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Aluminum salts in antiperspirants and breast cancer risk

A critical review of the literature

SCAHT report for FSVO

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Executive Summary

The question of a potential role of aluminium in the etiology of breast cancer is not a newly emerging issue, but a 16 years old debate that has drawn considerable media coverage and public attention over the years, and that has been largely discussed by risk assessment bodies worldwide. In 2001, it was hypothesized that aluminium-containing antiperspirants applied in the armpits may result in a deposition of aluminium in breast tissues, and may promote the development of breast cancer (Darbre, 2001). Since then, various scientific groups have been working on this hypothesis (e.g. Darbre 2003, 2016; Exley et al., 2007; Mandriota et al., 2016; Mannello et al., 2009, 2013; McGrath, 2003; Pineau et al., 2014; Sappino et al., 2012). Recently, it was reported that aluminium chloride could promote tumor development in normal breast tissue grafted into immunodeficient mice (Mandriota et al., 2016). However, the experimental and epidemiological data are not conclusive. While new experimental data has been gained in the last five years, giving rise periodically to a renewed interest into this question, there is a need for a closer look at the evidence. The Swiss Centre for Applied Human Toxicology (SCAHT) was requested by the Swiss Federal Food Safety and Veterinary Office (FSVO) to conduct a critical review of the literature to clarify a potential link between exposure to aluminium salts in antiperspirants with breast cancer.

This report summarizes the current state of literature elucidating this question, with a focus on aluminium hazards in term of skin irritation and sensitization, genotoxicity and carcinogenicity, and on aluminium exposure from application of antiperspirants to intact or compromised skin. It reviews the evidence for aluminium as a breast carcinogen from *in vitro*, *in vivo*, and human epidemiological studies, and discusses these findings in a weight-of-evidence approach.

SCAHT considers that the quality of the available data is insufficient, and provides no convincing evidence to support a causal association between the use of aluminium-containing antiperspirants and breast cancer. Our conclusions concur with the evaluations of major regulatory and scientific bodies (Afssaps, 2011; AGES, 2016; BfR, 2014; SCCS, 2014; SHC, 2015) and peer-reviewed studies (Namer et al., 2008; Willhite et al., 2014) which did not see a link between cancer and oral/dermal exposure to aluminium. Nonetheless, further data are needed to definitely exclude a role of aluminium in the aetiology of breast cancer. It remains to be established:

- *Whether the presence of aluminium in the breast has any adverse effect;*
- *Whether there is a disproportionate incidence of breast cancer in the upper outer breast quadrant;*
- *How much of the aluminium measured in breast tissue could have originated from antiperspirant use;*
- *To which extent aluminium from antiperspirants contributes to the exposure of the breast to aluminium in comparison to other sources of exposure (such as the diet or pharmaceuticals).*

SCAHT recommends in particular more research to be conducted in the following areas:

- *Robust mechanistic studies with clear working hypotheses and consideration of potential sources of bias;*
- *Additional in vitro testing in CHO/HPRT assays (e.g. OECD Test Guideline 476) to better characterize potential mutagenicity;*
- *Data on dermal permeation rates of aluminium salts in human skin (preferably with radiolabelled ^{26}Al);*
- *Data on the potential for accumulation of dermal and systemic aluminium after dermal exposure;*
- *Data on potential exposure to breast tissue via lymph and on aluminium levels in axillary lymph nodes.*

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Disclaimer

The present report reflects the views of the Swiss Centre for Applied Human Toxicology (SCAHT) only and does not necessarily reflect the official position of the Swiss Federal Food Safety and Veterinary Office (FSVO).

Abbreviations

ACL	Al chloride
ACH	Al chloride hexahydrate
Afssaps	French Agency for the Safety of Health Products (superseded by ANSM in 2012)
Al	Aluminium (spelled "aluminum" in U.S. English)
AGES	Austrian Agency for Health and Food Safety
ANSM	French National Agency for Medicines and Health Products (superseded Afssaps in 2012)
AZAG	Al-Zr chlorohydrate glycine complex
bw	Body weight
BfR	German Federal Institute for Risk Assessment
CHO	Chinese hamster ovary cells
EFSA	European Food Safety Authority
EMT	Epithelial-to-mesenchymal transition
ER	Oestrogen receptor
FAO	Food and Agriculture Organization of the United Nations
FSVO	Swiss Federal Food Safety and Veterinary Office
GPMT	Guinea pig maximisation test
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
ICD	International Classification of Disease
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LIQ	Lower inner quadrant of the breast
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOQ	Lower outer quadrant of the breast
MMP	Matrix metalloproteinase
NOAEL	No Observed Adverse Effect Level
OECD	Organization for Economic Cooperation and Development
PTWI	Provisional Tolerable Weekly Intake
ROS	Reactive oxygen species
SCCS	EU Scientific Committee on Consumer Safety
SED	Systemic Exposure Dose
SEER	U.S. National Cancer Institute's Surveillance Epidemiology and End Results Program
SHC	Belgian Superior Health Council
SNP	Single-nucleotide polymorphism
SSA	Skin Surface Area
TJ	Tight junction
TWI	Tolerable Weekly Intake
UIQ	Upper inner quadrant of the breast
UOQ	Upper outer quadrant of the breast
WHO	World Health Organization
yr	Year

1 Background and scope of the report

In 2001, it was hypothesized that aluminium-containing antiperspirants applied in the armpits ("axillary vault") may result in deposition of aluminium (Al) in breast tissues, and may promote the development of breast cancer (Darbre, 2001). Since then, various scientific groups have been working on this hypothesis (e.g. Darbre 2003, 2016; Mandriota et al., 2016; Mannello et al., 2013; McGrath, 2003; Pineau et al., 2014; Sappino et al., 2012). Some studies have reported higher Al concentrations in breast tissue of women with breast cancer than those without (Exley et al., 2007; Mannello et al., 2009, 2011). Several *in vitro* studies on human breast cancer cell lines have suggested that Al could promote the initiation, progression and dissemination of breast cancer (Bakir and Darbre, 2015; Darbre et al., 2013b; Sappino et al., 2012). Recently, it was reported that Al chloride (ACL) could promote tumor development in normal breast tissue of immunodeficient mice (Mandriota et al., 2016). However, the experimental and epidemiological data are not conclusive.

The question of a potential role of Al in the etiology of breast cancer has drawn considerable media coverage and public attention over the years, and has been largely discussed as well by risk assessment bodies worldwide. While new experimental data have been gained in particular in the more recent years, leading periodically to a renewed interest into this question, there is a need for a closer look at the available evidence.

SCAHT was therefore requested by the FSVO to conduct a comprehensive review of the literature to clarify a potential link between exposure to Al salts in antiperspirants with breast cancer.

In particular, the following topics are to be addressed:

i) *Human exposure to aluminium via skin*

ii) *Dermal absorption via intact versus razor-damaged skin*

iii) *Cosmetic products which contain aluminium*

iv) *Evidence for/against aluminium as a breast carcinogen from animal and epidemiology studies*

v) *Other tumor types which may be associated with aluminium exposure*

vi) *Hypothesised mechanisms if causality is suspected*

vii) *Thresholds for hypothesised mechanisms*

viii) *Possible other co-factors for observed effects*

ix) *Weight of evidence for aluminium as a breast carcinogen*

x) *Position of other competent authorities (SCCS, EFSA, JECFA, BfR, Afssaps, etc.), and by personal contact and direct consultation, of the Sappino group (University of Geneva; Mandriota et al., 2016)*

1.1 Structure of the report

The present report first presents brief background information on the anatomy and biology of axillary skin and breast, as well as a review of relevant physicochemical properties of the Al salts used in antiperspirants, their mode of action, and their occurrence in cosmetics.

The current state of knowledge on relevant hazards of Al in term of skin irritation and sensitization, genotoxicity and carcinogenicity is summarized, and the evidence for Al exposure from application of antiperspirants to intact or compromised skin is reviewed.

We then review the evidence for Al as a breast carcinogen from *in vitro*, *in vivo*, and human epidemiological studies, and discuss these findings in term of consistency and biological relevance, in a weight-of-evidence approach.

Last, we summarize the position of various European regulatory authorities, and formulate our conclusions and recommendations.

1.2 Methodology of the literature search

Our review of the peer-reviewed literature is based on PubMed and Web of Science database searches, to capture:

- Original mechanistic, *in vitro* and *in vivo* animal or human studies on dermal permeation and absorption of aluminium compounds;
- Original mechanistic, *in vitro* and *in vivo* animal or human studies on breast cancer following dermal exposure to aluminium compounds, as well as other type of exposure and cancer;
- Original epidemiological human studies on risk factors for breast cancer;
- Reviews as supporting information to better frame the problem, understand the anatomy, biology and physiology of the axillary skin and the breast, the biology and epidemiology of breast cancer, as well as knowledge gaps and areas of uncertainties;

Data collection strategy used the methodology of a Systematic Review which begins with the definition of the question(s), followed by the pre-determination of assessment criteria for data identification, screening and selection (refer to **Appendix I**).

- The primary search was conducted in January 2017 in PubMed and Web of Science according to predefined identification criteria. The search results from all the databases were pooled, and duplicates removed. Articles were screened for title and abstract relevance, full text availability, and were then assessed further for eligibility.
- Data were organized to separate reviews from original articles, which are further listed into several categories (mechanistic, *in vitro*, *in vivo*, human studies). Other sources were also considered as part of data selection process, such as additional articles that may have been missed by the initial electronic search through cross-referencing in selected papers or technical reports, direct search in relevant journals or online sources.
- Justification for included versus excluded articles were provided at each step of the data identification, screening and selection procedure to guarantee a reproducible and transparent literature-search strategy.

In addition, a targeted strategy was applied to retrieve regulatory documents, technical reports and scientific opinions on the toxicity profile of aluminium compounds, their use in antiperspirants and cosmetic products, risk assessments following their topical use, and competent authorities' positions regarding a potential association between Al-containing antiperspirants and the risk of breast cancer in women.

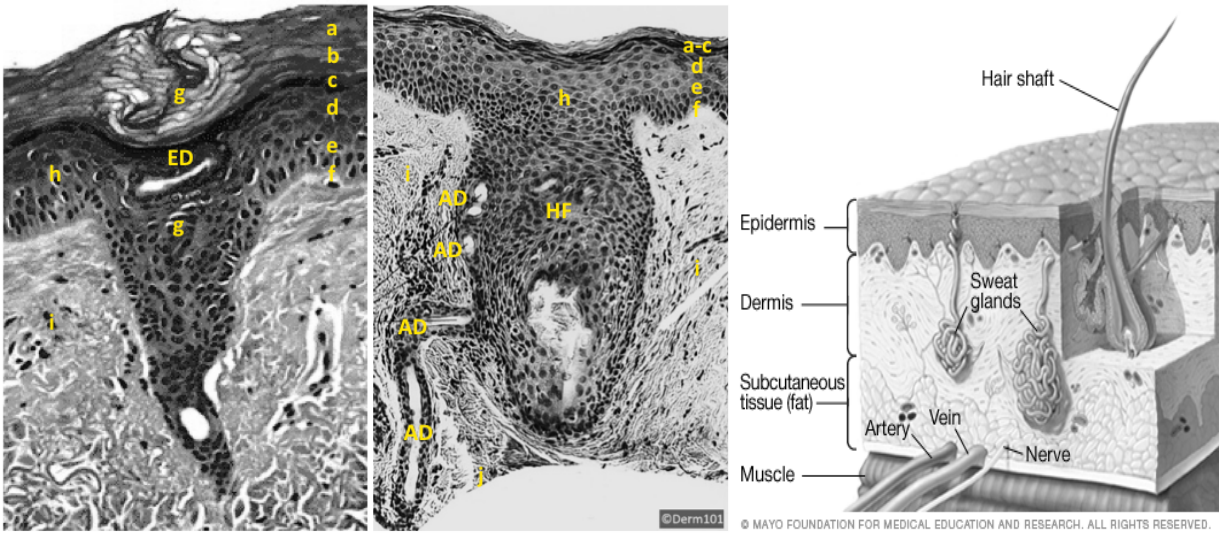
- ***Nota Bene**: SCAHT gives preference to public-access documents from vetted sources such as regulatory agencies and other government bodies or related scientific organizations, which benefit from an internal peer-review process by panels of experts, such as the U.S. Agency for Toxic Substances and Disease Registry (ATSDR), the European Food Safety Authority (EFSA), and the World Health Organization (WHO), as well as the EU non-food Scientific Committees (SCCS, SCHEER) and major EU Member States Health Authorities such as the Dutch National Institute for Public Health and the Environment (RIVM), the German Institute for Risk Assessment (BfR), etc.*

2 Background information on axillary skin and the breast

2.1 Axillary skin and sweat glands

The axillary skin contains two types of sweat glands (**Figure 1**): the eccrine glands (thermoregulation) and the apocrine glands (body odour/pheromones production). A third type of sweat gland (termed 'apoecrine', with mixed characteristics from both the eccrine and apocrine types) has been described in the literature but its existence is not well defined and somehow controversial (Bovell et al., 2007; Lonsdale-Eccles et al., 2003; Wilke et al., 2006). Eccrine sweat glands outnumber apocrine sweat glands about 25'000 in each axilla. Eccrine glands are typically located in the mid-dermis; eccrine ducts are long, forming a spiral in the intraepidermal portion of the sweat duct (i.e. the *acrosyringium*) that opens directly onto the skin surface. Eccrine sweat is abundantly and continuously produced as an odourless aqueous solution containing mainly electrolytes and small organic acids. Apocrine glands are located in the deeper dermis. The apocrine sweat is formed intermittently inside large acini in the apocrine secretory coil, and is excreted directly into adjacent hair follicles before reaching the skin surface; it is odourless, slightly viscous and milky, and is rich in proteins, steroids, lipids, as well as organic acids and electrolytes (Lonsdale-Eccles et al., 2003). Axillary sweat has a normal pH of 4.5-7.4, with increased sweating rates associated with higher pH values (Lansdown, 2011). The characteristic human axillary odour results from the biotransformation of odourless natural secretions from the apocrine glands into volatile odorous molecules by resident skin microflora, mainly *Staphylococcus*, *Corynebacterium*, *Propionibacterium* and *Micrococcus* spp. (James et al., 2012).

Figure 1: Sweat gland histology



*Histological sections of axillary skin showing an eccrine sweat duct (**left**) and apocrine sweat duct (**right**). (a) stratum corneum; (b) stratum lucidum; (c) stratum granulosum; (d) stratum spinosum; (e) stratum basale; (f) basement membrane; (g) acrosyringium; (h) epidermis; (i) dermis; (j) hypodermis; (AD) apocrine duct; (ED) eccrine duct; (HF) hair follicle.*

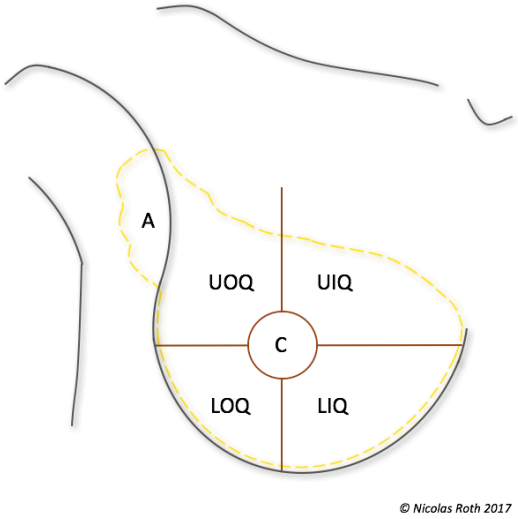
2.2 Breast anatomy

2.2.1 Major features of the breast

The breast lies in the superficial fascia (i.e. in the hypodermis) of the pectoral region, and is a modified sweat gland of the apocrine type, constituted of the mammary gland, which is subdivided in ca. 15-20 lobes, lactiferous ducts and lobules. The breast is divided into five major areas (UOQ, UIQ, C, LOQ, LIQ in **Figure 2**). The mammary tissue frequently extends beyond the apparent outline of the breast into the axilla as a narrow process, variable in size, called the axillary tail (or "*axillary tail of Spence*"; (A) in **Figure 2**) (Ampil et al., 2012; Macéa and Fragnani,

2006). The axillary tail is in direct contact with anterior axillary lymph nodes, and can be even palpable in some women and mistaken for enlarged axillary lymph nodes. However, breast cancer in the axillary tail appears to be rare (< 0.1%); carcinoma arising from this particular area is considered to be separate from that originating from the upper outer quadrant (UOQ) of the breast[1] (Ampil et al., 2012).

Figure 2: Quadrants of the breast and axillary tail



- *Upper outer quadrant (UOQ)*
- *Upper inner quadrant (UIQ)*
- *Central quadrant (C), further divided into nipple and areola*
- *Lower inner quadrant (LIQ)*
- *Lower outer quadrant (LOQ)*
- *Axillary tail (A)*

Source: SCAHT, University of Basel, 2017.

2.2.2 Lymphatic circulation and lymphatic drainage of the breast

The breast is drained by the lymphatic circulation, which forms a widespread network of lymphatic vessels that run in the deeper layer of the superficial fascia in close association with blood vessels. The skin over the breast is drained via superficial lymphatic capillaries, which ramify subcutaneously and converge towards the axillary lymph nodes (Krontiras and Bland, 2006; Macéa and Fregnani, 2006). The main lymphatic drainage of the breast occurs within the substance of the gland and not through the vessels on the superficial or deep surface (i.e. the dermis or the hypodermis). The main collecting trunks run laterally as they pass through the axillary fascia in the substance of the axillary tail (Bland et al., 2009). The major features of the lymphatic circulation in the breast are presented in **Figure 3**. Both the axillary and the internal thoracic lymph nodes receive lymph from all the breast quadrants, however the lateral quadrants have a certain tendency to drain into the former, and the medial quadrants into the latter. Since the axillary lymph nodes drain most of the breast (> 75%), they are also the main location for metastasis (Macéa and Fregnani, 2006).

As a rule, the lymph flows away from the tissues through a complex system of lymphatic capillaries into draining lymph nodes[2]. Mechanisms exist to prevent the absorbed lymph from leaking back into the insterstitial fluid or flowing back from lymphatic vessels thanks to a system of one-way valves (Murphy et al., 2008). However,

[1] Breast cancers in the UOQ and in the axillary tail are coded as SEER 50.4 and 50.6, respectively by the U.S. National Cancer Institute's Surveillance Epidemiology and End Results (SEER) Program (Ampil et al., 2012). The same coding is used by the WHO International Classification of Disease (ICD-10).

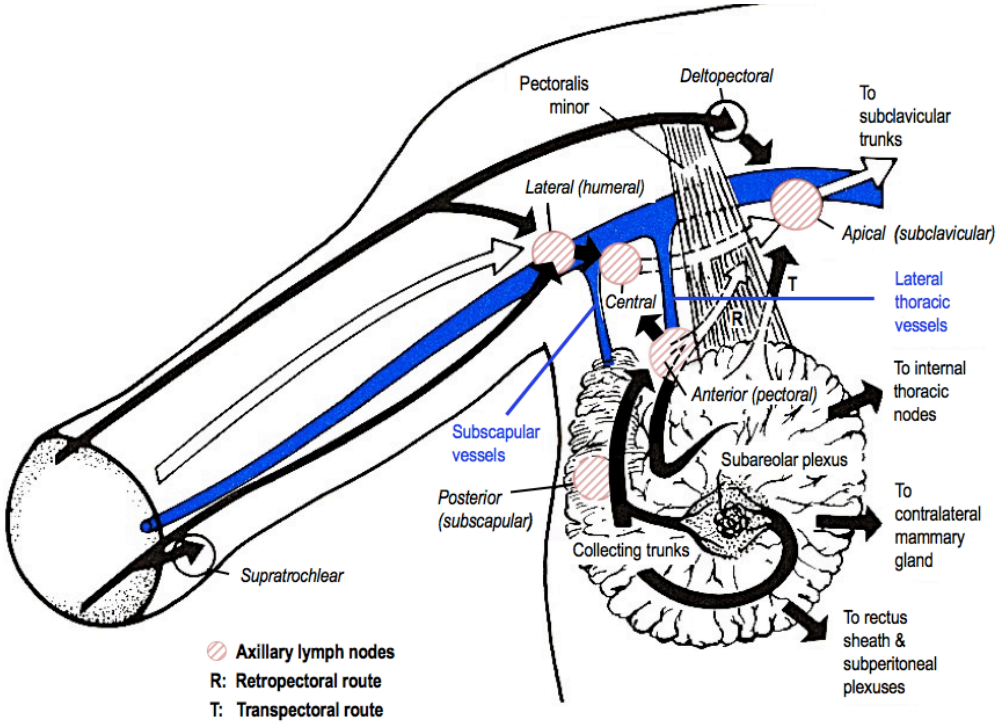
[2] The superficial, valveless and highly permeable lymphatic capillaries collect the extracellular fluid from the neighbouring tissues, and drain into larger contractile lymphatic vessels propelling the unfiltered lymph into larger afferent lymphatics, which drain into receiving lymph nodes. The filtered lymph leaves the lymph node via efferent lymphatic vessels, which may directly drain into the right or thoracic ducts, or may empty into another lymph node as its afferent lymphatic vessels (Murphy et al., 2008).

McGrath (2003) argued that "*unique valveless and bidirectional lymphatic flow exists between the breast and axillae. Shared is a rich anastomose*[3]*, which could easily provide direct and chronic exposure of breast tissue to underarm application of chemicals". [SCAHT notes: this view was confirmed by P.A. Sappino, personal communication].* These opinions are somehow difficult to reconcile with textbook knowledge:

> *"The lymphatic flow is predominantly unidirectional, except in subareolar and central regions of the breast, or in circumstances, in which physiological lymphatic obstruction occurs as a consequence of neoplastic, inflammatory, or developmental processes that initiate a reversal of flow with bidirectional egress of lymph."* (Bland et al., 2009).
>
> *"Lymphatic flow is typically unidirectional, except in the pathological state, and has preferential flow from the periphery toward larger collecting ducts."* (Krontiras and Bland, 2006).

- ***SCAHT notes: in the context of a normal, healthy breast there is a priori no reason to think that the lymphatic circulation in the axillary vault would be bidirectional. Provided that Al could penetrate in the epidermis, it would be resorbed along with the lymph via the skin superficial plexus and deep plexus, respectively, which drain into the axillary lymph nodes. It remains to be determined if and to which extent Al could be transported via the lymph from the axillary vault to the surrounding breast tissues.***

Figure 3: Lymphatic drainage of the breast



Five groups of axillary lymph nodes, distributed in the soft conjunctive tissue of the axillary cavity, are classically described in the breast: the anterior, posterior, lateral, central and apical lymph nodes (Macéa and Fregnani, 2006).

Source: Modified from: Basic Human Anatomy: A Regional Study of Human Structure. Dartmouth Medical School, https://www.dartmouth.edu/~humananatomy/figures/chapter_7/7-2.HTM. © O'Rahilly, 2009. Reproduced with permission from R.S. Swenson.

[3] Lymphatic capillaries ramify into four plexi: (i) two superficial plexi located in the dermis (cutaneous plexus) and in the superficial subcutaneous region (subcutaneous plexus); and (ii) two deep plexi, one located in the pectoralis major muscle fascia (fascial plexus), and the other in the mammary gland, including lobes and ducts (subareolar plexus), which plays a key role by draining the breast via collecting trunks into the axillary nodes (Fregnani and Macéa, 2009).

3 Aluminium salts in antiperspirants: chemistry, function and use

Aluminium (Al) is a ubiquitous, naturally-occurring element in the environment. Environmental Al may also arise through anthropogenic activities such as mining and industrial uses. It is used in a wide range of manufacturing products such as food contact materials, paper, dyes, pigments, paints, glass, fuels, textiles, cookware, cosmetics and pharmaceuticals; it is also used in water purification and oil refining processes (ATSDR, 2008).

3.1 Relevant physicochemical properties of Al salts

Aluminium (Al) is the third most abundant element on/in our planet, and is the most abundant metal in the earth's crust, mainly in the form of silicates, oxides and hydroxides, combined with other elements such as sodium and fluorine and as complexes with organic matter. Aluminium is a non-redox active metal, and has the highest charge-to-ionic radius ratio of all elements. It has very high chemical reactivity; the formation of the [Al-O] bond is recognized as one of the strongest in nature (a thin surface layer of aluminium oxide forms when the bare metal is exposed to air, effectively preventing further oxidation). Therefore, it is rarely found as the free Al^{3+} cation in nature, but in bound form, both inorganic (oxides, hydroxides, chloride, nitrate, sulfate, silicate) and organic (e.g. citrate or other carboxylic acids). In biological systems, Al binds strongly to organic acids and proteins, in particular transferrin (90%), Ca^{2+}-binding S100 proteins, sulfhydryls, nitrogen and carboxylates groups of keratin, serum proteins, etc. (Lansdown, 2011). Kinetic and structural studies show that the chemical and structural properties of ligands can be substantially altered by binding of Al. Free Al^{3+} can also displace essential metal protein cofactors, such as Mg^{2+}, Zn^{2+} or Ca^{2+}, and alter metalloproteins function (Lansdown, 2011).

