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REVIEW ARTICLE

Evaluation of human exposure to aluminum from food and food contact materials

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Abstract

Aluminum constitutes the third most common element in the earth's crust. In spite of this there is no evidence that it is essential for any living organism. It has been shown that uptake of aluminum in large amounts can have detrimental effects on the nervous system, bones and the hemopoietic system. Aluminum exposure in humans is generally the result of ingestion of foods that naturally contain aluminum, those treated with approved food additives and the result of migration from utensils and packaging. The tolerable uptake as derived by the European Food Safety Authority is 1 mg aluminum/kg body weight/week for all groups. Regional differences contribute to a large variation in worldwide uptake of aluminum. Evaluation of the results of various studies shows that individual dietary exposure to aluminum can vary greatly. For adults the average exposure amounted to 0.2–1.5 mg/kg body weight and week. As a result of their lower body weight, the maximum exposure for children and young people was found to be between 0.7 and 2.3 mg/kg body weight and week. This represents values between 14 and 105 mg aluminum/week for a 70 kg adult, and from 21 to 69 mg/week for a 30 kg child. These estimates show that for part of the human population enough aluminum can be taken up through diet to reach the tolerable weekly intake.

Keywords Aluminum · Aluminium · Uptake · Foods and food products · Food contact materials · Tolerable weekly intake · TWI

Introduction

Aluminum is the most common metal (8.3% by weight) and the third most common element in the earth's crust. The only other metals with comparative occurrences are iron (6.2%) and calcium (4.6%). In contrast to iron, zinc or magnesium no scientific studies have provided evidence that aluminum[1] is essential for a living organism. In only two older studies were there reports of aluminum being necessary for the growth of horsetails [35] and tee bushes [9] and in one study [2] aluminum is described as essential for goats. On the other hand uptake of aluminum can represent a health risk for the human organism, including the nervous system, bones and hemopoietic system [4]. In addition there have been suggestions indicating a relationship of aluminum to Alzheimer's dementia [29, 36]. A direct influence of increased aluminum uptake on the appearance of a disease has, however, only been shown in regard to few illnesses,

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[1] In the following the term "aluminum" will be used for the sake of simplicity, although strictly aluminum compounds are meant, in particular regarding foods of animal or plant origin as well as food additives.

including dialysis encephalopathy [8, 17], osteomalacia [8], anemia and aluminosis [4].

Inner exposure of humans is generally the result of consumption of unprocessed foods and drinking water that naturally contain aluminum (primary content), migration of aluminum-based contact materials (secondary content) as well as approved food additives (also secondary content) [14]. As a result of the accumulation of aluminum in the body a tolerable weekly intake (TWI) of 1.00 mg/kg body weight/week has been specified, based upon toxicological animal experiments on mice, rats and dogs. This value presently serves as the foundation for assessment of aluminum in foods and food products in the EU [12]. Based on our own and international studies the following report is an evaluation of human exposure to aluminum from food and food contact material taking in consideration this TWI.

Properties and potential applications

Due to its numerous positive technical, chemical and physical properties aluminum finds many practical industrial and household applications. A differentiation is made between material used in close or direct contact with, or proximity to the human body and others not in close contact. Examples of close contact materials are nanoparticles in sunscreens, tooth paste (e.g., AlF_3 prophylaxis of caries), pharmaceuticals (e.g., heartburn medications, pH-regulators), vaccines (adjuvants to increase immune reactions), cosmetics (e.g., deodorants—antiperspirants), food additives (e.g., coloring agents, stabilizers) as well as uptake of naturally occurring aluminum in foods of plant or animal origin (Table 3). Non-contact applications include, for example building materials as pure metal or in alloys (e.g., vehicle construction, aerospace technology, suitcases, tents), electrotechnical applications (e.g., electrical conductors), packaging and containers (beverage and canned goods, mocha pots, outdoor utensils and dishes, coffee capsules, aluminum foil for household use), fuel for solid–fuel rockets (up to 30% Al), pyrotechnics, pigments for paints, decorative articles and ornaments, organic syntheses ($LiAlH_4$: reducing agent) as well as polishing compounds (Al_2O_3: paste, suspension in MeOH or H_2O).

