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In vitro study of percutaneous absorption of aluminum from antiperspirants through human skin in the Franz™ diffusion cell ☆

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ABSTRACT

Aluminum salts such as aluminum chlorohydrate (ACH) are known for use as an active antiperspirant agent that blocks the secretion of sweat. A local case report of hyperaluminemia in a woman using an aluminum-containing antiperspirant for 4 years raises the problem of transdermal absorption of aluminum (Al). Only a very limited number of studies have shown that the skin is an effective barrier to transdermal uptake of Al. In accordance with our analytical procedure, the aim of this study with an in vitro Franz™ diffusion cell was to measure aluminum uptake from three cosmetic formulations of antiperspirant: the base for an "aerosol" (38.5% of ACH), a "roll-on" emulsion (14.5% ACH), and a "stick" (21.2%), by samples of intact and stripped human skin (5 donors). The Al assays were performed by Zeeman Electrothermal Atomic Absorption Spectrophotometry (ZEAAS). Following contacts lasting 6, 12 and 24 h, the Al assays showed only insignificant transdermal absorption of Al (≤0.07% of the quantity of Al deposited) and particularly low cutaneous quantities that varied according to the formulations (1.8 μg/cm² for "aerosol base" and "stick" — 0.5 μg/cm² for the "roll-on"). On stripped skin, for which only the "stick" formulation was tested, the measured uptake was significantly higher (11.50 μg/cm² versus 1.81 μg/cm² for normal skin). These results offer reassurance as regards to the use of antiperspirants for topical application of ACH-containing cosmetic formulations on healthy skin over a limited time span (24 h). On the other hand, high transdermal Al uptake on stripped skin should compel antiperspirant manufacturers to proceed with the utmost caution.

1. Introduction

Aluminum salts, such as aluminum chlorohydrate (ACH), a water-soluble aluminum complex [$Al_2(OH)_5Cl,2H_2O$], are the active ingredient of antiperspirants in most cosmetics. The mechanism consists of the precipitation of ACH inside the eccrine sweat glands to produce insoluble aluminum hydroxide, which plugs the gland and blocks the secretion of sweat [1,2]. In addition, aluminum (Al) alters sweating by constricting the dermal duct lumen by direct effect or via its anticholinergic action [3].

The skin onto which antiperspirants are applied constitutes the main entranceway into the organism of aluminum (Al), and transdermal uptake of Al salts may be harmful [4]. Aluminum overload can cause anemia, bone disease and dementia in patients with reduced kidney function [5]. Strong suspicions about the role of Al in neurodegenerative disorders such as Alzheimer's, Parkinsonism and Guam Parkinsonism/amyotrophic lateral sclerosis [6–8], and the potentially toxic role of this metal in antiperspirants have already been reviewed by Exley [9]. The questions put forward by this author [9] suggest that Al absorbed following the application of antiperspirants may act through novel non-systemic target sites, a hypothesis that has been confirmed by Darbre [10]. Indeed, the results of this study [10] demonstrate that Al in the form of aluminum chloride or ACH can interfere with the function of estrogen receptors of MCF7 human breast cancer cells both in terms of ligand binding and in terms of estrogen-regulated reporter gene expression. Given the wide exposure of the human population to antiperspirants, Al could play a role in the increasing incidence of breast cancer. Indeed, Exley et al. [11] have confirmed a higher content of Al in the outer breast from biopsies (in 17 breast cancer patients), a finding that may be explained by this region's closer proximity to the underarm, where maximal density of antiperspirant application may safely be assumed. However, these authors [11] do not go so far as to affirm that the Al measured in these breast biopsies originated from antiperspirants. In light of these studies, observations by McGrath [12] offer convincing evidence that a rise in chronic apocrine sweat gland obstruction due to antiperspirant use has been occurring with increased frequency throughout the world and parallels rises in breast and prostate cancer incidence and mortality rates.

☆ We wish to dedicate this article to the late Professor J.P. Marty.