The solubility of Al salts is strongly dependent on pH. Many are water-insoluble at neutral pH (roughly 6-8), but their solubility is increased at lower or higher pH. Aqueous Al chloride (ACL) has a pH < 4, Al chlorohydrate (ACH) pH = 4.38. Generally, decreases in solution pH to less than 5.5 result in exponential increases in Al^{3+} concentrations (McGrath, 2003). Aqueous Al hydroxide gel has a pH of ca. 6, at which the gel is positively charged and will absorb to the negative charge of most proteins (Guy et al., 1999).

Various Al salts have been used as active ingredients in antiperspirants (see **Appendix 2**), mainly chloride salts. These salts are unstable in the presence of an oxygen source such as water, and hydrolyse more or less slowly to form Al hydroxide, with release of hydrochloric acid (HCl). The chemical and biological reactivity of the ACL salts is largely driven by the formation of the Al-O bond, which is the thermodynamic driving force of this reaction. These properties govern the skin irritating properties of Al salts (see **section 4.2**). Three main ACL salts are used in antiperspirants:

- **Aluminium chloride** (ACL). Anhydrous $AlCl_3$ (*CAS 7446-70-0)* is very corrosive because the exothermic reaction with water releases HCl. The hexahydrate form $[Al(H_2O)_6^{(3+)} \cdot 3Cl^-]$ (*CAS 7784-13-6*) is less corrosive but ionizes in aqueous solution in $[Al(H_2O)_6]^{3+}$, a weak acid which eventually dissociates at neutral pH into stable Al hydroxides [$Al(OH)_3$, $Al(OH)^{4-}$] by releasing HCl.
- **Aluminium chlorohydrate** (ACH) *(CAS 12042-91-0)* and Al sesquichlorohydrate *(CAS 11089-92-2)*, of general formula $[Al_nCl_{(3n-m)}(OH)_m]$, are partially hydrolyzed, and are significantly less corrosive. Both compounds tend to form polynuclear aggregates positively charged which contribute to markedly lower their reactivity (and the reaction exothermicity); eventually they will also fully hydrolyze and release HCl. The most representative ACH compound is $[Al_2Cl(OH)_5]$ (*CAS 12042-91-0*).
- **Aluminium-Zirconium chlorohydrate glycine complex** (AZAG) contains a mixture of monomeric and polymeric zirconium (Zr^{4+}) and Al^{3+} complexes with hydroxide, chloride and glycine. Several representatives exist such as Al-Zr Tetrachlorohydrex Gly (*CAS 134910-86-4*), Al-Zr Pentachlorohydrex Gly (*CAS 125913-22-6*), or Al-Zr Octachlorohydrex Gly (*CAS 174514-58-0*). These salts form colloidal hydroxide gels in contact with sweat without releasing Zr, which is known to cause skin granuloma in hypersensitive individuals (Guy et al., 1999). Bretschneider et al. (1977) showed that the effectiveness of

Al-Zr complexes was independent of Al:Zr ratio (over a range: 0.5:1 to 6:1), and was less influenced by the chemistry of the cosmetic adjuvants/formulants.

3.2 Mode of action of Al-containing antiperspirants

Antiperspirants should not be confused with deodorants, as they exhibit different mechanisms of action.

- Antiperspirants aim at reducing the amount of sweat/perspiration, which in turn contributes to alter the microenvironment in which skin bacteria proliferate, and hence to control indirectly body odour.
- Deodorants directly target the odour production, either by reducing or masking unpleasant body odours, through the action of antibacterial agents (e.g. triclosan, or quaternary ammonium salts such as benzalkonium chloride). Their typical composition consists of perfume, antibacterial substances and substances that neutralize unpleasant odour, or a combination of these ingredients (SCCS, 2014).

⇒ **While antiperspirant formulations often possess some deodorant properties, deodorants never exhibit an antiperspirant action** (Benohanian, 2001).

Following dermal application, Al salts dissolve on the skin surface and to some extent diffuse down the skin appendages (or 'shunts', i.e. hair follicles and eccrine/apocrine sweat ducts), which offer low-resistance diffusion pathways for water-soluble electrolytes such as metals. Interestingly, salts of Zr, Zn, Cr, Fe, Ti, In, Ga, Sn, Mg, Cu, Be, and Sc have also shown antiperspirant activity, albeit less than that of Al compounds. With the exception of Zr, these other compounds are not used in consumer products (Guy et al., 1999).

The widely accepted theory is that Al hydrolyses and forms an obstructive plug in the outer part of the sweat ducts (i.e. the *acrosyringium*) due to amorphous Al hydroxide gel formation or protein precipitation, or both, that blocks or reduces the sweat flux reaching the skin surface (a mechanism referred to as 'ductal closure' or 'emphraxis') (Relier and Luedders, 1977; Lansdown, 2011). Hölzle and Kligman (1979) showed that since there is no precipitation of ACL in eccrine sweat duct at pH 5-6, tissue components may contribute to the formation of the plug. Acidic Al salts are gradually neutralized during their diffusion down the sweat duct in the presence of proteins or glycoproteins of the more alkaline sweat, or by interaction with proteins from the keratinized luminal epithelial cells, or the cellular debris from their sloughing off. The depth and extent of ductal obstruction are determined by the length of application, the concentration and the nature of antiperspirant applied to the skin. Epidermal regeneration is believed to remove the obstructive plug (Guy et al., 1999).

3.2.1 Supporting mechanistic information

The site of action of antiperspirant activity and the penetration depth of Al salts have been investigated *in vitro* and *in vivo* using human skin. Ductal diffusion and precipitation at varying depths have been demonstrated histologically. Comparative *in vivo* studies on animal and human skin demonstrated that the more acidic salts such as ACL or Al nitrate migrate further down the sweat duct, due to their smaller size and higher diffusion capacity, and produce a longer-lasting effect. ACL is able reach the upper and mid intra-dermal portion of the ducts, sometimes even right down to the eccrine or apocrine secretory coils (Lansdown, 1973a; Papa and Kligman, 1967; Quatrale et al., 1981a; Reller and Luedders, 1977). ACH and the polycationic AZAG form polymers with a slow diffusion rate, and are more restricted to the intra-epidermal ductal regions in the uppermost layers of the epidermis. ACH has been found in the whole *acrosyringium*, mainly in the upper portion, but also down to the level of the *stratum spinosum* and *stratum basale*. AZAG is mainly restricted to the *stratum corneum* (Quatrale et al., 1981a, 1981b, 1981c, 1985), but in one study could be identified as far as the upper intra-dermal duct (Quatrale et al., 1981c). The above pattern for all Al salts was observed in both forearm and axillary skin; however, penetration depth in axillary skin was found to be systematically greater (Quatrale et al., 1985).

➢ *[**SCAHT notes**: one important limitation to the series of experiments conducted by Quatrale et al., 1981a, 1981b, 1981c, 1985; and Strassburger and Coble, 1987 (all from the antiperspirant manufacturer Gillette) is that the fluorescence technique used to visualize the amorphous Al cast in the tissues used the*

Morin staining; it was subsequently shown that this stain is not suitable for detection of small amounts of Al in skin tissues because it also reacts with iron and copper. Lumogallion on the contrary is more reliable as it reacts specifically with Al to form a fluorescent complex and has been successfully used for sensitive detection of Al in epithelial tissues (Uchiumi et al., 1998; Yanagishita et al., 2012)].

Recently, using an *in vitro* tape stripping experiment, Mayeux et al. (2012) showed that the deposition of ACL on the forearm skin is not restricted to the eccrine sweat ducts opening, but is also seen on the skin plateau and inside the skin microrelief lines. Following topical application of ACH on palmar skin, Yanagishita et al. (2012) confirmed histologically the precipitation of an amorphous gel including keratin and polysaccharides in the sweat duct portion of the *stratum corneum*. Chen et al. (2016) studied the antiperspirant action of a 15% ACH roll-on at the single pore level on the palm using advanced photon fluorescence and imaging techniques. They demonstrated for the first time *in vivo* the formation of a 'plug' near the pore entrance, at the junction between the sweat pore and the spiral eccrine duct. The plug appeared as a disorganized structure mainly localized at 0-30 μm from the skin surface, with some cast visible down to 100 μm, but not at 130 μm. However, they could not confirm the physicochemical nature of the plug.

There are still many knowledge gaps relating to the precise and comprehensive description of Al interaction with sweat, or with the ducts and glands components (in particular of the apocrine type), as well of the long-term consequence of pore plugging on sweat secretion and reabsorption. Early observations and new insights into sweat gland anatomy and physiology have led some authors to challenge the generally accepted theory of plug formation as the sole mode of action of Al-containing antiperspirants (Burkhart and Burkhart, 2008). Additional biological effects of Al salts on the active transport in the eccrine duct (Hölze and Kligman, 1979; Hölzle and Braun-Falco, 1984), increased reabsorption ('leaky hose' hypothesis; Papa and Kligman, 1967), or on secretion processes in the secretory coil (McWilliams et al., 1987) have been described, but their interpretation is often complicated by the presence of cellular damage, ductal disruption and early signs of inflammation (see **section 4.1**). Burkhart and Burkhart (2008), based primarily on limited *in vitro* evidence, further hypothesized that Al may alter sweating by constricting the dermal duct lumen by a direct effect on the ductal membrane or via its anticholinergic action; or by affecting the eccrine secretory flow via inhibition of the sodium channel/Na^+K^+-ATPase system. All these views remain speculative.

In summary, although mechanical plugging of sweat gland ducts is plausible, the mode of action of Al salts in antiperspirants is still not entirely clear.

3.3 Use of Al in cosmetics

3.3.1 Al in antiperspirants

Al was first commercialized for its antiperspirant properties at the beginning of the 20th century (ca. 1902-1903) as Al chloride (Afssaps, 2011; McGrath, 2003; Stillians, 1916). Because ACL has a marked potential for local irritation and fabric damage due to its strong acidity (pH 2.5-3), less acidic and better tolerated Al salts (e.g. lactate, formate, sulfate, alum) were gradually introduced and formulations improved (e.g. by adding urea or glycine as buffers), albeit with reduced efficacy (Afssaps, 2011). Aqueous formulations of Al salts have been shown to be more efficient than anhydrous formulations. Efficacy differences are also observed as a function of vehicle. Progressive use of less acidic and more versatile ACH in the 1940s allowed for both irritation reduction and efficacy (Bretschneider et al., 1977).

The main active ingredient in antiperspirants since the early 1960s is ACH (BfR, 2014), mainly in aerosols and roll-on formulations. Glycinated complexes of ACH and Zr ("AZAG") such as Al-Zr tetra- or octa-chlorohydrex glycine were introduced in the late 1960s (Benohanian, 2001), and are most likely found in sticks, gels and other solid formulations. According to Afssaps (2011), BfR (2014) and VKM (2013), ACH is currently the main active

ingredient used in antiperspirants. This is also confirmed by our own informal survey of the different antiperspirants available on the Swiss market in January 2017[4].

According to the Swiss and European Regulations on cosmetics (Art. 54 of the Swiss Federal Ordinance on Foodstuffs and Utility Articles (LGV, 2016) and Annex III of the Cosmetic Regulation EC/1223/2009 (EC, 2009)), only Al-Zr hydroxychloro complexes are regulated with a maximal concentration of 20% of anhydrous Al-Zr chloride hydroxide. Other Al complexes, like ACL or ACH are not specifically regulated, but must comply with the general requirement for all cosmetic products: they have to be safe for human health.

Based on the European CosIng (2017) database, twenty-five Al compounds may be used in cosmetics products (see **Appendix 2**). In 2007, Afssaps conducted a survey of representatives of the cosmetic industry and reported concentrations of Al salts in antiperspirants ranging from 1% (Al capryloyl glycine) to up to 20% (ACH, AZAG); typical Al concentrations in ACH-containing antiperspirants were 5% in spray, 15% in roll-on, and 20% in stick formulations (Afssaps, 2011). According to BfR (2014), concentrations of 20% are typically encountered with ACH, which represents ca. 5% of Al on a w/w basis, but can be as high as 30% in antiperspirant creams (based on unreferenced data from the German Cosmetic, Toiletry, Perfumery and Detergent Association, IKW, cited in BfR 2014).

According to a Norwegian survey in 2011 (reported in VKM, 2013), about 90% of the antiperspirants on the Norwegian market contain the active ingredient ACH in concentrations up to 25%; median Al concentration in antiperspirants sold on the Norwegian market (n=8) was 4.1% (41 g Al/kg; range 28-71).

3.3.2 Al in other cosmetic products

Besides antiperspirants, Al is present in a wide range of other cosmetic products, including in particular lipstick/gloss and toothpastes (SCCS, 2014). A large variety of inorganic and organic aluminium compounds including salts, chlorohydrates, minerals, glasses and clays, colloidal 'lakes', carbohydrates and fatty acids are used as cosmetic ingredients, performing different functions in several product types. According to the CosIng Database, at least 16 functions can be attributed to Al compounds: *abrasive, absorbent, anticaking, antiperspirant, astringent, binding, buffering, bulking, cosmetic colorant, deodorant, emollient, emulsion stabilising, humectant, opacifying, skin protecting, and viscosity controlling*.

- Water-soluble Al-containing ingredients include: simple inorganic salts, simple organic salts, Al benzoate, and chlorohydrates. These ingredients can be used in skin care products. Functions reported in Cosing are *astringent, buffering agent, deodorant, antiperspirant* (SCCS, 2014).
- Water-insoluble Al-containing ingredients include: minerals, glasses and clays; Al lakes; carbohydrates; fatty acids salts. Insoluble minerals, glasses and clays are typically added to cosmetic products as *bulking agents, coloured pigments*, and sometimes as mild *abrasives*, e.g. in toothpastes where they provide shine/gloss benefit by polishing the enamel. Al colloidal 'lakes' are mainly used in lipsticks and toothpastes as colorants (SCCS, 2014; VKM, 2013). Al hydroxide can be used also as a coating agent for titanium dioxide nanoparticles in sunscreens.

Afssaps (2011) reported Al main uses and concentrations in different cosmetic products, based on a survey of cosmetic products in 2007 (see **Appendix 3**). Maximum Al salt concentrations range from 0.17% (skin care moisturizing creams, gels or oils) to 80% (face masks). SCCS (2014) reported that Al content in Al 'lakes' usually ranges from 0.01 to 10 %, but that a 'lake' with 18% Al content had also been found on the EU market. Whitening

[4] Aluminium chlorohydrate is used as main ingredient in antiperspirants spray and roll-on of brands on the Swiss market such as Addidas, Borotalco, Bourgeois, Dove, Fa, Fenjal, Garnier, Mum, Nivea, Rexona. A few roll-on and creams contain Al Zr tetra- or octa- chlorohydrex GLY (Addidas, Mitchum; Rexona). Certain products use combination of Al salts such as Fenjal (ACH + Al Mg Silicate) or *Nivea dry Comfort* (ACH + ASCH + Al Mg Silicate). One product was found mislabelled as a deodorant instead of antiperspirant (*Fenjal Vitality Deodorant Spray 24h)*.

toothpastes may contain high Al concentrations. According to SHC (2015), concentrations of Al hydroxide of 13% and 26% in whitening toothpastes are reported for the Norwegian market (NILU, 2011 in VKM, 2013). Aluminium concentrations in cosmetics were analysed by the Norwegian Institute for Air Research (NILU) in 2011 on request from the Norwegian Food Safety Authority (VKM, 2013). Aluminium concentrations in lipsticks were up to 0.28% (2.8 g/kg), and in lipglosses 0.08% (0.8 g Al/kg). Median concentrations in lipstick/lip gloss (n=11) was 0.77% (7.7 g/kg, range <LOD [0.35 mg Al/kg]-28), with a mean concentration of 0.87% and a 95th percentile value of 2.1% (SHC, 2015; VKM, 2013).

4 Hazard

The toxicity profile of Al compounds and their potential effects on human health have been reviewed in depth by various regulatory bodies (ATSDR, 2008; EFSA, 2008, 2011; SCCS, 2014; IPCS, 1997; JECFA, 2008, 2011), as well as in the peer-reviewed literature (e.g. risk assessment reviews by Krewski et al., 2007; and Willhite et al., 2014; endpoint-specific evaluations by Shaw et al., 2014; Yokel and McNamara, 2001; Tomljenovic, 2011; Walton, 2014; and Zhu et al., 2014). The present report focuses on skin irritation and sensitization, genotoxicity and carcinogenicity endpoints; the reader is referred to the aforementioned references for further, more complete information.

4.1 Aluminium toxicity endpoints

The most sensitive endpoints for Al toxicity are nervous system related (neurodevelopmental and chronic neurotoxicity). Health-based reference values (tolerable weekly intakes) derived by EFSA and JECFA are summarized in **Table 1**.

Table 1: Overview of health-based references values derived for Al

	Animal study	NOAEL/LOAEL[1] mg Al/kg bw/day	Uncertainty factor[2]	Additional uncertainty factor[3]	TWI[4] / PTWI[5] mg Al/kg bw/week	Comments
EFSA, 2008	Neurodevelop-mental toxicity in mice	NOAEL 10 LOAEL 50	100 100	- 3	1	The TWI is a rounded value of the TWI provided by the NOAEL approach (0.7 mg Al/kg bw/week) and the TWI provided by the LOAEL approach (1.2 mg Al/kg bw/week) from several studies.
JECFA, 2007	Various dietary studies in mice, rats and dogs	LOAELs 50–75	100	3	1	The lowest LOAELs were used as basis for the estimation of the PTWI due to the lack of an appropriate NOAEL.
JECFA, 2012	Developmental and chronic neurotoxicity in rats	NOAEL 30 LOAEL 100	100	-	2	The NOAEL of 30 mg/kg bw/day was considered an appropriate basis for establishing a PTWI.

[1]NOAEL – no observed adverse effect level, LOAEL – lowest observed adverse effect level.
[2]Uncertainty factor due to interspecies and intraspecies differences.
[3]Additional safety factor due to the use of LOAEL.
[4]TWI – tolerable weekly intake, term used by EFSA.
[5]PTWI – provisional tolerable weekly intake, term used by JECFA.

Source: VKM (2013), Table 3.

4.2 Skin irritation and sensitization

The potential for irritation or sensitization of Al salts is of interest, because inflammation can damage the skin and alter the skin barrier function, which could lead to an increase in dermal absorption of Al. A review of the historical literature on Al use in antiperspirants as well as in vaccines (as an immunogenic adjuvant) notes that the skin irritant and immunogenic properties of Al salts have been known for almost a century (Stillians, 2016; Baker and Gill, 1934). Mild irritations and itching were already well established as frequent side effects of the application of ACL antiperspirant solutions, but cases of contact dermatitis were only rarely observed (Stillians, 1916). Early studies in humans conducted on axillary, forearm or back skin consistently reported frequent cases of mild to strong irritation, soreness and allergic reactions following application of ACL (Ellis and Scurr, 1979; Hölze and Braun-Falco, 1984; Hölze and Kligman, 1979; Papa and Kligman, 1967; Reller and Luedders, 1977; Scholes et al., 1978) or Al sulfate (Sulzberger et al., 1949).

4.2.1 Skin irritation

In vitro and *in vivo* animal and human studies reported dermal inflammatory reactions after acute, subacute or subchronic topical exposure to Al-containing antiperspirants. Many observations reported a subclinical inflammatory reaction, in the absence of any visible irritation. Epidermal damage triggers the release of pro-inflammatory mediators and growth factors from epidermal keratinocytes and dermal mast cells (Evans et al.,

2012), with activation of the skin resident immune system frontline. Structural damage to the *stratum corneum*, sweat ducts and glands is sometimes associated with early signs of physiological changes in the eccrine sweat glands activity. While these effects have been observed for the shaved axilla, they have been mostly documented for intact forearm skin.

The irritation potential of Al compounds varies by type. Lansdown (1973a) exposed mouse, rabbit and pig skin to various concentrations of Al salts**.** He found that ACL (10%) and Al nitrate (10%) caused epidermal changes consisting of hyperplasia, microabscess formation, dermal inflammatory cell infiltration and occasionally ulceration in all three species treated, but no such effects from Al sulfate (10%), Al hydroxide (10%), Al acetate (10%) or ACH (10% or 25%). However, Mayeux et al. (2012) reported no skin irritation after daily administration of aqueous 5% ACL solution to the volar forearm of volunteers for 7 days.

ACL pro-inflammatory effects are often associated with early histological structural damage of sweat ducts (Hölzle and Braun-Falco, 1984; Hölze and Kligman, 1979; Reller and Luedders, 1977; Sulzberger et al., 1949), necrosis or death (Hölzle and Braun-Falco, 1984; Reller and Luedders, 1977), sometimes with degeneration of eccrine ducts, more rarely eccrine glands (Hölzle and Braun-Falco, 1984; Sulzberger et al., 1949). In isolated cases, eccrine ductal hypertrophy, rarely associated with rupture, was observed (Reller and Luedders, 1977; Sulzberger et al., 1949). A recurring observation was an inflammatory reaction in the absence of visible irritation, with histological periductal damage as well as lymphocytes and polymorphonuclear leukocytes infiltration around the intradermal duct at the epidermis-dermis interface. This was often interpreted as Al-induced *miliaria rubra*[5] (Hölze and Kligman, 1979; Papa and Kligman, 1967; Reller and Luedders, 1977; Sulzberger et al., 1949) due to sweat gland blockage. Many of these studies were performed under occlusion, with short exposure times (1-24hrs) and varying application duration (days-weeks) and frequency (once a day/week to several times a day/week). Higher exposure duration and frequency generally led to more severe effects (Hölzle and Braun-Falco, 1984; Reller and Luedders, 1977), but studies performed under occlusion (Hölze and Kligman, 1979; Papa and Kligman, 1967; Reller and Luedders, 1977) were not associated with an increase in the severity of effects compared to the studies conducted without occlusion (Hölzle and Braun-Falco, 1984; Sulzberger et al., 1949[6]).

ACH and AZAG are not classified as skin irritants. These two Al salts are known to be well tolerated as active ingredients of Al antiperspirants (Guy et al., 1999). Available animal and human studies with ACH and AZAG reported no epidermal damage (Lansdown, 1973a, 1973b; Quatrale et al., 1981b). Mechanistic studies on forearm or axillary skin showed that ACH and AZAG (whose distribution was primarily restricted to the acrosyringium = the intraepidermal part of the duct of the sweat gland nearest to the surface of the skin, with some deeper penetration occasionally seen for ACH) produced no gross damage to the reabsorptive duct (the terminal half of the sweat gland) nor subtle ultrastructural changes in epithelial cell membranes lining the duct (Quatrale et al., 1981b, 1981c). This was confirmed also by more recent studies ACH and AZAG on human palmar skin which did not show any skin damage or structural changes of eccrine sweat ducts and glands below the *stratum corneum* level (Chen et al., 2016; Yanagishita et al., 2012).

Studies in humans on Al-containing antiperspirants have reported incidence rate of visible irritation in the range 10-44% (Scholes et al., 1978; Ellis and Scurr, 1979; Streker et al., 2012; Swaile et al., 2012).

4.2.2 Skin sensitization

Al has been used since the 1930s for its immunogenic properties as an adjuvant in vaccines (Baker and Gill, 1934; Powell et al., 2015). However, aluminium compounds are not classified as skin sensitizers. No skin sensitization

[5] The common type of the skin rash called *miliaria* is sometimes referred to as prickly heat, or a heat rash, or a sweat rash. Miliaria occurs in some people when they sweat a lot. It can be very itchy. It is due to a blockage of the sweat ducts which causes sweat to seep into the skin cells.

[6] The Sulzberger et al. (1949) study on female axillary skin irritation mentioned shaving as part of the study design, but no attempt was made to link any of the observations with a potential impact of shaving on skin integrity.

was demonstrated in the mouse local lymph node assay (LLNA) test with ACL 25% (Basketter et al., 1999) or in the guinea pig maximisation test (GPMT) with ACH 25% (SHC, 2015). Cases of skin sensitization in humans are rare (0.2% according to 1922 patients tested by Hemmer et al., 1996). A few cases of axillary contact dermatitis have been reported, with ACL-based (Garg et al., 2010) or Al sulfate-based (*alum)* antiperspirants (Gallego et al., 1999 in SCCS, 2014; Leventhal et al., 2014). Other cases of Al contact allergies from various etiologies are described in the literature (e.g. Akyol et al., 2004; Purello-D'Ambrosio et al., 2000; Siemund et al., 2012). False-positive reactions with ACL 2% on routine patch testing or testing with the empty Finn Chamber® have also been described (Hemmer et al., 1996). There are some indications that subcutaneous injections of Al-containing vaccines may be weakly sensitizing (Krewski et al., 2007; Willhite et al., 2014). Siemund et al. (2012) showed that patch testing with ACL 10.0% solutions gave the highest number of positive Al reactions (14/21), compared to 2% (4/21) or 20% (non-specified) solutions. There were no positive reactions with the Finn Chamber®. Interestingly, they found that the allergy be verified by an intradermal test only in 16% (3/9) of those individuals with a strong Al contact allergy, and concluded that intradermal testing was less sensitive than patch testing in detecting Al allergy.

