

## ALUMINIUM ET ANTITRANSPIRANTS

*Dans un article publié dans le Journal of Applied Toxicology, le Dr. P. Darbre préconise des recherches supplémentaires sur l'existence d'une hypothétique corrélation entre l'aluminium présent dans les anti-transpirants et le cancer du sein. L'Oréal craint que ces propos n'inquiètent inutilement les consommateurs.*

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## ALUMINIUM ET ANTI-TRANSPIRANTS

|                             |  |
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*Dans un article publié dans le Journal of Applied Toxicology<sup>1</sup>, le Dr. P. Darbre préconise des recherches supplémentaires sur l'existence d'une hypothétique corrélation entre l'aluminium présent dans les anti-transpirants et le cancer du sein. L'Oréal craint que ces propos n'inquiètent inutilement les consommateurs.*

La santé et le bien-être de nos consommateurs sont une priorité du Groupe L'Oréal et une vigilance active est exercée sur toutes les questions relatives à la sécurité de nos produits cosmétiques et des ingrédients qui les composent.

L'article publié dans le *Journal of Applied Toxicology* ne comporte aucune donnée nouvelle étayant la théorie selon laquelle les ingrédients utilisés dans les anti-transpirants et les déodorants seraient cancérigènes. En outre, à l'exception de l'aluminium, aucun des métaux et composés métalliques mentionnés dans l'article n'entre dans la composition des cosmétiques. Certains d'entre eux sont même proscrits par la Directive Cosmétique Européenne.

Anti-transpirants et déodorants sont conçus pour agir en surface et ne seraient pas efficaces s'ils étaient absorbés par la peau en grande quantité. Par ailleurs, d'après la littérature et les études réalisées par l'industrie cosmétique, le risque que les sels d'aluminium pénètrent dans la peau est infime. En tout état de cause, la quantité absorbée serait insignifiante par rapport à ce que nous absorbons quotidiennement par l'alimentation<sup>2</sup>. Enfin, les sels d'aluminium entrent dans la composition de nombreux médicaments délivrés sans ordonnance, comme

<sup>1</sup> Darbre P D. Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. *Journal of Applied Toxicology*. 2006; DOI:10.1002/jat.1135

<sup>2</sup> Flarend et al., *Food Chem Toxicol*. Février 2001;39(2):163-8

les antiacides (Maalox®), qui n'ont ni effets indésirables connus sur la santé humaine, ni lien avec le cancer.

Le Dr. Pr. Darbre a déjà, dans le passé, allégué qu'il existait une corrélation entre les sels d'aluminium présents dans les anti-transpirants et les déodorants et le cancer du sein. Cette théorie n'a pas été corroborée. De plus d'après plusieurs grandes organisations de recherche sur le cancer, il n'existe pas de mécanisme plausible par lequel les anti-transpirants pourraient provoquer le cancer du sein.

Enfin, comme tous les cosmétiques, anti-transpirants et déodorants doivent respecter des critères de sécurité très stricts et font l'objet d'une batterie de tests extrêmement rigoureux avant d'être mis sur le marché. Seuls les produits présentant toutes les garanties de sécurité pour le consommateur sont commercialisés.

Nous sommes absolument convaincus de l'innocuité des ingrédients que nous utilisons et tenons à rassurer les consommateurs quant à la sécurité de nos produits.

## ALUMINIUM ET ANTITRANSPIRANTS

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### **1/ La présence de sels d'aluminium dans les antitranspirants constitue-t-elle un risque pour la santé ?**

Il n'y a aucun fondement scientifique à l'allégation selon laquelle l'utilisation d'antitranspirants contenant des sels d'aluminium puisse être associée à un risque pour la santé.

Les données disponibles garantissant la sécurité de cet ingrédient ont conduit les instances réglementaires à statuer :

- En Europe, l'emploi des sels d'aluminium est réglementé par la Directive européenne relative aux produits cosmétiques (Annexe III). Cette législation a fixé des concentrations maximales dans les produits finis, strictement respectées par le Groupe L'Oréal.
- Aux Etats-Unis, si les déodorants sont des produits cosmétiques, les antitranspirants ont été classés parmi les médicaments en vente libre et la Food and Drug Administration (FDA)<sup>1</sup> a confirmé en juin 2003 l'usage des sels d'aluminium dans cette catégorie.
- Par ailleurs, les études scientifiques les plus récentes montrent que les taux d'aluminium absorbés par la peau en liaison avec l'utilisation de produits antitranspirants sont négligeables et bien inférieurs à la limite maximale établie par l'Organisation Mondiale de la Santé (OMS).

<sup>1</sup> Instance qui réglemente aux Etats-Unis les aliments, les cosmétiques, les médicaments et les dispositifs médicaux

## **2/ Les sels d'aluminium sont-ils absorbés par la peau ?**

Dans l'hypothèse où une quantité infinitésimale de sels d'aluminium issue des produits cosmétiques pourrait pénétrer dans la peau, elle s'avèrerait négligeable eu égard aux autres sources d'absorption d'aluminium (eau, produits alimentaires, médicaments...).

