# The Paraben Debate

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*Review*

# Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks

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**ABSTRACT: This toxicology update reviews research over the past four years since publication in 2004 of the first measurement of intact esters of *p*-hydroxybenzoic acid (parabens) in human breast cancer tissues, and the suggestion that their presence in the human body might originate from topical application of bodycare cosmetics. The presence of intact paraben esters in human body tissues has now been confirmed by independent measurements in human urine, and the ability of parabens to penetrate human skin intact without breakdown by esterases and to be absorbed systemically has been demonstrated through studies not only *in vitro* but also *in vivo* using healthy human subjects. Using a wide variety of assay systems *in vitro* and *in vivo*, the oestrogen agonist properties of parabens together with their common metabolite (*p*-hydroxybenzoic acid) have been extensively documented, and, in addition, the parabens have now also been shown to possess androgen antagonist activity, to act as inhibitors of sulfotransferase enzymes and to possess genotoxic activity. With the continued use of parabens in the majority of bodycare cosmetics, there is a need to carry out detailed evaluation of the potential for parabens, together with other oestrogenic and genotoxic co-formulants of bodycare cosmetics, to increase female breast cancer incidence, to interfere with male reproductive functions and to influence development of malignant melanoma which has also recently been shown to be influenced by oestrogenic stimulation. Copyright © 2008 John Wiley & Sons, Ltd.**

KEY WORDS: paraben; oestrogen; androgen; cosmetics; endocrine disruption; breast cancer; melanoma; male reproductive disorders; esterase; skin; absorption; carcinogenesis

## Introduction

It has been suggested previously that chemicals with oestrogenic and/or genotoxic properties applied in bodycare cosmetics around the breast area could be a contributory factor in the rising incidence of breast cancer (Darbre, 2001; Darbre, 2003; Harvey and Darbre, 2004). Compelling evidence of a link comes from the disproportionately high numbers of female breast cancers which originate in the upper outer quadrant of the breast and which is the area to which underarm and bodycare cosmetics are targeted (Darbre, 2001, 2003). Analysis of the annual quadrant incidence of breast cancer in Britain published in 2005 showed not only that there were now 54% of breast cancers in the upper outer quadrant (subdivision of the breast into four quadrants and a central nipple area would be expected to give no more than 20% in each region from random distribution), but that the relative proportion in that region had risen linearly on an annual basis since 1979 (Darbre, 2005a). This is inconsistent with the dogma that the high incidence of breast cancer in the upper outer quadrant of the breast relates solely to the slightly greater amount of breast epithelial tissue in that region but would be consistent with the increasing use of cosmetic products in the underarm area (McGrath, 2003). Studies published in 2004 further challenged this dogma by showing increased levels of genomic instability in outer regions of the human breast in histologically normal tissue (Ellsworth *et al*., 2004a). Instability of the genome in human cells is an important contributor to genetic changes that drive tumorigenic processes (Lengauer *et al*., 1998) and in accordance with the cancer field theory could provide a milieu where genetically altered cells would then be more susceptible to the development of cancer (Slaughter *et al*., 1953). The underlying mechanism of genetic instability found in outer regions of the breast remains to be identified, but has been suggested to involve damage resulting from topically applied cosmetic chemicals (Ellsworth *et al*., 2004b).

A wide range of consumer products including underarm deodorants, antiperspirants, skin moisturisers, body creams, body sprays and suncare products are applied topically

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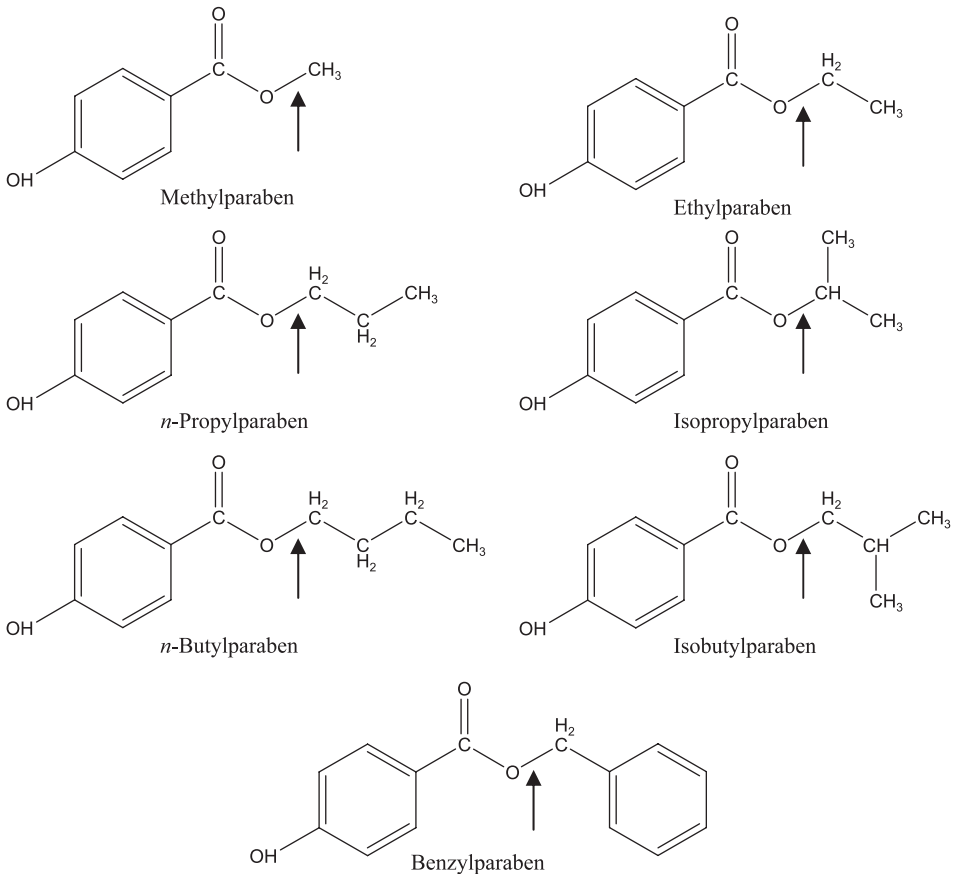


**Figure 1.** The chemical structures of seven alkyl esters of *p*-hydroxybenzoic acid (parabens) which are commonly used in consumer products. Hydrolysis of the ester linkage (arrow) gives the common paraben metabolite *p*-hydroxybenzoic acid

to the breast and upper chest region on a frequent basis and left on the skin, allowing for continuous dermal exposure, absorption and accumulation in underlying tissues (Harvey and Darbre, 2004; Darbre, 2006a). Over the past few years, chemical components used in these increasingly chemically complex products have been shown to possess oestrogenic properties and to be present in either human breast tissue or human milk (which has been secreted from breast epithelial cells; Darbre, 2006a; Donovan *et al*., 2007). The alkyl esters of *p*-hydroxybenzoic acid (parabens) are one such group of chemicals which are used extensively as preservatives in cosmetic consumer products, and the structures of the most commonly used esters are shown in Fig. 1. In 1984, it was estimated that parabens were used in 13 200 different cosmetic formulations (Elder, 1984) and a survey of 215 cosmetic products in 1995 found parabens in 99% of leave-on products and 77% of rinse-off products (Rastogi *et al*., 1995). More recent studies continue to show the presence of parabens, primarily methylparaben and propylparaben (also in combination with phthalates) in the majority of body care cosmetics analysed, including deodorants, creams and lotions (Shen *et al*., 2007). The European Union permits the use of parabens in cosmetic products with a maximum concentration of each one of 0.4% and a total maximum concentration of 0.8% (EU Cosmetics Directive 76/768/EEC). In 2004, the measurement in human breast cancer tissue of intact esters of the five commonly used parabens, methylparaben, ethylparaben, *n*-propylparaben, *n*-butylparaben and isobutylparaben (Darbre *et al*., 2004a), stimulated international discussion, and although the presence of a chemical in a tissue does not imply any functional role in disease processes, this finding did stimulate review of the safety of using parabens in so wide a range of consumer products (Harvey and Darbre, 2004; Bergfeld *et al*., 2005; Golden *et al*., 2005; Soni *et al*., 2005). Over the ensuing four years, there has been not only publication of literature reviews but also of original research, and this review attempts to summarize the new and significant findings which have added to the knowledge database on parabens and which have stimulated regulatory action for some uses.