- ***SCAHT notes: it is unknown if and to which extent a subclinical inflammatory context (i.e. a reaction in the absence of any visible irritation) may favour Al absorption in certain individuals (resorption through blood capillaries or superficial lymphatic circulation). Given Al strong binding capacity to proteins (see section section 3.1), accumulation in the epidermis seems possible, although the low rates of skin sensitization by Al salts could be an indication that Al does not reach a critical concentration in the epidermis. Skin regeneration processes through epidermal desquamation may represent a competing removal mechanism for Al bound to keratin. It can also be speculated that improved, less irritating formulations and replacement of ACL by the chlorohydrate salts have contributed to minimize allergic reactions over time.***

4.3 Genotoxicity and mutagenicity

Aluminium interacts directly with DNA *in vitro* and *in vivo*. This is likely due to its extremely high charge ($^{3+}$) to ionic radius (0.05 nm) ratio causing it to rapidly penetrate nuclear compartments, and bind tightly to nucleic acids, heterochromatin, linker histones, and DNA and RNA polymerase enzyme systems (see McGrath, 2003 for a discussion). Lower pH values appear to favour Al complexes with DNA (ATSDR, 2008). Main observed effects include cross-linking between chromosomal proteins and DNA, inhibition of cell division and interference with DNA synthesis (either inhibition or induction), and induction of chromosomal aberrations (EFSA, 2008).

Mutagenicity assays (reviewed in ATSDR, 2008; EFSA, 2008; Krewski et al., 2007) have mostly yielded negative results in various *in vitro* bacterial and mammalian test systems: (i) Ames test using several strains of *S. typhimurium*, and organic or inorganic salts of Al (chloride, lactate, fluoride, silicate). One positive result was apparently obtained in a study using dye-alumina complexes, but it is questionable since the effect was likely associated with the presence of impurities in the complexes rather than with Al per se (Brown et al., 1979 cited in Krewski et al., 2007); (ii) *E. coli* tryptophan (trp) reverse mutation assay, using ACL, Al fluoride, or Al silicate; (iii) *B. subtilis* rec (recombinant)-assay, using ACL, Al sulfate, or Al oxide; (iv) mouse lymphoma thymidine kinase (L5178Y locus) assay, using ACL; (v) Syrian hamster embryo transformation assay, using ACL.

- Sappino et al (2012) reported that ACL had no detectable mutagenic effect in two strains of *E. coli* (CC105, ML3), based on the number of Lac+ revertants (indicating AT to TA transversions) or rifampicin resistant (Rif^R) forward mutants (detecting all base pair substitutions) cells in CC105 (Lac^- Rif^S) cultures or of Lac+ revertants in ML3 (Lac^-) cultures. Mandriota et al. (2016) reported 48 point mutations affecting 43 genes regulating cellular proliferation, migration, metastasis and apoptosis (see **section 4.3.1.1**).

Other genotoxicity effects observed in some of these tests were DNA damage and effects on chromosome integrity and segregation *in vitro* (EFSA, 2008).

Indeed, numerous studies have shown that Al compounds induce both **structural and numerical chromosomal aberrations** (i.e. clastogenicity and aneugenicity, respectively) in various test systems both *in vitro* and *in vivo*, incl. human cell lines, mammals (mouse, rat, hamster), fish, zebrafish, insects, and plants (EFSA, 2008; García-Medina et al., 2011; Krewski et al., 2007; Pereira et al., 2013; Willhite et al., 2014): (i) *in vitro* studies in rats, mice, hamster peritoneal cells, showed chromosomal aberrations using Al hydroxide; (ii) *in vitro* studies in human blood lymphocytes, using mainly ACL or Al sulfate, collectively reported induction of micronuclei (consistently!), chromatid type aberrations, sister chromatid exchanges, apoptosis or inhibition of DNA repair; (iii) *in vivo* studies in rats and mice, by oral gavage or intraperitoneal injection, using primarily ACL or Al sulfate, showed induction of chromosomal aberrations, micronuclei, sister chromatid exchanges; (iv) no human studies were identified.

- Sappino et al. (2012) have reported an increase of DNA double strand breaks (DSBs) in MCF10A cells following exposure to ACL (at up to 300 μM). However, no clastogenic effects were detected in ACL-treated MCF10A cells.

Several indirect mechanisms for these genotoxic effects have been proposed (EFSA, 2008), in particular cross-linking of DNA with chromosomal proteins, interaction with microtubule filaments, damage of lysosomal membranes with liberation of DNAase, and induction of oxidative stress via the formation of iron-induced or superoxide-induced (Exley, 2004a; Mujika et al., 2011) reactive oxygen species (ROS).

An independent evaluation of the genotoxicity potential by Krewski et al. (2007) noted limitations in the experimental database (study design, cell culture conditions, lack of characterization of test system for cytotoxicity and of proper controls due to pH effects, high exposure doses, absence of or no clear dose-response), yielding controversial results. EFSA (2008) and SCCS (2014) both concluded that the observed clastogenic and aneugenic effects of Al salts are seen only at high exposure levels, due to non-specific indirect mechanisms. These effects are therefore probably not relevant at human dietary exposure levels (EFSA, 2008).

4.4 Carcinogenicity

4.4.1 Carcinogenicity other than breast cancer

Long-term chronic toxicity or carcinogenicity studies in laboratory animals with Al have been reviewed by ATSDR (2008), EFSA (2008), Krewski et al. (2007), SCCS (2014, Annex I) and IPCS (1997). Several limitations have been raised regarding the quality of the experimental database, namely: (i) the limited number of animal studies available; (ii) the poor quality of the reporting (often missing information about the type of Al compound used, the type of tissues investigated, the type and localization of the tumors observed, etc); (iii) the study design (use of only one species to a single exposure level and limited histological examinations); and (iv) the fact that most of them were old studies that were likely not conducted according to GLP (ATSDR, 2008; SCCS, 2014).

Epidemiological studies in the occupational setting have suggested that inhalation exposures to Al dusts during Al production and transformation processes may present an increased risk of *lung* and *bladder* cancers (IARC 1987; ATSDR, 2008). While the evidence is limited, causality is often confounded by co-exposures to polycyclic aromatic hydrocarbons, aromatic amines, nitro compounds, and fibres such as asbestos or silicates (Krewski et al., 2007). There is no evidence of an increased risk of cancer in persons not occupationally exposed, and IARC does not classify aluminium itself as a human carcinogen. Willhite et al. (2014) have recently reviewed additional epidemiological studies from occupational/inhalation exposure:

- Friesen et al. (2009) investigated associations between occupational exposures to alumina and bauxite dusts with cancer (among several other health conditions). Cumulative alumina exposures showed no notable associations or trends for cancer. Despite some limitations (statistical power and treatment of the data), the authors concluded that there were no significant associations between occupational Al exposures and cancer risk.

- Donoghue et al. (2014) investigated the human health effects of NO_2, SO_2, PM_{10}, arsenic and cadmium emissions from bauxite mining and alumina refineries; they found that arsenic accounted for 75% of the total incremental cancer risk (1.2×10^{-6}) 3 km downwind of these facilities. Al was not associated with an increased incidence of cancer.

4.4.2 Al in relation to breast cancer risk

The following section presents relevant in vitro, in vivo and epidemiological breast cancer studies in relation to Al exposure. Studies are briefly summarized and listed in a chronological order. For a full description of the study findings, as well as SCAHT critical evaluation of each of these studies, please refer to **Appendix 4**.

4.4.2.1 In vitro studies

Several relevant *in vitro* studies using murine and human normal or breast cancer cell lines have been located in the literature.

- **Darbre (2005b)** found that (i) ACL or ACH could weakly interfere with the binding of $1.6 \cdot 10^{-9}$M [3H]estradiol to cytosolic estrogen receptor (ER); (ii) estrogen-stimulated proliferation of MCF-7 human breast cancer cells grown for 14 days in monolayer culture with 10^{-8}M 17β-estradiol was further inhibited with 1 mM ACL (P=0.002), 300 μM ACH (P=0.007) or with 1 mM ACH (P<0.001); (iii) the overall estrogen induction of the chloramphenicol acetyl transferase (CAT) reporter gene activity was increased by nearly 3-fold with 100 μM ACL (2.81±0.10) and with 100 μM ACH (2.81±0.14). The CAT reporter gene activity was higher after 24-h exposure to 100 μM ACL or 100 μM ACH in the absence of 17β-estradiol (P=0.018; P=0.023, resp.) and in the presence of 17β-estradiol (P<0.001; P=0.014, resp.). There are however many limitations and insufficiencies to this study, e.g. a general lack of characterization of the test system incl. basic cell biology and toxicity experiments, inadequate exposure and control, missing experimental details and statistical treatment of the data.
- **Darbre et al. (2011)** aimed to compare proliferation and global gene expression profiles of MCF-7 human breast cancer cells grown for 21 weeks with or without ACH 100 μM in the presence of 10^{-8}M 17β-estradiol: (i) cell proliferation was assayed at time zero and after 21 weeks; there was no detectable effect of ACH on proliferation rate between these two time points; (ii) 21 weeks exposure of MCF-7 cells to ACH 100 μM lead to a ≥2-fold upregulation of 50 genes, and a ≥2-fold downregulation of 57 genes. The study only reports the results obtained with several S100 Ca^{2+}binding protein genes for which mRNA overexpression were up to ca. 2 to 4-fold. Other limitations relate to the quality of the reporting incl. missing statistics, the study design (exposure, dose), and the overall poor test system characterization.
- **Sappino et al. (2012)** found that (i) 6 weeks exposure of MCF10A normal primary breast epithelial cells to 100 μM ACL induced loss of contact inhibition and increased anchorage-independent growth; 1 week of MCF10A cells to 100 μM ACL or 300 μM ACL (P<0.0001) reduced the numbers of cells, but there were no signs of apoptosis; (ii) culture with 10, 100 or 300 μM ACL increased the percentage of senescence associated ß-galactosidase-positive cells in proliferating MCF10A cells after 1 week; (iii) exposure of primary human epithelial cells to 100μM or 300 μM ACL increased in a dose dependent manner the expression of p16/INK4a, a marker of senescence; (iv) 10, 100 and 300 μM ACL increased DNA DSBs in a dose- and time-dependent manner in proliferating MCF10A cells, but had little or no effect in HaCaT keratinocytes, and no influence on X-ray induced DSB repair in MCF10A cells; (v) ACL 300 μM was not detectably mutagenic in bacteria. This study suffers from many insufficiencies (e.g. study design and conduct, quality of the reporting, poor test system characterization).
- **Darbre et al. (2013a)** showed that long-term exposure to either ACL 100 μM or ACH 100 μM as single doses increases migratory and invasive properties of MCF-7 human breast cancer cells in culture. They found that: (i) 32 weeks but not 1 week exposure of MCF-7 cells to ACL or ACH significantly increased

cell motility of the cells using a wound healing assay after 0 and 21 h (n=10 independent wounds, P<0.001); (ii) cell migration rate and cell invasion rate were increased following 37 weeks exposure to ACL or ACH, as compared to controls, but not 1 week exposure which decreased the cell invasion rate; (iii) in addition, they reported (data not shown) that 1 week or 53 weeks exposure to ACL or ACH did not change the level of E-cadherin or β-catenin expression in MCF7 cells. N-cadherin and P-cadherin[7] were not detectable in these cells. There are several limitations to this study, incl. inadequate reporting of the results incl. statistics and missing data, lack of characterization of the test system, and insufficiencies with the study design (dose, exposure, controls).

- **Bakir and Darbre (2015)** reported that 20-25 weeks exposure to ACL or ACH 10 μM or 100 μM can: (i) increase migration and invasiveness of estrogen unresponsive MDA-MB-231 human breast cancer cells. Average cell motility was increased after 25 weeks exposure to 10 μM ACL (P=0.019) and to 10 μM ACH, but there was no effect to ACL 100 μM or ACH 100 μM. Effects on cell migration rate and invasion rate following 25 and 23 weeks exposure to ACL 10 μM were larger than ACL 100 μM, but effects with ACH 10 μM were smaller than ACH 100 μM in both experiments, compared to water control; (ii) increase matrix metalloproteinases[8] MMP9 and MMP14 expression at the mRNA and protein levels. An overall increase in MMP9 RNA and protein is observed for all the doses and compounds tested (often non-significant results), but some anomalies exist with the observed effects in relation to the exposure duration (1 week vs 21 weeks). Overall effects on MMP14 at the mRNA and protein level were weak, statistically non-significant, compared to control. These results are rather inconclusive, and difficult to use and interpret. Some inconsistencies are not discussed, and statistics are missing for some results. Many basic experiments are missing also (test system characterization, cytotoxicity, etc).

- **Farasani and Darbre (2015)** found that long-term (19-21 weeks) exposure of MCF10A cells to ACL 100 μM and ACH 100 μM significantly decreased levels of BRCA1 mRNA ($P < 0.001$) (by RT-PCR) and BRCA1 protein ($P < 0.001$) (Western immunoblotting). They also found significant reduced levels of other DNA repair genes BRCA2, Rad51, and CHK2 mRNAs ($P < 0.001$), as well as ATR mRNA ($P < 0.05$) A decrease was also seen for CHK1 mRNA, but it was only significant for ACH 100 μM ($P < 0.05$). Major limitations are a poor test system characterization, no control of pH and cell viability/cytotoxicity, single dose experiments, inadequate controls, limited exposure time points).

- **Mandriota et al. (2016)** showed that exposure to long-term (4-7 months) concentrations of ACL 10 μM and/or 100 μM *in vitro* transform normal murine mammary gland (NMuMG) epithelial cells compared to water control. A change in phenotype and morphology with scattered growth pattern reminiscent of epithelial-to-mesenchymal transition (EMT) was observed (63% decrease in E-cadherin and 3.7-fold increase in N-cadherin expression, two markers of EMT) and in the soft agar assay, where larger colonies were recorded compared to control (all positively significant results). Xenografting experiments based on subcutaneous injections into the flank of 6 weeks old female mice from three different mouse strains with decreasing immunodeficiency, namely, NOD SCID gamma (NSG) (severe), NOD SCID (moderate), or nude mice (low), revealed that *untreated* NMuMG cells form tumors and metastasize, to a limited extent, in the highly immunodeficient and natural killer (NK) cell deficient NSG strain, but not in the less permissive and NK cell competent NOD SCID or nude strains. In contrast, NMuMG cells transformed *in vitro* by ACL 100 μM form large tumors and metastases in all three mouse models. These effects were reported to correlate with a mutagenic activity of ACL. Mandriota et al found 48 mutations affecting 43 genes in ACL 100 μM treated versus control NMuMG cells, with mutations affecting genes regulating

[7] Cadherins are calcium dependent cell-to-cell adhesion molecules that are biomarkers of cancer invasion and metastasis, their absence being associated with a more aggressive cancer cell phenotype.

[8] Matrix metalloproteinases are zinc-dependent endopeptidases which degrade extracellular matrix and are often upregulated in metastatic cancers (Bakir and Darbre, 2015).

cellular proliferation, migration, metastasis and apoptosis, including Max-binding protein *Mnt* and T-lymphoma invasion and metastasis-inducing protein 2 *Tiam2*. Direct sequencing confirmed 13 mutations (n=14, 92.8%) Although ACL-treated NMuMG cells clearly exhibited a proliferative advantage in the soft agar and xenograft assays, ACL does not increase proliferation in the conventional 2D culture, therefore it was concluded that the observed mutations are not a simple consequence of increased proliferation. There are some limitations to this study (e.g. single dose experiments, controls, exposure time points, molecular toxicity endpoints), which would have benefited from a better characterization of the test system (incl. basic cellular biology and toxicity experiments).

- **Darbre (2016)** very briefly reported in an exploratory experiment the growth of MCF-10A human breast epithelial cells in a semi-solid methocel culture after 13 weeks of exposure to 100 μM ACL, 100 μM ACH or 10^{-8}M 17β-estradiol. Most of the experimental details are lacking which preclude the use of these data for any risk assessment purposes.

4.4.2.2 Human studies

A few human studies have attempted to determine Al concentrations in body fluids and cancer breast tissues in humans, and to correlate these findings with inflammatory markers (Mannello et al., 2011, 2013); or to correlate Al concentrations in breast cancer with genomic instability (Rodrigues-Peres et al., 2013):

- **Mannello et al. (2011)** investigated the correlation between Al levels and iron - binding proteins in nipple aspirate fluids (NAFs) collected from women with breast cancer and healthy women. The mean level of Al was significantly higher in the breast tumor NAF (268.4 ± 28.1 μg/l; n=19) than in the healthy control NAF (131.3 ± 9.6 μg/l; n=16; P < 0.0001). Mean ferritin level was higher breast tumor NAF (280.0 ± 32.3 μg/l) than in NoCancer NAF (55.5 ± 7.2 μg/l). Correlation between Al levels and iron-binding proteins: Al was significantly positively correlated with ferritin and transferrin (correlation coefficient R = 0.94 and R = 0.79, respectively; both P < 0.001) in NAF from breast cancer women, but Al was not significantly correlated with ferritin (R = 0.35, P = 0.18) and transferrin (R = -0.02, P = 0.93) in NAF from healthy women.

- **Mannello et al. (2013)** in a follow up study using the same NAF samples (Mannello et al., 2011) found significantly increased levels of protein oxidative carbonyls in cancer patients compared to healthy women (2.35 vs 0.41 nmol/mg protein, respectively; P < 0.0001), with Al content and carbonyl levels showing a significant positive linear correlation (r^2 0.6628, p < 0.0001). In cancer NAF samples (268.4 ± 28.1 μg/l; n=19), they found significantly increased levels of pro-inflammatory cytokines (IL-1β, IL-6, IL-12 p70, and TNF-α) and chemoattractant CC and CXC chemokines (IL-8, MIP-1α and MCP-1), compared to healthy NAF samples. In 63% (n=12/19) of invasive cancer NAF samples they found a significant positive linear correlation among Al, carbonyls and pro-inflammatory IL-6 cytokine (Y = 64.79x − 39.63, r^2 0.8192, P < 0.0005), as well as pro-inflammatory monocyte chemoattractant MCP-1 cytokine (Y = 2026x − 866, r^2 0.9495, P < 0.0001).

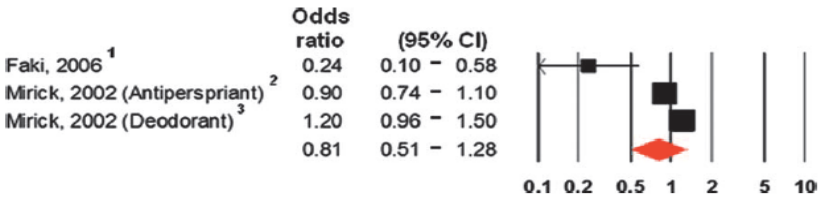
- **Rodrigues-Peres et al. (2013b)** in a cross-sectional study aimed to examine the relationship between Al concentrations in the peripheral and central areas of breast tumors with the instability of three key genes in breast cancer, ERBB2, C-MYC, and CCND1 and aneuploidy of the chromosomes harboring these genes. They analyzed in tumor samples collected from breast cancer patients (n=118) ERBB2, C-MYC, and CCND1 expressions and statuses of their respective chromosomes 17, 8, and 11. They found that amplification and/or aneuploid-positive statuses for ERBB2/CEP17, C-MYC/CEP8, and CCND1/CEP11 were detected in 24, 36.7, and 29.3 % of the tumors, respectively. Al levels of >2.0 mg/kg were found in 20.3 and 22.1 % of the central and peripheral breast tumor areas, respectively. They compared the mean Al concentrations according to the ERBB2, C-MYC, and CCND1 gene statuses first in the central and then in the peripheral tumor areas. They found that Al concentration was not related to these altered gene statuses (P = 0.09-0.59 for all experiments).

4.4.2.3 Epidemiological studies and breast cancer after dermal exposure to antiperspirants

To date, only four primary observational studies (Fakri et al., 2006; Linhart et al., 2017; McGrath, 2003; Mirick et al., 2002) and a meta-analysis (Hardefeldt et al., 2013) have investigated the effect of antiperspirant/deodorant use on breast cancer development:

- **Mirick et al. (2002)** conducted a population-based case-control (n=813 vs n=793) study to investigate a possible relationship between use of deodorant or antiperspirant applied for underarm perspiration and the risk for breast cancer in women aged 20-74 years old (1992-1995), based on structured telephone interviews. Specific questions relating to the type of product used and underarm shaving practice were included. They found that none of the factors evaluated had an influence on breast cancer risk: (i) regular use of antiperspirant (OR 0.9 [0.7-1.1] P=0.23); (ii) regular use of deodorant (OR 1.2 [0.9-1.5] P=0.19); (iii) use of antiperspirant within one hour after shaving (OR 0.9 [0.7-1.1] P=0.40); (iv) use of deodorant within one hour after shaving (OR 1.2 [0.9-1.5] P=0.16).
- **McGrath (2003)** conducted a population-based retrospective study (1993-2001) using a written questionnaire sent to surviving female breast cancer patients (n=437 eligible) to evaluate their underarm hygiene practices of antiperspirant/deodorant use and underarm shaving. He found that: (i) the extent of antiperspirant/deodorant use and axillary shaving are associated with an earlier age of breast cancer diagnosis; and (ii) beginning these habits at an earlier age was associated with a significantly earlier age of diagnosis. It was concluded that combined habits were necessary, whereas separately they were not associated with a significant earlier age of diagnosis.
- **Fakri et al. (2006)** conducted a population-based case-control study comprising 54 cases of breast cancer (mean age 43±8y) and 50 controls (mean age 41±15y) who were patients in the same hospital. Data were collected based on interviews (2002-2003). It was found that 82.0% of the controls used antiperspirants compared with 51.8% of cases ($P < 0.05$). The use of antiperspirants was not associated with the risk of breast cancer, but family history and use of oral contraceptives were.
- **Hardefeldt et al. (2013)** re-analysed the Fakri et al. and Mirick et al. data using a random effects model to calculate pooled odds ratio (OR) for the effects of antiperspirant and deodorant on breast cancer (see **Figure 4**). The pooled risk point estimate (in red in the figure) appears to be in the direction of a protective effect (OR=0.81, 95% CI=0.51-1.28). Results point at a no association between antiperspirants/deodorants use and risk of cancer in Mirick et al. (2002), harmful effects of antiperspirant use *versus* protective effects of deodorant use against cancer risk in Fakri et al. (2006).

Figure 4: Meta-analysis for antiperspirant/deodorant use and breast cancer risk



Source: Hardefeldt et al. 2013.

- **Linhart et al. (2017)**, in an age-matched hospital-based case-control study (n=209, 20-85 yrs old, 2013-2016), recently reported that self-reported (questionnaire-based) use of underarm cosmetic products was significantly (p=0.036) associated with breast cancer risk only in women who reported using these products several times daily before 30 yrs of age (adjusting for age, family history of breast cancer, family history of other cancer, history of benign breast disease, age at menarche, parity, age at birth of first child, age at menopause, menopausal status, hormone replacement therapy, average body mass index and alcohol consumption), increasing their risk for breast cancer by an OR of 3.88 (95% CI 1.03-14.66, p=0.0456). Al measured in defatted breast tissue was significantly associated with self-reported

underarm cosmetic products use (p=0.0269 for underarm cosmetic products use under the age of 30, p=0.0093 for underarm cosmetic products use during the last 5 years). Median (interquartile) Al concentrations were significantly higher (p=0.0014) in cases than in controls (5.8, 2.3-12.9 versus 3.8, 2.5-5.8 nmol/g). It was concluded that Al may accumulate in breast tissue and that starting application of these products at a younger age represents a risk factor for breast cancer.

4.4.2.4 Epidemiological studies and breast cancer after inhalation

- Pan et al. (2011) investigated residential proximity to Canadian Al smelters and risk of female breast cancer in a large population-based case-control study (n=21'000). Data from 2343 breast cancer cases and 2467 controls were used. Results indicated no increased risk of breast cancer among premenopausal or postmenopausal women living within 0.8-3.2 km of an Al smelter. After adjustment for several co-variables[9]: (i) the odds ratios were not statistically significant for premenopausal breast cancer among women living 0.8-3.2 km from Al smelters compared to the controls (8 breast cancer patients and 13 controls; OR=0.52 [0.21-1.31]) or for those living less than 0.8 km from smelters (two breast cancer patients and one control; OR=2.08 [0.18-23.72]); (ii) the odds ratios for postmenopausal breast cancer patients were not statistically significant for women living 0.8-3.2 km from Al smelters compared to the controls (19 breast cancer patients and 14 control; OR=1.06 [0.50-2.23]) or for those living less than 0.8 km from smelters (six breast cancer patients and six controls; OR=0.97 [0.27-3.41]); (iii) for both pre- and postmenopausal breast cancer patients, the odds ratios for those living greater than 3.2 km from an Al smelter were all unity (Willhite et al., 2014, as cited). Major limitations of this study are that it was based on a self-administered questionnaire, that there was no biomonitoring of Al or air sampling, and that results were mostly not statistically significant.

4.4.3 Mode of action of Al as a potential human breast carcinogen

Several hypotheses regarding potential mechanism(s) of action of Al as a human breast carcinogen have been proposed, however causality has not been established so far. Proposed modes of action have been based mostly on circumstantial evidence, or on hypotheses that have not been verified through testing, and which are therefore still largely speculative:

(i) Can Al interfere with the estrogen receptor and modulate* estrogen-regulated genes expression*?

