OXFORD Toxicology Research

Toxicology Research, 2022, **11**, 511–519
https://doi.org/10.1093/toxres/tfac029
Advance access publication date: 31 May 2022
Paper

Assessment of dermal absorption of aluminium from a representative antiperspirant formulation using a (^{26}Al)Al microtracer approach: a follow-up study in humans

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A follow-up study was performed in 12 healthy women to evaluate systemic exposure to aluminium following topical application of a representative antiperspirant formulation under real-life use conditions (part A) and to assess the local fate of topically applied aluminium by taking additional tape strips and skin biopsies (Part B). A simple roll-on formulation, containing the maximal possible radioactive dose, was prepared with [^{26}Al] aluminium-labeled chlorohydrate (ACH). The microtracer of [^{26}Al] was used to distinguish aluminium from the natural background, using accelerator mass spectrometry. [^{26}Al] aluminiumcitrate was administered intravenously to estimate the dermal fraction absorbed. Despite the 25-fold increase of the topical dose compared with the previous study, only 12 blood samples gave results above the lower limit of quantitation (0.118 fg/mL). The most reliable estimates of the dermal fraction absorbed are derived from noncompartmental analysis with the urine data. By using the intravenous dose to normalize the urinary excretion to 100% bioavailability, the best estimate of the fraction absorbed of [^{26}Al] from a topical application of [^{26}Al]-aluminium-labeled chlorohydrate in an antiperspirant formulation was 0.00052%. Part B of the study demonstrated that the majority of the aluminium in the formulation remained associated with the external layers of the skin without penetration through the skin.

Key words: microtracer research; accelerator mass spectrometry; dermal bioavailability of aluminium.

Introduction

Aluminium (Al) is a commonly occurring metal in the earth's crust and is naturally present in water and agricultural products. Humans are exposed to Al through food, drinking water, pharmaceuticals, and cosmetic products. Al salts, such as Al chlorohydrate (ACH), are widely used as antiperspirants and as treatment for hyperhidrosis.[1] In 2012, Joint FAO/WHO Expert Committee on Food Additives[2] established a provisional tolerable weekly intake of 2 mg/kg bw/week, based on a pivotal 12-month oral rat study that included a multigenerational and a developmental toxicity study with aluminium citrate.[3] Regulatory review in Europe[4,5] revealed no conclusive evidence of Al playing a role in cancer[6,7] and neurodegenerative disorders. Previous studies have shown that systemic exposure following dermal Al exposure is so low that sensitive analytical techniques such as accelerator mass spectrometry (AMS) are required.[8,9] To enable robust quantitative risk assessment, EU authorities requested an accurate measurement of the skin penetration of Al from antiperspirant use.[4]

Therefore, an absolute bioavailability study was performed with 12 healthy women evaluating systemic exposure to Al following topical application of a representative antiperspirant formulation under real-life use conditions, including single and repeated dosing and shaving of the axillae.[9] A [^{26}Al] microtracer was used to distinguish Al dosed from natural background. [^{26}Al]-Al-citrate was administered intravenously (IV) to estimate fraction absorbed (F_{abs}).[9] Following topical application, only 2 blood samples were just above

Received: December 1, 2021. **Revised:** March 21, 2022. **Accepted:** May 5, 2022

the lower limit of quantitation (LLOQ; 0.12 fg/mL). From urinary excretion data, a conservative mean F_{abs} (0.0094%) was estimated, using the half LLOQ-based method.[10] No apparent difference between the various use conditions (single and repeated dosing as well as shaving) was observed.[9] Having reviewed this study, the SCCS requested further experimental work to address residual data gaps, particularly referring to the local fate of Al and the ability to determine an F_{abs} value.

This follow-up study consisted of 2 parts (Part A and Part B), each with 6 females (12 females in total). Part A included additional features compared with the previous study:[9] (i) increased proportion of radiolabel (~25-fold) incorporated into the dermal dose to improve the chance to quantify absorption, (ii) collection of total urine throughout the first 24 h up to Day 11 to improve the estimates of Al excreted in urine, (iii) collection of feces until Day 11 to enrich data on recovery and excretion, (iv) analysis of [^{26}Al] on protective gauzes, T-shirts, and washes to recover as much of the applied dose as possible, and (v) tape stripping and skin biopsies.[9] The subjects also received an IV dose containing [^{26}Al] at Day 36 to determine the absolute dermal bioavailability analogous to our earlier study.[9] Part B, conducted in a separate cohort of 6 females, included tape stripping and skin biopsies at different time-points to obtain valuable information on the fate of the topically applied Al. This investigation was performed separately in order not to compromise the real-life consumer exposure scenario in Part A. Although the aluminium concentration in the test formulation and amount applied was identical for the cohort in Part B, the proportion of radiolabel within the test formulation could be reduced to 1 Bq [compared with part A (2500 Bq)] without losing analytical sensitivity. All 12 subjects received a single dermal dose after 2 weeks of daily shaving and use of a marketed Al-containing antiperspirant.