## **3/ Existe-t-il une association entre l'utilisation d'antitranspirants et le cancer du sein ?**

Selon l'American Cancer Society, de nombreuses études épidémiologiques solides sur les facteurs de risque de cancer du sein ont été publiées. Aucune d'elle n'a établi que l'utilisation d'antitranspirants serait un facteur de risque de cancer du sein ou d'autres cancers.

Le National Cancer Institute des Etats-Unis a récemment précisé que *"les scientifiques du National Cancer Institute n'ont connaissance d'aucune étude de recherche étayant l'existence d'un lien entre l'utilisation d'antitranspirants ou déodorants pour les aisselles et le développement ultérieur d'un cancer du sein"*.

La Food and Drug Administration (FDA)<sup>2</sup> a également précisé qu'il n'existe aucune preuve et aucune donnée de recherche étayant la théorie selon laquelle les composants des antitranspirants ou déodorants pour les aisselles provoqueraient le cancer et elle conseille *"d'encourager les personnes inquiètes du risque de cancer à parler à leur médecin"*.

De plus, l'Organisation Mondiale de la Santé a récemment examiné la sécurité d'emploi des sels d'aluminium et elle est arrivée à la conclusion selon laquelle *"il n'y a aucune indication de ce que l'aluminium serait cancérigène"*.

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<sup>2</sup> Instance qui réglemente aux Etats-Unis les aliments, les cosmétiques, les médicaments et les dispositifs médicaux.

# STAND-BY-STATEMENT

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- Developed by a Colipa Crisis Management Team -



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## Aluminium and antiperspirant products

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### **Background**

A new review article by P. Darbre<sup>1</sup> implies that aluminium salts used in antiperspirants could be capable of interfering with estrogen receptors and are therefore a potential cause for breast cancer. Media activity has been reported from several European countries and further activity can be expected.

### **Response**

Ingredients used in cosmetics are strictly regulated by the European Cosmetics Directive and are subject to rigid safety controls and scientific analyses at both European and national level. The cosmetic industry is therefore confident of the safety of underarm antiperspirants and deodorants.

The article contains no new scientific evidence or research data to support the theory that ingredients in underarm antiperspirants or deodorants cause cancer. Furthermore, the metals or metal-containing substances described in the article, except for aluminium, are not used in cosmetics or are banned under the EU Cosmetics Directive. The article merely raises hypotheses that are unsubstantiated by scientific facts and that may cause unnecessary concern among users of antiperspirants or deodorants. Finally, the article relies mainly on published data showing interaction of estrogen receptors with metals in vitro, i.e. in the test tube. It has been known for many years that metals may interact with all kinds of proteins under such conditions without any evidence of adverse effects under real life conditions. The article by Darbre provides no data that would lead to a change of this view..

Human nutrition contains a significant amount of aluminium. Published literature and industry in-house studies demonstrate a negligible potential of aluminium salts from deodorants to penetrate skin under use conditions. The amount of aluminium compounds penetrating the skin after application of a

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<sup>1</sup> Darbre P D. Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. Journal of Applied Toxicology. 2006; DOI:10.1002/jat.1135

deodorant is 40 times less than the amount typically taken up via the intestines from normal food over the same time period <sup>2</sup>.

According to a number of leading cancer research organisations, there is no plausible mechanism by which antiperspirants and deodorants could cause breast cancer. Thorough epidemiological studies of breast cancer risk found no association between antiperspirant use and the risk of breast cancer <sup>3</sup>.

Dr Sarah Rawlings, Head of Policy and Information at Breakthrough Breast Cancer says *"There is no reliable scientific evidence to suggest a link between deodorant or antiperspirant use - both on their own and in combination with shaving - and breast cancer. A large number of scientific studies have investigated breast cancer risk factors, however there is no reliable evidence to suggest deodorant or anti-perspirant use are two of them. This review does not provide any further proof"*,

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<sup>2</sup> Flarend et al., Food Chem Toxicol. 2001 Feb;39(2):163-8

<sup>3</sup> Mirick et al, 2002, JNCI Vol. 94, No. 20: 1578-1580

## Aluminium and Antiperspirants

CTPA is aware of an article to be published in the Journal of Applied Toxicology by Dr Philippa Darbre<sup>1</sup> calling for further research into a theoretical link between aluminium in antiperspirants and breast cancer, and we are concerned that this article will cause unnecessary worry amongst users of antiperspirant deodorants.

Understandably there is concern about the incidence of breast cancer and while we welcome any research that tries to determine a cause for breast cancer cases, the article may cause alarm if it is taken out of context. It is crucial to bear in mind that it is not based on new research or evidence. Darbre presents a theory and calls for further research. However a number of leading cancer research organisations have stated there is no plausible biological mechanism by which antiperspirants could cause breast cancer.

