A SINGLE CENTRE PILOT STUDY TO OBTAIN DATA ON THE CONCENTRATIONS OF PARABENS AND ALUMINIUM IN HEALTHY HUMAN BREAST TISSUE

A thesis submitted to the University of Manchester for the degree of MPhil
in the Faculty of Medical and Human Sciences

2011

George Metaxas

SCHOOL OF MEDICINE

CONTENTS

Final word count: 26,875

List of Tables figures and graphs

ABSTRACT

George Metaxas, MPhil Medicine, The University of Manchester , 27/09/2010

A single centre pilot study to obtain data on the concentration of parabens and aluminium in healthy human breast tissue.

Background: The rising incidence of breast cancer suggests lifestyle and environmental causes. During the last decade many chemicals, among which parabens and aluminium, have been shown to express estrogenic properties in in-vitro and animal studies. A causative relationship theory between chemicals and breast cancer has been proposed. Another theory has linked the use of underarm cosmetics with the increased prevalence of breast cancer in the upper outer quadrant.

Purpose: We conducted this study to investigate the presence and distribution of five commonly used esters of para-hydroxybenzoic acid and aluminium, in the female breast and their possible relationship with breast cancer development. We attempted to shed light into the link hypothesis between underarm cosmetics and breast cancer.

Materials and methods: Fourty breast cancer patiens who would undergo mastectomy, completed the study questionnaire with information about underarm cosmetics use and other lifestyle and epidemiological parameters. Histological information was retreived from their medical records. We obtained tissue samples from four different regions across every mastectomy specimen, from the axilla to the sternum and analysed them for parabens and aluminium.

Results: Parabens and aluminium were found intact in almost all samples. The distribution in the four regions across the breast from the axilla to the sternum was homogenous and independent of the patient's age, tumor location and hormone receptor status. There were no significant differences in the concentrations between women who had used underarm cosmetics and those who had not, or those who had used them in the past but have now stopped using them. There was no correlation between the length of underarm cosmetic use and the the concentration of parabens and aluminium across the breast.

Discussion: This is the first study to demonstrate the presence of five commonly used p-hydroxybenzoic acid esters and aluminium in the healthy female breast tissue. The measured concentrations are low and the pattern of distribution is homogenous but universal in the studied population, thus allowing the expression of their weak estrogenic properties for a lifelong period. We did not find a causative relationship with breast cancer. However, our findings can be added to those from other in-vitro, animal and human studies on the presence, properties, and combined effect of several widely used chemicals with hormonal properties. In the light of this relatively recent evidence there is an urge for developing biomonitoring techniques, determining the total chemical burden, and investigating the mixture effects in humans. Prevention policies should support the reduction of exposure to artificial chemicals, especially to those categorised as endocrine disrupters and for vulnerable groups.

Conclusions: Parabens and aluminium can be found intact in the healthy female breast. Underarm deodorants or antiperspirants do not constitute the main source of parabens and aluminium for the female breast and cannot be considered an independent risk factor for breast cancer. Further research is needed to identify the contribution of these chemicals to the total body burden and the possible effect of mixtures in the pathogenesis of breast cancer.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning;

Copyright statement

Acknowledgements

I would like to thank my supervisor Lester Barr who supported and guided me during this project, in parallel with overseeing my surgical training. I would also like to thank Phillipa Darbre and Julie Morris for their invaluable contribution to the completion of the study. I wish to express my gratitude to the Genesis Appeal that funded this project and generously covered my fees at the University of Manchester.

I dedicate this to my family. My wife Sofia, my daughter Vivian, my mother Melpomeni and my sister Kyriaki for their endless patience and support. To the memory of my father Stelios.

.... to all the patients, who despite the tough times they are going through, choose to participate in clinical studies, with the sincere intention to contribute in the fight against cancer.

1. THE EPIDEMIOLOGY OF BREAST CANCER

Breast cancer is the most common malignancy in women accounting for 32 per cent of all cancers in the U.K. It is the second most common cause of cancer death in women, after lung cancer. It is ninth among ten leading causes of death in countries with high income, being responsible for almost 0.15 million deaths per year (1.8%). Incidence rates of breast cancer are increasing worldwide and remain highest in developed countries (Fig1). In the U.K. the incidence rates have increased by 84 per cent between 1971 and 2005. (Source: Office for National Statistics)

1.1. Geographic variations in the incidence of breast cancer

More than 1.1 million cases of breast cancer are now diagnosed across the world each year, compared with about 500,000 cases in 1975. Half of the world's breast cancer cases occur in North America and Europe. The highest incidence rates are observed in white females in the U.S. In 2008, 182,500 women in the United States were diagnosed with invasive breast cancer, and 40,480 women died of the disease as it remains the demographic's second leading cause of cancer mortality[1]. Approximately one woman in ten in Europe will develop breast cancer at some point in her life. The lowest breast cancer rates are reported in Asian regions. In general, incidence is much lower in developing countries. Incidence rates in Japan are also low, which contradicts the role of the high economic level of a country being associated with high risk of breast cancer. The range of variation in incidence worldwide reaches a tenfold difference[2], and a part explanation for that, arises from the differences in the distribution of well known risk factors in the population.

Though breast cancer incidence has been increasing, screening practices for early diagnosis have contributed to keeping mortality at lower levels. The observed geographic differences of invasive and in situ breast cancer incidence underlines the need for targeted additional resources for promoting breast cancer screening to specific locations[3]. Previous studies have also demonstrated geographical variations in the primary treatment for early stage beast cancer[4], as well as in survival rates[5]. It has been suggested that geographical variation in endocrine function and differences in fertility

between populations may correlate with the variations in the incidence and the natural course of the disease[6].

Regional patterns may also reflect an aggregate of diverse factors including, for example, varying presence of hazards in the environment, demographics and lifestyle of a mobile population, subgroups of susceptible individuals, and changes and advances in medical practice and health care management[7, 8, 9]. Correlation studies have shown, that dietary factors can also explain part of the international variation, and most suspicion has fallen on dietary fat[10, 11]. Since the 1960s numerous epidemiological studies have been widely used to determine the contributing factors for the geographical variations in breast cancer rates, by focusing on the implication of dietary, reproductive, hormonal, cultural and environmental factors in the etiology of the disease[12, 13, 14, 15]. The use of age-standardized rate (ASRs)[1] allows comparisons of different regions or populations at different points in time as though there were no differences in the underlying age structures.

Country	Lifetime risk
UK, USA, Canada, Switzerlan, Argentina, Uruguay	1 in 10
Australia, New Zealand, Italy, Denmark, Sweden, Iceland Finland, Spain, France	1 in 11
Eastern Europe, Philippines, Saudi Arabia, Singapore	1 in 17
Northern Africa, South Africa, Brazil	1 in 25
Japan, Ethiopia, Angola, Columbia, Venezuela	1 in 35
China, India, East Africa	1 in 50

Table 1: Inernational differences in breast cancer rates – lifetime risk[15].

1.1.1. Incidence and Mortality rates of breast cancer[16]

In many nations, states, and regions cancer incidence is monitored by population-based registries, data from which are periodically summarized. Unfortunately, cancer

[1] Age-standardized rate is a summary measure of the cancer rate that a population would have if it had a standard age-structure. This statistical technique (age-standardization) is used to compensate for variations in age structures of different populations, or of changes in age structure across time.

incidence data are available for less of the world's population than are mortality data. Mortality trends can be affected by incorrect certification of death or coding of the underlying cause even in the case of cancer (Women who die of breast cancer in a given year will have been diagnosed and treated up to 10 years or more, earlier). Cancer mortality trends reflect earlier trends in incidence and survival, and cannot be interpreted sensibly without them. These indicators are not perfect or adequate on their own[17].

Figures 1 and 2 illustrate the wide variation of female breast cancer incidence and mortality rates worldwide (year 2002) and within EU countries (year 2008). The rate of mortality for the Chinese population (6.2 / 100,000) was less than a quarter that of the highest rate, reported for Denmark (26.4 / 100,000). Although the age-standardized rate for the United States (20.7 / 100,000) is based on the largest number of cases, it is 78% that of the rate in Denmark but nearly three times that of Japan (7.1 / 100,000).

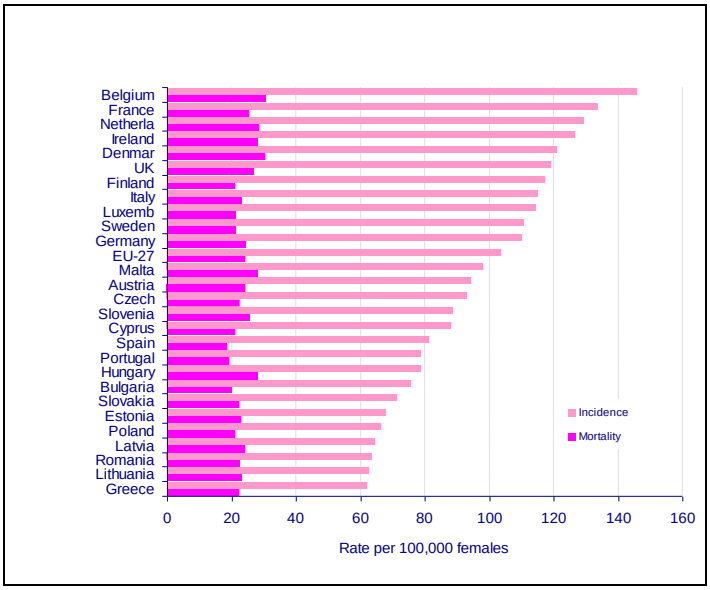


Fig. 1: Age standardised incidence and mortality rates, female beast cancer in EU countries, 2008 estimates (source: Cancer Research UK)

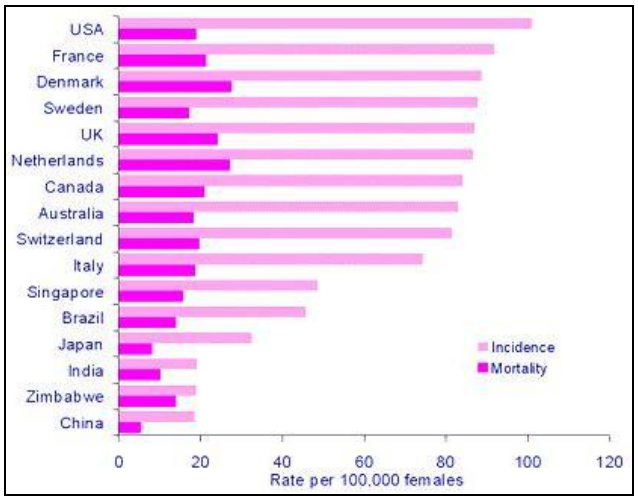


Fig. 2: Age stadardised incidence and mortality rates, female breast cancer in selected countries 2002 estimates (source: Cancer Research UK)

Kawamura et al.[18] demonstrated a decrease in the age-standardized mortality rates of breast cancer among women from the USA, the UK (fig. 3), France, and Italy, over the period 1960-2000, following a peak around 1990. However, during the same period, ASRs for Japanese women, which used to be much lower than those observed in the other countries, increased constantly and the difference has recently become smaller. This increasing trend was observed irrespective of age group, and the differences between age groups in Japan were small (fig. 4, 5).

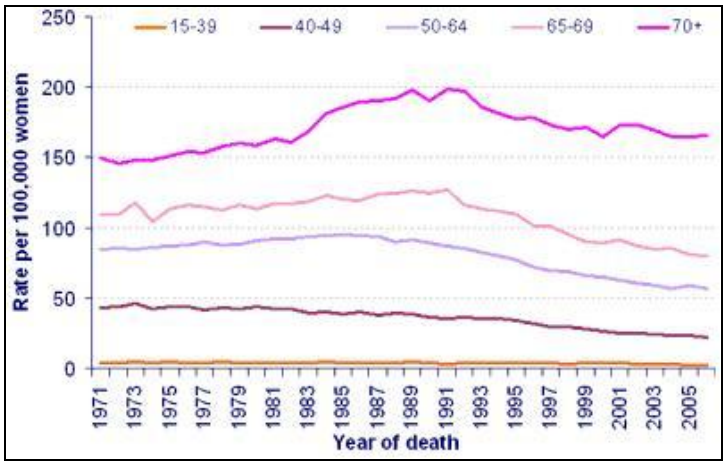


Fig. 3: Age-specific mortality rates, breast cancer, females, UK 1971 – 2006 (source: Cancer Research UK)

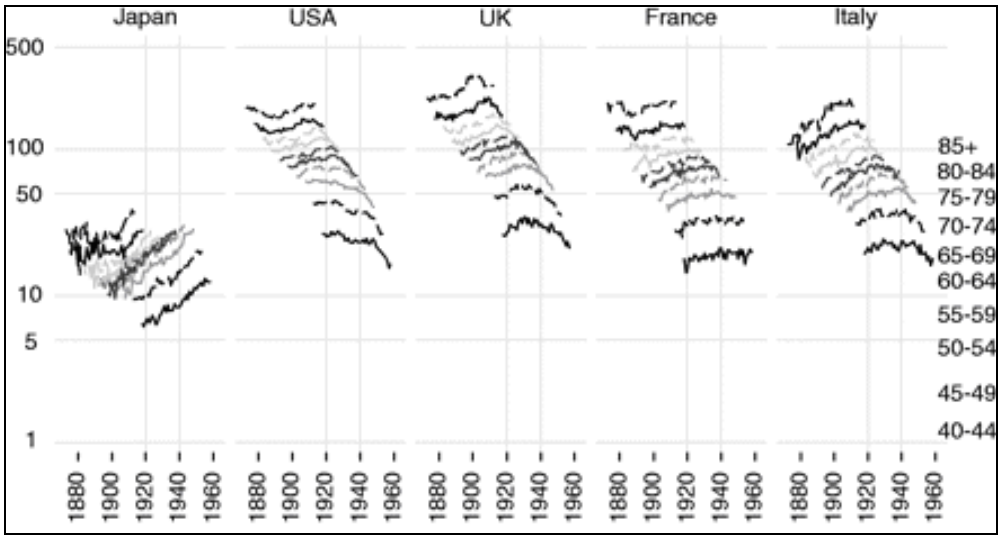


Fig. 4: Mortality for breast cancer by age group, year of birth (source WHO Motality Database)

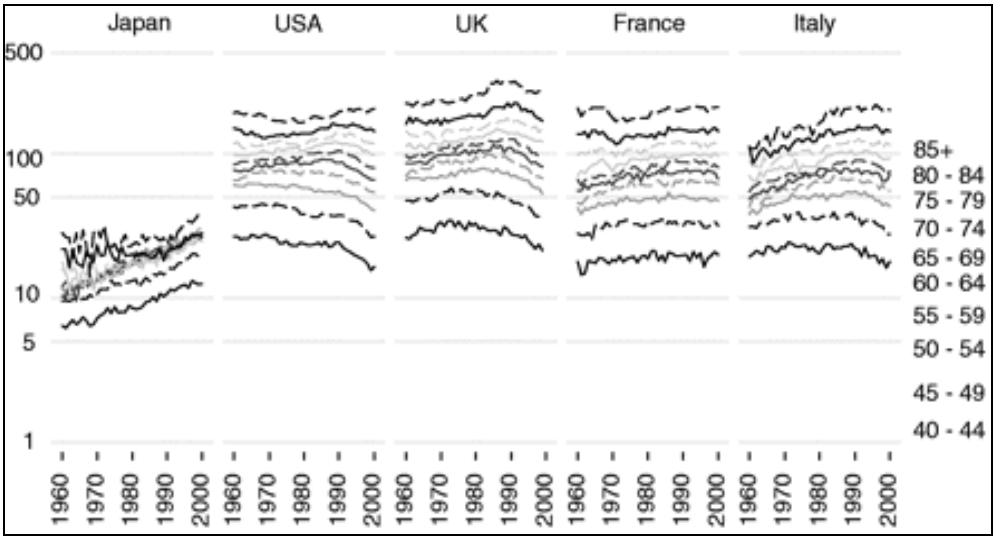


Fig 5: Mortality from beast cancer by age group, year of death (source WHO Motality Database)

The Netherlands are listed among the countries with the highest incidence and mortality rates in the EU. Figure 6 demonstrates the decreasing ASRs for mortality in this country for the period from 1980 to 2007 following the 1990 peak. There is also a decrease in the number of breast cancer cases as a percentage of the total cancer mortality burden for this country from 1997 to the first semester of 2007 (fig 7).

