Exposure and Health (2020) 12:863–868
https://doi.org/10.1007/s12403-020-00346-9

ORIGINAL PAPER

Aluminium in Brain Tissue in Non-neurodegenerative/Non-neurodevelopmental Disease: A Comparison with Multiple Sclerosis

C. Linhart[1] · D. Davidson[2] · S. Pathmanathan[2] · T. Kamaladas[2] · C. Exley[3]

Received: 5 October 2019 / Revised: 7 February 2020 / Accepted: 15 February 2020 / Published online: 25 February 2020

Abstract

Human exposure to aluminium is a burgeoning issue. The brain is a sink for systemically available aluminium and a putative target of neurotoxicity. An increasing number of studies continue to confirm the presence of aluminium in human brain tissue though primarily in relation to donors who have died of a neurodegenerative or neurodevelopmental disorder. Herein, we have measured aluminium in brain tissue in donors who died of a specific disease or condition though without showing any neurodegeneration. The donors were diagnosed as not suffering from multiple sclerosis. Herein, these novel data are compared with recent data on aluminium in brain tissue in multiple sclerosis. Brain tissues from all four lobes were obtained from the Multiple Sclerosis Society Tissue Bank. Tissues were digested using microwave-assisted acid digestion and their aluminium content was measured by transversely heated graphite furnace atomic absorption spectrometry. Both are established methods in our laboratory. Detailed statistical analyses were used to compare new data with recent data for multiple sclerosis. Aluminium was found in brain tissue in each donor with a high proportion of measurements (189/291) being below 1.00 µg/g dry weight. The data for all cases (median and IQR) were 0.74 (0.48–1.28), 1.23 (0.62–1.63), 0.84 (0.45–1.14) and 1.01 (0.62–1.65) µg/g dry weight for occipital, parietal, temporal and frontal lobes, respectively. There was a statistically significant positive correlation between aluminium content of brain tissue and the age of donor. Comparison of data for this non-multiple sclerosis group with brain aluminium data for donors dying with a diagnosis of multiple sclerosis showed that the latter had a statistically significant higher content of brain aluminium. The data reinforce a previous conclusion that the aluminium content of brain tissue in multiple sclerosis is elevated and support the suggestion that human exposure to aluminium may have a role to play in the aetiology of multiple sclerosis.

Keywords Human exposure to aluminium · Aluminium in brain tissue · Aluminium in multiple sclerosis · Aluminium and neurodegenerative disease · Aluminium and neurodevelopmental disease

Introduction

Human exposure to aluminium is burgeoning (Exley 2013; Klotz et al. 2017; Stahl et al. 2017). Humans are living in the aluminium age (https://www.hippocraticpost.com/mens-health/the-aluminium-age/) and everyday exposure is impacting upon daily life (Cabral Pinto et al. 2018, 2019; Stahl et al. 2018; Handra et al. 2019). Systemic aluminium is excreted in urine (Michalke et al. 2018) and sweat (Genuis et al. 2011) and retained in tissues and especially brain tissue (Exley and House 2011). The longevity of neurones predisposes them to the accumulation of aluminium though very little is understood as to whether higher concentrations of aluminium in brain tissue are due to its greater access or its increased retention (Exley 2014; Bondy 2016). Ageing

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12403-020-00346-9) contains supplementary material, which is available to authorized users.

✉ C. Exley
c.exley@keele.ac.uk

[1] Department of Medical Statistics, Informatics and Health Economics, Medical University of Innsbruck, Innsbruck, Austria