⇒ Binding (unspecified) to the ER, and modulation of the oestrogen-responsive ERE-CAT reporter gene (Darbre, 2005b)

(ii) Can Al increase oxidative stress and tumor-promoting activity in the breast microenvironment?

⇒ Promotion of the Fenton reaction and ROS generation, disruption of iron homeostasis via interference with ferritin and transferrin functions (Mannello et al., 2011)

⇒ May act through the formation of a stable Al-superoxide anion ($[AlO_2^{\bullet}]^{2+}$) (Exley, 2004a; Mujika et al., 2011)

⇒ Release of pro-inflammatory cytokines, chemokines and chemo-attractants (Mannello et al., 2013)

⇒ May act by inhibiting the NA^+/K^+-ATPase in the ductal apocrine epithelial cells (Mannello et al., 2006; Darbre et al., 2011)

[9] Co-variables considered for both premenopausal and postmenopausal women were: age, province of residence, education, smoking pack-years, alcohol consumption, number of live births, age at menarche, total energy intake, and employment in the industry under consideration.

(iii) Can Al trigger genomic instability in breast epithelial cells?

- ⇒ May act as an activated oncogene similar to *ras* (Sappino et al., 2012)
- ⇒ Up or downregulation of key genes involved in DNA repair such as *BRCA1/2*, Rad51, CHK1, CHK2 and ATR (Farasani and Darbre, 2015)
- ⇒ DSBs leading to point mutations in genes regulating cellular proliferation, migration, metastasis and apoptosis (Mnt, Tiam-2) (Mandriota et al., 2016)

(iv) Can Al increase the proliferation of breast epithelial cells?

- ⇒ Loss of contact inhibition, increase of anchorage-independent cell growth (Mandriota et al., 2016; Sappino et al., 2012)
- ⇒ Replicative immortality via bypassing p53/p21Waf1-mediated cellular senescence (Sappino et al., 2012)
- ⇒ Promotion of breast cancer development via disruption of calcium homeostasis and protein phosphorylation, and altered expression of S100 calcium-binding proteins (Darbre et al., 2011)**.**

(v) Can Al increase metastasis?

- ⇒ Increased cell motility and invasiveness (Bakir and Darbre, 2015; Darbre et al., 2013a)
- ⇒ Upregulation of matrix metalloproteinases (MMP) is often associated with cancer metastasis (*MMP9, MMP14)* (Bakir and Darbre, 2015)

In a recent review, Darbre (2016) suggested that Al can enable multiple hallmarks of cancer to develop in breast epithelial cells, and how these hypotheses are aligned on the 'hallmarks of cancer' proposed by Hanahan and Weinberg (2000, 2011)[10].

No thresholds for these **hypothesized mechanisms** have been identified by the authors of these experimental studies; this is primarily due to the absence or the lack of a clear dose-response relationship in these studies, which have often used a single dose, essentially in the higher dose range (100 μM to 1 mM), ACL 100 μM being the most used. Justification for using this particular concentration appears to be two-fold: (i) it corresponds to the highest concentration of Al which had previously been shown to have no detrimental effect on proliferation of human breast cells in the long term, as determined by Darbre (2005b), however she had originally determined this concentration *"as the highest level without any inhibitory effects on growth over 14 days"* (Darbre, 2005b); (ii) 100 μM represents the biological concentration at the upper end of measurements in human breast tissue, which has been reported to be in the range 0.8-87μM[11] (Farasani and Darbre, 2015).

[10] Hanahan and Weinberg (2000, 2011) have established a framework for understanding the complexity of cancer development by defining six basic hallmarks of cancer (*sustained proliferative signaling*, *evasion of growth suppression*, *resistance to cell death*, *replicative immortality*, *induction of angiogenesis*, *activation of invasion and metastasis*), together with two further enabling characteristics (*genomic instability*, *tumor-promoting inflammation*) and two emerging hallmarks (*reprogramming of energy metabolism*, *evasion of immune suppression*).

[11] Levels of Al in human breast tissue have been reported as ranging from 4-437 nmol/g dry weight (Exley et al., 2007): assuming tissue is about 80% water and 1 g of tissue has a volume of 1 ml, these concentrations equate to 0.80×10^{-6} M to 0.87×10^{-4} M (Farasani and Darbre, 2015; as cited).

In conclusion, SCAHT notes:

- ***There is to date no evidence that Al is carcinogenic either in humans or experimental animals, however the database is limited. There is no indication of carcinogenicity at high dietary doses (up to 850 mg Al/kg bw/day) in animal studies (SCCS, 2014). Similarly, Krewski et al. (2007) concluded that experimental studies in animals failed to demonstrate carcinogenicity to Al exposure alone. Regulatory bodies have concluded that Al is not carcinogenic and does not increased risk of breast cancer (ATSDR, 2008; Afssaps, 2011; SCCS, 2014).***

- ***Experimental studies with human or murine normal or transformed mammary cell lines present only circumstantial evidence that Al may play a role in breast cancer development. These studies suffer from numerous insufficiencies in the study design, conduct, reporting, and statistical analysis. Major limitations are: (i) a lack of proper characterization of the test system, as often many basic cellular biology and toxicity experiments are missing (e.g. how much Al enters the cell, what type of intracellular effects it may trigger, whether proliferating cells reach confluence, or whether high concentrations of Al used are cytotoxic); (ii) use of single high dose experiments, with limited exposure time points, and water as negative control which is not equimolar in H^+ and Cl^-; (iii) the lack of statistical significance for many of the data obtained or even the absence of a statistical analysis of the data for some of these studies. No thresholds for these hypothesized mechanisms have been identified, due to the absence or the lack of a clear dose-response relationship in these experimental studies.***

- ***The epidemiological database in relation with the use of antiperspirants (and/or deodorants) is very limited and contradictory. A major limitation of some of these studies relates to whether study subjects have been exposed to Al or not, when the study design does not allow to discriminate the use of antiperspirants from the use of deodorants.***

- ***Collectively, uncertainties with the experimental database lower the overall strength of evidence and make conclusions drawn from them difficult.***

5 Exposure and risk

Human exposure to Al is widespread through food, cookware and food contact materials, drinking water, pharmaceuticals and consumer products, including cosmetics. According to EFSA (2008) and JECFA (2012), the diet is the major contributor to the total Al exposure for the general population, with inhalation and dermal exposure playing only a minor role.

Dermal exposure to Al from cosmetics has been generally considered to be a minor pathway, however full quantitative risk assessments have not always been conducted, due to major data gaps on Al absorption and fate following dermal application, and on its possible contribution to the total Al exposure. This leads to high uncertainty when assessing risk from dermal exposure to Al (Afssaps, 2011; VKM, 2013). Given the current knowledge gaps, SCCS (2014) concluded that it was not feasible to conduct a dermal risk assessment.

5.1 Intact skin

It is assumed that Al exhibits low percutaneous penetration in intact skin, based on its ability to form complexes with skin proteins (in particular keratin), which tends to restrict Al penetration to the more superficial layers of the epidermis (Guy et al., 1999; Hostynek, 2003; Krewski et al., 2007; Lansdown, 2011), though deeper penetration has also been occasionally observed (see **section 3.2.1**). It is generally considered that the skin represents an efficient barrier to the dermal absorption of Al. This is based on the (often implied) assumption of an intact skin, with full structural and functional integrity of the *stratum corneum*. The epidermal binding and denaturation of the *stratum corneum* are considered to be transitory and of little physiological relevance, the Al-keratin plugs being somehow lost through normal physiological removal mechanisms (Strassburger and Coble, 1987; Lansdown, 2011).

5.1.1 Skin permeation routes

Several permeation routes for Al salts are possible once dissolved on the surface of the skin:

- **Transcellular pathway** through keratin-rich corneocytes and keratinocytes. Al has been shown to strongly bind -SH ligands in epidermal keratin, leading to the formation of Al-keratin complexes and progressive denaturation of epidermal keratin (Lansdown, 1973a, 2011). Hardly any Al is believed to diffuse into the *stratum corneum* of an intact skin via this route. Mayeux et al. (2012) studied *in vitro* and *in vivo* deposition patterns of ACL onto the skin and found that Al was restricted inside the skin microrelief lines and as annular deposits where acrosyringia are opening. Repetitive applications increased both the deposit area and the barrier function.
- **Paracellular pathway** through: **(i)** lipid bilayers or tight junction (TJ)-derived structures[12] in the *stratum corneum*. Watkinson et al. (2002) investigated the effect of Al-containing antiperspirants on the barrier functionnality of the axillary *stratum corneum*; they reported a reduced barrier function due to changes in the ceramides-to-cholesterol ratio. They hypothesized that this unique feature may allow for an increase in water flux, which may affect in turn the permeability of electrolytes such as Al and other metals; **(ii)** through TJs in the *stratum granulosum*[13], as well as in the intra-epidermal and intra-dermal epithelial cells lining the sweat ducts and coils. Wilke et al. (2006) suggested potentially weak points in the epithelial barrier function, that may favour Al absorption through the skin: (i) the *acrosyringium* lacks functional TJs, and is not completely keratinized, in particular below the *stratum spinosum*; (ii) the

[12] Haftek et al. (2011) observed TJ-like structures and associated protein claudin 1 in corneocytes, and suggested that their persistence in the *stratum corneum* could potentially increase lateral intercorneocyte cohesion.

[13] Tight junctions are complex epithelial transmembrane cell-cell junctions that form a functionnal barrier in the *stratum granulosum* (Brandner, 2016), due to the co-localization of TJ-associated transmembrane proteins occludin, claudin-1 and claudin-4, which is not the case in the *stratum spinosum* or in the *stratum basale* (Wilke et al., 2006).

dermal portion of the eccrine sweat duct in the superficial dermis, and the apical eccrine and apocrine coil in the deep dermis, possess a functional TJ barrier, but are not keratinized It has been hypothesized that since the dermal duct and the sweat glands provide a barrier which consists only of TJs, the latter could be a possible entry route for Al and other metal electrolytes, if alterations of TJs-associated proteins are present[14] (Brandner, 2016).

- **Shunt pathway** through hair follicles and sweat ducts. The latter is considered to be one, if not the predominant pathway for the diffusion of strong electrolytes across the skin (within minutes) (Hostinek, 2003). Since innermost ductal epithelial cells and hair follicles are keratinized, Al binding with epidermal keratin and other proteins will lead to the obstruction of skin shunts, i.e. through 'plugging' of the sweat ducts and hair follicles.

5.1.2 Factors that may influence dermal absorption of Al

Most metal salts which have been investigated traverse the *stratum corneum* barrier in dissociated state with apparent permeability coefficients (K_p) on the order of 10^{-5} to 10^{-4} cm/hr (K_p for water is about 10^{-3} cm/hr). Permeability coefficient for Al has not been established yet (Guy et al., 1999). Among the Key factors driving Al permeation skin rate are: (i) the nature of the counter ion; (ii) Al strong electrophilicity and reactivity due to the small ionic radius to high charge ratio, leading to protein binding[15], which will determine the depth of penetration, and in turn the efficacy and duration of the antiperspirant effect; (iii) pH, since different hydrolysis products are formed at different pH values, thus affecting Al hydroxide polymerization (Guy et al., 1999; Hostynek, 2003).

Electropositive metals have been shown to strongly interact with nucleophilic residues such as proteins in the epidermis, which may result in significant deposits and/or reservoirs (e.g. Ni, Cr) or form complexes with metallothioneins (e.g. Cd, Cu, Zn) (Guy et al., 1999). This has yet to be established for Al, and there is to date no evidence that Al can accumulate in the skin. The quantity applied (per area) also has an influence on the level of penetration. This is usually inversely correlated to penetration, i.e. for large quantities the penetration rate is lower than for small quantities (Bfr, 2014).

Little is known about the endogenous factors that may affect the dermal penetration of Al salts (Hostynek, 2003). Some are subject to marked interindividual variability, e.g. acidity of the skin, state of hydration or the level of sebaceous lipids present on its surface, or other physiological conditions such as the presence of homeostatic controls for metals, which may contribute to modulate Al absorption/diffusion in the skin, as observed for Zn^{2+} and Ca^{2+}) (Guy et al., 1999). Ageing has been associated with decreased lipid surface content, reduced skin diffusivity and vascularization.

Regional differences in *stratum corneum* thickness (e.g. palmo-plantar > forearm > axillary skin) and shunt density can markedly affect metal accumulation and penetration. Compared to other skin types such as palmo-plantar or forearm skin, it appears that human axillary skin possesses specific physiological features such as: (i) an hyper-hydration state due to the closed nature of the axillary vault; (ii) a relative high density of eccrine and apoecrine sweat glands; (iii) a high density of sebaceous glands; and (iv) elevated skin temperatures (Evans et al., 2012). Axillary skin is usually considered as one of the thinnest skin area of the human body, though comparative studies in the literature are scarce and limited primarily to some historical investigations (Lee and Hwang, 2002).

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[14] Loss of transmembrane proteins expression, of TJ integrity and of adhesion has been shown to promote breast cancer development and metastasis (Brennan et al., 2009; Martin et al., 2010; Salvador et al., 2016).

[15] Protein reactivity has been documented for Al, in particular towards keratin, albeit mainly in the sweat ducts. In vitro studies also suggest that Al has a high affinity for the S100 Ca^{2+}-binding proteins, which are ubiquitous epidermal proteins contributing to prevent losses of Ca^{2+} from the normal desquamation process (Sato et al., 1989 cited in Wilke et al., 2006; Saurat et al., 1981). S-100 proteins are abundantly expressed by the basal cells of the eccrine coil (Wilke et al., 2006).

- **Lee and Hwang (2002)** showed that axillary skin epidermis was the second thinnest out of 28 different types of skin biopsied from Korean women (n=6) with a mean of 54.6 μm, and were 36% thinner as compared to Korean men (n=6, 85.6 μm, P<0.01). Epidermis in the axillary vault was 32-44% thinner than in the forearm area (front of forearm, 80.3 μm; back of forearm, 97.1 μm). The study did not measure *stratum corneum* thickness.

It should be noted that most of the studies on skin application and penetration of Al salts evaluated eccrine glands in the forearm or palmar skin, which has been shown to differ markedly with axillary skin with regard to the *stratum corneum* composition (e.g. ceramides, cholesterol, fatty acids) (Wu and Kilpatrick-Livermore, 2011; Watkinson et al., 2002), and to a lesser extent, with regard to the epidermis thickness (Lee and Hwang, 2002). Watkinson et al. (2002) using transepidermal water loss and corneosurfametry reported a unique ceramides-to-cholesterol ratio in the axillary *stratum corneum* in Caucasian women (18-55 yrs old), which suggests a reduced barrier function as compared to other skin types. Axillary cornified envelopes have been found to be smaller than those found on forearm skin, indicative of a shorter *stratum corneum* turnover (Evans et al., 2012).

5.2 Razor-damaged skin

Historical investigations and mechanistic studies of dermal application of Al-containing antiperspirants in humans have hardly considered the impact of a weakened or compromised skin on the tolerance, efficacy or potential permeation of Al salts (Hölze and Braun-Falco, 1984; Hölze and Kligman, 1979; Papa and Kligman, 1967; Quatrale et al., 1981a, 1981b, 1981c, 1985; Reller and Luedders, 1977; Strassburger and Coble, 1987; Sulzberger et al., 1949).

5.2.1 Effect of shaving on skin

The effects of shaving on human skin integrity and percutaneous absorption are incompletely studied (Evans et al., 2012; Hamza et al., 2015; Gattu and Maibach, 2010, 2011). While direct physical damage to the *stratum corneum* around the hair follicles by scratching, and increased epidermal hyperproliferation after chronic shaving have been described (Marti et al., 2003), insight into the potential physiological impact of shaving on the epidermis and dermis is largely lacking. In particular, data on female axillary shaving are scarce, and are limited to two studies, conducted by Unilever (Marti et al., 2003; Tuner et al., 2007):

- **Marti et al. (2003**) were the first to assess the acute and chronic effects of shaving (dry razor) on the skin structure, biochemistry and function. Interestingly, a higher shaving frequency (once a day vs once a week over a 4-week period, with concomitant use of undisclosed antiperspirant roll-on) was associated with a higher level of visible irritation, increased dryness and scaling, but these changes were not reflected in the lipid barrier composition (i.e. in term of ceramide, cholesterol, fatty acids content) or with observed effects on the *stratum corneum* barrier function, with no corresponding further removal of tissue. They concluded that gentle shaving can prime the viable epidermis to produce an elevated inflammatory response (as demonstrated by the significant elevation of histamine-induced itch and -induced neurogenic flare) without physically breaking the skin barrier.

- **Turner et al. (2007)** assessed the impact of shaving (dry razor) on the *stratum corneum* thickness of the axillary vault (pilous) and fossa (non-pilous) and on skin inflammatory response. Results showed that axillary shaving removes the *stratum corneum*, with a mean value of 36.1 ± 21.7% w/w (mean ± SD, n=6 women) of the shaved debris being skin. Thickness of the axillary epidermis, as well as inflammatory response as evidenced by histamine-induced itching and flare, was higher in shaved areas than in unshaved areas, but flare in the axillary fossa was greater than in the axillary vault. They concluded that chronic damage to the epidermis could set off a cascade of pro-inflammatory mediators which may trigger keratinocytes hyper-proliferation leading to the development of a thicker epidermis - a mechanism which suggests adaptation to frequent shaving, yet not sufficient to protect the axilla from damage and irritation.

There are indications that the axillary skin may have a reduced barrier function compared with e.g. forearm or palmo-plantar skin, owing to its enhanced cholesterol-to-ceramide ratio (Watkinson et al., 2002; Wu and Kilpatrick-Livermore, 2011). It was suggested that the increased ceramide level in the axillary skin, observed primarily in younger women, may be related to increased cell proliferation and thickened epidermis in response to frequent shaving (Wu and Kilpatrick-Livermore, 2011). While more frequent shaving was associated with a higher level of visible irritation (Marti et al., 2003, Turner et al., 2007), no significant changes in the lipid barrier composition of the *stratum corneum* were observed.

It has been also hypothesized that antiperspirant formulations contain also emulsifying agents which can also contribute to alter further the lipid and protein content and reduce the *stratum corneum* barrier function. Such a case was observed by Sulzberger et al. (1949), who tested on previously shaved women's axillary skin two antiperspirant cream formulations both containing 20% Al sulfate (a less acidic and weak antiperspirant), but varying concentrations of the surfactant alkyl benzene sulfonate at 8% (A) and 4% (B). They reported a "*uniform and least expected*" strong inflammatory reaction in all study participants within an hour after a single application of formula A. Formula B caused only mild irritation, and blank vehicle control did not produce any visible sign of irritation. Swaile et al. (2012) noted that men with unshaven axillae experienced less local irritation than those with shaved skin because bound to underarm hair.

Available evidence consistently indicates that mild shaving already results in loss of *stratum corneum* barrier function, visible or sensory irritation, increased skin dryness and scaliness. Harsh shaving will likely create more severe abrasions, nicks or cuts, exposing directly the dermis and providing an easy access to the blood or lymph circulation. This is also supported by indirect evidence for other chemicals gained from *in vitro* and *in vivo* studies conducted on impaired or compromised human skin:

- **Gattu and Maibach (2010, 2011)** have reviewed human *in vitro* (n=8) and *in vivo* (n=15) studies measuring dermal absorption of xenobiotics through damaged or diseased skin. In most studies, they found a modest but clear enhancement in absorption in compromised skin compared to intact skin, generally in favor of hydrophilic molecules, and less so for lipophilic compounds. Only one *in vitro* study reviewed included a metal (nickel), but enhancement factor was reported to be zero. The authors concluded that while most methods of mechanical damage[16] produced a varying degree of physical, chemical or biological damage resulting in some penetration enhancement, this did not remove all skin barrier properties. They also noted the general need for additional studies, with more consistent study designs and measurement methodologies, as well as information on correlations between *in vitro* and *in vivo* data to fully elucidate how much absorption/penetration occurs through the many types and degrees of damaged skin.
- **Hamza et al. (2015)** recently reviewed the literature to assess the effect of shaving on percutaneous penetration of xenobiotics and skin function but could not identify additional human studies to the studies of Marti et al. (2003) and Turner et al. (2007). The other studies presented in their review were experimental animal studies in rodents, pigs and monkeys, which showed controversial results (positive and null findings) on percutaneous absorption, which appeared to be both species, location site and chemical specific. These studies differ largely in their designs, methods and exposure conditions, and are of poor relevance for the human axillary exposure scenario.
- **Guillard et al. (2004)** reported a case of a 43-year-old woman who applied about 1 g of an antiperspirant cream containing ACH on each underarm after washing and shaving every morning for 4 years (calculated to amount a daily external dose of 0.108 g corresponding to a cumulative dose of 157.30 g).

[16] 'Tape stripping' is the most widely used *in vitro* method to mimic the shaving (or waxing) scenario (removal of the *stratum corneum* to expose the *stratum granulosum* of the epidermis), but may also mimic impaired skin caused by eczema or other skin conditions (by increasing transepidermal water loss) (Gattu and Maibach, 2010, 2011).

She shaved her armpits on a regular basis, and never experienced skin irritation or rashes. Occupational exposure and exposure to Al-containing antacid pharmaceuticals could be excluded, but no information was given on other potential exposure sources to Al. The patient complained of bone pain and extreme fatigue, but had no abnormal neuropsychological or electroencephalographic findings. Average blood plasma Al level was 3.9 µM (normal plasmatic values = 0.37 µM) and urinary Al level was 1.71 µM/24 h (normal urinary values = 1.10 µM/24 h). Blood levels returned to the normal range in ca. eight months (0.1-0.3 µM) after discontinuation of antiperspirant use. No attempt was made to link the hyperaluminemia with breast cancer (further discussion of this case is given by Exley, 2004b).

In conclusion, there is sufficient evidence to indicate that shaving, especially in case of small injuries, will likely result in an increase of dermal absorption of Al compared to an intact skin. A partial or complete loss of the *stratum corneum* function will facilitate the access to, or directly expose the epidermis.

⇒ This contrast with the view of the German Cosmetic, Toiletry, Perfumery and Detergent Association (IKW, Industrieverband Koerperpflege- und Waschmittel e. V.) which stated that *"antiperspirants can be applied without any concerns on shaved skin, too"* (IKW, 2017). According to their own safety assessment [*SCAHT notes: unpublished*], data indicate that during typical shaving of the armpits, only a low amount of the upper layer of skin is removed as a rule, which corresponds to the amount of skin particles which are normally scrubbed from the skin in the course of a day (IKW, 2017).

⇒ Darbre (2013b, 2016) pointed out that current widespread cultural practices of shaving prior to antiperspirant application conflicts with EU and US regulations which do have warning statements that antiperspirants should not be applied to broken, damaged or irritated skin: *"Do not apply to broken skin. If rash or irritation develops, discontinue use."* (FDA 1982, *§350.50 Labelling of antiperspirant drug products*); "*do not apply to irritated or damaged skin*." (EEC, 1976).

5.3 Dermal absorption studies

We have located only four studies that have measured permeation rates of aluminium following dermal exposure *in vitro* or *in vivo*. These are briefly and critically reviewed below.

- ***Anane et al. (1995) Bioaccumulation of water soluble aluminium chloride in the hippocampus after transdermal uptake in mice.***

  Anane et al. (1995) found increased levels of Al in the urine of Swiss male mice exposed to 0.1 or 0.4 µg/day Al chloride (0.01-0.04 µg Al/day) applied daily to a 4 cm^2 shaved area for 130 days. Previously *in vitro* data were reported as part of this study leading to very high absorption rates (absorption rates of 98 % for the 50 ng dose and 45 % for the 100 ng dose). Concerns have been raised regarding the interpretation and the validity of the study findings due to multiple methodological bias (Afssaps, 2011; IAI, 2007; SCCS, 2014; VKM, 2013): (i) application on large area of back skin; (ii) no control measures to prevent oral exposure from grooming; (iii) the reported increase of brain aluminium suggests >100% bioavailability; (iv) not a GLP study; (v) it does not fulfil the SCCS requirements. This study is considered to be non-reliable.

- ***Flarend et al. (2001) A preliminary study of the dermal absorption of aluminium from antiperspirants using aluminium-26.***

  After repeated exposure under occlusion for 6 days to ACH 21 % (about 13 mg of aluminium) to each axilla under occlusive dressing in two volunteers (one man and a woman), on skin previously tape stripped twice, blood and urine samples were collected over 7 weeks. Aluminium was detected in the

blood 6 hours after the first application and remained detectable for 15 days. On the basis of the excreted quantity of isotope-marked aluminium over 14 days via the urine, the authors calculated a penetration rate of approximately 0.012 %. The highest value of absorbed dose was 0.052%. Main reported shortcomings are: (i) not a GLP study; (ii) poor statistical power as it was performed using 2 tests subjects only; (iii) data showed major differences between the test subjects; (iv) relevance of the exposure scenario (solution, occlusion conditions), which may overestimate the absorption rate. For these reasons, the only *in vivo* human study conducted so far is of limited value for regulatory risk assessment purposes (Afssaps, 2011; SHC, 2015; BfR, 2014; SCCS, 2014).