Toxicology

Aluminum and aluminum compounds were shown in animal studies to have neurotoxic properties as early as 1897 [10]. These results were later confirmed in additional animal and epidemiological studies [34]. The detailed mechanism of aluminum toxicity remains unknown, however, numerous experiments indicate that aluminum and its compounds produce or intensify inflammatory processes that can result in cell damage. Use of antacids for treatment of heartburn has also been shown to have detrimental effects in the form of bone disease and microcytic anemia [30]. Uptake of phosphorous by the intestines is inhibited by aluminum because it binds inorganic phosphorous compounds. This may result in damaged bone mineralization or even in osteomalacia (bone softening). In animal studies aluminum related embryonal damage, decelerated bone formation, and generalized retarded growth have been described [11]. There is a direct connection between aluminum exposition and the development of aluminosis (aluminum pneumoconiosis). As a result of conflicting data it is presently not possible to satisfactorily evaluate the potential role of aluminum in Alzheimer's disease. It has been suggested that the neurotoxic potential of aluminum and the possibility that aluminum in the form of Al^{3+}-ions may reach the brain due to its similar size to iron bound to transferrin. Increased aluminum concentrations were reported in certain brain regions of Alzheimer's patients [15].

Aluminum uptake

Since non-contact materials do not play a role in human internal uptake of aluminum they will not be further discussed here. Human internal exposure is a result of the following direct-contact applications:

- food
- household utensils
- food additives
- cosmetics
- pharmaceuticals.

Whereas exposure to aluminum through cosmetics (e.g., deodorants) and pharmaceuticals (e.g., heartburn medicines) could, as a matter of principle, be avoided, exposure through dietary uptake (total: uptake of foods and food products of plant and animal origin including utensils made of aluminum from which the metal ions can migrate and additives) is seemingly unavoidable. Although it would be possible to forgo use of any additives containing aluminum or utensils made of the metal (e.g., cookware, flatware, camping dishes, drinking bottles, grill pans) when dining at a restaurant it cannot always be completely determined which permissible additives are present in the dishes or beverages offered[2] or in which containers foods/beverages have been prepared or stored. In the following only foods and food products will be

[2] Snack bars, inns and restaurants do partially list food additives that have been used. The additive is either listed by name or by E-number.

Table 1 Typical aluminum concentrations in foods and food products. Source: Own representation of data from López et al. [21], EFSA [12], Stahl et al. [31]

Concentration range (aluminum in mg/kg foodstuff)	Foodstuff or foodstuff group
≤ 5.00	Most unprocessed foods
5.00–10.0	Bread, cake, pastry baking mixes, flour
	Vegetables: mushrooms, spinach, radish, chard, lettuce
	Candied fruits
	Animal products: milk products, sausage, offal, seafood
> 10.0	Tea leaves
	Cocoa or cocoa products
	Spices, herbs
	Coffee

considered with primary and/or secondary aluminum content (aluminum containing foods of plant or animal origin, aluminum from food contact materials and aluminum from approved food additives); the other paths of exposure noted above will not be considered.

Aluminum in foods and food products (primary content)

Since comparatively large amounts of aluminum are found in the soil due to the incidence of the metal in the earth's crust, it can be taken up by plants (carry over). The efficiency of uptake by plants is dependent upon the concentration of aluminum in, and composition of the soil. An acidic soil results in an increased uptake in the same plants as those growing on less acidic soil [21]. $Al_2(SO_4)_3$ is often used as a coagulant to physically remove particulate matter in the treatment of drinking water. By this means aluminum can be released into drinking water. Particularly large amounts of aluminum are found in tea, coffee, and cereals [1]. Tea leaves may contain as much as 945 mg/kg aluminum [23]. The reason for these large concentrations can be found in the ability of tea plants to grow in acidic soils from which large amounts of aluminum are available. Spices also belong to the group of foods with high concentrations of aluminum (compare Table 4). López et al. [21] analyzed the aluminum concentration of 72 samples of 17 different spices and herbs. The aluminum concentrations in all samples were above the limit of detection, with large variations between the various products, but also within the individual product groups. The values measured were between 0.74 and 56.5 mg/kg dry weight. Especially large concentrations were found in cinnamon, mustard, oregano and paprika [21]. Legumes and potatoes also take up aluminum from the soil. In chocolate the aluminum concentration is directly correlated with the cocoa content [18]. Table 1 is a summary of foods and food products in which aluminum have been detected.