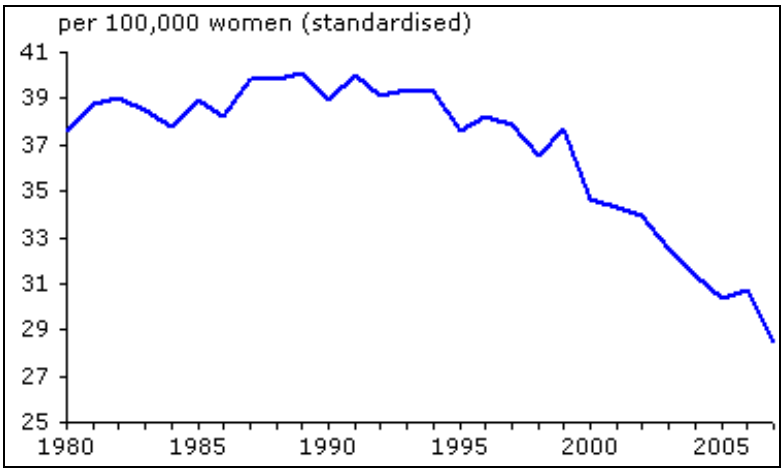


Fig 6: Age standardised mortality rate, female beast cancer (1980-2007), The Netherlands (source CBS)

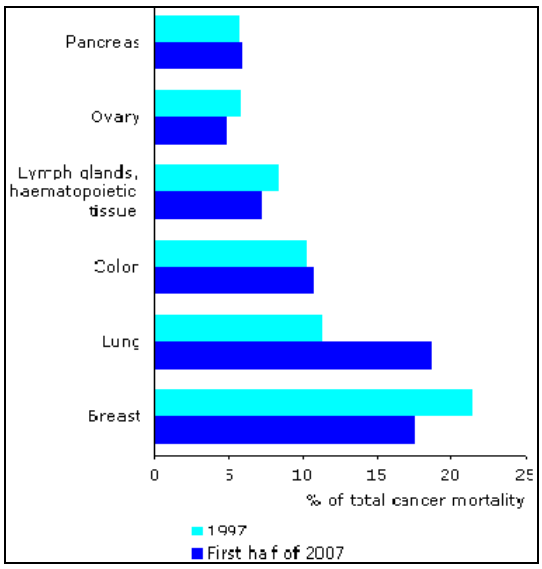


Fig 7: Number of cancer cases as a percentage of the total cancer mortality burden, The Netherlands (1997-2007) (source CBS)

1.2. The effect of screening on the incidence and mortality rates

The wide implementation of breast cancer screening in Europe during the 1990s was followed by a rise in the incidence, but this lasted only for a few years, after which incidence returned to pre-screening levels. Thus, a modest increase in breast cancer incidence due to screening would be expected in all studies during screening implementation period[19]. Screening mammography detects breast cancers earlier than those detected symptomatically, and so mammographically detected breast cancers tend to have better prognoses[20, 21] especially among patients ages 50-69 year. Detection of breast carcinoma via mammographic screening is associated with a lower risk of recurrence[22, 23]. The decline in breast cancer mortality has been attributed to mammography screening, adjuvant systemic therapy and the earlier detection of palpable tumours[24]. Despite the improved prognosis and more favorable tumor stage, even after the full implementation of the screening program the majority of invasive malignancies are still detected between screening rounds or in patients who do not participate in the program[25]. This could change in the future with the adaptation of more accurate breast-specific assays for the early diagnosis of cancer. In developed countries, the major investment made in early detection and treatment, has contributed to the longer survival of patients. On the other hand the increase of life expectancy together with the growing population, may partly explain the increase of almost all cancers incidence with age**.**

Waller et al. demonstrated the pattern of the incidence rise in the UK[26]. The authors found that the incidence of breast cancer in women aged 55-65 years rose sharply in 1989 and 1992 (explained by the introduction of a national breast cancer screening program in 1987-8). Among women aged 55-65 years, breast cancer incidence peaked in 1992-3. Rates then decreased steadily until 1996 before rising again, reflecting a second screening round, to a second peak in 2000. It was estimated that screening per se increases a woman's lifetime risk of being diagnosed with breast cancer from 7.8% to 8.6%.

Other research groups across Europe illustrated similar findings. An important question that arises from these studies regards the possibility of overdiagnosis (ie, the

detection of asymptomatic disease that would otherwise not have arisen clinically). Data from two Danish screening programs[27] suggest that the incidence of breast cancer has increased regardless of screening, while there is no evidence to demonstrate overdiagnosis of invasive breast cancer or if overdiagnosis was found to occur, it was only of limited magnitude. Data from Limburg screening program[28] in the Netherlands showed that the improved detection after 1995, and the lower than desirable decrease in large tumours, indicate a suboptimal screening performance before 1996. It can also explain the higher than expected rise in incidence after completion of the prevalent screening round.

Ductal carcinoma in situ (DCIS) accounts for approximately 20% of screen-detected cancers. It is usually impalpable and diagnosis is made commonly madde with mammography. The incidence of DCIS has significantly increased with the implementation of screening programs. There have been concerns that identifying DCIS is overdiagnosis of breast cancer, however there is strong evidence that the detection of high grade and necrotic DCIS by screening and its subsequent treatment prevents the development of invasive cancer with poor prognosis [29, 30, 31, 32, 33].

The incidence of breast cancer (invasive + DCIS) in Britain has increased from an age standardised rate of 74 per 100,000 women to 121 between 1975 and 2004. Table 2 demonstrates data for the period 1996-2006, when almost 16,000 breast cancer cases were detected, among almost two million women screened across the UK. It was estimated that the NHS Breast Screening Programme would save 1,250 lives every year until 2010 in England[34]. Of all invasive cancers diagnosed in England during 2007-2008, 52.3 per cent were 15mm or less which could have not been detected by hand. The World Health Organisation's International Agency for Research on Cancer (IARC) concluded that mammography screening for breast cancer reduces mortality[35, 36].

10 YEAR COMPARISON: NUMBER OF CANCERS DETECTED							
Year of data collection	Invasive cancers	Non- and Micro-invasive cancers	Total cancers	Number of women screened	CANCER DETECTION RATE PER 1000 Invasive	CANCER DETECTION RATE PER 1000 Non-invasive	CANCER DETECTION RATE PER 1000 Total
1996/97	5660	1468	7410	1340175	4.4	1.1	5.5
1997/98	6427	1726	8215	1419287	4.5	1.2	5.8
1998/99	6337	1634	8028	1308751	4.7	1.2	6.1
1999/00	7675	2076	9797	1550265	5.0	1.3	6.3
2000/01	7945	2080	10079	1535019	5.2	1.4	6.6
2001/02	7911	2218	10191	1507987	5.2	1.5	6.8
2002/03	8931	2416	11593	1579165	5.7	1.6	7.3
2003/04	10400	2868	13290	1685661	6.2	1.7	7.9
2004/05	11063	2953	14040	1748997	6.3	1.7	8.0
2005/06	12600	3317	15944	1942449	6.5	1.7	8.2

Table 2 Screen detected cancers in UK through a decade. Source: office for national statistics[37].

In 2004 there were around 36,900 new cases diagnosed (screen detected and symptomatic). This represented 32 per cent of all female cancers and a rate of 121 cases per 100,000 women. Four in five new cases were diagnosed in women aged 50 and over, with the peak in the 55 to 64 age group. Around 10,300 women died from breast cancer in England in 2004, a rate of 28 deaths per 100,000 women. Figure 8 demonstrates the incidence and mortality rates of breast cancer in the UK in relation to the implication of breast screening program for the period 1971-2004.

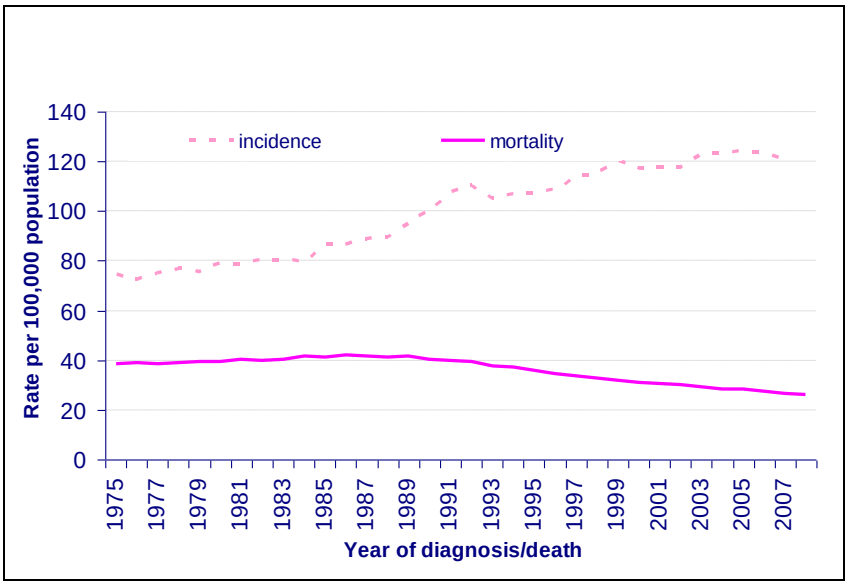


Fig. 8. Breast cancer incidence and mortality rates, U.K., (1975-2008), introduction of breast screening program

In 2007 there were 38.291 new cases

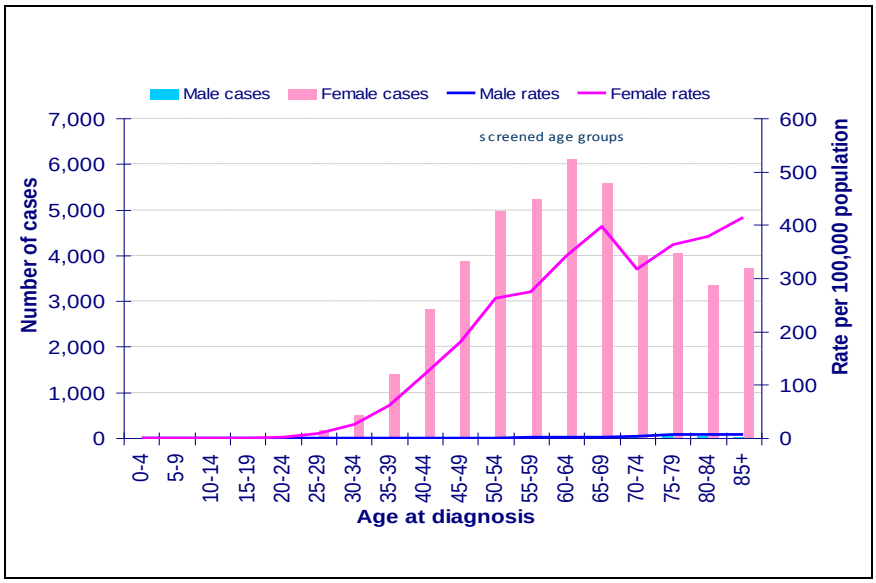


Fig. 9. Number of new cases and age-specific incidence rates, by sex, breast cancer, UK 2007

1.3. The Effect of population migration on breast cancer incidence

Age adjusted incidence and mortality for breast cancer vary by up to a factor of five between different countries. The expression of the genetic basis of breast cancer also varies between different racial and ethnic groups. Comparisons of cancer statistics at international level may be complicated by political and socioeconomic changes[38]. It is often not possible to determine whether changes in cancer rates are the result of truly changing risk in a region or changes in the population following large-scale migrations[2, 8, 39]. Migrant studies suggest that environmental factors are more important than genetic differences between populations. As a result of that, the rates of breast cancer reflect the rate in the host country within one or more generations. Several generations after migration of Japanese women in the United States they experience a similar breast cancer incidence risk as the Caucasians[40, 41, 42]. On the other hand, Japanese women who developed breast cancer were found to have significantly better survival than Chinese, Filipino and Caucasian[43]. Changes in lifestyle factors such as the use of oral contraceptives, cannot explain the elevated risk observed in recent migrants from Asian counties[44]. On the other hand, specific changes in the diet, physical activity and weight may have a significant impact on breast cancer risk[45]. Prevalence of established risk factors do influence breast cancer incidence, as breast cancer rates are found to be increased for more recently immigrated groups and decreased among more established groups[46]. A protective modulation by environmental factors on high-risk groups has also been suggested. Koifman et al. demonstrated a relatively lower than expected breast cancer mortality pattern in Ashkenazi women who live in Brazil. A higher frequency of BRCA1 and BRCA2 mutations is reported among these women in different countries[47].