- ***Pineau et al. (2012) In vitro study of percutaneous absorption of aluminum from antiperspirants through human skin in the Franz™ diffusion cell.***

  This study reports unpublished data performed by the cosmetic industry (Laboratoire PMIC) in 2007 at the request of Afssaps (2011). Dermal absorption of ACH from three antiperspirant formulations (spray 38.5%; roll-on 14.5%; stick 21.2%, intact vs stripped skin) was tested using human full abdominal skin biopsies (n=5), following 6, 12 and 24hrs exposure. The study was realized according to OECD TG 428 and the SCCS recommendations. They calculated penetration rates for the spray, roll-on and stick formulations of 0.65%, 0.18% and 0.96%, respectively. In the scenario of damaged skin by tape stripping, the penetration rate was 5.9%. This study suffers from several limitations: (i) the lack of an intact vasculature; (ii) results suggest that contamination from environmental Al sources are possible, since Al concentrations increase over time, regardless of the sample type; (iii) large variability in all measured Al samples (SD > 60%); (iv) large deviations in mass balance values (51±10% to 141±29%) falling outside the SCCS criteria for validity (100±15%). The mass balance values were omitted when the PMIC study was published, preventing public scrutiny of this key criterion for a valid study (SCCS, 2014). For these reasons, both the SHC (2015) and the SCCS (2014) rejected the Pineau study for risk assessment purposes. On the other hand, VKM (2013) concluded that it fulfilled most of the SCCS requirements.

- ***Mistry et al. (2013) Effect of occlusion on the percutaneous absorption of aluminium from antiperspirant products.***

  This *in vitro* study also used the Franz™ diffusion cell to test the dermal absorption of Al chloride and ACH of four antiperspirant formulations (aerosol, roll-on, stick), under occlusion or without occlusion. Human scrotal skin samples were obtained from surgery, immediately frozen at -30°C and stored up to a maximum of 4 months until the experiments. While the authors attempted seemingly to replicate the Pineau experiment, they did neither follow OECD guidelines nor SCCS recommendations. Several other limitations can be observed: (i) information of surface of diffusion is lacking; (ii) information on number of biopsies and patients demographics is completely lacking; (iii) no tissue viability; experimental conditions are not physiologically relevant (citric acid at pH 5.5); (iv) lack of quality control measures (contamination sources, skin thickness); (v) lack of experimental details (number of diffusion cells by experiment and donor for all the experiments); (vi) permeation rates of non-occluded skin found in some instances greater that with occlusion (0.022% vs 3.437%). This is a poor quality and non-reliable study. [*SCAHT notes: this study was not reviewed by the SCCS (2014) or the SHC (2015)*].

In conclusion, the available dermal absorption studies are of poor quality and do not meet current standards for use in regulatory risk assessment. Irrespective of their flaws and limitations, and in the absence of a better alternative, some regulatory bodies have still considered the Pineau et al. (2012) study to conduct a risk assessment of Al-containing antiperspirants (Afssaps, 2011; BfR, 2014; VKM, 2013) (see **section 5.4**).

Important knowledge gaps remain regarding the dermal bioavailability of Al, and to what extent it may be retained in the viable intact or compromised axillary epidermis and dermis, to reflect realistic exposure conditions. In particular, the permeation rates calculated by Pineau et al. (2012) based on occluded

administration may not be representative of the shaved underarm skin scenario (BfR, 2014). Indeed, skin occlusion can significantly affect the skin barrier function, the hydration level, lipids profile, as well as molecular and cellular turnover and processes (Zhai and Maibach, 2001). Pineau et al (2012) may have therefore overestimated permeability rates, however increase in moisture content is not always correlated with an increase of percutaneous absorption for topically applied chemicals (Zhai and Maibach, 2001). In addition, these four studies may reflect poorly (if at all) the toxicokinetics of Al in a chronic, long-term consumer exposure scenario.

- To address these knowledge gaps, a new industry study on aluminium skin absorption from antiperspirants should be available soon. Cosmetic Europe has been mandated in 2014 to produce a state-of-the-art study with dermal exposure to ^{26}Al in healthy volunteers to address the uncertainties from the PMIC and Flarend et al. studies. The study outline was presented by Cosmetic Europe at a BfR symposium in November 2014[17,18].

5.4 Dermal exposure assessments

- **Afssaps (2011)** used dermal absorption data from an unpublished study by Laboratoire PMIC (2007), commissioned by the industry Fédération des entreprises de la beauté (*In vitro* percutaneous absorption of ACH through human skin, PMIC, Antony, France, 2007). In this study, conducted in accordance with OECD Test Guideline 428, dermal passage ("absorption") of aluminium was measured *in vitro* in Franz cells after application of ACH by aerosol (38.5% ACH), roll-on emulsion (14.5% ACH) or stick (21.2% ACH) to intact skin, or for the stick, also to 3-tape stripped skin. Dermal absorption across intact skin was 5% for all 3 formulations, across stripped skin with the stick it was 18%. Based on these data, Afssaps calculated that antiperspirant use would lead to an additional exposure of 2.1 mg Al/kg bw/day for the first scenario, and of 75 mg Al/kg bw/day in the second scenario. In conclusion, the margin of safety is 11 in intact skin exposure conditions and less than 1 in the case of damaged skin exposure conditions.
- **BfR (2014)** used dermal absorption data from a study by Pineau et al (2012), which appears to be the peer-reviewed version of the unpublished Laboratoire PMIC (2007) study used by Afssaps (2011). In Pineau et al paper, the calculated penetration rates for the spray, roll-on and stick formulations were reported to be 0.65%, 0.18% and 0.96% across intact skin, respectively (for comparison, the PMIC data were reported by Afssaps 2011 as 5% for the three formulations). For tape-stripped skin, dermal transfer was 5.9% (versus 18% reported from the PMIC study by Afssaps). who reported *in vitro* (Franz cell) data (cited by VKM 2013 and BfR 2014). Thse data were used to calculate a systemic exposure dose (SED) of 10.7 (spray), 2 (roll-on) and 10.5 (stick) µg/kg bw/day. For skin damaged by 'tape stripping', the SED was 74.5 µg/kg bw/day. Given the TWI of 1 mg/kg and oral bioavailability of 0.1%, the corresponding SED is 0.175 µg/kg/day. BfR (2014) therefore considered that there is a risk of exceeding the TWI by use of Al-containing antiperspirant even for intact skin.
- **VKM (2013)** used the Pineau et al. (2012) data to estimate an absorption rate of 0.6% in intact (normal) skin and of 10.7% in tape-stripped skin (impaired skin). According to VKM (2013) *"the total exposure to aluminium from food and the use of cosmetic products was estimated as a 'systemic' exposure. Thus, for comparison, the TWI set by EFSA (2008) was recalculated to 1 µg Al/kg bw/week, while the PTWI set by JECFA (2012) was converted to 2 µg Al/kg bw/week, taking into account an oral bioavailability of 0.1% (EFSA, 2008) and*

[17] Mason D, Bury D, Cosmetics Europe. Presentation at BfR Symposium, 26-27 November 2014 available at http://www.bfr.bund.de/cm/343/cosmetics-industry-remains-confident-exposure-to-aluminium-from-cosmetic-products-is-negligible.pdf

[18] http://www.bfr.bund.de/de/presseinformation/2014/32/aluminium_im_alltag__ein_gesundheitliches_risiko_-192135.html

assuming similar toxicity following oral and dermal exposure to aluminium. These tolerable intakes are termed systemic TWI, and are estimates of the amount of aluminium that can be absorbed (oral and dermal absorption) weekly over a lifetime without appreciable health risks." (Deviations: mean body weight of adults in the Norwegian population as reported in the national dietary surveys is 77.5 kg).

- ***SCAHT note on tape stripping**: In all the above studies, it is assumed that tape stripping mimics shaving. Studies which specifically address axillary skin properties after shaving have reported no change in skin barrier properties (Marti et al., 2003), and epidermal thickening consistent with keratinocyte hyper-proliferation (Turner et al., 2007). These data cast some doubt on the relevance of stripped skin studies to quantify the dermal bioavailability of Al salts after shaving.*

5.5 Antiperspirants and other sources of dermal Al

Antiperspirants and deodorants are believed to be used by over 90% of the US population (Behohanian, 2001; Flarend et al., 2001; McGrath, 2003); similar use patterns probably apply to Europe and other industrialized countries worldwide. Unfortunately, possible misclassification or lack of distinction between antiperspirants and deodorants in some studies hampers interpretation (Fakri et al., 2006; Linhart et al., 2017; McGrath, 2003; Mirick et al., 2002).

Roll-on may be the most representative formulation used (Afssaps, 2011; VKM, 2013; SHC, 2015). The various formulations (spray, roll-on, stick, cream) may differ in term of exposed skin surface area (SSA) and frequency of application (SCCS, 2011), as well as kinetics upon skin deposition. For sprays, the deposition of Al salts will likely not be restricted to the axillae, but also to a small extent to the trunk, and there is also the possibility of inhalation exposure.

Other potential dermal sources of aluminium include cosmetics (e.g. sunscreens, lipsticks, toothpastes) and pharmaceutical products (e.g. antacids). It has been speculated that under certain conditions, Al could desorb from titanium oxide nanoparticles used in some sunscreens; BfR (2014) noted that aluminium-containing sun lotions, given their large skin surface application and pattern of use (several times a day), may also lead to higher Al exposure (BfR, 2014). According to Nicholson and Exley (2007), applying sun lotions five times a day on the body surface (ca. 1000 mg/day) could lead to a mean additional absorption of approximately 140 µg (0.14 mg), based on the Flarend et al. (2001) penetration rate of 0.014%. However, no information is provided by Nicholson and Exley to justify their assumptions and the reason to deviate from the recommended default values[19] proposed by the SCCS guidance (SCCS, 2011).

Dermal exposure is possible from pharmaceutical and cosmeceutical products. BfR (2014) noted that creams containing 5% ACH are available on the EU market to relieve sore and damaged/broken skin. Aluminium acetate tartrate solution is marketed in various products in Switzerland for superficial skin damage[20]. Aluminium diacetate is listed in the WHO Model List of Essential Medicines as an antiseptic agent used in wet dressings to assist healing of skin wounds[21].

[19] Recommended values for sunscreen lotions are a frequency of application of twice daily for a total daily application of 18.0 g (SCCS 2011).

[20] (http://www.pharmawiki.ch/wiki/index.php?wiki=Euceta&search=Aluminii%20acetatis%20tartratis#bottom).

[21] (http://apps.who.int/medicinedocs/en/d/Jh2918e/25.1.html).

6 Evidence for/against aluminium as a breast carcinogen

6.1 The 'Darbre hypothesis'

A few scientific groups have been working over the last decade on the **hypothesis proposed by P. Darbre (2001) that the topical application of Al-containing antiperspirants could increase the incidence of breast cancers in women** (Exley et al., 2007; Darbre 2001, 2003, 2004, 2006a, 2006b, 2016; Darbre et al., 2013b; Harvey, 2003; Harvey and Darbre, 2004; Mandriota et al., 2016; Mannello et al., 2011, 2013; Pineau et al., 2014; Sappino et al., 2012). Several lines of evidence, hypotheses, and experimental observations, and their further interpretations, have been presented the authors of these studies, in particular:

- *The high incidence of breast cancers as well as breast cysts and fibroadenomas observed in the UOQ region, AND the UOQ region is coincidentally close to the axillary skin area where antiperspirants are applied. [SCAHT notes: this is considered the strongest supporting evidence for a role for underarm cosmetics in breast cancer (Darbre, 2001, 2003, 2004, 2005a; Harvey and Darbre, 2004; etc), based on the consideration that the evidence for larger density in the UOQ region of the breast seems "largely anecdotal" (Darbre, 2001)].*
- *The parallel progression noted between antiperspirant sales AND the incidence of breast cancer, in association also with higher income and western axillary hygiene practices (Darbre, 2001; McGrath, 2003, 2009).*
- *The unexplained 50% increase in incidence rate of ductal carcinomas in the 1970's-1990's in the UK, AND the hypothesis of an unidentified missing environmental component, since over 50% of women with breast cancer have no major identified risk factors (McGrath, 2003; Darbre, 2011; Darbre 2013b).*
- *The early age of onset of cancers among users of antiperspirants (McGrath, 2003).*
- *Al is a metallestrogen AND estrogen is a risk factor for breast cancer (Darbre 2005b). [SCAHT notes: the claim that Al is a metallestrogen is based on a single ER-binding and gene expression assay in one study (Darbre 2005b). No mode of action has been proposed to characterize how Al binds and interferes with the ER]*
- *Antiperspirant use leads to Al accumulation in the underlying breast tissues due to its high absorption through the axillary skin, AND breast cancer patients have higher Al concentrations in breast tissues, NAF or BCF, compared to healthy individuals (e.g. Darbre, 2005a; Mandriota et al., 2016; Mannello et al., 2011, 2013; Sappino et al., 2012).*
- *Breast cancer arises from epithelial cells lining the breast ducts and lobules, AND Al can accumulate in the apocrine ductal epithelial cells and block their secretions (Darbre, 2016; Darbre et al., 2011, 2013b).*

Alternative explanations to the 'Darbre hypothesis' exist, but have been largely ignored by the proponents of this theory. Interpretation of these data needs to consider the complexity of the biology and epidemiology of breast cancer (**section 6.2**), including consideration of several factors which are well-established confounders of the proposed association (**section 6.3**), in particular:

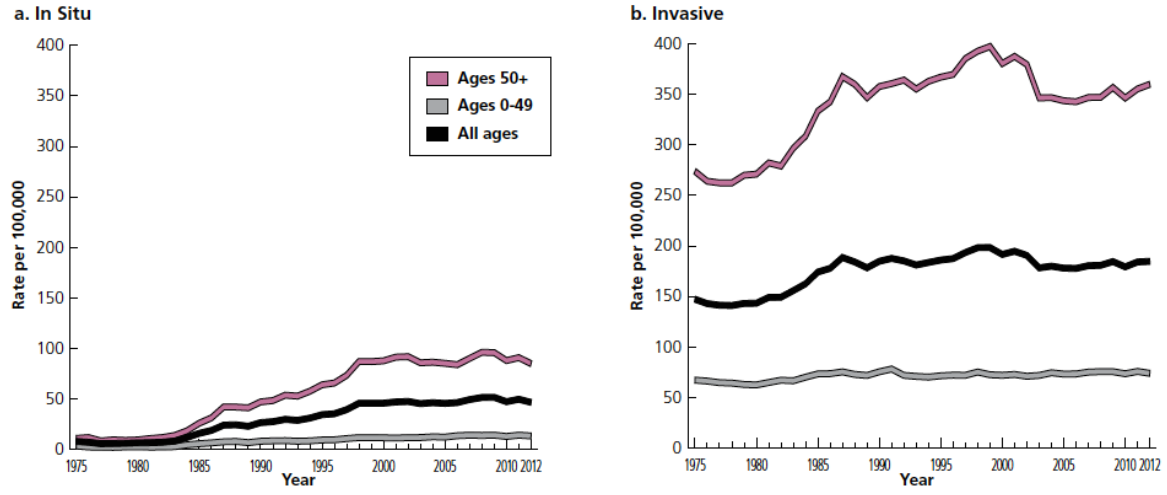
- *The higher parenchymal density of the breast tissue in the UOQ region compared to other breast areas may explain the higher observed incidence of pre-cancerous lesions and cancers in the UOQ;*
- *Many other well-established epidemiological risk factors may confound a potential association between Al and breast cancer in women, including reproductive, clinical, genetic and environmental factors (Namer et al., 2008; Kurian et al., 2010; Barnard et al., 2015).*
- *Many metals can accumulate in cancer breast tissues and have been shown to induce proliferation of human breast cancer cells in vitro (see **Appendix 7**).*

- *The possible influence of other factors that may explain many of the observed effects in these studies (such as the influence in cell culture media of free chlorine and low pH, known to generate ROS leading to genotoxicity and mutagenicity in in vitro cell cultures).*

6.2 Breast cancer epidemiology, biology, and risk factors

Recent epidemiological evidence demonstrates that despite the continued increase in sales of antiperspirants and deodorants over 1970-2000, the incidence of breast cancer has remained stable in premenopausal women; these data do not support the McGrath (2003) hypothesis of a parallel progression of antiperspirant sales with the incidence of breast cancer (Namer et al., 2008). A comparison of incidence rates in 2001 with those in 2004 in the United States demonstrates a decrease in annual age-adjusted incidence of 8.6% (95% CI 6.8-10.4). The decrease was evident only in women 50 years of age or older, and was more evident in ER-positive cancers than in ER-negative cancers. The observed decrease in breast-cancer incidence was attributed to the drop in the use of hormone-replacement therapy among postmenopausal women in the United States following the first report of the Women's Health Initiative (Radvin et al., 2007). Evolution of the annual incidence of breast cancer in US women (1975-2012) can be seen in **Figure 5** below.

Figure 5: Trends in In Situ and Invasive Female Breast Cancer Incidence Rates* by Age, US, 1975-2012



*Rates are age adjusted to the 2000 US standard population within each age group.
Source: Surveillance, Epidemiology, and End Results (SEER) Program, SEER 9 Registries, National Cancer Institute.

American Cancer Society, Inc., Surveillance Research, 2015

Source: American Cancer Society. Breast Cancer Facts & Figures 2015-2016. Atlanta: American Cancer Society, Inc. 2015.

Namer et al. (2008) pointed out that some facts and questions seem to have been overlooked when considering a potential association between Al and antiperspirant: *"If the link between the use of antiperspirants and breast cancer is real, how to explain the non-bilaterality of breast cancer? The non-increase of breast cancer in men? The fact that not all breast cancers are hormone dependent? And the fact that breast cancer is not the only cancer that can be hormone dependent?".* Breast cancers are mainly unilateral (97-99%) (Barranger, 2016 citing Nichol et al., 2011; Rummel et al., 2015).

Breast cancers are grouped by oncologists into four major subtypes (mainly based on the expression of hormone receptors (HR) and human epithelial growth receptor (HER2)): luminal A, luminal B, HER2-overexpressing and triple negative (which do not express estrogen receptor, progesterone receptor, or the oncogene *c-erbB2/HER2*) (Barnard et al. 2015). Most epidemiology studies and risk statistics consider breast cancer only as a single entity, but it is increasingly recognized that breast cancer subtypes vary in occurrence (especially by race/ethnicity), and

in their risk associations with other factors (Kurian et al., 2010), such as **anthropometrics**. **Height** is positively associated with risk of Luminal A breast cancer in postmenopausal women (Horn et al., 2014). **BMI** is positively associated with risk of all luminal subtypes and for the HER2 subtype in Norwegian postmenopausal women (Horn et al., 2014), but only with luminal A and not luminal B in Japanese postmenopausal women (Miyagawa et al., 2015). **Overweight and obesity** significantly increased the risk of triple negative subtype but decreased the risk of luminal breast cancer in premenopausal Turkish women (Turkoz et al., 2013). It has been suggested that there are unique **racial/ethnic specific incidence patterns** for breast cancer subtypes (Kohler et al., 2015). For example, the 'black-white crossover'[22] in breast cancer incidence refers to higher rates of triple-negative breast cancers and lower rates of HR(+)/HER2(-) breast cancers in black women as compared to white women (Clarke et al., 2012).

Major **risk factors** for the development of breast cancer include reproductive, genetic, and environmental variables, including lifestyle factors (Dieterich et al., 2014; Key et al., 2001; Kurian et al., 2010). Mutations in certain genes greatly increase breast cancer risk, but these account for a minority of cases (Key et al., 2001). Certain **susceptibility genes** are associated with high risk mutations (BRCA1/2, involved in DNA repair), and others with moderately penetrant mutations of genes (e.g. CHEK2) or with low-risk polymorphisms associated with modest effects sizes (Walsh et al., 2016). Many of the established risk factors are hormonal, in particular increased exposure to **endogenous estrogen** (associated with **early menarche**, **late menopause**, **late age of first pregnancy** or **lack of breast-feeding**, as well as use of **oral contraceptives** and **hormone substitution therapy**). Other risk factors are: **family history of breast cancer** (with first degree family member), **body mass index** (BMI), and **alcohol consumption** (Kurian et al., 2010, Barnard et al., 2015). **Breast density** (which is reflects variations in the amounts of collagen and number of epithelial and non-epithelial cells in the breast) is a major risk factor for breast cancer; only **age** and **BRCA carrier status** are associated with larger relative risks of breast cancer than percent of mammary density (Boyd et al., 2011; Gastounioti et al., 2016). Breast density and family history have been shown also as major breast cancer risk factors for women 40-49 years (OR>2), with nulliparity, oral contraceptive use and late parity playing a minor role (OR=1.0-1.5) (Nelson et al., 2012).

6.2.1 Quadrant-specific breast cancer distribution

Several clinical or population-based studies (Aljarrah and Miller, 2014; Darbre, 2005a; Lee, 2005; Rummel et al., 2015; Sohn et al., 2008) have compared female quadrant-specific breast cancer distribution and reported that the highest frequency of tumors[23] is in the upper outer quadrant (UOQ) and the lowest frequency is in the lower inner quadrant (LIQ) (see **Table 2**).

- **Darbre (2005a)** analyzed the annual incidence of female breast cancer for each quadrant recorded nationally in England and Wales between 1979 and 2000 and in Scotland between 1980 and 2001[24]. She found that the incidence of female breast cancer in the UOQ rose from 47.9% in 1979 to 53.3% in 2000 in England and Wales, and from 38.3% in 1980 to 54.7% in 2001 in Scotland. She further reported that the proportion of breast cancers in the UOQ is rising annually in a linear mode (with correlation coefficients R±SD=+0.71±0.01 and R±SD =+0.80±0.03 (both $p<0.001$) in UK & Wales and in Scotland, respectively). She also noted that this disproportionate proportion of breast cancers in the UOQ appears

[22] Breast cancer incidence is higher among black women than white women before age 40 years, but higher among white women than black women after age 40 years (Clarke et al., 2012).

[23] SCAHT note: "breast cancer" in most publications refers to malignant tumors = mammary carcinoma, as opposed to benign tumors such as breast cysts or other fibroadenoma. Yet possible misclassification/coding remains, in particular for multiquadrant overlapping tumors (see Bright et al., 2016).

[24] The data collected by Darbre (2005a) were categorized according to the ICD-10 classification (50.0 N+A; 50.1 C; 50.2 UIQ; 50.3 LIQ; 50.4 UOQ; 50.5 LOQ; 50.6 A), all corresponding to malignant mammary carcinoma.

to rise with year of publication up to a level of 60.7% in 1994 (Darbre, 2003; Darbre, 2005b).

- **Aljarrah and Miller (2014)** investigated two cohorts of women diagnosed with breast cancer in Scotland between 1957-1959 and 1997-1999. They found a statistically significant ($p<0.0001$) increase in proportion of malignant tumors located in the UOQ in the more recent cohort (53.4%) compared to the older cohort (40.5%).

The commonly accepted explanation for this differential distribution is that it reflects the higher epithelial density of the breast tissue in the UOQ compared to other breast areas (Lee, 2005; see below), which may have a higher fat content. However, alternative explanations have been proposed, such as (i) the use of antiperspirants/deodorants; and (ii) genomic instability (see **section 4.4.3**).

Some researchers have argued that the higher proportion of tumors in the UOQ may be related to the increasing use of cosmetic products (incl. antiperspirants, deodorants and other underarm cosmetics) applied to the axillary vault or breast area (Darbre, 2001, 2003, 2016; Harvey, 2003; Harvey and Darbre, 2004)

- **Lee (2005)** aimed to test the alternative hypothesis that the high proportion of mammary carcinoma arising in the UOQ of the breasts is a reflection of the greater amount of breast tissue in this quadrant, based on UK data. He found that the proportion of malignant to non-malignant histological findings between the different 4 quadrants and the retro-areolar region did not differ significantly. He showed that the distribution in the breast of normal, benign and malignant tissues were very similar. The number of core biopsies from the UOQ according to the histological diagnosis reported as 'normal' [67%, 95% CI, 59-74%], 'benign' [57%, 95% CI, 51-63%] or 'malignant' [62%, 95% CI, 57-67%] were similar. Lee concluded that the higher frequency of breast cancers in the UOQ was due to the larger proportion of breast tissue in this quadrant, thus supporting his hypothesis.

- **Bright et al. (2016**) analyzed female breast cancers based on US SEER cancer registry data (n=630'007, 1975-2013) and English cancer registry data (n=1'121'134, 1979-2013), to compare the trend in quadrant-specific breast cancer incidence between the two countries, and to determine whether a disproportionate UOQ increase is present. They found that: (i) English breast cancer incidence in the UOQ rose significantly from 13% to 28% from 1979 to 2013, whereas no significant increase was observed among SEER data; and (ii) incidence rose disproportionately in the UOQ compared to non-site-specific tumors (e.g. overlapping tumors) in England. The proportion of non-site-specific tumors was substantially higher in England than SEER throughout the study period (62% in England; 39% in SEER). Bright et al. (2016) concluded that breast cancer incidence in the UOQ increased disproportionately compared to non-site-specific tumors in England but not in SEER, likely due to the decrease in non-site-specific tumors observed in England over time. While these findings may reflect real epidemiological differences in breast cancer etiology between UK and US, they considered the explanation of an artefact of changing data collection methods and improvements in site coding (i.e. ICD classification) in either country to be much more likely.

Other researchers have argued that the higher proportion of tumors in the UOQ may be related to genomic instability in the UOQ region.