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Consequently, given the possibly negative effects of antiperspirants on human health, questions arise with regard to the transdermal uptake of Al [13]. Even though it has been reported that aluminum chloride can be absorbed through the skin of mice [14], in vivo animal models may not be relevant, since the epidermal structure of the mouse is based on only 2 or 3 cell layers, as opposed to 20 to 30 in humans. The one published in vivo study in human (2 healthy volunteers) was performed by Flarend et al. [15] with a single application of ACH with occlusive bandage using radiolabeled aluminum (^{26}Al). They showed [15] that only 0.012% of the applied aluminum was absorbed. In contrast, Guillard et al. [16] have reported uptake of Al from an underarm antiperspirant to a toxic level of about 4 μM in blood plasma, which returned to the normal range about eight months (0.1–0.3 μM) after discontinuation of 4 years of antiperspirant use. Along with hyperaluminemia in this woman, the authors observed symptoms of bone pain, extreme fatigue with normal neuropsychological and electroencephalographic tests and without any truly clear explanation concerning possibly overly high plasma aluminum [17].

As a result of these studies, the French Ministry of Health and FEBEA (Fédération des Entreprises de la Beauté, Paris, France) initiated an in vitro study of Al on the human skin as regards to the possible cutaneous penetration on healthy and stripped skin of the different commercial antiperspirant formulas.

Subsequent to the validation of the analytical procedure for the Al assay with different media on human skin using a Franz™ diffusion cell [18], the aim of this study was to measure the in vitro human percutaneous absorption and skin distribution of ACH incorporated at different concentrations in three cosmetic formulations: a base for “aerosol” (38.5% of ACH); a “roll-on” emulsion (14.5% of ACH); a “stick” (21.2% of ACH) on normal and on stripped skin. In more, calculation of the quantities of Al deposited on the skin in the Franz™ cell with respect to the physiological application conditions helps to render the experimentation relevant to human exposure.

This percutaneous absorption study was conducted according to the OECD (Organization for Economic Cooperation and Development, 428 guideline 2004, http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg428.pdf) and the SCCP (Scientific Committee on Consumer Products, guidelines 2003, http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out242_en.pdf). This in vitro technology is considered as a validated alternative test and accepted as such by the Scientific Committee on Consumer Products.

2. Materials and methods

2.1. Cosmetic formulations tested

All cosmetic formulations and their certifications of analysis were supplied by Unilever Laboratories (Seacroft, Leeds, UK); a base for aerosol (38.5% of ACH), a roll-on emulsion (14.5% of ACH) and a stick (21.2% of ACH).

2.2. Skin biopsies

The five skin biopsies were obtained from the abdominal skin of a bank of Caucasian humans ranging in age from 29 to 52 years through cosmetic surgery. Skin biopsies were cleared of adhering subcutaneous fat.

2.3. Reagents

2.3.1. Receptor Fluids

- Phosphate buffer saline pH 7.4 Sigma ref: P4417 (St. Louis, MO, USA) + 0.1% (w/v) sodium azide (Scharlau, Chemie, S.A., Spain) in ultrapure water.
- Phosphate buffer saline pH 7.4 + 0.1% (w/v) sodium azide + 5% (w/v) Brij 98 (Acros organics, Fisher Scientific, Illkirch, France) in ultrapure water.

2.3.2. Washing solutions

- Brij 98 to 5% (w/v) in ultrapure water.
- HNO_3 65% (Suprapur® Merck, Darmstadt, Germany) to 0.063% (v/v) in ultrapure water.

2.3.3. Analytical reagents

All chemicals for measurements of Al were suprapure quality and purchased from Merck (Darmstadt, Germany), Perkin-Elmer, (Norwalk, CT, USA), Sigma (St Louis, MO, USA) and VWR International (Strasbourg, France). Purified water with a specific resistance > 18 MΩ/cm was obtained with an osmosis system (Permo, Département Industrie, BWT France S.A.S.).

