (ATSDR, 2006).

Aluminium compounds have also shown no evidence of inducing gene mutations in mammalian cells *in vitro.* No induction of forward mutations were observed at the thymidine kinase locus in L5178Y mouse lymphoma assay with aluminium chloride when tested at concentrations up to 625 μg aluminium chloride/ml (Oberly *et al*., 1982, as quoted in ATSDR, 2006 and IAI, 2007).

In common with other agents producing protein: DNA cross-linking, Al^{3+} has however been demonstrated to be clastogenic *in vitro* and *in vivo* and to decrease cell division both in mammalian and plant cells. Chromosomal aberrations have been reported in peritoneal lavage cells obtained from rats, mice and Chinese hamsters exposed *in vitro* to aluminium hydroxide (Nashed, 1975 as reported in Bhamra & Costa, 1992, and WHO, 1997). In an early study, cultured human blood lymphocytes treated with 20 μg/ml aluminium sulphate showed positive responses for induction of micronuclei, chromatid type aberrations and sister chromatid exchanges (Roy *et al*., 1990).

The induction of micronuclei in human lymphocytes by Al salts was further investigated in cytokinesis-blocked cells: Migliore and coworkers demonstrated a significant but not dose-related increase in micronuclei formation at all dose levels in a study in which human lymphocytes were exposed to levels of 500, 1000, 2000 or 4000 μM aluminium sulphate for 48 h after PHA stimulation (Migliore *et al*., 1999).

In another study human peripheral blood lymphocytes were treated with 1, 2, 5, 10 and 25 μg/ml aluminium chloride at different stages of the cell cycle (G_0/G_1, G_2/S and during the whole cell cycle), and the formation of micronuclei and induction of apoptosis was assessed (Banasik *et al*., 2005). With all treatment schemes an increase in the frequency of micronuclei was observed initially, but a decrease was observed at high concentrations (10 and/or 25 μg/ml), correlated with an increase in apoptosis. The G_0/G_1 phase of cell cycle was found to be more sensitive than S/G_2 phases. The characterisation of micronuclei by in situ fluorescence hybridisation with centromeric DNA probes highlighted the induction of both centromere-negative and centromere-positive micronuclei by $AlCl_3$, indicating both clastogenic and aneugenic activity.

The same researchers have also examined DNA damage and apoptosis in human lymphocytes treated with aluminium chloride at concentrations of 1, 2, 5, 10 and 25 μg/ml for 72 h, using a comet assay with confirmation of apoptosis by flow cytometry (Lankoff *et al*., 2006). DNA damage was induced in a concentration-dependent manner up to a level of 10 μg/ml, while at 25 μg/ml DNA damage declined, accompanied by a high level of apoptosis, indicating selective elimination of damaged cells. DNA damage was significantly increased in presence of endonuclease III and formamidopyrine glycosilase, indicating the presence of oxidized purines and pyrimidines. Based on these findings the authors have suggested that oxidative stress may be a possible mechanism of aluminium-induced (Al^{3+}) DNA damage (Banasik *et al*., 2005; Lankoff *et al*., 2006), noting that aluminium (Al^{3+}) has been demonstrated to promote the generation of iron-induced reactive oxygen species (Zatta *et al*., 2002; Yousef, 2004).

The authors also examined the effect of aluminium (Al^{3+}) on DNA repair, by pre-treating human lymphocytes with 10 μg/ml aluminium chloride for 72 h followed by irradiation with 2 Gy. Decreased DNA repair capacity was demonstrated in the aluminium chloride-treated cells compared with non-treated controls (Lankoff *et al*., 2006). Such an effect, which is common to several heavy metals, was attributed by the authors to the inhibition of DNA repair enzymes by Al^{3+}, possibly secondary to the interaction of Al^{3+} with zinc-finger protein domains.

In another study cultured human lymphocytes were treated with 5, 10, 15 and 25 μM aluminum chloride during the G_1, G_1/S, S, and G_2 phases of the cell cycle. DNA damage was induced, as detected by comet assay, and structural chromosomal aberrations were observed in all phases of cell cycle, especially in the S phase. Aluminium chloride also induced endoreduplication and polyploidy in treatments performed during $G_1$and G_2 (Lima *et al*., 2007). However, all tested concentrations were cytotoxic and reduced the mitotic index significantly in all phases of the cell cycle.

Stimulation of DNA synthesis has been demonstrated in human dermal fibroblasts *in vitro* as measured by ^3H thymidine incorporation, determined by scintillation counting (Dominguez *et al*., 2002). Cells treated with 1.85-74 μM aluminium (aluminium nitrate) for 1, 2, 3, 4 and 5 days showed statistically significant increases in DNA synthesis at concentrations of 3.7 μM and above, from 2 days exposure onward. At 74 μM and 5 days exposure, synthesis increased by 322% over control. Stimulation of DNA synthesis was accompanied by increased fibroblast division at concentrations of 7.4-74 μM after 3 days incubation (Dominguez *et al*., 2002).

A clastogenic potential of aluminium has also been demonstrated in short-term *in vivo* genotoxicity studies in rodents. Chromatid aberrations, including breaks, translocation and ring

formations, were reported to be significantly increased in the bone marrow of mice injected intraperitoneally with 0.01, 0.05, or 0.1M aluminium chloride (1 ml/30 g. bw, equivalent to 44 mg/kg bw, 222 mg/kg bw, or 443 mg/kg bw), although a clear dose-response relationship was not apparent (Manna & Das, 1972, as reported in WHO, 1997 and ATSDR, 2006).

A dose-dependent inhibition of cell division and an increase in chromosomal aberrations was reported in the bone marrow of rats administered either aluminium sulphate (212, 265, 353, 530, 1060 or 2120 mg/kg bw, equivalent to 17, 22, 28, 43, 85 or 172 mg Al^{3+}/kg, administered orally by gavage) or potassium aluminium sulphate (764 mg/kg bw or 503 mg/kg bw, equivalent to 28 or 43 mg Al^{3+}/kg, administered orally by gavage) daily for 21 days (Roy *et al*., 1991b). Roy and co-workers also examined the induction of micronuclei in the bone marrow of Swiss albino mice given 2 intraperitoneal doses of aluminium sulphate (250 and 500 mg/kg bw (0.75 and 1.5 mmol Al/kg bw) 24 hours apart (Roy *et al*., 1992). The authors reported a dose related increase in micronuclei which attained statistical significance at the dose of 500 mg/kg b.w. (Roy *et al*., 1992). The same authors also demonstrated a dose-related induction of sister chromatid exchanges (SCEs) in bone marrow from male Swiss albino mice 24 h after a single intraperitoneal dose of aluminium sulphate (100, 200, or 400 mg/kg bw) (Dhir *et al*., 1993). An aluminium nitrilotriacetate complex given intraperitoneally to male Wistar rats (7 mg Al/kg bw, equivalent to 259 µmol/kg) did not however cause increased formation of 8-hydroxydeoxyguanosine in liver DNA (Umemura *et al*., 1990)

