Concentrations of Parabens in Human Breast Tumours

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Parabens are used as preservatives in many thousands of cosmetic, food and pharmaceutical products to which the human population is exposed. Although recent reports of the oestrogenic properties of parabens have challenged current concepts of their toxicity in these consumer products, the question remains as to whether any of the parabens can accumulate intact in the body from the long-term, low-dose levels to which humans are exposed. Initial studies reported here show that parabens can be extracted from human breast tissue and detected by thin-layer chromatography. More detailed studies enabled identification and measurement of mean concentrations of individual parabens in samples of 20 human breast tumours by high-pressure liquid chromatography followed by tandem mass spectrometry. The mean concentration of parabens in these 20 human breast tumours was found to be 20.6 ± 4.2 ng g^{-1} tissue. Comparison of individual parabens showed that methylparaben was present at the highest level (with a mean value of 12.8 ± 2.2 ng g^{-1} tissue) and represents 62% of the total paraben recovered in the extractions. These studies demonstrate that parabens can be found intact in the human breast and this should open the way technically for more detailed information to be obtained on body burdens of parabens and in particular whether body burdens are different in cancer from those in normal tissues. Copyright © 2004 John Wiley & Sons, Ltd.

INTRODUCTION

The alkyl esters of *p*-hydroxybenzoic acid (parabens) are used widely as preservatives in many thousands of cosmetic, food and pharmaceutical products (Elder, 1984). These simple esters have proved to be very effective antimicrobial agents, with antimicrobial activity increasing with the length of the alkyl grouping from methyl to *n*-butyl (Murrell & Vincent, 1950), and it is the simplicity and effectiveness of these compounds that have resulted in their widespread use. As such, the human population is exposed to parabens from a wide variety of sources on a daily basis. Parabens are permitted as preservatives in food up to 0.1% and the average daily intake of parabens from food by adult humans was estimated in 1984 to be 4–6 mg kg^{-1} (Elder, 1984). In cosmetics, parabens are permitted in concentrations of up to 1% (Elder, 1984). In 1984, it was estimated that parabens were used in 13 200 different cosmetic formulations (Elder, 1984) and a more recent

survey of 215 cosmetic products found that parabens were used in 99% of leave-on products and in 77% of rinse-off cosmetics (Rastogi *et al.*, 1995).

Animal studies have shown that parabens are rapidly absorbed, metabolized and excreted. Parabens are quickly absorbed from the gastrointestinal tract and from blood, hydrolysed to *p*-hydroxybenzoic acid, conjugated and the conjugate excreted in the urine (Jones *et al.*, 1956; Heim *et al.*, 1957; Tsukamoto & Terada, 1960, 1962, 1964; Derache & Gourdon, 1963; Phillips *et al.*, 1978; Kiwada *et al.*, 1979). Parabens also can be absorbed rapidly through intact skin (Whitworth & Jun, 1973; Fischmeister *et al.*, 1975; Komatsu & Suzuki, 1979) and this can be influenced by the presence of penetration enhancers found in cosmetic preparations (Kitagawa *et al.*, 1997). However, the presence of carboxylesterases in skin and subcutaneous fatty tissues results in varying hydrolysis to *p*-hydroxybenzoic acid (Lobemeier *et al.*, 1996) and this can also influence absorption (Bando *et al.*, 1997). However, the question remains as to whether any of the parabens can enter the body intact from the long-term, low-dose levels to which humans are exposed. Parabens have a high oil/water partition coefficient and water solubility decreases with increase in ester chain length (Elder, 1984). Therefore, if any parabens do enter the human body intact, they may be able to accumulate in fatty components of body tissues in a similar manner to that of other lipophilic

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pollutants that are known to bioaccumulate (Dobson et al., 1989; Dobson, 1993; Sonawane, 1995; Hardell et al., 1996; Guttes et al., 1998; Stellman et al., 1998, 2000; Darbre, 1998).

Most studies have indicated that parabens are not mutagenic (Elder, 1984), but there are reports that they can cause chromosomal aberrations (Ishidate et al., 1978), particularly in the co-presence of polychlorinated biphenyls (Matsuoka et al., 1979), and subcutaneous administration of methylparaben has been reported to cause mammary adenocarcinomas in rats (Mason et al., 1971). At a cellular level, parabens have been shown capable of disrupting cellular function through inhibiting secretion of lysosomal enzymes (Bairati et al., 1994) and causing mitochondrial dysfunction (Nakagawa & Maldeus, 1998). However, the recent discovery that parabens possess oestrogenic activity has challenged the concepts of their toxicity in new ways. Because parabens can bind to oestrogen receptors, they may be able to mediate unwanted effects at much lower concentrations and more specifically than through non-receptor mediated mechanisms.