[2] Life Sciences, The Huxley Building, Keele University, Stoke-on-Trent, UK

[3] Lennard-Jones Laboratories, The Birchall Centre, Keele University, Stoke-on-Trent, UK

is considered a major risk factor for the accumulation of aluminium in brain tissue (Roider and Drasch 1999) and in itself may underlie a higher content of aluminium in brain tissue in sporadic Alzheimer's disease (House et al. 2012; Yumoto et al. 2018). There are no other confirmed risk factors for the accumulation of aluminium in brain tissue though aluminium is shown to be elevated in a number of neurodegenerative and neurodevelopmental disorders including familial Alzheimer's disease (fAD) (Mirza et al. 2017) and autism spectrum disorder (ASD) (Mold et al. 2018a). Recently, we made the first analyses of aluminium in brain tissue in individuals who died with a diagnosis of the neurological condition, multiple sclerosis (MS) (Mold et al. 2018b). Our previous research had suggested that individuals with MS had a higher-than-expected body burden of aluminium (Exley et al. 2006; Jones et al. 2017) and this prompted us to look for aluminium in MS brain tissue. The aluminium content of tissues in MS was found to be universally high with every donor brain having at least one tissue where the measured content of aluminium was considered as pathologically significant (≥ 3.00 µg/g dry weight). Detailed statistical analyses revealed no relationships with gender or, perhaps surprisingly, age of donor. Age was not a risk factor for aluminium in brain tissue in MS. Previously, we have asked the question as to how much aluminium in brain tissue is too much (Exley and Mold 2019) and this question is equally applicable in considering MS. Do we have evidence that the aluminium content of brain tissue in MS is high due to increased uptake or higher retention? To help in answering this question, The Multiple Sclerosis Society Tissue Bank provided us with brain tissues from donors who did not die with a diagnosis of MS and additionally showed no neurodegeneration beyond that which could be attributed to normal ageing. However, all donors died following a period of (usually traumatic) disease and primarily a cancer-related condition. There are no data in the current scientific literature describing aluminium in brain tissue in individuals who died of a non-neurodegenerative/non-neurodevelopmental disease. Herein, we have made these measurements and compared the data with our previous data on MS. We aimed to provide further information on aluminium in brain tissue in disease and to test if data for MS are, as was previously suspected, unusually high.

Materials and Methods

Tissues

Brain tissues were obtained from the Multiple Sclerosis Society Tissue Bank, Imperial College, London, following ethical approval (NRES Approval No. 08/MRE09/31). Donors included three females and nine males between the ages of 35 and 88 years. Cancer had played a role in the death of at least eight donors. Neuropathological investigation of all brains revealed no specific neuropathology beyond that described as age related. None of the donors had multiple sclerosis.

Quantitative Measurements

The aluminium content of tissues was measured by an established and fully validated method (House et al. 2012) which herein is described only briefly. Samples of cortex, between 0.6 and 5.0 g in weight, were thawed at room temperature and cut using a stainless steel blade into sections approximately 0.3–0.5 g in weight. Tissues were dried for 48 h, to a constant weight, in an incubator at 37 °C. Dry and thereafter weighed tissues were digested in a microwave (MARS Xpress CEM Microwave Technology Ltd.) in a mixture of 1 mL 15.8 M HNO_3 (Fisher Analytical Grade) and 1 mL 30% *w/v* H_2O_2 (BDH Aristar). The resulting digests were clear with no fatty residues and, upon cooling, were made up to 5 mL volume using ultrapure water (cond. < 0.067 µS/cm). Total aluminium was measured in each sample by transversely heated graphite furnace atomic absorption spectrometry (TH GFAAS) using matrix-matched standards and an established analytical programme alongside previously validated quality assurance data (House et al. 2012). The latter included method blanks, detailed descriptions of which have been published recently (Exley and Mold 2019).

Statistical Analyses

Data for aluminium content of tissues were skewed and were not normally distributed. For descriptive summary statistics, the median and interquartile range were calculated for each donor and additionally per donor and lobe. For all test statistics and models, aluminium content data were log transformed. Due to unbalanced groups (non-MS donors = 14, MS donors = 12) and unbalanced numbers of samples per donor and lobe, mixed effect models including random effects for donors and lobe were used.

First, a model was calculated for the non-MS group to analyse differences between the factors lobes and gender and associations with the covariate age. The model contains all these factors as main terms without interactions. Additional random effects were nested and included the factor, number of samples, nested within lobe and donor.