6. Carcinogenicity

Epidemiological studies of occupationally-exposed populations have suggested an association between inhalation exposure to aluminium dust and aluminium compounds during production and processing and cancer in humans (ATSDR, 2006, IAI, 2007). While the International Agency for Research on Cancer (IARC, 1984: Straif *et al*., 2005) concluded that "the available epidemiological studies provide limited evidence that certain exposures in the aluminum production industry are carcinogenic to humans, giving rise to cancer of the lung and bladder.", aluminium exposure was confounded by exposure to other agents including polycyclic aromatic hydrocarbons, aromatic amines, nitro compounds and asbestos. IARC also concluded therefore that "A possible causative agent is pitch fume." There is no evidence of increased cancer risk in non-occupationally exposed persons and IARC did not implicate aluminium itself as a human carcinogen.

The carcinogenicity of some aluminium salts has been investigated in a limited number of studies in experimental animals (WHO, 1997; ATSDR, 1999; IAI, 2007). In a poorly reported oral drinking water study in Long-Evans rats exposed to aluminum potassium sulphate at a concentration of 5 mg/kg as aluminium (equivalent to 1.2 mg Al/kg bw/day for approximately 2-2.5 yr, Schroeder & Mitchner (1975a) reported a significantly increased incidence of gross tumours in male rats only, compared with controls. At gross necropsy, 13/25 (52%) aluminum-treated male rats were found to have tumors compared to 4/26 (15.4%) controls. Six of the tumors in the aluminum-treated males were malignant compared to two malignancies in the control rats. The types of tumours observed were not specified further. Aluminum levels in the base diet were not reported in these studies, although the animals were reported to be fed a low-metal diet in metal-free environmental conditions.

In another long-term study, Wistar rats (30 males and 30 females per group) were exposed for 2 years via the diet to a mixture of aluminium phosphide and ammonium carbamate, as a commercial product, Phostoxin®, which was used as a source of phosphine gas in the fumigation of cereal grains and other agricultural products (Hackenberg,1972). The level of Phostoxin® included in the diet in this study was 90 g/metric tonne of diet, 10 times the dosage

of aluminium phosphide recommended for cereal treatment and equivalent to 60 mg/kg diet of aluminium phosphide and 28 mg/kg added Al^{3+} (approximately 1.4 mg/kg bw/day), No increase in the incidence of neoplasms in either male or female rats receiving aluminium phosphide compared with controls was observed (Hackenberg, 1972). The Panel noted the low concentration of aluminium phosphide used in this study and the fact that aluminum levels in the base diet were not reported.

Schroeder and Mitchener also carried out a drinking water study with aluminium potassium sulphate in Swiss Webster mice, at a dose level of 1.2 mg/kg b.w. aluminium /day for 2-2.5 years (Schroeder & Mitchener (1975b). They reported a significantly increased incidence of gross tumors in the aluminium-treated female mice of 19/41 (46.3%) compared with 14/47 (29.8%) in controls. The incidence of "lymphoma leukemia" was also significantly increased (10/41 versus 3/47 in controls) in female mice. A dose-response relationship could not be determined for either species because only one aluminum dose was used and the types of tumors and organs in which they were found were not specified (ASTDR, 2006). According to ASTDR, very few study details were reported in this paper and it is unclear if the investigators grouped several types of tumors into the "lymphoma leukemia"

A more recent study in mice by Oneda *et al*. (1994) involved the administration of aluminium potassium sulphate (APS) to B6C3F1 mice (60 males and 60 females per group) at levels of 1, 2.5, 5 or 10% in the diet for 20 months (equivalent to 1500, 3750, 7500 or 15000 mg/kg bw/day of aluminium potassium sulphate and 85, 213, 427 or 853 mg Al/kg bw/day). Body weight gain was reduced in the mice receiving 10% APS and increased in the groups receiving 1 or 2.5%, while those receiving 5% showed similar weight gains to controls. Survival rates were marginally increased in all APS-treated mice compared with controls, and there was no evidence of Al-related toxicity in any of the treated groups. There was also no increase in the incidence of gross tumours, neoplastic lesions, or other proliferative lesions in treated mice compared with controls. Animals receiving 10% APS in the diet showed a significantly lower incidence in total tumours compared with controls, which was mainly attributable to a reduction in the incidence of hepatocellular carcinoma, although incidence of other tumours such as pulmonary adenocarcinoma and Harderian gland adenomas were also reduced. The lower tumour incidence was in turn attributed to the reduced body weight gain seen in animals at this dose level. The authors concluded that there was no evidence of tumourogenicity or any other toxic actions of APS in B6C3F1 mice in this study (Oneda *et al*. 1994).

A number of studies have examined the potential carcinogenicity of aluminium-containing fibres and particles following exposure of animals via the inhalation or intraperitoneal routes. Although there is some evidence of carcinogenicity in such studies, this is highly dependent on the physical properties of the particles/fibres and is not considered to be relevant to exposure of humans via the oral route.

In an *in vitro* cell transformation assay using Syrian hamster embryo cells in which a range of metal salts were tested, there was no evidence of induction of cell transformations after application of aluminium salts (not further specified) (Di Paolo and Casto, 1979, as quoted in ASTDR, 2006 and IAI 2007). This assay has been reported to detect cellular events that are relevant to carcinogenesis

7. Reproductive and developmental toxicity

7.1. Reproductive toxicity

7.1.1. Animal studies

Mice

Male Swiss mice (eight animals per dose) were administered 0, 50, 100, or 200 mg of aluminium nitrate nonahydrate/kg bw/day by intraperitoneal injection for 4 weeks (5 days/week). After the treatment period the males were mated with untreated females for 4 days. Ten days after the end of the mating period the females were killed and the uterus content examined. The males were killed at the end of the treatment period. Male body weights were significantly decreased in all aluminium treated groups. A significant decrease in the weight of testes and epididymis occurred in animals treated with 200 mg/kg bw/day, but no differences were observed in the relative organ weights. The pregnancy rate was significantly lower after 100 (25%) and 200 (18.8%) mg/kg bw/day compared to the controls (62.5%). The histological examination of the testis revealed necrosis of spermatocytes and spermatides in mice treated with 100 and 200 mg/kg bw/day. Testicular and epididymal sperm counts were significantly reduced at the highest dose, but the sperm motility was unaffected. No effects on fertility and no testicular alterations were observed at 50 mg/kg/day. This study shows that aluminium nitrate at high intraperitonal doses is toxic for the reproductive system of male mice (Llobet *et al.*, 1995).