- **Ellsworth et al. (2004)** analyzed 26 commonly altered chromosomal regions in breast cancer to evaluate genomic instability, using biopsies from women with breast cancer (n=21) collected at various stages of breast cancer development (premalignant and invasive ductal carcinomas at stage I, II, III). They found that outer quadrants (UOQ, LOQ) demonstrated significantly higher levels of genomic instability compared to inner quadrants (UIQ, LIQ) ($P=0.017$). One marker in particular related to the most frequently altered chromosomal regions in breast cancer, showed a significantly higher level of instability ($P=0.039$) in outer compared with inner quadrants. Levels of genomic instability in tissues located within the same quadrant as the primary tumor, but not immediately adjacent to the tumor, did not differ significantly ($P=0.363$). They concluded that greater genomic instability in outer quadrants can

partially explain the propensity for breast cancers to develop there, rather than simple volume-related breast tissue concepts (i.e. density of the breast parenchyma). It was further hypothesized that it may be related to the predominant axillary lymphatic drainage through the outer quadrants of the breast (see **section 2.2**).

- **Rodrigues-Peres et al. (2013b)** tested the 'genomic instability hypothesis' by examining the relationship between Al concentrations in either the central or peripheral breast tumor areas with the instability of three key genes known to be associated in breast cancer (ERBB2, C-MYC, CCND1) and aneuploidy of the chromosomes harboring these genes. Since Al concentration was not related to any of the gene statuses in either tumor region, they concluded that Al does not affect genomic stability in breast tissues.

Table 2: Incidence of breast cancers in the breast quadrants

Reference	*Study*	*Eligible malignant tumors*	*UOQ*	*UIQ*	*C*	*LOQ*	*LIQ*
Lee, 2005	*Nottingham City Hospital, UK (2001)*	*n=746, diagnostic breast needle core biopsies*	*62%*	*18%*	*6%[d]*	*9%*	*5%*
Rummel et al 2015	*Clinical Breast Care Project (2001–2013)*	*n=980[e], all breast tumors*	*52%*	*16%*	*11%*	*14%*	*8%*
Sohn et al 2008	*Department of Defense tumor registry (10yrs)*	*n= 13'984, retrospective cohort[a], lobular carcinoma + ductal adenocarcinoma*	*58%[b]*	*14%*	*9%[c]*	*10%*	*9%*
Darbre, 2005a	*National Regional Cancer for UK & Wales, and Scotland (1979 to 2000)*	*n=212,677 breast cancers, based on ICD-9 or ICD-10 classification*	*54%[b]*	*15%*	*15%[f]*	*10%*	*6%*
Aljarrah and Miller, 2014	*Scotland (1957-1959)*	*n= 1158 breast tumors*	*41%*	*19%*	*13%*	*7%*	*5%*
Aljarrah and Miller, 2014	*Scotland (1997-1999)*	*n= 1477 breast tumors*	*53%*	*11%*	*5%*	*10%*	*6%*

Abbreviations: C = central quadrant; LIQ = lower inner quadrant; LOQ = lower outer quadrant; UIQ = upper inner quadrant; UOQ = upper outer quadrant; [a] Multifocal disease, cancer involving more than one quadrant, and cancer without specific locations were excluded; [b] included axillary tail; [c] included the nipple and areola complex; [d] retroareolar; [e] multicentric disease or tumors spanning multiple quadrants were excluded; [f] results from central (excl. nipple and areola) and for nipple and areola were added here for comparability purposes - this study included large multiple tumor quadrants as well as small tumors localized at the interface between two quadrants, and treated them in a single category 'Q6' (15% vs 14% incidence in the old cohort vs the more recent one; they are not shown in this table). Numbers are rounded to the nearest percent.

Source: SCAHT, 2017.

6.2.2 Concentrations of Al measured in cancer breast tissues vs normal tissues

The hypothesis that antiperspirant topical use leads to Al accumulation and passive distribution through the lymphatic system in the underlying breast tissues (McGrath, 2003), and that breast cancer patients have higher Al concentrations in breast tissues compared to healthy individuals, is often argued to support the hypothesis that underarm cosmetics cause breast cancer (Darbre, 2001; Darbre, 2016).

Al can accumulate in experimentally-induced mammary carcinoma tumors in laboratory animals (Ogoshi et al. 1994 cited in BfR, 2014). BfR (2014) noted that: "*indications that Al is more likely to accumulate in tumor tissue as a consequence of cancer than being its cause is suggested by a study involving animal experiments (Ogoshi et al., 1994). By administering a carcinogenic substance (2,7-Dimethylbenz[a]anthracene) with the feed, tumors were*

triggered in the mammary gland of rats. Higher levels of Al were discovered in the breast tissue of animals with tumors compared to the healthy control animals". Several clinical studies have reported higher concentration of Al in cancer breasts or adjacent-to-tumor compared to normal or benign tumors (Exley et al., 2007; Ng et al., 1997; Millos et al., 2009; Mulay et al., 1971; Pasha et al., 2008; Romanowicz-Makowska et al., 2011) (see **Appendix 7**).

Other studies have reported increased Al concentrations in breast cysts fluids (BCF) or nipple aspirate fluids (NAF) (Mannello et al., 2009, 2011):

- **Mannello et al. (2009)** measured Al concentrations in BCF collected from women affected by gross cystic breast disease; they found significantly higher ($P < 0.0001$) median Al concentrations in *apocrine type I* BCF (n=27, 150 μg/l) compared to *transudative type II* BCF (n=21, 32 μg/l). In comparison, Al content found in milk was 25 μg/L (range 11-36), and for blood 6 μg/L (range 3-9).
- **Mannello et al. (2011)** measured Al concentrations in NAF collected from breast cancer patients and compared them to Al values in NAF from healthy women. Mean Al concentrations were significantly higher ($P < 0.0001$) in the breast tumor NAF (268.4 ± 28.1 μg/l; n=19) than in the healthy control NAF (131.3 ± 9.6 μg/l; n=16).

In contrast, House et al. (2013) and Rodrigues-Peres et al. (2013a) did not detect any differences in Al concentrations between malignant, tumor-to-adjacent or healthy tissues.

- **House et al. (2013)** tested the hypothesis of a regional distribution of Al across the breast in women with primary breast cancers (n=22). They found no statistically significant regionally specific differences in the content of Al across the breast (whole breast tissue only). Mean Al concentration across all breast quadrant tissues was 0.39 μg/g tissue (dry weight). Results for the four breast quadrants were (Mean ± SD): axilla 0.53 ± 1.22; lateral 0.40 ± 0.81; medial 0.36 ± 0.89; central 0.27 ± 0.54 μg/g tissue (dry weight).
- **Rodrigues-Peres et al. (2013a),** in a cross-sectional study (n=150, 2008-2010), found no Al gradient from normal to diseased breast tissue. They found no statistically significant differences in average Al concentrations in the central tumor area (1.88 ± 3.60 mg/kg), peripheral regions of breast tumors (2.10 ± 5.67 mg/kg, and in adjacent-to-tumor normal tissues (1.68 ± 11.1 mg/kg) (mean ± SD, $P>0.5$).

These studies cannot be compared easily one-to-one. Aluminium content in normal, adjacent-to-tumor and cancer breast tissues can vary markedly across and within studies, reflecting the biological variability. There are also technical challenges related to the measure of Al content in human breast tissues which contain a high content of fat, which makes sample preparation difficult. It was suggested that future studies should measure Al content in tissue and fat separately, since breast is a fatty tissue and there can be high inter-individual tissue variability in fat content Darbre et al. (2013b). House et al. (2013) and others have discussed potential sources of variability and uncertainty in Al measurements: (i) the type of biopsy sampling and preparation procedure (thawed or intact material, partially defatted vs whole tissue; direct use of freeze dried material vs microwave acidic digestion, etc); (ii) the type of analytical technique used to quantify the elements which vary markedly across studies[25]; (iv) whether control and correction for background contamination were applied systematically throughout all the experimental procedures (i.e. methods blank, reagents, reference solutions, lab material, atmospheric deposition, etc).

[25] in particular: X-ray fluorescence (XRF), particle-induced x-ray emission technique (PIXE), neutron activation analysis (NAA), flame atomic absorption spectrometry (FAAS), and more recently, inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), graphite furnace atomic absorption spectrometry (GFAAS), or end-heated graphite atomizer atomic absorption spectrometry (TGA AAS).

6.3 Possible other co-factors for observed effects

As noted in **section 6.1**, several potential factors can confound the association between Al with breast cancer development. Among those, we have in particular considered:

- **The role of epidemiological factors**. These were presented in **section 6.3**.
- **The role of other metals**. A large body of evidence demonstrates that metals are directly or indirectly involved in breast carcinogenesis, and that they accumulate in cancer breast tissues (e.g. Garg et al., 1994; Geraki et al., 2004; Millos et al., 2009; Mulay et al., 1971; Ng et al., 1997; Pasha et al., 2008; Piacenti da Silva et al., 2009; Raju et al., 2006; Rizk and Sky-Peck, 1984; Romanowicz-Makowska et al., 2011; Siddiqui et al., 2006; Silva et al., 2012) (see **Appendix 7**). Ogoshi et al. (1994) demonstrated that Al accumulated in experimentally induced mammary carcinomas in rats (as well as in cancers of the liver, stomach, and duodenum; cited in BfR, 2014). However, controversy still exists whether bioaccumulation of certain metals is a consequence of the disease rather than the reason for the development of the malignancy (Florea and Büsselberg, 2011; Raju et al., 2006). For instance, this accumulation correlates often well with the clinical stage of breast tumor development[26]. A general and common explanation is that metals accumulate in rapidly growing cancer cells because of the increased cellular and enzymatic activity, the need for blood supply, and the need to mobilize the immune and antioxidant defense systems (Millos et al., 2009; Majewska et al., 2007): (i) Cd, Zn, Cu, Fe and Ni have been shown to mimic estrogen action (Lappano et al., 2016); (ii) Fe, Cu, Cr and Cd promote tumorigenesis; (iii) Pasha et al. (2008) found that Cd, Cr, Co, Mn and Fe were strongly and significantly correlated in breast carcinogenesis; (iv) Ca microcalcifications plays a key role in the extension of tumors to adjacent tissues; (v) Mg, K and P are increased to respond to the higher rate of cell proliferation; (vi) Cu and Fe (III) are required for angiogenesis; (vii) furthermore Fe (II) promotes carcinogenesis through ROS formation and inhibition of macrophages tumoricidal activity (Majewska et al., 2007); (viii) increased levels of Cu and Cr lead to higher ROS and free radicals formation; (ix) Cu, Zn and Mn are cofactors of Cu/Zn SOD and Mn SOD dismustases which act as ROS scavengers, and inhibit cancer growth, but can play both a protective and harmful role, depending on their cellular ratio (Millos et al., 2009; Majewska et al., 2007). It should be noted that similar observations were made in other cancerous tissues as well. Significant differences in the concentration of selective metals are generally observed between specific cancerous tissues (Raju et al., 2006). All these metals can confound the association between Al and breast cancer throughout the various stages of cancer development.
- **The role of the experimental conditions in cell culture system: free H^+ and Cl^- as potential confounders.** A major limitation observed in all experimental studies reviewed as part of this work, is the inadequate nature of the controls. Solutions of Al salts are acidic, and upon hydrolysis release HCl (see section 3.1). The kinetics of this reaction is variable among Al salts (faster for ACL), but it is realistic to expect a decrease in pH in long-term experiment, given that fresh Al solutions are continuously (generally twice a week) diluted in the cell culture medium. Low acidic solutions have been shown to be particularly deleterious to cells cultured *in vitro*, and to induce mutagenicity and genotoxicity (Willhite et al., 2014). Unfortunately, control for pH seems not to have been done on a routine basis in most of these experiments. A notable exception is the study by Sappino et al. (2012) who noted that the adjunction of ACL 10-300 μM had little effect on the pH of the MCF10A cell culture medium (H_2O: pH = 7.50; ACL 10 μM: pH = 7.40; ACL 100 μM: pH = 7.41; ACL 300 μM: pH = 7.33), yet it is unclear if it was measured in fresh or long-term culture medium (which was changed twice a week with fresh ACL dilutions, with an "expected pH cell culture medium 7.2"). It is worth noting also that at pH 6-8, Al salts

[26] It has been observed that metal content varies the most in tumor stages II and III, which are the stages undergoing typically marked changes. Tumors are classified clinically according to their stage I-IV.

precipitate to form various gelatinous Al hydroxides. It is therefore surprising that these salts are not observed in the cell culture medium at ca. physiological pH by any of these studies (as explicitly reported in the Sappino study). It is possible, as noted by Willhite et al. (2014), that Al hydroxides may have formed in situ, and are responsible in part for the effects observed. In conclusion, negative control should have equimolar concentrations of H^+ and Cl^-, positive controls such as $GaCl_3$ and $InCl_3$ used in Sappino et al. (2012) should have equimolar concentrations of H^+.

7 Weight of evidence for Al as a breast carcinogen

7.1 Summary of the various lines of evidence

7.1.1 *In vitro* studies

Collectively, these studies suggest that Al is able to trigger aggressive biological features in both ER-positive (estrogen responsive) and ER-negative (estrogen-unresponsive) breast cancer cell lines (MCF7, MDB-BA-231) and normal mammary epithelial cells (MCF10A) such as loss of contact inhibition, migration, invasion and anchorage-independent growth (Bakir and Darbre, 2015; Darbre et al., 2013b; Sappino et al., 2012). The anchorage-independent cell growth suggests that these cells are transformed, yet it is not clear if these cells simply clump together, rather than being transformed to a more aggressive phenotype with the ability to migrate and invade.

The hypothesis by Darbre (2005b) that Al promotes tumor development via its interference with the estrogen receptor (ER) and estrogen-regulated gene expression has not been verified. As rightly noted by Namer et al. (2008) ER inhibitors like tamoxifen are able to bind to the receptor but do not induce cell proliferation. Two studies suggested that Al may trigger genomic instability, by up/down regulating gene expression of genes involved in DNA repair in humans (BRCA1/2, ATR, Rad51, CHK1, CHK2) (Farasani and Darbre, 2015) or in immunocompromised mice models (e.g. Mnt, Tiam2, SFI1) (Mandriota et al., 2016). Sappino et al 2012 suggested that Al is not generically mutagenic but that, similarly to an activated oncogene, it induces proliferation stress, DNA double strand breaks, and senescence in normal mammary epithelial cells, and that long-term exposure to ACL generates and selects for cells able to bypass p53/p21Waf1 mediated cellular senescence.

Eight relevant experimental studies investigated the effects of ACL or ACH, two of the main compounds used in antiperspirants, on human and murine normal breast epithelial cells or breast cancer cells. All these studies were in favor of an association between Al exposure and an increased risk of breast cancer (Bakir and Darbre, 2015; Darbre, 2005b, 2016; Darbre et al., 2011, 2013a; Farasani and Darbre, 2015; Mandriota et al., 2016; Sappino et al., 2012), lending support to the 'Darbre hypothesis'. These studies are consistent across the four cell line models used and the laboratories who conducted these experiments. However, most of these studies provide at best circumstantial evidence that Al has oncogenic potential. None of them attempted to study the effects of Al in other cancer cell types, and failed to take into account other possible co-factors into consideration. These studies suffer from numerous insufficiencies in the study design, conduct, reporting, and statistical analyses, with a lack of proper characterization of the test system, and an absence or no clear dose-response relationships, due primarily to the use of single dose experiments. A major drawback is the absence of control of the pH in the culture media and of basic cytotoxicity testing which hamper the interpretation of the evidence. It is therefore possible that all these in vitro studies have directly observed the effects of H^+, Cl^- or OH^- in lieu of observing direct genotoxic effects of Al. This weakens a potential causal implication of Al. Collectively, all these limitations lower the quality and confidence in the results obtained by these studies, and contribute to weaken the overall strength of evidence and the conclusions that can be drawn from these studies.

7.1.2 Human studies

Only three relevant human studies in humans have investigating the association between Al exposure and breast cancer risk. Two *in vivo* studies reported a positive association between Al exposure and an increased risk of breast cancer (Mannello et al., 2011, 2013), one did not (Rodrigues-Peres et al., 2013b). Breast cyst fluids (BCF) and nipple aspirate fluids (NAF) collected from breast cancer-affected women have been shown to have increased Al concentrations compared to healthy women (Mannello et al., 2009, 2011, 2013). Significantly positive correlations were reported between Al content in NAF with (i) ferritin and transferrin concentrations in Mannello et al. (2011), and (ii) levels of pro-inflammatory cytokines, chemokines and carbonyls (Mannello et al., 2013). These studies support a pro-oxidant action of Al in the breast microenvironment, but the pathways of toxicity remain unclear. Darbre (2016) speculates that these results could be interpreted as a disruption of iron homeostasis leading to iron-driven breast carcinogenesis (see **section 4.4.3**). However, Rodrigues-Peres et al.

(2013b) did not find any association between Al content in NAF and gene expression of key genes involved in breast carcinogenesis, which argues against the hypothesis of genomic instability.

7.1.3 Epidemiological studies

The epidemiological evidence is very limited, of moderate quality, and contradictory. To date, only four primary observational studies (Fakri et al., 2006; Linhart et al., 2017; McGrath, 2003; Mirick et al., 2002) and one meta-analysis (Hardefeldt et al., 2013) have investigated the effect of regular antiperspirant/deodorant use on breast cancer development. Two epidemiological studies reported a positive association between antiperspirants/deodorants and a risk of breast cancer (McGrath, 2003; Linhart et al., 2017), three reported no or negative association (Fakri et al., 2006; Hardefeldt et al., 2013; Mirick et al., 2002). With the exception of Linhart et al. (2017), these studies were based on indirect measurements of Al exposure, so it is not possible to link these results with internal concentrations and exposure. The quality of evidence is generally low, except for the two methodologically rigorous case-control studies by Mirick et al. (2002) and Linhart et al. (2017), who accounted for other potential well-established epidemiological co-factors. The cohorts of these two studies are not easily compared with one another: (i) Mirick et al., (2002) included breast cancer patients diagnosed in the early 1990s, whereas cases in Linhart et al. (2017) were diagnosed around 20 years later (2013-2016); (ii) these women were exposed to different generations of antiperspirant products (formulation, Al salt, other cosmetic ingredients, concentrations used, etc). The meta-analysis of Hardefeldt et al. (2006) pooled data from only two studies (Mirick and Fakri), which limits the strength of their conclusion in favor of a negative association between Al-containing antiperspirants and an increased risk of breast cancer.

These studies cannot definitively exclude an association between antiperspirant use and breast cancer. It remains to be determined if Al may represent an additional risk factor for breast cancer development, and if so, how important it is relative to existing well-established risk factors. Further studies should aim for a clear and unequivocal distinction between antiperspirant and deodorant use, as well as achieving adequate study power. An inherent difficulty resides in finding proper negative control groups, i.e. women that do not use Al-containing antiperspirants, however formulations that contain 'zero aluminium salts' are available today on the market. A foreseeable difficulty is that many women who use these products have a highly variable use pattern due to the moderate-to-low efficacy of these products, so discontinuing their use or changing the product may be common practice.

7.2 Synthesis

7.2.1 Hazard

Aluminium is a genotoxicant that interacts directly with DNA *in vitro* and *in vivo*. It is not mutagenic but can induce both structural and numerical chromosomal aberrations. The experimental database suffers from many insufficiencies (e.g. study design, cell culture conditions, lack of characterization of test system for cytotoxicity and of proper controls due to pH effects, high exposure doses, absence of or no clear dose-response relationship) and contradictory. Furthermore, the genotoxic effects of Al salts are seen only at high exposure levels, which is probably not relevant at human dietary exposure levels, and are not expected at exposure levels which are achieved via cosmetic use.

Experimental studies with human or murine (either normal or transformed) mammary cell lines present only circumstantial evidence that Al may play a role in breast cancer development. These studies suffer from numerous insufficiencies in the way they have been carried out. No thresholds for these hypothesized mechanisms have been identified, due to the absence or the lack of a clear dose-response relationship in these studies. Collectively, uncertainties with the experimental database lower the overall strength of evidence and make conclusions drawn from them difficult. The epidemiological database in relation with the use of antiperspirants (and/or deodorants) is very limited and contradictory. A major limitation of some of these studies relates to whether study subjects have been exposed to Al or not, when the study design does not allow to

discriminate the use of antiperspirants from the use of deodorants. The evidence available to date, though limited, suggests that Al is not carcinogenic in experimental animals and humans and that Al does not increase the risk of breast cancer.

7.2.2 Exposure

Important knowledge gaps remain regarding the dermal bioavailability of Al, and to what extent it may be retained in the viable intact or compromised axillary epidermis and dermis, to reflect realistic exposure conditions. Most of the studies on skin application and penetration of Al salts evaluated eccrine glands in the forearm or palmar skin, which differs markedly with axillary skin with regard to the *stratum corneum* composition. Whether intact axillary skin may have a reduced barrier function as compared to other skin types remains to be clarified, as well as what would be the implications in term of Al absorption. There is however sufficient experimental evidence to indicate that shaving, especially in case of small injuries, will likely result in an increase of dermal absorption of Al compared to an intact skin. The available dermal absorption studies are generally of poor quality and do not meet current standards for use in regulatory risk assessment. The fact that none of these studies reported a change in skin barrier properties and epidermal thickening using tape stripping, questions the relevance of stripped skin studies to quantify the dermal bioavailability of Al salts after shaving.

7.2.3 Supporting information (skin penetration, mechanistic and dermal absorption studies)

Al salts can penetrate and precipitate at varying depths of the sweat gland apparatus. While mechanical plugging of sweat gland ducts has been largely accepted, the mode of action of Al salts in antiperspirants is still not entirely clear. Due to the strong binding properties of Al with skin proteins, this is more likely to happen in the stratum corneum and the upper epidermis, but deeper penetration in the lower epidermal or upper dermal sweat ducts is possible, though apparently rare, especially for the latter. There are still many knowledge gaps relating to the precise and comprehensive description of Al interaction with sweat, or with the ducts and glands components, as well of the long-term consequence of pore plugging on sweat secretion and reabsorption, and what it means in term of a potential Al absorption through the different skin layers.

Accumulation in the epidermis seems in theory possible, although it can be hypothesized that Al may be efficiently removed via the normal skin regeneration and desquamation process. Furthermore, the low rates of skin sensitization by Al salts could be an indication that Al does not reach a critical concentration in the epidermis. There is yet no conclusive evidence that Al is able to efficiently penetrate the intact skin. However, a partial or complete loss of the stratum corneum function will facilitate the access to, or directly expose the epidermis, as well as providing an easy access to the epidermal blood capillaries or lymphatic circulation (which will drain into the axillary lymph nodes), which will both eventually end up in the systemic blood circulation. In the context of a normal, healthy breast there is a priori no reason to think that the lymphatic circulation in the axillary vault would be bidirectional. It remains to be determined if and to which extent Al could be transported via the lymph from the axillary vault to the surrounding breast tissues.

7.2.4 Causality

No causal mechanism(s) of action have been demonstrated so far to support the 'Darbre hypothesis'. Several hypotheses regarding possible mode(s) of action have been proposed, however they have been based on indirect or inconclusive evidence, or have not been verified yet through testing, and are therefore still largely speculative. No thresholds have been identified by P. Darbre and colleagues to support their hypotheses. This is primarily due to the lack of a dose-response relationship in most of these experimental studies, owing to their exploratory character (incl. lack of statistical power, of proper test system characterization, of sufficient endpoints, doses, and time points investigated, and of reporting of experimental conditions). Most of these experimental studies need further testing (in particular work should be replicated by other laboratories) to confirm or refute the proposed hypotheses. Alternatives hypotheses exist that point to a possible **reverse causation** effect, due to the biology of breast cancer and the tumor propensity to accumulate many metals over the course of its

development, incl. Al.

7.2.5 Strength of the association

There are many possible other co-factors for the observed effects, incl. well-established epidemiological risk factors that may confound a possible association between Al and an increased risk of breast cancer, which contributes to weaken a possible causal link between Al and breast cancer.

7.2.6 Biological plausibility and relevance

- ***Relevance of the dose.*** The standard concentration used in the experiments, 100 μM, is 260-1430x higher than the 0.07-0.38 μM Al median blood levels of healthy individuals (Willhite et al., 2014). The dose of 1 mM used by Darbre in some experiments corresponds to 2600-11430x the normal plasma values.

- ***Relevance of the in vitro models.*** MCF7, MDA-MB-231 (both tumorigenic), and MCF10A (non-tumorigenic) are well-established human breast cell lines in cancer research, and are arguably the most commonly used cell lines worldwide (see **Appendix 7**). Nonetheless, such models have inherent limitations in fully recapitulating normal human breast and breast cancer development (Qu et al., 2015). In this respect, 3D culture such as in the soft agar assay (Mandriota et al., 2016; Sappino et al., 2012) is a better predictor of cell behaviour and function in vivo, in comparison with 2D monolayer culture (all studies from Darbre and colleagues). Most of the in vitro studies reviewed have used cell lines with a fairly high or extremely high passage number (98-390). When continually cultured in vitro, cell lines are prone to genotypic and phenotypic drift, which may lead them to differ from the original tumor they were derived from (Burdall et al., 2003). It should be noted also that MCF7 and MDA-MB-231 cell lines are not derived from primary breast tumors, but from aggressive metastatic tumors of pleural effusions. As Burdall et al. (2003) noted, *"research that relies on such lines will be biased toward more rapidly progressive types of breast carcinoma and to late-stage disease, rather than lower grade and earlier stage breast cancers"*. Similarly, while xenograft models provide a whole organism environment for tumor growth, immunocompromised mice (e.g. NSG, NOD SCID) may enhance cell response towards a more aggressive tumor formation and progression. The poor relevance of the animal subcutaneous injection route (as used in Mandriota et al., 2016) has also been questioned in breast cancer research, based on the distinct difference between the stroma of human and mouse mammary tissue, which influence breast tumor cells (Holliday and Speirs, 2011).