The oestrogenic activity of parabens was first reported in 1998 (Routledge et al., 1998). Since then, parabens have been shown to bind to oestrogen receptors from different sources, including rodent uterus (Routledge et al., 1998; Blair et al., 2000; Fang et al., 2001) and MCF7 human breast cancer cells (Byford et al., 2002; Darbre et al., 2002, 2003). They have been shown to regulate oestrogen-responsive reporter gene expression in yeast cells (Routledge et al., 1998; Jin-Sung et al., 2000; Nishihara et al., 2000) and in human breast cancer cells (Byford et al., 2002; Darbre et al., 2002, 2003), and expression of the endogenous oestrogen-regulated genes pS2 (Byford et al., 2002) and progesterone receptor (Okubo et al., 2001) in breast cancer cells. Parabens can increase the growth of MCF7 human breast cancer cells (Okubo et al., 2001; Byford et al., 2002; Darbre et al., 2002, 2003), which can be blocked with the antioestrogen ICI 182 780 (faslodex) (Byford et al., 2002; Darbre et al., 2002, 2003), demonstrating the growth effects to be oestrogen-receptor-mediated. Their oestrogenic activity has been demonstrated also in animal models in vivo in fish (Pedersen et al., 2000) and in increasing uterine weight in immature rats (Routledge et al., 1998) and immature mice (Darbre et al., 2002, 2003). In line with other environmental oestrogens, butylparaben has been shown also to be able to alter reproductive function in male rats, including reduction in sperm counts (Oishi, 2001). In general, the oestrogenic and antimicrobial activities of the parabens increase with the length and branching of the alkyl ester (Darbre et al., 2002, 2003).

Because oestrogen is known to influence the incidence of breast cancer (Lipworth, 1995) and ablation of oestrogen action remains the preferred treatment for hormone-sensitive breast tumours (Miller, 1996), the presence of oestrogenic chemicals in the breast area could potentially influence both the incidence and treatment of breast cancer. Parabens are used as preservatives in a range of cosmetics applied to the underarm and breast area and it has been suggested that regular application of such oestrogenic chemicals could influence breast cancer development (Darbre, 2001, 2003; Harvey, 2003). However, the outstanding question remains as to whether parabens can enter and accumulate in the human breast. Previous studies have identified other environmental oestrogenic chemicals that can accumulate in fatty tissue of the breast (Dobson, 1993;

Hardell et al., 1996; Guttes et al., 1998; Stellman et al., 1998, 2000). This study has aimed to investigate whether parabens also can be detected in human breast tissue, using available breast tumour material. Initial experiments enabled the extraction of total parabens from human breast tissue to be visualized by thin-layer chromatography. More detailed studies enabled identification and measurement of individual parabens in human breast tumour samples by high-pressure liquid chromatography (HPLC) followed by tandem mass spectrometry (MS/MS).

MATERIALS AND METHODS

Human breast tumour material

Samples of human breast tumour material were collected at the Edinburgh Breast Unit and stored in liquid nitrogen.

Chemical standards

Methylparaben, ethylparaben, n-propylparaben, n-butylparaben and benzylparaben were purchased from Sigma (Poole, UK). Isobutylparaben was a gift from Nipa Laboratories (Mid-Glamorgan, UK). All compounds were made as stock solutions of 0.1 M in ethanol.

Extraction of parabens from human breast material and analysis by thin-layer chromatography

All glassware was pre-washed in 0.1 M NaOH and extractions were performed using sterile polycarbonate tubes (Falcon). Samples of human breast tissue (1 g) were chopped finely with a sterile razor and homogenized in 5 ml of hexane using a hand-homogenizer. Samples were left in a sealed polycarbonate tube with mixing for 1 h and then spun at 1500 rpm in a bench centrifuge at room temperature for 2 min. The supernatant was placed in a sterile polycarbonate tube, 5 ml of 0.1 M potassium bicarbonate was added and the tube was inverted 40 times by hand. The mixture was spun at 1500 rpm at room temperature for 2 min to separate the phases. The upper yellow hexane layer containing phenolic compounds was placed in a new sterile polycarbonate tube, 5 ml of 0.1 M potassium carbonate was added and again the tube was inverted 40 times by hand. The mixture was spun at 1500 rpm at room temperature for 2 min to separate the phases. The lower aqueous layer containing the phenols as potassium salts was taken into a new sterile polycarbonate tube and acidified by the addition of 300 µl of concentrated hydrochloric acid to give a pH in the 1–3 range (checked with pH paper). The free phenolic compounds released on acidification were extracted into 5 ml of diethyl ether by inverting the tube by hand 40 times (Pope et al., 1990). The mixture was spun at 1500 rpm at room temperature for 2 min to separate the phases. The upper ether layer was removed and evaporated to dryness under nitrogen overnight in a fume hood.