A second model included the factor group to analyse differences between the MS and the non-MS groups (Zuur et al. 2009). Data on the hippocampus lobe exist only for the non-MS group and were therefore excluded from the mixed effect model comparing MS and non-MS donors. We considered a *p*-value smaller than 0.05 to be statistically significant. To obtain pairwise differences between lobes

and disease groups, post hoc tests with Tukey's correction were performed with the function *glht* from the R package multcomp. For univariate and descriptive analysis, SPSS Statistics v.22 (IBM Analytics, Armonk, NY, USA) was used; for the mixed effect model, RStudio Version 1.1.463 © 2009–2018.

Results

Aluminium Content of Brain Tissues

The aluminium content of all tissues ranged from 0.01 (limit of quantitation) to 17.26 µg/g dry weight (Supplementary Table 1). The latter being a significant outlier and originating from the temporal lobe of a 35-year-old male who died of cancer of the tongue. The majority of measurements across all four lobes (and occasionally the hippocampus) for all individuals were below 1.00 µg/g dry weight (189/291) though 59, 24 and 19 measurements were in the range, 1.00–1.99, 2.00–2.99 and ≥ 3.00 µg/g dry weight, respectively. There were statistically significant associations relating to brain aluminium content and age in non-MS donors ($r_{(289)} = 0.2$, $p = 0.008$, $n_{(\text{samples})} = 12$, $n_{(\text{samples})} = 291$, Table 2). However the effect of age was weaker in the multivariate mixed effect model (mixed effect model, $p = 0.014$, $n_{(\text{donors})} = 12$, $n_{(\text{samples})} = 291$, Table 2). There were no significant differences in brain aluminium content of males and females (mixed effect model, $p = 0.864$, $n_{(\text{donors})} = 12$, $n_{(\text{samples})} = 291$, Table 2). There were no statistically significant differences in aluminium content between lobes (mixed effect model, df = 4, $p = 0.203$, $n_{(\text{donors})} = 12$, $n_{(\text{samples})} = 291$, Table 2). Regarding the latter, the data for all cases (median and IQR) were 0.74 (0.48–1.28), 1.23 (0.62–1.63), 0.84 (0.45–1.14) and 1.01 (0.62–1.65) µg/g dry weight for occipital, parietal, temporal and frontal lobes, respectively (Table 1). There was a significant trend for higher brain aluminium content in individuals who died with a diagnosis of cancer (8 individuals) compared to other conditions (4 individuals) (mixed effect model, df = 1, $p = 0.004$, $n_{(\text{donors})} = 12$, $n_{(\text{samples})} = 291$, Tables 1 and 2).

Comparison with Brain Aluminium Content in Multiple Sclerosis

The non-MS group and MS group were gender matched (Fisher's exact test, $n = 26$, $p = 0.13$) though the former were older (*t* test, $p = 0.003$, $n = 26$). There was no statistically significant association relating to brain aluminium content and age for the MS group ($r(330) = 0.03$, $p = 0.611$, $n = 14$, observations = 332). The aluminium content across all lobes were significantly higher in MS donors (mixed effect model, $n(\text{samples}) = 615$, $N(\text{donors}) = 26$, $p = 0.004$, Tables 3 and

Table 1 Descriptive summary statistics for the non-MS group per lobe, showing median, interquartile range (IQR) and sample size per donor (*n*)

