
EDITORIAL

Significance of the Detection of Esters of *p*-Hydroxybenzoic Acid (Parabens) in Human Breast Tumours

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This issue of *Journal of Applied Toxicology* publishes the paper *Concentrations of Parabens in Human Breast Tumours* by Darbre *et al.* (2004), which reports that esters of *p*-hydroxybenzoic acid (parabens) can be detected in samples of tissue from human breast tumours. Breast tumour samples were supplied from 20 patients, in collaboration with the Edinburgh Breast Unit Research Group, and analysed by high-pressure liquid chromatography and tandem mass spectrometry. The parabens are used as antimicrobial preservatives in underarm deodorants and antiperspirants and in a wide range of other consumer products. The parabens also have inherent oestrogenic and other hormone related activity (increased progesterone receptor gene expression). As oestrogen is a major aetiological factor in the growth and development of the majority of human breast cancers, it has been previously suggested by Darbre that parabens and other chemicals in underarm cosmetics may contribute to the rising incidence of breast cancer. The significance of the finding of parabens in tumour samples is discussed here in terms of 1) Darbre *et al.*'s study design, 2) what can be inferred from this type of data (and what can not, such as the cause of these tumours), 3) the toxicology of these compounds and 4) the limitations of the existing toxicology database and the need to consider data that is appropriate to human exposures. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

This issue of *Journal of Applied Toxicology* publishes the paper 'Concentrations of Parabens in Human Breast Tumours' by Darbre *et al.* (2004) which reports that esters of *p*-hydroxybenzoic acid (parabens) can be detected in samples of tissue from human breast tumours. Breast tumour samples were supplied from 20 patients in a collaboration with the Edinburgh Breast Unit Research Group, and analysed by high pressure liquid chromatography and tandem mass spectrometry. The parabens are used as antimicrobial preservatives in underarm deodorants and antiperspirants, and in a wide range of other consumer products. The parabens also have inherent oestrogenic activity (briefly reviewed in the next section) and oestrogen is a *major* aetiological factor in the growth and development of human breast cancer. It has previously been suggested that chemicals in underarm cosmetics may contribute to the rising incidence of breast cancer (Darbre, 2001; 2003; and see Harvey, 2003) and the significance of the finding of parabens in tumour samples is therefore highly topical.

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OESTROGEN AS A COMMON FACTOR IN BREAST CANCER AND PARABEN TOXICITY

It has been known for many years that oestrogen is the major aetiological factor in the development of breast cancer and, indeed, modern therapies continue to use pharmacological receptor blockade and synthetic suppression (e.g. aromatase inhibition) in clinical treatments (McPherson *et al.*, 1994; Wiseman, 1994; Elledge & Osbourne, 1997; Walker, 1999; Lønning, 2001; Beral *et al.*, 2003). Given this, it is logical to suggest that application of oestrogenic agents to areas adjacent to the breast may be an unnecessary risk in some women (in this context it has been suggested that first-degree relatives of breast cancer patients and peri-adolescent females would be at most risk of continued exposure to oestrogenic chemicals). The ubiquitous use of underarm deodorants and antiperspirants throughout the Western world means that millions of women have applied a range of chemicals to the axilla of the arm and it is surprising that only recently have some of these chemical ingredients been screened for the toxicologically important endpoints of inherent oestrogenic and hormonal activity.

There are now numerous reports that various parabens are oestrogenic. Lemini *et al.* (1997) reported that subcutaneous administration of *p*-hydroxybenzoic acid produced vaginal cornification and increased uterine weights (both classic effects of the action of endogenous oestradiol)

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in mice. Routledge *et al.* (1998) reported that butylparaben competed with [³H]oestradiol in an oestrogen receptor binding assay, that methyl-, ethyl-, propyl- and butylparaben were weakly positive in a yeast oestrogen assay and that butylparaben was positive in an immature rat uterotrophic assay by the subcutaneous (but not oral; see later) administration route. 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 and also that butylparaben and isobutylparaben increased progesterone receptor gene expression. Interestingly, oestrogen-progestagen hormone replacement therapy confers the greatest risk of breast cancer (Beral *et al.*, 2003) and the parabens show activity to both oestrogen and progesterone receptors. Darbre *et al.* (2002, 2003), using both MCF7 and ZR-75-1 human breast cancer cell lines, report oestrogenic activity for isobutylparaben and benzylparaben. These latter studies also reported 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). Parabens also have structures predicted to bind to the oestrogen receptor (Hong *et al.*, 2002).

Harvey (2003) provides a perspective on the dose levels reported to produce effects in short-term *in vivo* animal studies (i.e. while dose levels are relatively high, convention dictates that risk assessments would apply safety factors of at least 100-fold to data from animal studies when extrapolating to safe human exposures) and the relative lack of activity by the oral route (presumably due to metabolic breakdown, an effect that apparently does not occur with the more direct subcutaneous or topical administration routes in animal studies) as factors particularly relevant to risk assessments specific for cosmetic use, and the possibility of inappropriate extrapolation from the existing parabens animal toxicology database. In reviewing the various reports of paraben oestrogenicity, potency ranges from 500-fold less than oestradiol (reported by Lemini *et al.* (1997) in a rat uterotrophic assay following subcutaneous administration of *p*-hydroxybenzoic acid) to 10 000-fold less potent as reported by Routledge *et al.* (1998) for butylparaben in the *in vitro* yeast assay. Clearly there is a need to place human exposures of the parabens into perspective: contributions to the total body burden of oestrogenic agents include endogenous oestrogen and a variety of xenobiotics (e.g., resorcylic acid lactone residues in food; Everett *et al.*, 1987). Parabens represent just one class of these oestrogenic materials, all of which need to be considered both in terms of inherent oestrogenic potency as well as their actual concentrations in human tissues. Although oestrogenic potency of the parabens is relatively weak, the use patterns of underarm cosmetics and parabens in other products can result in long-term exposures.

