JOURNAL OF APPLIED TOXICOLOGY
J. Appl. Toxicol. **24**, 167–176 (2004)
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/jat.978

Endocrine Disrupters and Human Health: Could Oestrogenic Chemicals in Body Care Cosmetics Adversely Affect Breast Cancer Incidence in Women?

A Review of Evidence and Call for Further Research

Philip W. Harvey[1,*] and Philippa Darbre[2]

[1] Department of Toxicology, Covance Laboratories Ltd, Otley Road, Harrogate, North Yorkshire HG3 1PY, UK
[2] Division of Cell and Molecular Biology, School of Animal and Microbial Sciences, University of Reading, Reading RG6 6AJ, UK

Key words: endocrine disruption; parabens; xenoestrogen; breast cancer; women; cosmetics; Directive 76/768/EEC; *p*-hydroxybenzoic acid esters; carcinogenesis; body care; human health.

In the decade that has elapsed since the suggestion that exposure of the foetal/developing male to environmental oestrogens could be the cause of subsequent reproductive and developmental effects in men, there has been little definitive research to provide conclusions to the hypothesis. Issues of exposure and low potency of environmental oestrogens may have reduced concerns. However, the hypothesis that chemicals applied in body care cosmetics (including moisturizers, creams, sprays or lotions applied to axilla or chest or breast areas) may be affecting breast cancer incidence in women presents a different case scenario, not least in the consideration of the exposure issues. The specific cosmetic type is not relevant but the chemical ingredients in the formulations and the application to the skin is important. The most common group of body care cosmetic formulation excipients, namely *p*-hydroxybenzoic acid esters or parabens, have been shown recently to be oestrogenic *in vitro* and *in vivo* and now have been detected in human breast tumour tissue, indicating absorption (route and causal associations have yet to be confirmed). The hypothesis for a link between oestrogenic ingredients in underarm and body care cosmetics and breast cancer is forwarded and reviewed here in terms of: data on exposure to body care cosmetics and parabens, including dermal absorption; paraben oestrogenicity; the role of oestrogen in breast cancer; detection of parabens in breast tumours; recent epidemiology studies of underarm cosmetics use and breast cancer; the toxicology database; the current regulatory status of parabens and regulatory toxicology data uncertainties. Notwithstanding the major public health issue of the causes of the rising incidence of breast cancer in women, this call for further research may provide the first evidence that environmental factors may be adversely affecting human health by endocrine disruption, because exposure to oestrogenic chemicals through application of body care products (unlike diffuse environmental chemical exposures) should be amenable to evaluation, quantification and control. The exposure issues are clear and the exposed population is large, and these factors should provide the necessary impetus to investigate this potential issue of public health. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

Harrison (2001) and Sharpe and Irvine (2004) have recently summarized the hypotheses and status of evidence implicating endocrine disruption and adverse impacts on human health. Writing in the *British Medical Journal*, both Harrison (2001) and Sharpe and Irvine (2004) have outlined that in men hypospadias, cryptorchidism, prostate cancer, testicular cancer and semen quality, and in women breast cancer, cystic ovaries and endometriosis have all been suggested as indicators of adverse trends in reproductive health. Harrison (2001) further summarized: 'the idea that these trends are real and connected with environmental pollution is gaining credence internationally'. If breast cancer is indeed one such indicator, the incidence of the disease has certainly increased in recent decades (Quinn & Allen, 1995; data for England and Wales) and because only approximately 5% of breast cancer is due to highly penetrant dominant genes (see Quinn & Allen, 1995) the majority of cases probably involve epigenetic lifestyle or environmental factors (see also Darbre, 2001, 2003), with oestrogen generally considered the major aetiological factor (discussed later).

Although the evidence is reasonably clear that endocrine disruption, particularly exposure to oestrogenic chemicals, has produced effects in aquatic species, there has been

* Correspondence to: Philip W. Harvey, Department of Toxicology, Covance Laboratories Ltd, Otley Rd, Harrogate, North Yorkshire HG3 1PY, UK.
E-mail: philip.harvey@covance.com

Received 6 February 2004
Revised 23 February 2004
Accepted 1 March 2004

Copyright © 2004 John Wiley & Sons, Ltd.

only conjecture that 'humans also live in a sea of oestrogens' and that 'apparent increases in the incidence of certain reproductive conditions may be due to exposure to chemicals in the environment' (see Harrison, 2001). The main hypothesis is the so-called Sharpe–Skakkebaek hypothesis for reproductive abnormalities in men due to increased exposure to oestrogens *in utero* (Sharpe & Skakkebaek, 1993). In this hypothesis it was postulated that synthetic chemicals present in the environment were the prime source of excessive oestrogenic stimulation of the foetal male. Harrison (2001) discussed the phthalates and bisphenol-A as potential candidates for general endocrine disrupters because of their ubiquitous nature, the perceived potential for human exposure and also introduced additional mechanisms to include potential effects of antiandrogens.

In the decade that has passed since Sharpe and Skakkebaek's hypothesis for effects in men, science has barely moved on and there is still no conclusive evidence that endocrine disrupters are linked, or not, to adverse health effects in humans, largely because the necessary work has not been undertaken (see discussion in Sharpe & Irvine, 2004, concerning the lack of definitive human data, difficulties and considerations). There have been a multitude of reports quantifying the oestrogenic potential of chemicals in a variety of assays and although this is necessary in the process of hazard assessment there has been no development of what this means to human health. For many oestrogenic chemicals potency is relatively low (compared with oestradiol) but of most importance, the opportunity for human exposure in the general population to many of these compounds is also low and in many cases this has mitigated concerns. Research efforts have been diverted by issues of synergy (McLachlan, 1997) but also directed at ultralow dose and hormesis-type effects that could have a bearing on the negligible human exposure scenarios of some of the chemicals implicated as weak xenoestrogens.

At the same time there are genuine issues concerning effects of long-term exposures to low doses and mixtures that, for various reasons, have not been researched. The statement that there is no evidence that human health has been adversely affected by endocrine-disrupting chemicals remains technically correct because the appropriate work has not been conducted (see also Sharpe & Irvine, 2004). Indeed, there are no data on the effects of long-term, low-dose exposures to environmental oestrogenic chemicals, and therefore human health effects and levels of risk are unknown.

The development of standardized regulatory approaches for endocrine disrupter screening and testing by the US Environmental Protection Agency (USEPA) has not solved the question of what the generated data actually mean for human health. Harvey and Johnson (2002) suggested a framework for assessing endocrine toxicity data in the context of all available toxicity data, and discussed the practical irrelevance of very-low-potency oestrogens in the contexts of probable low opportunity for actual human exposures and other more critical toxicity endpoints. Ashby (2001) has also cautioned that the developmental effects of endocrine disrupters detected in rodent studies currently cannot be extrapolated to humans (the argument being the uncertainty of applying results across species, together with the absence of a control database and variability of parameters across strains and in protocols). Thus, research into endocrine disrupters and human health appears to have stalled over data extrapolation to humans and the practical consideration of real-life exposure issues being irrelevant for the majority of chemicals.