ID	Subgroup	Occipital			Parietal			Temporal			Frontal			Hippocampus		
		Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*	Median	IQR	*n*
CO26	Cancer	0.90	0.46–1.16	13				1.01	0.71–1.13	5	0.97	0.47–1.11	5			
CO28	Cancer	0.43	0.36–0.91	6							0.23	0.14–0.33	4			
CO32	Cancer	1.48	0.94–2.66	14	0.90	0.47–2.23	8	0.22	0.01–0.64	5	2.65	1.40–3.78	4			
CO37	Cancer	0.89	0.53–1.01	9	0.95	0.52–1.85	4	1.18	1.07–1.26	6	1.08	0.87–2.03	5	0.56	0.01–2.57	3
CO54	Cancer	0.85	0.44–1.62	9	0.22	0.04–7.49	4	0.03	0.03–0.03	5	0.38	0.12–0.52	5			
CO45	Cancer	0.53	0.47–1.01	6	0.88	0.67–1.99	13	1.39	0.50–2.40	9	0.38	0.22–0.73	5			
CO48	Cancer	2.18	1.64–3.31	5	1.44	0.23–2.43	3	0.79	0.62–0.96	6	0.21	0.21–1.39	5			
CO25	Cancer	0.21	0.03–0.23	5	1.04	0.32–1.31	10	0.01	0.01–0.13	5	0.55	0.30–1.15	5			
CO29	Other	0.61	0.39–0.94	8	0.32	0.20–0.37	9	0.16	0.02–0.40	10	1.17	0.58–3.06	4			
CO36	Other	0.24	0.16–0.76	9	0.09	0.01–0.57	5				0.75	0.75–1.07	5	0.34	0.10–0.60	5
CO64	Other	0.41	0.38–0.44	2	0.30	0.01–1.00	6	0.14	0.01–0.71	8	1.22	0.08–2.03	5			
CO76	Other	0.48	0.17–0.56	3	0.45	0.10–0.99	16				1.92	0.01–2.17	5			

Aluminium data are µg/g dry weight tissue

Table 2 Mixed effect model for aluminium content in non-MS donors with the fixed factors disease group (*non-cancer* as reference), *lobe* (*occipita*l as reference), *gender* (*female* as reference) and the covariate *age*

Predictors	Al levels in brain of all non-MS donors		
	Estimates	CI	*p*-value
(Intercept)	− 0.26	− 1.87 to − 0.65	< 0.001
Disease group	0.37	0.14 to 0.59	0.004
Lobe	− 0.59	− 0.79 to 0.40	0.203
Age	0.01	− 0.00 to 0.02	0.014
Gender	0.02	− 0.25 to 0.29	0.864
Random effects			
Donor: lobe: sample	0.08		
n (donor)	12		
n (lobe)	5		
n (sample)	16		
n (total measurements)	291		
Marginal R^2/conditional R^2	0.11/0.24		

The random effects include *donor, lobe* and number of *observations*. Random effects show the veffects of the nested and unbalanced factors: donor, lobe and sample. The marginal R^2 considers only the variance of the fixed effects, while the conditional R^2 takes both the fixed and random effects into account

CI confidence interval, *df* degrees of freedom

Table 3 Descriptive statistics of aluminium contents according to the disease groups. Number of donors is shown in brackets, IQR (interquartile range). Data for the lobe hippocampus ($n_{(samples)} = 8$) are excluded in this table

Group	Number of samples	Min	Median	IQR	Max
MS ($n = 14$)	332	0.01	1.20	0.44–3.00	132.6
Non-MS ($n = 12$)	283	0.01	0.61	0.21–1.31	17.3
Cancer ($n = 8$)	188	0.01	0.83	0.34–1.61	17.3
Other ($n = 4$)	95	0.01	0.38	0.09 -0.93	5.0

4) than non-MS donors (Fig. 1). The single mixed effect models per lobe showed a significant difference between MS donors and non-MS donors for the frontal lobe ($p = 0.012$, Table 4, Supplementary Table 2, Fig. 2).