Materials and methods

Production and analysis of [^{26}Al]-labeled dose formulations

The full worldwide stock of purified [^{26}Al]Al isotope was purchased from Los Alamos National Laboratory, USA. For Part A, the [^{26}Al]-labeled ACH was prepared and incorporated into an antiperspirant formulation as previously described,[9] including further downsizing of the batch and increasing dose (2,500 Bq versus ~100 Bq[9]). In short, aluminium powder, aluminium chloride solution, [^{26}Al]Al (concentrated by evaporation), and water were mixed and heated to initiate the reaction. The [^{26}Al]-labeled ACH used in this study was comparable to specifications for commercially available antiperspirant actives used in marketed products. For part B of the study, this material was diluted 2,500× with commercial ACH (Elementis, USA) to obtain the required dose of ~1 Bq.

The solution for intravenous (IV) administration was prepared according to the Principles of Good Manufacturing Practice (GMP) at the GMP hotlab of the department of Radiology & Nuclear Medicine of the Free University Medical Center (Amsterdam, The Netherlands).[9] The procedures were fully described in the Investigational Medicinal Product Dossier, which was approved by the ethical committee prior to administration. For logistic reasons, 2 batches of IV solution were manufactured.

Details of the dose formulations are given in Table S1 (see online supplementary material for a color version of this table).

Sample analysis

All samples were analyzed for [^{26}Al] content using AMS. The AMS methods[9] were (re)qualified for the analysis of blood, urine, fecal homogenates, and extracts in 0.1 M HCl. The methods were accepted as they fulfilled the requirements for linearity, accuracy, and precision. Each analytical batch consisted of study samples together with 6 calibration samples in duplicate and 3 QC samples, analyzed in triplicate.

Urine samples were analyzed for ^{27}Al content by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) using Rhodium (m/z 103) as the internal standard.[9] The analytical method was qualified for selectivity, linearity, accuracy, precision, and LLOQ.

Data analysis

^{26}Al/^{27}Al isotope ratios of the samples were converted to mBq/mL by plotting these on the linear calibration line, using a weighing factor or $1/x^2$. Concentrations in mBq/mL were then converted to fg Aluminium/mL based on the specific activity for [^{26}Al]Al of 722 Bq/μg. Blood concentration-time area under the curves (AUCs) were determined using the trapezoid method (linear up, log-linear down method).[9] $AUC_{0:672}$ for each subject was used to calculate the dermal bioavailability (F_{abs}) as the ratio of AUC dermal to IV, then averaged over subjects.

Study design and subject characteristics

The study was designed as a single-centre, open-label, 2-period, fixed sequence study, conducted in the Netherlands (Center for Human Drug Research, Leiden). Twelve healthy women (18–43 years of age, body mass index within 18.1–27.3 kg/m^2, 6 in each cohort) were included. Subjects were familiar with frequent wet shaving using an appropriate female safety razor. Exclusion criteria included clinically significant abnormality of the axilla (e.g. scars, tattoos, and/or dermal abnormalities); use of Al-containing medications; and axillary hyperhidrosis. Both parts of the study were approved by the Medical Ethics Review Board Brabant (The Netherlands); informed consent was obtained from all subjects. A schematic overview of the study design can be found in Fig. 1.

Two treatment periods were included (Part A): 1 topical application (~2,500 Bq) and 1 IV administration (~0.1 Bq).