In a study of aluminum in various foodstuff groups, Stahl et al. [31] examined the aluminum content of 1431 different products in Germany, in particular plant-derived foods and drinks. Absolute aluminum concentrations of the products were determined and were found to range from 0.4 to 737 mg/kg food product. The large range of variation suggests that the differences are the result of secondary sources. The results of this study can be summarized as follows: 77.8% of the foods were found to have an aluminum content of less than 10.0 mg/kg, 17.5% were within the range of 10.0–100 mg/kg and only very few (4.60%) had a content greater than 100 mg/kg [31].

Aluminum from food contact materials

As early as the 1890s dishes, drink containers, pots and pans were being manufactured out of aluminum due to its positive technological properties. The first widespread use was in the military field where dishes, pots and canteens made of aluminum were used. New methods of food preparation, storage and packaging led to an increased use of aluminum in the food sector [7]. Today it is impossible to imagine the food sector without aluminum. Table 2 illustrates the various uses of aluminum in the range of food contact materials.

In 2013, the European Council published guidelines for food contact materials made of metal: metals and alloys used in food contact materials and articles [13]. These included suggested specific release limits (SRL) for metals and alloy constituents, including aluminum. These SRL, however, are only recommendations. The limit for release of aluminum in foods and food products was specified at 5.00 mg/kg foodstuff. In spite of their widespread usage or potential usage (see Table 2) comparatively few studies exist on the transfer of aluminum to food from cooking utensils.

The studies of Pennington [24] on the transfer of aluminum to foods show that the release of aluminum is in particular dependent upon pH value of the contents, duration of cooking and addition of sugar and salt. Comparisons were made between cooking in a glass pot and cooking in an aluminum pot. Preparation of acidic substances such as fruit juices in aluminum pots resulted in an increase in aluminum

Table 2 Summary of aluminum food contact materials. Source: Own representation of data from: BMG [7]

Packaging of food products	Cookware and cooking utensils
Aerosol cans (e.g., for spray cream)	Baking tins
Aluminum foil	Bread boxes
Containers for convenience foods	Lids
Lids for yogurt containers	Coffee pots
Beverage cans	Cooking utensils, stirring spoons, ladles
Coffee capsules	Pans
Tubes for mustard, mayonnaise, sauces	Pots
Packaging for candy	
Composite materials for beverage cartons and coffee packaging	
Supplementary materials for PET bottles	
Food preparation	Domestic appliances
Aluminum tanks for wine, juices, oils, milk	Ice cream machines
Baking sheets	Juicers
Meat and sausage hangars	Espresso machines
Machine parts	Coffee percolators

concentration of more than 1000% over the original content measured [24]. Müller et al. [22] studied the migration of aluminum from packaging material and cooking utensils. The authors came to the conclusion that the preparation of acidic products such as tomato sauce in unlined aluminum containers resulted in release of large amounts of aluminum. The rate of transfer showed a negative correlation with the frequency of use of the utensil [22]. Bassioni et al. [3] examined the use of aluminum foil in the preparation of foods and determined an increase in aluminum migration with increasing cooking temperatures, pH value and salt content. Ranau et al. [25] demonstrated an increase in aluminum content in fish after preparation of fish filets in aluminum foil. According to the authors, factors that affect the migration are the chemical composition of the filets and the pH value of the food, preparation conditions and the presence of other substances (e.g., organic acids and salts [25]). Turhan [37] examined the transfer of aluminum to meat that was prepared in aluminum foil and determined an increase in content from 76 to 378%. The author came to the conclusion that the temperature of preparation has a greater influence on the migration than the time of preparation. In addition, he suggests that fat content is an influential factor [37]. The German Institute for Risk Assessment (BfR) detected high aluminum concentration in soft pretzels and in fruit juices. The pretzels are dipped in an aqueous solution of sodium hydroxide and were then baked on aluminum sheets. The fruit juices were stored in uncoated aluminum tanks. In both cases increased concentrations of aluminum were detected in the products. Consequently, the BfR has issued recommendations that aluminum containers do not come into contact with acidic beverages and that baked goods treated with lye solution should not be baked directly on aluminum surfaces [5]. Systematic studies of the transfer of aluminum to foods and food products from food contact materials have been reported by Stahl et al. [32, 33]. The results of these studies are presented in more detail in "Tolerable weekly intake (TWI)—influence of contact materials (secondary content)" in regard to exceeding the TWI.