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## Measurement of Parabens in Human Tissues

### Environmental Exposures

If a chemical component of body care cosmetics is to influence human breast cancer incidence, then continuous dermal exposure must translate into absorption of the chemical through the dermal layers and into underlying breast tissues. Publication of results describing the measurement of paraben esters in human breast cancer tissue (Darbre *et al*., 2004a) caused substantial discussion because this was the first time parabens had been shown to be present as intact esters within the human body and specifically within the human breast. Although critiques underlining the limitations of this pioneering study have been extensive from cosmetic industry associations (Golden and Gandy, 2004; Jeffrey and Williams, 2004; Flower, 2004; and see the discussion in Harvey, 2004 and reply Darbre *et al*., 2004b), the systemic absorption of parabens from environmental exposures has now been confirmed by other groups through the measurement of intact esters of parabens in raw sewage (Lee *et al*., 2005; Canosa *et al*., 2006a) and in human urine (Ye *et al*., 2006a; see Table 1). Although presence in raw sewage can be from various sources, from human excretion to wash-off products (and non-cosmetics) entering the waste water system, the presence in urine confirms human systemic absorption, and because intact paraben esters were found, these compounds have escaped metabolism by either skin esterases if exposure was dermal, or by intestinal and liver metabolic systems if exposure was oral (discussed later). In the Canadian study, methylparaben, ethylparaben, *n*-propylparaben and *n*-butylparaben were all detected in all sewage influent samples with methylparaben (up to 1.47 μg $ml^{-1}$) and *n*-propylparaben (up to 2.43 μg $ml^{-1}$) being detected at the greatest levels (Lee *et al*., 2005). Similar results were obtained in the Spanish study with methylparaben detected in sewage up to 2.92 μg $ml^{-1}$ and *n*-propylparaben up to 1.22 μg $ml^{-1}$ (Canosa *et al*., 2006a). The same group has also detected di-chlorinated forms of methylparaben and propylparaben in raw sewage water samples (Canosa *et al*., 2006b) and it is currently unknown how halogenation affects toxicity. The parabens detected in human urine at the highest levels were also methylparaben and *n*-propylparaben at median concentrations of 43.9 and 9.05 ng $ml^{-1}$ respectively (Ye *et al*., 2006a). In another report by the same group, methylparaben, ethylparaben and propylparaben were detected mostly as conjugated species in 22 urine samples from adults (Ye *et al*., 2006b), again confirming both systemic exposure and detection of intact esters that escaped metabolism. Evidence that parabens can enter the human body as intact esters has therefore now been confirmed in several studies, and over all six studies the parabens detected at highest levels were consistently methylparaben and *n*-propylparaben (which is now subject to regulatory withdrawal for food uses, discussed later). Whilst this may reflect the greater use of methylparaben in cosmetics (Rastogi *et al*., 1995), more recent work has shown that, of the commonly used parabens, methylparaben penetrates the skin to the greatest extent (El Hussein *et al*., 2007), despite having the lowest lipophilicity. As a general note, recent work predicting intestinal absorption in Caco-2 cells has shown that various parabens were metabolized by esterases to *p*-hydroxybenzoic acid and the authors concluded that pre-systemic intestinal metabolism of orally ingested parabens may limit systemic exposure to paraben esters *in vivo* (Lakeram *et al*., 2007). The significance of this is that if intact paraben esters are detected in human tissue or urine, it is less likely that exposure was via the oral route (since oral ingestion results in both intestinal and liver metabolism, reducing the probability that paraben esters will survive intact) and implicates dermal exposure as a potentially important exposure route. The nature of systemic species of paraben esters will influence toxicity, not least the endocrine disrupting potency of the different esters as one example of a relatively well-researched toxicological endpoint, and this must be taken into account in toxicological evaluations.

**Table 1.** Levels of parabens measured in human tissues

| | Human breast tissue mean ng $g^{-1}$ tumour *n = 20* | Human urine (USA) median ng $ml^{-1}$ *n = 100* | Human serum mean peak level (3 h) ng $ml^{-1}$ *n*-butyl applied as topical cream *n = 26* | Human urine mean level mg 24 $h^{-1}$ *n*-butyl applied as topical cream *n = 26* |
|---|---|---|---|---|
| Methylparaben | 12.8 | 43.9 | | |
| Ethylparaben | 2.0 | 1.0 | | |
| *n*-Propylparaben | 2.6 | 9.1 | | |
| *n*-Butylparaben | 2.3 | 0.5 | 0.135 | 2.6 |
| Isobutylparaben | 0.9 | | | |
| Benzylparaben | 0.0 | 0.0 | | |
| *Reference* | *Darbre et al., 2004* | *Ye et al., 2006a* | *Janjua et al., 2007* | *Janjua et al., 2008a* |



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## Dermal Absorption

Measurement of parabens in human tissues then poses the question of the origin of the absorbed compounds and whether parabens from topically/dermally applied cosmetic products could be a source of the body burdens found. Parabens are known to be readily absorbed through the skin and it has been suggested that hydrolysis of parabens by skin esterases could be incomplete in the context of increasing cosmetic useage and inter-individual variations (Darbre *et al*., 2004a; Harvey and Darbre, 2004). *In vitro* studies have shown that 30% of applied propylparaben penetrates the skin intact in rat skin (Bando *et al*., 1997), and after 8 h contact, penetration of some esters can be even higher with up to 60% of methylparaben and 40% of ethylparaben crossing rabbit skin intact (Pedersen *et al*., 2007). In humans, variations between individuals in hydrolysis of parabens have now been shown in the case of human liver esterases (Jewell *et al*., 2007), although studies are still lacking for skin esterases. Furthermore, recent work has also revealed that hydrolysis of parabens by esterases is slower in human skin than in rat skin (Prusakiewicz *et al*., 2006; Harville *et al*., 2007), suggesting that predictions based on rat skin metabolism data may significantly underestimate the level of paraben esters that can be absorbed from topical application into underlying tissues of human skin. Another study (Ishiwatari *et al*., 2007) has demonstrated that methylparaben is not hydrolysed completely by esterases of human skin. At 12 h after application of a test formulation of 0.15% methylparaben to human volunteers, the concentration in the stratum corneum was 10 pmol $cm^{-2}$ (detection limit given as 0.02 pmol $cm^{-2}$) or 0.028% of the application, and repeated application (twice a day for one month) caused accumulation of methylparaben in the stratum corneum to 20 pmol $cm^{-2}$ after 1 week and 120 pmol $cm^{-2}$ after 4 weeks. Just 2 days after cessation of use, levels of methylparaben decreased to <10 pmol $cm^{-2}$. This is of importance when considering current useage of cosmetics where there can be repeat applications of a product during the day and/or multiple applications of different products each containing parabens.

Further studies have demonstrated that variations in product formulation can also influence dermal permeation (Mbah, 2007) and in particular the presence of alcohol (which is also a common cosmetic formulant) can act to inhibit esterase breakdown of parabens *in vitro* (Lakeram *et al*., 2006). Additionally, ethanol enhances dermal penetration of methylparaben in guinea pig skin *in vitro* (Kitagawa *et al*., 1997), and ethanol not only enhances methylparaben transport across excised Yucatan micropig skin, but inhibits hydrolysis of methylparaben to *p*-hydroxybenzoic acid (the common metabolite of all paraben esters) and promotes the transesterification conversion of methylparaben to butylparaben (Oh *et al*., 2002). This transesterification of methylparaben to butylparaben in skin is significant because butylparaben has more potent oestrogenic activity, and together with propylparaben, butylparaben has been subject to regulatory withdrawal (discussed later). Thus common co-ingredients in cosmetic formulations may interact in mixtures, resulting in higher skin and body burdens of parabens [and other endocrine active compounds common in cosmetics, e.g. phthalates (Jobling *et al*., 1995; Harris *et al*., 1997; Okubo *et al*., 2003), polycyclic musks (Gomez *et al*., 2005; Schreurs *et al*., 2005), UV filters (Schlumpf *et al*., 2001; Inui *et al*., 2003; Janjua *et al*., 2004; Koda *et al*., 2005), aluminium chlorhydrate (Darbre, 2006b), triclosan (Gee *et al*., 2008) and cyclosiloxanes (McKim *et al*., 2001; He *et al*., 2003)].