2.4. Equipment

2.4.1. Cleaning of the glassware

All materials were washed and soaked overnight in HCl 36 M solution (Suprapur® Merck Darmstadt, Germany) diluted at 10% (v/v) with ultrapure water. Next, they were rinsed five times with ultrapure water and then checked for Al contamination [18].

2.4.2. Franz™ diffusion cell

Percutaneous absorption was studied quantitatively, in vitro, on human full skin biopsies mounted in Franz™ diffusion cell (Fig. 1) (Laraspiral, Dijon, France) with an offered surface for diffusion of 1.76 cm^2. The dermal part of the skin was bathed with a saline solution in which the substance absorbed through the skin was assayed over time.

The skin biopsy was mounted in horizontal position between the two parts of the cell demarcating two compartments, one on each side of the sample: the “donor” compartment consisted of a glass cylinder with a surface of 1.76 cm^2 accurately defined, applied to the upper side of the skin; a “dermal receptor” compartment (containing the Receptor Fluid) applied to the lower side of the skin, consisted of a 6 mL fixed volume compartment, with a sampling port for analysis.

The two compartments were held in place by a horseshoe clamp, and water tightness was provided by the skin maintained between them. The “dermal” receptor compartment was filled with a Receptor Fluid made of phosphate buffer saline (pH 7.4) containing 0.1% sodium azide as a preservative and 5% of Brij 98 polyoxyethylene oleyl ether as a non-ionic solubilizer. Aluminum chlorohydrate is soluble in this solution and tissue viability conditions have been fully respected. The “dermal” compartment was immersed in a water bath in order to maintain the epidermal surface temperature at about + 32 ± 2 °C.

Homogeneous distribution of the temperature of the dermal compartment was maintained by a magnetic stirring bar, with the diffusion cell mounted on a stirring device.

Samples of the fluid contained in the “dermal” compartment (Receptor Fluid) were collected from the sampling port during the study and at the end of the diffusion time. During the contact of the product with the skin, the upper portion, i.e. the “donor epidermal” compartment, was open to the air, thereby exposing the epidermis to the ambient conditions of the laboratory environment, or was under occlusion with Parafilm® “M”, when the artificially damaged skin was studied.

Quality control of skin thickness was performed for each skin sample before mounting on a diffusion cell using a specific device (calibrated plastic wedge of 513 μm with a thickness control system and a reading to 1/100 mm). Skin samples were then randomized and mounted in the diffusion cells without being subject to any other treatment.