The extract was taken up in 50 µl of ethanol and aliquots were run against paraben standards (50–400 ng per track) on thin-layer chromatography plates (DC-Alufolien Kieselgel 60 F254, Merck; ca. 6 cm wide × 8 cm high) using a solvent of 5% (v/v) ethanol–95% (v/v) chloroform. Parabens were visualized under ultraviolet light. For quantitation, the image under ultraviolet light was captured digitally

and relative levels of bands were analysed by image analysis using the software packages Transform 3.4 (Fortner) and Origin 6.0.

Extraction of parabens from human breast tumour material and analysis by HPLCMS/MS

Samples of human breast tumour material (0.25 g) were chopped finely with a sterile razor and homogenized in a mixture of 6.25 ml of ethanol and 6.25 ml of acetone. This mixture was left with periodic shaking overnight in a sealed glass Corex tube. The next day, the mixture was spun at 2500 rpm for 10 min on a bench centrifuge at room temperature. The supernatant was removed to a clean Corex tube. The pellet was re-extracted with a further 1.5 ml of ethanol and 1.5 ml of acetone, spun and the two supernatants pooled. The total supernatant was dried under nitrogen at room temperature. To the residue was added 6 ml of 70% (v/v) aqueous methanol; the mixture was vortexed and then incubated overnight at −20 °C. The next day, the mixture was spun at 3200 rpm for 20 min at 4 °C and the supernatant was removed to a clean Corex tube. The pellet was re-extracted with a further 1 ml of 70% (v/v) aqueous methanol by vortexing and spun again at 3200 rpm for 20 min at 4 °C. The two supernatants were pooled and dried under nitrogen for analysis by HPLCMS/MS.

The extracts were dissolved in HPLC mobile phase (0.25 ml) and the paraben concentration determined by HPLCMS/MS. Samples (20 µl) of the final extracts were chromatographed on a Hypersil Elite C18 column (150 × 2.1 mm; 5 µm) at a flow rate of 0.3 ml min^{-1} and eluted with a linear binary gradient of 15 mM ammonium acetate pH 4.5 (A) and acetonitrile (B) ($t = 0$ min A 70%, $t = 15$ min A 40%, $t = 16$ min A 70%, $t = 25$ min next injection). The HPLC retention times for the paraben standards are provided in Table 1. The parabens were detected with a Sciex API 2000 triple quadrupole mass spectrometer equipped with a heated nebulizer probe operated in the negative ion mode. Optimal setting of the instrument for detection by mass reaction monitoring (MRM) was established empirically by infusion of paraben standards (1 µg ml^{-1}). The mass transitions selected for MRM detection utilized the fragmentation of the deprotonated molecular ion and are listed in Table 1. Chromatographic peaks corresponding to individual parabens were detected automatically and the mass of analyte calculated after interpolation from calibration curves prepared over the working range 1–300 ng ml^{-1} using the Analyst™ (PE Biosystems) software package.

Extractions were performed in groups such that each group of two to five tumour extractions had one blank

Table 1—Paraben standards: HPLC retention times and mass transition for MRM detection

Analyte	HPLC retention time (min)	Mass transition (Q1–Q3; m/z) for MRM detection
Methylparaben	4.6	151.1–92.1
Ethylparaben	7.3	165.1–92.1
n-Propylparaben	10.6	179.1–92.1
Isobutylparaben	13.4	193.1–92.1
n-Butylparaben	13.7	193.1–92.1
Benzylparaben	14.0	227.3–92.1

extraction carried out alongside, with all procedures identical except for the omission of tumour material. However, analysis by HPLCMS/MS was carried out for all samples on the same day sequentially. Final paraben concentrations were calculated by subtraction of the values obtained from the corresponding blank extraction. Because the blank values showed variation, statistical analysis was performed using the paired t-test method (Snedecor & Cochran, 1980).