Confirmation of the ability of parabens to be absorbed systemically from topical application to human subjects has now been published in a study where levels of parabens could be shown to increase in both blood and urine of 26 healthy young men following topical application of parabens in a cream cosmetic formulation (Janjua *et al*., 2007, 2008a). From whole body topical application of 2 mg $cm^{-2}$ of a cream containing 2% *n*-butylparaben (average 800 mg total applied), *n*-butylparaben was detectable in serum 1 h after the first application with a mean peak level of 135 μg $l^{-1}$ after 3 h (Janjua *et al*., 2007) and in urine with a peak value after 8–12 h and a mean level of 2.6 mg 24 $h^{-1}$ (Janjua *et al*., 2008a). The majority of the butylparaben detected in urine was conjugated to glucuronide (2.1% unconjugated; Janjua *et al*., 2008a). From the serum concentration, equivalent to 135 ng $ml^{-1}$, the oestrogenic equivalence of butylparaben can be calculated: butylparaben is considered to be 10 000 times less potent than 17$\beta$-oestradiol (EFSA, 2004) and thus this concentration would be equivalent to 13.5 pg $ml^{-1}$ of oestradiol, which in turn can be related to endogenous concentrations for example in normal tissue (approximately 55 pg $ml^{-1}$ in breast; Clarke *et al*., 2001). A similar calculation has been conducted based on parabens concentrations in breast rather than serum (Harvey and Everett, 2006). Janjua *et al*. (2007) demonstrate that parabens can be rapidly absorbed through the skin into the human body even from a single dose of body care product and that esterase levels in the skin are not sufficient to hydrolyse all paraben esters to completion. Real-life exposure would be lower (since 0.4% of a single ester is permitted in Europe rather than 2% as used by Janjua *et al*., 2007), but would involve repeated applications of numerous products over time and the potential for accumulation. Indeed, studies using an *in vitro* model have shown that permeation of parabens through human skin can increase with repeated doses (El Hussein *et al*., 2007), emphasizing the need for further work to assess absorption of parabens under conditions of long-term repeat-dose topical applications such as would be relevant to daily multiple applications of cosmetic products to the skin. It is also important to



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note that Janjua and colleagues have shown other oestrogenic chemicals commonly found in bodycare cosmetics to also be absorbed systemically from whole body topical application of cosmetic creams. This includes phthalates (Janjua *et al*., 2007) and the sunscreens benzophenone-3, octyl-methoxycinnamate and 3-(4-methyl-benzylidene) camphor (Janjua *et al*., 2004). Thus, real-life repeated dermal exposures to the range of paraben esters, and other oestrogenic co-formulants in body care cosmetics, could provide significant additional oestrogenic stimulation in the human body.

## Oestrogenic Activity of Parabens and its Main Metabolite *p*-hydroxybenzoic Acid

### Oestrogen Agonist Properties

Epidemiological, clinical and experimental studies over more than a century confirm that oestrogen plays a central role in the development, progression and treatment of breast cancer (Miller, 1996; Lonning, 2004), which brings into question potential interactions from environmental chemicals which can enter the human breast and which can mimic oestrogen action. All the widely used paraben esters have now been shown to possess oestrogenic activity in assay systems *in vitro* and *in vivo* and a list of the many studies from different laboratories was published in 2004 (Harvey and Darbre, 2004), updated in 2006 (Harvey and Everett, 2006) and has been further updated here in Table 2.

The molecular basis of the genomic action of oestrogen involves binding of ligand to oestrogen receptor (ER), ER$\alpha$ or ER$\beta$, receptor–ligand dimerization, binding to oestrogen response elements (ERE) in the DNA and transactivation of gene expression (Oettel and Schillinger, 1999). Strategies for determining whether a ligand possesses oestrogenic activity therefore begin with investigation of the ability to bind to ER and the ability to induce oestrogen-regulated gene expression, and then progress to the question of whether physiological responses can be induced in cells in culture or in whole animal models (Soto *et al*., 2006; Clode, 2006). Growth of oestrogen-dependent cells, especially of the MCF7 human breast cancer cell line, has provided a sensitive and reliable measure of cell growth response (Soto *et al*., 2006) and increase in uterine weight in the immature female rodent has provided a reliable indicator of oestrogenic activity in a whole animal model (Clode, 2006). The majority of studies shown in Table 2 report oestrogen agonist activity of parabens, a minority of studies (one out of 25 *in vitro* studies, and seven out of 30 *in vivo* studies in Table 2) reported negative findings in oestrogenicity assays. These reports tended to be studies of the shorter alkyl group parabens such as methylparaben and ethylparaben, but were notably more frequent in the *in vivo* studies, which probably reflects variations in methodology, animal strain and dose between studies. In this respect, the database is small for *in vivo* work on methylparaben and ethylparaben, and it would be useful to conduct more systematic, consistent and controlled studies, using a range of dose levels and dose routes to establish both no-effect-level and lowest-effect-level doses for all the paraben esters.

Oestrogenic activity of parabens is known to increase with increasing length of the linear alkyl chain from methylparaben to *n*-butylparaben (Routledge *et al*., 1998; Byford *et al*., 2002) and with branching in the alkyl chain from *n*-propylparaben to isopropylparaben (Okubo *et al*., 2001) or from *n*-butylparaben to isobutylparaben (Darbre *et al*., 2002). Extension of the alkyl chain of methylparaben with a structure containing an aromatic ring in benzylparaben also increased oestrogenic activity (Darbre *et al*., 2003). Further studies over the past 3 years have been published confirming the oestrogenic activity of these esters in yet more diverse *in vitro* and *in vivo* assays which are summarized in Table 2. In addition, studies have shown that the common metabolite of paraben esters *p*-hydroxybenzoic acid (see Fig. 1) also possesses oestrogenic activity in both *in vitro* and *in vivo* assays (Table 2). Thus, whilst shortening of the alkyl group in the paraben ester was already known by 2004 to reduce oestrogenic activity, more recent work has shown that complete removal of the alkyl grouping reduces activity still further but does not eradicate all oestrogenicity. This questions current contention that metabolic hydrolysis of parabens to their common metabolite, *p*-hydroxybenzoic acid acts to eradicate oestrogenic body burdens from paraben exposure in consumer products.

### Should Parabens be Termed 'Weak Oestrogens'?

Whilst their ability to mimic oestrogen action is now well documented, the extent to which parabens can be labelled as 'weak oestrogens' (e.g. Golden *et al*., 2005) needs further consideration. Receptor-mediated mechanisms of ligand activity are dependent on two fundamental events: ligand affinity and ligand efficacy (Strange, 2008). In the specific case of oestrogen action, ligand affinity for the ER can be estimated through *in vitro* techniques such as ligand binding assays (Green and Leake, 1987), and the weaker the ligand binding affinity for ER, the higher the concentration of ligand needed to saturate the ER or to compete out radiolabelled oestradiol from binding to ER. Ligand efficacy refers to the ability of the ligand to influence receptor-mediated signalling pathways which in the case of genomic mechanisms of oestrogen action could involve the ability of ER to bind to chaperone Hsp proteins, to dimerize (to homodimers or heterodimers with ER$\alpha$ and ER$\beta$), to translocate to the nucleus, to bind to the ERE in DNA and to transactivate gene expression (Oettel and Schillinger, 1999). Displacement of radiolabelled