Table 4 Full mixed effect model for aluminium content in all donors with the fixed factors *disease group* (*non-MS* as reference), *lobe* (*occipita*l as reference), *gender* (*female* as reference) and the covariate *age*

Al levels in brain of all non-MS and MS donors			
Predictors*	Estimates	CI	*p*-value
(Intercept)	− 0.63	− 1.22 to − 0.04	0.040
MS	0.38	0.13 to 0.62	0.004
Lobe	− 0.49	− 0.51 to 0.38	0.071
Gender	− 0.04	− 0.26 to 0.18	0.730
Age	0.00	− 0.00 to 0.01	0.211
Random effects			
Donor:lobe:sample	0.16		
$n_{(donor)}$	26		
$n_{(lobe)}$	4		
$n_{(sample)}$	28		
$n_{(total\ measurements)}$	615		
Marginal R^2/conditional R^2	0.081/0.284		

The random effects include *donor, lobe* and number of *samples*. Random effects show the effects of the nested and unbalanced factors: donor, lobe and sample. The marginal R^2 considers only the variance of the fixed effects, while the conditional R^2 takes both the fixed and random effects into account. Data for the lobe hippocampus ($n_{(samples)} = 8$) are excluded from this analysis

CI Confidence interval, *df* degrees of freedom, *ICC* Interclass correlation coefficient

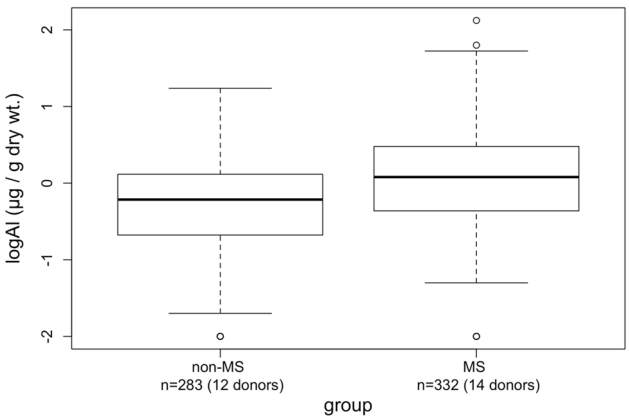

Fig. 1 Boxplots for aluminium measurements stratified by lobes for non-MS and MS groups

Discussion

We report the first measurements of aluminium in human brain tissue in donors who died of a non-neurodegenerative or non-neurodevelopmental disorder and whose brain showed no recognisable signs of neurodegeneration. These are not 'healthy' donors, they all died from a serious medical condition, primarily (8 of the 12 donors) relating to cancer. Data covered a wide range of tissue aluminium content and while a high proportion of tissues, ca 65%, could be considered as within a normal or non-pathogenic range (< 1.00 µg/g dry weight) *ca* 7% of tissues had an aluminium content considered as pathologically significant (≥ 3.00 µg/g dry weight) (Exley and Mold 2019). The donor's age varied between 35 and 88 years and a statistically significant association showed higher brain aluminium content with