In general, groups with recent immigration histories carry a higher burden of cancers that are not commonly observed at high rates in western countries. On the contrary, groups with older immigration histories, have a higher burden from cancers that are commonly observed in western countries (e.g., breast, colorectal)[48]. This indicates that they may have acculturated to the western lifestyle and succumbed to risk factors associated with these cancers. In addition, immigrants often seem to face barriers to medical care, decreased quality of care and delay in breast cancer diagnosis and

treatment, which also reflects in worse survival. Rates of mammography vary by race - ethnicity and are markedly lower among women with lower levels of education, without health insurance, and in recent immigrants. The above cannot be considered only a result of linguistic and cultural differences[49, 50]. Access to, utilization and affordability of cancer care facilities from migrants in developed countries is important for reducing mortality rates of these populations[51].

Geographical variations, time trends, and studies on populations migrating from low- to high-risk areas, which show that the incidence in such populations approaches that of the host country in one or two generations, clearly suggest an important role of environmental factors in the etiology of breast cancer.

1.4. Breast cancer risk factors

Gender

It has been recently estimated that the lifetime risk of developing breast cancer is 1 in 1,014 for men and 1 in 9 for women in the UK[52]. Being a woman is one of the biggest risk factors for developing breast cancer. Women have many more breast cells than men and are constantly exposed to the growth-promoting effects of the female hormones estrogen and progesterone. In 2007, 277 new male breast cancer cases were diagnosed in the UK. On average most of men with breast cancer are diagnosed in their early 70s. It is suggested that male breast cancer incidence is rising in urban UK and USA and there is some evidence that weight control and exercise may be protective factors[53].

Age

The risk of breast cancer increases with age. The table below shows the percentage of women (%) who will get breast cancer over different time periods in the U.S. For example, the table suggests 3.5% of women who are now 60 years old will get breast cancer sometime during the next 10 years (by the age of 70).

Current age	10 years	20 years	30 years
30	**0.4**	**1.8**	**4.2**
40	**1.4**	**3.9**	**7.0**
50	**2.5**	**5.8**	**8.9**
60	**3.5**	**6.9**	**8.8**
70	**3.9**	**6.1**	**N/A**

Table 3: Breast cancer incidence prediction in relation to age. Source: National Cancer Institute. Data are from 17 Surveillance, Epidemiology, and End Results (SEER) registries covering 25% of the U.S. population.

The risk of dying from breast cancer also increases with age. The table below demonstrates the percentage of women (%) who will die from breast cancer over different time periods. The table shows that 0.7% of women who are now 60 years old will die from breast cancer during the next 10 years. That is, about 1 woman out of 100 women who are 60 years old today will die from breast cancer by the age of 70.

Current age	10 years	20 years	30 years
30	**0.1**	**0.3**	**0.7**
40	**0.2**	**0.6**	**1.2**
50	**0.4**	**1.1**	**1.8**
60	**0.7**	**1.4**	**2.2**
70	**0.9**	**1.7**	**N/A**

Table 4: Breast cancer mortality prediction in relation to age. Source: National Cancer Institute. Data from the National Center for Health Statistics (NCHS). SEER Fast Stats.

Over 80% of all breast cancer cases in the UK are in women over the age of 50. Breast cancer is less common in women under the age of 40. However, approximately 20% of breast cancers are diagnosed in women younger than 50 years because those women represent 73% of the total female population.

Genetic Risk – Family History

About 5% to 10% of breast cancer cases are thought to be due to inherited susceptibility, resulting directly from inherited genetic alterations (mutations). The most common inherited mutations are those of the BRCA1 and BRCA2 genes[2]. Women with an inherited BRCA1 or BRCA2 mutation have up to an 80% chance of developing breast cancer during their lifetime [54, 55, 56]. Other genes that do not impart the same level of breast cancer risk as the BRCA genes have been cloned. The ATM gene normally helps repair damaged DNA. Certain families with a high rate of breast cancer have been found to have mutations of this gene. The CHEK2 gene increases breast cancer risk about two-fold when it is mutated. In women who carry the CHEK2 mutation and have a strong family history of breast cancer, the risk is increased. Inherited mutations of the p53 tumour suppressor gene (Li-Fraumeni syndrome) can also increase the risk of developing breast cancer [57]. The Peutz Jeghers syndrome patients (germline mutation of STK11/LKB1 gene in most cases) have been found to have a very high risk of breast cancer, which increases by age [58]. The PTEN gene normally helps regulate cell growth. Inherited mutations in this gene cause Cowden syndrome, a rare disorder in which people are at increased risk for both benign and malignant breast tumors, as well as growths in the digestive tract, thyroid, uterus, and ovaries.

Increased mammographic density

Breast density depends on the relative amounts of fat, connective tissue and epithelial tissue. Stroma and epithelium attenuate x-rays more than fat and appear light on a mammogram, while fat appears dark**.** Breasts with a higher proportion of fatty tissue are less dense. Increased density is one of the factors that can affect the efficacy of mammography by decreasing its sensitivity. Breast cancer, is less easily detected in denser breasts. Younger women tend to have denser beasts.

[2] BRCA1, BRCA2 (breast cancer 1 & 2 susceptibility genes) belong to a class of genes known as tumor suppressors, which maintain genomic integrity to prevent uncontrolled proliferation.

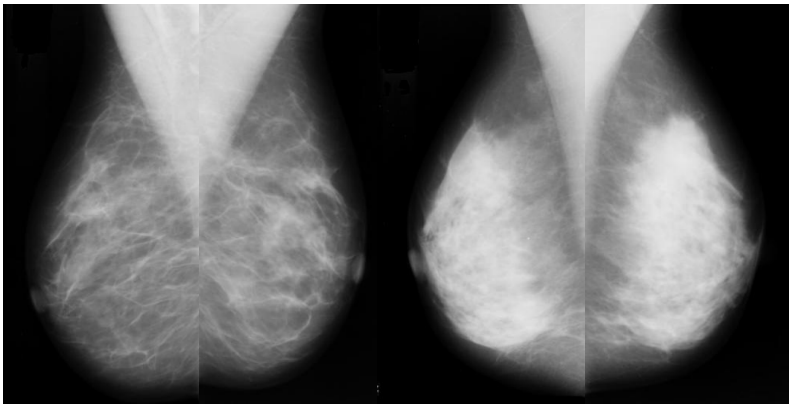


Fig 10: Mammograms of fatty (left) and dense breasts (right)

There is extensive evidence that mammographic density is an independent risk factor for breast cancer. A high percentage of dense parenchyma on mammographic images appears to confer a 4 - fold risk of developing breast cancer[59]. Extensive mammographic density is strongly associated with the risk of breast cancer detected by screening or between screening tests. A substantial fraction of breast cancers can be attributed to this risk factor[60]. There is growing evidence that the more important determinant of breast density is inherited[61, 62, 63, 64, 65]. Some authors who have identified this important risk factor for pre-and post - menopausal women suggest that breast density should be accounted in the basis of the Breast Cancer Risk Assessment Tool in order to obtain higher accuracy[66, 67]. It has also been suggested that the absolute size of the dense areas appears to be a better measure of breast cancer risk than the relative intensity of the density[68]. In postmenopausal women that do not use hormone replacement treatment (HRT) a positive association has been reported between the level of endogenous sex hormones and mammographic breast density[69]. The use of HRT (dose related and especially regarding progestin) has been demonstrated to increase breast density leading to a decreased sensitivity of mammography. Authors suggest that this group of women could be offered an alternative plan of surveillance or method of screening[70, 71, 72]

Diabetes type II

In a recent meta-analysis of case control and cohort studies Larsson et al reported findings that strongly indicate an association between diabetes type II and an increased risk of breast cancer[73]. Other researchers who identified the association, report no significant influence of diabetes type II on mammographic density[74].

Race - Ethnicity

Women who have different ethnic backgrounds have been found to be at different risk of developing breast cancer[75]. White women are slightly more likely to develop breast cancer than are African-American women. African-American women however, tend to have more aggressive tumors (high-grade with negative ER status) and are therefore more likely to die of the disease. Asian, Hispanic, and Native-American women have a lower risk of developing and dying from breast cancer. These differences in risk are likely to be partly due to different cultures and lifestyle risk factors such as the age women have their first child, and number of births. However, a genetic basis for this difference in risk cannot be completely ruled out until the genetic variation between different ethnic groups is fully understood[76, 77].

Hormonal risk factors

Age at menarche - **Late menopause**: Early age at menarche and late menopause are associated with an increased risk of breast cancer. Relative risk for premenopausal breast cancer is reduced by an estimated 7% for each year that menarche is delayed after the age of 12 years, and by 3% for postmenopausal breast cancer. China has a later average age at menarche (16-17 years). There is approximately 3% increase in breast cancer risk for each year menopause is delayed. The risk of breast cancer is lower for postmenopausal women compared to premenopausal women of the same age. This is true for both natural menopause and menopause induced through surgery[78].

Parity - Age at first birth: The effect of parity on reducing the risk of breast cancer has long been recognized. In the 18th century Bernado Ramazzini (1633-1714) reported the high rate of breast cancer in nuns compared with married women. In one meta-analysis nulliparity was associated with a 30% increase in risk compared with parous women. Women having a first child before 20 years of age have a 50% reduction in lifetime breast cancer risk when compared with women who do not have children. The risk for beast cancer is associated with the age at first birth and the total number of full term pregnancies. There is a reduction in risk of 7% for each birth after the first, in the absence of breast feeding[79]. A possible protective mechanism has been suggested in recent studies[80, 81, 82], which show that a full-term pregnancy imprints a specific epigenetic signature in the breast epithelium of postmenopausal parous women

that is significantly different from nulliparous women and those who have developed cancer.

Breastfeeding: There are many important benefits associated with breastfeeding for both mother and child. Some studies suggest that breast-feeding may slightly lower breast cancer risk, especially if breast-feeding is continued for 1.5 to 2 years.The lack of or short lifetime duration of breastfeeding typical of women in developed countries makes a major contribution to the high incidence of breast cancer in these countries. The longer women breast feed the more they are protected against breast cancer[83]. The Department of Health (UK) recommends that women breastfeed for the first six months of an infant's life as it provides all the nutrients a baby needs.

Oral Contraceptives (OC): Oral contraceptives were first approved for use in the United States in 1960. Usage varies widely by country, age, education, and marital status. One quarter of women aged 16–49 in Great Britain currently use the pill[84] compared to only 1% of women in Japan. The use of oral contraceptives (OCs) slightly increases the risk of breast cancer in current and recent users, but there is no significant excess risk ten or more years after stopping use. These estimates are based on a collaborative analysis of 54 studies in 25 countries, with data on over 50,000 women with breast cancer[85]. Cancers diagnosed in women who have used OC seem less likely to be advanced clinically than those diagnosed in women who have never done so. OC users are generally younger women whose breast cancer risk is comparatively low, so the small excess risk in current users will result in a relatively small number of additional cases. Duration of use, age at first use, dose and type of hormone seem to have no effect on risk. However, effects in older age groups (over 50) should only be beginning to appear now depending on the extend of use of OC across population of each country.

Hormone Replacement Therapy (HRT): Hormone replacement theapy is used by over 20 million women in western countries to counteract menopausal symptoms. Taking HRT can increase the risk of breast cancer. The risk depends on the length of use and it is suggested that short-term use (less than five years) has small effect. However, a woman’s chance of developing breast cancer will be about the same as if she had never taken HRT, within five years of stopping it. HRT use increases breast

density and reduces the sensitivity of mammography. The risk effect is greater for combined oestrogen-progestagen than for oestrogen only HRT. It is estimated that in the UK 20,000 extra breast cancer cases have occurred over the past ten years among women aged 50-64 as a result of HRT use. Of these additional breast cancers 75% are due to the use of combined oestrogen – progestagen therapy[86].

Endogenous Hormones and breast cancer risk: Analyses of prospective studies have found a statistically significant increased risk of breast cancer in postmenopausal women with higher levels of sex hormones[87, 88]. The risk was approximately double for women whose oestradiol levels were in the top quintile compared with women whose oestradiol levels were in the bottom quintile Evidence for premenopausal women is inconclusive. Similar data are provided by other recent studies, supporting the role of circulating hormones as a risk factor either independently or in direct association with mammographic breast density[89, 90]. It has also been suggested that the increase in breast cancer risk with increasing body mass index among postmenopausal women is largely the result of the associated increase in estrogens, particularly bioavailable estradiol[91].

In vitro fertilisation (IVF) treatment: It has been suggested that IVF may increase the risk of breast cancer since it increases levels of female hormones such as oestrogen. So far, there is no clear evidence of a link. Currently, women receiving IVF treatment are not considered to be at a greater risk of breast cancer[92, 93, 94, 95].

Benign Breast Conditions

A few benign and uncommon breast conditions have been linked to an increased breast cancer risk[96, 97]. These include:

Proliferative Lesions Without Atypia

The following seem to raise a woman's risk of breast cancer slightly (at least 1 ½ to 2 times normal):

- usual ductal hyperplasia (without atypia)
- complex fibroadenoma
- sclerosing adenosis
- several papillomas or papillomatosis
- radial scar

- palpable cysts in women younger than 45

Proliferative Lesions With Atypia

These conditions have a stronger effect on breast cancer risk, raising it 4 to 5 times higher than normal:

- atypical ductal hyperplasia (ADH)
- atypical lobular hyperplasia (ALH)

Anthropometric risk factors

Genetic or early shared environmental factors may affect risk estimates in studies of anthropometric measures and cancer risk, but do not seem to explain observations of increased cancer risks related to BMI or height[98]. Epidemiological evidence suggest possible mechanisms that explain the influence of anthropometric factors on breast cancer risk. These mechanisms implicate:

- Genetic predisposition to obesity and to specific body fat distributions
- Increased levels of circulating endogenous sex hormones, insulin and insulin-like growth factors.
- Storage of toxins in fat tissue that can serve as a source of carcinogens
- Lifestyle factors which are related to greater adult height and weight
- The fact that after the menopause, fat tissue becomes the main source of the female oestrogen hormone.

Height: Height is determined by the interaction of genes, nutrition and hormone levels. Being tall slightly increases the risk of developing breast cancer, irrespective of menopausal status[99].