However, there is one current topical case of potential endocrine disruption and a potential human health effect that certainly satisfies the exposure issues in terms of widespread use and direct dermal exposures (as well as laboratory findings of oestrogenicity and other endocrine and reproductive effects) and appears to be generating interest in human epidemiology: the use of underarm and body care cosmetics with oestrogenic chemical excipients (particularly the parabens) and the hypothesized association with breast cancer incidence, particularly in women. It must be noted that the type of cosmetic product is irrelevant (e.g. antiperspirant/deodorant versus body lotion, moisturisers or sprays versus creams) and attention must focus on issues of actual exposure to chemicals through continued dermal application of body care products and the endocrine/hormonal activity and toxicity of the chemicals in the formulations.

BODY CARE COSMETICS, PARABENS AND BREAST CANCER: A HYPOTHESIS

The hypothesis for a role of underarm or body care cosmetics, more specifically their chemical excipients (Darbre, 2003; Harvey, 2003), in the rising incidence of breast cancer in women in recent decades has been placed on a scientific basis (Darbre, 2001; Mirick *et al.*, 2002; Darbre, 2003; McGrath, 2003; Darbre *et al.*, 2004; see also Harvey, 2003; Harvey & Everett, 2004). There is a correlation between the growth in the use of body care products in the Western world over recent decades and the increasing incidence of breast cancer, but other lifestyle changes also have occurred during this period. Parabens have been suggested as the agents in body care formulations potentially involved in breast cancer (Darbre, 2003; see also Harvey, 2003) because of their ready absorption through the skin (e.g. Soni *et al.*, 2001, 2002) as intact esters (Bando *et al.*, 1997), their hormonal activity and their reproductive toxicity (discussed later), but other suggestions are for a role of aluminium (Darbre, 2003; McGrath, 2003). Additionally, the siloxanes or cyclosiloxanes are present in high proportions in personal care products (octamethylcyclotetrasiloxane is present at 40–60% by weight in such products as antiperspirants and cosmetics, Luu & Hutter, 2001). The cyclosiloxanes are reported to concentrate in ovaries and uterus of mice following a single subcutaneous injection and also have an 'affinity' for the oestrogen receptor (Kala *et al.*, 1998; see also Hayden & Barlow, 1972; Levier & Jankowiak, 1972).

The hypothesis that chemicals in body care cosmetics may influence breast cancer incidence certainly requires further research but meets the following suggested criteria required for an endocrine disrupter to affect the human population:

(i) Frequent or near-continuous, long-term and direct exposures (in this case, regular application to the skin of the chemicals in body care formulations).
(ii) Exposure of a large population, including potentially sensitive higher risk sub-groups.

Copyright © 2004 John Wiley & Sons, Ltd.

(iii) Endocrine and hormonal activity of the chemicals (e.g. oestrogenicity of the parabens as ingredients in such formulations).
(iv) Absorption into the body: parabens have been detected as intact esters in breast tumour tissue (Darbre *et al.*, 2004), suggesting a direct non-oral route, and are readily absorbed through the skin (Soni *et al.*, 2001, 2002) as intact esters (Bando *et al.*, 1997). Dermal absorption can imply a direct effect on the adult, but the issues of exposure of the foetus and subsequent effects in later life are also a primary concern in endocrine disrupter research (Sharpe & Irvine, 2004). However, exposure issues are more difficult to quantify and cause-and-effect relationships are difficult to study, not least because of the time lag.

There is limited epidemiological evidence (McGrath, 2003) suggesting a potential link between underarm cosmetics and breast cancer in women, but Mirick *et al.* (2002) have not detected any differences in breast cancer rates with antiperspirant use (discussed later). Neither study design is conclusive and each has its limitations, therefore further research is a priority. Similarly, a causal link between either the cosmetics or parabens used on the axilla or adjacent body areas and breast cancer has not been established. The hypothesis requires rigorous testing and the appropriate research has to be conducted to provide definitive evidence either for or against such a link.

The evidence leading to the postulation that underarm and body care cosmetics, particularly containing weakly oestrogenic formulation excipients, may be associated with breast cancer is briefly outlined below.

HUMAN EXPOSURE TO COSMETICS AND PARABENS: OCCURRENCE AND CONCENTRATIONS IN BODY CARE FORMULATIONS

Parabens have been used for 50 years in cosmetics, food and other consumer products. In cosmetics they are used in a variety of products designed to be applied to the skin, particularly the axilla and breast, and include moisturisers and body lotions. Parabens have antimicrobial and preservative properties and extend shelf-life. Parabens are readily absorbed through the skin and gastrointestinal tract and metabolism involves hydrolysis to *p*-hydroxybenzoic acid (see reviews on propylparaben and methylparaben by Soni *et al.*, 2001, 2002). Bando *et al.* (1997) report that in an *in vitro* rat skin model 30% of applied propylparaben and 4% of applied butylparaben penetrated the skin intact without being metabolized by skin esterases to *p*-hydroxybenzoic acid.

In determining paraben exposure, it is useful to have data on the extent of the use of parabens in cosmetic formulations and scientifically robust confirmation of actual concentrations. Rastogi *et al.* (1995) analysed 215 cosmetic products for the methyl-, ethyl-, propyl-, butyl- and benzylparaben esters as part of a monitoring programme to assess Danish and EEC regulation compliance. The results showed that 77% of the products investigated contained parabens, with concentrations ranging from 0.01% to 0.87% (a 125 ml container would therefore contain 1 g of paraben esters in a product with 0.87% paraben content). They further found that nearly all (99%) of the leave-on products and 77% of rinse-off products contained parabens. Maximum concentrations of 0.32% methyl- and propylparabens, 0.19% ethylparaben and 0.07% butyl- and benzylparabens were present in these samples, with mixtures of parabens increasing the total contents. The order of use preference was methyl-/ethyl-/propyl-/butyl-/benzylparaben.

Gruvberger *et al.* (1998), working in Malmo University Hospital, Sweden, analysed 100 dermal moisturizer products by high-performance liquid chromatography. They also report that parabens were the most common preservatives in the formulations. They compared their analyses with information supplied by manufacturers as required for labelling and report that information was incorrect on preservatives contained in the cosmetics in 10.2% of cases. When the products were analysed for chemicals that should not be present in the moisturizers according to the manufacturers, at least one chemical was detected in 17% of formulations. This incorrect chemical composition information was discussed in terms of the risk to individuals with allergies inadvertently applying contraindicated formulations.

More recent surveys of body care products have continued to show that products do not comply with the EC Cosmetics Directive with respect to labelling. Rastogi (2000) reports that incorrect ingredient labelling with respect to paraben content was found in 10% of investigated products, that a total of 45% of the investigated skin creams had incorrect labelling and that parabens were used extensively, with one or more parabens found in 87% of the investigated products. Hydroxybenzoic acids were also detected; these bear structural similarity to the parabens and have been shown to be weakly oestrogenic (Lemini *et al.*, 1997; see later discussion).

Clearly, the incorrect labelling of body care formulation ingredients and excipients reported in these studies is an EC Directive compliance issue. The detection of compounds not declared by manufacturers is also a health concern. Acute exposures of sensitized individuals to certain chemicals in body care cosmetic formulations could provoke an allergic response, but these data also suggest that the oestrogenic burden from cosmetics may be greater than is apparent to the general public and scientists alike from product labelling.