RESULTS

Extraction of parabens from breast tissue and detection by thin-layer chromatography

In initial exploratory experiments it was possible to detect parabens in human breast tissue using the extraction procedures described in the Materials and Methods section, followed by thin-layer chromatography against paraben standards. Aliquots (10–400 ng) of methylparaben, ethylparaben, n-propylparaben, n-butylparaben and isobutylparaben were run on thin-layer plates and could be detected under ultraviolet light. Under these conditions all the paraben standards ran to the same position, which was, on average, 0.47 ± 0.03 of the distance to the solvent front. Extracts of human breast tissue contained compounds visible under ultraviolet light at the same relative position as the paraben standards. From rough comparison by eye of the relative levels of paraben standards, it was estimated over six separate extractions that the samples contained in the region of 10–50 ng paraben per g breast tissue. Figure 1 shows the results of one experiment in which three aliquots (97, 194 and 388 ng) of n-butylparaben standards were run on thin-layer plates alongside the extract of 1 g of breast tissue. The relative intensities of the resulting bands under ultraviolet light were subjected to image analysis and plotted as a standard curve shown in Fig. 1. The relative intensity of the paraben band extracted from 1 g of tissue was 11 730, which corresponded to 47.1 ng paraben g^{-1} tissue.

It was on the basis of these preliminary results that we then proceeded to more detailed identification of individual parabens by HPLCMS/MS

Extraction of parabens from human breast tumours and analysis by HPLCMS/MS
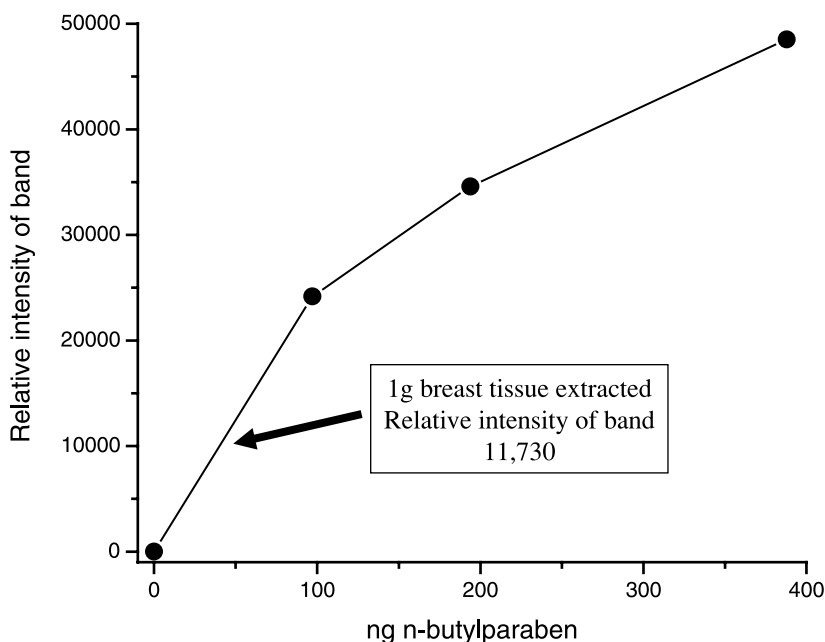
Retention times and mass transition for MRM detection for the six paraben standards are shown in Table 1.

Parabens were extracted from a sample of each of 20 human breast tumours and extracts were analysed by HPLCMS/MS against paraben standards as described in the Materials and Methods section. Chromatographic peaks due to methylparaben, ethylparaben, n-propylparaben, n-butylparaben and isobutylparaben were seen in breast tumour extracts and were well resolved from one another. No peaks due to benzylparaben at its retention time of 14.0 min were seen in any of the tumour extracts. A typical chromatogram is shown in Fig. 2.

At a practical level, extractions were performed in small groups such that each group contained between two and five tumour samples together with one blank extraction. The blank extraction was performed with all procedures identical, except for the omission of tumour

Figure 1. Detection of parabens from human breast tissue by thin-layer chromatography. Three aliquots of *n*-butylparaben (97, 194 and 388 ng) were run as standards on thin-layer plates alongside the extract of 1 g of breast tissue, and the relative intensities of the resulting bands under ultraviolet light were subjected to image analysis. The relative intensities of the bands for the three aliquots of *n*-butylparaben were plotted as a standard curve as shown. The relative intensity of the paraben band extracted from 1 g of tissue was 11 730, which calculated to an equivalent of 47.1 ng of paraben.

material. The concentrations of parabens in the 20 tumours as measured by HPLCMS/MS were corrected by subtraction of the corresponding blank value. Results are shown in Table 2. Because the blank values showed variation, the statistical significance of the mean corrected concentrations of each paraben in the 20 tumour extracts was tested by the paired *t*-test method, thus enabling the confidence limits of these mean values to be calculated (Table 3).