Dr Sarah Rawlings, Head of Policy and Information at Breakthrough Breast Cancer says *"There is no reliable scientific evidence to suggest a link between deodorant or antiperspirant use - both on their own and in combination with shaving - and breast cancer. A large number of scientific studies have investigated breast cancer risk factors, however there is no reliable evidence to suggest deodorant or anti-perspirant use are two of them. This review does not provide any further proof"*, while the US National Cancer Institute says that *"The US Food and Drug Administration.... does not have any evidence or research data to support the theory that ingredients in underarm antiperspirants or deodorants cause cancer"*.

In the past, Darbre has also alleged a causal link between the method of action of aluminium in antiperspirants and breast cancer - a theory which has not been substantiated. Whereas a study<sup>2</sup> examining the relationship between antiperspirant and deodorant use and breast cancer concluded that their findings did not support the hypothesis that antiperspirant use increases the risk for breast cancer; and in their recent opinion<sup>3</sup> the SCCP (the European Commission's Scientific Committee on Consumer Products) also concluded there are insufficient data to establish a clear link between the use of underarm cosmetics and breast cancer.

Safety is the number one priority for our industry. All ingredients used in cosmetic products are strictly regulated by the European Cosmetics Directive (76/768/EEC). These laws require cosmetic products must be safe to use, and each product is subject to a rigorous safety assessment by a duly qualified professional before placing on the market. The cosmetic industry is therefore confident of the safety of underarm antiperspirants and deodorants and we take any suggestion that we might be compromising safety by failing to invest in further research very seriously.

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## Aluminium and Antiperspirants (Cont)

### Editors Notes

The paper<sup>1</sup> claims:

*"Aluminium salts in antiperspirants are a major source of exposure to aluminium in humans."*

Aluminium is the third most naturally abundant element in the environment, found in food, water, pharmaceutical as well as a wide range of consumer products.

The overwhelming mass of toxicity data available does not indicate any risk of harmful effects from using any cosmetic products that contain aluminum.

*"Evidence is mounting that the aluminium-based compound can break through the skin."*

Antiperspirants and deodorants are designed to work on the surface of the skin, and so the products would not work if there was a significant amount absorbed. Published literature and industry in-house studies demonstrate a negligible potential for aluminium salts to penetrate the skin<sup>4</sup>. If a small amount were absorbed, this would be tiny in comparison to the amounts we consume in the foods we eat daily.

*"...once in the body it could mimic oestrogen."*

There is no evidence that this can harm human health. Many substances have the ability to mimic oestrogen – and these are found at much higher concentrations in the foods we eat. In practice, just because something has the potential to mimic a hormone (in this case oestrogen), it does not mean that it can cause harm to human health.

For further information, please contact the CTPA. We are always happy to discuss the science behind our industry's products. We want to ensure that the facts are clear and that the science is fully understood.

<sup>1</sup> Darbre P D. Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast. Journal of Applied Toxicology. 2006, In press

<sup>2</sup> Mirick *et al.*, JNCI, 2002, Vol. 94, No. 20: 1578-1580

<sup>3</sup> SCCP/0874/05 Extended Opinion on Parabens, underarm cosmetics and breast cancer. 28 January 2005

<sup>4</sup> Flarend *et al.*, Food Chem Toxicol. 2001 Feb;39(2):163-8

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# Metalloestrogens: an emerging class of inorganic xenoestrogens with potential to add to the oestrogenic burden of the human breast

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**ABSTRACT:** Many compounds in the environment have been shown capable of binding to cellular oestrogen receptors and then mimicking the actions of physiological oestrogens. The widespread origin and diversity in chemical structure of these environmental oestrogens is extensive but to date such compounds have been organic and in particular phenolic or carbon ring structures of varying structural complexity. Recent reports of the ability of certain metal ions to also bind to oestrogen receptors and to give rise to oestrogen agonist responses *in vitro* and *in vivo* has resulted in the realisation that environmental oestrogens can also be inorganic and such xenoestrogens have been termed metalloestrogens. This report highlights studies which show metalloestrogens to include aluminium, antimony, arsenite, barium, cadmium, chromium (Cr(II)), cobalt, copper, lead, mercury, nickel, selenite, tin and vanadate. The potential for these metal ions to add to the burden of aberrant oestrogen signalling within the human breast is discussed. Copyright © 2006 John Wiley & Sons, Ltd.

**KEY WORDS:** oestrogen; metalloestrogen; environmental oestrogen; xenoestrogen; cadmium; aluminium; smoking; antiperspirant; breast cancer