In considering the current exposures to parabens, which are still used extensively in body care products, historical exposures cannot be ignored. Past exposures may or may not be significant in terms of total or cumulative body burdens but they should not be ignored for lack of data, for example, on accumulation, persistence and tissue clearance. Cancer represents a risk over a lifetime and the effects of long-term, low-level exposures to weak environmental xenoestrogens on human health are unknown. With some products containing as much as 0.87% parabens (equivalent to 1 g in 125 ml, which is still below the maximum permitted concentrations internationally) the variability between low and high user rates (see McGrath, 2003 and later discussion) and consideration of the oestrogenic potency of parabens (see below) and their dermal absorption, it is possible to estimate and place the oestrogenic challenge to axilla or breast from this source into perspective. Harvey and Everett (2004) discuss systemic oestrogenic inputs in terms of total body burden, but the point should be made that there may be an issue of

Copyright © 2004 John Wiley & Sons, Ltd.

specific tissue sensitivity due to route and direct exposure (i.e. parabens absorbed dermally would contribute to total body burdens, but systemic exposure would be secondary to the translocation from skin, subcutis, adipose and breast tissues following external application to upper body, breast, chest or axilla areas). Furthermore, long-term and historical exposures and the impact on sensitive subgroups or those at elevated risk due to age, genetic–familial or other environmental factors are also important in considering issues of exposure at the population level and consequent risk (Darbre, 2003; Harvey, 2003). It should be considered also that any action may not be against a blank xenoestrogenic background. Other environmental chemicals, such as organochlorine agrochemicals and polychlorinated biphenyls with oestrogenic activity, also have been detected in the human breast (Falck *et al.*, 1992; Aronson *et al.*, 2000; Snedeker, 2001; Starek, 2003; Darbre *et al.*, 2004) and any effects of mixtures in endocrine disruption remain unknown.

PARABENS AND ENDOCRINE DISRUPTION: OESTROGENICITY

The role of oestrogen in the growth and development of breast cancer is reviewed in the next section. There are recent reports from a number of laboratories that various paraben esters are oestrogenic in a variety of test systems. Methyl-, ethyl-, propyl- and butylparabens are positive in the yeast oestrogen assay (Routledge *et al.*, 1998). In human MCF7 breast cancer cells, Byford *et al.* (2002) have shown that methyl-, ethyl-, *n*-propyl- and *n*-butylparaben are oestrogenic. Okubo *et al.* (2001) reported similar findings with ethyl-, propyl-, butyl-, isopropyl- and isobutylparaben in human MCF7 breast cancer cells and also that butylparaben and isobutylparaben increased progesterone receptor gene expression. Darbre *et al.* (2002, 2003), using both MCF7 and ZR-75-1 human breast cancer cell lines, report oestrogenic activity for isobutylparaben and benzylparaben. The structures of parabens compared with oestradiol are given in Fig. 1.

There are also reports of oestrogenicity of the parabens *in vivo*. Routledge *et al.* (1998) reported that butylparaben was positive in an immature rat uterotrophic assay by the subcutaneous but not oral administration route. Darbre *et al.* (2002, 2003) also report oestrogenic activity *in vivo*: isobutylparaben resulted in a uterotrophic response in immature mice following subcutaneous administration, but of most significance benzylparaben induced a uterotrophic response following topical administration (application to dorsal skin of 33 mg per mouse per day for 3 days; Darbre *et al.*, 2003). Additionally, Lemini *et al.* (1997) reported that subcutaneous administration of *p*-hydroxybenzoic acid (the major metabolic product of paraben esters) produced vaginal epithelial cell cornification and increased uterine weights (both classic effects of the action of endogenous oestradiol) in mice. One study so far has failed to show an oestrogenic response following subcutaneous dosing of various parabens at doses up to 100 mg kg^{-1} day^{-1} to the immature mouse, however 600 mg kg^{-1} day^{-1} administered subcutaneously to rats increased the uterine weights (Hossaini *et al.*, 2000).

It must be stated that the oestrogenic potency of the parabens is relatively weak in comparison to oestradiol. However, it must be stated also that the effects of long-term exposures (compared with the short treatment periods in the animal assays) to weak xenoestrogens on human health is unknown. Harvey (2003) discusses dose extrapolation from animals to humans in the context of risk assessment convention, including the application of safety factors to take into account limitations in animal models. Individual systemic oestrogenic inputs can be considered in terms of total body burdens (Harvey & Everett, 2004), notwithstanding the oestrogenic impact on particular tissues due to route, direct exposure, local tissue accumulation and concentration gradient issues that may be relevant to local dermal exposure and absorption.

It is interesting to note the oestrogenic activity of the parabens by the dermal route and that some studies have failed to show activity by the oral route compared with, for example, the subcutaneous route (presumably this is due to metabolic transformation of orally administered parabens). This may have a bearing on the relevance of using oral data to predict the complete toxicological profile of dermally applied compounds.

However, studies have shown adverse reproductive effects by the dietary route. It is worth noting that dietary administration can result in a different toxicokinetic profile compared with bolus oral dosing, characterized by more prolonged exposure to a lower achieved systemic dose: because paraben esters are rapidly absorbed, metabolized and excreted as urinary conjugates (Soni *et al.*, 2001, 2002), the more continuous inputs from the dietary route may produce the exposures necessary to detect effects. Oishi (2002a) reports adverse effects on male reproductive developmental parameters following dietary exposure of rats to 0.1% or 1% propylparaben. Oishi (2001, 2002b) also reports similar findings with 0.1% and 1% dietary butylparaben in rats and mice. Additionally, Kang *et al.* (2002) report adverse reproductive effects persisting in adult male offspring from rats treated with butylparaben subcutaneously during pregnancy, and Routledge *et al.* (1998) have shown that butylparaben is oestrogenic in female rats, indicating the potential mechanism.

On reviewing the significance of endocrine endpoints in toxicity data packages, a tiered weight of evidence approach can be adopted and this is useful in incomplete data packages and the identification of data gaps (Harvey & Johnson, 2002). The first tier contains structure–activity relationships and criteria are well developed for oestrogenicity, with phenolic structures being a major determinant of activity (see Dodge, 1998; Hong *et al.*, 2002; and Fig. 1 compares the phenolic structure of seven parabens with oestradiol). The next levels of data are the *in vitro* oestrogen assays (where it can be argued that human cells provide the most relevant models) followed by short-term mammalian *in vivo* assays such as the rat uterotrophic assay. The strongest evaluations of reproductive and endocrine endpoints would also include regulatory guideline-compliant reproductive toxicology studies conducted to Good Laboratory Practice: the USEPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) and the Organization for Economic Co-operation and Development (OECD) have relevant test guidelines and appropriate toxicity tests for reproduction toxicity would be OPPTS 870.3800, OECD 415 and OECD 416 and appropriate guidelines for prenatal developmental effects would be OPPTS 870.3700 and OECD 414 with the more recent screening protocols for

Copyright © 2004 John Wiley & Sons, Ltd.