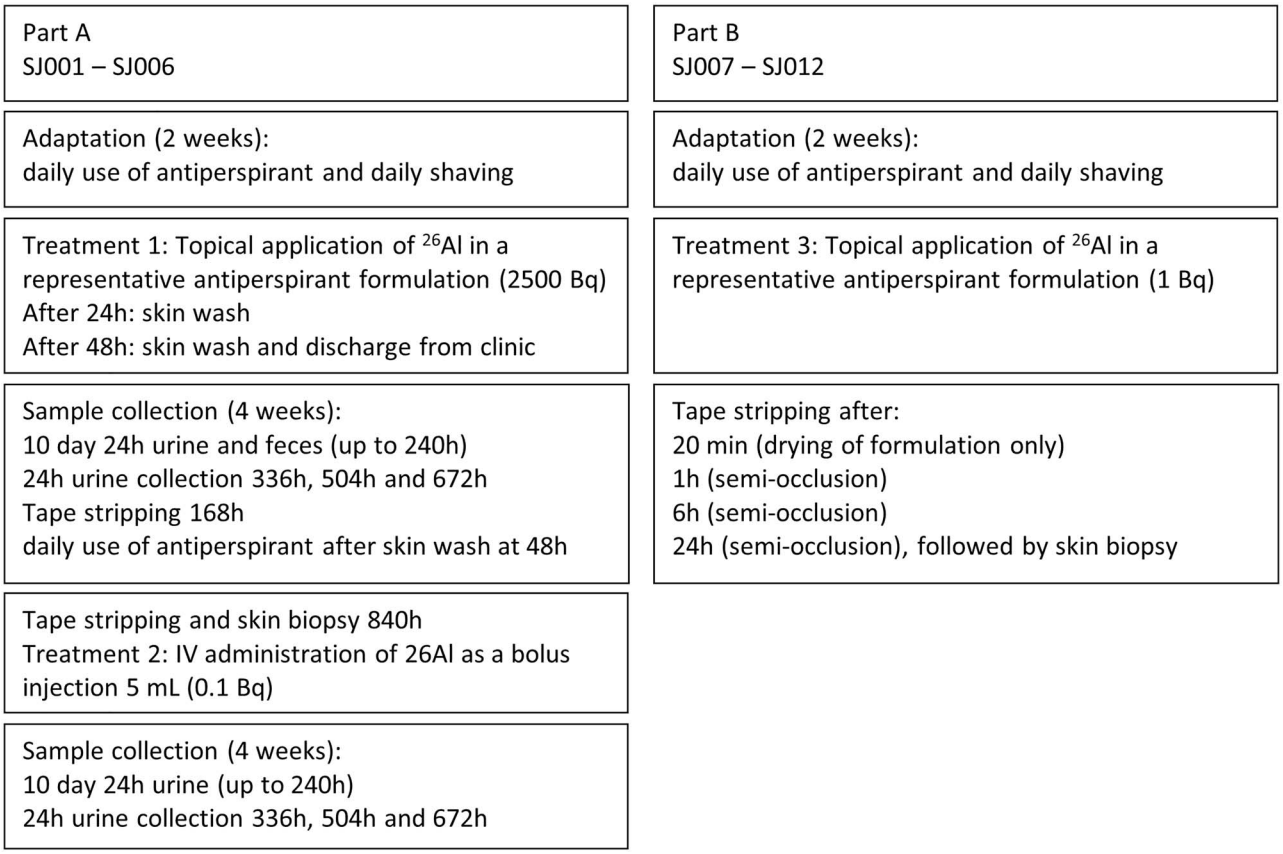

Fig. 1. Study design.

Part B consisted of a single topical application (~1 Bq). Each topical application was preceded by an adaptation period of 2 weeks [daily shaving of the armpits and application of antiperspirant product with Al (as ACH)]. The [^{26}Al]-labeled formulation was applied to both axillae within a delineated area of ~100 cm^2 per armpit. After the formulation had dried (~15–20 min), an air permeable gauze was applied over the axillae and a cotton T-shirt was worn to avoid loss of radiolabel to the environment, and 24 and 48 h after application, the axillae were washed using cotton gauze and a mild soap solution. The axillae were covered in-between with a fresh gauze and T-shirt. Subjects were released from the unit after the 48-h washing procedure. All T-shirts, gauzes, and washings were collected for AMS. Blood samples were collected at various time points up to 28 days after application. All urine and feces were collected up to 240 h postdose. Additionally, selected urine was collected up to 28 days postdose. On Day 8 (168 h postdose), the skin in the axillae was stripped (D-squame ® discs, CuDerm, USA) until the shiny interface of the viable epidermis became visible. On Day 36 (840 h postdose), the procedure was conducted in the opposite armpit. After stripping, a skin biopsy (3 mm) was taken within the stripped area. After the skin biopsy was taken, the IV solution was administered as a bolus injection of 5 mL into a suitable antecubital vein. Blood and urine samples were collected up to 28 days after IV administration.

In part B, the armpits were divided into 4 designated areas for tape stripping (just left and right of the middle part). The formulation (~1 Bq) was applied and allowed to dry for 20 min. Thereafter, one designated area was stripped until the shiny interface of the viable epidermis became visible. After stripping, an air permeable gauze was applied over both axillae and the subjects wore T-shirts. After 1, 6, and 24 h following dermal application, tape strips were taken from the remaining spots. At 24 h, a skin biopsy (3 mm) was taken within the stripped area.

Results

No clinically relevant changes in vital signs, laboratory, or ECG measures were observed throughout the study. All subjects completed the study without any relevant complaint and topical treatments were well tolerated. Mild discomfort was commonly observed upon IV administration of the [^{26}Al]-labeled solution; 1 subject experienced moderate discomfort, most likely due to subcutaneous (mis)dosing. A total number of 32 adverse events (AEs) were reported during the study period (Table S2, see online supplementary material for a color version of this table). All AEs considered to be possibly or probably related to study treatment (including the semi-occlusive conditions) were of moderate (n = 2) or mild severity (n = 10, Table S2, see online supplementary material for

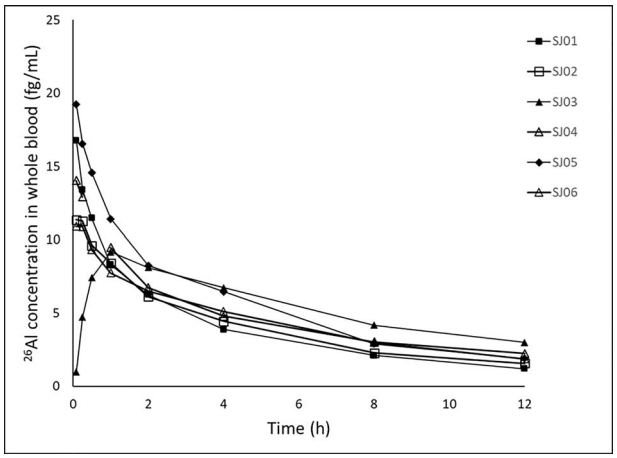

Fig. 2. Part A: [^{26}Al] concentrations in whole blood after IV injection (zoom 0–12h).