## Introduction

Over recent years, many compounds in the environment have been shown able to mimic or to interfere with the actions of physiological oestrogens and as such have been termed environmental oestrogens (Makela *et al.*, 1999; Darbre, 2002; Petrovic *et al.*, 2004). The origin of these compounds in the ecosystem is diverse and the number of such compounds identified extensive, comprising both natural and man-made compounds. Phytoestrogens are found in nature as organic components of plants (Woods, 2003). Natural and synthetic steroidal oestrogens have been released into the environment as both parent drug and metabolites through extensive use of the contraceptive pill and hormone replacement therapy. Xenoestrogens are generally acknowledged to be man-made non-steroidal organic chemicals which have been released into the environment from agricultural spraying, industrial processes, urban waste or consumer products and include organochlorine pesticides, polychlorinated biphenyls, bisphenol A, phthalates, alkylphenols and parabens (Makela *et al.*, 1999; Darbre, 2002; Petrovic *et al.*, 2004; Harvey and Darbre, 2004). The effects of these compounds on

endocrine disruption in wildlife are being measured (Matthiessen, 2003) but the impact on human health remains a subject for research (Darbre, 2006a). However, to date, compounds with oestrogenic activity have been organic molecules, often phenolic or ring structures (the molecular basis of oestrogenic structure–activity relationships is discussed later) and only recently has it been emerging that metal ions are also capable of interfering with oestrogen action, so defining a class of inorganic xenoestrogens now termed metalloestrogens (Safe, 2003). These include both cations and anions. Some of the metals have known physiological roles but others have no known physiological function. The organometal compound tributyltin, introduced into antifouling paints in the 1970s, has been known for some time to cause endocrine disruption in bivalves and gastropods (Matthiessen, 2003) but the fact that inorganic metal ions also possess oestrogen mimicking properties raises a novel mechanism of endocrine disruption, notable also for the known potential of such materials for significant human exposure.

## Mechanisms of Action *In Vitro*

The molecular basis of oestrogen action begins with the interaction of a ligand with intracellular receptors which are zinc finger transcription factors and which are thus dependent on protein–metal interactions for their function. The DNA-binding domain (DBD) of the oestrogen

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receptor (ER) consists of two zinc finger motifs, each formed by the side chains of two pairs of cysteine residues coordinated with a single zinc atom. On entering the cell, oestrogen binds to the ligand binding domain (LBD) of the ER and ligand–receptor dimers then bind through their zinc finger motifs to specific nucleotide sequences in the DNA termed oestrogen response elements (ERE) (Oettel and Schillinger, 1999). Early experiments using affinity chromatography showed that the ER could bind to certain metals attached to the matrix of the column, notably Zn(II), Ni(II), Co(II) and Cu(II) but not Fe(II) or Cd(II) (Medici *et al.*, 1989). Further work revealed that certain metals could substitute for zinc in the zinc fingers of ER and in so doing alter the ability of the DBD to bind to an ERE (Predki and Sarkar, 1992).

In addition to binding to the DBD, metals have been shown capable of binding to the LBD of the ER $\alpha$  and to block the binding of 17 $\beta$ -oestradiol to this domain. Cadmium has been shown to block the binding of [<sup>3</sup>H]-oestradiol to ER $\alpha$  and to do so through specific interactions with the LBD (Stoica *et al.*, 2000a). Chromium Cr(II), cobalt, copper, lead, mercury, nickel, tin and vanadate could all displace [<sup>3</sup>H]-oestradiol from ER $\alpha$  (Martin *et al.*, 2003) as could arsenite (Stoica *et al.*, 2000b) and selenite (Stoica *et al.*, 2000c). Binding studies using radiolabelled metals showed that <sup>57</sup>Co and <sup>63</sup>Ni can bind to recombinant ER $\alpha$  with equilibrium dissociation constants of  $3 \times 10^{-9}$  M and  $2 \times 10^{-9}$  M, respectively (Martin *et al.*, 2003) which are not dissimilar to the range of dissociation constants for [<sup>3</sup>H]-17 $\beta$ -oestradiol from ER $\alpha$  of various sources (Green and Leake, 1987). More recently, aluminium in the form of aluminium chloride or aluminium chlorhydrate has been shown to displace [<sup>3</sup>H]-oestradiol from cytosolic ER $\alpha$  of MCF7 human breast cancer cells (Darbre, 2005). When attached to oestradiol, metal ions (palladium and platinum) have been found to increase the relative binding affinity of ligand to receptors of MCF7 cells and the authors noted that 'it is unprecedented for a higher ER binding affinity to be observed for a cationic complex than for its metal-free ligand' (Jackson *et al.*, 2001).

