Human body is exposed to aluminium from diet, vaccination adjuvant, personal care products.

As underarm antiperspirant, it is applied to the breast area.

This upper outer quadrant of the breast is the site of greatest cancer incidence.

Aluminium has been measured in several human breast structures:
- Human breast tissue
- Milk
- Nipple aspirate fluid
- Breast cyst fluid
RISK FACTORS FOR BREAST CANCER

• GENETICS
  - Female
  - Loss of function of the BRCA1 / BRCA2 genes (loss of DNA repair)

• DIET / OBESITY / ALCOHOL / RADIATION

• HORMONAL EFFECTS (lifetime exposure to oestrogen)
  - PHYSIOLOGICAL (age of puberty, menopause)
  - CHILDBIRTH (age of first child, breastfeeding)
  - PERSONAL DECISIONS (oral contraceptive, HRT)

• OESTROGENIC CHEMICALS FROM THE ENVIRONMENT WHICH CAN ENTER THE HUMAN BREAST?
  - POLLUTION OF DIET / WATER
    - Persistent organochlorine pollutants (POPs)
  - POLLUTION OF THE DOMESTIC ENVIRONMENT
    - Polybrominated diphenylethers (flame retardants)
    - Phthalates, bisphenol A (plastics)
    - Alkyl phenols (detergents)
  - DERMAL ABSORPTION OF COSMETIC CHEMICALS
    - Parabens, triclosan (preservatives, deodorant)
    - Cyclosiloxanes (conditioning, spreading)
    - UV screens (absorb UV light)
    - Polycyclic & nitro musks, Lilial, benzyl salicylate/benzoate (fragrance)
    - ALUMINIUM (ANTIPERSPIRANT) (metalloreostrogen)
THE CASE FOR AN INVOLVEMENT OF COSMETIC CHEMICALS IN BREAST CANCER

• Exposure
  - Applied to underarm and breast area
  - Left on skin allowing for continuous exposure
  - Antiperspirants, deodorants, body lotions, body creams, body sprays, moisturising creams, breast firming/enhancing creams, tanning creams, sun-care creams.

• Chemical overload / individual susceptibility
  - Used with increasing frequency & quantity
  - Used by ever younger children & babies

• Molecular basis
  - DNA damage
  - Cell growth – oestrogenic chemicals
  - Enable “Hallmarks of Cancer”

• Chemical toxicity
  - Long-term low dose exposure
  - Exposure to mixtures of chemicals
  - Absorption, metabolism, clearance
  - Consideration of timing of exposure

Darbre 2001 Eur J Cancer Prev 10, 389-393
Darbre 2003 J Appl Toxicol 23, 89-95
Darbre 2006 Best Pract Res Clin Endo Metab 20, 121-143
Darbre 2009 Breast Cancer Research 11 (S3)
Darbre 2010 Anticancer Research 30, 815-828
EVIDENCE FOR AN INVOLVEMENT OF UNDERARM COSMETICS

- Proximity of application to the breast region

- Disproportionate incidence of breast cancer in the upper outer quadrant of the breast
  - Studies in the 1920–30s reported 31%, now over 50%
  - Disproportionate incidence is increasing linearly each year
    - England & Wales 47.9% in 1979→53.3% in 2006
    - Scotland 38.3% in 1980→57.0% in 2006
  - This is inconsistent with the disproportionality relating solely to more epithelial tissue in that region

- Genomic instability in outer breast quadrants
  - Tissues from breast quadrants of 21 patients
  - Assayed 26 chromosomal regions commonly deleted in breast cancer
  - 17 regions showed more genomic instability in outer regions

- Two epidemiological studies with conflicting results
THE CASE FOR AN INVOLVEMENT OF ALUMINIUM ANTIPERSPIRANT SALTS

- Aluminium salts are applied at high levels
  - Aluminium chlorohydrate 20% w/v
  - Aluminium zirconium chlorohydrate glycine complexes 25% w/v
  - Antiperspirant is left on skin allowing continuous exposure
  - Shaving may enhance entry to underlying tissues

- Aluminium chlorohydrate is absorbed through the skin
  - Using $^{26}\text{Al}$ underarm in human subjects
    - (Flarend et al, Food Chem Toxicol 39, 163, 2001)
  - High plasma Al (4mM) by transdermal uptake from antiperspirant
  - Aluminium absorbed through intact skin 1.81µg/cm² but more through stripped (shaved) skin 11.5µg/cm².
    (Pineau et al, J Inorg Biochem 110, 21, 2012)

