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Parabens can enable hallmarks and characteristics of cancer in human breast epithelial cells: a review of the literature with reference to new exposure data and regulatory status

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ABSTRACT: A framework for understanding the complexity of cancer development was established by Hanahan and Weinberg in their definition of the hallmarks of cancer. In this review, we consider the evidence that parabens can enable development in human breast epithelial cells of four of six of the basic hallmarks, one of two of the emerging hallmarks and one of two of the enabling characteristics. In Hallmark 1, parabens have been measured as present in 99% of human breast tissue samples, possess oestrogenic activity and can stimulate sustained proliferation of human breast cancer cells at concentrations measurable in the breast. In Hallmark 2, parabens can inhibit the suppression of breast cancer cell growth by hydroxytamoxifen, and through binding to the oestrogen-related receptor gamma may prevent its deactivation by growth inhibitors. In Hallmark 3, in the 10 nM–1 μM range, parabens give a dose-dependent evasion of apoptosis in high-risk donor breast epithelial cells. In Hallmark 4, long-term exposure (>20 weeks) to parabens leads to increased migratory and invasive activity in human breast cancer cells, properties that are linked to the metastatic process. As an emerging hallmark methylparaben has been shown in human breast epithelial cells to increase mTOR, a key regulator of energy metabolism. As an enabling characteristic parabens can cause DNA damage at high concentrations in the short term but more work is needed to investigate long-term, low-dose mixtures. The ability of parabens to enable multiple cancer hallmarks in human breast epithelial cells provides grounds for regulatory review of the implications of the presence of parabens in human breast tissue. Copyright © 2014 John Wiley & Sons, Ltd.

Keywords: Paraben; oestrogen; hallmarks of cancer; cosmetics; personal care products; endocrine disruption; breast cancer

Introduction

Evidence is accumulating to support the concept that dermal absorption of oestrogenic chemicals applied in personal care products to the underarm and breast region might be involved in the development of breast cancers (Darbre, 2001, 2003; Darbre and Charles, 2010; Darbre and Fernandez, 2013). One such group of chemicals are the alkyl esters of *p*-hydroxybenzoic acid (parabens), which are used as preservatives, are known to possess oestrogenic activity and have been measured as entering human breast tissue as intact esters (Darbre and Harvey, 2008; Harvey and Darbre, 2004). The most commonly used esters are methylparaben, ethylparaben, *n*-propylparaben, *n*-butylparaben and isobutylparaben, although isopropylparaben and benzylparaben are also used less frequently (chemical structures and CAS numbers are given in Table 1). Their widespread use in consumer products (Andersen, 2008; Guo and Kannan, 2013; Karpuzoglu *et al.*, 2013; Loretz *et al.*, 2006) has led to measureable levels across the global ecosystem in recent years (Brausch and Rand, 2011), including surface waters in China (Yu *et al.*, 2011), India (Ramaswamy *et al.*, 2011), Japan (Yamamoto *et al.*, 2011; Terasaki *et al.*, 2012), Spain (González-Mariño *et al.*, 2009), Switzerland (Jonkers *et al.*, 2009) and the USA (Renz *et al.*, 2013) and in sediment in Japan, Korea and the USA (Liao *et al.*, 2013b) and in soil in Canada (Viglino *et al.*, 2011) and Spain (Ferreira *et al.*, 2011). Since the first detection of parabens in human breast tumour tissue in 2004 (Darbre *et al.*, 2004), a more recent study has confirmed their ubiquitous presence in all regions of the human breast (Barr *et al.*, 2012) and other studies have shown them to be measureable in human milk (Ye *et al.*, 2008; Schlumpf *et al.*, 2010). Further recent studies are revealing their ubiquitous presence in many human body tissues, including blood, placenta, seminal fluid and extensively in urines from around the world (see Table 2). Parabens have been the subject of several recent reviews (Boberg *et al.*, 2010; Karpuzoglu *et al.*, 2013), but the potential for parabens to contribute specifically to the development of breast cancer was last reviewed by us in 2008 (Darbre and Harvey, 2008) and this

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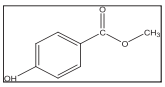
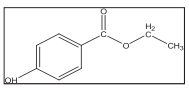
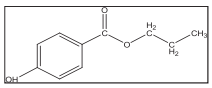
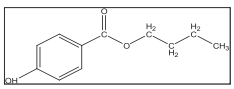
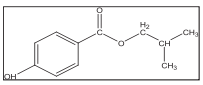
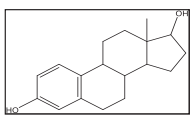
Table 1. Comparison of the relative binding affinity to human breast cancer cell oestrogen receptors and efficacy in stimulating human breast cell proliferation for the five parabens used most commonly in consumer products and measured as present in human breast tissue

Paraben	CAS no.	Chemical structure	Relative binding to oestrogen receptors of human breast cancer cells[a]	Efficacy in stimulating proliferation of MCF-7 human breast cells[b]	
			Molar excess for 50% inhibition of ^{3}H-oestradiol binding	Molar concentration for 50% of response with 10^{-8} M 17β-oestradiol (M)	Lowest observed effect concentration (M)
Methylparaben	99-76-3		3 000 000 ×	1×10^{-4}	6×10^{-5}
Ethylparaben	120-47-8		500 000 ×	1×10^{-5}	2×10^{-6}
n-Propylparaben	94-13-13		300 000 ×	2×10^{-6}	8×10^{-7}
n-Butylparaben	94-26-8		100 000 ×	2×10^{-6}	7×10^{-7}
isoButylparaben	4247-02-3		40 000 ×	1×10^{-6}	4×10^{-7}
17β-oestradiol	50-28-2		3×	5×10^{-12}	1×10^{-12}

[a]Numbers for relative binding to human oestrogen receptors from MCF-7 human breast cancer cells are taken from Darbre (2006).
[b]Numbers for efficacy in stimulating MCF-7 human breast cancer cell proliferation over 7 days are taken from Charles and Darbre (2013).

review collates research reported over the past 5 years, which adds further weight of evidence.

A framework for understanding the complexity of cancer development has been established by Hanahan and Weinberg when they described six hallmarks of cancer (Hanahan and Weinberg, 2000) and a further two enabling characteristics and two emerging hallmarks (Hanahan and Weinberg, 2011). These hallmarks illustrated in Fig. 1 document a range of altered gene expression and signalling pathways, which can lead to the cellular and molecular changes observed in cancer cells and their microenvironment, which together enable development of the diversity of changes that drive cancer development. The six basic hallmarks are sustained proliferative signalling, evasion of growth suppression, resistance to cell death, replicative immortality, induction of angiogenesis and activation of invasion and metastasis. Genomic instability and inflammation are two enabling characteristics needed as underlying events. Two more recently emerging hallmarks are the reprogramming of energy metabolism and evasion of immune suppression. In this review, we consider the evidence that parabens can enable the development of several of these hallmarks or characteristics in human breast epithelial cells.