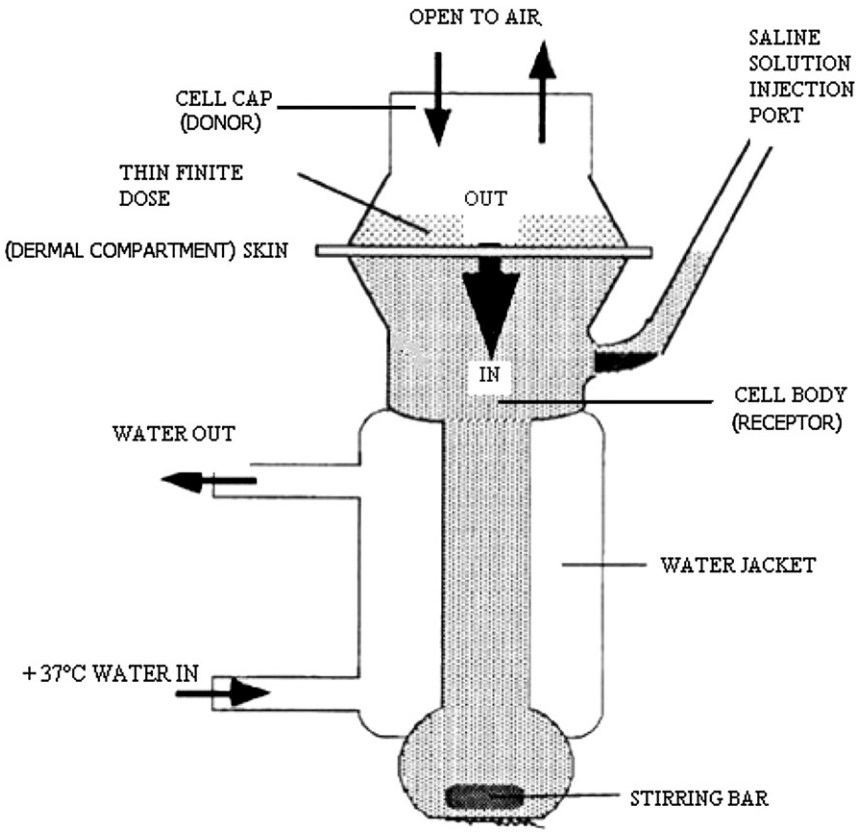

Fig. 1. Franz™ diffusion cell (static type).

The integrity of the skin barrier and the water tightness of the experimental model were verified for each diffusion cell before application of the cosmetic products, by measuring transepidermal water loss (TEWL). The measurement was performed directly on the epidermal compartment using an evaporimeter (Tewameter™, Courage & Khazaka, Köln Germany). The probe was left in place on the skin surface for 2 min; the TEWL was then recorded for one minute. Any cell presenting an abnormal TEWL, compared to the other cells used for the test and to data from the literature [19], was recorded in the laboratory notebook.

2.4.3. Apparatus

2.4.3.1. Digestion Device. Tissues (epidermis, dermis or strips) were digested with DigiPREP apparatus purchased from SCP Science (Courtaboeuf, France).

2.4.3.2. Spectrophotometer. The entire system was from Perkin-Elmer Corp. (Norwalk, CT06856, USA). Analyses were performed on a Zeeman Longitudinal-effect flameless atomic absorption spectrophotometer, model Aanalyst 600, equipped with an AS 800 autosampler (ZEAAS). The wavelength of the hollow cathode lamp (Lumina® Lamp) was 309.3 nm and the slit width was 0.7 nm. The injected volume was 20μL on the platform. All results are based on peak area measurements.

2.5. Methodology

As Al is an ubiquitous element, great care was taken at all stages of preparation to avoid any risk of sample contamination. Details of procedure have been described by Pineau et al. [20] concerning sampling conditions for trace elements.

The in vitro study was conducted in a clean laboratory environment. Tissues digestion was conducted in fume cupboards and Al measurement in a laminar flow bench, both in a clean laboratory.

2.5.1. Skin test

The three cosmetic formulations were applied on each sample of normal skin. A "stick" formulation was also studied, under occlusion with Parafilm® "M", on the same skin samples artificially damaged by tape stripping (stripped skin). The tape strip, 2.5 cm wide 3 M Micropore™, was applied 10 times consecutively under a constant controlled pressure of 135 g/cm², using a 500 g brass weight device.

For each formulation, 10 diffusion cells (2 cells/donor, 5 donors for all tests) were prepared on the day of application of the cosmetic formulations. Control of skin sample quality was then performed, and the accuracy of the weighing procedures was verified with certified weights. The procedure consists in weighing a precise quantity of the tested formulation, in applying it to the surface of the skin, and converting the weight into a quantity per cm².The receptor fluid liquid is continuously maintained under magnetic stirring. For the "aerosol" base (38.5% of ACH): 4.56 ± 0.50 mg (amount exactly known) of the formulation (corresponding to 2.59 ± 0.28 mg/cm²) was applied on the entire surface of the epidermis circumscribed by the cylinder. For the "roll-on" emulsion (14.50% ACH): 8.01 ± 0.50 mg of the formulation (4.55 ± 0.28 mg/cm²) was applied and for the "stick" (21.2% ACH) involving normal skin: 5.46 ± 1.13 mg of the formulation (3.10 ± 0.64 mg/cm²) was applied. For the stripped skin: 6.35 ± 1.27 mg of the stick of the formulation (3.61 ± 0.72 mg/cm²) was applied.

All formulations were applied accurately using a plastic spatula. The skin surface was left open in contact with the atmosphere of the laboratory (non-occluded situation). For stripped skin, the skin surface was occluded with Parafilm® "M".