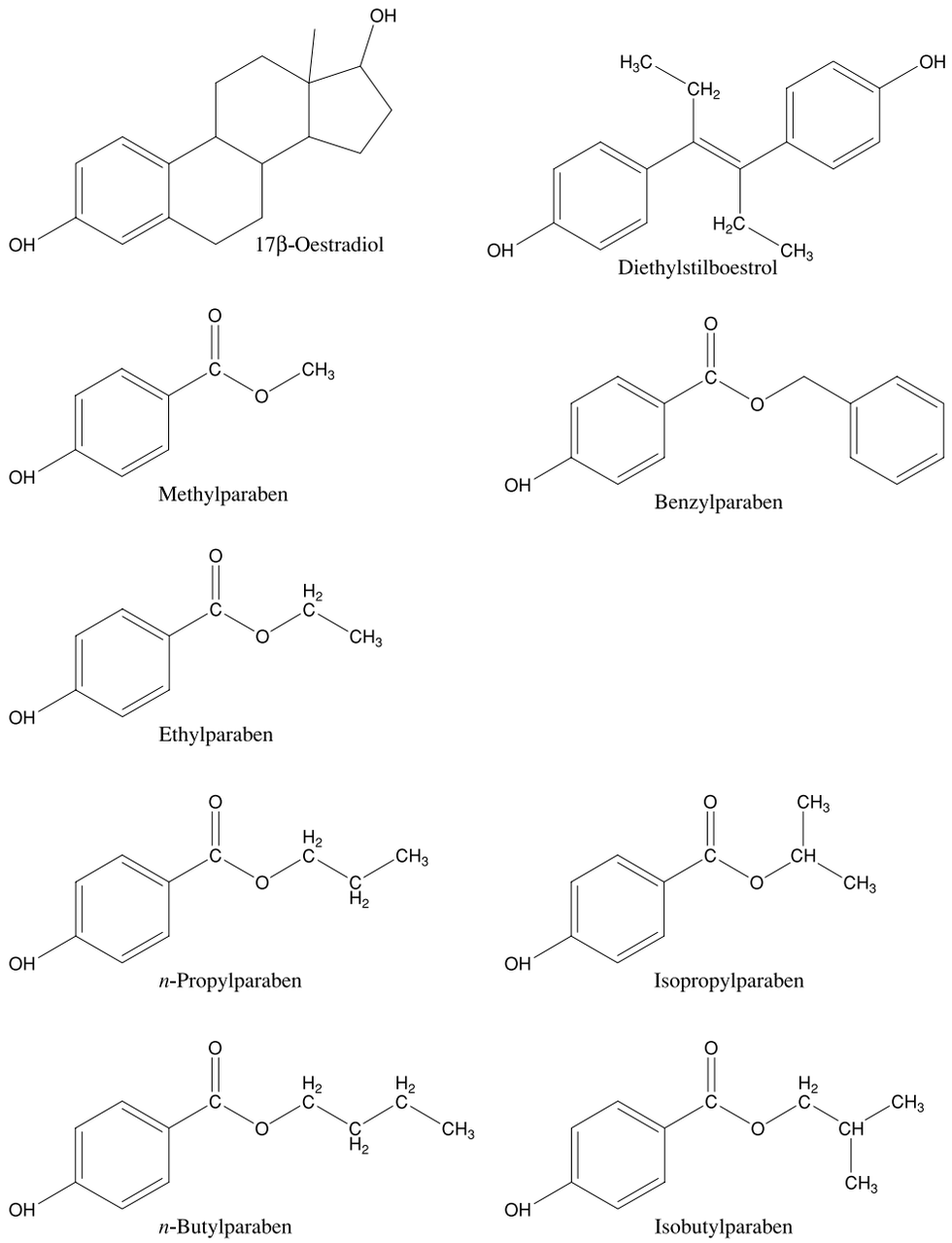

Figure 1. Chemical structure of seven parabens used in consumer products. The similarity in structure to part of 17β-oestradiol is apparent. Molecular modelling has suggested that parabens with straight chain alkyl groups could bind as pairs into the ligand binding domain of the human oestrogen receptor alpha with the hydroxyl groups of each paraben mimicking the positioning of the two hydroxyl groups of 17β-oestradiol (Byford *et al.,* 2002).

reproductive and developmental effects (OPPTS 870.3550 and OECD 421) providing useful starting points. There are also a number of dedicated endocrine tests recommended by the USEPA Endocrine Disrupter Screening and Testing Advisory Committee (see EDSTAC, 1998).

That the parabens as a group are deficient in toxicology data has been demonstrated (see also later discussion on regulatory status and data uncertainties). In their final report on the safety assessment of isobutylparaben and isopropylparaben, the Cosmetic Ingredient Review (CIR) Expert Panel indicated the absence of data on critical toxic endpoints necessary for adequate assessment of these two parabens (Willis, 1995). Consequently, the CIR referred to and extrapolated from data from the other related compounds: methyl-, ethyl-, propyl-, and butylparaben. It is logical to consider structurally related compounds in toxicological evaluations but it demonstrates the lack of complete data packages for individual parabens, and branching in the alkyl chain (from *n*- to iso-) now has been shown to enhance oestrogenic activity (Okubo *et al.*, 2001; Darbre *et al.*, 2002), which could affect the validity of certain extrapolations. Although literature reviews confirm the low general toxicity of both methylparaben and propylparaben (Soni *et al.*, 2001, 2002), they have not considered the more recent data on oestrogenicity. Thus, the recent findings of paraben oestrogenicity (and indeed reproductive and developmental toxicity) probably have not been considered in risk assessments to date.

Copyright © 2004 John Wiley & Sons, Ltd.

ROLE OF OESTROGEN IN BREAST CANCER

There is a wealth of information on the role of oestrogen, and other hormones, on the growth and development of breast cancer. Indeed the evidence is clear that even low doses of oestrogen designed as hormone replacement therapy (HRT) advance the onset of breast cancer (Bilimoria *et al.*, 1999), that HRT is recommended to be contraindicated following breast cancer diagnosis (Prasad *et al.*, 2003) and that reduction of oestrogens by new-generation aromatase inhibitors is being considered in a chemoprevention scenario (Goss & Strasser-Weippl, 2004). Furthermore, information on genetic and familial breast cancers suggests that only a small minority of cases have genetic aetiology: the UK cancer registry suggests that ca. 5% of breast cancers are due to highly penetrant dominant genes (Quinn & Allen, 1995). The majority of breast cancers therefore have an epigenetic environmental and lifestyle cause, with oestrogen probably *the* major factor in breast cancer aetiology. Lifestyle factors (including obesity, early age at menarche and delayed first pregnancy) are considered to affect breast cancer risk via oestrogen-mediated mechanisms and, indeed, modern clinical therapies for this cancer continue to use pharmacological oestrogen receptor blockade and synthetic suppression by aromatase inhibition (McPherson *et al.*, 1994; Wiseman, 1994; Elledge & Osbourne, 1997; Walker, 1999; Lønning, 2001; Goss & Strasser-Weippl, 2004). In short, preclinical, molecular and epidemiological evidence supports a role for oestrogen in all stages of breast tumour development (Goss & Strasser-Weippl, 2004).