- During my lecture I want to make three main points:
  
  1. Aluminium has been measured in a range of human breast tissue structures
  2. Aluminium is a metalloestrogen and oestrogen is a risk factor for breast cancer development
  3. Aluminium can influence migration of breast cancer cells and tumour spread is the main reason for mortality from breast cancer
1. Aluminium has been measured in a range of human breast tissue structures.
ALUMINIUM CAN BE MEASURED IN HUMAN BREAST TISSUE
- VARIED FROM 4-437 nmol/gm DRY WT

Aluminium content of the outer regions (axilla+lateral) was higher than inner regions (mid+medial) ($P=0.033$)

Exley et al., 2007 J Inorg Biochem 101, 1344-1346
NIPPLE ASPIRATE FLUID

• Biological fluid secreted from ductal / lobular epithelial cells
• Reflects breast microenvironment
• Can be collected non-invasively through nipple aspiration
• Contains secreted proteins and cells shed from ductal and lobular epithelium
• Used to identify biomarkers in women at higher risk of developing breast cancer

• Aluminium measured in nipple aspirate fluids
  • from healthy women (NoCancer)
  • from women affected by breast cancer (Cancer)

Mannello, Tonti, Medda, Simone, Darbre, 2011 J Appl Toxicol 31, 262
MEASUREMENT OF ALUMINIUM IN HUMAN NIPPLE ASPIRATE FLUID

Al 5.6  Ferritin 41.0  Transferrin 2.9  Serum (n=15)
Al 24.8  Ferritin 25.2  Transferrin 2.9  Milk (n=45)

NoCancer n=16
Cancer n=19

Mannello, Tonti, Medda, Simone, Darbre, 2011 J Appl Toxicol 31,262
ANTIPERSPIRANT USE AND BREAST CYSTS

GROSS CYSTIC BREAST DISEASE

• Common benign breast disorder
• Can be associated with increased risk of breast cancer
• Greater incidence in the upper outer quadrant of the breast

• Breast cysts result from blocked breast ducts
• Antiperspirants designed to block sweat ducts of the axilla

• Aluminium measured in breast cyst fluids
MEASUREMENT OF ALUMINIUM IN HUMAN BREAST CYST FLUID

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Median level of aluminium (µg/L)</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCF Type I</td>
<td>150 (80-330)</td>
<td>27</td>
</tr>
<tr>
<td>BCF Type II</td>
<td>32 (11-39)</td>
<td>21</td>
</tr>
<tr>
<td>Human serum</td>
<td>6 (3-9)</td>
<td>30</td>
</tr>
<tr>
<td>Human breast milk</td>
<td>25 (11-36)</td>
<td>45</td>
</tr>
</tbody>
</table>

Mannello, Tonti, Darbre, 2009 J Appl Toxicol 29, 1-6
CONCLUSIONS 1.

- Aluminium measured in human breast tissue
  - May be higher in outer than inner regions
  - Widely distributed but patchy

- Aluminium measured in nipple aspirate fluid
  - Higher in Cancer NAF than No-Cancer NAF
  - Correlation between raised levels of aluminium and iron-binding proteins in Cancer NAF

- Aluminium measured in breast cyst fluid
  - Higher in Type1 BCF

- SOURCE OF ALUMINIUM CANNOT BE IDENTIFIED

- WHY IS THERE SO MUCH ALUMINIUM IN HUMAN BREAST STRUCTURES AND IS IT RELATED TO THE DISEASE STATES?
2. Aluminium is a metalloestrogen and Exposure to oestrogen is a risk factor for breast cancer
OESTROGEN ACTS BY BINDING TO INTRACELLULAR RECEPTORS
- receptors function as ligand-activated transcription factors
HOW COULD ALUMINIUM INTERFERE IN THE OESTROGEN ACTION?

Oestrogen receptor

Oestrogen

BINDING TO ER

DNA

ERE

mRNA

Protein

OESTROGEN-REGULATED GENE EXPRESSION

OESTROGEN REGULATED CELL GROWTH
Can aluminium chlorohydrate interfere with $[^3\text{H}]$-oestradiol binding to oestrogen receptor of MCF7 human breast cancer cells?

-competitive binding assay
Can aluminium chlorohydrate interfere with expression of an oestrogen-responsive reporter gene (ERE-CAT) in MCF7 human breast cancer cells?