The reasons for the blank values for parabens, and their variation, are not clear. The MS data indicated that the blank values were genuinely parabens and not other contaminating compounds. The blank values did not come from the HPLCMS/MS procedure because blanks through the equipment were entirely negative. The blank values came from the extraction procedure itself. In a series of 30 blanks carried out on individual parts of the extraction procedure, it was not possible to identify any one specific reagent or procedure contributing to the blank value. However, when blank values were subtracted from the corresponding tumour extract values, 18/20 tumour extractions showed values of total paraben above the blank values. Values for total paraben present in the 20 tumour samples were 0–54.5 ng g^{-1} tissue, with an overall mean value of 20.6 ng g^{-1}. Methylparaben was present at the highest level, with an average value of 12.8 ± 2.2 ng g^{-1} tissue. This represented 62% of the total paraben recovered in the extraction. Benzylparaben was not detected in any tumour extract.

Estimates of recovery of parabens from the extraction procedure were made by spiking samples with benzylparaben, because this was the only paraben not detected in any blank or tumour extract. Analysis by HPLCMS/MS of three extraction blank samples, each spiked with 200 ng of benzylparaben, gave an average recovery of this paraben of 48.5% ± 4.8%.

DISCUSSION

Mean concentrations of each of six parabens in extracts of 20 human breast tumours (in the range 0–12.8 ng g^{-1} tissue; Table 3) have been measured with acceptable confidence. The reasons for the analytical blank values for parabens in these studies have not been identified definitively but probably relate to the ubiquitous use of parabens as preservatives even in laboratory detergents and personal care products of the operators. Analogous problems have been encountered with the measurement of phthalate esters because of their common use as plasticizers and their ubiquitous dispersal as impurities in solvents, water, glassware and many items of clinical and analytical laboratory equipment (Lopez-Aviva *et al.*, 1990; Leung & Giang, 1993; Colon *et al.*, 2000). More recent work in these laboratories (unpublished) has shown that immersion of all glassware in 1.0 M aqueous sodium hydroxide, followed by copious rinsing with double-distilled water, prior to use of this glassware in tissue extraction greatly reduces the blank values of paraben concentrations as measured by HPLCMS/MS. This addition to the analytical procedure is therefore recommended for use in further studies on paraben concentrations in tissues.