Interestingly, in one of the largest studies to date (the so-called Million Women Study) investigating hormonal inputs (hormonal replacement therapy) and breast cancer incidence and risk, oestrogen–progestagen hormone replacement therapy was found to confer the greatest risk of breast cancer, followed by oestrogen therapy alone (Beral *et al.*, 2003). As mentioned previously, Okubo *et al.* (2001) reported that butylparaben and isobutylparaben increased progesterone receptor gene expression, as well as being oestrogenic.

The relevance of this is that, because oestrogens are such a major factor in breast cancer, could synthetic xenoestrogens such as the parabens contribute to the incidence of breast cancer or growth and the development of existing cancers? Further, are the parabens a special case because of the clear potential for significant, regular and direct dermal exposures?

PARABEN DETECTION IN THE HUMAN BREAST

Darbre *et al.* (2004) recently have reported the detection of various paraben esters in human breast tumour tissue. Methylparaben accounted for ca. 60% of the total parabens detected, with the remainder composed of ethyl-, *n*-propyl-, *n*-butyl and isobutylparaben esters. Parabens are readily absorbed through the skin and gastrointestinal tract and metabolism involves hydrolysis to *p*-hydroxybenzoic acid (see reviews on propylparaben and methylparaben by Soni *et al.*, 2001, 2002). On the basis that intact esters probably would not survive metabolic transformation by the liver, it has been suggested that the route of disposition into the breast was likely to be local dermal absorption. Skin esterases can be expected to hydrolyse the parabens to their main metabolite *p*-hydroxybenzoic acid (which also shows weak oestrogenic activity; e.g. Lemini *et al.*, 1997), but enzymes can be saturated in an acute overload situation. Indeed, Bando *et al.* (1997), using an *in vitro* rat skin model, have shown that 4% of butylparaben and 30% of propylparaben penetrated the skin intact and were not metabolized to *p*-hydroxybenzoic acid. The significance of these data is that paraben esters can be absorbed into the body in intact form but confirmation of route, accumulation and persistence in breast tissue (from Darbre *et al.*, 2004) and characterization of potential harmful effects warrant further research.

The potential source of parabens from underarm and body care cosmetics formulations has been discussed previously in terms of the concentrations of these chemicals in a variety of formulations. It is important to note that the type of cosmetic, e.g. an underarm deodorant versus a body lotion or moisturiser, is irrelevant. What is important is the chemicals used in the formulations and the frequent application to the skin covering the upper body, chest, breast or axilla. The lymphatic drainage of the breast is a consideration and, although the principal route is drainage out of the breast and to the axilla, any drainage pattern from any quadrant of the breast can occur (Tanis *et al.*, 2001). Lymphatic anatomy is not fully understood, although tracer studies have assisted in the lymphatic mapping of, in particular, sentinel nodes in breast cancer (e.g. Tanis *et al.*, 2001). The path into the breast of chemicals such as the parabens (which are readily dermally absorbed (Soni *et al.*, 2001, 2002) as intact esters (Bando *et al.*, 1997) and are lipid soluble) following dermal application to the upper body, chest and breast has not been studied specifically. The relevance of lymphatic drainage under frequent chemical overload is unknown. Persistent dermal absorption of chemicals through the skin could exceed both the capacity of tissue enzymes and any lymphatic drainage, with the net result of chemical deposition into underlying tissues, including those of the breast. It is realistic to assume that concentration gradients will exist from where the chemicals in formulations are applied to the skin surface, through the subcutis, adipose and other tissues within the breast area. Local accumulation, persistence, metabolism and clearance of chemicals, via lymphatic drainage or otherwise, remain to be studied. It is also worth noting that the balance between internal and external pressures in a lymphatic channel can be influenced by massage in a negative or positive way to affect lymph flow (Tanis *et al.*, 2001) and the application of moisturisers and lotions involves such tissue massage. Although the presence of organochlorines in human breast tissue (Falck *et al.*, 1992; Aronson *et al.*, 2000; Snedecker, 2001) is likely to have arisen through oral exposure to environmental residues, the presence of intact paraben esters in the breast equally could have occurred by local dermal absorption; this requires specific confirmation (see Darbre *et al.*, 2004).

The detection of paraben esters in breast tumour tissue should not be taken to imply causality of the individual tumours, and discussion of these issues is given by Harvey and Everett (2004). However, Darbre *et al.* (2004) make the point that the concentrations detected can be compared

Copyright © 2004 John Wiley & Sons, Ltd.

with the concentrations of parabens shown to be oestrogenic in human breast cancer cells *in vitro*.

Darbre *et al*'s (2004) study is a contribution to a body of literature that reports chemicals in human breast tissue, with the suggestion that these compounds may be carcinogenic (Falck *et al.*, 1992; Snedeker, 2001). Breast organochlorine concentrations have been suggested to be correlated with increased cancer risk (Aronson *et al.*, 2000; Starek, 2003) and this has been related to organochlorine oestrogenicity (Starek, 2003).

It is interesting to note that there is a recent report that some medications contain phthalates (Hauser *et al.*, 2004). These are also compounds implicated in endocrine disruption and, compared with diffuse environmental exposures, the contribution from pharmaceuticals could be significant. Pharmaceuticals may also contain parabens as preservatives. Data from the US Food and Drug Administration (USFDA) and drug manufacturers on excipients (which are listed not least because of allergy issues) for four main breast cancer drugs (tamoxifen [Nolvadex], letrozole, anastrozole and exemestane [Aromasin]) reveal that only Aromasin (USFDA, 1999) was found to contain a paraben, the paraben being methylparaben. Aromasin was not the source of the parabens in the tumour samples from the patients reported in Darbre *et al.* (2004). Furthermore, Darbre *et al.* (2004) detected intact esters, which would probably not survive oral administration (*p*-hydroxybenzoic acid being the common metabolite of paraben esters). Obviously this raises the concern that there are oestrogenic inputs into potentially high-risk patients from pharmaceutical sources, but this may not be significant when all sources are considered.

EPIDEMIOLOGY OF UNDERARM COSMETICS USE AND BREAST CANCER

A recent attempt to examine if antiperspirants or deodorants affected breast cancer incidence was reported by Mirick *et al.* (2002). They conducted a population-based case-controlled study to investigate the relationship between the use of products applied for underarm perspiration and the risk of breast cancer in women aged 20–74 years, by retrospective interview of 813 case patients and 793 controls. They reported no increase in risk of breast cancer following the use of antiperspirants/deodorants and no effect of shaving.

In stark contrast, McGrath (2003) very recently has reported dramatically earlier ages of onset (measured by age of diagnosis) of breast cancer in women who use underarm antiperspirants/deodorants. McGrath conducted a survey of 437 women diagnosed with breast cancer and gave a detailed analysis, examining the age of starting the use of products and shaving, as well as the intensity or frequency of hygiene practice. Once grouped by frequency of underarm hygiene habits, the mean age of diagnosis was the primary endpoint. Both the frequency and earlier age of starting the use of antiperspirants/deodorants with underarm shaving were associated with an earlier age of breast cancer diagnosis (differences in age between frequent users and non-users was in the range 14.7–22 years, depending on comparisons indicating a marked effect). These results clearly suggest a chemical exposure dose–response effect (although specific toxic agents were not identified) and critical sensitivity at a younger age of exposure. It is interesting to note that oestrogen is also known to advance the onset of breast cancer, as measured by earlier diagnosis (Bilimoria *et al.*, 1999).