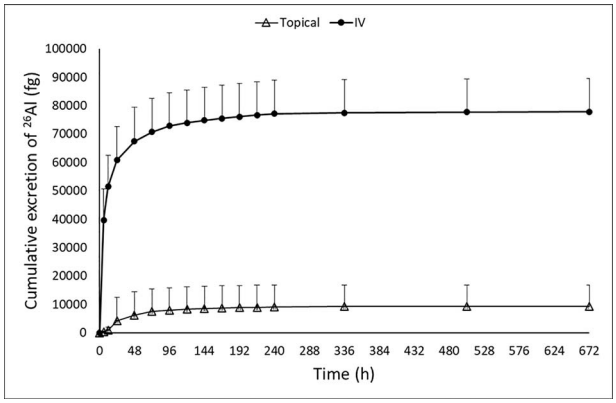

Fig. 3. Part A: Cumulative urinary excretion of [^{26}Al] after topical application (open triangles, 2500 Bq) or IV injection (black circles, 0.1 Bq).

a color version of this table). No serious AEs occurred in this study.

In Part A, 186 blood samples were analyzed for [^{26}Al] content. Albeit the topical dose of [^{26}Al]-ACH was increased 25-fold compared with the previous study,[9] only 12 whole blood samples (from 2 subjects) contained a concentration above the LLOQ of 0.118 fg/mL. These concentrations were well below 1 fg/mL and thus very low compared with the nominal dose given (~3.73 μg [^{26}Al]Al). After IV administration of 0.1 Bq (~120 pg [^{26}Al]Al), [^{26}Al] could be detected in blood up to 72 h. The analysis for the IV administration is based on only 5 subjects due to the inadvertent mis-dosing of one subject, who showed a clearly different PK profile, resembling intramuscular or subcutaneous injection (Fig. 2). All values below LLOQ were replaced with the LLOQ value to provide conservative estimates of exposure. This approach resulted in a calculated averaged percentage absorbed of less than 0.0021% (n = 5), principally driven by samples that were below LLOQ.

The entire urine output was collected up to 240 h postdose (24-h intervals); the first 24 h were divided into 0–6, 6–12, and 12–24 h collections. In urine samples (192 in total), [^{26}Al] was detected up to 144 h (topical application) or up to 672 h (IV administration). The cumulative excretion of [^{26}Al] in urine from both routes is presented in Fig. 3; the urinary excretion represents 0.00026% of the topical dose applied and 68.8% of the IV dose. The dermal fraction absorbed was calculated by the ratio of the total fraction excreted in urine following the topical dose to the total fraction excreted following the IV dose. Using the same conservative strategy of replacing values <LLOQ with LLOQ,[9] the average fraction absorbed was found to be at least smaller than 0.00052% (n = 5). This means that of 200,000 Al atoms applied on the skin, only maximally 1 atom was actually absorbed.

All urine samples were also analyzed for the total amount of Al present ([^{27}Al] and [^{26}Al]) using ICP-MS and found vary day-to-day within normal ranges.[11–13] With these results and taking into account the urinary output, the total urinary excretion of Al [from all possible routes (oral, inhalation, dermal)] was calculated and is presented in Fig. 4 (open symbols). To assess the contribution of antiperspirant use to this total excretion of Al in urine, the following approach was taken. First, the ratio between [^{26}Al] and Al was calculated. The amount of Al in the dose was calculated to be 0.9166 g Al; the amount of [^{26}Al] in the dose was calculated to be 25.55 μg, resulting in a [^{26}Al]: Al ratio of 1: 35,868. Since absorption of both isotopes in the antiperspirant formulation would be the same, this ratio was used to convert the radiolabel in the urine, measured as [^{26}Al]/mL (i.e. pg/L) to the equivalent μg total Al/L (Fig. 4, closed symbols). Importantly, since antiperspirants may be used on a daily basis, one might expect that exposure on preceding days would also contribute to aluminium excretion; therefore, to account for the fact that [^{26}Al] was administered only once, the exposure from [^{26}Al] was extrapolated to a multiple dose by adding up the amount of each preceding day (values below LLOQ were set to $^1/_2$ LLOQ, i.e. representing 0.0020 μg/L) and this is presented by the solid line in Fig. 4. When comparing the solid line with the open symbols in Fig. 4, it can be concluded that, even in the daily use scenario, only a minor part of the Al excreted in urine originally derived from the topically administered antiperspirant formulation.

In this study, every practical effort was taken to recover as much of the [^{26}Al] dosed as possible by collecting: skin wash samples (containing gauzes, cloths, and razor blades) and the T-shirts worn by the subjects. The average recovery from these samples was 70% (59–77%, Table 1). Recovery in remaining samples (urine, fecal homogenates, skin biopsies, and tape strips) represented 0.021% (Table 1). In fecal samples between 24 and 240 h after dermal application, Al was detected at low levels (mean: 0.0017%, Table 1). The amount of [^{26}Al] recovered from the tape strips after 168 and 840 h was rather similar.