Aluminum from food additives (secondary content)

As defined by Regulation no. 178/2002 "foods and food products" are substances that are designated, whether in processed, partially processed or unprocessed form, to be eaten by human beings [26]. Equivalent to foods and food products are any coverings, encasements or other wrappings that are destined to be consumed with the food or which can be foreseen as being consumed. According to Regulation no. 1333/2008 additives are substances that are added to foods for technological reasons during preparation, processing, treatment, packaging, transport or storage [27]. Aluminum and aluminum compounds are added to foods and food products as colorants, separating, stabilizing, and firming agents as well as vehicles for vitamins. In addition, certain food colorants may also be used as aluminum varnish in the preparation of specialty foods. To reduce aluminum exposure some aluminum containing food additives have been limited, or their maximum amounts reduced by Regulation no. 380/2012 [28]. At present, aluminum (E 173) may only be used as a coating for confectionary products and pastries, aluminum sulfate (E 520–523) only for candied cherries, acidic sodium aluminum phosphate (E 541) only for sponge cake, aluminum sulfate (E 520) for liquid egg white, sodium aluminum silicate (E554) only for table salt used in surface treatment of ripened cheeses. For all aluminum varnishes there is a limit for the maximum allowable aluminum content (Regulation no. 1333/2008). In addition, potassium aluminum silicate (E 554) is approved for vitamin preparations as is aluminum starch octenylsuccinate (E 1452) for dietary supplements with corresponding maximum permissible amounts (Regulation no. 1333/2008). To the best of our knowledge no data exits regarding human exposure to aluminum via food additives and for that reason additives will not be included in the following TWI discussion.

Bioavailability of aluminum

The bioavailability of orally ingested aluminum is estimated to be 0.1–0.3% [24]. Bioavailability increases when dietary uptake is low [16]. More than 95% of orally ingested aluminum is eliminated by the kidneys [12]. In rats the uptake of aluminum through nutrition is increased by two to fivefold in the presence of citric acid [19]. Phosphate and fluoride bind aluminum in foods, possibly making it less absorbable in the gastrointestinal tract [1].

Tolerable weekly intake (TWI)—dietary influence (primary content)

The European Food safety authority derived a TWI of 1 mg/kg body weight/week, based on toxicological studies on rats, mice and dogs [12]. This TWI is considerably lower than the previous TWI of 7 mg/kg body weight/week as was derived from other estimates and had been valid until 2008. Based on the present TWI of 1 mg/kg body weight/week an adult weighing 70.0 kg may ingest 70.0 mg and a child weighing 30 kg 30.0 mg of aluminum/week. This value represents the sum total of aluminum uptake from the three close contact substances, food, food contact materials and additives[3]. The TWI can be reached or exceeded depending upon the amount or frequency in which foods and food products containing aluminum are consumed. Examples of foods and food products that have been examined in our own studies are presented in Table 3. It is possible to calculate the percentage of TWI reached for adults (70.0 kg body weight) and children (30 kg body weight) according to the aluminum concentration and amount consumed per day of the individual foods. The various foods are listed in descending order according to the concentration of aluminum detected per kilogram or liter.

From Table 3 it is apparent that the aluminum concentration varies greatly within the various food groups, however, the data shown in Table 1 is useful for orientation purposes. The highest concentration was 945 mg/kg, found in tea leaves, followed by cocoa powder (165 mg/kg) and chocolate (48 mg/kg). These are in turn followed by fine bakery ware (19 mg/kg), confectionery (17 mg/kg), salt pretzels and similar savory biscuits (13 mg/kg). Meat and sausage, venison and fish contain much lower amounts of aluminum as do beverages. The TWI can be reached by the consumption of tee alone. For children the consumption of tea (made from tea leaves under the assumption that the total aluminum or aluminum compounds from the tea leaves are released into the water) can result in greatly exceeding the TWI (221%). The situation is similar with the consumption of chocolate (112%). For adults consumption of tee can result in 94.5% of TWI, chocolate 48.5% (Table 3). Examples of consumption of the other foods listed in the table are 0.17% TWI for an adult consuming venison, as much a 19% for fine bakery ware and for children 0.39% (venison) and 44.3% (fine bakery ware). In summary it must be noted that consumption of foods and food products that naturally contain aluminum can result in reaching or even exceeding the TWI.