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**Table 2.** Summary of *in vitro* and *in vivo* studies published on the oestrogenic activity of parabens

| Paraben | Result *in vitro* | Result *in vivo* | Reference |
|---|---|---|---|
| Methylparaben | + ve (yeast + receptor binding) | | Routledge *et al*., 1998; Nishihara *et al*., 2000; Miller *et al*., 2001; Schultis and Metzger, 2004; Morohoshi *et al*., 2005 |
| | + ve (human MCF7) | | Byford *et al*., 2002; Schultis and Metzger, 2004; Pugazhendhi *et al*., 2005, 2007; Vanparys *et al*., 2007 |
| | − ve (rat uterus receptor binding) | | Lemini *et al*., 2003 |
| | + ve (rat uterus receptor binding) | | Blair *et al*., 2000 |
| | | − ve (rat uterotrophic) | Hossaini *et al*., 2000 |
| | | − ve (rat dietary repeat dose reproductive toxicity study) | Oishi, 2004 |
| | | + ve (rat uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 2003 |
| Ethylparaben | + ve (yeast + receptor binding) | | Routledge *et al*., 1998; Nishihara *et al*., 2000; Miller *et al*., 2001; Schultis and Metzger, 2004; Morohoshi *et al*., 2005 |
| | + ve (human MCF7) | | Okubo *et al*., 2001; Byford *et al*., 2002; Schultis and Metzger, 2004; Vanparys *et al*., 2007 |
| | + ve (rat uterus receptor binding) | | Blair *et al*., 2000; Lemini *et al*., 2003 |
| | + ve (human HeLa overexpressing ER) | | Gomez *et al*., 2005 |
| | | − ve (rat uterotrophic) | Hossaini *et al*., 2000 |
| | | − ve (rat dietary repeat dose reproductive toxicity study) | Oishi, 2004 |
| | | + ve (rat uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 2003 |
| *n*-Propylparaben | + ve (yeast + receptor binding) | | Routledge *et al*., 1998; Nishihara *et al*., 2000; Miller *et al*., 2001; Schultis and Metzger, 2004; Morohoshi *et al*., 2005 |
| | + ve (human MCF7) | | Okubo *et al*., 2001; Byford *et al*., 2002; Schultis and Metzger, 2004; Vanparys *et al*., 2007 |
| | + ve (rat uterus receptor binding) | | Blair *et al*., 2000; Lemini *et al*., 2003 |
| | + ve (human HeLa overexpressing ER) | | Gomez *et al*., 2005 |
| | | − ve (rat uterotrophic) | Hossaini *et al*., 2000 |
| | | + ve (rat dietary repeat dose reproductive toxicity study) | Oishi, 2002a |
| | | + ve (rat uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (rainbow trout) | Bjerregaard *et al*., 2003 |
| | | + ve (male medaka fish) | Inuie *et al*., 2003 |
| | | + ve but antagonist (zebrafish) | Mikula *et al*., 2006 |
| *n*-Butylparaben | | − ve (rat teratogenicity study) | Daston, 2004; Harvey 2005 |
| | + ve (yeast + receptor binding) | | Routledge *et al*., 1998; Nishihara *et al*., 2000; Miller *et al*., 2001; Schultis and Metzger, 2004; Morohoshi *et al*., 2005 |
| | + ve (human MCF7) | | Okubo *et al*., 2001; Byford *et al*., 2002; Schultis and Metzger, 2004; Pugazhendhi *et al*., 2007; Vanparys *et al*., 2007 |
| | + ve (rat uterus receptor binding) | | Blair *et al*., 2000; Lemini *et al*., 2003 |
| | + ve (human HeLa overexpressing ER) | | Gomez *et al*., 2005 |
| | | + ve (rat uterotrophic) | Routledge *et al*., 1998 |
| | | + ve (rat uterotrophic) | Hossaini *et al*., 2000 |
| | | + ve (rat dietary repeat dose reproductive toxicity study) | Oishi, 2001 |
| | | + ve (mouse dietary repeat dose reproductive toxicity study) | Oishi, 2002b |
| | | + ve (rat development and reproductive toxicity study) | Kang *et al*., 2002 |
| | | + ve (rat uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (rainbow trout) | Alslev *et al*., 2005 |
| Isopropylparaben | + ve (human MCF7) | | Okubo *et al*., 2001 |
| | + ve (yeast + receptor binding) | | Morohoshi *et al*., 2005 |
| Isobutylparaben | + ve (human MCF7) | | Okubo *et al*., 2001 |
| | + ve (human MCF7; ZR-75-1) | | Darbre *et al*., 2002 |
| | + ve (yeast + receptor binding) | | Morohoshi *et al*., 2005 |
| | | + ve (mouse uterotrophic) | Darbre *et al*., 2002 |
| | | + ve (rat uterotrophic) | Koda *et al*., 2005 |
| Benzylparaben | + ve (human MCF7; ZR-75-1) | | Darbre *et al*., 2003; Schultis and Metzger, 2004 |
| | + ve (yeast + receptor binding) | | Miller *et al*., 2001; Schultis and Metzger, 2004; Morohoshi *et al*., 2005 |
| | + ve (rat uterus receptor binding) | | Blair *et al*., 2000 |
| | | + ve (mouse uterotrophic) | Darbre *et al*., 2003 |
| *p*-hydroxybenzoic acid (main metabolite) | + ve (human MCF7) | | Pugazhendhi *et al*., 2005 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 1997 |
| | | − ve (rat uterotrophic) | Lemini *et al*., 2003 |
| | | + ve (mouse uterotrophic) | Lemini *et al*., 2003 |



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oestradiol in competitive ER binding assays requires higher concentrations of parabens than physiological oestrogens and some other xeno-/phyto-oestrogens (see references in Table 2; summarized for the paraben esters in Darbre, 2006a), demonstrating that parabens have lower binding affinity to ER than some other oestrogenic ligands. However, this does not result in reduced efficacy if sufficient concentration of paraben is present. Indeed, in whole cell assays, with sufficient concentration, the parabens give responses in terms of increased gene expression and cell proliferation in human breast cancer cells of the same magnitude as 17$\beta$-oestradiol (see references in Table 2, especially Byford *et al*., 2002; Darbre *et al*., 2002, 2003). Parabens are not partial agonists, as might be implied by the term 'weak', but give full agonist responses in whole cells. Furthermore, the fact that, in cell-based assays, the parabens might achieve full agonist response at lower concentrations given more time, has not been a considered parameter in any of the published studies on parabens. Oestrogenic activity is usually defined relative to the concentration required to achieve maximal (or half-maximal) effect in a set time in any given assay system, such that compounds with 'weaker activity' require higher concentrations to achieve the response in the set time frame of the assay. The principle that partial agonist effects can be enhanced over a longer time period has been shown in the case of the oestrogen agonist properties of triclosan (Gee *et al*., 2008), and this should be repeated with the paraben esters since it is of high importance to environmental situations where the compound would be present over the long term and not only for a set time frame. The operational label of 'weak oestrogen' therefore needs to be reconsidered in the context of whole cells and environmental studies.

## How closely do Parabens Mimic Oestrogen Action?

Another uncertainty which has surrounded the oestrogenic properties of parabens is the extent to which paraben action is identical to that of 17$\beta$-oestradiol. Studies published up to 2004 have shown that parabens can upregulate a few single genes (reporter genes, pS2, progesterone receptor) in a manner similar to that of oestradiol (see references in Table 2), but global gene expression profiling has revealed that oestradiol regulates the expression of many hundred genes and that more genes are downregulated by oestradiol than upregulated (Frasor *et al*., 2003). Recent expression microarray studies have shown some similarities in global gene expression patterns between parabens and oestradiol when parabens were used at concentrations sufficient to stimulate a growth response (Terasaka *et al*., 2006; Pugazhendhi *et al*., 2007), but not all genes were regulated in an identical manner (Pugazhendhi *et al*., 2007). Using a 20K expression array, some genes were found to respond differently to parabens from oestradiol whilst other genes could be regulated to different extents even between individual parabens (Pugazhendhi *et al*., 2007). Furthermore, since methylparaben was found to influence the expression of a greater number of genes than *n*-butylparaben (Pugazhendhi *et al*., 2007), this further questions the classification of methylparaben as 'weaker' than *n*-butylparaben.