Weight: More recent research studies have shown that an increased risk of breast cancer is found with increasing levels of all the anthropometric variables including height, weight, body mass index, waist-hip ratio (WHR), waist circumference and weight gain[100, 101, 102, 103]. For premenopausal women, breast cancer risk increases with increasing height, but decreases with higher weight or body mass index. Some authors consider WHR more specific indicator of breast-cancer risk than BMI[104] while others suggest that central and not general obesity in pre-menopausal women is specifically

associated with an increased risk of breast cancer[105]. Obesity at the time of diagnosis is thought to be significant as a poor prognostic factor in both pre- and post-menopausal women with breast cancer[106, 107]. At present there is enough evidence to suggest that weight management should be a part of breast cancer prevention strategy

Environmental factors

The term environmental factors refers to the non-genetic breast cancer risk factors. These include life style factors, artificial chemicals, any substances with possible estrogenic or carcinogenic effect that exist in air water and food, diet and radiation. Part of the prevention startegies concentrate on actions to reduce involuntary exposure to these factors that are considered avoidable.

The relative contribution of genetically heritable and environmental factors to the incidence of breast cancer can be estimated more precisely in populations of twins and families that carry the BRCA genes mutations. It has been demonstrated that the environmental factors that are not common between a pair of twins accounted for the 67% of the susceptibility variation to breast cancer, despite the identical genetic background[108]. A Scandinavian study demonstrated that, among women who carry the mutated tumour suppressor genes BRCA1 / BRCA2, those who were born after 1940 were found to be in a much higher risk (67%) for breast cancer by the age of 50 than those who were born earlier (24%)[109]. Another population study showed that the penetrance of the Icelandic BRCA2 founder mutation increased nearly four-fold from 1920 to 2000, whereas the death risk before the age of 70 years increased approximately two-fold[110].

Radiotherapy for Hodgkin's Lymphoma: Breast cancer is the most common solid tumour among women treated for Hodgkin's lymphoma (HL)[111]. History of HL in women diagnosed subsequently with breast cancer is also a negative prognostic factor[112]. The risk for women who have had the most common type of chest radiotherapy is related to radiation dose and age at diagnosis and is similar to that for women with a strong family history of the disease. Since 2003 the Department of Health in the UK offers annual screening to these women as the potential benefit from that is increased[113, 114].

Diagnostic ionising radiation and/or radiotherapy: Small exposure to ionising radiation is greatly outweighed by the benefits associated with having mammograms or other types of X-rays, such as finding and treating breast cancer early. Previous studies demonstrated little if any risk of radiation-induced breast cancer associated with exposure of breast tissue to low-dose radiation (e.g., from mammographic X rays or adjuvant radiotherapy)[115]. Recent studies though support the hypothesis that low-dose ionising radiation, and particularly exposures during childhood, increases breast cancer risk[116, 117]. It is suggested that there is a subgroup in the female population with increased susceptibility to radiation-induced breast cancer[118]. Other authors suggest that radiotherapy for a previous cancer or diagnostic ionising radiation exposure from chest X-rays may be associated with a significantly increased breast cancer risk among women who carry BRCA1 or BRCA2 pathogenic germline mutations[119, 120]. There is also increasing evidence that carriers of pathogenic alleles in DNA repair and damage recognition genes may have an increased risk of BC following exposure to ionising radiation, even at low doses[121]. It can be concluded that identification of women susceptible to radiation damage would contribute to the risk/benefit assessment of radiation therapy versus alternative therapeutic options.

Radiotherapy on the contralateral breast: Data on the incidence of contralateral breast cancer fail to demonstrate an oncogenic effect of irradiation to date[122, 123].

Radiologists – Occupational exposure: No excess risk has been reliably demonstrated, from occupational exposure to medical radiation among physicians[124, 125, 126, 127].

Airline crew exposure to cosmic radiation: Airline flight personnel work in an environment with exposure to known or suspected carcinogenic factors, including ionizing cosmic radiation, aircraft generated magnetic fields and disturbance of the circadian rhythm. Studies' results in different countries have been controversial. Significantly increased risks for breast cancer and malignant melanoma among female flight attendants in Iceland and Japan have been related to occupational risk factors[128, 129, 130]. There was no clear evidence though that these factors studied affected breast cancer risk among Scandinavian flight attendants. Authors suggest that differences in reproductive history and factors related to lifestyle influence the incidence of breast

cancer among this group of women[131, 132, 133].

Atomic bomb, Chernobyl accident, nuclear weapon tests: People who have been exposed to high amounts of ionising radiation, such as an atomic bomb explosion or radiation accident, are at increased risk of many types of cancer, including breast cancer. A significant 2-fold increase in risk was observed, during the period 1997-2001, in the most contaminated districts after the Chernobyl accident. The risk was highest among women who were younger at the time of exposure. It is considered unlikely that this excess could be entirely due to the increased diagnostic activity in these areas[134]. A significant trend with dose was also observed for female breast cancer in relation to Soviet nuclear weapons testing[135]. Findings from the Life Span Study (LSS) of the health effects of exposure to atomic bomb radiation have also documented a strong dose-response relation between radiation exposure and breast cancer incidence in both sexes[136, 137]. Results from a prospective study of atomic-bomb survivors in Japan support the evidence that daily consumption of fruit and vegetables reduces the risk of total cancer[138].

Diet: The role of diet for the risk of breast cancer is of great interest as a potentially modifiable risk factor[139]. The controversial association between diet and breast cancer risk is also based on the fact that diet is closely related to other lifestyle factors. (cultural background, lack of exercise, smoking, stress etc.). Most of the research on diet and breast cancer has focused on fat, meat and fish, dairy products, fruit, vegetables, phyto-oestrogens and alcohol. Among the prospective epidemiologic studies conducted on diet and breast cancer incidence and gene-diet interactions and breast cancer incidence, to date there is no consistent, strong, and statistically significant association[140, 141]. Similar findings are reported in UK regarding the possible effect of vegetarian diets or dietary isoflavone intake on breast cancer risk in a population of British women with heterogeneous diets[142].The following general dietary considerations have been implicated and/or remain under investigation:

Moderate alcohol consumption increases breast cancer risk. Drinking, on average, one unit of alcohol per day increases a woman's risk of breast cancer by about 6%[143]. A healthy weight may reduce the risk for breast cancer after menopause and women who drink alcohol also should take sufficient folate, which can mitigate this excess risk[144,

[145]. Traditional Mediterranean diet[3] significantly reduces endogenous estrogens and therefore acts as a dietary preventive measure for breast cancer[146], while a typical western diet[4] seems to increase breast cancer risk in postmenopausal women[147]. The higher intakes of protein and meat in early to mid-childhood may lead to earlier menarche. This may have implications for the lifetime risk of breast cancer and osteoporosis[148]. High dietary intakes of plant lignans and high exposure to enterolignans in a Western population have been associated with reduced risks of ER- and PR-positive postmenopausal breast cancer[149,] and a possibly reduced breast cancer risk in post-menopausal women[150]. Dietary fat intake is directly associated with the risk of postmenopausal invasive breast cancer[151]. A high glycemic diet may increase the risk of breast cancer in women[152, 153, 154]. Dietary fiber intake from fruit and cereal vegetables and soybeans may play a role in reducing breast cancer risk[155, 156, 157, 158, 159]. A diet rich in fruits and vegetables may also reduce the risk of fibroadenoma formation[160]. There is no good scientific evidence to recommend that women alter their consumption of dairy products in order to reduce their risk of breast cancer. Studies have suggested that dairy products, particularly low-fat products, might decrease the risk of the disease. The possible mechanisms implicated include a calcium content or a correlated component and vitamin D intake[161, 162, 163].

Exposure to light at night – Disruption of circadian rythm: Studies have suggested that women who are regularly exposed to light at night (for example, women who do night shift work) may have an increased chance of developing breast cancer than women who are not[164, 165, 166, 167, 168]. The suggested mechanism for this involves the disturbance of the circadian rhythm through melatonin pathway. Melatonin is a hormone produced in the pineal gland that shows potential oncostatic activity and is acutely suppressed by light exposure[169].

[3] The Mediterranean diet is a nutritional model inspired by the traditional dietary patterns of some of the countries of the Mediterranean Basin. Based on food patterns typical of Crete, much of the rest of Greece, and southern Italy this diet emphasizes olive oil as the principal source of fat, abundant plant foods, fresh fruit, dairy products (cheese and yogurt), and fish and poultry consumed in low to moderate amounts, zero to four eggs consumed weekly, red meat consumed in low amounts, and wine consumed in low to moderate amounts.

[4] The 'Western diet' is a popular dietary pattern in the developed countries, characterized by high intakes of red meat, high-sugar products (drinks and desserts), high fat, refined grains, high-fat dairy products, and eggs. This pattern is increasingly being adopted in developing countries

Environmental oestrogens: (Endocrine disruptors, endocrine modulators, environmental hormones, endocrine active compounds). These are naturally occurring compounds or man-made chemicals that may interfere with the production or activity of hormones of the endocrine system leading to adverse health effects[170, 171].

1.5. Clinical association does not prove causation.

Many experimental and clinical studies investigate the carcinogenic potential of several chemical substances that act by expressing estrogenic properties, damaging DNA, stimulating the development of a tumour or altering the development of the human breast. Although there is strong evidence that many chemicals can disrupt normal physiological processes, it is very difficult to prove a causal relationship between those and breast cancer. Ruthan et al reported 216 chemicals that can cause breast tumours mostly by DNA mutation in animal studies. Despite the significant discrepancy between humans and animals, regarding the dose and length of exposure effect, this is a clear indication for further clinical research[172]. Most of the studies in humans that measured levels of one or more chemicals are unable to investigate exposure in early life or even in utero which may be of crucial importance. Moreover, it is very difficult to assess the exposure to combinations of chemicals which would be more realistic. It is also difficult to find control groups of unexposed humans as most of the chemicals are widespread in use and can be attained from multiple sources. There are several examples of false or unproved causational link hypotheses between possible risk factors and breast cancer occurrence in literature.

The example of Soya: Phyto-estrogens are a group of plant-derived substances that are structurally or functionally similar to estradiol. There are several different types of phyto-oestrogens found in plant foods. Soy contains isoflavones which are phyto-estrogens that have displayed cancer-fighting activity in laboratory tests. During the last decades soy products captured media and consumers' attention because epidemiologic data suggested an association between high intake of soy foods and low breast cancer risk in Asian countries. According to a Canadian study[173], foods with the highest relative phytoestrogen content in a Western diet are nuts and oilseeds, followed by soy products, cereals and breads. The highest concentrations of isoflavones are

found in soy beans, whereas lignans are the primary source of phytoestrogen found in nuts and oilseeds cereals, fruits and vegetables.

A meta-analysis of the relation between soy consumption and breast cancer risk in 18 epidemiologic studies[174], conducted from 1978 to 2004, has demonstrated highly variable results. Experimental data suggest that soy constituents can be estrogenic and potentially risk enhancing.The results from laboratory studies and clinical trials remain equivocal [175, 176, 177, 178, 179, 180, 181] and announcements in the media have been rather controversial (table 5).

BBC News 4-2000: A soya-rich diet can reduce the levels of harmful cholesterol, lowering the chance of developing heart disease
The Observer 8-2000: A health warning was sounded last night over the dangers of eating soya after two senior American government scientists revealed that chemicals in the product could increase the risk of breast cancer in women, brain damage in men and abnormalities in infants.
Sunday telegraph 1-2007: Cancer patients are being warned to avoid foods rich in soy because they can accelerate the growth of tumours. The Cancer Council NSW will issue guidelines today, warning about the dangers of high-soy diets and soy supplements for cancer patients and those people in remission from cancer.
Reuters UK 4-2007: Using cells in a lab dish, researchers at the University of California, Los Angeles, found that diindolymethane (DIM), a compound resulting from digestion of cruciferous vegetables, and genistein, an isoflavone in soy, reduce the production of two proteins needed for breast and ovarian cancers to spread.
Cancer research UK 10-2007: How does soy affect breast cancer? We don't know yet

Table 5. News reports: Soy effect on breast cancer risk

Another population based case-control study has demonstrated a reduced risk of endometrial cancer for women that regularly consume soya foods[182]. Other studies have proposed a minor potential effect of phytoestrogens intake in male fertility[183, 184] and a possible protective role in prostate cancer prevention[185].

The existing animal and human data do not allow definitive conclusions to be drawn about the effect of soyfoods or isoflavones on breast cancer risk and on the survival of breast cancer patients. A 2006 review article[186] stated that there is need to evaluate, at cellular level, the impact of isoflavones on breast tissue in women at high risk for

breast cancer. Interestingly some studies suggest adverse effects from soy constituents [187, 188], and therefore no recommendations can be made for high-dose isoflavones as an intentional supplement rather than a normal dietary intake to prevent breast cancer or prevent its recurrence.

The example of western practice of bra wearing: During the last decades there have been concerns regarding a possible link between wearing a bra and breast cancer. Two mechanisms have been suggested for this link. The first one involves the hypothesis that bras prevent normal lymphatic flow and lead to tissue anoxia, which has been related to fibrosis and increased cancer risk. The second mechanism suggests a link between increased temperature of the breasts (caused by bras) with alterations of hormonal function, which have been widely linked to breast cancer. In 1995 a study by S. Singer and S. Grismaijer was published in their book "Dressed to Kill: The Link Between Breast Cancer and Bras," claiming that the more hours per day that a bra is worn, the higher the rate of breast cancer (up to 125 fold higher) and that women who do not wear bras have a dramatically reduced rate of breast cancer[189]. Some authors also declare that there is no positive evidence that bra wearing is good for the breast. A Japanese study demonstrated that a bra can actually increase breast sagging, rather than the opposite[190].

In 1991 Hsieh et al. published a multicenter study on breast cancer risks involving bra cup size and handedness. As a side issue of their paper, they surprisingly found that premenopausal women who did not wear bras had half the risk of breast cancer compared with bra users. The authors suggest that the reason for this is possibly the body and bra size (thinner women with smaller breasts). Among bra users, larger cup size was associated with an increased risk of breast cancer, although the association was found only among postmenopausal women and was accounted for, in part, by obesity. These data suggested that bra cup size (and conceivably mammary gland size) and not bra–wearing may be a risk factor for breast cancer[191]. Another group of researchers in Japan demonstrated in their study that wearing a girdle and bra lowers the levels of the hormone melatonin -which is believed to have anti-cancer activity-, by 60 percent[192]. Advice offered on bra wearing by the media usually include: wearing the correct bra size, making sure it's not too tight, not sleeping with a bra on and wearing it as less as possible.