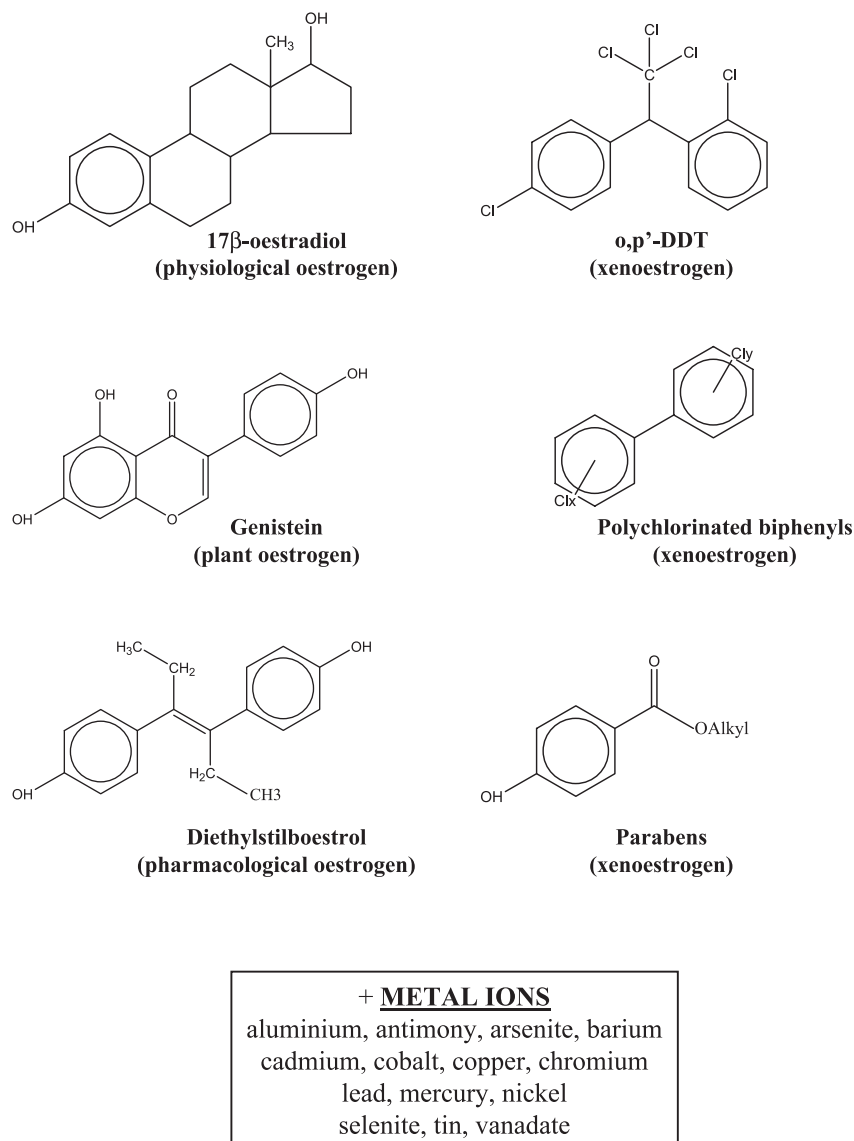
However, in addition to blocking the binding of 17 $\beta$ -oestradiol to ER present in cytosolic extracts, these metalloestrogens can also give rise to oestrogen agonist responses in whole cells. The addition of metalloestrogens on their own could result in down-regulation of ER levels, increased oestrogen-regulated gene expression (including both endogenous genes and reporter genes) and increased proliferation of cells dependent on oestrogen for growth (Garcia-Morales *et al.*, 1994; Stoica *et al.*, 2000a,b,c; Choe *et al.*, 2003; Martin *et al.*, 2003; Darbre, 2005). The effects on gene expression by these metalloestrogens could be blocked with antioestrogen implying that mechanisms were ER-mediated (Garcia-Morales *et al.*, 1994; Stoica *et al.*, 2000a,b,c; Martin *et al.*, 2003). Metal cations and metal anions now shown

to possess these properties include aluminium, antimony, arsenite, barium, cadmium, chromium Cr(II), cobalt, copper, lead, mercury, nickel, selenite, tin and vanadate; published studies are summarised in Table 1. Interestingly, metalloestrogens do not appear to antagonise the actions of 17 $\beta$ -oestradiol on gene expression and cell proliferation, and on the contrary, in some cases act to enhance the agonist action of 17 $\beta$ -oestradiol. Either copper or cobalt increased the proliferation of MCF7 human breast cancer cells in the presence of 17 $\beta$ -oestradiol (Martin *et al.*, 2003). Cadmium (Garcia-Morales *et al.*, 1994), chromium, copper, cobalt, lead, nickel, mercury, tin, vanadate (Martin *et al.*, 2003) and aluminium (Darbre, 2005) given in the presence of 17 $\beta$ -oestradiol could increase the expression of specific genes to levels greater than that found with oestradiol alone.

Figure 1 illustrates the diversity in the chemical structure of organic molecules which can bind to the oestrogen receptor and give rise to oestrogenic responses. However, study of the crystal structure of the ER $\alpha$  has shown that the binding of ligand into the LBD does have specificity (Brzozowski *et al.*, 1997; Tanenbaum *et al.*, 1998). The crystal structure of the human ER $\alpha$  has shown how the LBD is partitioned from the external environment and how the physiological ligand 17 $\beta$ -oestradiol is positioned by hydrogen bonding of its 3- and 17-hydroxyl groups to amino acid side chains and by hydrophobic bonding of the remainder of the steroid ring system to other amino acid side chains (Brzozowski *et al.*, 1997; Tanenbaum *et al.*, 1998). Other organic molecules can also be accommodated within the LBD provided that they are mainly hydrophobic molecules but have at least one relatively unhindered phenolic group with the hydroxyl as a *para*- or *ortho*-substituent (Fang *et al.*, 2001). Molecular modelling has demonstrated that the smaller paraben molecules can bind either singly or in pairs into the LBD in a mode in which their phenolic hydroxyl groups bind similarly to those of *meso*-hexoestrol and by inference also of 17 $\beta$ -oestradiol (Byford *et al.*, 2002). The metals also show specificity in their binding patterns to ER $\alpha$ . Transfection and binding assays with ER $\alpha$  mutants have identified cysteine381, cysteine447, glutamic acid523, histidine524 and aspartic acid538 as possible interaction sites of cadmium with the ligand binding domain (Stoica *et al.*, 2000a). The divalent metal cations copper, cobalt, nickel, lead, mercury, tin and chromium appear to activate ER $\alpha$  through a mechanism also involving cysteine381, cysteine447, histidine524 and the negatively charged side chains of glutamic acid523 and aspartic acid538 (Martin *et al.*, 2003). The metal anion vanadate also requires cysteine381, cysteine447 and histidine524 but consistent with its negative charge interacts with the positively charged side chains of asparagine532 and lysines 529 and 531 instead of the negatively charged side chains of amino acids (Martin *et al.*, 2003).