Guo *et al.* (2005) administered 0, 7, or 13 mg/kg bw/day of aluminum chloride to 10 CD-1 male mice/group subcutaneously for 14 days. After the exposure to aluminium each male was housed daily with three untreated females. The mated females were replaced by new virgin females and this procedure was carried out continuously for 9 weeks. The average body weights of the male mice were unaffected by the treatment. A reduction of the libido (i.e. number of females showing evidence of mating/number of females placed with each male) appeared at week 4 and was most marked at weeks 4-6 in both treated groups. All treated animals had completely recovered at the end of the experiment (week 11). The fecundity index was significantly reduced at weeks 4 and 5. No clear dominant lethal effects were observed.

In a second experiment, male mice treated for 2 weeks as in the first experiment (0, 7, or 13 mg/kg bw/day of aluminum chloride), were sacrificed at weeks 3, 5 and 11 of the experimental period to measure serum and testicular aluminium levels and for histological examination of testes. Testicular aluminium levels increased significantly after aluminium exposure, and this elevation lasted at least 2 weeks after termination of the aluminium administration. Histological examination showed necrosis of spermatids and spermatozoa at week 5, but not at week 11. This study demonstrates that aluminium chloride is able to alter the fertility of male mice when administered subcutaneously for 2 weeks (Guo *et al.,* 2005).

Rats

Male Sprague Dawley rats (number per group not reported) were treated by gavage for 60 days with 0, 180, 360, or 720 mg/kg bw/day of aluminium nitrate nonahydrate corresponding to 0, 13, 26, or 52 mg aluminium/kg bw/day. Female rats were treated by the same route and with the same doses for 14 days prior to and throughout the mating period, and through gestation, delivery and lactation. Males and females were mated according to the respective dose levels. Treatment with aluminium did not affect the survival of the adult rats. No other toxicity data and data related to potential effects on the male reproductive organs were reported. The

percentage of pregnant females was similar in all groups. The average number of corpora lutea and implantations were not significantly different except for a lower number of corpora lutea in the highest dose group, which was attributable to an unusually high number of corpora lutea in the control group. This paper gives very little information about the reproductive toxicity of aluminium, but indicates that a dose of 52 mg aluminium/kg bw/day did not interfere with male and female rat fertility (Domingo *et al.*, 1987a).

Female Sprague Dawley rats (11 to 17 per group) were exposed to aluminium nitrate nonahydrate in the drinking water at doses delivering 0, 50, and 100 mg aluminium/kg bw/day for 15 days before mating with untreated males. The aluminium exposure was maintained during gestation and lactation. Citric acid was added to the drinking water of the animals exposed to 50 and 100 mg aluminium/kg bw/day in order to enhance the gastrointestinal absorption (355 and 710 mg citric acid/kg bw/day, respectively). Control females received water containing 710 mg citric acid/kg bw/day. There was a reduction of food consumption and maternal body weight gain during gestational days 7 -15 in the groups exposed to aluminium compared to the control group. There were no differences among the groups in the length of gestation, mean number of pups per litter, viability index, and pup body weight at birth. (Colomina *et al.*, 2005).

Rabbits

Four groups of six male New Zealand rabbits each received ascorbic acid (AA 40 mg/kg bw, group 2), aluminium chloride (34 mg $AlCl_3$/kg bw every other day, group 3) or a combination of the two (group 4). Group 1 served as negative control and received 0 mg/kg of $AlCl_3$ and 0 mg/kg of AA. The substances were administered by gastric tube every other day for 16 weeks. Semen collection occurred weekly over the 16 weeks of the study. All rabbits were killed at the end of the treatment period. Body weight, feed intake and relative weights of testes and epididymis were significantly decreased in rabbits treated with $AlCl_3$. Treatment with $AlCl_3$ caused a statistically significant decrease in the overall means of semen ejaculate volume (- 13.4%), sperm concentration (- 13.1%), total sperm output (- 23.9%), (%) sperm motility (- 9,2%), total motile sperm per ejaculate (- 27.5%), and libido (by increasing reaction time from 2.04 sec. to 4.30 sec.). AA improved the semen quality showing that it was able to antagonize the effects induced by $AlCl_3$. As $AlCl_3$ is able to produce reactive oxygen species, the protective effects of AA may be related to its antioxidant properties (Yousef *et al.*, 2005).

Dogs

Groups of 4 male and 4 female beagle dogs were fed diets containing 0, 3000, 10000, or 30000 mg KASAL (basic sodium aluminium phosphate) per kg for 26 weeks. A sharp, transient decrease in food consumption and a concomitant decrease of body weight were observed in the high dose males. No effects on food consumption were observed in females. In the high dose males there was a decrease of testicular weight; furthermore two animals of this group had moderate seminiferous tubule germinal epithelial cell degeneration and atrophy. The authors' conclusion that the testicular changes appeared to be secondary to the reduction in food consumption and body weight due to palatability problems is questionable. The dietary concentrations of KASAL were equal to average doses of 4, 10, 27 or 75 and 3, 10, 22 or

80 mg Al/kg bw per day for male and female dogs, respectively suggesting a NOAEL of 27 mg Al/kg bw in male dogs (Pettersen *et al.*, 1990).

1

2 Table 11. **Summary of reproductive toxicity studies**

Species	Route	Compound	Dose of compound (Mg/kg bw/day)	Doses of aluminium (Mg/kg bw/day)	Duration	NOAEL/LOAEL	Reference
Sprague Dawley rats	Drinking water	Aluminium nitrate nonahydarate plus citric acid		0, 50, or 100*	Females 15 days before mating and during gestation and lactation. Males were not treated.	Reduced food consumption and body weight gain (50 and 100 mg aluminium/kg bw/day). No effects on female fertility.	Colomina *et al.* 2005
Sprague Dawley rats	Gavage	Aluminium nitrate nonahydarate	0, 180, 360, or 720	0, 13, 26, or 52*	Males 60 days before mating. Females 2 weeks before mating until end of lactation	No effects on male and female fertility. NOAEL: 52 mg Al/kg bw/day.	Domingo *et al.*, 1987a
New Zealand rabbits	Gavage	Aluminium chloride alone plus citric acid	34	6.4*	16 weeks	Reduction of semen quality. NOAEL not identified	Yousef *et al.* 2005
Beagle dogs	Diet	SALP, basic (KASAL)	0 – 112 – 390 – 1,143	4, 10, 27, or 75*	26 weeks	Reduced testes weight, epithelial germinal cells degeneration. NOAEL: 27 mg Al/kg bw/day	Pettersen *et al.*, 1990