Hormone replacement treatment and coronary heart disease risk: Several epidemiological studies showed that women who were taking combined hormone replacement therapy also had a lower incidence of coronary heart disease (CHD) suggesting that HRT was protective against CHD. Controlled trials though, demonstrated that HRT caused a small but significant increase in risk of CHD. Re-analysis of the data showed that women undertaking HRT were more likely to be from socio-economic groups A, B and C1 (professionals to non manual occupations with higher income), with better than average diet and exercise regimes. The two were coincident effects of a common cause, rather than cause and effect as had been supposed[193]. In 2003 one third of women in UK between 50 and 64 were using HRT[194]. Health authorities now consider that risk-benefit considerations do not favour the use of HRT for prevention of cardiovascular diseases and bone fractures in postmenopausal women[195]. Also, it has already been suggested in some studies that the reduction in HRT use in some areas has contributed to the reduction of breast cancer and ductal hyperplasia incidence[196, 197, 198].

2. ENDOCRINE DISRUPTERS AND BREAST CANCER

The term “endocrine disrupters” describes the environmental oestrogens, also known as xenoestrogens, which are compounds that present estrogenic properties and include:

- Natural hormones. These can be released into the environment from any animal.
- Natural chemicals. These include toxins produced by components of plants (phytoestrogens, such as genistein or coumestrol) and certain fungi. It has been demonstrated in experimental studies that exposure to such compounds during gestation could contribute to the development of hypospadias[199].
- Man-made chemicals and by-products released into the environment. These include some pesticides (e.g. DDT and other chlorinated compounds), dioxins, chemicals in some consumer and medical products (e.g. some plastic additives), and a number of industrial chemicals (e.g. polychlorinated biphenyls - PCBs). Also, synthetically produced pharmaceuticals that are intended to be highly hormonally active, e.g. the contraceptive pill and treatments for hormone-responsive cancers may also be detected in sewage effluent.

Many of these chemicals have been linked with developmental, reproductive, neural, immune, and other problems in wildlife and laboratory animals[200, 201]. While effects of exposure to xenoestrogens on aquatic wildlife are well documented, the experimental evidence for impairment of reproductive behavior and physiology in mammals has been debated. The arguments against such studies have been that the route, time course and intensity of exposure did not simulate environmental exposure and that the chemicals tested have additional non-estrogenic toxic effects, hindering generalization of actual "xenoestrogenic" effects. Some scientists think these chemicals also are adversely affecting human health in similar ways although their hormonal activity is many times weaker than the body's own naturally present hormones. Possible results of these effects include declined fertility and increased incidences or progression of some diseases including endometriosis and cancers including breast cancer. Environmental chemicals with oestrogenic activity are the most well studied however chemicals with anti-oestrogen, androgen, anti-androgen, progesterone, or thyroid-like activity have also been identified.

Data from the Seveso Women's Health Study demonstrated that serum dioxin concentration is significantly related with breast cancer incidence among women in the study cohort[202]. However, there is currently no good evidence that normal exposure to environmental chemicals increases the risk of breast cancer. The difficulty to make a direct association is based on the fact that it takes many years for most breast cancers to develop and therefore it is practically impossible to work out what chemicals women have been exposed to, over previous decades before their breast cancer is detected. It is also hard to isolate the effects of every individual chemical on breast cancer risk. Endocrine disruptors can act additively at concentrations which are individually harmless[203].

Disruption of the endocrine system can occur in various ways:

- By mimicking a natural hormone, fooling the body into over-responding to the stimulus.
- By blocking the effects of a hormone from receptors
- By directly stimulating or inhibiting the endocrine system and causing overproduction or underproduction of hormones.

Effects suggested as being related to endocrine disruption have been reported in molluscs, crustacea, fish, reptiles, birds and mammals in various parts of the world. The clearest example of an endocrine disrupter in humans is diethylstilbestrol (DES), a synthetic oestrogen prescribed in the 1950s and 1960s to five million pregnant women for the prevention of spontaneous abortion. It was found that some of the children who had been exposed in the uterus had developmental abnormalities, and that some of the girls developed an unusual form of vaginal cancer when they reached puberty. As a consequence, DES was banned in the 1970s.

In The U.K. about 300.000 women used the drug diethylstilbestrol (DES) to avoid miscarriages between 1953 and 1971. Later studies showed that the daughters of those had a double than normal breast cancer risk. As the later reach the menopausal age the risk is expected to increase. This example highlights the importance of exposure prevention to oestrogens at the early stages of development[204].

Although numerous human epidemiological studies have been conducted to determine whether environmental EDs may contribute to an increased risk of breast cancer, the

results remain inconclusive. Overall, the current scientific evidence (from human and animal studies) do not support a direct association between exposure to environmental EDs and increased risk of breast cancer. However, all the studies published to date have measured ED exposure levels in adult women. The claim that the time of life when exposure takes place (e.g., prenatal, neonatal, childhood, adolescence) may be the most critical factor is supported by human data on radiation and smoking and by basic research in animal models[205]. The later provide examples of the growing scientific field termed "the developmental origins of adult disease" and suggest new targets of abnormal programming by endocrine disrupting chemicals[206]. Adult women currently at risk for breast cancer may have been exposed to exogenous EDs in utero or during infancy, childhood, and adolescence in the mid-1900s when contaminant levels of organochlorines were higher[163]. Water is the surrounding medium of a large number of water-breathing species (e.g., fish, aquatic invertebrates) and is consumed by humans and terrestrial species. A variety of pesticides, industrial chemicals, and natural hormones have been detected in surface waters[170]. Chemicals may be dissolved in water and/or bound to particulate matter. In water-dwelling species, uptake can occur through direct contact via the gills or as they feed. Fish and/or mussels have been shown to accumulate halogenated phenols[207].

Exposure to potential mammary carcinogens is widespread from chemicals found in consumer products, air and drinking water pollution, food, and women's workplaces. Epidemiologic studies have included a small number of chemicals identified as mammary carcinogens or as hormone disruptors, which may have implications for breast cancer, however, evidence is emerging for associations between breast cancer and polychlorinated biphenyls, polycyclic aromatic hydrocarbons, and organic solvents[208,209].

2.1. Environmental chemicals in breast tissue and breast cancer risk

The human breast is composed of adipose, epithelial and connective tissue. Adipose tissue comprises for the 10% to 100% of the total breast weight depending on age and body habitus[210]. Breast cancer arises from the epithelial cells that are distributed unevenly with the upper outer quadrant containing more epithelial tissue than the other areas of the breast. The proportion of epithelial to fatty tissue determines the density of the breast which is an important risk factor for breast cancer. The adipose tissue can act like a store for chemical compounds in the breast in close proximity to the epithelial tissue. Such distribution of potentially carcinogenic chemicals supports the plausibility of a causational link with breast cancer[211]. Martin et al. supported this plausibility hypothesis by demonstrating that mammary lipid from healthy women who underwent breast reduction, contained genotoxic constituents[212]. Hu et al. (1997) performed an in vitro study on breast epithelial cells derived from high risk family history patients who underwent risk reducing mastectomy. They demonstrated that genetic predisposition was associated with increased susceptibility to environmental chemical carcinogens, by treating the cultures with the latter. They noticed early stage neoplastic changes that were not seen in cells cultures from women without a family history risk[213]. It is therefore important to determine the biologically active substances that may play a role in human breast carcinogenesis by genetic damage and the level and significance of environmental exposure to them. One has to keep in mind that the serum, tissue or milk samples timing may not reflect the exposure-related aetiology for breast cancer developement[214]. There is conflicting evidence on the correlation between the blood serum levels and tissue levels of some chemicals and therefore the serum levels may not be considered a reliable index of the tissue burden[215, 216, 217]. Bioaccumulation of chemical compounds has been correlated with the body mass index age and diet pattern[218].

Polychlorinated Biphenyls (PCBs) and Dichloro-Diphenyl-Trichloroethane (DDT), are highly lipid soluble and thus capable of accumulating in the breast. PCBs were widely used as coolants and insulating fluids for transformers and capacitors, stabilizing additives in flexible PVC coatings of electrical wiring and electronic components,

pesticide extenders, etc.[5] Their production was banned in the 1970s due to the high toxicity of most PCB congeners and mixtures. PCBs are classified as persistent organic pollutants which bioaccumulate in animals. They are very stable compounds and do not degrade readily. They may be extremely difficult to destroy, and there is the risk of creating extremely toxic dibenzodioxins and dibenzofurans through partial oxidation. The United States Environmental Protection Agency (EPA) and the International Agency for Research on Cancer (IARC) have determined that PCBs are probably carcinogenic to humans. PCBs are also classified as probable human carcinogens by the National Cancer Institute, World Health Organization, and the Agency for Toxic Substances and Disease Registry. Recent research by the National Toxicology Program has confirmed that PCB126 (Technical Report 520) and a binary mixture of PCB126 and PCB153 (Technical Report 531) are carcinogens. Studies of PCB workers found increases in rare liver cancers and melanoma. There has been no sufficient evidence of direct relation between PCB's and breast cancer incidence[219]. DDT is a well known synthetic pesticide that was first produced in 1874. DDT was used with great effect to combat mosquitoes spreading malaria, typhus, and other insect-borne diseases DDT was made available for use as an agricultural insecticide. Until 1980s, agricultural use of DDT was banned in most developed countries. In the United Kingdom DDT was not banned until 1984. The use of DDT in vector control has been largely replaced by less persistent, and more expensive, alternative insecticides. Previous studies did not support the hypothesis that exposure to DDT is an important risk factor for breast cancer. However, exposure to DDT during critical periods of human development and individual variations in metabolizing enzymes of DDT are still important areas to be researched. Cohn et al studied blood samples that were collected from young California mothers in the 1960s while DDT was still in use, and tracked their breast cancer status[220, 221]. Interestingly, they found a strong association between exposure to the p,p-isomer of DDT early in life and breast cancer later in life.

Organochlorine pesticides and PCBs remain under investigation as possible risk factors for breast cancer because of their oestrogenic properties and widespread presence in the environment. Measurable levels have been reported in human and

[5] Commercial PCB mixtures were marketed as Clophen by Bayer in Germany, Aroclor by Monsanto in USA, Kanechlor by Kanegafuchi in Japan, Santotherm by Mitsubishi in Japan, and Phenoclor and Pyralene by Prodolec in France.

animal tissue for several decades. They have also been found in breast milk, maternal blood and cord blood[219]. They have been found in countries where these chemicals have been banned. Diet is a major factor that influences breast milk levels of persistent organic pollutants, with patterns in fish consumption playing a particularly significant role. Some countries like Sweden and Germany have ongoing breast milk monitoring programs. Regional differences in levels of xenobiotics in breast milk are related to historical and current local use patterns[222]. Adipose or serum assays can be useful measures of human body burden of environmental organochlorinated compounds in epidemiological studies of breast cancer[219].

In 1976, researchers reported for the first time higher levels of DDT and PCBs in the cancer tumours compared with the adjacent mammary and adipose tissue in nine cancer patients[223]. In 1992, other researchers found significantly high levels of PCBs, DDT, and DDE (Dichlorodiphenyldichloroethylene – byproduct of DDT) in the breast fat of women with cancer suggesting a role for these compounds in the etiology of breast cancer[224]. In aother study, Beta-HexachloroCycloHexane (b-HCH), a synthetic pesticide, was correlated with breast cancer risk when the tissue levels exceeded 40.1mg/kg of fat[225]. Other studies also report correlation of breast cancer risk with PCBs[226, 14, 227] and pesticides aldrin and lindane[227]. Organochlorine pesticides have also been related to benign breast disease[228]. A few studies have found, but could not explain, either positive[14] or negative[229] associations between the concentration of chemicals and hormone receptor negative status.

There are several studies however, than have not found any correlation between the above or other chemicals and breast cancer risk[230, 231, 232, 13]. According to recent reviews of epidemiologic evidence, the hypothesis of an association of environmental exposure to PCBs and risk of breast cancer cannot be supported in adulthood in the general population[233, 234]. This conclusion is based on the lack of correlation with breast-cancer mortality in studies involving almost 9,000 women with occupational exposure to PCBs. However, there are still uncertainties for selected subgroups of women or individual PCB congeners.

3. CURRENT TRENDS IN BREAST CANCER RESEARCH AND PREVENTION

3.1. Screening strategies and risk assessment tools

The ideal means of effecting cancer before treatment control is primary prevention. Screening may be applicable if it has been shown to reduce mortality from the disease. The wide-ranging individual susceptibility and lifetime risk levels for breast cancer have important implications for screening and detection. Screening does not prevent from breast cancer, but reduces the mortality rates by 15-48%, especially in women between 40 and 70 years of age[235, 236, 237]. This reduction is attributed to early stage detection and improved multidisciplinary management. For those women who are known gene mutation carriers, or have a strong family history of the disease, the national guidelines suggest an altered surveillance plan and offer risk reduction options. In the U.K., women who do not fall in the high risk group, are invited by the British National Breast Cancer Screening Program for a screening mammogram on a three yearly basis from the age of 47 to 73 years. The guidelines in the United States recommend annual mammograms for every woman over the age of 40. The primary goal of national screening programs has been to maximize the number of women who receive regular mammograms. However, in some states screening programmes are poorly implemented or non-existent and many women, especially those in lower socioeconomic groups, are not screened, resulting in advanced stage diagnosis and poorer outcomes[238, 239]. The implementation of a screening strategy that allows an individualized approach, requires accurate classification of women according to their level of risk. Various risk prediction techniques that associate genetic, biologic, hormonal, lifestyle and other factors are currently used for the development of risk assessment tools. These are based on mathematical models that relate all the risk parameters with the characteristics of the disease treatment and outcomes. Some of the most widely used risk assessment tools are available online such as Adjuvant online, The Gail model, BOADICEA, BCRA, etc. Independent validation of such models has produced variable results and the knowledge of how to best integrate data into these models needs to be improved and revalidated[240]. The goal of improved risk assessment

is not to increase the use of screening mammography, but to identify optimal strategies and for some women, that might mean fewer mammograms.

3.2. Prevention and avoidable environmental factors

Studies that investigate the synergistic potential of hormone disrupting chemicals, have demonstrated that a mixture can cause adverse effects even when the individual substances are at a level that should not cause any problem[241, 242]. Early exposure to these chemicals during the intrauterus period or later in puberty may have a serious impact in later life. In industrialized societies, there is a widespread exposure to chemicals that may potentially increase risk for breast cancer[208]. These have either been released into the environment in the past decades, or are currently being used with unknown longterm health consequences. Various levels of xenoestrogens have been measured in the body fat or the breast adipose tissue of breast cancer patients. It is very difficult to determine chemical exposures that occurred one or more decades before a breast cancer is detected.