Oestrogenic Activity of Parabens

The central role of oestrogen in the development of breast cancer has been established through epidemiological, clinical and experimental studies (Miller, 1996), and the effective use of endocrine therapy, which is based on antagonizing oestrogen action (antioestrogens) or inhibiting oestrogen synthesis (aromatase inhibitors) to reduce tumour growth (Lonning, 2004) further underlines the strong association between the presence of oestrogen and breast cancer prognosis. As breast cancer incidence is highest in postmenopausal women (Key *et al*., 2001) and rates of oestrogen responsive cancers are higher in postmenopausal women (Li CI *et al*., 2003), which is a time

Table 2. Published measurements of parabens in human tissues

Human tissue		Collection	Country	n	Units	Mean/ median	Methylparaben	Ethylparaben	*n*-Propylparaben	*n*-Butylparaben	isoButylparaben	Reference
Milk	General population	2004–2006	Switzerland	54	ng ml-1	Mean	2.18	1.26	1.42	0	0	Schlumpf *et al.*, 2010
Milk	General population	2007	USA	4	ng ml-1	Range	0.5-3.0		0-0.3			Ye *et al.*, 2008
Breast	Cancer tissue	1980s	Scotland	20	ng g-1	Mean	12.8	2.0	2.6	0.9	2.3	Darbre *et al.*, 2004
Breast	Unaffected tissue adjacent to a cancer	2005–2008	England	160	ng g-1	Median	16.6	3.4	16.8	2.1	5.8	Barr *et al.*, 2012
Blood	General population	2005	Norway	332	ng ml-1	Median	9.4	<3	<2	0	0	Sandanger *et al.*, 2011
Placenta	General population		Spain	50	ng g-1	Median	1.6	0.4	0.5	0.5		Jimenez-Diaz *et al.*, 2011
Seminal fluid	General population	2006	Denmark	60	ng ml-1	Median	1.0	0.14	0.7	0.06		Frederiksen *et al.*, 2011
Urine	General population	2003–2005	USA	100	ng ml-1	Median	43.9	1.0	9.1	0.5		Ye *et al.*, 2006
Urine	General population	2005–2006	USA	2548	µg L-1	Median	63.5	0	8.7	0	0	Calafat *et al.*, 2010
Urine	Infertility clinic (men)	2000–2004	USA	194	µg L-1	Median	27.4		3.45	0	0	Meeker *et al.*, 2011
Urine	General population	2006	Denmark	60	ng ml-1	Median	17.7	1.98	3.6	0.19		Frederiksen *et al.*, 2011
Urine	Pregnant women	2004–2008	Spain	120	ng ml-1	Median	191.0	8.8	29.8	2.4		Casas *et al.*, 2011
Urine	4-year old boys	2004–2008	Spain	30	ng ml-1	Median	150.0	8.1	21.5	1.2		Casas *et al.*, 2011
Urine	Fertility clinic (men & women)	2005–2010	USA	2721	µg L-1	Median	112		24.2	0.7		Smith *et al.*, 2012
Urine	Pregnant women	2010–12	Puerto Rico	105	ng ml-1	Median	153		36.7	0.4		Meeker *et al.*, 2013
Urine	Pregnant women	2007–2010	Japan	111	ng ml-1	Median	75.8	7.5	20.2	0.6		Shirai *et al.*, 2013
Urine	Children (9-10years)	2012	China	70	ng ml-1	Median	3.7	0.6	1.5	0.03		Wang *et al.*, 2013
Urine	Adults	2010	China	26	ng ml-1	Median	19.5	0.09	4.3	0		Wang *et al.*, 2013
Urine	Young adults 20years old (men/women)		China	68/41	ng ml-1	Median	3.8/10.0	1.4/1.6	2.2/7.0			Ma *et al.*, 2013
Urine	Pregnant women (22-29years / 30-39years old)	2011	Korea	46	µg L-1	Median	209.1/169.9	21.9/65.6	28.8/6.3	0/0		Kang *et al.*, 2013
Urine	2.5-87 years old	2012	Greece	100	ng ml-1	Average	49.2	6.8	34.8	5.5		Asimakopoulos *et al.*, 2014
Urine	Mother/child pairs	2011	Denmark	288	ng ml-1	Median	14.0/3.0	0.9/0.4	1.7/0			Frederikesn *et al.*, 2013
Urine	General population		Belgium	25	ng ml-1	Median	36.2	3.3	0.8	0		Dewalque *et al.*, 2014
Urine	Urban community (women)	2011	Canada	28	µg L-1	Median	25.5	10.2	2.8	0.3	0.2	Genuis *et al.*, 2013
Urine	Urban community (men)	2011	Canada	11	µg L-1	Median	26	10.4	3.1	0.4	0.2	Genuis *et al.*, 2013

The units are given as published but assuming 1 g of tissue has a volume of 1 ml, then all units are directly comparable.
n, number of samples in the study.

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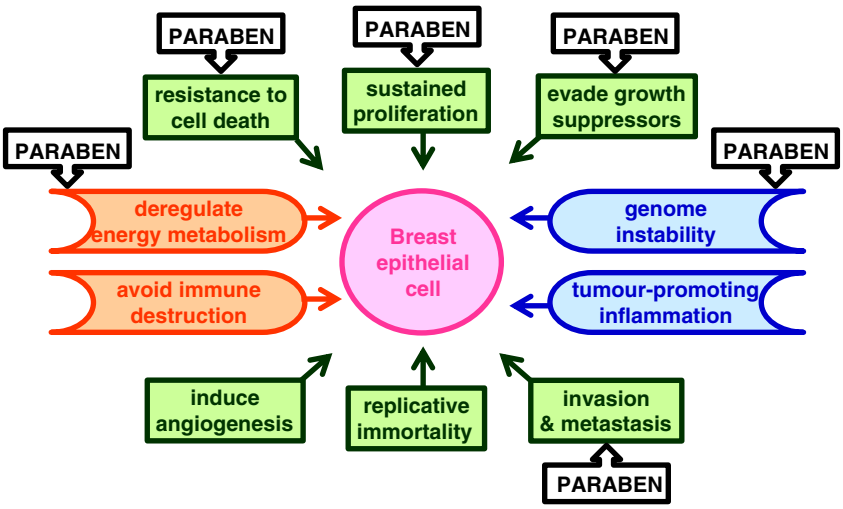

Figure 1. The hallmarks of cancer, which have been shown to be influenced by one or more paraben in human breast epithelial cells. Six basic hallmarks (green); two enabling characteristics (blue); two emerging hallmarks (orange) as defined by Hanahan and Weinberg (2011).

when endogenous oestrogen synthesis from the ovary has dropped, the question has to be asked as to what is fuelling the growth of these oestrogen-responsive tumours. As all the paraben esters widely used in consumer products have been shown to possess oestrogenic activity in assay systems *in vitro* and *in vivo* (Darbre & Harvey, 2008), it has been suggested that their presence in human breast tissue (Barr *et al*., 2012; Darbre *et al*., 2004) might play a functional role in influencing the development of breast cancer (Darbre and Harvey, 2008; Harvey and Darbre, 2004; Harvey and Everett, 2006).