## Role of ER$\alpha$ and ER$\beta$

The molecular basis of oestrogen action involves binding of the ligand to intracellular receptors which function as ligand-activated transcription factors (Oettel and Schillinger, 1999), but two oestrogen receptors (ER$\alpha$ and ER$\beta$) have now been characterized which vary in their tissue distributions and in their ligand binding affinities (Kuiper *et al*., 1997; Imamov *et al*., 2005). Whilst ER$\alpha$ levels are relatively higher in mammary and uterine tissue, and most notably in ER positive breast cancers, ER$\beta$ is more ubiquitously distributed and may mediate oestrogenic actions across non-reproductive tissues. Furthermore, ER$\beta$ is now known to act as a growth inhibitor and the relative proportion of ER$\beta$ in breast cancer cells is thought to influence the outcome in breast cancer (Speirs *et al*., 2004), which suggests that altering ER$\beta$ actions could be as detremental as enhancing ER$\alpha$ activity. Recent work has suggested that individual parabens may vary in their relative binding affinity for ER$\alpha$ and ER$\beta$ (Okubo *et al*., 2001; Gomez *et al*., 2005), which in turn suggests that there could be equally varied responses of the different parabens in different tissues and during development of different breast cancers. Whether the parabens can also bind to the oestrogen-related receptors will also be important in the light of the recent description of the binding of bisphenol A to human oestrogen-related receptor-$\gamma$(Okada *et al*., 2008).

## Effects on Oestrogen Metabolism

Although many of the effects of environmental oestrogens are mediated via ER$\alpha$ and ER$\beta$, it is now known that some xenoestrogens may be able to exert endocrine disrupting properties through interfering with metabolic enzymes responsible for the synthesis of physiological oestrogens or for modification of their availability in free, unconjugated form (Whitehead and Rice, 2006). In this respect, a recent report that parabens may be able to inhibit sulfotransferases (Prusakiewicz *et al*., 2007) is another illustration of their varied oestrogen disrupting actions. Oestrogen action *in vivo* is regulated through a balanced interaction between sulfotransferase enzymes (SULTs) which catalyse sulfate conjugation and sulfatases which release free oestrogens. Many environmental oestrogen disrupters, such as hydroxylated polyhalogenated aromatic



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hydrocarbons, are known to function as SULT inhibitors (Kester *et al*., 2002) and that parabens can also inhibit sulfation of oestrogens through inhibition of SULTs (Prusakiewicz *et al*., 2007) suggests that parabens may also indirectly enhance oestrogen effects through elevation of free oestradiol levels.

## Antiandrogenic Properties of Parabens and Male Reproductive Disorders

Many environmental chemicals which possess oestrogenic properties have also been shown to display antiandrogenic activity. Certain pesticides, the fungicide vinclozolin, bisphenol A, some phthalates and triclosan can antagonize the action of androgens in assays *in vitro* and in animal models (Kelce and Wilson, 1997; Sohoni and Sumpter, 1998; Gee *et al*., 2008). It is therefore interesting that recent reports have documented the ability of several parabens to bind to human androgen receptor (Satoh *et al*., 2005) and an antiandrogenic activity for all parabens tested in antagonizing the action of testosterone on reporter gene expression (Satoh *et al*., 2005; Chen *et al*., 2007; see Table 3 for details). Although male reproductive abnormalities resulting in animal models from exposure to endocrine-disrupting compounds have been attributed to the oestrogenic activity of the chemicals, the relevance of antiandrogenic properties is now receiving more serious consideration (Bay *et al*., 2006; Sharpe, 2006; Filby *et al*., 2007). Repeat oral dosage of propylparaben and butylparaben in diet to juvenile rodents has been reported to result in alterations to male reproductive functions including spermatogenesis, testosterone secretion and epididymal weights (Oishi, 2001, 2002a,b), and this has been assumed to result from oestrogenic activity of the parabens. However, it is also possible that antiandrogenic actions of parabens through AR-mediated pathways could have contributed to the effects observed, which brings into question whether this data should be included under Table 2 or Table 3 or both.

Over the past 50 years, male reproductive disorders have been documented as increasing in the human population, and four male reproductive health problems, cryptorchidism, hypospadias, reduced semen quality and testicular cancer, are considered indicators of the testicular dysgenesis syndrome with a suspected origin in fetal or early postnatal life (Bay *et al*., 2006). However, whether causality involves exposure to oestrogenic and/or antiandrogenic chemicals at this sensitive time frame and the source of such exposure remain uncertain. Evidence has been documented for exposure to oestrogenic/antiandrogenic chemicals causing endocrine disruption in aquatic species (Matthiessen, 2003) and for exposure to phthalates producing male reproductive abnormalities in laboratory rodent models similar to those observed in humans (Sharpe, 2006). Maternal exposure to butylparaben during gestation and lactation has been shown to result in reproductive disorders in male offspring by postnatal day 49 (Kang *et al*., 2002). Whether the effects resulted from butylparaben crossing the placenta during gestation or passing in milk during suckling, or a combination of both, remain to be determined, but the sensitivity of early postnatal male rodents to development of reproductive disorders when exposed to butylparaben via the dietary route has been confirmed in subsequent studies (Oishi, 2001). Since parabens are now known to penetrate skin (see earlier), it remains in question as to whether similar consequences might result from topical application of parabens to early

**Table 3.** Summary of *in vitro* and *in vivo* studies published on the antiandrogenic activity of parabens

| Paraben | AR binding assay | Reporter gene assay (antagonist activity) | *In vivo* assay | Reference |
|---|---|---|---|---|
| Methylparaben | − ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| | | + ve (transfected HEK 293 cells) | | Chen *et al*., 2007 |
| | | | − ve (rat dietary repeat dose male reproductive toxicity study) | Oishi *et al*., 2004 |
| Ethylparaben | − ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| | | | − ve (rat dietary repeat dose male reproductive toxicity study) | Oishi, 2004 |
| *n*-Propylparaben | + ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| | | + ve (transfected HEK 293 cells) | | Chen *et al*., 2007 |
| | | | + ve (rat dietary repeat dose male reproductive toxicity study) | Oishi, 2002a |
| *n*-Butylparaben | + ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| | | + ve (transfected HEK 293 cells) | | Chen *et al*., 2007 |
| | | | + ve (rat dietary repeat dose male reproductive toxicity study) | Oishi *et al*., 2001 |
| Isopropylparaben | + ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| Isobutylparaben | + ve (recombinant hAR) | + ve (transfected CHO-K1 cells) | | Satoh *et al*., 2005 |
| *p*-Hydroxybenzoic acid (main metabolite) | | − ve (transfected HEK 293 cells) | | Chen *et al*., 2007 |



DOI: 10.1002/jat

**Table 4.** Oestrogen and androgenic activity of chemical constituents of cosmetics applied to the underarm and breast area and evidence for their absorption into the human body

| Cosmetic chemical | Function in cosmetic | Measurement in human body | Penetration of human skin *in vitro* | Systemic absorption into humans from topical application | Oestrogenic activity (agonist and/or antagonist) | Androgenic activity (agonist and/or antagonist) |
|---|---|---|---|---|---|---|
| Parabens | Preservative | Breast, urine (see Table 1) | Prusakiewicz *et al*., 2006; Harville *et al*., 2007; Ishiwatari *et al*., 2007 | Dorsal skin into blood (Janjua *et al*., 2007) and urine (Janjua *et al*., 2008a) | see Table 2 | see Table 3 |
| UV filters | Absorb UV light | Urine (Hayden *et al*., 1997; Felix *et al*., 1998), milk (Hany *et al*., 1995) | Treffel and Gabard,1996; Hayden *et al*., 1997; Chatelain *et al*., 2003 | Dorsal skin into blood (Janjua *et al*., 2004) and urine (Janjua *et al*., 2008b) | Schlumpf *et al*., 2001; Inui *et al*., 2003; Koda *et al*., 2005; Heneweer *et al*., 2005; Kunz and Fent, 2006 | |
| Polycyclic musks | Fragrance | Adipose tissue (Kannan *et al*., 2005), serum/milk (Kuklenyik *et al*., 2007); milk (Reiner *et al*., 2007) | | | Gomez *et al*., 2005; Schreurs *et al*., 2005; Mori *et al*., 2007 | Schreurs *et al*., 2005; Mori *et al*., 2007 |
| Aluminium chlorhydrate | Antiperspirant | Breast tissue (Exley *et al*., 2007) | | Underarm skin into blood (Flarend *et al*., 2001; Guillard *et al*., 2004) | Interfere with binding of oestrogen to ER and oestrogen-regulated gene expression (Darbre 2005b) | |
| Cyclosiloxanes | Conditioning, spreading | | Zareba *et al*., 2002 | | Hayden and Barlow, 1972; McKim *et al*., 2001; He *et al*., 2003 | |
| Triclosan | Deodorant/ preservative | Milk, blood (Adolfsson-Erici *et al*., 2002; Hovander *et al*., 2002; Allmyr *et al*., 2006) | Moss *et al*., 2000 | Cosmetic use into plasma/milk (Allmyr *et al*., 2006) | Gee *et al*., 2008 | Gee *et al*., 2008 |
| Phthalates | Plasticizer | Milk (Calafat *et al*., 2004); amniotic fluid (Silva *et al*., 2004); saliva (Silva *et al*., 2005); urine (Calafat and McKee, 2006) | | Cosmetic use into urine (Duty *et al*., 2005); dorsal skin into blood (Janjua *et al*., 2007) and urine (Janjua *et al*., 2008a) | Jobling *et al*., 1995; Harris *et al*., 1997; Okubo *et al*., 2003 | Sohoni and Sumpter, 1998; Lee and Koo, 2007 |