The fate of [^{26}Al] on the skin and within the stratum corneum was studied in more detail by collecting tape strips from the axilla at 4 different time points (Fig. 5,

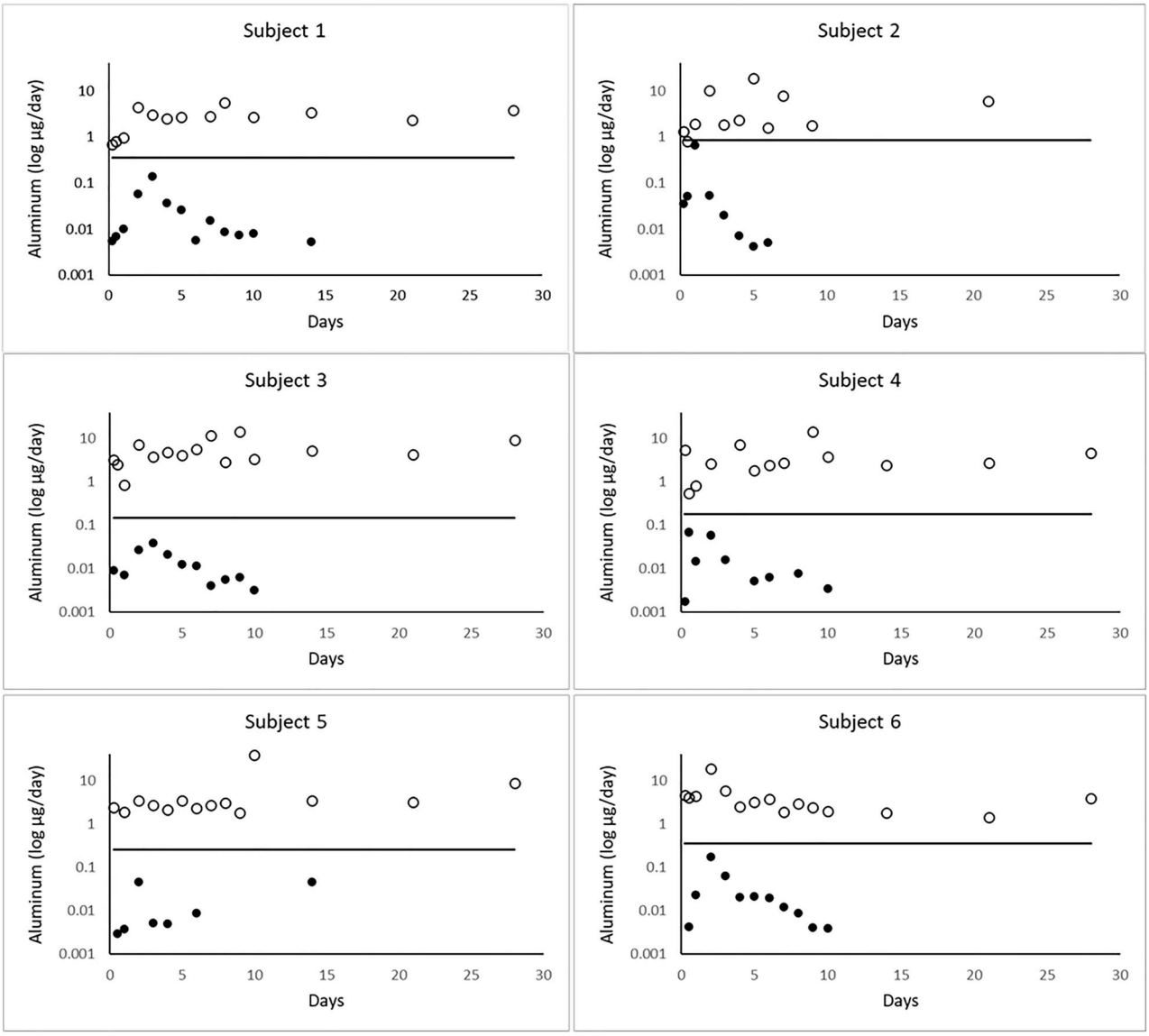

Fig. 4. Al excretion in microgram for all subjects (black circles Al derived from antiperspirant (AP), open circles total Al excretion, solid line cumulative Al excretion from AP).

Table 1. Overview of average % of dose recovered in all study samples in part A (n = 6).