Routledge *et al*. (1998) were the first to show that parabens possess oestrogenic activity, and in 2008 we reviewed the evidence for oestrogenic activity of methylparaben, ethylparaben, propylparaben, butylparaben and benzylparaben together with their common metabolite *p*-hydroxybenzoic acid in *in vitro* and *in vivo* assays (Darbre and Harvey, 2008). Oestrogens act in target cells by binding to intracellular oestrogen receptors (ER), which function in a genomic mechanism as ligand-activated transcription factors to influence patterns of gene expression (Hah and Kraus, 2014) or in non-genomic mechanisms through interaction with growth factor signal transduction pathways (Banerjee *et al*., 2014). Assay systems *in vitro* have demonstrated oestrogenic activity of parabens through their ability to bind to ERs in a competitive binding assay, to influence expression of oestrogen-regulated genes and to increase proliferation of cells dependent on oestrogen for their growth, and this was reviewed by us in 2008 (Harvey & Darbre, 2008). The gold standard *in vivo* assay has been to measure an increase in uterine weight in either immature or ovariectomized rodents following subcutaneous/dermal/oral administration, although some other biomarkers of oestrogen action have been used in other organisms (Boberg *et al*., 2010; Harvey & Darbre, 2008). The relative binding affinity for parabens to human ER is 10 000–1 000 000-fold lower than for 17β-oestradiol (Darbre, 2006) but increases with linear length of the alkyl chain from methylparaben to *n*-butylparaben (Byford *et al*., 2002; Routledge *et al*., 1998) and with branching in the alkyl chain from *n*-butylparaben to isobutylparaben (Darbre *et al*., 2002). All *in vitro* assays show dose–responses that correlate with the binding affinity to ER. The *in vivo* uterotrophic assay also shows higher doses needed for a response to paraben than 17β-oestradiol but there are variations between species (immature mice are more responsive than ovariectomized mice or immature Wistar rats; Lemini *et al*., 2003) and between routes of administration (subcutaneous, oral or topical; Boberg *et al*., 2010). Recently, benzylparaben has been shown to increase uterine weight in immature SD rats at a particularly low dose of 0.16 mg (kg body weight)$^{-1}$ (Hu *et al*., 2013).

Exposure of Human Tissues to Parabens

If parabens are suspected to exert a functional role in the human breast, then the first considerations must be of the extent to which parabens can enter the human breast as biologically available intact esters from environmental exposures.

Source of Human Exposure

Owing to their effective antimicrobial properties, parabens are used as preservatives in an extensive range of consumer products to which the human population is exposed, including personal care products, foods and pharmaceuticals (Andersen, 2008; Karpuzoglu *et al*., 2013; Loretz *et al*., 2006; Yazar *et al*., 2011). However, their use as effective preservatives is now extending into other applications, including preservation of paper products and absorption from handling paper cannot be ignored as an exposure route (Liao and Kannan, 2014). Furthermore, in addition to exposure through identifiable products, it seems that parabens are becoming ubiquitously distributed across indoor air making the unventilated indoor environment another source of exposure (Canosa *et al*., 2007; Rudel *et al*., 2010; Weschler and Nazaroff, 2014). The common metabolite of the paraben esters, *p*-hydroxybenzoic acid, has been measured in some fruits (Mattila *et al*., 2006) and vegetables (Kang *et al*., 2008), but intact esters in plant extracts seem likely to have originated from the commercial processing of the plant material (Li *et al*., 2003) or from uptake of parabens from soil fertilized with municipal biosolids (Sabourin *et al*., 2012). Although some bacteria have been reported to possess some paraben esters (Quevrain *et al*., 2009), the widespread detection of parabens across the ecosystem would seem to be

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originating from the extensive use of synthesized paraben esters added as preservatives to consumer products.

Although systemic exposure to parabens may occur through their use as preservatives in medical products administered subcutaneously, through inhalation/transdermal absorption of parabens in indoor air (Weschler and Nazaroff, 2014) or through dermal/oral exposure from handling paper products (Liao and Kannan, 2014), it seems probable that the main routes of exposure are either oral from food or dermal from topical application of personal care products. Based on paraben concentrations measured in foods and per capita daily ingestion rates of foods, a recent estimated daily intake of total parabens in the USA has been calculated as 940, 879, 470 and 307 ng (kg body weight)$^{-1}$ day^{-1} for infants, toddlers, children and adults respectively (Liao *et al*., 2013). Based on the amount and frequency of use of personal care products and measured median paraben concentrations in products, the total dermal intake doses of parabens have been calculated to be 31.0 μg (kg body weight)$^{-1}$ day^{-1} for adult females and this rose to between 58.6 and 766 μg (kg body weight)$^{-1}$ day^{-1} for infants and toddlers (Guo and Kannan, 2013) suggesting that exposure through personal care products can be substantial. The same conclusion was reached also some years ago by Harvey and Everett (2006) who calculated that a significant oestrogenic challenge to breast tissue could be achieved from dermal absorption of parabens in a single application of a body care lotion to the breast/chest area. Using the same conservative absorption factors and oestrogenic potency as published by Harvey and Everett (2006), their calculations have been extended to individual paraben esters and are shown in Table 3. Using no more than maximal current European Union (EU) recommended levels, the oestrogenic stimulus generated from a single application of lotion is biologically meaningful even for single esters alone and values in Table 3 in bold are those $>$ 20% of the endogenous oestrogen levels of 55.3 pg ml^{-1} g^{-1} tissue (Clarke *et al*., 2001). Comparison to the concentrations of each of the paraben esters measured in human breast tissue (Barr *et al*., 2012) as converted to oestrogen equivalents, it can be seen that even the highest concentrations measured in human breast tissue could be achieved by very few such applications of lotion (Table 3) and this should be considered in the context of exposure of a large global population where on average each consumer would use not one but multiple personal care products on a daily basis.

Absorption into Human Tissues

Over the past 5 years, parabens have been measured in a wide range of human urine samples from across the globe where parabens are reported as ubiquitously present in almost all samples in almost all studies, but with considerable variation necessitating levels to be reported as medians rather than means (Table 2). Measurements in other body tissues have been fewer but one study did report a correlation of paraben levels within individuals between urine, serum and seminal plasma (Frederiksen *et al*., 2011) suggesting paraben absorption is distributed systemically. The measurement of intact paraben esters in human tissues and fluids so widely demonstrates that at current exposure levels these compounds are escaping metabolism either by skin esterases if exposure was dermal or by intestinal and liver metabolic processes if the exposure was oral.