The above combined facts are strong enough to require precautionary action. All women, especially when pregnant, should try to minimize their exposure to avoidable environmental factors particularly, chemicals with estrogenic properties. The European Union and the local governments need to act to ensure safety controls, and effective regulation of chemicals, as well as implementation of policies that promote a safer environment. In the year 2000 the Royal Society in the UK stated that: "*In view of the magnitude of the potential risks, we strongly believe that scientific uncertainty should not delay precautionary action for risk reduction.*" In 2005, the Prague Declaration on Endocrine Disruption, signed by more than 200 scientific experts from across Europe and the USA, called for measures to reduce the risks associated with endocrine disrupting chemicals. Diet and lifestyle recommendations quoted below have been implemented as part of the European Prevention Policy guidance to all citizens:

- To eat plenty of fruit and vegetables
- To buy organic food wherever possible

- To avoid unnecessary exposure to chemicals particularly garden and indoor pesticides, homecare products such as paints and detergents and personal care products including cosmetics.
- To not microwave food in plastic containers or wrapping
- To express concerns to government representatives or Members of the European Parliament,about hormone disrupting chemicals and their links to breast cancer.
- To ask for tighter controls over synthetic chemicals that disrupt our hormone systems.

3.3. Chemoprevention

Breast cancer prevention studies aim to determine whether pharmaceutical agents are able to prevent breast cancer in women who carry an increased risk e.g. have a strong family history or have been diagnosed as BRCA1 and BRCA2 gene mutation carriers. The following prevention studies have been completed since 1986:

- The Royal Marsden Hospital Tamoxifen Chemoprevention Trial.
- The National Surgical Adjuvant Breast and Bowel Project
- The Italian Tamoxifen Prevention Study
- The Breast Cancer Prevention Trial
- the Multiple Outcomes of Raloxifene Evaluation
- The Study of Tamoxifen and Raloxifene
- The International Breast Cancer Intervention Study.

These randomized trials have investigated the use of tamoxifene, and raloxifene in reducing the incidence of breast cancer in high risk pre- and postmenopausal women. Tamoxifen and raloxifene have both proved to be equally effective in the prevention of breast cancer in all studies. Raloxifene may have less adverse effects. However, in the U.K. both agents are not licenced for risk reduction unless used in the settings of a clinical trial. It has also been demonstrated that although tamoxifen does not reduce the incidence of ER-negative breast cancers, it may delay their detection by approximately 1 year[243]. The effect of the preventive treatment on the mortality of the later diagnosed breast cancers is not clear. The most important adverse effects of preventive medication included an increased rate of venous thromboembolic episodes and endometrial cancer. Currently, other studies are investigating the preventive value of

aromatase inhibitors anastrazole, letrozole and exemestane which are used effectively in the treatment of postmenopausal breast cancer patients.

3.4. Risk reducing surgery

Women who carry a high breast cancer risk may consider the risk reduction option of preventive surgery. Existing data suggest that preventive bilateral mastectomy in women at high risk due to BRCA1 or BRCA2 gene mutations, may reduce by about 90% the chance of developing breast cancer. Preventive salpingo-oophorectomy also reduces the risk of developing breast cancer by 50% and gynaecological cancer (ovarian, phallopian, primary peritoneal) by 80% in the same group of women[244]. The decision to participate in a clinical trial, take preventive medication, or undergo preventive surgery is an individual one and should be a result of informed consent. Both the benefits and the risks of the treatment must be discussed. The perceptions and the uptake of information varies widely among high risk women in different countries or centres within a country and depends much on the quality of risk counselling[245, 246].

3.5. Priorities in breast cancer research

The European Code Against Cancer[247] emphasises the importance of "*elucidating the natural course of disease progression and identifing disease subgroups with distinctive risk profiles and treatment susceptibilities"***.** Priorities for breast cancer research include, the identification of biomarkers, the molecular analysis of the transition from pre-invasive to invasive disease, and the need for extensive databases so data can be assimilated and exploited for maximum benefit. High priority should also be given to research aimed at the study of pharmacological and natural compounds that could potentially prevent the development of breast cancer in susceptible patients[248]. More research is needed to identify potential chemicals, measure the exposure to them and explore their roles individually in the pathogenesis of breast cancer during and after breast development. Therefore, new techniques of accurate detection of multiple chemicals should be developed.

During the last two decades research has concentrated in identifying biomarkers and developing aggregate profiles of breast cancer in specific genes and proteins. This will allow in the future, the tailoring of therapy to individual molecular profiles, and tumour microenvironments. It is also important to communicate the implications of breast cancer risk, to both physicians and individual women in such a way that they can make informed decisions about screening and participating in clinical trials. As large population studies are needed to obtain reliable data, researchers and organisations should also identify and overcome barriers to participation of patients or healthy individuals in studies, especially those that similarly to our study require provision of biologic specimens.

4. THEORETICAL REASONS FOR QUESTIONING THE SAFETY OF UNDERARM COSMETICS

4.1. Deodorants and antiperspirants, mechanism of action and ingredients

Deodorants and antiperspirants are among the most commonly used cosmetic products. The first commercial deodorant was launched in 1888. A recent report (Mintel market research 2007) states that over 94 per cent of women and 89 per cent of men use these products in the UK, which is higher than any other country in Europe. The market for antiperspirants and deodorants reached $1.9 billion in the U.S (2005 Mindbranch market research) while in the UK (Mintel market research 2006) it was valued at £459 million. Western lifestyle is increasingly adopted in eastern countries such as India China and Japan, were deodorants market presents a strong growth assisted by the leading companies of the cosmetic industry of the west.

Most consumers use these products on a daily basis as a lifetime habit.They are applied in the armpit area until they are rinsed off with a bath which is usually followed by new application either on intact or most likely on shaved skin. Women are adviced though not to use cosmetics over broken skin (e.g. after shaving). There are significant differences in the way these products work. Antiperspirants reduce sweating and therefore can be used under the arm only. Deodorants aim to prevent odour by reducing the levels of bacteria, but do not reduce sweating. Both of the products may contain perfumes and fragrances to minimize and cover body odour. However, deodorants can be used not only under the arm but also all over the body. Antiperspirants control underarm sweating by blocking the pores and preventing discharge of perspiration. Beacause of their effect in the physiological body functions, in the United States, antiperspirants are certified as drugs.

There are no recommendations regarding dosage and age safety especially before puberty. Animal studies have demonstrated that the tumorigenic effect of chemical carcinogens is maximal in younger subjects in which the mammary gland is undifferentiated and highly proliferating[249]. In aerosol products the on-product label

advises the user to spray onto the skin surface from a minimum distance. However none of the products labels determines either the quantity to be applied or the effectiveness in realtion to the way of application. Consequently, the applied quantity depends on how long the user sprays, or rolls the product and therefore can vary significantly between different users. Concerns have been raised for the safety of several chemical ingredients that are contained in both deodorants and antipersirants. As a respond to that many companies are increasingly offering "natural" alternatives. The most common ingredients found in deodorants and antiperspirants and their intented actions are listed below:

- Perfumes and fragrances: they mask body odour
- Emollient oils: they prevent water loss.
- Glycerine or vegetable derived oils: they moisturise the skin
- Masking oils: they minimise stains.
- Alcohol: it dissolves the active ingredients
- Silica*[6]: it clears the oiliness of sweat
- PEG-8 Distearate: it makes easier to wash of the product
- Structure providers: water, cyclomethicone*, disteardimonium hectorite, hydrogenated Castor Oil, 18-36 Acid Trygliceride, Stearath**[7]
- BHT antioxidant: it keeps ingredients in optimal state until use.
- Butane, Isobutane and Propane: Aerosol propellants that produce a spray.
- Talc*
- Propylene glycol

Aluminium

Aluminium salts are the main active antiperspirant agents and include aluminium chlorhydrate and aluminium – zirconium chlorhydrate glycine complexes. Some natural deodorants also use a crystal form of aluminium (ammonium alum). Their mechanism of action involves the formation of a physical plug at the top of the sweat duct, which prevents the escape of sweat onto the body surface[250].

Aluminum is the most widely distributed metal in the environment comprising with its compounds about 8% of the Earth's surface. Aluminium metal, powders and oxides,

[6] * ingredients listed as high priority for review by CIR
[7] ** identified by the CIR Expert Panel as 'may need reconsideration - revalidation'

are widely used in construction, transportation and packaging industry as well as in the manufacture of several industrial and household products and food. Aluminium hydroxide is used in pharmaceutical and personal care products[251]. Occupational exposure to aluminium has been related to an increased bladder cancer risk and the "production of aluminium" has been classified as carcinogenic by the International Agency for Research on Cancer (IARC)[252] in 1987. Limits have been introduced in several countries for the maximum exposure to aluminium dust and aluminium oxide. Food, water and medical preparations such as antacids are the main sources of aluminium intake in the absence of occupational exposure. Although the theory that links aluminium with the pathogenicity of Alzheimer's disease has been mostly discarded, there are still numerous ongoing studies about its possible neurotoxic and other effects on human tissues[253, 254]. Aluminium salts have been linked to the development of granulomas. It has been demonstrated that aluminium in the form of aluminium chloride or aluminium chlorhydrate can interfere with the function of oestrogen receptors of MCF7 human breast cancer cells. Due to this type of oestrogenic effect it has been categorized as a metalloestrogen[255]. Aluminium has been measured in breast cysts fluid[256].

Triclosan

Triclosan is a broad spectrum antimicrobial agent that has been used extensively for almost four decades in several consumer products including underarm cosmetics. Clinical and experimental studies that had been perfomed in the past to determine its possible toxicity, mutagenicity, carcinogenicity and effect on reproduction had shown that triclosan could be considered safe for use[257]. Although remaining under investigation, it is approved for use in dentifrice and mouthrinse products (toothpastes, oral dentifrices for plaque control etc) in many European countries since the early nineties[258]. The chemical structure of triclosan resembles known non-steroidal estrogens (e.g. DES, bisphenol A). Foran et al (2000) studied its potential action as an endocrine disruptor. Results from their study did not support the estrogenic hypothesis but suggested potential weak androgenic effect[259]. Due to Triclosan's common use in industry, it is often detected in waste-water effluent. Researchers that investigated its effects on various life stages and reproduction of fish, concluded that it is highly toxic on the early life stages, and that its metabolite may have weak estrogenic properties[260]. Triclosan has also been found present, among other pollutants and known endocrine

disrupters, in other locations and environments were changes due to estrogenic effects have been demonstrated in fish[261]. Veldhoen et al (2006), demonstrated that exposure to low levels of triclosan induces changes in thyroid hormone-mediated processes proving its disrupting effect on North American bullfrog[262]. The negative effects of triclosan on the environment have led the Swedish Society for Nature Conservation (http://www.snf.se) to recommend not using triclosan in toothpaste. Triclosan and its metabolites have been found in higher concentrations in breast milk of women who use personal care products, suggesting multiple sources of systemic exposure as well as the need for further studies to assess the possible negative effects on breastfed babies[263, 264]. A recent study by Gee et al (2008), demonstrated clearly that triclosan possesses oestrogenic and androgenic properties which warrants further investigation for a possible impact on human health[265].

Parabens

Alkyl esters of p-hydroxybenzoic acid (parabens) are a group of chemicals with bactericidal and fungicidal properties that have been widely used as preservatives in the cosmetic pharmaceutical and food industries, because of the strong record of efficacy, stability, low cost, and rapid excretion from the body[266]. Preservatives are necessary components in most cosmetics and skin care products because they keep them sterile and prolong the length of the product use and expiring date. The antimicrobial effect of parabens and their use as preservatives were first established in 1924 by Sabalitschka. In 1981, the Food and Drug Administration reported that four parabens compounds were used as preservatives in more than 13,200 formulations, including most cosmetic products[267]. This number has raised to 22,000 in 2008 according to the FDA report. Almost 90% of all cosmetics contain one or more paraben ingredient. Parabens are often used in combination with other types of preservatives to provide preservation against a broad range of microorganisms[268, 269]. Products that contain parabens include: deodorants, makeup, moisturizers, hair care products, and shaving products shampoos, cleansing gels, personal lubricants, topical - parenteral pharmaceuticals and toothpaste. They are also used as food additives [270, 271].

Common parabens include methylparaben (E218), ethylparaben (E214), propylparaben (E216), and butylparaben (E215). Less common parabens include isobutylparaben (E217), isopropylparaben (E218), benzylparaben and their sodium salts. Parabens can

be found naturally in raspberries and blackberries. Exposure to parabens may occur through ingestion, eye or skin contact and inhalation. They are completely absorbed from the gastrointestinal tract and through the skin. They are hydrolyzed to p-hydroxybenzoic acid, conjugated, and the conjugates are rapidly excreted in the urine[272, 273, 274]. After oral administration, parabens are hydrolyzed by nonspecific esterases, widely distributed in the body, including the gut[275]. After dermal exposure, parabens can also be hydrolyzed by esterases present in human skin and subcutaneous fat tissue[276]. Unhydrolyzed parabens may be also be excreted in urine after exposure. It has been believed for decades that parabens contained in cosmetics are safe, because of the low doses involved and the fact that they are unlikely to penetrate into the tissue, remain intact, and accumulate there[277]. However, recent studies have highlighted the fact that these chemicals are not so readily excreted when applied directly to the skin. They have also been found in samples taken from breast tumors, suggesting that at least a fraction of the parabens can be absorbed without hydrolysis. The accumulative effect of applying these chemicals to our skin on a daily basis is not clear[267]. The epidemiology, structure, and properties of parabens have been evaluated in several studies that investigated:

- Effects on enzymes and other biochemical parameters
- Toxicological effects, acute and subchronic, Cytotoxicity
- Skin permeation
- Carcinogenicity
- In vitro immunosupression
- Embryotoxicity
- Mutagenesis effects
- Antimicrobial effects
- Immunotoxicity and sensitivity
- Estrogenicity

Parabens proved in all studies non-mutagenic, non-teratogenic and non-carcinogenic. It is established that they can cause skin irritation and contact dermatitis in a small percentage of the general population who present paraben allergies. Sensitization has occurred when medications containing parabens have been applied to damaged or broken skin[277] or intravenously. A recent review on butyl-paraben studies illustrates

irritation of the GI tract in large doses, amyloidosis in rats, cytotoxicity in vitro, adverse effects on reproductive system of male rats after maternal exposure, but no mutagenicity or carcinogenicity[278]

In vivo and in vitro assessment tests of a compound's estrogenic activity include:

- Binding activity to estrogen receptor assays
- Estrogen and progesterone receptors expression assays
- Regulation of CAT gene expression
- Proliferation of MCF-7 cells.
- Uterotrophic assays
- Male reproductive-tract effects

4.2. Regulation of chemicals used in cosmetic products

The legislation in UK includes a list of 769 chemicals which may not be included in cosmetics and it sets out maximum concentrations of 56 chemicals which can be used as preservatives, together with other restrictions on their use. All cosmetics and household products, and their ingredients, must be tested for safety. Since June 2007, companies which import or manufacture chemicals, including those used in cosmetics and household products, have been required to provide safety data on all their products, together with an assessment of risk, in order to register them under an EU-wide system for testing the effects of chemicals on human health and the environment[279].