Sample	Recovery in % of dose (range)
Skin wash 24 h	62.0 (54.1–73.6)
T-shirt 24 h	6.0 (1.1–14.6)
Skin wash 48 h	1.6 (0.8–3.0)
T-shirt 48 h	0.09 (0.07–0.15)
Subtotal (non-absorbed dose)	69.7 (58.7–76.7)
Urine	0.0003 (0.0001–0.0007)
Feces	0.0017 (0.0008–0.0057)
Skin biopsy (840 h, n = 2, remaining <LLOQ (n = 4))	0.00003–0.00004
Tape strips (168 h)	0.0097 (0.0019–0.0417)
Tape strips (840 h)	0.0090 (0.00004–0.0525)
Subtotal (potential absorbed dose)	0.021 (0.004–0.095)

Table S3, see online supplementary material for a color version of this table). At 20 min, most of the recovered dose was found in the outer tape strip. The percentage of the applied dose decreased substantially with each sequential tape strip. This does not necessarily represent formulation that is within the stratum corneum, since

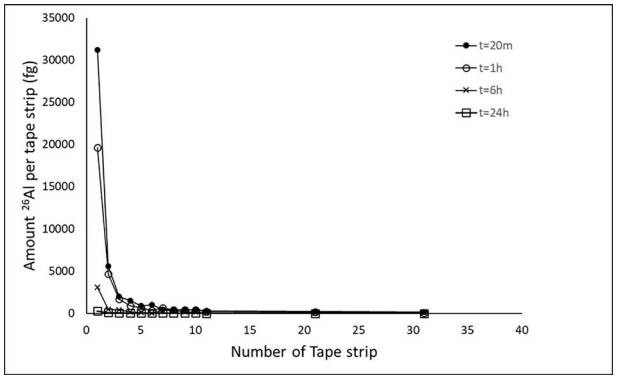

Fig. 5. Part B: Representation of the average amount [^{26}Al] (in fg) recovered from tape strips.

these tape strips may still contain formulation sticking in the troughs/furrows of the heavily crenulated axilla skin. By 24 h, the amount recovered decreased to less than 2% of the normalized dose applied. Importantly, the profile within the stratum corneum did not change over time; the majority of the recovered Al was in the initial tape strip and diminished rapidly with each strip. In skin biopsies (taken at 24 h), 0.08% of the dose applied was recovered (n = 4, 2 samples were found to be <LLOQ). All results were normalized for the total amount of [^{26}Al] recovered after 20 min of application, since this first tape strip contained more than 100% of the applied dose in all subjects. This was likely due to the uneven surface of the axillae which prevented homogeneous distribution within the designated area. The first time-point, where the axilla was undisturbed, should best reflect the maximum dose applied.

Discussion and conclusion

This follow-up study (Part A) on the assessment of dermal absorption of Al from a representative antiperspirant formulation under real-life conditions represents a refinement of our previous study[9] and supports a more precise quantitative assessment of consumer exposure. Part B, performed separately, not to compromise the real-life consumer exposure scenario of Part A, was conducted to generate more detailed data on the fate of [^{26}Al] after dermal application to strengthen the limited "mass balance" data generated in part A.

Previously, no apparent difference was observed between the various treatments (shaving, single, or daily use).[9] Thus, this new investigation was simplified to a single treatment (daily shaving and product use). Several improvements were included in the study design to enhance data interpretation, such as increased proportion of radiolabel in the topical dose, collection of total urinary output, feces, and "mass balance"-like samples. A detailed list of the differences in the design of both studies is given in Table 2. It should be noted that both studies have been conducted in the Caucasian population only. While no people of color (PoC) have been included in the study, at a mechanistic level, this would not be expected to impact on the results. Antiperspirants function by interacting with bicarbonate buffered sweat and ductal mucins to temporarily occlude sweat ducts. This mechanism of action relies on skin physiology that is not significantly different between Caucasian and PoC, including pH on the skin surface and within the skin. The major difference between Caucasian and PoC skin resides in the pigmentation; however, it would not be expected to alter the action of aluminium in sweat ducts.

In theory, blood concentrations would provide an absolute bioavailability value. However, despite a maximal increase of the dermal radioactive dose (25-fold using the full worldwide stock) and the use of the most sensitive AMS techniques, the majority of measures in blood remained below LLOQ and the dermal AUC could not be derived using noncompartmental analysis. Instead, a very conservative scenario was applied by replacing all values below LLOQ with the LLOQ value, which resulted in an estimated average dermal bioavailability of ~0.0021%, principally driven by samples below LLOQ. This estimate was markedly lower when compared with our initial study (0.0116%, upper estimate, average of all treatments), representing a substantial refinement to the conservative approach taken previously.[9] To strengthen this estimate of ~0.0021%, we focused on the urinary excretion following IV and dermal dosing.

As the topical dose had 25-fold more radioactivity compared with our first study,[9] more urine samples were above LLOQ. Also, the measurement of urinary Al excretion was considerably refined by collecting 3 samples within the first 24 h and the complete 24 h urine output for the first 10 days. Again, values below LLOQ were replaced with LLOQ, resulting in a conservative mean estimated fraction absorbed of 0.00052% (0.00026–0.00108%). These results confirm that our previous approach using the half LLOQ-based method[10] provided a conservative estimate (average fraction absorbed was 0.0094%[9]). Since estimates of the fraction absorbed are driven by LLOQ, the 25-fold increase in the topical dose (sensitivity) resulted in a comparable 20-fold reduction in estimated dose.