* not clear that aluminium content of feed has been defined

7.2. Developmental toxicity

7.2.1. Animals

Mice

Swiss Webster mice (number not reported) were treated subcutaneously on days 3, 5, 7, 9, 11, 13, and 15 of pregnancy with 10, 20, or 40 mg aluminium/kg bw as aluminium lactate. Controls were treated with the solvent (phosphate buffered saline). Dams were killed on day 18 of gestation. Maternal mortality and food intake were not affected by aluminium treatment. Necrotic skin lesions near the injection site were observed in the low (7%), mid (64%) and high (100%) dose groups. There was a dose-related decrease in the percentage of successful pregnancies: control 80%, low dose 71%, mid dose 57%, high dose 25%. Because of the high frequency of skin lesion and low incidence of complete pregnancy the high dose group was discontinued. The maternal body weights at day 18 of the pregnancy were not affected by the treatment. Fetal and placental weights were not affected by the treatment and no major malformations were observed in any group. However, minor anomalies (dilated cerebral ventricles, dilated renal pelvis, or haemorrhage in ventricles) were increased after 10 and 20 mg Al/kg bw in comparison to the control (12%, 23%, 33%). No other signs of embryotoxicity were observed (Golub *et al.*, 1987).

Sixteen pregnant Swiss Webster mice divided into three groups were fed on semipurified diets containing 25, 500, or 1000 mg aluminium/kg as aluminium lactate. The experimental diet began on day 0 of gestation and continued throughout pregnancy and lactation. The maternal aluminium intakes were reported to be equivalent to 5, 100 and 200 mg aluminium/kg bw/day, respectively at the beginning of the pregnancy and 10, 210 and 420 mg aluminium/kg bw/day, near the end of lactation. There were no effects of dietary aluminium on maternal mortality, weight gain, food intake or other signs of toxicity. There were no group differences in pregnancy rate, litter size, birth weight, or perinatal and postnatal pup mortality (Donald *et al.*, 1989).

Female Swiss mice (20 per group) were treated by gavage on days 6 – 15 of gestation with 0, 66.5, 133, or 266 mg aluminium hydroxide /kg bw/day, corresponding to 0, 23, 46, or 92 mg aluminium/kg bw/day. The dams were killed on day 18 of gestation. No treatment related signs of maternal toxicity were observed. No signs of embryotoxicity, including morphological abnormalities, were observed (Domingo *et al.*, 1989)

Swiss (CD-1) mice (10 to 13 per group) were given daily doses of aluminium hydroxide (166 mg/kg bw), aluminium lactate (627 mg/kg bw), aluminium hydroxide (166 mg/kg bw) plus lactic acid (570 mg/kg bw), lactic acid (570 mg/kg bw), or distilled water (control) by gavage on gestational days 6 – 15. A reduction of body weight gain (not related to food consumption) was observed in dams treated with aluminium hydroxide + lactic acid and aluminium lactate. The postimplantation loss was similar in all treated and control groups. Treatment with aluminium lactate was associated with a significant reduction of fetal body weights. Cleft palate and delayed fetal ossification were also recorded in this group (Colomina et al., 1992).

Swiss mice (number per group not reported) were given daily doses of aluminium hydroxide (300 mg/kg bw), ascorbic acid (85 mg/kg bw), aluminium hydroxide (300 mg/kg bw) + ascorbic acid (85 mg/kg bw), or distilled water by gavage on gestational days 6 – 15. The body weights were comparable among the groups during the exposure period and during the overall gestation period, whereas the maternal food consumption was significantly reduced during the overall gestation period in the dams receiving aluminium hydroxide alone or aluminium

hydroxide plus ascorbic acid. No differences were observed among the groups in percentage of postimplantation loss, fetal body weight, incidence of major malformations and minor anomalies (Colomina *et al.*, 1994).

Five groups of CD-1 mice were given a single dose of 995 mg/kg bw of aluminium nitrate nonahydrate by gavage on one of the days 8 – 12 of gestation. Control animals received deionized water by gavage. One female died in the 8, 9, 10, and 12 day groups; one female aborted in the 8 and 12 day groups; one female resorbed all litter in the 8 day group. A reduction of body weight gain was observed in all treated groups. The average fetal body weight was reduced in all treated groups in comparison to control. The most common morphological anomalies associated with aluminium exposure were reduced ossifications in all treated groups (Albina *et al.*, 2000).

Rats

Benett *et al*. (1975) administered aluminium chloride to pregnant Holtzman rats by intraperitoneal injection. Two different experiments were performed. In the first study (acute treatment) 8 and 9 dams respectively were treated with 40 mg aluminium chloride/kg bw on either gestational day 9 or 13. In the second study (repeat dose treatment) 5 to 10 dams per group received 0, 75, 100, or 200 mg aluminium chloride/kg bw/day on days 9 -13 or 14 -18 of pregnancy. The acute treatment with 40 mg/kg bw resulted in no maternal toxicity or embryotoxicity. With the chronic treatment, some animals did not survive the treatment at dose levels of 100 and 200 mg/kg bw/day. Autopsy of the mothers revealed abundant ascites, extensive adhesions between organs and perihepatic granuloma. The mean weight of fetuses was less in all treated groups in comparison with the controls with the exception of the animals treated with 75 mg/kg on days 14 -18 of pregnancy. The incidence of resorptions was significantly higher in animals treated with aluminium chloride at 75 mg/kg bw/day (days 9 -13), 100 mg/kg bw/day (days 14 -18) and 200 mg/kg bw/day (days 9 -13). The number of dead fetuses was significantly higher than in controls only at the highest dose level (treatment on days 9 – 13). Due to the high maternal and embryofetal mortality very few fetuses were available for examination in the two highest dose groups. A significantly high incidence of abnormal fetuses was recorded from animals treated with aluminium chloride at 100 mg/kg bw on days 14 – 18, three fetuses with abnormal digits and seven fetuses with wavy ribs.

Pregnant Wistar rats (6 to 12 animals per group) received from day 1 of gestation to parturition the following doses of aluminium with the diet: 0, 100, 300, or 400 mg Al/kg bw/day as the chloride salt or 0, 100, 200, or 400 mg Al/kg bw/day as the lactate salt. Food and water consumption were not affected by the treatment. A reduction of maternal body weight gain was recorded in animals treated with the mid and high doses of aluminium chloride and with the high dose of aluminium lactate. The average litter size at birth was similar in all treated and control groups but the postnatal mortality was very high in the mid and high dose groups of animals treated with aluminium chloride and in the high dose group of aluminium lactate. The pup weights at birth and during the postnatal development were significantly reduced in comparison to controls in the mid and high dose aluminium chloride and in the high dose group of aluminium lactate (Bernuzzi *et al.*, 1989b).