SIGNIFICANCE OF THE DETECTION OF PARABENS IN BREAST TUMOURS

Darbre *et al.* (2004) have shown that a common group of chemicals used in underarm deodorant and antiperspirant

formulations and other consumer products, previously generally regarded as safe but recently shown to possess oestrogenic activity in a wide variety of assays, can be detected in human breast tumour tissue. This finding would logically be a significant prerequisite criterion to the hypothesis that these compounds may be involved in, or in some way contribute to, the incidence of breast cancer (which has steadily risen over recent decades in the UK and elsewhere, paralleling, for example, underarm cosmetic usage; see Darbre, 2003) in that there would obviously need to be cellular exposure to these chemicals in order to induce any direct carcinogenic response. *If* the source of these chemicals was prior historical use of underarm cosmetics containing these ingredients (at present it is not known what the half-life or clearance of these chemicals from human breast tissue is, or the contribution from sources other than underarm cosmetics — see below), then such data suggest that these chemicals can be absorbed dermally and probably persist in human breast tissue. Several points must be made in discussing Darbre *et al.*'s (2004) findings:

- (i) The detection of parabens in breast tumour tissue should not be taken to imply causality of the individual cancer, because the findings are essentially coincidental in nature.
- (ii) 'Normal' breast tissue, and other tissue, was not analysed. Although the question remains of what levels occur in such control tissue, it should be recognized that apparently normal tissue at the *time* of biopsy may later develop a tumour (this is important because cancer represents a risk over a lifetime and not a single time point) and there are questions of what would be an appropriate control for this type of data.
- (iii) The obvious route of entry into the breast tissue is local absorption from the underarm (because esters were detected rather than metabolites) and the source is probably therefore underarm cosmetics. However, the source needs to be confirmed and Darbre *et al.* make it clear that their study does not identify route.
- (iv) Although the data could be consistent with local absorption, it would be interesting to establish what the levels of parabens are in other tissues (e.g. blood, adipose and those also sensitive to oestrogen).
- (v) It is obvious that extraneous synthetic organic chemicals serve no useful function in the human breast but, the question is, have they caused harm?
- (vi) Related to this, Darbre *et al.*'s (2004) study analysed parabens because of interest in their use in underarm cosmetics: other chemicals also may be present (because these types of study are records of single time points, the levels of a variety of extraneous chemicals could increase or decline over a lifetime).
- (vii) Darbre *et al.*'s (2004) study shows the presence of parabens in breast tumour tissue: although it has been emphasized that the significance of this should not be overinterpreted, their route of disposition and possible effects on the breast are worthy of further investigation.
- (viii) In the general context of the hypothesis, any response of cells in the breast will depend on the properties of the chemicals, the timing and relative duration of exposure (consider potential differences of effect

between adolescent exposure with the developing breast and exposure in later life), the dose and the interaction with other genetic and environmental factors.

GENERAL CONSIDERATIONS AND CONCLUSION

The findings of parabens in tumour samples are additional results in line with the general hypothesis that there may be a link between oestrogenic compounds commonly used in underarm cosmetics and other consumer products and breast cancer. The results alone, however, do not suggest that these chemicals caused the tumours in these patients. Darbre *et al.*'s findings invite several questions: how did the parabens get into the breast, are they persistent and could they do harm? The answers require further research.

The hypothesis that underarm cosmetics may contribute to the incidence of breast cancer has obvious implications, not least because of the size of the population potentially exposed. The role of oestrogen in breast cancer is clear. It is also now clear that the parabens are weakly oestrogenic and thus there is logic to the hypothesis when combined with other lines of evidence (Darbre, 2003). However, apparently little is known of any side-effects associated with long-term, low-level exposures to synthetic xenoestrogens. The use of underarm cosmetics presents a special case because of the direct application of the compounds to skin. Darbre *et al.*'s (2004) study indicates that paraben esters are detectable in breast tumour tissue, which could feasibly result from a previous history of cosmetic use, local dermal absorption and some degree of residue persistence, but the route also could be from other sources,

such as orally if there was no metabolic transformation of the parent compound.

The hypothesis forwarded that underarm cosmetics may be implicated in the incidence of breast cancer (Darbre, 2003) has been discussed also in terms of the potential toxicity of oestrogenic formulation ingredients (Harvey, 2003). Although recent efforts have been made to examine 'antiperspirant use and the risk of breast cancer' (see Mirick *et al.* (2002), who report no association based on retrospective interview), there is a need for research that carefully focuses on chemical toxicity issues (i.e. the specific formulation ingredients and not simply underarm cosmetics *per se*). Research also should consider sensitive population subgroups (especially adolescents and first-degree relatives of breast cancer patients) and requires designs with the sensitivity to elucidate any effects of long-term, low-level exposures to mixtures. As far as toxicological reviews and risk assessments of the parabens are concerned, they apparently have not taken into account recent evidence of inherent oestrogenic and hormonal activities (Soni *et al.*, 2002; Willis, 1995) and there is a perceived need to conduct up-to-date risk assessments on the suitability of each type of paraben specifically for their use in underarm cosmetics. Finally, 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), particularly breast organochlorine concentrations correlated with increased cancer risk (Aronson *et al.*, 2000) and related to oestrogenicity (Starek, 2003). Whether underarm cosmetics will prove to be a special case because of their direct application or not, unlike diffuse environmental exposures, individual use is preventable and the removal of oestrogenic formulants would effectively resolve at least one potential mechanistic factor central to this hypothesis.

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