Table 1. Summary of the published studies on steroid agonist activity of certain metal ions

| Metal  | Test system                                    | Oestrogenic response: <i>in vitro</i>  | Oestrogenic response: <i>in vivo</i>  | Reference  |
|--|--|--|---|--|
| Cobalt, copper, nickel   | Affinity chromatography                        | ER bound to column and eluted with chelating agents  |   | Medici <i>et al.</i> , 1989                                  |
| Cadmium, cobalt  | Reconstituted ER                               | DNA binding in a gel shift assay   |   | Predki and Sarkar, 1992                                      |
| Cadmium  | MCF7 human breast cancer cells                 | ER reduced, E-regulated gene expression increased, cell growth increased   |   | Garcia-Morales <i>et al.</i> , 1994                          |
| Cadmium  | MCF7 human breast cancer cells/<br>COS-1 cells | 109Cd binds to ER, inhibits 3H-E binding to ER, reporter gene expression increased, use of ER mutants to define the binding site                       |   | Stoica <i>et al.</i> , 2000a                                 |
| Arsenite   | MCF7 human breast cancer cells                 | Inhibited 3H-E binding to ER, ER reduced, E-regulated gene expression increased, cell growth increased   |   | Stoica <i>et al.</i> , 2000b                                 |
| Selenite   | MCF7 human breast cancer cells                 | Inhibited 3H-E binding to ER, ER reduced, E-regulated gene expression increased  |   | Stoica <i>et al.</i> , 2000c                                 |
| Copper, chromium, cobalt, lead, mercury, nickel, tin, vanadate | MCF7 human breast cancer cells                 | ER reduced, E-regulated gene expression increased, cell growth increased.  |   | Martin <i>et al.</i> , 2003                                  |
| Antimony, barium   | MCF7 human breast cancer cells                 | E-regulated gene expression increased, cell growth increased.  |   | Choe <i>et al.</i> , 2003                                    |
| Cadmium  | T47D human breast cancer cells                 | E-regulated gene expression increased  |   | Wilson <i>et al.</i> , 2004.                                 |
| Cadmium  | Rat uterotrophic assay                         |  | Increased uterine weight, increased growth and development of mammary glands, <i>in utero</i> exposure gives early puberty  | Johnson <i>et al.</i> , 2003                                 |
| Cadmium  | Rainbow trout                                  |  | Alterations to E-regulated gene expression <i>in vivo</i>   | LeGuevel <i>et al.</i> , 2000; Veillard and Baillhache, 2005 |
| Cadmium  | Amphibian embryos                              |  | Enhanced oestradiol-induced lethality   | Fridman <i>et al.</i> , 2004                                 |
| Aluminium  | MCF7 human breast cancer cells                 | Inhibited 3H-E binding to ER, interference in E-regulated gene expression  |   | Darbre, 2005   |
| Cadmium  | LNCaP human prostatic cancer cells             | <b>Androgenic response: <i>in vitro</i></b><br>Inhibited R1881 binding to AR, AR reduced, A-regulated gene expression increased, cell growth increased | <b>Androgenic response: <i>in vivo</i></b><br>In castrated rodents, increased weight of prostate and seminal vesicles, increased probasin expression in prostate. | Martin <i>et al.</i> , 2002                                  |
| Cobalt, copper, nickel   | Affinity chromatography                        | <b>Progestogenic response: <i>in vitro</i></b><br>PR bound to column and eluted with chelating agents  |   | Medici <i>et al.</i> , 1989                                  |
| Cadmium, selenite  | Receptor binding assay                         | <b>Glucocorticoid response: <i>in vitro</i></b><br>Inhibit ligand binding to GR  |   | Simons <i>et al.</i> , 1990                                  |