Tolerable weekly intake (TWI)—influence of contact materials (secondary content)

The German Federal Institute for Risk Assessment (BfR) has determined that the use of uncoated aluminum meal trays in the so-called cook and chill technique of catering for shared accommodations such as kindergartens, schools, businesses, nursing homes as well as out-of-home catering can result in a comparatively large release of aluminum into foods. The BfR came to the conclusion in this study that the contribution to aluminum uptake by occasional food consumption from uncoated aluminum trays is minor, whereas daily consumption may result in a significant increase [6]. Daily consumption of a meal (about 200 g acidic food) processed and stored warm (release: 4 mg aluminum/200 g), would result in an adult (70.0 kg) ingesting an additional 28 mg aluminum/week, which would amount to 40% of the TWI. For a child, consumption of the same amount (200 g/day) would amount to 93.3% TWI. In addition to aluminum trays, drinking bottles and moka pots made of aluminum are very popular with consumers. Storage of beverages and preparation of coffee in moka pots can also result in transfer of aluminum to the product. In our own study of drinking bottles made of aluminum it was shown that drinking tea from an unlined bottle would result in 72.6% TWI for a 30 kg child. Use of aluminum moka pots, on the other hand would only result in about 4% TWI by consuming 3 l/week, even if the pot was washed in the dishwasher, against the explicit warning of the manufacturer. Aluminum camping cookware is preferred for, e.g., outdoor activities due to its light weight and excellent heat conduction. Preparation in aluminum dishes or camping utensils can result in transfer

[3] In cases in which pharmaceuticals containing aluminum are ingested or cosmetics containing aluminum are used this sum is increased accordingly.

Table 3 Aluminum concentration in various foods and food products, uptake daily and weekly and percentage TWI

Product	Mean value (mg/kg)	Daily uptake (kg) foodstuff[a]	Weekly uptake (kg) foodstuff	Uptake of aluminum/week (mg)[b]	Percentage TWI adult (70 kg)	Percentage TWI child (30 kg)	References
Tea leaves (prepared as beverage)	945	0.01	0.07	66.2	94.5	221	Nanda et al. [23]
Cocoa powder	165	0.005	0.035	5.8	8.25	19.3	Stahl et al. [31]
Chocolate	48.0	0.1	0.7	33.6	48.0	112	Stahl et al. [31]
Fine bakery ware	19.0	0.1	0.7	13.3	19.0	44.3	Stahl et al. [31]
Confectionery	17.0	0.05	0.35	5.95	8.5	19.8	Stahl et al. [31]
Salt pretzels and similar savory biscuits	13.0	0.1	0.7	9.1	13.0	30.3	Stahl et al. [31]
Pastas	10.0	0.1	0.7	7.0	10.0	23.3	Stahl et al. [31]
Bread	3.0	0.2	1.4	4.2	6.0	14.0	Stahl et al. [31]
Ready-cooked meals	3.0	0.03	0.21	0.63	0.9	2.1	Stahl et al. [31]
Cheese	2.76	0.1	0.7	1.9	2.76	6.44	Junk [20]
Meat and sausages	2.2	0.15	1.05	2.31	3.3	7.7	Junk [20]
Wine and fruit wine	2.0	0.5	3.5	7	10.0	Not calculated[c]	Stahl et al. [31]
Herring filets marinated (canned)	1.99	0.2	1.4	2.8	3.98	9.29	Junk [20]
Meat and meat products (canned)	0.779	0.15	1.0	0.82	1.17	2.73	Junk [20]
Beer and mixed drinks containing beer	0.5	0.5	3.5	1.75	2.5	Not calculated[c]	Stahl et al. [31]
Tuna fish (canned)	0.344	0.15	1.05	0.36	0.52	1.2	Junk [20]
Venison	0.11	0.15	1.05	0.12	0.17	0.39	Junk [20]
Mineral water	0.01	2.5	17.5	0.18	0.25	0.58	Stahl et al. [31]

Which foods and food products significantly contribute to human inner exposure to aluminum. Unpublished data

[a]Basis: National study of food consumption, report of results, Part 2, German national study of young people and adults, Max Rubner Institute Federal Research Institute for Nutrition and Food 2009; rounded uptake and estimates