Previous reviews have suggested that dermal rather than oral exposure is more likely to have resulted in the parabens entering human tissue (Darbre and Harvey, 2008; Harvey and Everett, 2012). Personal care products applied on a frequent basis and left on the skin allows for continuous dermal exposure and therefore over a long period may result in absorption and accumulation into underlying tissues. Confirmation of the ability of parabens to be absorbed systemically from dermal application of cosmetic cream to human subjects has been demonstrated (Janjua *et al*., 2007, 2008), with paraben esters measurable in blood after as little as 1 h from dermal application (Janjua *et al*., 2007). Notwithstanding the presence of esterases in skin, some of the parabens must therefore be evading metabolic breakdown through the dermal route. Previous studies have shown parabens to be readily absorbed through animal skin (Darbre and Harvey, 2008) but absorption kinetics (ElHussein *et al*., 2007) combined with lower rates of metabolism in human skin (Harville *et al*., 2007) suggests that absorption through human skin is higher than through animal skin. Several studies have now reported a positive correlation between the amount of one or more personal care products used and levels of parabens measured in human blood (Sandanger *et al*., 2011) or urine (Braun *et al*., 2014; Meeker *et al*., 2013). The reported correlation between urinary levels of parabens in mother and child pairs in rural and urban regions of Denmark (Frederikesn *et al*., 2013) and between mothers and their newborn infants in Korea (Kang *et al*., 2013) is also indicative of an environmental link within families. Higher levels of parabens in urine from women than men has been interpreted as related to a higher use of cosmetic products in women (Calafat *et al*., 2010; Ma *et al*., 2013; Smith *et al*., 2012). Likewise, higher levels of parabens in African Americans than Caucasians may also relate to patterns of personal care product usage (Calafat *et al*., 2010; Smith *et al*., 2012).

Measurement in Human Breast Tissue

Publication in 2004 of measurements of intact paraben esters in 20 samples of human breast cancer tissue (Darbre *et al*., 2004) caused substantial discussion because this was the first time parabens had been shown to be present as intact esters in the human body (see Harvey and Everett, 2004). Their known ability to stimulate growth of human breast cancer cells through their oestrogenic properties (Byford *et al*., 2002; Darbre *et al*., 2002, 2003) in the context of oestrogen as an established risk factor for breast cancer (Miller, 1996) sparked debate as to the potential for their presence in the human breast to influence breast cancer development (Darbre and Harvey 2008; Harvey and Darbre 2004; Harvey and Everett 2006). However, at that time, there remained a gap between measured paraben concentrations in breast tissue and the higher amount of any one ester needed *in vitro* to stimulate growth of human breast cancer cells maximally. This gap between measured tissue paraben levels and concentrations needed for *in vitro* assays has now been closed. This has occurred partly through more recent measurements of higher concentrations of parabens in human breast tissue (Barr *et al*., 2012) but mainly through the realization that lower concentrations of parabens can also stimulate growth of human breast cancer cells in culture over a longer assay time and, furthermore, that mixtures of five paraben esters can add together at even lower concentrations to stimulate human breast cancer cell proliferation (Charles and Darbre, 2013).

The more recent and larger set of measurements of paraben esters in 160 samples of human breast tissue taken from four serial locations across the breast from axilla to sternum from 40

Table 3. Calculated potential for human dermal absorption of paraben esters in a body lotion and potency of oestrogenic action as related to relative oestrogen receptor binding affinity. Calculations are as described by Harvey and Everett (2006). Application assumes a bottle of 125 ml body lotion is used up in 1 month by one daily application of 4.2 ml

Application		Absorption	Relative binding affinity to oestrogen receptor	Oestrogen equivalents absorbed		Range of concentrations measured in human breast tissue[a]	
Paraben content of lotion[c]	Paraben in one daily application (4.2 ml)	Dermal absorption (10% of application)	Relative oestrogenic potency of parabens (fold lower than oestradiol)[d]	Oestrogen equivalents[b] of absorbed paraben	Oestrogen equivalents[b] absorbed per day assuming an area 500 cm^2 [e] (pg g^{-1})	ng g^{-1} measured in breast tissue	Oestrogen equivalents[b] of measured paraben (pg g^{-1})
0.87% mixed parabens[f]	36.3 mg[f]	3.6 mg[f]	100 000[f]	36 ng[f]	**72**[f]	Median 85.5[g]	0.9
0.4% methylparaben	16.8 mg	1.7 mg	1 000 000	1.7 ng	3.4	0–5102.9[g]	0–5.1
0.4% ethylparaben	16.8 mg	1.7 mg	167 000	10.1 ng	**20.1**	0–499.7[g]	0–3.0
0.19% propylparaben	8.0 mg	0.8 mg	100 000	8.0 ng	**16.0**	0–2052.7[g]	0–20.5
0.19% butylparaben	8.0 mg	0.8 mg	33 000	24.2 ng	**48.5**	0–95.4[g]	0–2.9
0.19% isobutylparaben	8.0 mg	0.8 mg	13 000	61.5 ng	**123.1**	0–802.9[g]	0–61.8

[a]Calculated values are given in oestrogen equivalents and compared to concentrations measured in human breast tumour tissue.
[b]Endogenous concentrations of oestradiol in breast tissue: 0.203 nM oestradiol (55.3 pg ml^{-1} g^{-1} tissue) has been reported in normal breast adipose and an average of 1.28 nM (348 pg ml^{-1} g^{-1}) in human breast tumours (Clarke *et al.*, 2001): values in bold are > 20% of the oestrogen in normal tissue.
[c]Contents in lotion are maximal levels recommended under the EU Cosmetics Directive 76/768/EEC.
[d]Relative binding affinities taken from Darbre (2006).
[e]cm^3 ml^{-1} g^{-1} tissue are equivalent.
[f]Calculation for total paraben taken from Harvey and Everett (2006).
[g]Measured concentrations as published by Barr *et al.* (2012).

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patients undergoing mastectomy for breast cancer has confirmed widespread distribution of parabens both across individual breasts and between women (Barr *et al*., 2012 see also discussion in Harvey and Everett, 2012). One or more paraben ester was detected in 158 of 160 (99%) of the tissue samples and 96 of 160 (60%) contained all five of the esters measured (methylparaben, ethylparaben, *n*-propylparaben, *n*-butylparaben and isobutylparaben) (Barr *et al*., 2012). In line with measurements in other body tissues (see Table 2), methylparaben and propylparaben were the two parabens detected at highest levels. Cell culture studies demonstrated that proliferation of human breast cancer cells could be increased by exposure to these five parabens either alone or in combination at some of the measured breast tissue concentrations (Charles and Darbre, 2013). Forty-three of 160 (27%) human breast tissue samples contained at least one paraben at a concentration above that needed for an observed effect on proliferation (lowest observed effect concentration). For the 22 tissue samples taken at the site of oestrogen-responsive (positive for oestrogen and progesterone receptors ER+PR+) primary cancers, 12 contained a sufficient concentration of one or more paraben in combination to stimulate proliferation of MCF-7 human breast cancer cells in culture (Charles and Darbre, 2013). This demonstrates that parabens, either alone or in combination, are present in some human breast tissues at functional concentrations and that assessment must take into account not one but all esters present.

Distribution of parabens across the human breast is also an important question into trying to understand the basis for the disproportionate incidence of breast cancer in the upper outer quadrant of the breast, which now exceeds 50% in the UK (Darbre, 2005; Darbre and Charles, 2010). As most breast cancers start in epithelial cells of the breast, this disproportionality has long been assumed to be due to a greater amount of epithelial tissue in that region (Haagensen, 1971). However, this assumption has been questioned more recently by the hypothesis that environmental chemicals such as parabens might be disproportionately distributed into that region either because they are applied directly to the adjacent underarm and upper chest region or because physiological mechanisms, such as blood circulation or lymphatic drainage, deposit chemicals into that region (Darbre, 2001, 2003). Measurements of parabens across four serial locations of the human breast revealed higher levels of *n*-propylparaben in the outer axilla region compared with inner regions (Barr *et al*., 2012) but further studies are needed to ascertain the full significance of this.