With such apparent differences in results there is a need to extend this line of research, and future designs must have adequate depth of enquiry and control comparisons, not be limited to product type (the chemicals actually applied to the skin are the primary concern) and take into account exposure/dose issues (frequency and duration of use) as well as age of exposure commencement. The issue is one of toxicology of chemicals, and these are basic questions.

An interesting fact discussed by Darbre (2003) is that there is a disproportionately large number of primary tumours affecting the upper outer quadrant, and also the left breast. If this cannot be explained by inherent physiological or anatomical differences, it may be taken as evidence of a lifestyle effect: relative chemical dose applied to this area in right-handed women (who form the larger proportion of the population) has been suggested as a possible reason.

REGULATORY STATUS OF PARABENS AND DATA UNCERTAINTIES

Although the use of parabens in cosmetics in the European Community falls under Directive 76/768/EEC dating from 1976, the toxicology of the compounds has had more recent regulatory evaluations in Europe for their safety in food.

The Health and Consumer Protection Directorate of the European Commission, Scientific Committee on Food (SCF), has clearly indicated concerns over the toxicology of the parabens (SCF/CS/ADD/CONS/53 Final, 4 April 2003) dating back to reviews in 1994 and again in 2000, with withdrawal of the acceptable daily intake (ADI) currently pending (SCF, 2003). Further studies on parabens in the rat to investigate cell proliferation in the forestomach and developmental toxicity have been requested. The SCF statement clearly indicates that 'the available data showed some inadequacies and uncertainties'. Although the proliferative lesions in the forestomach of the rat are of equivocal toxicological significance, the developmental toxicology is of more concern. Indeed, several recent studies report adverse effects of dietary propylparaben (Oishi, 2002a) and butylparaben (Oishi, 2001, 2002b) on the development of the male reproductive system in rats and mice. Kang *et al.* (2002) also reported adverse reproductive effects persisting in adult male offspring from rats treated subcutaneously with butylparaben during pregnancy. Clearly these findings, together with the recent information on the oestrogenicity of a variety of paraben esters *in vitro* and *in vivo*, could have a bearing on the existing developmental toxicity concerns of the SCF.

The Joint World Health Organization and United Nations Food and Agriculture Organization (WHO/FAO) Expert Committee on Food Additives (JECFA) evaluated the paraben toxicology database in 1966 and 1974 on their safety for use in food. An ADI was set for the ethyl, methyl and propylparaben esters of 0–10 mg kg^{-1} day^{-1} (WHO, 1974). This appears not to have been updated since. The toxicology studies on which this ADI was based were

Copyright © 2004 John Wiley & Sons, Ltd.

conducted in the 1950s and 1960s. As well as lacking any meaningful carcinogenicity studies, the database also lacked any reproductive toxicology data (studies likely to detect effects on oestrogen-sensitive target organs and tissues) or developmental toxicity studies. Because no adequate studies were available on butylparaben, a toxicological evaluation was deemed impossible (WHO, 1966a). The database is also lacking for propylparaben (WHO, 1966b). There are no other current initiatives within this WHO/FAO framework to evaluate the parabens, except to establish the current use of parabens in foodstuffs.

Of most importance, there are few toxicology data available to make any judgement of either toxicity or risk. The lack of any meaningful toxicology database for the SCF and JECFA is probably not an issue if parabens are no longer used in foods, because this is the extent of their remit. It is of more importance that there is no regulatory standard toxicology database that can be applied to the safety of human exposures in general.

The paucity of this database is clear even as applied to oral exposures. The available data would now be considered inadequate (indeed, the SCF judge the data that are available to be inadequate; SCF, 2003) and cannot be extrapolated readily to the dermal route (because of the differences in metabolism and oestrogenic effects that occur with dermal exposures). Evaluations of parabens specifically for their use in cosmetics have also noted toxicology data gaps (Willis, 1995). The emerging findings of oestrogenicity and reproductive toxicity from a number of laboratories require incorporation into risk assessments as a matter of priority, and such assessments should especially consider exposures and potential effects of parabens applied dermally to model the use patterns of personal and body care formulations containing these chemicals (Harvey, 2003).

CALL FOR FURTHER RESEARCH

The issues reviewed are complex but the basic question is: what is the reason for the rising rates of breast cancer in women? The evidence is consistent with an environmental cause and, given the role of oestrogen in breast cancer, there is logic in the hypothesis that environmental exposure to xenoestrogens is a factor. In developing this hypothesis further and examining potential sources of xenoestrogenic stimulation, direct application to the skin and local absorption of oestrogenic chemicals in body care cosmetics (moisturizers, lotions, cremes, deodorants, etc.) is suggested as a significant potential route of exposure. Such xenoestrogenic stimulation is likely to exert any effects in combination with other known contributory factors and the relative importance of each, or any interactions, is currently unknown.

It is suggested that body care cosmetics are a potentially important source of oestrogenic chemicals, and consequently that body care cosmetics may be associated with the rising incidence of breast cancer in women (Darbre, 2001, 2003; Harvey, 2003). The parabens are one group of chemicals that are used extensively in body care formulations, are oestrogenic in a variety of assays, are readily absorbed dermally and have been detected in human breast tumour tissue. At present there is no proven causal link between parabens as oestrogenic chemicals currently and historically used in underarm and body care formulations and breast cancer (Bradford-Hill criteria are discussed below). Given the recent evidence of the oestrogenicity of parabens, together with the fact that they are readily absorbed, the question of whether they are suitable to apply regularly to the skin of the general population is questioned, especially in light of an inadequate toxicology database (SCF, 2003). The potential effects of the parabens on human health, particularly the incidence of breast cancer, is promoted to warrant further study (as indeed are other hormonally active chemicals also fitting high-exposure patterns).

A related question is whether underarm and body care cosmetics *per se* are linked to breast cancer: the current database of two studies is conflicting. One study asked breast cancer patients about their use of antiperspirants and compared answers with those from healthy controls (i.e. healthy controls at the time of the study, as women can go on to develop breast cancer), finding no differences. A second study grouped breast cancer patients by the intensity and frequency (i.e. considered dose and exposure) with which they used such products and compared this with breast cancer patients who had never used such products, finding a difference in age of diagnosis. These data indicated a dose–response relationship.

Clearly, there are several issues that deserve further investigation, not least because the identification of plausible factors contributing to breast cancer incidence must be considered a research priority. The issue is focused because body care cosmetics present a very clear scenario in terms of exposure (or dose) and the size of the population involved. The widespread use of, and direct exposure to, body care cosmetics, in contrast to diffuse environmental exposures, should provide a large and diverse sample to study and be amenable to evaluation, sub-sample selection (e.g. to match age, other lifestyle or risk factors), quantification and control.