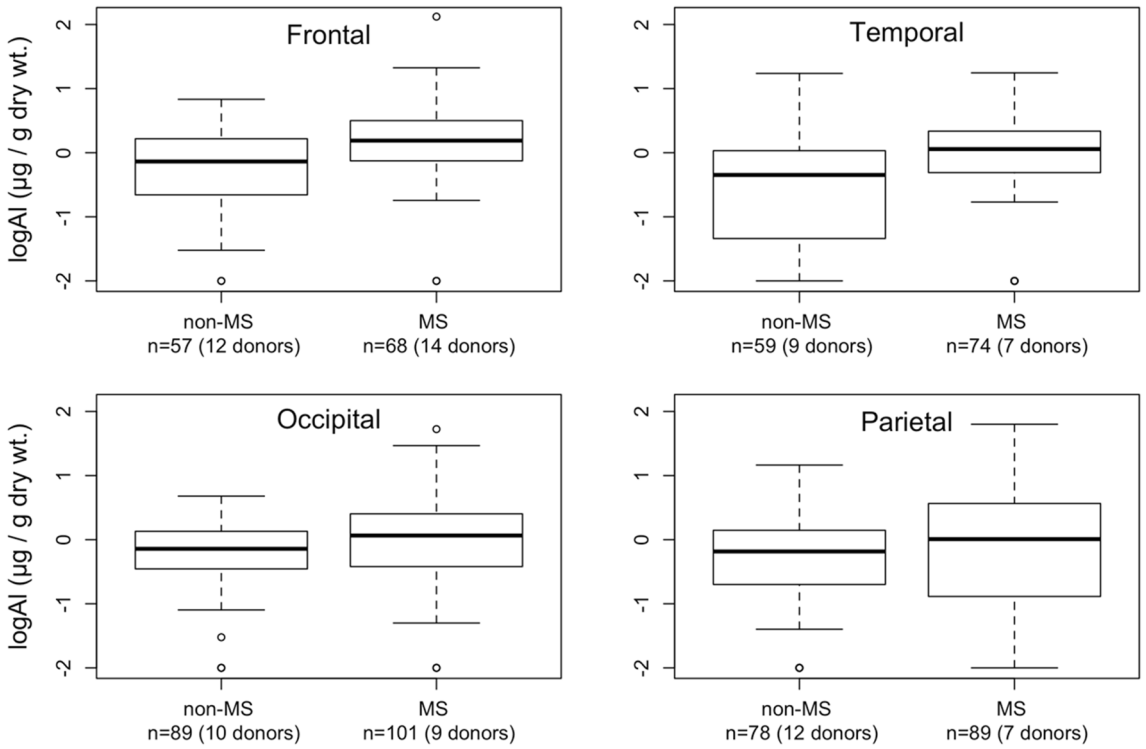

Fig. 2 Boxplots for aluminium measurements stratified by lobes for non-MS and MS groups

increased age. This finding was opposite to that previously observed in MS (Mold et al. 2018b). While it confirmed a previous observation of age as a risk factor for brain aluminium content in a non-neurologically affected population (Roider and Drasch 1999), it also suggests that a correlation with age may not be significant in neurologically impaired populations. There was a clear significant trend for higher brain aluminium content in the eight cancer-related cases compared with the four non-cancer-related cases (Tables 1 and 2). The Multiple Sclerosis Society Tissue Bank provided the brain tissues investigated herein as possible control tissues for comparing with aluminium in brain tissue in MS. It is certainly true that none of the donors had MS or signs of neurodegeneration and while they were slightly older than the MS donor group, they were gender matched. Detailed statistical analyses showed unequivocally that the content of aluminium in brain tissue in MS was significantly higher than that in non-MS control group. This is the first unequivocal confirmation that aluminium is increased in brain tissue in MS (Mold et al. 2018b) and it further supports the contention that the body burden of aluminium is elevated in MS compared to individuals without MS (Exley et al. 2006; Jones et al. 2017). The data do not prove a role for aluminium in MS, but a higher content of a neurotoxin and powerful pro-oxidant (Verstraeten et al. 1997; Exley 2004) in brain tissue in MS cannot be discarded as a putative aetiological factor in a disease with both genetic and environmental components (Thompson 2017). The results also have translational aspects in that they support previous research in which silicon-rich mineral waters were suggested as potential long-term therapies in treating MS (Jones et al. 2017).

Acknowledgements Indirect funding to support tissue acquisition and some laboratory work came from a grant to Keele University (CE) from the Children's Medical Safety Research Institute (CMSRI), a registered charity based in Washington DC, USA. The Multiple Sclerosis Society Tissue Bank, funded by the Multiple Sclerosis Society, registered charity 207495, supplied tissue samples and the associated clinical and neuropathological data.

Compliance with Ethical Standards

Conflict of interests The authors have no conflicts of interest to report.

References

Bondy SC (2016) Low levels of aluminium can lead to behavioral and morphological changes associated with Alzheimer's disease and age-related neurodegeneration. Neurotoxicol 15:222–229

Cabral Pinto MMS, Marinho-Reis P, Almeida A, Ordens CM, Silva MM, Freitas S, da Silva EAF (2018) Human predisposition to cognitive impairment and its relation with environmental exposure to potentially toxic elements. Environ Geochem Health 40:1767–1784

Cabral Pinto MMS, Ordens CM, de Melo MTC, Inácio M, Almeida A, Pinto E, da Silva EAF (2019) An inter-disciplinary approach to evaluate human health risks due to long-term exposure to contaminated groundwater near a chemical complex. Expo Health. https://doi.org/10.1007/s12403-019-00305-z