Biological Availability

Although compounds may be present in human tissue, their biological availability has to be taken into consideration in any assessment of potential for effects. Binding of parabens to human serum albumin has been reported as weak, suggesting circulating parabens would probably be in a free form in the blood available to reach tissues (Greige-Gerges *et al*., 2013). However, as for physiological oestrogens, paraben availability could also be influenced by conjugation. While conjugation is generally assumed to remove oestrogenic activity, this is not always the case and some sulphates (Pugazhendhi *et al*., 2008) or glucuronides (Zhang *et al*., 1999) of plant phytoestrogens have been shown to retain oestrogenic activity. Nothing is known about the oestrogenic activity of conjugates of parabens, but although measurements of parabens in human urine have reported the esters to be mainly conjugated as glucuronide or sulphate (Ye *et al*., 2006; Dewalque *et al*., 2014), the parabens measured in human milk were reported as mainly in unconjugated form (Ye *et al*., 2008; Schlumpf *et al*., 2010). Since milk is secreted from the epithelial cells of the breast and these are the main target cells for cancer, this would suggest that parabens in the breast cancer cells are biologically available in unconjugated form.

The Hallmarks of Cancer

As it is not possible to study the effects of parabens directly *in vivo* in the human breast, the next best approach to investigating the implications of the presence of parabens in human breast tissue would seem to be to study effects of parabens on human breast epithelial cells (transformed and nontransformed) in cell culture systems using concentrations of individual esters and mixtures of esters at concentrations that have environmental relevance in having been measured in samples of human breast tissue (Barr *et al*., 2012). The conceptual framework of the hallmarks of cancer (Hanahan and Weinberg, 2011) then offers a focus on which to assess the overall ability of parabens to influence processes leading to cancer development in breast cells. The ability of parabens to bind to ER and so mimic oestrogen action has been a focus of research effort but their mechanisms of action may not necessarily be limited to ER-mediated mechanisms. Microarray studies have shown that although some genes are influenced by exposure to parabens in a similar way to oestradiol, most genes are not regulated in the same way by paraben and oestradiol, suggesting parabens can imprint unique gene signatures on to cells (Pugazhendhi *et al*., 2007). Differences in gene expression have been observed across a range of cellular functions, which could potentially impact on the hallmarks of cancer if validated at a protein level. Specific upregulation by parabens of mRNA for a homologue of a BRCA1-interacting protein (a component of the DNA damage response network) could be indicative of cellular response to genomic insult, the failure of some parabens to increase mRNA for interleukin 24 (known to induce apoptosis) could result in resistance to cell death and failure of some parabens to reduce mRNA for adrenomedullin (known to play a role in tumour angiogenesis) could impact on vascularization of the tumour (Pugazhendhi *et al*., 2007).

Hallmark 1: Sustaining Proliferative Signalling

A fundamental trait of breast cancer cells is their ability to undergo sustained proliferation. Control mechanisms would normally ensure regulated entry and progression through the cell cycle but loss of response to control signals results in overproliferation and disruption to tissue architecture/function. Oestrogen is one main enabling signal for proliferation of breast epithelial cells and sustained oestrogen signalling is a feature of the growth of oestrogen-responsive breast cancer cells (Darbre, 2012). The oestrogenic activity of parabens specifically demonstrated in human breast cancer cells (Byford *et al*., 2002; Darbre *et al*., 2002, 2003) and their presence in human breast tissue (Barr *et al*., 2012; Darbre *et al*., 2004) indicate the potential for them to drive sustained proliferation of breast epithelial cells that possess ER, and the majority of breast cancers are ER+ (Li *et al*., 2003; Lonning, 2004; Miller, 1996).

Stimulation of human breast cancer cell proliferation in monolayer culture. Parabens have been shown to increase proliferation of several lines of oestrogen-responsive human breast cancer cells in monolayer culture (Byford *et al.*, 2002; Darbre *et al.*, 2002, 2003; Okubo *et al.*, 2001; Wrobel and Gregoraszczuk, 2013). As the proliferative response can be inhibited by antioestrogen (Byford *et al.*, 2002; Darbre *et al.*, 2002: Darbre *et al.*, 2003), this suggests that the mechanism is ER-mediated. This increased proliferation is observed not only in the absence of oestrogen but also in the presence of low levels of oestrogen (Darbre, 2009) such as could occur *in vivo* after menopause or low oestrogen stages in the menstrual cycle (Wright *et al.*, 1999). Furthermore, low levels of individual parabens also add together to give increased responses both in the absence of oestrogen and in the presence of low levels of oestrogen (Darbre, 2009).

Of the five parabens most commonly used in personal care products, competitive binding studies to human ER have shown that isobutylparaben binds most strongly to the ER followed by *n*-butylparaben > *n*-propylparaben > ethylparaben > methylparaben (Table 1). Comparison to effects on cell proliferation show that the relative amount of each paraben needed to increase proliferation correlates with its relative binding affinity to ER (Table 1), implying an ER-mediated mechanism. However, it should be noted that all parabens are full agonists in terms of increasing proliferation of human breast cancer cells (Fig. 2). The term 'weak' continues to be applied to parabens but this relates only to their ER binding affinity, which is lower than for 17β-oestradiol and does not relate to their efficacy in terms of stimulating proliferation of human breast cancer cells. Higher concentrations of parabens are needed to stimulate cell proliferation compared to oestradiol because their ER binding affinity is lower but when sufficient concentration is present they are full agonists with the same efficacy as oestradiol as shown in Fig. 2. The main consideration therefore becomes not whether their ER binding is of low affinity but the concentration of paraben present in the target breast tissue. The range of concentrations of the five parabens measured in human breast tissue is indicated on Fig. 2 in relation to the concentrations needed to drive proliferation of the human breast cancer cells in culture and there is evidently some overlap for single parabens alone. Mixtures of five paraben esters and longer time-frames have shown that lower doses of individual parabens can also combine to drive proliferation in the longer term (Charles and Darbre, 2013). This is highly relevant to the environmental reality of exposures in the human breast *in vivo*. At least one paraben ester was measured in 99% of human breast tissues assayed (Barr *et al.*, 2012) showing that human breast epithelial cells are ubiquitously exposed long-term to parabens *in vivo*. Furthermore, all five of the paraben esters were measured in 60% of the breast tissues (Barr *et al.*, 2012) showing that in the human breast, the cells are frequently exposed not to one but to all five esters in combination. An assessment of the ability of parabens to drive sustained proliferation of human breast epithelial cells must therefore now be based on the combination of all five esters at the concentrations of each as measured in human breast tissue and furthermore must take into consideration the ability of low doses to stimulate proliferation when left for a longer time-frame (Charles and Darbre, 2013).