Contact between the skin and the formulations was maintained for 24 h after the application. Samples of the Receptor Fluid of the "dermal" compartment were collected at 6 h, 12 h and 24 h.

At the end of the 24-hour diffusion period, the surface of the skin and the glass donor part of the cell were washed to remove the excess of formulation as follows: three times 1 mL of 5% Brij 98 solution followed by wiping with two dry q-tips. The residual products in these cotton buds and liquids were extracted by contact during 48 h in 0.063% nitric acid solution. These solutions were named "washing liquids". After washing of the skin surface, the cells were dismantled, and the different skin layers were separated.

2.5.2. Tissue separation

The horny layer was first removed by tape stripping with 3 M Micropore™ adhesive tape applied on the skin for a few seconds under a constant controlled pressure (33 g/cm²) with a 100 g brass weight. Three strips were collected (S_1 to S_3). The epidermis and dermis were then separated mechanically, using forceps. They were put respectively in vials and in a dry-heat oven (+50 °C) for 24 h. Before being assayed, all samples (washing liquids, horny layer, epidermis, dermis and Receptor Fluids) were stored at −20 °C.

2.5.3. Aluminum measurement

2.5.3.1. Tissue preparation. Tissues (epidermis, dermis or strips) were digested in Sarstedt (Nümbrecht, Germany) tubes with 1 mL (v/v) HNO_3 65% (12 M) (Suprapur® Merck, that is to say 14 N) left overnight at room temperature. After 24 h, tissues were heated at +100 °C (DigiPrepApparatus) for 1 h, keep cooling and then completed with 4 mL ultrapure water containing 1% (v/v) Triton X-100 (Sigma). The clear and homogeneous solutions, which left no deposit, were analyzed by ZEAAS. Each selected tissue was digested according to the method of Van Ginkel et al. [21] as modified for dry-weight samples.

2.5.3.2. Preparation of working standards.

- Receptor Fluid and washing liquid preparation

An aluminum standard solution [1000 µg/L with 0.1 N HNO3 (v/v)] was prepared in Sarstedt tubes by appropriate dilution of the stock 1 g/L (Perkin-Elmer) in 2% (v/v) HNO_3 (Merck). From this standard solution, all working standards (20–40–80 µg/L and 50–100–200 µg/L) were made in Sarstedt tubes, by dilution with phosphate buffer saline, 0.1% (w/v) sodium azide, 5% (w/v) Brij 98 in ultrapure water. Aluminum measurements were performed in triplicate analysis (mean ± 1 SD).

- Epidermis, dermis and strips

Solutions were prepared in the different Sarstedt tubes.

2.5.3.3. Graphite furnace program. The basic settings used for the graphite furnace for Receptor Fluid, washing liquid, epidermis, dermis and strips are given in Guillard et al. [18]. The graphite furnace program was adapted to the "Stabilized Temperature Platform Furnace" (STPF) concept [22].

2.5.3.4. Quality assurance. Each Al calibration was monitored by controls carried out in the laboratory for each matrix (Receptor Fluid, washing solution, dermis, epidermis or strips) [18]. In addition, analytical performance was monitored by participation in two interlaboratory surveys, the Quebec Toxicology Center Interlaboratory Comparison Program [23] and the Worldwide Interlaboratory Aluminum Quality Control [24].

2.5.4. Statistical analysis

Analyses of variance were calculated on all the parameters for each formulation using the computer Software STATVIEW (Apple Macintosh, USA). Descriptive statistics were calculated on all the parameters for each formulation using the computer software Excel™ 2000 (Microsoft, USA). To detect any statistically aberrant value within the general "population" of the diffusion cells, all data obtained were tested by the Dixon test. The statistical comparison between all the formulations was done by an analysis of variance (ANOVA, Scheffe test). $p<0.05$ was considered statistically significant.