In the European Union, parabens are regulated by Cosmetic Directive 76/768/EEC, Annex VI, part 1, reference 12. They can be used as a preservative up to a maximum concentration of 0.4 % in the finished product for 1 ester and up to 0.8 % for mixtures of esters. The most recent findings on the oestrogenic effects and the effects on the male reproductive system, were reviewed in 2005 by the Scientific Committee on Consumer Products (European Comission)[280]. It was concluded that more information would be needed before making a final statement on the maximum concentration of propyl, isopropyl, butyl and isobutyl paraben allowed in cosmetic products. The maximum authorized concentrations remained unchanged for the methyl and ethyl parabens. Data were requested before end of March 2005 regarding in vitro

percutaneous absorption studies, as well as studies of the reproductive and developmental toxicity of propyl, isopropyl, butyl and isobutyl paraben. In 2006 and 2008 the main conclusions of SCCP remained unchanged as the further data provided were inconclusive[281, 282].

Cosmetic products and ingredients in the U.S.A (with the exception of color additives) are not subject to FDA (Food and Drug Administration) premarket approval authority. Any ingredient may be used in the formulation of a cosmetic provided that the ingredient and the finished cosmetic are safe, the product is properly labeled, and the use of the ingredient does not otherwise cause the cosmetic to be adulterated or misbranded under the laws that FDA enforces. According to 2000 FDA statistics, 89 percent of the 10,500 ingredients used in personal care products have not been evaluated for safety by regulatory agencies or review panels, or anyone else. The FDA though, can only have a product removed from the market if they can prove it harmful in a court of law.

…"FDA believes that at the present time there is no reason for consumers to be concerned about the use of cosmetics containing parabens. However, the agency will continue to evaluate new data in this area. If FDA determines that a health hazard exists, the agency will advise the industry and the public, and will consider its legal options under the authority of the FD&C Act in protecting the health and welfare of consumers". (March 2006)

The Cosmetic Ingredient Review (CIR) program was established in 1976 by the Cosmetics, Toiletry, and Fragrance Association, with the support of FDA and the Consumer Federation of America (CFA). CIR performs independent, expert reviews to determine if ingredients used in cosmetics are safe. In 1984 review of the safety of methylparaben, propylparaben, and butylparaben concluded they were safe for use in cosmetic products at levels up to 25%. (parabens are used at levels ranging from 0.01 to 0.3%) . Up to June 2005, safety assessments were performed on only about 10% of the ingredients allowed in cosmetic and personal care products, including deodorants. Only nine out of almost 1200 of those have been deemed unsafe for use in cosmetics and the safety issue has been described. The available data were found insufficient to support the safety of 114 ingredients[283]. In 2003, the CIR began the process to reopen the safety assessments of methylparaben, ethylparaben, propylparaben, and

butylparaben in order to offer interested parties an opportunity to submit new data for consideration. In September 2005, the CIR decided to re-open the safety assessment for parabens to request exposure estimates and a risk assessment for cosmetic uses. In December 2005, after considering the margins of safety for exposure to women and infants, the Panel determined that there was no need to change its original conclusion that parabens are safe as used in cosmetics[284].

4.3. Estrogenic properties and other effects of parabens

In 1998 Routledge et al were the first to present findings from in vitro and in vivo (uterotrophic) studies which confirmed the weak estrogenic properties of parabens[285]. They suggested that particular attention should be paid to estimating the actual levels of systemic exposure of humans to these chemicals. Other experiments on animals, yeast and human cells have also confirmed that parabens have weak estrogenic activity, acting as xenoestrogens[286, 287, 288, 289, 290, 291, 292, 293]. Their lower activity in vivo compared to in vitro, may be attributed to their metabolism to non-estrogenic metabolites. The estrogenic activity of parabens is also significantly lower when compared to a control oestrogen, leading to the assumption that there is a large margin of safety between the average exposure to these chemicals from underarm cosmetic use and the doses that cause estrogenic effect[294]. Whether endocrine active chemicals with weak activity have similar risks to known estrogens remains a question that needs further investigation. The estrogenic activity of parabens is dose-related and increases with the length of the alkyl group (methyl>ethyl>propyl>butyl). The fact that some estrogens are known to drive the growth of tumours has raised questions about the possible carcinogenic effect of the weak estrogenic activity of parabens contained in cosmetics[295]. Golden et al (2005) compared parabens to 17beta-estradiol and diethylstilvestrol and concluded that parabens could not increase the risk of estrogen-mediated side-effects. The risk was demonstrated to be even less comparing to exposure to naturally occurring endocrine active compounds in the diet[296].

The permeation of parabens through human skin and their accumulation in skin layers or other tissues has been studied in in-vivo, ex vivo and in vitro studies[288, 297, 298]. These indicated that parabens have significant increasing permeations in skin layers,

thus promoting accumulation, which may be associated with skin toxicities and carcinogenicity. The extent of penetration depends more on paraben characteristics (solubility, lipophilicity) rather than on the composition of the applied cosmetic. Janjua et al[299] demonstrated that parabens as well as some other chemical compounds can be systemically absorbed in man after topical application. In their study, systemic absorption though did not influence the levels of reproductive and thyroid hormones. Side-effects evaluation studies of cosmetic preservatives, have shown that parabens have similar skin irritation potential at the minimal inhibitory concentration of each preservative[300]. Chronic topical application of parabens may lead to prolonged estrogenic effects in skin. Skin anti-aging benefits of many topical cosmetics and pharmaceuticals could be derived, in part, from the estrogenicity of parabens[301]. Whilst an oestrogenic environment is known to influence breast cancer incidence promotion growth and progression it remains uncertain as to its role in the genetic changes associated with initiation of cancer[302]. In vitro and animal studies have also demonstrated antiandrogenic and hepatotoxic properties of parabens[303, 304].

4.4. Public awareness, the media and advertising policies on the safety of parabens and aluminium

We conducted an online review of the three top selling newspapers in the UK for the year 2002, to retrive articles that would contain references on parabens and aluminium link with breast cancer. A total of four articles were found in one newspaper only (Daily mail**)** for the whole period 1997 - 2003. These articles referred to the usage of parabens as preservatives in consumer products, as well as their oestrogen mimicking activity, skin irritation side effects and queried on their possible carcinogenic properties. They also commented on the unknown effect of a combined product use. The first study to raise public awareness on parabens through the media reports was published two years later by Dr Darbre et al[304]. The number of article references in the media increased dramatrically, and from 2003 to 2010 67 articles were found in the same newspaper.

As parabens became increasingly a popular and controversial subject in the news during the last decade, some organizations objected to their everyday use and declared the need for further safety assessments. Cosmetic companies that use them in the

manufacture of products, often interpret the absence of evidence as evidence of safety. However, although it is claimed that they are totally safe, many manufacturers advertise their products' safety by declaring the absence of parabens and other "suspicious" chemicals in them or stating the potential dangers that can arise from their usage[308]. Typical examples of online advertisements are listed below:

Unilever: *"The antiperspirants we make are self-preserving. This means that they do not need preservatives like parabens to work effectively. Parabens are, however, used safely to preserve a wide range of other everyday products, and do not cause breast cancer. .... All deodorants and antiperspirants are thoroughly assessed for safety and meet strict legal safety requirements".*

Lonza: **"***...announces the launch of a new product to meet the growing demand for alternative cosmetic preservatives. With growing concerns over traditional preservatives such as parabens, the new product provides a single solution to companies seeking alternatives to traditional preservatives."*

Avea**:** *"Buy paraben, petroleum, phthalate and SLS free organic skin care and cosmetics online....*

Purist Company: *..."Parabens are not used in any products... Many cosmetic ingredients…are preserved with parabens. When a cosmetic manufacturer uses paraben-preserved ingredients in their formulations, they are not required to list parabens in the label ingredient listing. They are classified as "incidental ingredients" when used in this way, and are excluded from the usual ingredient disclosure rules… We also avoid using ingredients that have been preserved with parabens.***"**

4.5. Studies that investigate the link hypothesis between underarm cosmetics and breast cancer

The relation between deodorants and breast cancer had circulated in the internet before any clinical epidemiological study was performed. The rising incidence of breast cancer suggests a link between the disease and lifestyle. For example, the application of antiperspirants or deodorants following axillary shaving has been an increasingly

adopted lifestyle factor during the last 5 – 6 decades raising concerns about its possible effect on the incidence of breast cancer.

The first study with epidemiological evidence on this hypothesis was conducted by Mirick et al who investigated the possible relation between the use of underarm products and breast cancer risk[309]. They also investigated whether armpit shaving could be a possible contributor to absorption of harmful chemicals contained in deodorants and antiperspirants. The authors concluded that the use of the above products did not increase the risk of breast cancer.

Mc Grath et al, made the hypothesis that between women who had survived breast cancer, those who regularly used deodorants and antiperspirants and shaved their underarms would be expected to have an earlier age of diagnosis. An earlier age of diagnosis would also be expected in those starting to use deodorants and shaving at an earlier age. They investigated the mean age of diagnosis in relation with the overall frequency of product usage. The study results suggested that frequency and earlier onset of antiperspirant / deodorant usage with underarm shaving were associated with an earlier age of breast cancer diagnosis implying a possible link between antiperspirant - deodorant use and breast cancer[310].

Parabens were found intact in the human breast tumours[304]. This is in line with the general link hypothesis between oestrogenic compounds used in cosmetics and breast cancer. However, the association alone, does not prove causation in these patients[311]. This study did not compare with control samples, either from healthy individuals or from healthy parts of the breast. More information needs to be obtained on whether body burdens are different in cancer from those in normal tissues. Methylparaben was detected in greatest amounts reflecting either the more widespread use of methylparaben or its greater ability to be absorbed into body tissues and to resist hydrolysis.

Sharpe and Irvine state that the greatest concern of health effects from endocrine disruption is through exposure during pregnancy and foetal exposure in utero, and they suggest ways to minimize risk in individuals by life style changes in women seeking to become pregnant (stopping smoking, reduced use of cosmetics and body creams)[312].

The upper outer quadrant of the breast is the area closest to the armpit were underarm cosmetics are applied and the most frequent site for incidence of breast cancer. It has been believed that this could be explained by the higher concentration of epithelial tissue in that region. This is inconsistent with the fact that the reported incidence in this quadrant appears to rise with year of publication. Other authors have demonstrated greater genomic instability in outer quadrants which could also partially explain the propensity for breast cancers to develop there[313]. Identification of the reasons for disproportionate site-specific increase could provide clues as to causative factors in breast cancer[314].

Despite the evidence on endocrine disrupting activity of many chemical components, most of them have not been individually tested and revalidated under new safety guidelines. Also, most of the safety assessment studies ignore any possible synergistic effect of other xenoestrogens and phytoestrogens. Most studies have investigated single factors for their safety in vitro. However it remains unknown whether the levels reached in humans, can be sufficiently high to exert similar biological actions. Silva et al (2002) studied the effects of multicomponent mixtures of xenoestrogens with each component at concentrations below its individual NOEC (No Observed Effect Concentration). They concluded that the mixture of estrogenic agents can produce significant effects when combined at concentrations below their NOECs. Similarly This finding highlights the limitations of single agents investigation studies. Consequently, safety assessments that ignore the possibility of joint action of chemical mixtures can lead to underestimations of risk[242, 315]. Up to date there is no sufficient information on whether parabens can affect human health on a long-term use basis by themselves or in combination with other chemicals.

4.5. Conclusions

- While the role of oestrogen in the development and progression of breast cancer is well established, there is a continuous exposure to a wide range of chemicals with proved oestrogenic effects. These compounds have been isolated in several studies from tissue and serum samples.

- Most of the risk assessments on the safety of parabens were conducted before it was known that they could act as environmental estrogens and be detected in human tissue.
- The ability of parabens to permeate human skin and has been demonstrated
- There is lack of information on
    - long term low level absorption and accumulation effects
    - multiple chemicals concentrations and effects in sample tissues – micture effects.
- There is a need for risk assessment practice modification. Revalidation of individual compounds and mixtures should be considered in some cases.
- Until today there are no safety guidelines that comment on the quantity and pattern of usage of underarm cosmetics especially according to individual susceptibility, age, or genetic profile.
- There is a need to investigate the molecular basis of a possible link between environmental oestrogens and breast cancer.

5. A STUDY OF PARABENS AND ALUMINIUM CONCENTRATION WITHIN HUMAN BREAST TISSUE

5.1. Introduction

Although the incidence of breast cancer has been increasing worldwide over the past thirty years, no specific causes have been found yet. The factors that may promote the development tumour remain under investigation, but as with all cancers breast cancer is a result of gene mutations and is therefore considered a genetic disease. The inherited gene mutations are responsible for almost 25% of all breast cancer and cases while the rest occur sporadically[316]. Within the group of familial cancers up to 5% are associated to BRCA1 and BRCA2 genes mutations[317]. The rest are attributed to multiple genes with varying levels of biologic activity and lower penetrance. The susceptibility of the individuals to the disease depends on the gene polymorphism the number of genes present and the way they interact with each other[318, 319]. Breast cancer has also been associated a group of rare familial cancer syndromes that include Cowden, Li-Fraumeni, Peutz-Jehger, ataxia-telangiectasia and Muir-Torre syndrome[316]. Lifestyle and environmental factors can play a role in the expression of abnormal genes and may influence the susceptibility to breast cancer, not only between different individuals or populations, but also between different generations within the same families. These factors also influence the risk for sporadic cancers as well as the outcome of the disease[320].