**Figure 1.** Diversity in the chemical structure of compounds which, through binding to oestrogen receptor, can result in oestrogen agonist action. Such compounds include the physiological oestrogen 17 $\beta$ -oestradiol, the phytoestrogen genistein, the synthetic pharmacological oestrogen diethylstilboestrol, xenoestrogens o,p'-DDT, polychlorinated biphenyls, parabens and metalloestrogens. There are 209 congeners of polychlorinated biphenyl with varied numbers of chlorine substitutions at the 2,3,4,5,6 positions of each ring as indicated by 'Clx' and 'Cly'. Common alkyl substitutions in the parabens include methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl or benzyl

Thus, whilst phenolic steroids and other organic structures depend for their oestrogenic activity on hydrogen bonding to amino acid side chains, the cationic and anionic metalloestrogens appear to depend for their oestrogenic activity on ionic bonds with acidic or basic amino acid side chains, respectively, and by coordination of amino acid side chains to metal atoms. This may explain why the only amino acid residue common to the binding of both physiological oestrogens (Brzozowski *et al.*, 1997; Tanenbaum *et al.*, 1998) and metalloestrogens (Stoica *et al.*, 2000a; Martin *et al.*, 2003) is histidine524, since this amino acid is capable of both types of bonding.

Nevertheless, it is suggested that metalloestrogens act to activate ER $\alpha$  through the formation of a complex within the LBD of the receptor and which may result in the repositioning of helix 12 of the receptor, an event central to ER function (Martin *et al.*, 2003).

### Evidence of Oestrogenic Activity *In Vivo*

Oestrogenic effects of cadmium have been demonstrated *in vivo* in the rodent uterus and mammary gland (Johnson *et al.*, 2003). In the uterotrophic assay, a single

intraperitoneal dose of cadmium of 5–10  $\mu\text{g kg}^{-1}$  body weight could increase uterine weight by 1.7–1.9 fold, and this was accompanied by proliferation of the endometrium and induction of progesterone receptor (PR) and complement C3. In the mammary gland, cadmium promoted an increase in the formation of side branches and alveolar buds and the induction of casein, whey acidic protein, PR and C3. Exposure to cadmium *in utero* on days 12 and 17 of gestation resulted in female offspring experiencing an earlier onset of puberty, and, as measured on postnatal day 35 at the rapid growth phase of the gland, an increase in the epithelial area and the number of terminal end buds in the mammary gland (Johnson *et al.*, 2003). These effects (at 5–10  $\mu\text{g kg}^{-1}$ ) were observed in rats at doses that are comparable to the provisional tolerable weekly intake (PTWI) recommended by the World Health Organization to be 7  $\mu\text{g kg}^{-1}$  per week for humans (WHO, 1989).

Further studies with supporting evidence for endocrine disrupter actions of cadmium in animal models have been published. In rainbow trout, cadmium can act as an endocrine disrupter through alterations to oestrogen-regulated gene expression (LeGuevel *et al.*, 2000; Vetillard and Bailhache, 2005). In amphibian embryos, exposure to oestradiol can be lethal and this effect could also be found following exposure to cadmium, and furthermore, exposure to cadmium and oestradiol simultaneously produced an additive effect on lethality (Fridman *et al.*, 2004). These reports justify further research into the endocrine disrupting properties of cadmium and *in vivo* studies on other metalloestrogens are now needed. Previous estimates of the PTWI for cadmium (WHO, 1989) were set before reports of its oestrogenic activity, and as for all endocrine disrupting chemicals, identification of receptor-mediated mechanisms can alter risk assessment, due to the lower concentrations needed and specific targeting of effects when operating through cellular receptors. It is therefore important that more studies be conducted on the oestrogenic potency of these metal ions in *in vivo* models, in order to determine the relevance of the *in vitro* data to the whole animal.

### Can Other Steroid Hormone Receptors be Affected?

Although most information centres around oestrogen action, it is apparent that androgen action is also influenced by cadmium (Martin *et al.*, 2002). Cadmium has been reported to block the binding of androgen to its receptor, to increase expression of androgen-regulated genes in LNCaP cells and to give rise to *in vivo* androgenic responses in rodents which were blocked by an antiandrogen (Martin *et al.*, 2002). The early work with the ER showed that PR had similar metal-binding properties to the ER (Medici *et al.*, 1989). Cadmium and

selenite have been reported to inhibit ligand binding to the glucocorticoid receptor (GR) (Simons *et al.*, 1990). This suggests that the function of all members of the steroid hormone nuclear receptor superfamily may be influenced by metals, although more research is needed before firm conclusions can be drawn.

### Relevance for the Human Breast

The question then arises as to the implications of the oestrogenic actions of metals for animal and human health, or whether the oestrogenic activities are only a matter of academic curiosity. Pollution of the ecosystem with heavy metals is widespread (Jarup, 2003) and there is potential for endocrine disruption throughout the animal kingdom which warrants investigation. However, there are some special situations in relation to human breast cancer which deserve more specific consideration. Since oestrogen is known to be involved in the development and progression of human breast cancer (Miller, 1996), any components of the environment which have oestrogenic activity and which can enter the human breast could potentially play a functional role. Cadmium and aluminium come into this category.