The low Al levels observed in fecal samples between 24 and 240 h were unexpected since the elimination via feces is very unlikely.[14] Thus, these low levels were considered secondary to contamination, possibly by small quantities dropping from the T-shirt or being ingested following hand to mouth contact. They seem an artifact that should be interpreted with caution and no comparable samples from the IV dose are available. However, these would not account for environmental cross contamination anyway. Albeit fecal excretion is unlikely, this assumption represents an uncertainty in the exposure calculation.

Achieving a good mass balance of Al is challenging,[8] particularly without disturbing the desired real-life consumer scenario by strict measures such as occlusion. This study was not designed to be a classical mass

Table 2. Differences in study design between de Ligt (2018,[9]) and Part A of the current study.

	De Ligt, 2018[9]	**Current study Part A**
Number of subjects	12	6
Dose	100 Bq [^{26}Al]-ACH in a representative topical formulation	2500 Bq [^{26}Al]-ACH in a representative topical formulation
Application site	Both axillae (50 Bq [^{26}Al] each)	Both axillae (1250 Bq [^{26}Al] each)
Antiperspirant use	Single and repeated*	Repeated
Application details	Nonocclusion: subjects were wearing non-occlusive T-shirts during the first 24 h and to avoid loss to the environment	Semi-occlusion: the application site was covered with gauzes loosely attached under the arms and subjects were wearing non-occlusive T-shirts during the first 48 h and to minimize loss to the environment
Shaving regimen	Adaptation period of 4 weeks with either daily wet shaving[a] or no shaving at all	Adaptation period of 2 weeks with daily wet shaving[a]
Urine collection	Morning spot urine samples (24, 48, 72, 168, 336, 504, and 672 h)	2- h interval urine samples (0–6, 6–12, 12–24, and 24 h intervals up to 216–240, 312–336, 480–504, and 648–672 h)
Feces collection	Not done	24-h intervals for 10 days
Samples to assess the local fate of [^{26}Al]	Not done	Skin wash (including gauzes) and T-shirt samples (24 and 48 h), tape strips (at 168 and 840 h), skin biopsy (at 840 h)

[a]Last shaving was performed on the morning prior to [^{26}Al]-ACH application at the clinical site. [b]Dosing after adaptation period without antiperspirants considered to represent a single dose of ACH and dosing after adaptation period with daily use of antiperspirants considered to represent repeated dosing.

balance study, since the necessary occlusion would have created an entirely artificial exposure scenario, not resembling real-life consumer use conditions. Besides, absolute bioavailability studies are the first choice study design for the estimation of internal exposure, while mass balance studies are assessing excretion (routes). As demonstrated by Flarend *et al.*,[8] attempts to occlude/tape-strip the axillae of subjects results in rapid damage of the axilla skin, which impacts skin absorption and the harm caused to the subjects is considered to be unethical. Here, the application sites were semi-occluded, which is why a significant amount of [^{26}Al] was found in all T-shirts collected after 24 h.

Overall recovery of the topically applied [^{26}Al] in this study was ~70% and significantly greater compared with the previously published study, where recovery was below 50%.[8] Recovery was predominantly in samples considered as nonabsorbed: skin washes and T-shirts. Based on the significant amount of [^{26}Al] on the T-shirt, loss to the environment is the most plausible explanation for the proportion not accounted for. This is further supported by the Al found in the 48 h samples (skin wash and T-shirt), which, after 2 days, still contained measurable amounts of Al (more than 1% of the dose applied). Also, all control tape strip samples taken from the upper back at 168 h (part A) or 24 h (part B) after application contained measurable levels of [^{26}Al], demonstrating that transfer occurred to other areas of the body via contamination of the environment. It is clear that the mean recovery from the biological samples—skin biopsy, tape strips, urine, and feces together—is only 0.021% and this shows that an extremely small amount crosses the skin barrier (Table 1). Moreover, the amount of [^{26}Al] recovered from the tape strips after 168 and 840 h was rather similar, which indicates that the [^{26}Al] remained on the skin surface and did not penetrate to lower skin layers. This is further supported by the results of part B, which demonstrate that most of the formulation remained external on the skin surface (Fig. 5). Virtually, all the radioactivity was removed in the first few tape strips, indicating that the applied labeled substance was predominantly associated with external layers of the skin without absorption. Also, the similarity of the tape strip profiles at the various time points shows no evidence of inward distribution *within* the stratum corneum and lower skin layers over the time-course of part B, as one might expect for substances that penetrate into the skin.[15]

Our data are consistent with the hypothesis that the soluble Al salts form insoluble gels of Al hydroxide at physiological pH on the skin surface; then, the insoluble Al precipitate forms superficial plugs in the openings of sweat ducts[16–18] and strongly associates with proteins on the surface of the stratum corneum. These temporary plugs are lost from the skin surface through natural sloughing of the stratum corneum. In reality, loss of the antiperspirant formulation to the environment is expected to be even greater. To have a controlled, yet conservative, exposure scenario, we used semi-occlusive gauze and a standard T-shirt 20 min after application. However, in normal life, consumers would likely dress soon after applying antiperspirant, which would remove some of the freshly applied formulation from the surface of the axilla. Furthermore, the presence of the gauze would have protected the test formulation from the gentle abrasion of the fabric on the skin which might further dislodge adherent formulation.