Aluminium nitrate was administered by gastric intubation to four groups of ten pregnant Sprague Dawley rats at doses of 0, 180, 360, or 720 mg/kg bw/day from gestation days 14 to 21. No data on maternal toxicity were reported. The dams delivered and pups were observed during the postnatal period. The number of litters and the number of live young per litter were lower for all treated groups in comparison to the control group, but no significant dose–

dependent differences were noted. The mean pup body weight was significantly lower in the group treated with the highest dose (Domingo *et al.*, 1987b).

Three groups of ten pregnant Sprague Dawley rats received intragastrically a daily dose of 180, 360, or 720 mg/kg of Aluminium nitrate dissolved in distilled water on days 6 – 14 of gestation. The control group received demineralised water. Cesarean section was performed on day 20 of gestation. In all groups given aluminium nitrate the dams gained significantly less body weight than the controls. The number of runt fetuses increased dose-dependently in all treated groups. Fetal body weight was significantly reduced in all aluminium treated groups. Severe signs of delayed ossification were present in all aluminium treated groups where an increase of congenital malformations, minor anomalies and variations were also recorded (Paternain *et al.*, 1988).

Pregnant Wistar rats (6 to 9 animals per group) received 400 mg aluminium/kg bw/day as aluminium lactate in the diet for either the first week of pregnancy (GD 1 -7), first and second weeks (GD 1 – 14), or from day 1 to parturition. The maternal body weight was significantly decreased only on days 16 and 19 in the group treated for the whole gestational period. No effects of treatment on litter size, mortality rate and weight of pups were noted (Muller *et al.*, 1990).

Three groups of 18 – 19 pregnant Wistar rats were given by gavage doses of 192, 384, or 768 mg/kg bw/day of aluminium hydroxide dissolved in distilled water divided in two equal administrations on days 6 -15 of gestation. A fourth group of 19 dams treated with distilled water served as control. Dams were killed on day 20 of gestation. No maternal deaths or signs of maternal toxicity were observed during the study. There were no treatment related effects on percentage of resorptions, mean number of live fetuses, or fetal body weight. There were no differences between control and treated groups in the incidence of major malformations or developmental variations (Gómez *et al.*, 1990).

Aluminium chloride was administered to pregnant Wistar rats mixed with the standard diet from day 8 of gestation to parturition. The doses were 160 (14 dams) or 200 (13 dams) mg Al/kg bw/day. The control group (12 dams) received standard diet. The weight gain of pregnant rats and the food consumption were similar in the control and the treated groups. There was an effect of prenatal treatment on postnatal pup surviving but this effect was not dose-dependent. The mean pup weight on day 1 post-partum was significantly reduced in the treated groups in comparison to the controls, but the body weights on the following days did not differ between the three groups. Also the age of appearance of eye opening showed no difference between the three groups (Bernuzzi *et al.*, 1986).

Pregnant Sprague Dawley rats (10 to 17 animals per group) were exposed to aluminium in the drinking water for 15 days before mating, and during gestation and lactation. The aluminium was administered in the drinking water as aluminium nitrate nonahydrate providing doses of 0, 50, or 100 mgAl/kg bw/day. In order to enhance the gastrointestinal absorption, 355 and 710 mg/kg bw/day of citric acid were added to the drinking water of the groups exposed to 50 and 100 mg Al/kg bw/day. The controls received water supplemented with 710 mg/kg bw of citric acid. The exposure to aluminium resulted in reduced food consumption (days 7 –15 of gestation) and reduced maternal weight gain (days 15 – 21). There were no differences among groups in the length of gestation, mean number of fetuses per litter, viability and lactation index. On postnatal days 12, 16 and 21 male and female pups of dams exposed to 100mg Al/kg bw/day showed a reduced body weight gain (Colomina *et al.*, 2005).

Table 12. **Summary of developmental toxicity of aluminium**

Species	Route	Compound	Doses of compound (mg/kg bw/day)	Doses of aluminium (mg/kg bw/day)	Duration	NOAEL/LOAEL	Reference
Swiss Webster mice	Diet	Aluminium lactate		7.5, 155, or 310	Day 0 of pregnancy to end of lactation.	The NOAEL was 310 mg Al/kgbw/day	Donald *et al*., 1989.
Swiss CD-1 mice	Gavage	Aluminium lactate	627	57.4	Days 6 to 15 of gestation	Reduced fetal weight, cleft palate, delayed ossification. No NOAEL obtained.	Colomina *et al*., 1992.
Swiss mice	Gavage	Aluminium hydroxide	0, 66.5, 133, or 266	0, 23, 46, or 92	Days 6 to 15 of gestation	No effects seen. The NOAEL was 92 mg Al/kg bw/day	Domingo *et al*., 1989.
Swiss mice	Gavage	Al hydroxide alone or plus ascorbic acid	300	103	Days 6 to15 of gestation	No effects seen. The NOAEL was 103 mg Al/kg bw/day	Colomina *et al*., 1994.
Swiss CD-1 mice	Gavage	Aluminium hydroxide alone or plus lactic acid	166	57.4	Days 6 to 15 of gestation	No effects seen. The NOAEL was 57.4 mg Al/kg bw/day	Colomina *et al*., 1992.
CD-1 mice	Gavage	Aluminium nitrate nonahydrate	995	71	On single day from day 8 to 12 of gestation	Delayed fetal development. The LOAEL was 71 mg Al/kg bw	Albina *et al*., 2000.
Wistar rats	Diet	Aluminium lactate	0, 100, 200, 400	0, 9, 18, 36	Day 1 of pregnancy to parturition.	Postanatal mortality; reduced pup weight. The NOAEL was 18 mg Al/kg bw/day	Bernuzzi *et al*., 1989b.
Wistar rats	Diet	Aluminium lactate		0, 400	Days 1 -7; 1-14, or 1 to parturition.	No effects on pups weight, litter size or pup mortality. The NOAEL was 400 mg/kg bw/day	Muller *et al*., 1990.
Sprague Dawley rats	Gavage	Aluminium nitrate nonahydrate	0, 180, 360, or 720	0, 13, 26, or 52	Day 1 to 14 of gestation	Reduced fetal weight, increase of fetal abnormalities. The LOAEL was 13 mg Al/kg bw/day	Paternain *et al*., 1988.
Sprague Dawley rats	Gavage	Aluminium nitrate nonahydrate	0, 180, 360, or 720	0, 13, 26, or 52	Day 14 to 21 of gestation	Reduced pup weight, increased postnatal mortality. The LOAEL was 13 mg Al/kg bw/day	Domingo *et al*., 1987
Wistar rats	Diet	Aliminium chloride	0, 100, 300, or 400	0, 20, 60, or 80	Day 1 of pregnancy to parturition	Postnatal mortality; reduced pup weight. The NOAEL was 20 mg Al/kg bw/day	Bernuzzi *et al*., 1989a.
Wistar rats	Diet	Aluminium chloride		0, 160, or 200	Day 8 of pregnancy to parturition	Reduced pup weight at birth. The LOAEL was 160 mg Al/kg bw/day.	Bernuzzi *et al*, 1986.
Wistar rats	Gavage	Aluminium hydroxide	0, 192, 384, or 768	0, 66, 132, or 264	Days 6 -15 of gestation	No embryotoxicity. The NOAEL was 264 mg Al/kg bw/day.	Gómez *et al*., 1990.