The total mean paraben level was found to be of the order of 20 ng g^{-1} tissue. This adds parabens to the list of environmental oestrogenic chemicals that can be found to accumulate in the human breast and already includes polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) (Dobson, 1993, Hardell *et al.*, 1996; Guttes *et al.*, 1998; Stellman *et al.*, 1998, 2000). Comparisons between the relative levels of parabens and other pollutants are not easy because several factors have to be
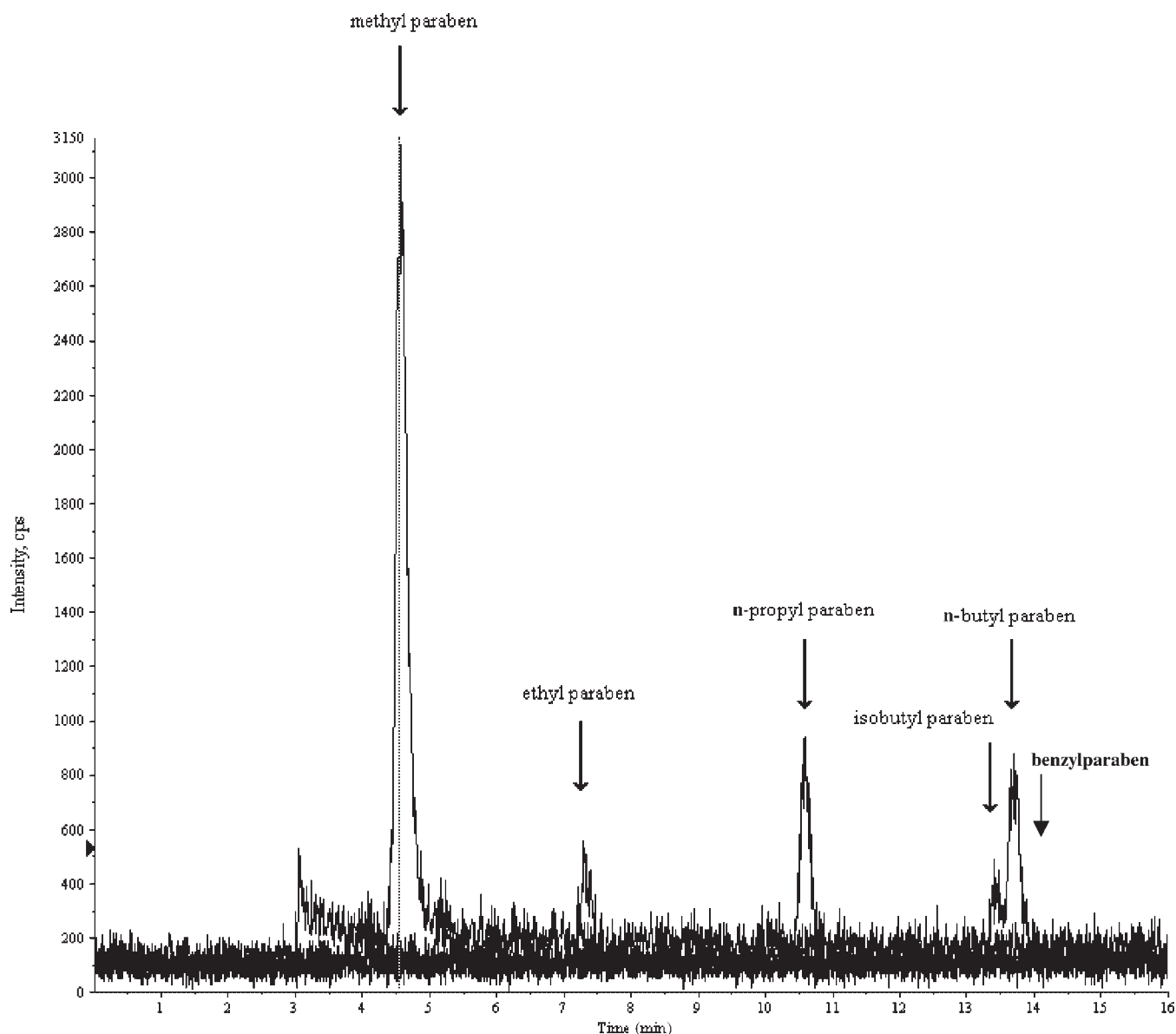
Figure 2. The HPLCMS/MS chromatograms for methylparaben, ethylparaben, *n*-propylparaben, isobutylparaben and *n*-butylparaben in a human breast tumour extract. Tumour tissue was extracted as described in the text, chromatographed on a Hypersil Elite HPLC column and detected by tandem mass spectrometry in the mass reaction monitoring mode. The annotated arrows indicate the identity of the peaks evident in the chromatograms. Benzylparaben was not seen.

taken into account, including the source of tissue used and the number of isomers or congeners. Most studies of bioaccumulation of pollutant chemicals are carried out by using serum or urine and studies using breast adipose tissue are few. Furthermore, for parabens there are only six commonly used forms whereas for PCBs there are 209 congeners. Studies of breast adipose tissue from women in Long Island, New York, without breast cancer showed a mean body burden for 14 PCB congeners of 267 ng g^{-1} and for seven OCPs of 707.5 ng g^{-1} (Stellman *et al.*, 1998). However, Table 4 shows that levels in breast tissue of individual pesticide residues and PCB congeners vary substantially. Although knowledge of total body burdens of these compounds is far from complete, the accumulation of parabens in breast tissue does fall within the broad range of these other compounds.

In the present study, paraben concentrations measured in tumours were unequivocally of the esters themselves.

This demonstrates that at least a proportion of the parabens present in cosmetic, food and pharmaceutical products can be absorbed and retained in human body tissues without hydrolysis by tissue esterases to the common metabolite *p*-hydroxybenzoic acid. These results complement earlier studies in which there was evidence that the oestrogenic properties of these parabens in culture of human breast cancer cells were also due to the esters themselves and not to a common metabolite (Byford *et al.*, 2002; Darbre *et al.*, 2002, 2003). However, these studies cannot identify either the source of the parabens or whether they entered the human body by an oral or by a topical route. Nor can they identify whether the parabens entered the human breast by a systemic route or through non-systemic mechanisms involving simply local absorption and diffusion from chemical overload of topical preparations applied to the breast area. Recent evaluation of parabens in uterotrophic assays has shown them to give oestrogenic responses in immature

Table 2—The HPLCMS/MS analysis of parabens in 20 human breast tumours[a]