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postnatal male rodents. This is an important question because parabens are used in an extensive array of baby wipes and baby creams where the products would be left on the skin of the genital area of baby boys, allowing for absorption and accumulation (especially following skin abrasions from nappy/diaper rash) into underlying tissues of the reproductive organs throughout this sensitive time frame.

## Parabens and Skin Cancer

Although the oestrogenic properties of parabens have been extensively discussed in relation to the development of breast cancer and in particular from the topical application of paraben-containing cosmetics around the breast area, many other body tissues apart from the mammary gland are also sensitive to oestrogen action, not least reproductive organs, skin, bone and the cardiovascular system. With the continued use of parabens in so wide a range of skincare products (Elder, 1984; Rastogi *et al*., 1995; Shen *et al*., 2007) and with such ubiquitous distribution of parabens in the domestic environment that they can now be detected not only in body tissues but in house dust (Canosa *et al*., 2007), it is important to consider whether there are wider implications, particularly for skin, which is the body tissue with which the topical cosmetics will be in immediate contact. Oestrogens have long been known to have a profound influence on skin development and composition, and the reduction in oestrogen levels at menopause results in changes associated with aging (Thornton, 2002; Hall and Phillips, 2005; Verdier-Sevrain *et al*., 2006). These aging effects on the skin can be reduced with topical treatment of skin with oestrogen-containing creams (Sator *et al*., 2004). Use of parabens in so many cosmetic products which are applied topically to skin should be expected therefore to have an influence on both the epidermis and the dermis of skin. Whether such an influence is positive or negative at different life stages and at different concentrations needs urgent investigation. Recent research has reported that methylparaben can indeed influence the aging and differentiation of keratinocytes (Ishiwatari *et al*., 2007), since long-term application of methylparaben to keratinocytes could influence proliferation rate, cell morphology and expression of hyaluronan synthases and type IV collagen. Further work has shown that methylparaben and ethylparaben can induce oxidative stress in skin after reaction with singlet oxygen ($^1O_2$) in visible light to produce glutathione conjugates of hydroquinone (Nishizawa *et al*., 2006). However, the report that methylparaben potentiates UV-induced damage of skin keratinocytes including reactive oxygen species (ROS) and nitric oxide (NO) production and lipid peroxidation (Handa *et al*., 2006) poses more serious questions concerning potentially genotoxic effects of methylparaben applied in cosmetics to human skin when exposed to sunlight. This is important in the context of the use of methylparaben in sunscreen products and the continued uncertainty as to whether there is a positive or negative association between sunscreen use and development of human skin cancers (Diffey, 2005; Francis *et al*., 2006).

There are two main forms of skin cancer: melanoma and non-melanoma skin cancer. Non-melanoma skin cancers, including basal cell and squamous cell carcinomas, are the most common form of skin cancer, and in 2004, there were at least 72 000 new cases registered in the UK (Office of National Statistics, London). However, they pose a lower clinical problem than the melanomas due to success rates of early treatment (Neville *et al*., 2007). By contrast, melanoma has become a major public health problem in many countries and since the 1960s has risen by 3–8% per year in most European countries (Thompson *et al*., 2005) with an annual incidence rate in the UK in 2004 of 13.0 [age standardized rate (European) per 100 000 population], which is equivalent to 9000 new cases registered in 2004 in the UK (Gavin and Walsh, 2005). Melanoma affects younger people more than most cancers, with about 40% of cases in the under 50s (Gavin and Walsh, 2005), and there is currently a striking increase in incidence in youth (Strouse *et al*., 2005; Downard *et al*., 2007) and a strong inverse relationship with social deprivation (Gavin and Walsh, 2005). The natural history of human malignant melanoma has suggested that oestrogen might influence the incidence and development of these tumours (Schmidt *et al*., 2006), partly because the incidence in females is low before puberty, rising steeply through the reproductive years (Strouse *et al*., 2005), and survival may vary between men and women (Reintgen *et al*., 1984; Gavin and Walsh, 2005). Numerous studies have attempted to identify a role for ER$\alpha$ as a biomarker in melanoma (Tanemura *et al*., 2007), in the way that ER$\alpha$ serves as a prognostic marker in breast cancer (Miller, 1996), but to date this has been without clear success and the spurious distribution of ER$\alpha$ in melanoma cells has obscured the role of oestrogen in melanoma. However, recent studies show that ER$\beta$, and not ER$\alpha$, is the predominant oestrogen receptor type in melanocytic lesions, with ER$\beta$ being detected ubiquitously where ER$\alpha$ was not (Schmidt *et al*., 2006). The discovery that ER$\beta$ immunoreactivity was increased in severely dysplastic nevi and lentigo malignas (*in-situ* melanoma) but decreased in melanomas progressively deeper in the dermis suggests that ER$\beta$ may be playing a role in the biology of these cancers and might serve as a useful prognostic marker (Schmidt *et al*., 2006). In the light of an involvement of ER$\beta$ on melanocytic pathophysiology (Schmidt *et al*., 2006), the ability of methylparaben to potentiate UV-induced damage in keratinocytes (Handa *et al*., 2006) and the ability of parabens to act via ER$\beta$ (Okubo *et al*., 2001; Gomez *et al*., 2005), a potential involvement of parabens (alone or together



DOI: 10.1002/jat

with other oestrogenic chemicals in cosmetics including UV filters) should now be considered in studies of the development of malignant melanoma. The higher rate of melanoma in younger people (Gavin and Walsh, 2005; Strouse *et al*., 2005; Downard *et al*., 2007), the increasing incidence in youth (Strouse *et al*., 2005; Downard *et al*., 2007) and the inverse relationship with social deprivation (Gavin and Walsh, 2005) could all correlate with greater use of paraben-containing skincare/suncare products, be it through more lavish amounts at each application, more frequent applications or a lifestyle where products are required more often and at higher levels. It is interesting to note the recently reported left-sided excess of invasive cutaneous melanoma in six different countries (Brewster *et al*., 2007), which echos remarkably the similar findings of left-sided excess of breast cancers (Darbre *et al*., 2003). This contrasts to the deficit of left-sided tumours at many other sites (Brewster *et al*., 2007). Handedness in application of skincare products may yet explain this phenomenon in both cases (Darbre, 2003; Brewster *et al*., 2007).