8. Neurotoxicity and neurodevelopmental toxicity

8.1. Introduction

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

The neurotoxicity of aluminium can be grouped according to the presence or absence of certain key features, including: (a) the induction of cytoskeletal pathology in the form of neurofilamentous aggregates, (b) alteration in cognition and behaviour in the absence of cytoskeletal pathology but with significant neurochemical and neurophysiological modifications, and (c) the developmental stage of the host (e.g. maternal exposure with consequent fetal effects) (Strong *et al*. 1996).

Aluminium can induce neurofilamentous aggregates by several mechanisms from the level of gene expression to catabolism of neurofilament but no single mechanism for the neurotoxicity has been established. Aluminium also possesses a potent ability to crosslink neurofilaments in both phosphorylated and nonphosphorylated states, which may change their solubility and susceptibility to proteolysis. A rough association between the extent of neurofilamentous aggregation and the severity of the clinical deficit can be determined albeit variability has been observed among the studies, even when the study designs were comparable (Strong *et al*., 1996).

Although rodents fail to develop cytoskeletal pathology in response to aluminium salts, in most cases aluminium lactate and in a few studies aluminium chloride or aluminium nitrate have induced behavioural abnormalities including changes in learning, memory and locomotor activity. In these animal models aluminium has been shown to affect cholinergic activity, glucose metabolism, signal transduction pathways, agonist-stimulated inositos phosphate accumulation, free radical-mediated cytotoxicity, and protein phosphorylation but no single mechanism for the neurotoxicity of aluminium has been established (Strong *et al*. 1996; Yokel 2000). The understanding of the mechanism for neurodevelopmental effects of aluminium is also limited, but there is evidence for aluminium transfer across the placenta and impairments in neurodevelopment in offspring exposed during gestation and/or lactation (Strong *et al*., 1996).

Aluminium may also affect the uptake of other metal ions. It has been demonstrated *in vitro* that 15 μM aluminium stimulates uptake of nontransferrin bound iron in a human glial cell line. Aluminium may thereby disrupt iron hemeostasis in the brain by mechanism including the transferrin receptor, a nontransferrin transporter, and ferritin. A decreased level of ferritin may result in an increased concentration of unbound intracellular iron. As iron is redox active this may result in increased oxidative damage. (Kim *et al*., 2007)

8.2. Humans

Aluminium has been associated with neurotoxicity in dialysis patients. In a study with 55 patients suffering from dialysis encephalopathy in six dialysis centres using a uniform clinical classification, the incidence of dialysis encephalopathy rose significantly with increasing cumulative exposure to aluminium via the dialysate (Schreeder *et al*., 1983;WHO 1997).

A number of epidemiological studies have been conducted, mostly focussing on a postulated association of aluminium exposure with Alzheimer disease and cognitive impairment. These studies have considered exposure from drinking water and from antacids and are not conclusive, as some suggest an association (Martyn *et al*., 1989;Neri & Hewitt 1991) and others do not (Wettstein *et al*., 1991). The studies mainly adopt assumptions about exposure based on concentrations of aluminium in the water supply and do not include estimates of additional dietary exposure (JECFA 2007). The Panel concluded that these studies are not informative for a safety assessment of aluminium from dietary intake.

The German Federal Institute for Risk Assessment in an updated statement on aluminium and Alzheimer disease concluded "so far no causal relationship has been proven scientifically between elevated aluminium uptake from foods including drinking water, medical products or cosmetics and Alzheimer disease. Amyloid deposits in the brain are typical for Alzheimer. However, an above-average frequency was not observed either in dialysis patients or in aluminium workers – two groups of individuals who come into contact with aluminium on a larger scale"(BfR, 2007).

In a study on miners inhaling finely ground aluminium powder (McIntyre powder consisting of 15 % elemental aluminium and 85 % aluminium oxide) as a prophylactic agent against silicotic lung disease, there was no significant difference on self or proxy reported neurological disorder between exposed and non-exposed miners. However, cognitive test scores and proportions were impaired on at least one test indicating a disadvantage for exposed miners. The relative risk of impairment of cognitive function among exposed miners was 2.6 (Rifat *et al*., 1990).

8.3. Animals

8.3.1. Studies on juvenile and adult animals

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

The effects of subchronic exposure (90 days) to aluminum chloride were analysed in 3, 10 and 24 month old male Wistar rats (n=270) by investigating the function of the vestibulo-ocular reflex (VOR). The animals were tested after 30, 60 and 90 days of exposure. Aluminium chloride was added to the drinking water and from measurement of the individual intake of water the intake of aluminium in the three dose groups were 11.1, 21.5 and 43.1 mg Aluminium/kg bw per day from this source. In addition, analyses showed that the animal diet contained 90 mg aluminium/kg providing an additional dose of 9 mg aluminium/kg bw per day. Therefore, the total doses from food plus water were approximately 20, 30, and 52 mg

aluminium/kg bw per day. There was a significant effect on post-rotatory nystagmus in animals given 52 mg Aluminium/kg bw per day, which was established as a LOAEL and 30 mg Aluminium/kg bw per day can be considered as a NOAEL (Mameli *et al*., 2006).

Groups of 6 week-old female Swiss Webster mice (numbers not given) were given either 3 or 1000 mg aluminium/kg diet as aluminium chloride (equivalent to <1 mg/kg bw/day and 50 mg/kg bw/day) for 5 or 7 weeks. There were no changes in thermal sensitivity, negative geotaxis or auditory startle between the groups after 5 or 7 weeks. Aluminium affected air puff startle at 5 and 7 weeks and also forelimb and hind limb grip strength were decreased in the high dose group (Oteiza *et al*., 1993).