A question raised regarding the interpretation of the first study[9] was the potential difference in excretion kinetics of the Al species immediately after IV dosing with Al (in citrate-buffered saline), which might be subtly different to the kinetics of the topically applied

formulation (with ACH). Normally, approximately 10–20% of Al in plasma is bound to citrate and is therefore potentially more accessible for renal filtration, whereas 80–90% of Al in the plasma is bound to transferrin and unfilterable.[19] There is an equilibrium between the amount of Al bound to plasma citrate and the amount of Al bound to plasma transferrin; the kinetics of this equilibrium are known to be relatively rapid.[20] It is reasonable to assume that any dermally applied Al reaching the systemic circulation would have reached equilibrium and behave in a similar manner to endogenous Al ions (i.e. being carried predominantly by transferrin). However, following the IV administration, there is suddenly a relatively large amount of Al complexed with citrate. Based on a validated PBPK model for Al distribution,[20,21] the equilibrium between Al citrate and Al bound to transferrin was estimated to stabilize within only 15 min after the IV administration (see also Fig. S1, see online supplementary material for a color version of this figure).

Although equilibrium is reached relatively quickly, Al complexed with citrate might be more rapidly excreted than transferrin bound Al. For the brief period (15 min[20] and Fig. S1, see online supplementary material for a color version of this figure) following IV dosing with Al (in citrate-buffered saline), more rapid excretion may overestimate the total fraction absorbed until equilibrium with transferrin is established. Consequently, the dermal fraction absorbed might be underestimated due to a more rapid excretion of Al during these first 15 min following IV administration. This subtle difference in excretion kinetics would only have an impact on the IV phase of the study for the time following dosing, until the equilibrium between citrate and transferrin has been reached.

To quantify the uncertainty associated with this period of citrate:transferrin equilibration, the fraction absorbed was also estimated by excluding the first urine sample (0–6 h). The assumption that the Al in the first urine sample was completely excreted within 15 min resulted in only a modest increase in the estimate of mean fraction absorbed to 0.00068% (vs. 0.00052% calculated including total urine from 0 h). Thus, the impact of subtle differences in excretion for the initial 15 min post IV dose can be considered negligible. This is also supported by the observation that the AUC of the blood profile from the "misdosed" subject was comparable to the other 5 subjects. On the other hand, as fecal excretion is unlikely, this represents a slight uncertainty in the exposure calculation.

Taken together, the most reliable estimates of the dermal fraction absorbed are derived from noncompartmental analysis with the urine data; these data are supported by the noncompartmental analysis using the limited whole blood data. Furthermore, by using the IV dose to normalize the urinary excretion to 100% bioavailability, this study provides sufficiently robust data to support a reliable estimation of the fraction of Al absorbed after topical application of a representative antiperspirant formulation. The SCCS acknowledged our interpretation of the current data in their updated risk assessment.[22]

In conclusion, the best estimate of the fraction absorbed of [^{26}Al] from a topical application of [^{26}Al]-ACH in an antiperspirant formulation is considered to be 0.00052%. Moreover, the vast majority of the Al in the formulation remains associated with the outer layers of the stratum corneum and does not penetrate the skin, but appears to be lost from the skin surface to clothing and the environment.

Supplementary material

Supplementary material is available at TOXRES Journal online.

Acknowledgments

We would like to acknowledge the United States Department of Energy Isotope Program because the isotope ^{26}Al used in this research was supplied by the United States Department of Energy (US DOE) Isotope Program, managed by the Office of Isotope R&D and Production. R.d.L. and W.V. agree and acknowledge the US DOE Isotope Program as the provider of the isotope. We thank Elementis (USA) for the provision of reagents used to prepare ACH formulations.

Authors' contributions

R.d.L. and W.V. designed the research and wrote the manuscript.
D.G., J.W., T.B., R.R., K.B., and A.W. performed the research.
R.d.L., S.T., G.P., B.W., D.B., D.M., and W.V. analyzed the data.

Abbreviations

ACH: Aluminium chlorohydrate AMS: Accelerator Mass Spectrometry; Al: Aluminium; AUC: Area Under the Curve; ECG: electrocardiogram; GMP: Good Manufacturing Practice; ICP-MS: Inductively Coupled Plasma Mass Spectrometry; JECFA: Joint FAO/WHO Expert Committee on Food Additives; LLOQ: Lower Limit of Quantitation; mBq: milli-Becquerel; TNO: Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek (Netherlands Organization for applied scientificresearch; US DOE: United States Department of Energy.

Funding

This study at TNO, The Netherlands was 100% funded by Cosmetics Europe.
Conflict of interest statement: R.d.L., J.W., D.G., T.B., R.R., K.B., A.W., and W.V. declared no competing interest for this work.

S.T., G.P., B.W., D.B., and D.M. are employed by their respective companies, each of which use Aluminium compounds in cosmetic products.

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