[b]Calculations based on the arithmetic means; Values have been rounded off for the sake of clarity

[c]As a rule, children do not consume alcoholic beverages. Therefore, the percent TWI was not calculated

of aluminum to the food. The experiments showed that with food simulants oil and tap water the limit of 5.00 mg/kg or mg/l is not exceeded. In contrast, in 0.5% citric acid results show that for a 30 kg child[4] the TWI would be exceeded by 149% and for a 70 kg adult 63.8%. Marinating fish with lemon juice in an aluminum camping container would result in the TWI being exceeded by 436% for a child and by 187% for an adult (calculation based on Stahl et al. [32, 33]). The aluminum concentrations in various foods after contact with aluminum utensils and containers are shown in Table 4. In addition, the resulting percentages of the TWI are shown.

In summary it is worth noting that the TWI can be reached or exceeded by consumption of foods that were stored in containers (tea, citric acid) or prepared in (acidic foods, coffee, marinated fish patties) contact materials made of aluminum.

[4] Table 4: the results shown are for a child weighing 30 kg and an adult weighing 70 kg based on a daily portion of a 10 ml portion of citric acid over a period of 1 week (7 days).

Conclusions

Estimates of the total exposure (primary and secondary content) to aluminum were made, based on studies from various European countries. As a result of cultural differences various foods and food products are consumed in different amounts in different regions and countries. Regional differences in soil and in cultivation techniques further contribute to a large variation in worldwide uptake of aluminum. Evaluation of the results of the various studies shows that individual dietary exposure to aluminum can vary greatly. For adults the average exposure amounted to 0.2–1.5 mg/kg body weight and week. As a result of their lower body weight, the maximum exposure for children and young people was found to be between 0.7 and 2.3 mg/kg body weight and week [12]. This represents values

Table 4 Aluminum concentrations and percentage TWI resulting from consumption of various foods after contact with aluminum containers and utensils

Beverage in aluminum bottle	Mean concentration (mg/kg or mg/l)	Aluminum uptake (mg/week)	Percentage TWI child	Percentage TWI adult
Acidic foodstuffs[a]	60.0	28.0	93.3	40
Tea from bags[b]	6.22	21.8	72.6	31.1
Coffee made in moka pots[c]	0.795	2.79	Not calculated[d]	3.98
Citric acid[e]	638	44.7	149	63.8
Fish patty[f]	74.6	131	436	187

[a]Results are for a child weighing 30 kg and an adult weighing 70 kg based on a daily uptake of 200 g acidic foodstuffs for a period of 1 week (7 days)

[b]Data for a child weighing 30 kg and an adult weighing 70 kg consuming a daily portion of 500 ml for a period of 1 week (7 days)

[c]The weekly uptake is based on an adult weighing 70 kg and a daily uptake of 500 ml coffee over a time period of 1 week (7 days)

[d]Since children do not generally drink coffee this calculation was not made

[e]The results shown are for a child weighing 30 kg and an adult weighing 70 kg based on a daily portion of a 10 ml portion of citric acid over a period of 1 week (7 days)

[f]Results are for a child weighing 30 kg and an adult weighing 70 kg based on a daily uptake of 250 g fish patties for a period of 1 week (7 days)

between 14 and 105 mg aluminum/week for a 70 kg adult, and from 21 to 69 mg/week for a 30 kg child. These estimates show that for part of the human population enough aluminum can be taken up through diet to reach the TWI. It is not possible to determine whether the source of aluminum is natural or secondary by analysis of prepared foods and food products. Total diet studies might provide an improved approach to the evaluation of consumer exposure to aluminum. In such studies it would be necessary to collect data on the consumption habits of specific consumer groups such as school children, out-of-home consumers, residents of nursing homes, patients in hospitals, vegetarians, vegans, flexitarians, adults (male/female differences), students, fast-food enthusiasts, etc., and to analyze the total aluminum uptake of the collective diets. If, in addition to the various consumer groups different regional as well as cultural differences are to be accounted for this kind of study would be very comprehensive, both in regard to cost and to logistics. There is no question, however, that it would provide a great improvement in available information on possible aluminum exposure.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Compliance with ethical requirements This article does not contain any studies with human or animal subjects.

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