3. Results and discussion

3.1. Skin quality control

The thickness and TEWL of each skin sample were measured before the application of the formulations. The overall thickness of the normal human skin samples was 1406 ± 331 µm, and 1362 ± 302 µm for the stripped skin (Table 1). The mean TEWL of all the normal human skin samples was 4.33 ± 1.40 g/m².h. The usefulness of such a measurement in vitro of TEWL consists in its verifying, in comparison with physiological values, the quality of the skin biopsies used for the diffusion tests, and the efficacy of the tape stripping. This value is classical for the human healthy skin obtained from the abdomen. The mean TEWL of all the stripped human skin samples was 12.38 ± 5.12 g/m².h.

3.2. Aluminum chlorohydrate percutaneous absorption

With regards to the aerosol base (38.5% of ACH), the substance possibly absorbed through the skin was assayed over time. On account of the viscosity of the aerosol, it was not possible to deposit more than 4.56 ± 0.50 mg of aerosol (2.59 ± 0.28 mg/cm²), corresponding to 248.5 ± 27.09 µg/cm² of Al.

The quantities of deposited Al expressed in mg/cm² d'ACH and in µg/cm² of Al are shown in Table 1. Analysis of the results reveals highly significant differences between the quantities of Al deposited in the form of aerosol base, roll-on emulsion or stick ($p<0.0001$). By contrast, the difference between stick normal skin and stick stripped skin is not significant.

The percutaneous absorption of Al may be expressed in µg/cm² as recovered in horny layer, viable epidermis, dermis and Receptor Fluid (Table 2). Analysis of the results involving the viable epidermis shows relative homogeneity as concerns the quantities of Al retained on normal skin, irrespective of the formulation. On the other hand, with stick formulation, stripped skin retains more Al, a finding that entails highly significant differences between tests on normal skin and tests on stripped skin ($p<0.005$).

As concerns the dermis (Table 2), the same observation holds true with $p<0.0001$, even for amounts of Al that are 2 to 5 times lower. As for the Receptor Fluid taken after 24 h of contact, the measured amounts of Al are negligible (<0.1 µg/cm²) and quite close to the figures recorded with blank samples. With the estimated level of absorption of Al from ACH of 0.012% (about 1/10.000) as described by Flarend et al. [15], we can evaluate Al concentration in Receptor Fluid at about 7.3 µg/L for the aerosol. In the framework of our experimental conditions for roll-on and stick in normal skin, the theoretical results involving the two formulations are close to 4.8 µg/L. Lastly, as concerns the stick on stripped skin, the expected concentration in the Receptor Fluid comes to 5.5 µg/L. Such low Al concentrations come close to constituting an unusually accurate analytical performance (LOD: 1 µg/L; LOQ: 3 µg/L).

The amount of Al observed in the stripped skin at the levels of both the viable epidermis and the dermis underscores quantities significantly higher than those measured in the same tissues in the normal skin (Table 2). These observations justify a targeted analysis of the horny layer, which is divided into three strips (S_1 to S_3) (Table 3). Irrespective of the cosmetic formulation (normal skin), the Al quantities (µg/cm²) in the horny layer invariably exceed those observed in the epidermis. Moreover, horny layer S_1 systematically displays a maximally high amount of Al. Sequential analysis of

Table 1
Quality control of the diffusion cells [mean ± SD, n = 10 cells/formulation (2 cells/donor, 5 donors)].