Tumour extract	1	2 as 1	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean	SEM
Benzylparaben	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isobutylparaben	5.2	0.1	5.2	3.3	2.8	3.5	2.5	1.9	4.9	2.2	1.7	3.9	2.2	2.3	1.2	2.0	1.7	0.0	1.0	0.0	2.4	0.4
n-Butylparaben	15.3	7.2	29.5	22.4	14.3	15.5	9.8	6.7	10.3	7.4	2.4	14.7	7.1	6.7	7.4	7.2	5.3	3.1	5.7	3.2	10.1	1.5
n-Propylparaben	6.5	7.9	18.6	18.4	10.9	17.9	10.0	10.5	11.6	6.9	9.1	16.5	10.2	17.2	10.1	15.4	4.2	5.3	5.5	5.6	10.9	1.1
Ethylparaben	7.2	3.0	9.6	6.3	4.6	2.3	1.9	1.8	7.0	3.2	1.8	6.4	2.1	4.5	1.1	2.2	1.8	0.7	2.0	2.1	3.6	0.6
Methylparaben	34.2	20.6	53.0	49.9	34.4	37.6	27.3	19.6	35.7	16.0	17.2	36.4	21.6	39.6	18.5	28.8	36.7	8.2	12.0	10.7	27.9	2.8
Total paraben	68.3	38.8	115.9	100.3	67.0	76.9	51.4	40.4	69.5	35.7	32.1	77.9	43.1	70.3	38.3	55.6	49.7	17.3	26.2	21.6	54.8	5.8

Blank value	1	2 as 1	3	4 as 3	5 as 3	6	7 as 6	8 as 6	9 as 6	10	11 as 10	12 as 10	13 as 10	14 as 10	15	16 as 15	17 as 15	18	19 as 18	20 as 18	Mean	SEM
Benzylparaben	0.0		0.0			0.0				0.0					0.0			0.0			0.0	0.0
Isobutylparaben	0.0		0.0			2.2				2.1					2.3			1.1			1.3	0.4
n-Butylparaben	6.5		18.0			9.3				4.0					8.2			2.2			8.0	2.3
n-Propylparaben	2.0		13.4			12.0				6.8					8.4			4.8			7.9	1.8
Ethylparaben	1.9		2.2			1.9				1.9					0.0			1.6			1.6	0.3
Methylparaben	10.1		27.8			20.5				10.3					11.6			9.9			15.0	3.0
Total paraben	20.5		61.4			45.9				25.0					30.6			19.6			33.8	6.8

Tumour less blank	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean	SEM
Benzylparaben	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Isobutylparaben	5.2	0.1	5.2	3.3	2.8	1.3	0.3	-0.3	2.7	0.2	-0.4	1.8	0.1	0.2	-1.1	-0.3	-0.6	-1.1	-0.1	-1.1	0.9	0.4
n-Butylparaben	8.8	0.7	11.5	4.4	-3.7	6.2	0.5	-2.6	1.0	3.4	-1.6	10.7	3.1	2.7	-0.8	-1.0	-2.9	1.0	3.5	1.0	2.3	1.0
n-Propylparaben	4.5	5.9	5.2	5.0	-2.5	5.9	-2.0	-1.5	-0.4	0.2	2.3	9.7	3.4	10.4	1.7	7.0	-4.3	0.5	0.7	0.8	2.6	0.9
Ethylparaben	5.3	1.2	7.4	4.1	2.4	0.5	0.0	-0.1	5.1	1.3	-0.2	4.5	0.2	2.6	1.1	2.1	1.8	-0.9	0.4	0.5	2.0	0.5
Methylparaben	24.1	10.5	25.2	22.1	6.6	17.1	6.8	-0.9	15.2	5.7	6.9	26.1	11.3	29.3	6.9	17.2	25.1	-1.8	2.1	0.8	12.8	2.2
Total paraben	47.9	18.4	54.5	38.9	5.6	31.0	5.5	-5.5	23.7	10.7	7.1	52.8	18.1	45.3	7.7	25.0	19.1	-2.3	6.6	2.0	20.6	4.2

[a] Paraben extractions were performed in small groups such that each group contained between two and five tumour samples together with one corresponding blank extraction. The blank extraction was performed with all procedures identical except for the omission of tumour material. Results are shown in ng g^{-1} tumour for the 20 extractions and for the corresponding blank values. The concentrations of parabens in the 20 tumours were then each corrected by subtraction of the corresponding blank value.