Similarly, recent evidence has altered the knowledge base of the toxicological profile of the parabens as *one* extremely common class of ingredients in body care cosmetics. It is not the purpose of this review to dictate what work should be done but to suggest the inadequacies of the regulatory toxicology database upon which the use of parabens have historically relied and to indicate that there is evidence supporting a hypothesis that oestrogenic compounds, and other chemicals, found in personal and body care formulations may be linked to the rising incidence of breast cancer. Certainly the supplementation of the existing paraben toxicology database, and the study of whether cosmetic use is associated with breast cancer and what the active chemical principles may be, are to be encouraged.

The step must be taken from hypothesis to rigorous testing in order to establish if this is the first case of environmental endocrine disruption to adversely affect human health. Additional epidemiology studies would be worthwhile and could include basic assessment of cross-cultural incidences of breast cancer and body care hygiene practices, e.g. high cosmetic user versus low user groups, but adequate consideration must be devoted to recording the chemicals actually present in formulations applied to the body (regardless of whether these are sprays, lotions or creams, antiperspirants or moisturizers) and indicators of dose–response relationships. Application of Bradford-Hill criteria for establishing association or causality (strength, consistency, specificity, temporality, biological gradient,

Copyright © 2004 John Wiley & Sons, Ltd.

i.e. dose response, plausibility, coherence, experimental evidence; Bradford-Hill, 1966; Shakir & Layton, 2002) provides a basis for evaluation of this hypothesis and for the generation of new data, and indicates data already fitting these criteria and where more are needed.

Although the use of body care formulations and dermal absorption of the chemical ingredients obviously provides direct exposure of the adult, it should be recognized that this may contribute ultimately to systemic burdens and exposure of the developing foetus *in utero*, potentially resulting in health consequences in later life. Sharpe and Irvine (2004) suggest that, in the field of endocrine disruption and human health, maternal exposure in pregnancy (resulting in possible effects on the health of the foetus in later life) is the greatest concern. They suggest ways to minimize risk in individuals, e.g. by 'life style changes in women seeking to become pregnant (stopping smoking, reduced use of cosmetics and body creams)'. Finally, given the uncertainties in the toxicology of the parabens (coupled with the large population exposed to these compounds through their ubiquitous use in a wide variety of body care product formulations and their propensity for dermal absorption), it would be prudent to adopt a precautionary principle until such regulatory data or appropriate risk assessments can be generated to prove their safety in use.

REFERENCES

Aronson KJ, Miller AB, Woolcott CG, Sterns EE, McCready DR, Lickley LA, Fish EB, Hiracki GY, Holloway C, Ross T, Hanna WM, SenGupta SK, Weber JP. 2000. Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.* **9**: 55–63.

Ashby J. 2001. Testing for endocrine disruption post-EDSTAC: extrapolation of low dose rodent effects to humans. *Toxicol. Lett.* **120**: 233–242.

Bando H, Mohri S, Yamashita F, Takakura Y, Hashida M. 1997. Effects of skin metabolism on percutaneous penetration of lipophilic drugs. *J. Pharm. Sci.* **86**: 759–761.

Beral V, Million Women Study Collaborators. 2003. Breast cancer and hormone replacement in the million women study. *Lancet* **362**: 419–427.

Bilimoria MM, Winchester DJ, Sener SF, Motykie G, Sehgal UL, Winchester DP. 1999. Estrogen replacement therapy and breast cancer: analysis of age of onset and tumor characteristics. *Ann. Surg. Oncol.* **6**: 200–207.

Bradford-Hill A. 1966. The environment and disease: association or causation. *Proc. R. Soc. Med.* **58**: 295.

Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. 2002. Oestrogenic activity of parabens in MCF7 human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* **80**: 49–60.

Darbre PD. 2001. Underarm cosmetics are a cause of breast cancer. *Eur. J. Cancer Prev.* **10**: 389–393.

Darbre PD. 2003. Underarm cosmetics and breast cancer. *J. Appl. Toxicol.* **23**: 89–95.

Darbre PD, Byford JR, Shaw LE, Horton RA, Pope GS, Sauer MJ. 2002. Oestrogenic activity of isobutylparaben *in vitro* and *in vivo*. *J. Appl. Toxicol.* **22**: 219–226.

Darbre PD, Byford JR, Shaw LE, Hall S, Coldham NG, Pope GS, Sauer MJ. 2003. Oestrogenic activity of benzylparaben. *J. Appl. Toxicol.* **23**: 43–51.

Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. 2004. Concentrations of parabens in human breast tumours. *J. Appl. Toxicol.* **24**: 5–13.

Dodge JA. 1998. Natural and anthropogenic environmental oestrogens: the scientific basis for risk assessment. Structure/activity relationships. *Pure Appl. Chem.* **70**: 1725–1733.

EDSTAC. 1998. *Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) Final Report*, August 1998. United States Environmental Protection Agency. Http://www.epa.gov/scipoly/oscpendo/history/finalrpt.htm

Elledge RM, Osborne CK. 1997. Oestrogen receptors and breast cancer. *British Medical Journal* **314**: 1834.

Falck F, Ricci A, Wolff MS, Godbold J, Deckers P. 1992. Pesticides and polychlorinated biphenyl residues in human breast lipids and their relation to breast cancer. *Arch. Environ. Health* **47**: 143–146.

Goss PE, Strasser-Weippl K. 2004. Aromatase inhibitors for chemoprevention. *Best Pract. Res. Clin. Endocrinol. Metab.* **18**: 113–130.

Gruvberger B, Bruze M, Tammela M. 1998. Preservatives in moisturisers on the Swedish market. *Acta. Derm. Venereol.* **78**: 52–56.

Harrison PTC. 2001. Endocrine disrupters and human health. Current research will establish baseline indices. *British Medical Journal* **323**: 1317–1318.

Harvey PW. 2003. Parabens, oestrogenicity, underarm cosmetics and breast cancer: a perspective on a hypothesis. *J. Appl. Toxicol.* **23**: 285–288.

Harvey PW, Everett DJ. 2004. Significance of the detection of esters of *p*-hydroxybenzoic acid (parabens) in human breast tumours. *J. Appl. Toxicol.* **24**: 1–4.

Harvey PW, Johnson I. 2002. Approaches to the assessment of toxicity data with endpoints related to endocrine disruption. *J. Appl. Toxicol.* **22**: 241–247.

Hauser R, Duty S, Godfrey-Bailey L, Calafat AM. 2004. Medications as a source of human exposure to phthalates: a case report. *Environmental Health Perspectives* available online 29 January 2004, doi: 10.1289/ehp.6804 [http://dx.doi.ora]

Hayden JF, Barlow SA. 1972. Structure–activity relationships of organosilicones and the female reproductive system. *Toxicol. Appl. Pharmacol.* **21**: 68–79.

Hong H, Tong W, Fang H, Shi L, Xie Q, Perkins R, Walker JD, Branham W, Sheehan DM. 2002. Prediction of estrogen receptor binding for 58,000 chemicals using an integrated system of a tree based model with structural alerts. *Environ. Health Perspect.* **110**: 29–36.