## Genotoxic Activity of Parabens

Whilst the ability of oestrogen to influence the incidence, growth, progression and metastasis of breast cancer is well established (Miller, 1996), the potential for oestrogen to act through genotoxic mechanisms to initiate breast cancer has been recognized only more recently (Russo and Russo, 2006). Several mechanisms have been postulated, including increasing the error rate of DNA replication through stimulation of cell proliferation via oestrogen-mediated activity and more direct genotoxicity through cytochrome-P450-mediated metabolic activation producing genotoxic metabolites. The identification of adverse effects of some environmental oestrogens which cannot be explained solely on the basis of ER-mediated endocrine disruption alone has prompted studies to consider whether xenoestrogens might also possess genotoxic activity. Notwithstanding parabens being inactive in classical assays for mutagenicity and carcinogenicity (Soni *et al*., 2005), recent research has reported the ability of propylparaben and butylparaben to cause DNA damage detectable in Comet assays and induction of chromosome aberrations together with sister-chromatid exchanges (Tayama *et al*., 2008). Although the effects observed were reported at high concentrations in the millimolar range in the CHO cells used, it remains to be ascertained whether there could be effects at lower concentrations over the longer term in mammary cells, whether there could be additive/inhibitory effects from multiple genotoxic chemicals present in the human breast (from other cosmetic chemicals and/or diet), or whether there might be particular windows of sensitivity to such genotoxic activity such as in the prepubertal breast (Darbre, 2006a).

## Regulatory Status of Parabens

Cosmetics are less stringently tested and receive less regulatory attention compared with other types of chemicals to which the general population is exposed (see discussion in Harvey and Everett, 2006). Despite this, cosmetics and bodycare products represent potentially the commonest exposure scenario to chemicals in individuals and the population as a whole. Recent reports of cosmetics safety in use show a surprisingly high percentage of adverse reactions to products, with 26.5% of women and 17.4% of men reporting an adverse event/reaction to cosmetic use (Di Giovanni *et al*., 2006). Of the reactions reported, 95.9% involved the skin and 4.1% were systemic reactions, and of these reports of systemic reactions, headache was the most common (40.3%) followed by nausea (24.2%).

The parabens are a group of chemicals that have been, and continue to be, used extensively in cosmetics and bodycare products. Elder (1984) estimated that parabens were used in 13 200 different cosmetic formulations and independent analyses of cosmetic products found parabens in 99% of leave-on products (Rastogi *et al*., 1995). Recent analyses confirm the presence of methylparaben and propylparaben in the majority of types of cosmetics tested including deodorants, creams and lotions (Shen *et al*., 2007). Despite this, the regulatory toxicology data package does not have adequate carcinogenicity or reproductive toxicology studies to meet modern regulatory standards. This situation, and the current regulatory status of the parabens as a group, is discussed below.

The European Union permits the use of parabens in cosmetic products with a maximum concentration of each one of 0.4% and a total maximum concentration of 0.8% (EU Cosmetics Directive 76/768/EEC) and they are also registered for use in foods. The latter use is more highly regulated and recent regulatory reviews have resulted in the withdrawal of ADIs (Acceptable Daily Intake) for several paraben esters on grounds of reproductive and endocrine toxicity. For example JECFA 2007 [The Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Committee on Food Additives] has recommended 'that in view of the adverse effects in male rats [reproductive toxicity associated with endocrine effects] propylparaben should be excluded from the group ADI for parabens used in food', recommending its withdrawal. The same evaluation noted the withdrawal of specification for butylparaben on similar grounds. Similarly, the EC Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority (EFSA) reviewed propylparaben (EFSA, 2004) and was unable to establish a no-observed-adverse-effect-level for reproductive and endocrine toxicity, and consequently an ADI, effectively recommending its exclusion/withdrawal from food use. These regulatory evaluations involve oral exposures but it has been previously suggested that



DOI: 10.1002/jat

dermal application of paraben esters may represent a special case because of a higher probability of escaping limited skin esterase action (Bando *et al*., 1997; Oh *et al*., 2002; Prusakiewicz *et al*., 2006; El Hussein *et al*., 2007; Harville *et al*., 2007; Janjua *et al*., 2007) and local subcutaneous tissue accumulation (see Harvey and Darbre, 2004), indicating a need for regulatory risk assessment harmonization. Harmonization would require withdrawal of butylparaben and propylparaben from cosmetics.

With regard to cosmetics uses, the European Commissions Scientific Committee on Consumer Products (SCCP) provided an extended opinion on the safety evaluation of parabens in 2005, noted the deficiencies in the toxicology database of the parabens and requested more information, specifically on 'full descriptions of available in vitro percutaneous absorption studies' and 'a complete dossier with regard to the reproductive and developmental toxicity of propyl, isopropyl, butyl and isobutyl paraben, with a special focus on the male reproductive system'. The European Cosmetic Toiletry and Perfumery Association (COLIPA) provided a submission in response to this request, but SCCP (2006) evaluated these data and concluded that the submission contained 'too many shortcomings to be scientifically valid' and therefore there still remain inadequacies and deficiencies in the paraben safety dataset, and concerns over paraben endocrine and reproductive toxicity.

Concerning the specific hypothesis of the role of parabens and breast cancer, the European Commissions Scientific Committee on Consumer Products (SCCP) concluded in the light of the current state of knowledge (in 2005) that there was no evidence of a demonstrable risk for the development of breast cancer following the use of underarm cosmetics containing parabens (SCCP, 2005). However, to date there are only two studies that have looked at breast cancer risk with the use of 'underarm' cosmetics and the data set is therefore too sparse to form any conclusions. Mirick *et al*. (2002) conducted a retrospective interview-based case–control study on a relationship between use of products for underarm perspiration and the risk for breast cancer, and found risk of breast cancer did not increase with use of antiperspirant (OR = 0.9; $P = 0.23$) or with deodorant (OR = 1.2; $P = 0.19$). Although this was a sizeable population study of breast cancer case patients ($n = 813$) and control subjects without breast cancer ($n = 793$), the weakness remains in the nature of the accuracy of the self-reported results, and in particular whether all subjects were clear about the difference between the ingredients added as antiperspirant and as deodorant in the commercial products which they had used, not least in view of the numbers who claimed to use antiperspirant in the absence of deodorant, products which are not freely available (usual combination is deodorant alone or both together). Accurate reporting could have been ascertained from a group of subjects who had never used any such products, but unfortunately such a group was not included in the study. By contrast, McGrath (2003) addressed the issue of frequency (intensity) of underarm product use within a cohort of breast cancer patients and their age of diagnosis, and reported that frequency and earlier use of antiperspirant/deodorants together with underarm shaving were associated with an earlier age of breast cancer diagnosis (up to 19 years earlier). Neither study identifies particular chemicals/ingredients in the vast range of products that can be used.

Harvey and Darbre (2004) and Harvey and Everett (2006) have noted that all types of bodycare cosmetics applied to the skin (not just underarm cosmetics) can be a source of local oestrogenic chemical input to the breast and should be considered in risk assessments. Furthermore, there are also an increasing number of other ingredients in various cosmetics that have been shown to be endocrine active or oestrogenic [for example polycyclic musks (Gomez *et al*., 2005; Schreurs *et al*., 2005), UV filters (Schlumpf *et al*., 2001; Inui *et al*., 2003; Koda *et al*., 2005), aluminium chlorhydrate (Darbre, 2006b), triclosan (Gee *et al*., 2008), phthalates (Jobling *et al*., 1995; Harris *et al*., 1997; Okubo *et al*., 2003), cyclosiloxanes (McKim *et al*., 2001; He *et al*., 2003)] and risk assessments should take into account mixture and combined repeated exposure effects.

## Conclusions and Further Research Needed

The principle first documented in 2004 that parabens can enter the human body as intact esters measurable in human breast cancer tissue (Darbre *et al*., 2004a) has now been confirmed through the measurement of paraben esters also in normal human urine (Ye *et al*., 2006a), and the principle that the measured parabens could be derived from topical application of cosmetic products (Harvey and Darbre, 2004) has been vindicated through the demonstration that parabens can penetrate into the human circulatory system from a single topical cosmetic application to a human subject (Janjua *et al*., 2007). With the continued use of parabens in the majority of bodycare cosmetics (Shen *et al*., 2007) and this evidence for systemic absorption of parabens following topical cosmetic application to human subjects (Janjua *et al*., 2007; 2008a), there is a need to now ascertain total blood and urine levels for all the paraben esters and their common metabolite *p*-hydroxybenzoic acid in order to more clearly understand whole body burdens in the population. A risk assessment of the oestrogen equivalents of paraben absorbed from a single daily application of a paraben-containing lotion has been calculated to be significant (Harvey and Everett, 2006), and Ye *et al*. (2006a) noted that, in a demographically diverse group of 100 US male and female adults with no known unusual exposure to parabens, methylparaben and propylparaben were detected in 96% of the samples. With such widespread presence of parabens in urines across the



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population, there is a need to equally understand distribution in all body tissues, and beyond breast, to now investigate the distribution of parabens across all endocrine-sensitive tissues which might be influenced through topical exposure to the parabens, not least male reproductive organs in the early years of life and the skin itself.