- ***Test system reliability and relevance.*** In vitro conditions may not reflect the in vivo situation and the complex biology of breast cancer. Inadequate culture conditions can have an impact at multiple levels (e.g. morphology, cell-cell interaction, cell polarity, cell differentiation, cellular signaling, gene **expression**) (Holliday and Speirs, 2011). Qu et al. (2015) found that MCF10A cells in 2D culture exhibit a basal-like phenotype but concomitantly express luminal markers, a situation not seen in 3D culture. They showed that MCF10A cells may not represent phenotypically normal luminal, basal, or progenitor/stem cells, thus questioning the relevance of MCF10A as a normal mammary epithelial model. Qu et al (2015) suggested that MCF10A may be a luminal-type cell line undergoing EMT transition. If this is true, it means that the observations of Mandriota et al. (2016) may not be relevant.

7.3 SCAHT evaluation

A large literature on Al and breast cancer has been generated since 2001, including a significant proportion of secondary literature (i.e. reviews, mini reviews, short commentaries, brief communications, opinions, hypothesis papers and editorials, mainly with no novel evidence and sometimes contradictory hypotheses). The overall strength of evidence is generally low, except for the case-control study from Mirick et al. (2002), which reported no association between the use of antiperspirants/deodorants and the risk of breast cancer. All of the papers reviewed in favor of the 'Darbre hypothesis' show a lack of critical evaluation of the available scientific information. We agree with the view of Barranger (2016): *"Partir d'hypothèses non vérifiées et extrapoler pour finalement conclure en nuançant ses propres observations est loin d'être une démarche scientifique rigoureuse".*

SCAHT considers that the database quality, in particular derived from experimental studies, is insufficient to establish a clear relationship between the use of Al-containing antiperspirants and breast cancer. In the light of current scientific knowledge, there is no convincing evidence to support such an association. No causal mechanism(s) of action have been demonstrated so far as most of the proposed hypotheses supporting a causal link between Al and breast cancer presented in **section 4.3** and **section 6.1** have not been verified; some of them appear to be based on disputable science or to be speculative, and more convincing alternative hypotheses exist.

Our conclusions concur with the evaluations of major regulatory and scientific bodies as well as peer-reviewed studies which did not see a link between cancer and oral/dermal exposure to Al. In particular, SCCS (2014) explicitly concluded that carcinogenicity is not expected at exposure levels which are achieved via cosmetic use. Nonetheless, further data are needed to definitely exclude a role of Al in the aetiology of breast cancer.

8 Position of other competent authorities (EFSA, BfR, ANSES etc.)

- ***Afssaps (2011):*** *"…Comme cela est discuté dans la partie « cancérogenèse », sur la base des données actuelles disponibles, l'exposition à l'aluminium par voie cutanée ne peut pas être considérée comme présentant un risque cancérogène."*

- ***AGES (2016)****: "Nach heutigem Wissensstand wird (…) über die intakte Haut sehr wenig aufgenommen und zwar wesentlich weniger als dies beispielsweise über die Nahrung der Fall ist. Insgesamt ist daher zu sagen, dass aufgrund der unterschiedlichen Ergebnisse weiterer Forschungsbedarf gegeben ist, um die Aufnahme von Aluminium nach dermaler Anwendung besser zu verstehen und eine etwaige Rolle von Aluminium bei Brustzellveränderungen abzuklären. Wenn auch zum gegenwärtigen Zeitpunkt eine absolute Vermeidung der Anwendung von Antitranspirantien zur Senkung eines eventuellen Brustkrebsriskos nicht empfohlen werden muss, kann aber die individuelle Aluminiumaufnahme reduziert werden. Zur Gesamtaufnahme tragen aluminiumhaltige Kosmetika, wie Antitranspirantien oder Cremes, bei. Die Aluminiumaufnahme durch Antitranspirantien wird vor allem dadurch gesenkt, indem diese nicht unmittelbar nach der Rasur bzw. bei geschädigter Achselhaut auf die Haut aufgebracht werden. Zudem sind Deodorantien ohne Aluminiumsalze im Handel erhältlich."*

- ***BfR (2014):*** *"Ein kausaler Zusammenhang zwischen der erhöhten Aluminiumaufnahme durch Antitranspirantien und der Alzheimer-Krankheit bzw. Brustkrebs konnte trotz einer Reihe entsprechender Studien aufgrund der inkonsistenten Datenlage wissenschaftlich bisher nicht belegt werden."*

- ***SCCS (2014):*** *"There was no indication of carcinogenicity at high dietary doses (up to 850 mg Al/kg bw/day) in animal studies, and SCCS considers that carcinogenicity is not expected at exposure levels which are achieved via cosmetic use".*

- ***SHC (2015):*** *"L'analyse critique des données épidémiologiques et des études chez l'animal n'a pas permis de mettre en évidence un lien entre cancer et exposition à l'aluminium par voie orale. De plus, aucun élément pertinent ne permet non plus de considérer l'exposition par voie percutanée à l'aluminium comme présentant un risque cancérogène. En particulier les données sont insuffisantes pour établir une relation claire entre l'utilisation d'antitranspirants, à base d'aluminium, au niveau des aisselles et le cancer du sein.*

9 Conclusions and recommendations

A large literature on Al and breast cancer has been generated since 2001, including a significant proportion of secondary literature (i.e. reviews, mini reviews, short commentaries, brief communications, opinions, hypothesis papers and editorials, mainly with no novel evidence and sometimes contradictory hypotheses). The overall strength of evidence is generally low, except for the case-control study from Mirick et al. (2002), which reported no association between the use of antiperspirants/deodorants and the risk of breast cancer. Only one other study reported a negative association between Al and genomic instability. All of the papers reviewed in favor of the 'Darbre hypothesis' show a lack of critical evaluation of the available scientific information. We agree with the view of Barranger (2016): *"Partir d'hypothèses non vérifiées et extrapoler pour finalement conclure en nuançant ses propres observations est loin d'être une démarche scientifique rigoureuse".*

SCAHT considers that the database quality, in particular derived from experimental studies, is insufficient to establish a clear relationship between the use of Al-containing antiperspirants and breast cancer. In the light of current scientific knowledge, there is no convincing evidence to support such an association. No causal mechanism(s) of action have been demonstrated so far as most of the proposed hypotheses supporting a causal link between Al and breast cancer have not been verified; some of them appear to be based on disputable science or to be speculative, and more convincing alternative hypotheses exist.

Our conclusions concur with the evaluations of major regulatory and scientific bodies as well as peer-reviewed studies which did not see a link between cancer and oral/dermal exposure to Al. In particular, SCCS (2014) explicitly concluded that carcinogenicity is not expected at exposure levels which are achieved via cosmetic use. Nonetheless, further data are needed to definitely exclude a role of Al in the aetiology of breast cancer.

Uncertainties remain about:

- *whether the presence of aluminium in the breast has any adverse effect;*
- *whether there is a disproportionate incidence of breast cancer in the upper outer breast quadrant;*
- *how much of the aluminium measured in breast tissue could have originated from antiperspirant use;*
- *to which extent aluminium from antiperspirants contributes to the exposure of the breast to aluminium in comparison to other sources of exposure (such as the diet or pharmaceuticals);*
- *the contribution from aluminium-containing antiperspirants to the total exposure to aluminium.*

SCAHT recommends in particular more research to be conducted in the following areas:

- *robust mechanistic studies with clear working hypotheses and consideration of potential sources of bias;*
- *additional in vitro testing in* CHO and HPRT assays *(e.g. OECD Test Guideline 476) to better characterize potential mutagenicity;*
- *adequately controlled experimental conditions (adequate negative and positive controls, investigation of influence of incubation medium, etc.);*
- *evaluation of the effects of Al in other cancer cell lines and cancer tissues;*
- *data on dermal permeation rates of aluminium salts in human skin (preferably with radiolabelled ^{26}Al);*
- *data on the potential for accumulation of dermal and systemic aluminium after dermal exposure;*
- *data on potential exposure to breast tissue via lymph and on aluminium levels in axillary lymph nodes.*

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Appendix 1 Methodology used by SCAHT

I. Problem formulation: defining the study objectives and key question(s)

- *Can Al-containing antiperspirants contribute to the development of breast cancer in women?*
- *How much Al can be absorbed through the human axillary skin? Are differences observed between intact vs damaged skin? To what extent can shaving increase the dermal absorption of Al salts? If skin permeation is observed, can Al be distributed directly via the lymph vessels to the underlying breast tissues?*

II. Definition of assessment criteria for data identification, screening and selection

- *A list of primary and secondary search terms is developed for database searching but not limited to: 'alumin*'; 'deodorant'; 'antiperspirant'; 'cosmetics'; 'skin absorption'; 'dermal absorption'; 'mode of action'; 'breast cancer'; 'mammary carcinoma'; 'tissue'; 'cells'; 'breast density'; 'distribution'; 'tumor location'; 'risk factor'; 'epidemiology'; 'exposure'; etc. These terms are then combined to capture relevant studies, using the Boolean operators AND, OR.*

III. Definition of eligibility criteria for data screening and selection

- *Inclusion criteria are based on: (i) relevance of title and abstract; (ii) primary information sources (original articles; reviews) with full text availability; (iii) languages are en, d, f; (iv) time period 1900-present. Exclusion criteria are based on: (i) secondary information (editorial, conference abstract, book chapter); (ii) languages are not en, d, f; (iii) non-relevant topics (e.g. radiography, intervention studies).*

IV. Web of Science search (January 2017)

Nota bene: the methodology used for the literature search in Web of Science is shown below as an example. A similar approach was used for searching the Pubmed database (not shown).

Combination of terms used:

#1 alumin[all] AND skin[all] AND absorption[all] -- 197 hits*

#2 alumin[all] AND dermal[all] AND absorption[all] -- 22 hits*

#3 alumin[all] AND deodorant[all]) -- 40 hits*

#4 alumin[all] AND antiperspirant[all] -- 123 hits*

#5 alumin[all] AND breast[all] AND cancer[all] -- 208 hits*

#6 alumin[all] AND mammary[all] AND carcinoma [all] -- 28 hits*

#7 alumin[all] AND breast[all] AND cells[all] -- 126 hits*

#8 alumin[all] AND sweat gland[all] -- 57 hits*

combine #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 -- 549 hits

⇒ *465 records excluded based on title/abstract, 84 records included based on title/abstract*

84 records included are analyzed for their relevance and further selection:

- *1 record excluded: language is not en, f, d — record # 16*
- *8 records excluded: full text not available incl. secondary information or wrong referencing — records # 10, 37, 40, 41, 65, 68, 77, 81,*
- *12 records excluded: full text assessed: lack of relevance; other topics — records # 2, 11, 13, 15, 30, 36, 39, 64, 79, 80, 82, 83*
- *48 records excluded: duplicates removed — records # 1, 4, 5, 6, 7, 8, 12, 14, 17, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 38, 42, 43, 45, 46, 48, 49, 51, 52, 53, 54, 55, 56, 57, 58, 60, 61, 62, 63, 66, 67, 74, 75, 84*
- *15 records selected: full text availability — records # 3, 9, 18, 23, 44, 47, 50, 59, 69, 70, 71, 72, 73, 76, 78*

Appendix 2 Aluminium compounds that may be used in cosmetics according to CosIng

Table 3: Aluminium that may be used in cosmetics/antiperspirants according to the European CosIng database

Non-restricted Ingredients (concentrations are not restricted)	**Restricted Ingredients (max 20%) (Annex III EC/1223/2009)**
Aluminum Bromohydrate	Aluminum Zirconium Octachlorohydrate
Aluminum Chloride	Aluminum Zirconium Octachlorohydrex Gly
Aluminum Chlorohydrate	Aluminum Zirconium Pentachlorohydrate
Aluminum Chlorohydrex	Aluminum Zirconium Pentachlorohydrex Gly
Aluminum Chlorohydrex Peg	Aluminum Zirconium Tetrachlorohydrate
Aluminum Chlorohydrex Pg	Aluminum Zirconium Tetrachlorohydrex Gly
Aluminum Citrate	Aluminum Zirconium Trichlorohydrate
Aluminum Dichlorohydrate	Aluminum Zirconium Trichlorohydrex Gly
Aluminum Dichlorohydrex Peg	
Aluminum Dichlorohydrex Pg	
Aluminum Sesquichlorohydrate	
Aluminum Sesquichlorohydrex Peg	
Aluminum Sesquichlorohydrex Pg	
Aluminum Sulfate	
Ammonium Alum	
Sodium Alum	
Sodium Aluminum Chlorohydroxy Lactate	

Source: COSING Database
http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.results&function=9&search

Appendix 3 Use of Al compounds in various cosmetic products

Table 4: Use concentration range of Al compounds in various cosmetic products based on a survey by Afssaps of cosmetic sector industry representatives for the year 2007 (modified from Afssaps, 2011, Table 7).

Catégorie de produits cosmétiques	Sel d'aluminium Nom INCI	Usages	Concentrations max. en sel
Déodorants et antisudoraux	Aluminium chlorohydrate	Antitranspirant	spray: 5 % roll-on: 15 % stick: 20 % crème: 30 %[a]
	Aluminium capryloyl glycine[b]	Antitranspirant	1 %
	Potassium alum (Sulfate d'aluminium et de potassium)	Antitranspirant	NC
	Aluminium sesquichlorohydrate (de 2,5 à 3 %) + aluminium capryloyl glycine[b] (de 1 à 2 %)	Antitranspirant	3,5 à 5 %
	Hydroxychlorure d'aluminium	Déodorant	5 %
Produits pour le rasage	Potassium alum	Agent apaisant	NC
Crèmes, émulsions, lotions, gels et huiles pour la peau	Aluminium Starch Octenylsuccinate	Agent de viscosité	1 à 5 %
	Aluminium sulfate	Astringent	0,17 %
	Magnésium aluminium silicate	Agent de viscosité	2 %
Crèmes de soin pour le corps et le visage	Stéarates d'aluminium	Agent épaississant	3 %
Produits de soins pour le visage et le corps	Silicate d'aluminium (argile)	Agent abrasif, absorbant et hydratant	10 %
Fonds de teint	Magnésium aluminium silicate	Epaississant	1,3 %
Produits de maquillage et démaquillage du visage et des yeux	Magnésium aluminium silicate	Epaississant	0,8 à 2,4 %
Produit de maquillage du visage	Hydroxychlorure d'aluminium	Agent astringent- visage	1,5 %
	Oxyde d'aluminium (Alumina)	Agent de support des laques de colorants	ongles: 2 % visage + lèvres: 3 % yeux: 10 %
	Oxyde d'aluminium (Alumina)	Hydratant, agent de support des poudres- visage	2 %
Masque de beauté	Aluminium starch octenylsuccinate	Absorbant	20 %
	Silicate d'aluminium (argile)	ND	60 à 80 %
Produits solaires	Hydroxyde d'aluminium	Agent d'enrobage du titane dioxyde	1,5 %
Produits pour soins dentaires et buccaux	Oxyde d'aluminium (Alumina)	Abrasif	3 à 5 %
	Hydroxyde d'aluminium	Abrasif, dentifrices extra-blanchissants	Jusqu'à 13-26%[c]
Dentifrices fluorés	Fluorure d'aluminium	NA	1 %
Colorant	Hydroxyde d'aluminium	NA	1 %
	Oxyde d'aluminium	NA	1 %
	Silicate d'aluminium	NA	1 %

[a] BfR (2014), based on IKW survey in 2012; [b] aluminium capryloyl glycine: capryloyl glycine+aluminium hydroxyde; [c] for whitening toothpastes (SHC, 2015), based on a NILU survey in 2011 (see VKM, 2013); NC: non communiqué; ND: information non disponible; NA: non applicable. $Al_2Cl(OH)_5 \cdot 2H_20$ = 210 g/mol, Al = 26,98 g/mol, so Al represents 25.7% Aluminum chlorhydrate.

Appendix 4 Review of *in vitro* studies

Table 5: Full summary results of in vitro studies

Hypothesis	*Postulated mode of action*	*Test system*	*Experimental conditions*	*Observed Effects (compared to - ctrl otherwise specified)*	*Reference*	*SCAHT evaluation*	*SCAHT assessment Conformity (GLP, TG), Ranking*
Al is a metallo-estrogen which alters the breast micro-enviroment	Interference with ER modulates transcriptional activity (ER, ERE, post)	MCF-7	ACL + ACH molar excess ACL 100 µM, 300 µM, 1mM, ctrl (water)	weak inhibition 17β oestradiol - ER binding ↓ growth MCF7, [NS] ↑ gene expression ERE-CAT, [NS]	Darbre 2005b	Exploratory study; test system poorly characterized, basic cell biology and toxicity experiments missing (lack of cytotoxicity control for pH, chlorine, inadequate negative controls water only, not equimolar HCl concentrations used); high Al exposure (dose 0.1-1mM); mechanism of binding, intracellular concentration, and cellular effects are unknown; were steps taken to prevent Al contamination from the lab environment (tools, material, air, etc)?	In favor of the hypothesis Not TG Not GLP Low quality Not reliable
	Proinflammatory action via ROS (Fenton reaction) and Al-superoxide formation	MCF7	ACH 100 µM, with or without 17βoestradiol Ctrl (culture medium without ACH) 21 weeks	Ø cell proliferation at 21 weeks (↑) to ↑ gene expression S100 Ca-binding proteins, [NS]	Darbre et al 2011	Proliferation assay: poor characterization of the test system, basic cell biology and toxicity experiments are missing (lack of cytotoxicity control for pH, chlorine; single dose experiment, no dose response relationship; exposure two time points (0, 21 weeks) only; statistical treatment of the data was not reported (significance?); were measures taken to prevent Al contamination from the lab environment (procedures, tools, vials, air)? Quality of the reporting - missing data: negative control without Al in cell culture experiment, reported only gene expression profile for S100 but claimed that a total of 107 genes were either up or down regulated. Results are inadequately reported experimental details, incl. statistics are missing.	In favor of the hypothesis Not TG Not GLP Low quality Not reliable
Al promotes metastasis	Increases migration and invasiveness reduced adherence capacity	MCF7	ACL 100 µM or ACH 100 µM, ctrl (water) 1 week (short) 32 or 37 weeks (long-term)	↑cell motility all*, only long term (↑) or ↑ cell migration all [NS], long-term (↑) or ↑ cell invasiveness all [NS], long-term (↓) to ↓ cell invasiveness, all [NS] short-term Ø E-cadherin, Ø β-catenin, all [NS], 1 week or 53 weeks	Darbre et al 2013a	Very high passage number of the MCF7 cells (obtained at passage 390); control water; no cytotoxicity/cell viability measurements; only 2 time points for time-lapse microscopy and wound healing assays; null results for E-cadherin/b-catenin assay but data not reported; molecular mechanism unknown, no explanation is given for the observed effects, in particular the protective effects after 1 week in the cell invasion assay. No statistical treatment of the data reported for migration and invasion assays, presumed to be statistically non-significant results. No attempt is made to measure how much Al gets into the cells and what intracellular effects it may trigger; measures taken to prevent Al contamination from the lab environment (procedures,	In favor of the hypothesis Not TG Probably not GLP Low quality Not reliable

						tools, material, air)? need better characterize test system, basic cell biology and toxicity experiments are missing.	
		MDA-MB-231	ACL 10 or 100 µM, ACH 10 or 100 µM, ctrl (water) 1 week (short) 23 or 25 weeks (long-term)	↑cell motility 10 µM ACL*, 10 µM ACH, [NS], only long term, other ø ↑ to↑↑ cell migration, all [NS] ↑ to ↑↑ cell invasiveness, all [NS] ↑MMP9 mRNA, *only for ACH 10µM and 100 µM, other[NS], 21 weeks (↑) MMP14 mRNA, 10 µM ACL [NS], 10 µM ACH [NS] (↑) to ↑ MMP9 protein, * for 10 µM and 100 µM ACL, other[NS], 1 week (↑) MMP14 protein, 10 µM ACL[NS],10 µM ACH [NS], 25 weeks	Bakir and Darbre 2015	Lack of characterization of the test system, basic cell biology and toxicity experiments are missing, Control water (not equimolar in H+ and chlorine), no cytotoxicity/cell viability measurements; no attempt is made to measure how much Al gets into the cells. In the migration and invasion experiments reported overall positive trend in migratory and invasion effects of Al salts, but do not discuss the discrepancies observed in the dose response (ACL 10 mM is much more effective that ACL 100 mM, but ACH 10 mM is less effective than ACH 100 mM). Statistical treatment of the data is not reported in the migration and invasion experiments (presumably all non-statistically significant results); also lack of significance in the RT-PCR experiments may explain some inconsistencies regarding the dose-response pattern observed with upregulation of MMPs gene expression. MMPs experiments results are difficult to use and to interpret, incl. statistics with often large standard deviations; were measures taken to prevent Al contamination from the lab environment (procedures, tools, vials, air)? .	In favor of the hypothesis Not TG Probably not GLP Low quality Not reliable
Al promotes genomic instability	Direct genotoxicity through double strands breaks Loss of repair genes function leads to DNA damage Cell death (Apoptosis) Epigenetic changes?	<u>MCF10A</u> Primary human mammary epithelial cells (only	10, 100, 300 µM ACL, 10 µM ACH, - ctrl (water), + ctrl GaCl3, InCl3, 1, 2, 5, 6, 9, 10, 14, 17 weeks	↑ cell proliferation, ACH 10 µM**, 9-14 weeks, (↓) ACL 100 µM [NS] 1 w, ↓ 300 µM*** 1 w, Ø cell proliferation HaCaT keratinocytes, ACL 300 µM, 17 weeks [NS] Ø C26Ci human colonic fibroblasts, ACL 300 µM, 17 weeks [NS] ↑ loss contact inhibition ACL 100 µM ↑ colony formation, [ACL] all**, + ctrl Ø [NS] Ø apoptosis[a], [ACL] all [NS] ↑ senescence[b], [ACL] all**** ↑ DSBs, [ACL] all P<0.02 ↑ DNA synthesis ACL 100 µM P < 0.003, ACL 300 µM**** ↑ expression p53/p21 protein, [ACL] all Ø inhibition DNA repair[c] ACL 300 µM[NS]	Sappino et al 2012	Lack of characterization of the test system for cytotoxicity: control water, not equimolar in HCl; positive ctrl GaCl3 and InCl3, pH not tested; study did control for pH, but unclear "the addition of ACL had little effect on the pH of the MCF10A cell culture medium (H2O: pH = 7.50; ACL 10 mM: pH = 7.40; ACL 100 mM: pH = 7.41; ACL 300 mM: pH = 7.33) but it is not clear if it was measured in fresh or long-term culture medium (which was changed twice a week with fresh ACL dilutions, with an *"expected pH cell culture medium 7.2"*), no precipitates were seen at this pH; high cell passage number (obtained at passage 98, used 99-125, arrived at non specified number); tumor growth assay, could have looked at other cell markers than EdU (e.g. cell cycle); senescence: seeding density should have been higher (contact!) also for DSBs; no experiment to show when cells are reaching confluence; statistics: 2 experiments only, at least 4 needed; reporting: some experimental details are missing or are unclear, e.g. cell culture experiments and single cells vs number of colonies: lack of information about number of experiments, how many images were taken, what methods were used for the colony	In favor of the hypothesis Not TG Probably not GLP Low quality Not reliable

						count, was blind counting applied to ensure unbiased results?; discontinuation with ACH for ACL not explained; cell culture exposure durations: numbers keep changing (1, 2, 5, 6, 9, 10, 14, 17 weeks), the logic behind it is not explained; variation of the Ames test, why not performing the TG original test?; needs more support information regarding test system characterization/basic cellular biology and toxicity experiments; were measures taken to prevent Al contamination from the lab environment (procedures, tools, material, air)?.	
		MCF10A	ACL 100 μM, ACH 100 μM, ctrl (water) 19-21 weeks	↓ BRCA1 + BRCA2 mRNA, ***, long-term ↓ BRCA1 protein, ACL*, ACH** long-term ↓to↓↓ Rad51 mRNA*** ↓CHK2mRNA***, all ↓ATR mRNA*, all ↓CHK1mRNA, ACH*; ACL^{NS}	Farasani and Darbre 2015	Inadequate control (water), poor test system characterization (no cell viability/cytotoxicity control, no basic cell biology experiments; intracellular Al concentrations and cell effects?; single dose experiments; exposure, only two time points (0 and 19 or 20 or 21 weeks); were measures taken to prevent Al contamination from the lab environment (procedures, tools, vials, air)?	In favor of the hypothesis Not TG Probably not GLP Low quality Not reliable
		Proliferation assays: NMuMg (two cell providers, p1; p2 (ATCC)) Xenografting: NSG, NOD SCID, Nude mice strains	ACL 10, 30, 100 μM ctrl (water) 4, 6, 7, 8 months	↓E-cadherin§, ↑N-cadherin§, ACL 100 μM^{d}, 6 m ↑colony forming, ACL 100 μM, 4m (p1); ↑colony forming, ACL 10 μM ^{e}, ACL 100 μM^{f}, 7m (p2) ↑tumorigenesis (100%) NSG, ACL 100 μM°, 6 m, associated with ↓ weight° in all animals and ↑ tumor size° ↑tumorigenesis NOD SCID (100%), ACL 100 μM^{g}, 8 m, (p1) ↑tumorigenesis Nude (100%), ACL 100 μM ^{h}, 8 m, (p1) ↑mutations, Tiam2 (1), Mnt (0.998), Sfi1 (0.999), Epha2 (1), Nes (0.944), Kcna5 (0.968), Ephb6 (0.934)^{f}, duration not specified	Mandriota et al 2016	Exposure 4-7 months to 100 mM is quite high, but no control for pH or cytotoxicity; negative control is water (should have added equimolar concentrations of H+ and chlorine), prepared stock solutions to perform 3 doses experiment 10, 30, 100 mM ACL but mostly single dosing experiments, no dose-response relationship, mostly (or only?) single time points; soft agar assay: quantification method not clear (no blinding); statistics: one injection per mouse (n=5); need better characterization of the test system, e.g. include other proteins involved in epithelial-to-mesenchymal transition (here only E-cadherin and N-cadherin); include doses with no effect concentrations; how much Al enters the cell? intracellular effects? in general, need more support information regarding test system/basic cellular biology and toxicity experiments.	In favor of the hypothesis Not TG Probably not GLP Moderate quality Reliable with restrictions