Mechanisms by which oestrogens increase proliferation can include genomic and non-genomic signalling pathways (Darbre, 2012). By the genomic mechanism, oestrogens act through interaction with intracellular ERs (ERα, ERβ), which function as ligand-activated transcription factors orchestrating a global change in expression of hundreds of genes (Hah and Kraus, 2014). Non-genomic mechanisms can result in more rapid actions through alteration to cell signalling phosphorylation cascades involving tyrosine kinase receptors (Banerjee *et al.*, 2014) or the G-protein coupled ER (GPER) (Lappano *et al.*, 2013; Soltysik and Czekaj, 2013). More recent use of ER and GPER inhibitors has suggested that propylparaben can also influence

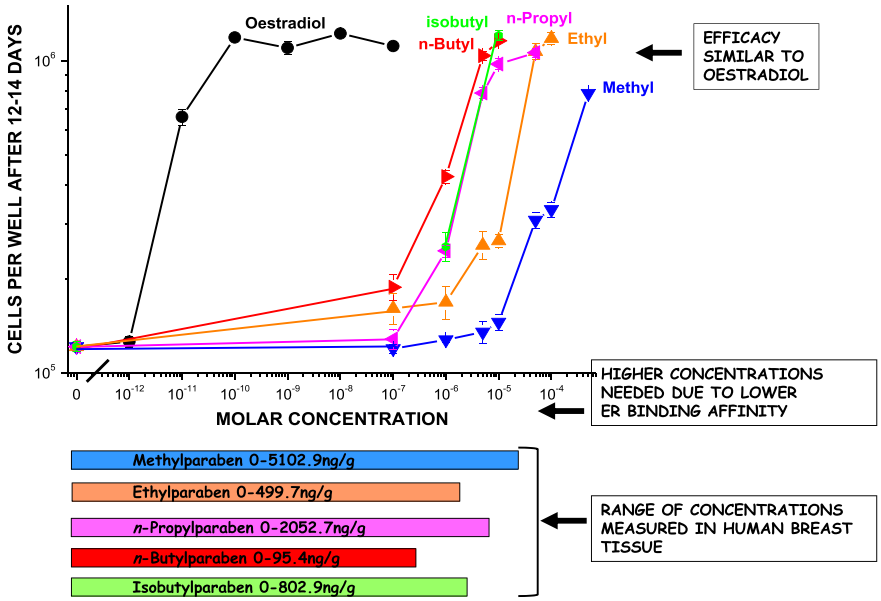

Figure 2. The efficacy of five parabens in stimulating the proliferation of MCF-7 human breast cancer cells *in vitro* as compared with 17β-oestradiol. The efficacy is similar for all compounds provided sufficient concentration is present: higher concentrations are needed for compounds with lower ER binding affinity. Data are amalgamated from four publications (Byford *et al.*, 2002; Darbre *et al.*, 2002, 2003; Pugazhendhi *et al.*, 2005). The range of concentrations of the five parabens measured in human breast tissues are added for comparison (Barr *et al.*, 2012). ER, oestrogen receptor.

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proliferation of MCF12A non-transformed breast epithelial cells through combined ER- and GPER-mediated mechanisms (Marchese and Silva, 2012). The recent report that parabens can also increase expression of the aromatase gene (*CYP19A1*) in both transformed and non-transformed breast epithelial cells suggests yet a further mechanism of action of parabens on cell proliferation whereby the parabens can act indirectly by increasing endogenous synthesis of oestradiol in the cells (Wrobel and Gregoraszczuk, 2013).

Over recent years, model culture systems have been developed to assay proliferation of breast epithelial cells under more physiological conditions where the cells are grown in a reconstituted basement membrane matrix such as matrigel rather than on a plastic surface. Growth of MCF12A breast epithelial cells (which possess ER) in such a 3D model allows for some assessment of breast glandular structures, in particular organized acini with deposition of basement membrane and hollow lumen (Marchese and Silva, 2012). Using this model system, exposure to propylparaben resulted in deformed acini and filling of the lumen, which could be arrested with inhibitors of ER and GPER showing that sustained proliferation by paraben could also result in overgrowth of cells within a glandular structure by ER- and GPER-mediated mechanisms (Marchese and Silva, 2012).

Enabling of non-transformed human breast epithelial cells to proliferate in suspension. The ability of anchorage-dependent epithelial cells to grow under anchorage-independent conditions (suspension culture) has long been acknowledged as a property of cells *in vitro*, which correlates with transformation *in vivo* (Shin *et al*., 1975), and suspension growth of immortalized non-transformed human breast epithelial cells has recently been established as a model system in which to identify the transforming ability of steroidal oestrogens (Russo and Russo, 2006; Russo *et al*., 2006). Use of this model system has revealed that parabens can also enable growth of MCF10A non-transformed immortalized human breast epithelial cells in suspension culture (Khanna and Darbre, 2013) indicating an ability of parabens to enable alterations towards loss of anchorage dependence for proliferation. MCF10A cells do not possess detectable levels of either ERα or ERβ protein but overexpression of ERα (not ERβ) can enhance suspension growth by oestradiol in these cells (Pugazhendhi and Darbre, 2010), so it remains to be determined as to whether parabens might act by increasing levels of ERα or whether the mechanism is non-ER-mediated. Figure 3 summarizes these results (Khanna and Darbre, 2013) in comparison with concentrations of the paraben esters measured in human breast tissues (Barr *et al*., 2012) and poignantly illustrates that some individual concentrations of parabens in human breast tissue are sufficient to enable these phenotypic changes.

Hallmark 2: Evading Growth Suppressors

The ability to reduce the growth of oestrogen-responsive breast cancer cells either by antagonizing oestrogen action at its receptor or by inhibiting aromatase from synthesizing oestradiol has provided a targeted therapy that has offered therapeutic benefit (Lonning, 2004). As clinical experience has shown the antioestrogen tamoxifen to be very effective at holding down tumour growth, tamoxifen is now being trialled for the prevention of breast cancer in high-risk women (Hollander *et al*., 2013). It is therefore unfortunate that methylparaben has been recently shown to inhibit the active metabolite of tamoxifen, hydroxytamoxifen, from suppressing breast cancer cell growth (Goodson *et al*., 2011). Interestingly another study has shown that several of the parabens (methylparaben, ethylparaben, propylparaben and butylparaben) can bind to the oestrogen-related receptor gamma (ERRγ) (Zhang *et al*., 2013), which is a nuclear receptor capable of modulating oestrogen signalling of proliferation in breast cancer cells (Ijichi *et al*., 2011). Since bisphenol A has been shown to bind strongly to ERRγ thereby preserving its deactivation by growth inhibitors such as hydroxytamoxifen (Matsushima et al., 2008),