	Sample thickness (μm)	TEWL (g/m².h)	ACH concentration (%)	Aluminum concentration (Al^{3+}) (%)	Formulation applied (mg/cm²)	Amount of aluminum (μg/cm²)
«Aerosol» base Normal Skin	1424 ± 438	4.42 ± 1.16	38.5	9.59	2.59 ± 0.28	248.47 ± 27.09[a,b]
«Roll-on» emulsion Normal Skin	1424 ± 363	4.14 ± 1.36	14.50	3.61	4.55 ± 0.28	164.30 ± 10.21
Stick Normal skin	1357 ± 250	4.72 ± 1.84	21.20	5.28	3.10 ± 0.64	163.80 ± 33.77
Mean values on 3 formulations Normal skin	1402 ± 348	4.43 ± 1.45			3.41 ± 0.94	192.19 ± 47.46
Blank samples Normal skin	1433 ± 236	3.76 ± 0.96		<LOD	<LOD	<LOD
Mean values on 3 formulations Normal skin + blanks	1406 ± 331	4.33 ± 1.40				
Stick Stripped skin	1341 ± 299	13.69 ± 5.38		5.28	3.61 ± 0.72	190.50 ± 37.95
Blank samples Stripped skin	1403 ± 339	9.76 ± 3.70		<LOD	<LOD	<LOD
Mean values on stick formulation Stripped skin + blanks	1362 ± 302	12.38 ± 5.12				

[a] p<0.0001, compared to «roll-on» emulsion.
[b] p<0.0001, compared to stick normal skin.

each formulation of the horny layer (S_1, Table 2) compared to the dermis (Table 3) indicates a sizable and steady diminution. Observation has shown a decrease of 6.6 (2.70 vs. 0.41 μg/cm²) for the stick, and 7.9 and 8.6 for aerosol and roll-on respectively. As concerns the tested formulations, the single significant difference ($p<0.05$) involves the antiperspirant stick and the roll-on emulsion for the horny layer (S_1) as well as the full horny layer (S).

For the same formulation (stick), the total amount of Al in normal skin (6.14 μg/cm²) is lower than on the stripped skin (11.43 μg/cm²) (NS), a finding which indicates that the presence of the horny layer diminishes the quantity of this metal immediately diffusible at the levels of the epidermis and the dermis.

It should be noted that in this study, we have worked on biological samples coming from a relatively heterogeneous population (i.e., age, range of donors was 29–52 years), a factor that likely explains the large-scale scattering. The analytical results for blank samples (Table 3) remained very low and homogeneous, hence ruling out the possibility that the widespread scattering observed on skin tests with a Franz™ cell was due to contamination.

Subsequent to consideration of these results, it is important to discuss Al's transfer and kinetics in human skin. As is illustrated in Table 3, irrespective of the formulation, the horny layer showed increased Al concentration. Physiologically, this metal may be eliminated through desquamation, and it is possible to delay its transfer towards the epidermis and the dermis. On the other hand, alteration of the horny layer and, all the more so, stripping, suppresses the resultant protective screen and impedes the delay. At the level of the epidermis and the dermis, the quantity at hand may precipitate inside the eccrine sweat glands to produce insoluble aluminum hydroxide [3], or else be propelled into the circulatory system and diffused throughout the organism.

As is shown in Table 2, analysis of total skin absorption corresponding to the overall quantity of Al/cm² measured in the viable epidermis, dermis and Receptor Fluid confirms the existence of a significant difference ($p<0.005$) between stripped skin (11.50 μg/cm²) and normal skin (1.81 μg/cm²) for the stick. The blanks remain very low (<0.32 μg/cm²). Variably rapid Al transfer entails delayed diffusion. As a result, the kinetics of Al transfer from percutaneous antiperspirant application towards the blood pool is conditioned by a large number of parameters that may be cosmetic-dependent (pH, pKa, formulation, size-grading or granulometry) and tissue-dependent (thickness, integrity, vascularization, temperature) [25,26].

In this study, using human skin in Franz™ diffusion cell, the fact that transfer kinetics cannot be comprehensively explored may be explained by the short duration of the experiment (24 h) and the

Table 2
Total amounts of aluminum (μg/cm²) recovered [mean ± SD, n = 10 cells/formulation (2 cells/donor, 5 donors)].