Table 3—Confidence limits of mean concentrations (ng g^{-1}) of parabens in the 20 human breast tumours of Table 2

Tumour minus blank	Mean	Confidence limit
Benzylparaben	0.0	0.0–0.0 (95%)
Isobutylparaben	0.9	0.1–1.7 (90%)
n-Butylparaben	2.3	0.3–4.3 (95%)
n-Propylparaben	2.6	0.7–4.5 (95%)
Ethylparaben	2.0	1.0–3.0 (95%)
Methylparaben	12.8	8.2–17.4 (95%)
Total paraben	20.6	11.8–29.4 (95%)

rodent uterus only when administered subcutaneously or topically but not orally (Routledge et al., 1998; Hossaini et al., 2000; Darbre et al., 2002, 2003), which suggests that skin penetration may be an important route for entry to the body.

A major issue in studies of accumulation of environmental pollutants in body tissues is whether the levels reached could be sufficiently high to exert any biological action. In four of the 20 tumours, total paraben concentration was more than twice the average level and, allowing for a 50% recovery of parabens through the analytical procedure, the corrected average level of parabens was ca. 100 ng g^{-1} tissue. This concentration may be compared with the level (ca. 150 ng ml^{-1}; 10^{-6} M) in culture medium at which n-propylparaben, n-butylparaben and isobutylparaben stimulated growth of oestrogen-dependent MCF7 human breast cancer cells (Okubo et al., 2001; Byford et al., 2002; Darbre et al., 2002, 2003). It is therefore not inconceivable that the levels of parabens measured in this study could exert oestrogenic effects on

epithelial cells in the human breast. Although in rodent uterotrophic assays the levels of parabens were administered at a higher range of 0.1–10 mg g^{-1} body weight (Routledge et al., 1998, Darbre et al., 2002, 2003), these studies did not incorporate any measurements of paraben levels reached in the uterus at the time of response, which prevents assessment of the concentrations needed for physiological response.

It is interesting that the paraben detected in greatest amounts was methylparaben. This may reflect the more widespread use of methylparaben in consumer products (Rastogi et al., 1995). Alternatively, it may reflect the greater ability of methylparaben to be absorbed into body tissues and to resist hydrolysis by esterases of human skin and subcutaneous fat tissue (Lobemeier et al., 1996). By contrast, benzylparaben was not found in any of the 20 breast tumours and this may similarly be attributed to its less frequent use in consumer products.

These measurements of paraben concentrations in breast tumours open the way technically to more detailed determinations of paraben levels in human body tissues. This study used 20 breast tumour samples because of the availability of the material. However, it will now be important to measure levels in corresponding normal tissue to determine whether there is any difference between normal and cancer tissues. Larger studies also are needed to give more representative values for body burdens in different tissues and across the human population. A main problem with human breast tumour samples is the varied infiltration of the tumour with fatty tissue and blood vessels and it will be important in future work therefore to have more precise histological information on the tumours in order especially to be able to relate results to fatty versus non-fatty tissue. It would be informative to ascertain whether there are any gradients in the accumulation of

Table 4—Summary of mean concentrations (ng g^{-1}) of individual pesticide residues and PCB congeners in human breast adipose tissue from control and breast cancer patients from two published studies

Pesticide or PCB	Control	Breast cancer	Control	Breast cancer
HCB	206	343	16	18
βHCH	72	84	16	20
Oxychlordane			39	46
trans-Nonachlor			40	51
p,p′-DDE	450	838	374	419
o,p′-DDD			13	16
p,p′-DDT	24	30	12	12
PCB 74			27	30
PCB 99			14	19
PCB 118	58	85	24	30
PCB 138	176	241	22	29
PCB 146			7	9
PCB 153	437	664	63	76
PCB 156	61	64	9	11
PCB 167			1	2
PCB 170	229	259	11	14
PCB 172			2	2
PCB 178			3	4
PCB 180	258	400	34	42
PCB 183			4	6
PCB 187			13	16
Reference	Guttes et al. (1998)	Guttes et al. (1998)	Stellman et al. (2000)	Stellman et al. (2000)
Location	Hesse, Germany	Hesse, Germany	New York, USA	New York, USA
Number of samples	n = 20	n = 45	n = 323	n = 232

parabens across the human breast from axilla to sternum in case the topical application of cosmetic at one place influences the levels of parabens detectable. It will also be important to know whether there is any difference between levels detectable in breast tumours compared with adjacent non-tumour material in order to determine whether higher levels of paraben accumulation might be present in the tumours. Such information, taken together with that of concentrations in tissues of endogenous steroid hormones and other xenoestrogens, should enable assess-

ment to be made of the impact of these weakly oestrogenic parabens on human health, and whether paraben accumulation from currently permitted levels in cosmetics, foods and pharmaceuticals remains acceptable.

Acknowledgements

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