At the current time it remains unknown as to whether the paraben levels measured in human tissues result from continuous exposure or accumulated chemicals, but with the use of multiple cosmetic products on a daily basis (Loretz *et al*., 2006), many of which may contain parabens as preservatives, there is a need to investigate the potential for paraben accumulation following repeated dermal applications. *In vitro* models suggest the potential for accumulation in underlying regions of skin (Ishiwatari *et al*., 2007), and recent unpublished data were broadcast on UK television (screened 11 October 2007. http://www.unrealitytv.co.uk/reality-tv/beauty-addicts-how-toxic-are-you-channel-4) indicating that body burdens of parabens could be reduced by eradicating use of cosmetics containing parabens. The ability to reduce body burden through alteration to cosmetic exposure needs to be substantiated by a controlled scientific study in a statistically viable group of subjects. A systematic examination in human and rat skin models of the rates of absorption and hydrolysis for all the paraben esters would provide scientific grounding for understanding the extent of absorption and escape from esterase metabolism at current environmentally relevant exposures. Furthermore, there is a need to investigate the degree to which there can be variation between individuals in the potential for paraben absorption and tissue accumulation following repeated applications in more substantial studies which Janjua and coworkers have initiated (Janjua *et al*., 2007).

A variety of studies have now demonstrated the ability of parabens to disrupt physiologically important functions in both *in vitro* cell culture systems and *in vivo* animal models and these are summarized in diagrammatic form in Fig. 2. The most extensive disrupting activity to be described has been that resulting from the property of parabens to bind to human ER and then to act via ER-mediated mechanisms to regulate gene expression and cell growth in oestrogen-responsive cells (see Table 2). Further endocrine disrupting activity has been demonstrated in the ability of parabens to antagonize AR-mediated events in androgen-responsive cells and to act as SULT inhibitors. Other reports have suggested parabens can influence the secretion of lysosomal enzymes in lymphocytes (Biarati *et al*., 1994), can impair mitochondrial function in rat hepatocytes (Nakagawa and Moldeus, 1998), can cause DNA damage in CHO cells (Tayama *et al*., 2008), and can potentiate UV-induced damage including ROS and NO production in keratinocytes (Handa *et al*., 2006). Given that intact paraben esters have been measured in human breast tissue (Darbre *et al*., 2004a), the possibility that their oestrogenic activity could influence the growth of oestrogen responsive breast cancers is evident. However, there is also the potential for the paraben esters to influence male reproductive functions through a combination of their oestrogenic and antiandrogenic properties. It is also a possibility that parabens could influence the development

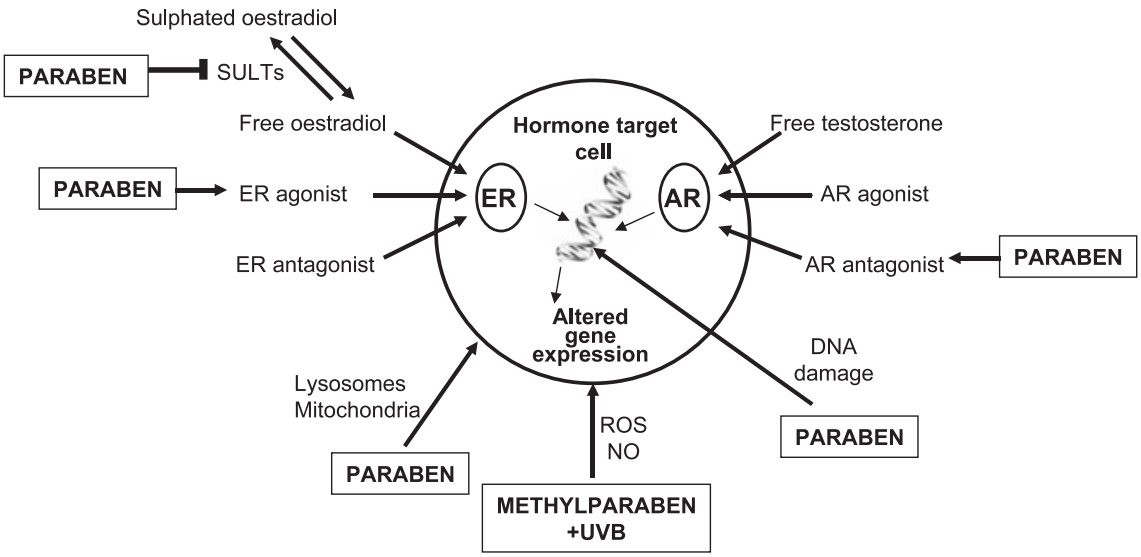


**Figure 2.** Parabens have now been shown to influence several molecular pathways within cells. As endocrine disrupting chemicals, they can act as oestrogen receptor (ER) agonists, as androgen receptor (AR) antagonists or as inhibitors of sulfotransferase enzymes (SULT). On wider cellular functions, they can disrupt lysosomal and mitochondrial functions, can cause DNA damage and can potentiate UVB-induced damage through production of reactive oxygen species (ROS) and nitric oxide (NO)



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of malignant melanomas through oestrogenic and genotoxic activity. It may be that the influence of parabens (together with other cosmetic chemicals) on human tissues is rather wider than has been hitherto anticipated and research is needed to ascertain how wide the implications may actually be.

Finally, there remains the need for controlled and detailed evaluation of breast cancer risk from bodycare cosmetics, taking into account product chemical ingredients, effect of formulations and total quantities applied, especially in potentially highly sensitive subgroups such as babies and children. There are still only two epidemiological studies in the database (Mirick *et al*., 2002; McGrath 2003) and no reported studies in animal models. It is unfortunate that under current UK regulations, cosmetic products can no longer be tested in animal models within the UK but breast cancer is a global problem and the potential for bodycare cosmetic formulations to cause breast cancer under defined conditions are in urgent need of investigation. Furthermore, at the current time, there remains a wide gap between knowledge about the oestrogenic activity of single paraben esters on their own (Table 2) and the environmental reality where body tissues are exposed to a mixture of oestrogenic chemicals including a mixture of all the paraben esters combined [all esters except benzylparaben were measured in both human breast tissue (Darbre *et al*., 2004a) and human urine (Ye *et al*., 2006a)] and a mixture of paraben esters together with other oestrogenic chemicals of both dietary or cosmetic origin (Darbre, 2006a). Recent research has demonstrated that environmental oestrogens can act in an additive manner alone or in combination with physiological oestrogens to give responses at concentrations where each alone would have little or no effect in either *in vitro* (Rajapakse *et al*., 2002) or *in vivo* (Brian *et al*., 2005) assays. Butylparaben has been shown specifically to give oestrogenic responses which are additive when combined with either the physiological oestrogen, oestradiol or the xenoestrogens, nonylphenol or Bisphenol A (Kyung-Sun-Kang *et al*., 2002). Research is now needed to define clearly whether there are additive or even inhibitory effects in human breast cancer cells of combinations of all the paraben esters and combinations of all the paraben esters together with other cosmetic oestrogens (see above) and also environmental oestrogens known to enter the breast through diet (Darbre, 2006a). Breast cancer epidemiology must face the reality of combined exposures from environmental sources (Kortenkamp, 2006) and this deserves to be incorporated into regulatory risk assessments. A full risk assessment taking into account the total oestrogenic burden of all chemicals in the human breast will require a profile of measurements of the oestrogenic chemicals in an average human breast today. To date, only limited and inadequate measurements have been made of individual chemicals (and only one set of measurements remain in the database for parabens in breast) and there are no data on profiles of chemical content in individual human breast samples. Furthermore, in view of the disproportionate number of breast cancers in the upper outer quadrant, it would seem appropriate to investigate variations in regional distribution of chemicals across the human breast such as has been described recently for aluminium (Exley *et al*., 2007).

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