Groups of 10-12 female mice aged 3-4 weeks were given aluminium as aluminium lactate in the diet for 13 weeks. The aluminium concentrations were 25 and 1000 mg aluminium/kg diet, equivalent to 2.5 and 100 mg/kg bw/day. The high dose group showed slightly increased growth, decreased motor activity, decreased grip strength and decreased startle responsiveness, but no significant changes in temperature sensitivity or footsplay (Golub *et al*., 1992a).

Groups of 42-day-old male Swiss Webster mice were given aluminium as aluminium lactate in the diet for 4 or 8 weeks. The aluminium concentrations were 7, 100, 500, 750 and 1000 mg aluminium/kg diet, equivalent to 0.7, 10, 50, and 100 mg/kg bw/day. Decreased brain weights were only found in the 4 weeks study in the top dose group. In contrast to the 13-week study, consistent, dose-dependent aluminium effects on grip strength and auditory startle were not found in the 4-week exposure or 8-week exposure cohorts (Golub & Keen 1999).

8.3.2. Studies on animals exposed during gestation or preweaning

Swiss Webster mice were fed aluminium as lactate in the diet at 25 (control), 500 or 1000 mg aluminium/kg diet from conception through weaning. The maternal intakes of aluminium were reported to be equivalent to 5, 100 and 200 mg aluminium/kg bw/day, respectively at the beginning of pregnancy and 10, 210 and 420 mg aluminium/kg bw/day, near the end of lactation. Weights, food intake and signs of toxicity were recorded at regular intervals and pregnancy outcome evaluated. Pups were assessed for growth, neurobehavioral development and signs of toxicity prior to weaning. They were assessed immediately after weaning and 2 weeks after weaning. No maternal or reproductive toxicity was detected and there were no group differences in pup mortality, growth or neurobehavioral development prior to weaning. Dietary aluminium was associated with a dose-related greater foot splay at 500 mg aluminium/kg diet at day 21 and 35, decreased sensitivity to heat at 1000 mg aluminium/kg diet at day 25 and 39, forelimb grip strength was increased at 1000 mg aluminium/kg diet but decreased at day 39 at 500 mg aluminium/kg diet. Hind limb grip strength was increased at both doses at day 25 whereas no change was observed at day 39. In the pups the doses were equivalent to 4, 75 or 150 mg aluminium/kg bw/day (Donald *et al*., 1989).

Groups of 20 female Swiss Webster mice were given 7 (control), 500 or 1000 mg aluminium/kg diet (equivalent to <1, 50 or 100 mg aluminium/kg bw/day) as aluminium lactate from conception until weaning or from conception through adulthood. There were no effects on pregnancy outcome, pup survival, body or organ weights. There was an increase in cage mate aggression in the 1000 mg group. Forelimb and hind limb grip strength were reduced in both dosed groups. The decrease in air puff startle was only statistically significant in the 500 mg group. Temperature sensitivity, negative geotaxis, and auditory startle were not affected by the diet. There were no differences in grip strength or auditory startle between mice exposed continuously to aluminium vs. those exposed only before weaning. It was concluded that

developmental exposure to 500 and the 1000 mg Al/kg diets had distinctive long-term effects on behavioural measures that were not dose dependent and were not further intensified by continuing exposure as adults (Golub *et al*., 1995).

Groups of 20 Swiss Webster mice received diets containing 7 (control), 100, 500 or 1000 mg aluminium/kg diet as aluminium lactate, throughout development (conception to 35 days of age) and were subjected to behavioural tests as adults (> 90 days of age). The authors considered these dietary doses to be equivalent to <1, 10, 50 and 100 mg aluminium/kg bw/day in adult mice, i.e. at the beginning of and during the pregnancy. During the lactation the doses were estimated to be <1, 42, 210, and 420 mg aluminium/kg bw/day. The basal diet contained the same percent of recommended dietary amount of phosphate, calcium, iron, magnesium, and zinc as young women usually consume. Females were evaluated in a Morris water maze at 3 months of age and males were evaluated in a motor test battery at 5 months of age. By weaning both males and females in the 500 or 1000 mg aluminium/kg groups weighed significantly less than controls. One offspring from each litter was used for behavioural testing. Subtle deficits in several neuroparameters were observed. These included impaired learning for the females in a maze in the 1000 mg aluminium/kg diet group and poorer cue utilisation in the maze in both the 500 and 1000 mg aluminium/kg diet groups. Performance of the males on the rotarod test was impaired in the 1000 mg aluminium/kg diet group. A reduction in hind limb grip strength was reported in approximately 15% of animals in the 1000 mg aluminium/kg diet group; this was no longer significant after adjustment for body weight. A dose-related and statistically significant difference between controls and rats given the 500 or 1000 mg aluminium/kg diet were observed on wire suspension fall latency. The dose of 100 mg aluminium/kg diet, equivalent to 10 mg/kg bw/day, was without any effect and can be considered a NOAEL These data suggest that developmental Al exposure under normal, but less than optimal, dietary conditions can lead to subtle but long-term effects on growth and brain function in adulthood (Golub & Germann 2001).

Female Swiss Webster mice at 6-8 weeks of age were given a diet containing 25 or 1000 mg aluminium/kg (equivalent to 2.5 or 100 mg/kg bw/day) as aluminium lactate from conception though pregnancy and lactation. At birth litters were fostered within or between low and high aluminium groups. Exposure to high aluminium during gestation, lactation, or both, significantly reduced offspring body weight from day 10 postnatally. Forelimb grip strength was decreased in offspring exposed during gestation to high aluminium. Hind limb grip strength decreased and temperature sensitivity was reduced in offspring exposed to high aluminium during gestation and lactation, and negative geotaxis latency was longer in offspring exposed to high aluminium during lactation (Golub *et al*., 1992b).

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

Groups of 13-14 Wistar rats received 0, 160 or 200 mg/kg bw/day of aluminium as $AlCl_3$ from gestation day 8 to parturition. In the offspring, the performance of the righting reflex and negative geotaxis were better in control animals compared to aluminium-dosed animals

whereas there were no differences in grasping reflex or locomotor coordination (Bernuzzi *et al*., 1986).

Pregnant Wistar rats received either aluminium chloride (100, 300 or 400 mg aluminium/kg bw/day) or aluminium lactate (100, 200 or 400 mg aluminium/kg bw/day) in diet from day 1 of gestation to parturition. No information on aluminium concentration in the diet was given. Maternal food and water consumption was not affected by treatment. A 5-10% deficit in maternal body weight was reported at day 18 of gestation in the mid- and high-dose groups treated with aluminium chloride and the high dose group treated with aluminium lactate, but not at earlier times. No effect of treatment on litter size was detected, but increased mortality was reported during the first week. This effect was significant in the groups receiving 300 mg aluminium/kg bw/day as aluminium chloride and in the 400 mg aluminium/kg bw/day as aluminium lactates. The neuromotor maturation of surviving pups treated with aluminium showed impairment during the first two weeks of life, with grasping reflex being significantly affected in all three aluminium lactate treatment groups and in all but the low dose aluminium chloride treated animals (Bernuzzi *et al*., 1989a).