Al induces proliferation	induce anchorage-independent growth	MCF10A	ACL or ACH 100 µM, ctrl (water)	↑to↑↑ cell proliferation, all [NS]	Darbre 2016	Exploratory study for which adequate reporting of experimental details is largely lacking. The reader is referred to another paper (Khanna and Darbre, 2013) for the methodology, however many aspects are unclear: inadequate control (ethanol vs water, not equimolar in HCl), inadequate number of experiments; replicates, was any statistical treatment was applied to the data? Number of colonies counted; were pH and cell viability/cytotoxicity controlled for?; exposure, only two time points (0 and 13 weeks; were measures taken to prevent Al contamination from the environment (procedures, materials, air)? Test system characterization (basic cell biology and toxicity experiments are missing) is basically lacking.	In favor of the hypothesis Not TG Not GLP Low quality Not reliable

*Abbreviations: ACL: Al chloride; ACH: Al chlorohydrate; DSB: DNA double strand break; w week; [a] measured as Annexin content; [b] measured as SAβGAL senescence-associated β galactosidase content; [c] measured as persistence of γ-H2AX foci-induced by X-rays, an indirect measure of interference with DNA repair process; [NS] statistically non-significant result; ø null result (no effect); * P<0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; [ ] concentration; (↑) weak increase; ↑ moderate increase; ↑↑ high increase; (↓) weak decrease; ↓ moderate decrease; choice of concentrations: the 10 µM was chosen as the average concentration measured in nipple aspirate fluid (Mannello et al., 2011), while the 100 µM concentration corresponds to the highest concentration of Al which had previously been shown to have no detrimental effect on proliferation of human breast cells in the long term. However, originally: "This concentration of Al salts was chosen as the highest level without any inhibitory effects on growth over 14 days" Darbre, 2005b). m: months; [°] P = 0.01; [#] P < 0.0023; [§] P < 0.00038; [d] P = 0.0067; [e] P = 0.02; [f] P= 0.00028; [g] P values were (treated animals vs control, tailored t-test): after 7 days, P = 0.37; 13 day, P = 0.02; 24 days: P = 0.001; 32, 39 and 45 days: P<0.001; [h] P values were (treated animals vs control, tailored t-test): after 7 days: P = 0.11; 14 days: P = 0.0049; 21 days: p = 0.0017; 28 and 35 days: p<0.003; values in brackets correspond to the PolyPhen2 score: the closer the value is to the number of 1, the more deleterious the mutation is.*

Appendix 5 Review of human studies

Table 6: Full summary results of human studies

Hypothesis	*Postulatedmode of action*	*Test system*	*Experimental conditions*	*Observed effects or association*	*Reference*	*SCAHT evaluation*	*SCAHT assessment of conformity (GLP, TG), Ranking*
Al is a metallo-estrogen which alters the breast micro-enviroment	Proinflammatory action via ROS (Fenton reaction) and Al-superoxide formation	NAF, Milk, Serum	NAF maca (n=19, median 40y, 31-58) NAF healthy (ctrl) (n=19, median 56y, 48-77)	Al in NAF maca: + Ferritin***, + transferrin*** Al in NAF ctrl: Ø ferritin, Ø transferrin	Mannello et al 2011	Al measurements, selection criteria unclear, descriptive stats/demographics inadequate, no stratification; Controls noCancerNAF, n=10 were healthy, n=6 had benign hyperplastic lesions, and the rest?; CancerNAF n=4 had in situ ductal breast carcinoma, n=13 had invasive breast carcinoma, what about the remaining two? Not described anywhere in the paper; NAF n=19, blood n=15, milk n= 45 (divided according to lactation stage), no justification for the number samples collected, why the discrepancies? Cases and controls were age-adjusted, missing data; large variations with NAF volumes collected (median 800 µL, range 130–1500 µL), not discussed; were measures taken to prevent Al contamination from the environment (procedures, tools, vials, lab air, etc)?.	In favor of the hypothesis GLP? Moderate quality Reliable
		NAF, Milk, Serum	NAF maca Al vs NAF healthy (ctrl)	↑Carbonyls**** ↑Cytokines IL-1β, IL-6, IL-12 p70, TNF-α*** ↑ CC, CXC IL-8, MIP-1α, MCP-1** Positive correlation between AL, carbonyl, IL-6, and MCP-1***(*)	Mannello et al 2013	Reporting, patients information section is unclear, follow up experiment to Darbre et al 2011, but discrepancies (volumes of NAF samples collected, molecular analyses from another 2005 study; used same patients demographics as in Darbre 2011 were measures taken to prevent Al contamination from the environment (procedures, tools, vials, lab air, etc)?.	In favor of the hypothesis GLP? Moderate quality Reliable

Al promotes genomic instability		Breast cancer tissue (n=118)	Central tumor area Peripheral tumor area Tumor-to-adjacent (normal) glandular area	↑ Al levels in C + P areas Ø ERBB2, C-MYC, CCND1, all[NS]	Rodrigues-Peres et al 2013b	Methodology, statistics and reporting are adequate, quality control; the gene/centromere statuses were assessed by a single blinded observer; GFAAS quantification: analytical curves with R values < 0.995 were excluded; for each group, two reagent blanks were prepared, ensuring that the contamination originating from reagents and from the laboratory environment was minimized; accuracy/validation: Al recovery (96-111%).	Against the hypothesis GLP? High quality Reliable

*Abbreviations: ACL: Al chloride; ACH: ACH; [NS] statistically non-significant result; Ø null result (no effect); * P<0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001; (↑) weak increase; ↑ moderate increase; ↑↑ high increase; (↓) weak decrease; NAF nipple aspirate fluid, collected non-invasively from breast cancer-effected women or healthy women; + positive effect (in favor of the association)*

Appendix 6 Review of epidemiological studies

Table 7: Full summary results of epidemiological studies

Reference (author, year)	*Study type, location, period*	*Age, sample size*	*Study design, exposure type, sampling*	*Observed effects or association*	*SCAHT evaluation*	*SCAHT assessment & overall quality rating*
Mirick et al., 2002	Case-control, population-based, 1992-1995	Cases (n=813) controls (n=793), women aged 20-74 yrs	deodorant or antiperspirant use, based on structured telephone interviews	All variable evaluated Ø, [NS] Ø regular use of antiperspirant [OR= 0.9, 95% CI, 0.7-1.1; P=0.23); Ø regular use of deodorant [OR=1.2, 95%CI, 0.9-1.5; P=0.19]; Ø use of antiperspirant within 1 hr after shaving [OR 0.9, 95% CI, 0.7-1.1; P=0.40]; Ø use of deodorant within 1 hr after shaving [OR 1.2, 95% CI, 0.9-1.5; P=0.16).	Methodologically valid study, yet the strength of the results may be limited by: the application of these underarm products was analyzed in a dichotomous way, the lack of more detailed information on specific patterns of product use (the study did not take into account intensity and duration of antiperspirant application as well as consider different age categories of exposure, so that effects at younger age may not be accounted for) and by the self-reported nature of the data. Other risk factors were not investigated; collected information on the type of products used and shaving habits but how did they discriminate exactly between use of deodorants and use of antiperspirants?	Against the hypothesis High quality Reliable
McGrath, 2003	Retrospective, population-based, 1993-2001	surviving female breast cancer patients (n=437)	Self-reported questionnaire	↑ Antiperspirant/deodorant use and axillary shaving, with an earlier age of breast cancer diagnosis	Information was collected on underarm hygiene practices of antiperspirant/deodorant use and underarm shaving An internal control group was not included, which weakens the findings of this study. Other risk factors were not investigated.	In favor of the hypothesis Moderate quality Reliable with restrictions
Fakri et al., 2006	Case-control, hospital-based, 2002-2003	Cases (n=54, mean age 43±8yrs) Controls (n=50, mean age 41±15yrs)	Questionnaire-based	82.0% of the controls used antiperspirants compared with 51.8% of cases (P < 0.05) Ø antiperspirants use ↑ deodorants use	Underpowered study; use of antiperspirants/deodorants was dichotomous, categorized only as 'use' vs 'no use'.	Against the hypothesis Low quality Not reliable
Hardefeldt et al., 2013	Meta-analysis	Re-analysis of Mirick and Fakri data	random effects model to calculate pooled OR	Fakri et al. [OR=0.24, 95% CI, 0.10-0.58][NS]; Mirick et al. (antiperspirants) [OR=0.90, 95% CI, 0.74-1.10][NS]; Mirick et al. (deodorants) [OR=1.20, 95% CI, 0.96-1.50][NS] Pooled risk point estimate [OR=0.81, 95% CI, 0.51-1.28][NS]	Very small meta-analysis, and one can argue about the validity of pooling the data from only two studies, which limits the strength of their conclusions and the usefulness for risk assessment.	Against the hypothesis Moderate quality Reliable with restrictions

Linhart et al., 2017	Case-control study, hospital-based, age-matched (1:1), Innsbruck, Austria, 2013-2016	Cases (n=209, mean age 51.9 ± 12.0 yrs) Controls (n=209, mean age 51.8 ± 12.1 yrs)	Questionnaire-based, self-reported use of underarm cosmetic products Al measurements concentration in breast tissue: cases (n=100, from a single breast mastectomy), controls (n=52, from both breast reduction mammoplasty). Al concentrations from three tissue sampling locations (UOQ, C, LIQ, three per case and six per control) were averaged per women, summarized with medians and IQR for cases and controls and stratified by UCP application. Subgroup analysis for Al measurements were performed separately for cases with UOQ tumors in and tumors in other quadrants.		Though a rather good epidemiological study by the quality of its methodology, design and conduct (i.e. power calculation, model adjusted for breast cancer risk factors, use of well-defined sample collection strategy and Al measurement procedure), and discussion of potential sources of bias, this study suffers from a certain number of limitations and potential bias, in particular regarding: **(i) exposure,** most women interviewed were not able to discriminate antiperspirants from deodorants, so these two products are NOT differentiated in this study (merged as a single 'underarm cosmetic products' variable). Consequently, women may have been exposed to Al or NOT; **(ii) causation**, a reverse causation effect cannot be excluded, i.e Al accumulate in the breast and in particular in the UOQ because breast carcinoma are bioaccumulators for metals incl. Al. But also: (iii) selection bias, comparability between cases mastectomy vs reduction mammoplasty for the Al breast measurement experiments, are cases and controls really matched in term of breast size, mammary tissue density, sampling?; (iv) recall bias, self-reporting information may be incomplete or inaccurate and may differ between cases and control, in particular because there is a mix of incident and prevalent cases in the study; besides, interviewers were medical school students, which are hardly "well-trained interviewers", and somehow inconsistent with "*we tried to reduce reporting and measurement bias by performing personal interviews with well-trained interviewers"*; (v) selective/outcome reporting bias, reporting of the results limited only to one age category and Al levels in UOQ. Given the many anomalies and inconsistencies (in relation with UCP use frequency and Al concentrations measured in breast tissues in cases vs controls, tabulated in tables 3, 4a and 4b) which are not explained and discussed, this require further clarifications and proper discussion (e.g why do median Al levels in women exposed before age 30 increase with UCP use frequency in cases, but not in controls [never > several times day, Table 3], which can be similarly observed in the table 4a for the UOQ stratified results, and elsewhere in table 4a and 4b; (vi) confounding bias, other metals were not accounted for; (vii) other potential bias that may threaten internal validity, e.g. statistical analyses? mismatch between number of case vs control in the UCP use experiments (n=209, vs n=209) vs tissue measurement experiments (n=100 vs n=52), limited sample size of the study leads to relatively small numbers in the sub-categories of the main exposure variable - though significant, the result concerning UCP use several times per day is based on a few cases only. Similarly, cases and controls were matched on age, but the subgroup for tissue sampling is not age matched (however Al levels did not correlate with age (r=0.028, p=0.7291).	In favor of the hypothesis Moderate to high quality Reliable with restrictions

Abbreviations: [NS] statistically non-significant result; Ø null result (no effect); ↑ positive association; CI Confidence interval; IQR interquartile range; OR odds ratio; UCP underarm cosmetic products (antiperspirants and deodorants)

Appendix 7 Al measurements in breast tissues

Table 8: Studies that have measured Al in cancer and healthy breast tissues

Reference	Methodology	Breast tissue extract analysed, tumor type, etc	Measured Al levels in Breast tissue (mean value± SD (range), otherwise specified)				Measured Al levels in breast fat (mean value, otherwise specified)			Other metals measured alongside Al (cancer vs healthy breast tissues)
			Normal (healthy)	Benign tumor	Adjacent-to-tumor (normal)	Cancerous (malignant)	normal	adjacent-to-tumor (normal)	cancer	
Mulay et al 1971	Paired samples [c] ES	Breast tissue (defatted) n=8 biopsies sc (n=4, 39-49y) dc (n=4, 40-62y)	n.a.	n.a.	0.51 (0.1-1.2) ppm [a, d]	Mean 3.75 ppm (range <0.5-8.1) [a, d]	n.a.	n.a.	n.a.	↑Cu,Mg,Mn,Zn* ↑Ca, Fe ∅ Ba, Cr, Sr, Sn n.d. Be, Bi, Cd, Co, Ga, Pb, Mo, Ni, S, V, Y, Zr (cancer vs adjacent-to-tumor tissues)
Ng et al 1997 (reported in House et al 2013a)	Not paired [c], freeze dried extracts analyzed directly by NAA	Breast tissue (total) n=46 (1 tissue sample per case)	n.a.	n.a.	14.28±1.48 (10-36) [f] median 12.0 µg/g	22.16±1.83 (10-78) [f] median 19.5 µg/g	n.a.	n.a.	n.a.	↑Al, Br, Ca, Cl, Cs, Fe, K, Mn, Na, Rb, Zn**** ↑Co*** (cancer vs adjacent-to-tumor tissues)
Exley et al 2007	Paired samples [c] Dried extract, acidic digestion, dilution, HGA AAS, mean method blanks substracted	Breast tissue (defatted) (n=68) vs breast fat (n=52) [b]	n.a.	n.a.	4-437 nmol/g [f] (0.11-11.8 µg/g)	n.a.	n.a.	3-192 nmol/g oil (fat) (0.08-5.18 µg/g)	n.a.	n.a.
Pasha et al 2008	Open wet digestion, partially thawed and defatted tissues, FAAS Method blanks measured but application not specified	Malignant maca (n=53, 35-64y) Benign (n=61, 36-59y)	n.a.	Mean 10.1 µg/g [e], SD 4.78 µg/g Median 10.2 µg/g Range 0.25-20.1 µg/g	n.a.	Mean 8.94 µg/g [e], SD 4.89 µg/g Median 8.62 µg/g, Range 0.18-20.8 µg/g	n.a.	n.a.	n.a.	↑Cd, Co, Cr, Cu, Fe, Mn, K, Ca, Zn****(cancer vs benign)

Reference	Methodology	Breast tissue extract analysed, tumor type, etc	Measured Al levels in Breast tissue (mean value± SD (range), otherwise specified)				Measured Al levels in breast fat (mean value, otherwise specified)			Other metals measured alongside Al (cancer vs healthy breast tissues)
			Normal (healthy)	Benign tumor	Adjacent-to-tumor (normal)	Cancerous (malignant)	normal	adjacent-to-tumor (normal)	cancer	
Millos et al 2009	Freeze dried, microwave digestion; ICO-OES; method blanks measured but application not specified	Breast biopsies n=47 Cancer group (n=27, 29-91y) Healthy group (n=20, 21-67y)	19.2 (1.0-76.1) [f] median 13 µg/g	n.a.	24.2 (8-48) [f] median 18.5 µg/g (n=8)	82.8 (12.1-297.0) [f] median 50.0 µg/g (n=19)	n.a.	n.a.	n.a.	↑ Al, Ca, Cu, K, Mg, Mn, P, Zn* (cancer vs healthy tissues) ∅ Fe, S (cancer vs healthy tissues) ↑ Al, Ca, Cu, K, Mg, Mn, P, Zn* (cancer vs benign tissues) ↑ Al, Mg, P, Zn* (cancer vs adjacent-to-tumor tissues) ↑ Ca* (tumor-to-adjacent adjacent vs healthy tissues)
Romanowicz - Makowska et al 2011	Paired samples [c] FAAS	Cancer breast tissue (total) dc (n=67) Adjacent-to-tumor(n=16) (n=67 women, aged mean (SD) 52.8± 6.4), range 32-78 y)	n.a.	n.a.	3.63 ± 1.00 (0.32-6.59) µg/g dry tissue	4.40 ± 1.82 (0.28-8.32) µg/g dry tissue	n.a.	n.a.	n.a.	(↑) Cd*, ↑ Al*, ↑ Ni [NS] (cancer vs adjacent-to-tumor tissues)

Reference	Methodology	Breast tissue extract analysed, tumor type, etc	Measured Al levels in Breast tissue (mean value± SD (range), otherwise specified)				Measured Al levels in breast fat (mean value, otherwise specified)			Other metals measured alongside Al (cancer vs healthy breast tissues)
			Normal (healthy)	Benign tumor	Adjacent-to-tumor (normal)	Cancerous (malignant)	normal	adjacent-to-tumor (normal)	cancer	
House et al 2013	Whole breast tissue (tissue and oil were not separated) Microwave (wet) digestion, THGA AAS Method blanks were used to estimate background levels ofcontamination (14.80 μg/L). Four different breast regions were extracted from the outer (axilla and lateral) to the inner (central and medial) breast (n=88)	Breast tissues (n=22), mastectomy for primary breast cancer (n=203 breast tissue digests)	n.a.	n.a.	n.a.	Median (n = 88) 0.01 μg/g dry wt. (IQR 0.22 μg/g dry wt., max 4.37 g/g dry wt.) mean 0.39 μg/g dry wt. (SD 0.89 μg/g dry wt.). (Axilla) median 0.01 μg/g (IQR, 0.17, 4.37), mean (SD) 0.53 μg/g (1.22) (Lateral) median 0.01 μg/g (IQR, 0.33, 3.02), mean (SD) 0.40 μg/g (0.81) (Medial) median 0.01 μg/g (IQR, 0.19, 3.81), mean (SD) 0.36 μg/g (0.89) (Central) median 0.02 μg/g (IQR, 0.29, 2.33), mean (SD) 0.27 μg/g (0.54)	n.a.	n.a.	n.a.	n.a.

Reference	Methodology	Breast tissue extract analysed, tumor type, etc	Measured Al levels in Breast tissue (mean value± SD (range), otherwise specified)				Measured Al levels in breast fat (mean value, otherwise specified)			Other metals measured alongside Al (cancer vs healthy breast tissues)
			Normal (healthy)	Benign tumor	Adjacent-to-tumor (normal)	Cancerous (malignant)	normal	adjacent-to-tumor (normal)	cancer	
Linhart et al 2017	Samples were not paired, cases (cancer) vs control (healthy) Al concentrations from three tissue sampling locations (UOQ, C, LIQ, three per case and six per control)[i] Tissue sampling procedure: see Exley et al 2007 Measurement procedure: see House et al 2013	Breast tissue (defatted) cases (n=100) vs controls (n=52) Mean age cases vs controls (52±12 yrs, age matched)	0 to 367.38 nmol/g dry weight[j] median 3.8 IQR 2.5-5.8 nmol/g	n.a.	n.a.	0 -367.38 nmol/g dry wt[j] median 5.8 nmol/g (IQR, 2.3-12.9)	n.a.	n.a.	n.a.	n.a.

Abbreviations: dc=ductal carcinoma; maca = mammary carcinoma; n.a.= non applicable; sc=scirrhous carcinoma; y = years old; [a] no statistically significant differences between cancerous and non-cancerous tissues; [b] biopsies were taken from the four regions of the breast in 17 tissue vs 13 fat patients; [c] paired samples of normal and cancer breast tissues were taken from the same patient; [d] values are dry mass (% ash); [e] values are wet mass; [f] values expressed in dry weight; [g] tissue values for the four breast quadrants were: 4-196 nmol/g (axilla), 10-182 nmol/g (lateral), 14-248 nmol/g (middle); 7-437 nmol/g (medial); [h] oil (fat) values for the four breast quadrants were: 3-192 nmol/g oil (axilla), 3-170 nmol/g oil (lateral), 3-51 nmol/g oil (middle), 3-122 nmol/g oil (medial); [i] Al concentrations were averaged per women, summarized with medians and IQR for cases and controls and stratified by UCP application. Subgroup analysis for Al measurements were performed separately for cases with UOQ tumors in and tumors in other quadrants; [j] raw data for these results are missing in the study; (↑) slight increase (accumulation); ↑ increase (accumulation); ∅ = null effect (no accumulation); * p<0.05; **p<0.01; *** p<0.005; ****p< 0.001; *****p<0.0001; [NS] statistically non significant result; n.c.= non communicated; n.d.= non detected; ES= Emission spectroscopy; FAAS = flame atomic absorption spectrophotometry; GFAAS = graphite furnace atomic absorption spectrometry; HGA= heated graphit eatomizer; ICP-OES= inductively coupled plasma optical emission spectrometry; NAA= neutron activation analysis; T(H)GA AAS= traditional end-heated graphite atomizer atomic absorption spectrometry; UCP=underarm cosmetic products (antiperspirants and deodorants).

Appendix 8 Description of the cell lines used

MCF-7 are spontaneously transformed, estrogen-responsive invasive ductal mammary carcinoma (primary tumor) cell lines obtained from a metastatic pleural effusion in a 69 yold Caucasian woman. MCF7 co-express both ERα and ERβ.

MDA-MB-231 are human invasive ductal mammary carcinoma (primary tumor) cell lines. They are considered ER-negative metastatic cells, (derived from a pleural effusion of a 51-year old woman with a breast adenocarcinoma), although some studies report low levels of both ERα and ERβ.

MCF10A normal human breast epithelial cells are an immortalized but non-transformed (not tumorigenic) cell line, derived from benign proliferative breast tissue and spontaneously immortalized. MCF10A or MCF10F only express ERβ, so the ER is not functional (Soule et al 1990). It has been shown that these cells exhibit a basal-like phenotype but share many features of mesenchymal cancer cell lines. MCF10A cells express basal/myoepithelial and luminal markers in 2D culture (Qu et al., 2015).

NMuMG cells are spontaneously immortalized and do not form malignant lesions in nude mice or in the mouse strain they were originally isolated from (NAMRU)

Table 9: Molecular classification of breast carcinoma

Classification	Immunoprofile	Other characteristics	Example cell lines (adapted from [13,22])
Luminal A	ER^+, $PR^{+/-}$, $HER2^-$	Ki67 low, endocrine responsive, often chemotherapy responsive	MCF-7, T47D, SUM185
Luminal B	ER^+, $PR^{+/-}$, $HER2^+$	Ki67 high, usually endocrine responsive, variable to chemotherapy. $HER2^+$ are trastusumab responsive	BT474, ZR-75
Basal	ER^-, PR^-, $HER2^-$	$EGFR^+$ and/or cytokeratin $5/6^+$, Ki67 high, endocrine nonresponsive, often chemotherapy responsive	MDA-MB-468, SUM190
Claudin-low	ER^-, PR^-, $HER2^-$	Ki67, E-cadherin, claudin-3, claudinin-4 and claudinin-7 low. Intermediate response to chemotherapy	BT549, MDA-MB-231, Hs578T, SUM1315
HER2	ER^-, PR^-, $HER2^+$	Ki67 high, trastusumab responsive, chemotherapy responsive	SKBR3, MDA-MB-453

EGFR, epidermal growth factor receptor; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Source: Qu et al. (2015)