![Bar chart showing CAT activity in dpms (dpm) for different conditions: no Al, AlCl$_{10}^{-4}$M, and Alchlor $10^{-4}$M with and without E.](chart.png)

Can aluminium chlorohydrate interfere in oestrogen regulation of growth of MCF-7 human breast cancer cells?

![Graphs showing cell growth over time with and without aluminium chlorohydrate added.]
Can aluminium chlorohydrate inhibit oestrogen-stimulated growth of MCF7 human breast cancer cells?

Aluminium chloride induces anchorage-independent growth of MCF10A human non-transformed, immortalised breast epithelial cells


Photographs of results deleted for copyright reasons, please see reference.
CONCLUSIONS 2

• Aluminium can displace $^3$H-oestradiol from oestrogen receptors

• Aluminium can increase expression of oestrogen regulated genes in the absence or presence of oestradiol

• Aluminium does not alter growth regulation of MCF-7 human breast cancer cells by oestradiol

• Aluminium does induce anchorage-independent growth of MCF10A human mammary epithelial cells
3. Aluminium can influence migration and invasion of human breast cancer cells
COULD ALUMINIUM INFLUENCE THE EARLY PROCESSES OF METASTASIS WHERE CELLS BREAK AWAY FROM THE INITIAL TUMOUR MASS?

Alterations to cell behaviour:
- Decreased adhesion
- Increased motility
- Increased migration
- Invasive properties

Diagrams of the processes of metastasis deleted for copyright reasons
MOTILITY OF CELLS CAN BE STUDIED USING TIME-LAPSE MICROSCOPY

- Cells are plated at low density in a 12-well culture dish
- Placed on an automatic stage in a 37°C/5%CO₂ chamber
- Photographs are taken of each well every 15 minutes for 24 hours
- Cells can be tracked using ImageJ software
USE OF TIME-LAPSE MICROSCOPY TO DETERMINE THE EFFECT OF ALUMINIUM ON MOTILITY OF MCF-7 HUMAN BREAST CANCER CELLS

Pictures show the final of 96 time points. Coloured lines show the movement from the first point.

Control                                     Al Chlorohydrate 32 weeks
EFFECT OF ALUMINIUM ON MOTILITY OF MCF-7 HUMAN BREAST CANCER CELLS

Cumulative length (pixels)

Control  AlCl3  AlChlor  AlCl3  AlChlor

1week  32 weeks

MOTILITY OF CELLS CAN BE STUDIED USING A WOUND-HEALING OR SCRATCH ASSAY

Picture of the microscope deleted for copyright reasons

0hr

24hr

48hr
USE OF A WOUND HEALING ASSAY TO DETERMINE THE EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

T=0

T=21hr

Control

AlCl3 32 weeks
EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

USE OF XCELLIGENCE TECHNOLOGY TO DETERMINE THE EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

Cells in serum-free medium

Medium with FCS

Cells pass through 8μm pores by chemotaxis and are detected on gold electrodes on the underside of the membrane.

Picture of the machine deleted for copyright reasons
EFFECT OF ALUMINIUM ON MIGRATION OF MCF-7 HUMAN BREAST CANCER CELLS

USE OF XCELLIGENCE TECHNOLOGY TO DETERMINE THE EFFECT OF ALUMINIUM ON INVASION OF MCF-7 HUMAN BREAST CANCER CELLS

Upper membrane surface coated with matrigel

Picture of the machine deleted for copyright reasons
EFFECT OF ALUMINIUM ON INVASION OF MCF-7 HUMAN BREAST CANCER CELLS

CONCLUSIONS 3.

- Aluminium can increase motility/migration of MCF-7 cells
  - as measured using time-lapse microscopy
  - as measured using the wound healing assay
  - as measured using xCELLigence technology

- Aluminium can increase invasive activity of MCF-7 cells
  - as measured through matrigel using xCELLigence technology

- Molecular mechanisms remain to be determined
CONCLUSIONS

• The human breast is exposed to aluminium

• Aluminium has been measured in human breast tissue structures

• Aluminium is a metalloestrogen

• Aluminium can turn human breast epithelial cells into a transformed phenotype in culture

• Aluminium can influence migratory and invasive properties of human breast cancer cells in culture

Vielen Dank für Ihre Einladung
Danke für Ihre Aufmerksamkeit