Pregnant rats received 400 mg aluminium /kg bw/day as aluminium lactate in the diet for either the first week (gestation day (GD) 1-7); first and second (GD 1-14); or from GD 1 to parturition. No information on aluminium concentration in the diet was given. Maternal body weight was significantly decreased on GD 16 and 19 by 26% and 35%, respectively, in rats given aluminium from GD 1 to parturition, but not in the other dose groups. No effect of treatment on litter size, mortality rate and weight gain of pups was noted. Performance of the pups in a negative geotaxis test from the second two dosing regimes was diminished. Also locomotor coordination was decreased in dosed animals. No differences were apparent in grasping and righting reflexes (Muller *et al*., 1990).

SUMMARY OF NEURODEVELOPMENTAL TOXICITY AND NEUROTOXICITY OF ALUMINIUM

Species	Route	Compound	Doses	Duration	NOAEL/LOAEL	Reference
Mouse (Swiss CD1, 10-13/group)	Diet	Al lactate	25 (control), 500 and 1000 mg Al/kg diet (concentration according to bodyweight, see text)	GD 0 to weaning	LOAEL: Highest dose (increased landing foot splay, increased hindlimb grip strength, decreased temperature sensitivity)	(Donald *et al*., 1989)
Swiss Webster Mice (males and females, 8/group)	Diet	Al lactate	7 (control), 500 or 1000 mg Al/kg diet 50 or 100 mg Al/kg bw/day	Conception through weaning or; Conception through adulthood	LOAEL: 50 mg/kg bw/day (reduced grip strength) (100 mg/kg bw/day)	(Golub *et al*., 1995)
Swiss Webster Mice (20/group)	Diet	Al Lactate	7 (control), 100, 500 or 1000 mg/kg diet <1, 10, 50 or 100 mg/kg/bw/day	Conception to 35 days age	LOAEL: 50mg/kg/bw per day (weighed significantly less than controls (only at 11 weeks)) 100mg/kg/bw per day. Impaired learning in a maze in high dose	(Golub & Germann 2001)
Swiss Webster mic(female)	Diet	Al lactate	25 mg/kg or 1000 mg/kg diet.	Conception through weaning	Decreased forelimb grasp strength	(Golub *et al*., 1992b)
Swiss Webster mice (males and females, n = 18)	Diet	Al lactate	7 (control) and 1000 mg Al /kg diet <1 and 100 mg/kg bw/day in adults	Conception through lifespan	LOAEL: 100mg/kg red eyes, fur loss, circling)	(Golub *et al*., 2000)
Wistar rats (13-14/group)	Diet	$AlCl_3$	160 or 200 mg Al/kg bw/day [a]	GD 8 to parturition	LOAEL: 160 mg/kg/day (pre-weaning mortality, delay in neuromotor development)	(Bernuzzi, Desor, & Lehr 1986)
Wistar rats (6-12/group)	Diet	Al lactate or $AlCl_3$	100, 200 or 300 mg Al/kg bw/day ($AlCl_3$) 100, 200 or 400 mg Al/kg bw/day (Al lactate)	GD 1 - 21	LOAEL: 200 mg Al/kg bw/day as $AlCl_3$ (grip strength) 100 mg Al/kg bw/day as Al lactate (grip strength)	(Bernuzzi, Desor, & Lehr 1989a)
Wistar rats (6-9/group)	Diet	Al lactate	400 mg Al/kg bw/day [a]	GD 1-7; or GD 1-14; or conception to parturition	LOAEL: 400 mg Al/kg bw/day (locomotor co-ordination)	(Muller et al. 1990)

Wistar rats (25-38/group)	Oral gavage	Al lactate	100, 200 or 300 mg Al/kg bw/day [a]	Postnatal day 5-14	LOAEL: 100 mg Al/kg bw/day (negative geotaxis test)	(Bernuzzi, Desor, & Lehr 1989b)
Wistar rats (4/group; multiple groups per dose)	Oral gavage	Al lactate	100 or 200 mg Al/kg bw/day [a]	Postnatal days 5-14	LOAEL: 200 mg/kg bw/day (increased brain Al, decreased choline acetyltransferase & general activity)	(Cherroret *et al.*, 1992)
Swiss Webster Mice (femal 10-12/group)	Diet	Al lactate	25 (control) or 1000 mg Al/kg 2.5 and 100 mg/kg bw/day	90 days 3-4 weeks at start	LOAEL: 100 mg/kg bw/day (Decreased grip strength)	(Golub *et al.*, 1992a)
Swiss Webster Mice (female, numbers not given)	Diet	$AlCl_3$	3 mg/kg or 1000 mg/kg (0.3 or 100 mg/kg bw/day)	5-7 weeks Animals were 42 days of age at start	LOAEL: Al chloride 100 /mg/kg bw/day. Decreased grip strenght	(Oteiza *et al.*, 1993)
Swiss Webster Mice (males 10/group, 22 in control)	Diet	Al lactate	7 (control), 100, 500 or 1000 mg/kg diet <1, 10, 50 or 100 mg/kg/bw/day	4 or 8 weeks. Animals were 45 days of age at start	LOAEL 100 mg/kg bw/day Decrease brain weight (4 weeks only). Grip strength affected but no dose-response	(Golub & Keen 1999)
Wistar rats males in total 270 animals	Drinking water	AlCl3	20, 30 and 52 mg Al/kg bw/day (measured)	30, 60and 90 days starting at a age of 3, 10 and 24 month	LOAEL 52 mg/kg bw/day Effects on the vestibulo-ocular reflex. NOAEL 30 mg/kg bw/day	(Mameli *et al.*, 2006)

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GLOSSARY / ABBREVIATIONS

AD	Alzheimer's disease
AMS	Accelerator Mass Spectrometry
BBB	Blood Brain Barrier
bw	body weigth
CSF	Cerebrospinal fluid
ECF	Extracellular fluid
FEEDAP	Panel on additives and products or substances used in animal feed
GD	Gestation day
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MCT	Monocarboxylate transporter
NOAEL	No Observed Adverse Effect Level
LOAEL	Lowest Observed Adverse Effect Level
SALP	Sodium Aluminium Phosphate
Tf	Transferrin
TfR-Me	Transferrin-receptor mediated endocytosis