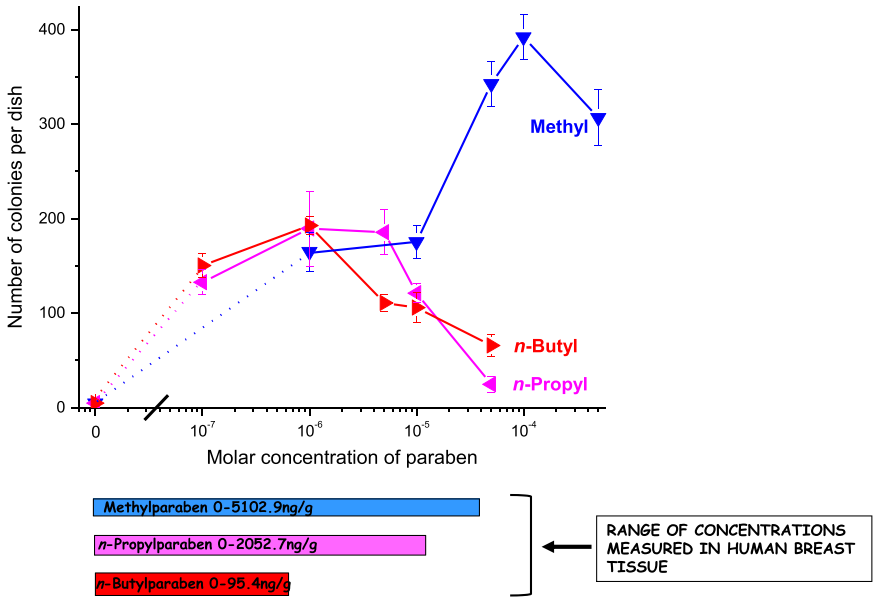

Figure 3. Comparison of the concentrations of three paraben esters needed for growth under non-adherent conditions (suspension culture) compared with the range of concentrations measured in human breast tissues. Suspension data taken from Khanna and Darbre (2013); tissue concentrations taken from Barr *et al*. (2012).

the inhibitory action of methylparaben on growth suppression by hydroxytamoxifen reported by Goodson *et al*. (2011) might be suggestive also of an ERRγ-mediated mechanism. As all these paraben esters have been measured as present in human breast tissue (Barr *et al*., 2012), this has therapeutic implications and poses questions as to whether parabens might inhibit growth suppressors more widely.

Hallmark 3: Resisting Cell Death

The destruction of irreparably damaged cells by programmed cell death (apoptosis) ensures their removal before the possibility of them progressing into cancer cells. In this way, apoptosis serves as a natural prevention for cancer, and factors that can inhibit apoptotic mechanisms have the potential to enhance risk of cancer development. Parabens have been shown to induce cell death by apoptosis in several cell types *in vitro*, including in human skin keratinocytes (Handa *et al*., 2006; Ishiwatari *et al*., 2007), in human dermal fibroblasts (Carvalho *et al*., 2012), in human hepatoma HepG2 cells (Khanal *et al*., 2012) and in rat pheochromocytoma (adrenal) PC12 cells (Egawa *et al*., 2012). However, all these studies were carried out at high doses of parabens in the 100 μM or above range, which are well above any concentrations measureable in human breast tissue (Barr *et al*., 2012). Work that is more recent has shown that at lower doses in the 10 nM to 1 μM range, exposure of human high-risk donor breast epithelial cells to methylparaben gives the opposite effect with a dose-dependent evasion of apoptosis (Goodson *et al*., 2011). Concentrations in this 10 nM (equivalent to 1.5 ng g^{-1} tissue) to 1 μM (equivalent to 152 ng g^{-1} tissue) range are within the levels measured in human breast tissue, which ranged for methylparaben from 0 to 5102.9 ng g^{-1} tissue (Barr *et al*., 2012).

Hallmark 4: Activating Invasion and Metastasis

The processes by which cancer cells progress to phenotypes with reduced adhesion, increased motility and increased invasive activity are central to enabling the spread of breast cancer cells and are a further hallmark of cancer cells. This is of special importance for breast cancer because mortality results from tumour growth at metastatic sites rather than at the primary site in the breast. Recently, long-term exposure to parabens has been shown to increase migratory and invasive properties of human breast cancer cells in culture (Khanna *et al*., 2014). Long-term exposure (20 weeks) of MCF-7 human breast cancer cells to methylparaben, *n*-propylparaben or *n*-butylparaben was found to increase migration measured using a scratch assay, time-lapse microscopy or xCELLigence technology (ACEA BioSciences, San Diego, CA, USA): invasive properties were found to increase in matrix degradation assays and migration assays through matrigel on xCELLigence (Khanna *et al*., 2014). It is interesting to note that the alterations developed over long-term (20 weeks) rather than short-term (1 week) exposure, implying that multiple events are needed over a period of weeks, but it is an environmental reality that as parabens are measured ubiquitously in breast tissue (Barr *et al*., 2012) that any human breast cancer cells would be exposed long-term *in vivo* and breast cancer has a long time course of development. Molecular mechanisms remain to be fully defined but the reduced levels of E-cadherin and β-catenin in long-term paraben-exposed MCF-7 cells (Khanna *et al*., 2014) are consistent with other studies showing a link between loss of these adhesion-related proteins and epithelial-to-mesenchymal transition, one mechanism consistently associated with metastasis (Scheel and Weinberg, 2012).

Enabling Characteristics: Genomic Instability

The ability of cells to detect and repair damage to DNA is fundamental to maintaining the accuracy of information stored in the genome for future generations of cells, and factors that can impair pathways of DNA damage detection or repair can lead to genomic instability and accumulation of mutations that may be selected for if they confer a growth advantage. Although parabens have been generally considered as non-mutagenic (Andersen, 2008), some studies do report research showing that exposure to parabens may enable damage to DNA and impede repair processes in certain specific circumstances.

Work published in 2006 showed that while methylparaben itself was without adverse effects in human keratinocytes, combination with exposure to ultraviolet B light could increase cell death, oxidative stress, nitric oxide production, lipid peroxidation and NFκB activation (Handa *et al*., 2006). Further work then reported that metabolites of methylparaben produced in the keratinocytes following ultraviolet exposure had DNA damaging activity in an *in vitro* assay measuring formation of oxidized guanine in calf thymus DNA, which is a measure of oxidative DNA damage (Okamoto *et al*., 2008). In Chinese hamster ovary (CHO-K1) cells, treatment with propylparaben or butylparaben for 1 h increased DNA fragmentation (DNA strand break) as measured using a comet assay, induced chromosome aberrations and increased sister-chromatid exchanges (Tayama *et al*., 2008). In the Vero cell line derived from green monkey kidney, exposure to propylparaben for 24 h caused cell cycle arrest at the G_0/G_1 phase of the cell cycle rather than loss of cell viability and this was associated with induction of DNA double-strand breaks and oxidative damage demonstrated using immunodetection techniques (Martin *et al*., 2010). A correlative study of human urinary exposure to parabens and markers of male reproductive health in the USA showed a link between urinary level of butylparaben and sperm DNA damage (Meeker *et al*., 2011). Beyond DNA damage, a repeated 28-day oral toxicity study of butylparaben in rats showed sperm DNA to be hypermethylated, which suggests an ability also to cause epigenetic alterations (Park *et al*., 2012).