	Viable epidermis (E) [a]	Dermis (D) [a]	Receptor Fluid (RF) 24 h [a]	Total skin absorption (E + D + RF)[a]
	μg/cm²	μg/cm²	μg/cm²	μg/cm²
Normal skin				
«Aerosol» base	1.49 ± 2.09	0.28 ± 0.18	0.07 ± 0.01	1.84 ± 2.23
«Roll-on» emulsion	0.30 ± 0.36	0.16 ± 0.05	0.07 ± 0.01	0.53 ± 0.38
Stick	1.30 ± 1.25	0.41 ± 0.27[†]	0.10 ± 0.05	1.81 ± 1.45
Blank samples (n = 5)	0.022 ± 0.004	0.13 ± 0.04	0.082 ± 0.006	0.23 ± 0.04
Stripped skin				
Stick	9.42 ± 7.82[‡‡]	2.01 ± 1.14[‡‡‡]	0.07 ± 0.03[‡]	11.50 ± 8.90[‡‡]
Blank samples (n = 5)	0.05 ± 0.02	0.18 ± 0.05	0.09 ± 0.01	0.32 ± 0.07

[‡]p<0.01, [‡‡]p<0.005, [‡‡‡]p<0.0001 compared to stick normal skin.
[a] In all the samples (n = 10 diffusion cells/formulation). Aluminum amounts were detected and quantified.

Table 3
Total amounts of aluminum (μg/cm²) recovered [mean ± SD, n = 10 cells/formulation (2 cells/donor, 5 donors)].

	Horny layer (S1) [a]	Horny layer (S2–S3) [a]	Full horny layer (S) [a]	Total skin quantity (S + viable epidermis + dermis)
	μg/cm²	μg/cm²	μg/cm²	μg/cm²
Normal skin				
«Aerosol» base	2.20 ± 2.14	1.78 ± 1.76	3.98 ± 3.89	5.75 ± 6.17
«Roll-on» emulsion	1.36 ± 1.24	0.88 ± 0.65	2.24 ± 1.87	2.69 ± 2.27
Stick	2.70 ± 1.15*	1.73 ± 0.71	4.43 ± 1.79*	6.14 ± 3.31*
Blank samples (n = 5)	0.000 ± 0.000	0.044 ± 0.017	0.044 ± 0.017	0.20 ± 0.06
Stripped skin				
Stick				11.43 ± 9.00
Blank samples (n = 5)				0.32 ± 0.08

[a] In all the samples (n = 10 diffusion cells/formulation). Aluminum amounts were detected and quantified.
* p<0.05 compared to «roll-on» emulsion normal skin.

absence of vascularization, even following verification of tissue integrity through measurement of TEWL (Table 1).

Moreover, simple percutaneous application of antiperspirants containing Al in conjunction with experimentation involving Franz™ diffusion cell undoubtedly underestimates the transfer capacity of Al. Indeed, the clinical case described by Guillard et al. [16] in a woman using ACH antiperspirant cream on each shaved underarm every morning for 4 years showed hyperaluminemia at about 4 μM (normal values in plasma < 0.37 μM) and urinary Al concentration of 1.71 μM/24 h (normal values in urine < 1.10 μM/24 h). The woman's hyperaluminemia may be explained by the existence of a diminished horny layer resulting from regularly performed shaving of the ACH application area and from constantly repeated applications of this metal (all told, approximately 157 g) over a period of 4 years. Using optical coherence tomography in a woman, Turner et al. [27] have already shown that underarm shaving removes stratum corneum, increasing permeability to cosmetics.

The use of Al salt-based antiperspirant remains widespread all over the world and more particularly in the USA (>90% of the population) [12]. In addition to large-scale transcutaneous Al penetration and suspected neuro-degenerative pathologies [6–8], a number of authors (Darbre [10], Exley et al. [11] and McGrath [12]) have mentioned a possible connection between increased antiperspirant use and heightened incidence of breast and prostate cancer. Given these observations, the French Ministry of Health has been applying the precautionary principle by discussing with manufacturers about how to end use of this metal in antiperspirants.

Abbreviations

ACH	aluminum chlorohydrate
LOD	limit of detection
LOQ	limit of quantification
TEWL	Trans Epidermal Water Loss
ZEAAS	Zeeman Electrothermal Atomic Absorption Spectrophotometry

Declaration of interest

The authors declare no conflict of interest.

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