Hossaini A, Larsen JJ, Larsen JC. 2000. Lack of oestrogenic effects of food preservatives (parabens) in uterotrophic assays. *Food Chem. Toxicol.* **38**: 319–323.

Kala SV, Lykissa ED, Neely MW, Lieberman MW. 1998. Low molecular weight silicones are widely distributed after a single subcutaneous injection in mice. *Am. J. Pathol.* **152**: 645–649.

Kang KS, Che JH, Ryu DY, Kim TW, Li GX, Lee YS. 2002. Decreased sperm number and motile activity on the F1 offspring maternally exposed to butyl *p*-hydroxybenzoic acid (butyl paraben). *J. Vet. Med. Sci.* **64**: 227–235.

Lemini C, Silva G, Timossi C, Luque D, Valverde A, Gonzalez-Marti M, Hernandez A, Rubio-Poo C, Chavez Lara B, Valezuela F. 1997. Estrogenic effects of *p*-hydroxybenzoic acid in CD1 mice. *Environ. Res.* **75**: 130–134.

Levier RR, Jankowiak ME. 1972. The hormonal and antifertility activity of 2, 6-*cis*-diphenylhexamethylcyclotetrasiloxane in the female rat. *Biol. Reprod.* **7**: 260–266.

Lønning PE. 2001. Aromatase inhibitors and inactivators in breast cancer. *British Medical Journal* **323**: 880–881.

Luu HMD, Hutter JC. 2001. Bioavailability of octamethylcyclotetrasiloxane (D4) after exposure to silicones by inhalation and implantation. *Environ. Health Perspect.* **109**: 1095–1101.

McGrath KG. 2003. An earlier age of breast cancer diagnosis related to more frequent use of antiperspirants/deodorants and underarm shaving. *Eur. J. Cancer Prev.* **12**: 479–485.

McLachlan JA. 1997. Synergistic effect of environmental estrogens: report withdrawn. *Science* **277**: 462–463.

McPherson KM, Steel CM, Dixon JM. 1994. ABC of breast diseases: breast cancer-epidemiology, risk factors and genetics. *British Medical Journal* **309**: 1003–1006.

Copyright © 2004 John Wiley & Sons, Ltd.

Mirick DK, Davis S, Thomas DB. 2002. Antiperspirant use and the risk of breast cancer. *J. Natl. Cancer. Inst.* **94**: 1578–1580.

Oishi S. 2001. Effects of butylparaben on the male reproductive system in rats. *Toxicol. Ind. Health.* **17**: 31–39.

Oishi S. 2002a. Effects of propylparaben on the male reproductive system. *Food Chem. Toxicol.* **40**: 1807–1813.

Oishi S. 2002b. Effects of butylparaben on the male reproductive system in mice. *Arch. Toxicol.* **76**: 423–429.

Okubo T, Yokoyama Y, Kano K, Kano I. 2001. ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ER alpha and PR. *Food Chem. Toxicol.* **39**: 1225–1232.

Prasad R, Boland GP, Cramer A, Anderson E, Knox WF, Bundred N. 2003. Short-term biologic response to withdrawal of hormone replacement therapy in patients with invasive breast carcinoma. *Cancer* **98**: 2539–2546.

Quinn M, Allen, E. 1995. Changes in incidence of and mortality from breast cancer in England and Wales since the introduction of screening. *British Medical Journal* **311**: 1391–1395.

Rastogi SC. 2000. Analytical control of preservative labelling on skin creams. *Contact Derm.* **43**: 339–343.

Rastogi SC, Schouten A, de Kruijf N, Weijland JW. 1995. Contents of methyl-, ethyl-, propyl-, butyl- and benzylparaben in cosmetic products. *Contact Derm.* **32**: 28–30.

Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. 1998. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicol. Appl. Pharmacol.* **153**: 12–19.

SCF. 2003. *Statement of the Scientific Committee on Food on the Parabens*. European Commission, Health and Consumer Protection Directorate, Scientific Committee on Food SCF/CS/ADD/CONS/53 Final 2 April 2003. http://europa.eu.int/comm/food/fs/sc/scf

Shakir SAW, Layton D. 2002. Causal association in pharmacovigilance and pharmacoepidemiology — thoughts on the application of the Austin Bradford-Hill criteria. *Drug Safety* **25**: 467–471.

Sharpe RM, Skakkebaek NE. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **341**: 1392–1395.

Sharpe RM, Irvine DS. 2004. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *British Medical Journal* **328**: 447–451.

Snedeker SM. 2001. Pesticides and breast cancer risk: a review of DDT, DDE and dieldrin. *Environ. Health Perspect.* **109**: (Suppl. 1): 35–47.

Soni MG, Burdock GA, Taylor SL, Greenberg NA. 2001. Safety assessment of propyl paraben: a review of the published literature. *Food Chem. Toxicol.* **39**: 513–532.

Soni MG, Taylor SL, Greenberg NA, Burdock GA. 2002. Evaluation of the health aspects of methyl paraben: a review of the published literature. *Food Chem. Toxicol.* **40**: 1335–1373.

Starek A. 2003. Estrogens and organochlorine xenoestrogens and breast cancer risk. *Int. J. Occup. Med. Environ. Health* **16**: 113–124.

Tanis PJ, Nieweg OE, Valdes Olmos RA, Kroon BB. 2001. Anatomy and physiology of lymphatic drainage of the breast from the perspective of sentinel node biopsy. *J Am. Coll. Surg.* **192**: 399–409.

USFDA. 1999. Aromasin (exemestane) tablets, product label. Available at [www.fda.gov/cder/foi/label/1999/20753lbl.pdf.

Walker RA. 1999. Hormonal mechanisms in breast cancer. In *Endocrine and Hormonal Toxicology*, Harvey PW, Rush KC, Cockburn A (eds). John Wiley; Chichester; 487–506.

WHO. 1966a. *Butyl* p-*Hydroxybenzoate. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment agents, Acids and Bases*. Joint FAO/WHO Expert Committee on Food Additives. http://www.inchem.org/documents/jecfa/jecmono/40abcj03.htm

WHO. 1966b. *Propyl* p-*Hydroxybenzoate. Toxicological Evaluation of Some Antimicrobials, Antioxidants, Emulsifiers, Stabilizers, Flour-treatment Agents, Acids and Bases*. Joint FAO/WHO Expert Committee on Food Additives. http://www.inchem.org/documents/jecfa/jecmono/40abcj06.htm

WHO. 1974. *Hydroxy Benzoate:* p-*Ethyl, Methyl, Propyl Esters. WHO Food Additives Series 5. Toxicological Evaluation of Some Food Additives, including Anticaking Agents, Antimicrobials, Antioxidants, Emulsifiers and Thickening Agents*. Joint FAO/WHO Expert Committee on Food Additives. http://www.inchem.org/documents/jecfa/jecmono/v05je13.htm

Willis L. 1995. Final report on the safety assessment of isobutylparaben and isopropylparaben. *J. Am. Coll. Toxicol.* **14**: 364–372.

Wiseman H. 1994. Tamoxifen. Molecular basis of use in cancer treatment and prevention. John Wiley: Chichester.

Copyright © 2004 John Wiley & Sons, Ltd.