There has been very little work carried out in breast cells specifically. Studies using immortalized non-transformed MCF10A human breast epithelial cells have shown that some parabens can increase DNA fragmentation in a comet assay and increase formation of micronuclei (Charles, 2011). The ability of parabens to enable MCF10A cells to grow in suspension (Khanna and Darbre, 2013) is also suggestive of the development of transforming characteristics in the cells. Growth of MCF10A cells in suspension culture following exposure to aluminium-based antiperspirant salts has been shown to be directly associated with DNA damage (Sappino *et al*., 2012) and similar studies would be useful to identify whether DNA damage was associated with the paraben-induced growth in suspension culture.

Overall, published data demonstrate the potential for individual parabens to cause DNA damage at high concentrations in the short term. It remains to be established whether DNA damage could also result from long-term low-dose exposure to mixtures of paraben esters in the human breast, or whether for certain short time frames, parabens (or their

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metabolites; Okamoto *et al*., 2008) might reach higher concentrations in the human breast (for example, at early times after cosmetic application to shaved skin).

Emerging Hallmarks: Reprogramming Energy Metabolism

The uncontrolled sustained proliferation of cancer cells places demands on energy generation, which result in alterations to regulation of metabolic pathways. It has long been recognized that even under aerobic conditions, cancer cells tend to rely on glycolysis with excess lactate production when normal cells would use mitochondrial pathways of the tricarboxylic acid cycle and oxidative phosphorylation (Warburg, 1956). The mammalian target of rapamycin (mTOR) is a key regulator that integrates signals from growth factors with sensory systems for nutrient, oxygen and energy levels, and upregulation of mTOR has been associated with cancer development (Strimpakos *et al*., 2009). Interestingly, methylparaben has been shown recently to increase mTOR in human breast epithelial cells, which implicates methylparaben in influencing changes to energy metabolism (Goodson *et al*., 2011).

Regulatory Status of Parabens

Over the past decade, parabens have been the subject of regulatory review for their use in both food and cosmetics. The Joint Food and Agriculture Organization and World Health Organization Expert Committee on Food Additives has recommended the withdrawal of an acceptable daily intake level for propylparaben and butylparaben on the grounds of reproductive and endocrine toxicity (JECFA, 2007). The EC Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food of the European Food Safety Authority (EFSA) were previously also unable to establish a no-observed-adverse-effect-level for propylparaben again on the grounds of endocrine toxicity (EFSA, 2004). Within the European Union, ingredients used in cosmetics are subject to recommendation rather than regulation under the European Union Cosmetics Directive (EU Cosmetics Directive 76/768/EEC). Under this directive, parabens have been recommended for use 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). However, more recent reviews have recommended reduction in the levels of *n*-propylparaben and *n*-butylparaben in cosmetic products to a combined maximum concentration of 0.19% with recommendation still pending for isopropylparaben, isobutylparaben and benzylparaben (SCCS, 2010). On 21 March 2011, Denmark notified the Commission that it had banned propylparaben, butylparaben, their isoforms and salts in cosmetics for children under the age of 3 years on the grounds of reproductive toxicity. This prompted further review by the EU (SCCS, 2013) who confirmed concern for use of parabens in leave-on products especially in the nappy area and a public consultation by the EU remains open at the current time.

As it stands, regulatory review has centred around adverse effects on male reproductive endpoints, but there is justification to review the use of parabens in leave-on products applied around the female breast based on evidence discussed in this review and especially when such leave-on products are applied after shaving, a procedure that can create nicks in the skin allowing even easier access for chemicals. Studies on absorption of aluminium chlorohydrate (used as antiperspirant in cosmetics) showed aluminium absorption of 1.81 $\mu g\ cm^{-2}$ for intact human skin but this was increased to 11.5 $\mu g\ cm^{-2}$ for stripped skin (a procedure equivalent to shaving) (Pineau *et al*., 2012) and such studies need to be conducted for parabens also.

Perspectives and Further Research Needed

This review has summarized evidence that parabens can lead in human breast epithelial cells to development of four of six of the hallmarks, one of two of the emerging hallmarks and one of two of the enabling characteristics of cancer cells as defined by Hanahan and Weinberg (2011) and can do so at concentrations that are not incompatible with levels measurable in some human breast tissues (Barr *et al*., 2012). The ability of parabens to cause sustained proliferation at lower doses if combined as mixtures of several esters is relevant to the environmental situation where exposure would not be to one ester alone and is especially poignant when using specific mixtures at concentrations as measured in a single human breast tissue sample (Charles and Darbre, 2013). Embracing the environmental reality of exposure to parabens being in the human breast over the long term has also shown parabens can influence hallmarks such as increased migration and invasion, which would have been missed if studies had been limited to the usual 1 day or 1 week of assay time. Although parabens have been defined as relatively non-irritating and no data exist on inflammation in relation to cancer, a number of studies have reported that parabens in cosmetic products can induce allergic contact dermatitis and skin inflammation in paraben-sensitive individuals (Karpuzoglu *et al*., 2013) and so further research into the enabling characteristic of tumour-promoting inflammation is justified. Current evidence suggests that individual parabens can cause DNA damage at high concentrations in the short term but further studies are needed to assess the environmentally relevant question of whether low doses of mixtures of esters could impact on the enabling characteristic of genetic instability in the longer term. To our knowledge, no data exist on whether parabens can influence angiogenesis, replicative immortality or avoidance of immune destruction and research into whether parabens can influence development of these hallmarks is needed.

Review of the implications of the presence of parabens in human breast must, however, also be considered in the context of the many hundred other environmental chemicals that have been measured as entering human breast tissue, including also other chemicals from personal care products (Darbre and Charles, 2010; Darbre and Fernandez, 2013). The ability of parabens to influence more than one hallmark of cancer cells and to act on different hallmarks at different doses suggests an already increasing complexity but this may be further magnified by considering the potential for also many other chemicals to combine through actions on different hallmarks and through additive effects enabling even lower doses of individual chemicals to act. This bigger picture explains why no single chemical has been linked consistently with breast cancer causation and probably never will be. What is now needed is further understanding of how multiple chemicals beyond just five paraben esters can combine to bring about common hallmark endpoints either through enabling efficacy for lower doses of single chemicals due to the same mechanism of action such as binding to ER or through complementary actions needed for overall realization of one hallmark or multiple

hallmarks. While it has been demonstrated that sufficient paraben was present in some human breast tissue samples to enable sustained proliferation, this was not the case for all (Charles and Darbre, 2013) and although epidemiological studies might conclude therefore that parabens play no functional role, the environmental reality is probably more complex in that different environmental exposures (even different personal care products) lead to different chemical burdens in the human breast and it is the total burden that counts. This should not lead to dismissal of any chemical as insignificant but more of an appreciation of the complexity of the action of chemical mixtures, which could be anticipated to act with non-monotonic dose–responses (Vandenberg *et al*., 2012) on endpoints that involve multiple changes such as cancer development. If regulation becomes too complex due to the plethora of chemical ingredients, then a strategy for prevention of breast cancer would seem better founded on recommendations for overall reduction in chemical exposure through reducing overall usage particularly of personal care products.

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