Cadmium is a ubiquitous environmental pollutant affecting human health through chronic low-level occupational or environmental exposure (Satarug and Moore, 2004). Accumulation occurs in humans with age because cadmium is excreted from the body only very slowly due to a lack of an active biochemical mechanism for elimination coupled with renal reabsorption: in newborns, the amount of cadmium found in the body is negligible, but the body burden increases with age and especially with smoking (Satarug and Moore, 2004; Henson and Chedrese, 2004). Cadmium has been classified as a human carcinogen (Waisberg *et al.*, 2003; Waalkes, 2003), and cadmium exposure has been linked to lung (Waalkes, 2003), renal (Il'yasova and Schwartz, 2005), prostatic and testicular (Goyer *et al.*, 2004) cancers. A role for cadmium has been proposed in adverse female reproductive outcomes (Henson and Chedrese, 2004; Satarug and Moore, 2004; Mlynarcikova *et al.*, 2005) and in breast cancer (Garcia-Morales *et al.*, 1994; Stoica *et al.*, 2000a; Johnson *et al.*, 2003; Satarug and Moore, 2004). Cadmium has been measured in the human mammary gland where levels are varied but can reach high concentrations (0.1–160.4  $\mu\text{g g}^{-1}$  breast tissue) (Antila *et al.*, 1996). Cadmium has also been found in human milk, which presumably reflects its passage through the breast, especially since the measured levels in the milk reflected maternal cadmium exposure (Nishijo *et al.*, 2002; Honda *et al.*, 2003). An association has been reported recently between high levels of urinary cadmium and high serum levels of testosterone (Nagata *et al.*, 2005), which could be of concern in the context that high total testosterone

levels have been associated with breast cancer risk (Somboonporn and Davis, 2004). Although cadmium is only one constituent of tobacco smoke, smoking does increase body cadmium burden (Johnson *et al.*, 2003; Henson and Chedrese, 2004; Satarug and Moore, 2004), and recent data show that smoking can increase the risk of breast cancer in premenopausal years and can have an adverse effect on prognosis in postmenopausal years (Fentiman *et al.*, 2005). The exposure of the human breast to cadmium (Satarug and Moore, 2004) and the known carcinogenic profile of cadmium (Waisberg *et al.*, 2003; Waalkes, 2003), together with its reported ability to mimic oestrogen action both *in vitro* and *in vivo* (see Table 1 for references) warrants further investigation into a potential role for cadmium in breast cancer.

Aluminium salts are added as the active antiperspirant agents in cosmetics at concentrations of up to 25% w/v (Laden and Felger, 1988) and as such form a major source of aluminium exposure in humans (Yokel and McNamara, 2001). However, the effects of widespread, long-term and increasing use of these cosmetics remain unknown, especially in relation to the breast, which is the general vicinity of application. In their use as underarm antiperspirants, aluminium salts are left on the skin around the underarm and breast area, allowing through continuous exposure for absorption through the dermis and chemical deposition in underlying local tissues. Furthermore, Western culture has resulted in frequent use of shaving prior to cosmetic application and this can damage the integrity of the stratum corneum, allowing direct chemical access. Whilst it had been assumed that unbroken skin would be an effective barrier to transdermal uptake of aluminium, dermal absorption of topically applied antiperspirant aluminium salts has now been demonstrated through intact mouse skin (Anane *et al.*, 1995, 1997) and human underarm skin (Flarend, 2001; Flarend *et al.*, 2001; Guillard *et al.*, 2004; Exley, 2004). The genotoxic profile of aluminium (Exley, 2001) together with its newly reported oestrogenic properties (Darbre, 2005) should be a cause for future research into its potential involvement in the development of human breast cancer.

There is no doubt that the human breast is now subject to a wide range of environmental oestrogenic insults (Darbre, 2006b). Of these, some are ingested by choice, for example, in contraceptives or hormone replacement therapy, but by far the greater number are xenoestrogens, taken inadvertently from the environment in diet or from application of cosmetics to human skin, and although weaker in oestrogenic potency, exposures may be relatively high and potential adverse effects are unknown. A range of organochlorine pesticides and polychlorinated biphenyls with oestrogen-mimicking properties have been measured in human breast adipose tissue and in human milk (Darbre, 2006b). These enter the human breast from varied environmental contamination of food, water and

air and due to their lipophilic properties can accumulate in breast fat. However, a more direct breast exposure to xenoestrogens occurs through the application of oestrogenic chemicals commonly used in bodycare cosmetics to the underarm and breast area (Harvey and Darbre, 2004). Such cosmetic xenoestrogens include parabens (Harvey and Darbre, 2004), cyclosiloxanes, triclosan, ultraviolet screens and phthalates (Darbre, 2006b), and now the aluminium salts can be added to this list (Darbre, 2005). Most studies to date have considered single chemicals in isolation, which alone may or may not reach levels in the human breast which are equivalent to those needed for measurable effects *in vitro*. However, the multitude of oestrogenic insults may summate together to be more significant than when considering each chemical in isolation. Cadmium and aluminium have the potential to add a novel dimension to the summation of this additive burden of aberrant oestrogen signalling in the human breast.

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