Exley C (2004) The pro-oxidant activity of aluminium. Free Radic Biol Med 36:380–387

Exley C (2013) Human exposure to aluminium. Environ Sci 15:1807–1816

Exley C (2014) Why industry propaganda and political interference cannot disguise the inevitable role played by human exposure to aluminium in neurodegenerative diseases including Alzheimer's disease. Front Neurol 5:212

Exley C, House E (2011) Aluminium in the human brain. Monat Chem 142:357–363

Exley C, Mold MJ (2019) Aluminium in human brain tissue: how much is too much? J Biol Inorg Chem 24:1279–1282

Exley C, Mamutse G, Korchazhkina O, Pye E, Strekopytov S, Polwart A, Hawkins C (2006) Elevated urinary excretion of aluminium and iron in multiple sclerosis. Mult Scler 12:533–540

Genuis SJ, Birkholz D, Rodushkin I, Beesoon S (2011) Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements. Arch Env Contam Toxicol 61:344–357

Handra CM, Ghita I, Ulmeanu A, Enache AM, Epureanu F, Coman OA, Coman L, Fulga I (2019) Depressive clinical manifestations associated with professional aluminum exposure. Rev Chim 70:2162–2167

House E, Esiri M, Forster G, Ince PG, Exley C (2012) Aluminium, iron and copper in human brain tissues donated to the medical research council's cognitive function and ageing study. Metallomics 4:56–65

Jones K, Linhart C, Hawkins C, Exley C (2017) Urinary excretion of aluminium and silicon in secondary progressive multiple sclerosis. EBioMedicine 26:60–67

Klotz K, Weistenhofer W, Neff F, Hartwig A, van Thriel C, Drexler H (2017) The health effects of aluminium exposure. Deutsches Arzteblatt Int 114:653

Michalke B, Kramer MF, Brehler R (2018) Aluminium (Al) speciation in serum and urine after subcutaneous venom immunotherapy with Al as adjuvant. J Trace Elem Med Biol 49:178–183

Mirza A, King A, Troakes C, Exley C (2017) Aluminium in brain tissue in familial Alzheimer's disease. J Trace Elem Med Biol 40:30–36

Mold M, Umar D, King A, Exley C (2018a) Aluminium in brain tissue in autism. J Trace Elem Med Biol 46:76–82

Mold M, Chmielecka A, Rodriguez MRR, Thom F, Linhart C, King A, Exley C (2018b) Aluminium in brain tissue in multiple sclerosis. Int J Environ Res Public Health 15:1777

Roider G, Drasch G (1999) Concentration of aluminium in human tissue – investigations on an occupationally non-exposed population in southern Bavaria, Germany. Trace Elem Electrolytes 16:77–86

Stahl T, Falk S, Rohrbeck A, Georgii S, Herzog C, Wiegand A, Hotz S, Boschek B, Zorn H, Brunn H (2017) Migration of aluminium from food contact materials to food-a health risk for consumers? Part I of III: exposure to aluminium, release of aluminium, tolerable weekly intake (TWI), toxicological effects of aluminium, study design, and methods. Environ Sci Europe 29:19

Stahl T, Falk S, Taschan H, Boschek B, Brunn H (2018) Evaluation of human exposure to aluminium from food and food contact materials. Eur Food Res Technol 244:2077–2084

Thompson AJ (2017) Challenge of progressive multiple sclerosis therapy. Curr Opin Neurol 30:237–240

Verstraeten SV, Golub MS, Keen CL, Oteiza PI (1997) Myelin is a preferential target of aluminium-mediated oxidative damage. Arch Biochem Biophys 344:289–294

Yumoto S, Kakimi S, Ishikawa A (2018) Colocalization of aluminium and iron in nuclei of nerve cells in brains of patients with Alzheimer's disease. J Alzh Dis 65:1267–1281

Zuur AF, Ineo EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, New York, p 574