Changes of the female breast are partly a result of the fluctuation of estrogens. Factors that increase estrogen exposure throughout lifetime, like early menarche, late menopause, use of OCP and HRT, raise the risk of developing breast cancer, among pre- and postmenopausal women. Estrogens may be involved in the initiation of a tumor development either by oxidative damage to DNA from their metabolic byproducts or by altering gene expression that stimulates growth and proliferation epithelial cells. Artificial chemicals, especially those with evidence of estrogenic action, may exert a similar action, by damaging DNA and promoting growth of damaged breast cells. The most extensively studied organochlorine pesticides, as well

as other chemicals that have been banned for decades, are persistent environmental pollutants that concentrate in the food chain and can be detected consistently in tissue blood and milk samples as they. Current research trends highlight the importance of determining how many environmental chemicals people are exposed to on a daily basis and how many of those are present in the human body.

Underarm cosmetics contain a cocktail of chemicals that are applied frequently to the body, without question of toxicity, on the area directly adjacent to the breast. They are not rinsed off each time, thus allowing for local accumulation to occur and may penetrate continuously through the skin without invoking any major physiological carrier, such as blood or lymphatics.The main active ingredients of these cosmetics are antiperspirant agents, deodorants and preservatives. These are a wide range of chemicals known to exert a variety of toxic effects.

Alkyl esters of *p*-hydroxybenzoic acid (parabens) are widely used as preservatives in cosmetics and other products, owing to their high antimicrobial acivity and these are known to possess oestrogen-mimicking properties in human breast cancer cells. Although parabens have been considered safe for many decades it has been recently suggested that they can accumulate in tissues and they have been identified within breast cancer tissue samples. The pattern of possible accumulation and concentrations within the female breast is currently unknown. The Aluminium-zirconium salts and aluminium chlorhydrate are the main antiperspirant components. Their mechanism of action involves the formation of a physical plug at the top of the sweat duct, which then prevents the escape of sweat onto the body surface.

One theory, amongst many, for the observed trend of cancer incidence in the upper outer quadrant is that this area lies closest to the axilla, and hence to where underarm cosmetics are applied and their ingredients may accumulate in higher concentrations. This hypothesis has not been supported by scientific evidence. However, the diversity in usage of cosmetics and the range of different products available provides ample possibility for breast cancer to arise through issues of quantity used, through pattern of usage or through individual susceptibility to specific product formulations.

5.2. Purpose

The purpose of our study was to investigate the presence of parabens and aluminium in healthy breast tissue obtained from mastectomy specimens of breast cancer patients. We also aimed to determine whether there is a gradient of concentration across the breast, in four different regions from the axilla to the sternum. We studied the plausibility of a causative relationship between parabens, aluminium and breast cancer. Finally, we attempted to shed light on the link hypothesis between the use of underarm cosmetics and breast cancer.

5.3. Population and methods

We recruited 40 breast cancer female patients from the Nightingale and Genesis Prevention Centre at Wythenshaw hospital in Manchester, UK. Inclusion Criteria for this study were: a) age 18 years or above. b) Subjects who would require single or bilateral mastectomy for their primary breast cancer and c) Subjects who would be able to give voluntary, written informed consent to participate in the study and from whom consent was obtained. Women who did not / were unable to give voluntary written informed consent and those who would not have a mastectomy as part of their primary treatment were excluded.

Each subject considered eligible for entry into this study and from whom voluntary, written informed consent was obtained, had a screening assessment in the form of a questionnaire with the following information recorded: Age, age at Menarche, if the subject is right or left handed, if they are vegetarian, if they live in a rural or urban environment, whether they have ever used underarm cosmetics, whether they regularly use antiperspirants/ deodorants or a combination, age at which the subject first started using underarm cosmetics.

Breast tissue samples were obtained as follows. A single sample of tissue was obtained from four different regions of each removed breast on a transect from the outer to the inner (axilla, lateral, central and medial). The purpose of this was to evaluate the hypothesis of concentration gradient in the measured parabens from the axilla to the

sternum. Each of the samples was divided in four smaller samples - two of which were kept for future studies - which were immediately stored in cryovials and deeped in liquid nitrogen. The samples were stored and assigned a code as to the exact site within the breast from where they came. This would blind the laboratory personnel testing for the chemical concentration until the last sample had been analysed. A total of 16 cryovials containing breast tissue from each patient were store in -80^{o}C at the laboratory of pathology in the Nightingale centre.

The samples were then transported in two groups, inside sealed boxes, containing dry ice to the University of Reading to continue witht the extraction of chemicals. The samples were analysed for the concentrations of a series of paraben esters (methylparaben, ethylparaben, *n*-propylparaben, *iso*-butyl-paraben, *n*-butylparaben). All sample extraction was carried out by the University of Reading (Dr Philippa Darbre), with dried samples being supplied by courier to M-Scan Ltd Geneva. The samples were stored frozen (ca. – 18°C) except when being analysed.

Chemical standards

Methylparaben, ethylparaben, n-propylparaben and benzylparaben were purchased from Sigma (Poole, UK). Isobutylparaben was a gift from NIPA laboratories (Mid-Glamorgan, UK). 13C6-n-butylparaben (Cambridge Isotope Laboratories Inc, MA, USA) was prepared in methanol.

Extractions of parabens from human breast tissue

All glassware was soaked overnight in concentrated sulphuric acid, rinsed at least 6 times in HPLC-quality water, soaked overnight in 1M aqueous NaOH, again rinsed at least 6 timesin HPLC-quality water and left to air-dry. No plasticware was used for any extractions, only glass homogenisers, glass tubes and glass pipettes were used and all glassware went through the same treatment as above. Weighed samples of human breast tissue (100-700mg) were homogenised in 6.25ml ethanol/acetone (1:1 vol/vol) in a glass homogeniser. This mixture was left with periodic shaking overnight at room temperature in a glass Corex tube. The next day, the mixture was centrifuged at 2,500rpm for 10 min in a bench centrifuge at room temperature. The supernatant was transferred to a clean Corex tube. The pellet was re-extracted with a further 1.5mm ethanol: acetone (1:1), centrifuged at 2,500rpm for 10 min at room temperature and the

resulting supernatant combined with the initial supernatant. The combined supernatants were evaporated to dryness under a stream of clean air at room temperature for 2-3h in a fume hood. The dried extract was taken up in 3mm 70% (v/v) aqueous methanol, vortexed well and placed overnight at -20^{0}C. The next day, the mixture was centrifuged at 3,200rpm (rotor Sorvall SW50) for 20min at 4^0 in a precooled rotor/centrifuge. The supernatant was collected and transferred to a clean glass tube. The fat pellet was washed with a further 0.5ml ice-cold 70% (v/v) aquous methanol, recentrifuged under the same conditions and the supernatants combined. The combined supernatants were mixed and were divided into two equal samples in new screw-cap autosampler vials (Agilent tecnologies) and each dried down in a Speedivac.

Analysis by HPLC MS/MS

Analysis was performed in the laboratories of MScann in Geneva, Switzerland using an Agilent 1100 HPLC system and ABI/Scieux API3000 triple quadrople mass spectrophotometer operating in the on-line Liquid Chromatography-Atmospheric Pressure Chemical Ionisation-Mass Spectrometry-Collisionary Activated Dissociation-Mass Spectrometry (LC/ACPI-MS-CAD-MS) mode. Extracts were taken up in 15mM ammonium acetate pH4.5 and samples (10µl) chromatographed on a reversed phase YMC-UltraHT Pro C18 column (50 x 2.0 mm) at a flow rate of 400 µl/min at 25^0 C and eluted with a linear binary gradient of 15mM ammonium acetate pH4.5 (solvent A) and actonitrile (solvent B) (ι =0 B 305; ι =4 B 70%; ι =5 B 70%; ι =5.1 B 30%; ι =11.0 B 30%). A set of twelve calibration standards were prepared in solvent A at concentartions of 0, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0, 10, 25, 50, 100 and 250ng/ml of each paraben ester (methylparaben, ethylparaben, n-propylparaben, n-butylparaben, isobutylparaben, benzylparaben and 25ng/ml of the $^{13}C_6$-methylparaben. Five quality control (QC) standards were also prepared at 0.5, 1.0, 5.0, 10 and 50 ng/ml of each paraben ester and 25ng/ml of the $^{13}C_6$-methylparaben.

These calibration standards were analysed and used to generate calibration curves. The QC standards were also analysed.

The samples were prepared as follows. 250 µl of a solution of the internal standard (25ng/ml) in buffer A was added to each vial. The vials were vortexed (10 seconds), sonicated (1 minute) and centrifuged (5 minutes at 16,000 RCF). The resulting supernatants were transferred to fresh HPLC vials before injection. An example of calibration curve to obtain data for the parabens, chromatogram from the samples and

standards analysed, and composite chromatogram for the parabens is shown in figures 11, 12 and 13.

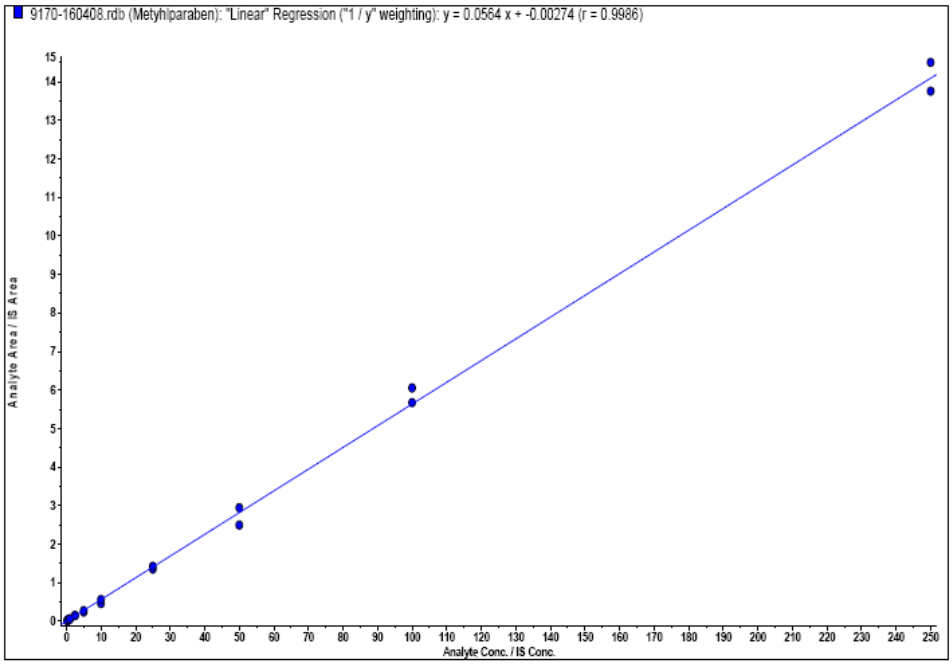


Fig 11. Example calibration curve for methyl-paraben

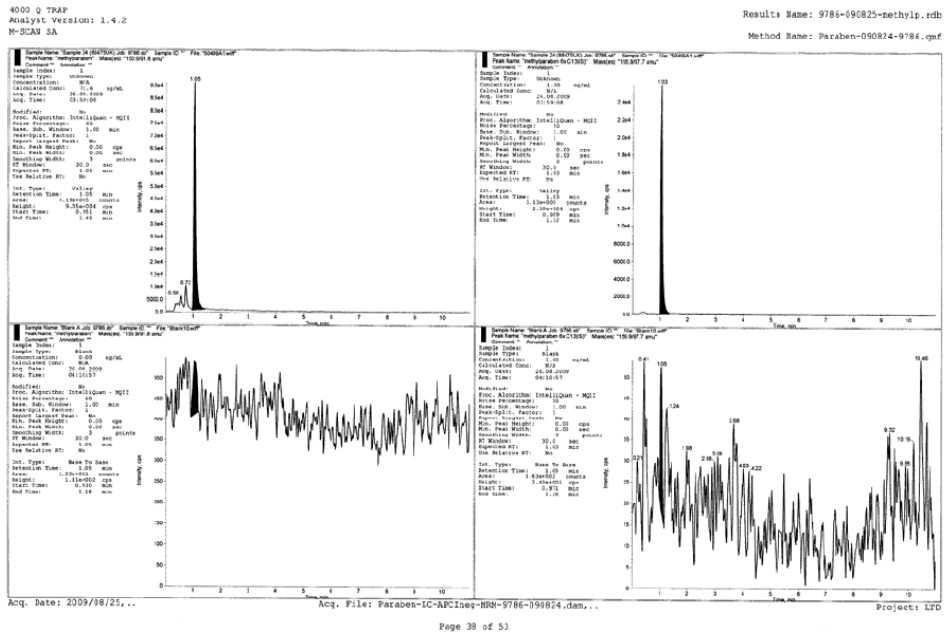


Fig 12: Analyses of parabens, example chromatogram

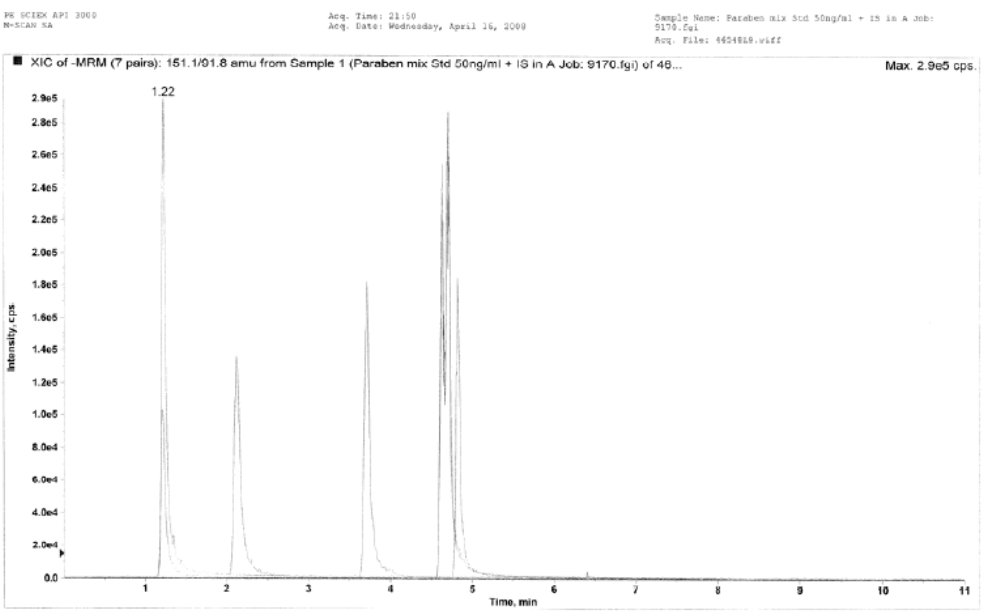


Fig 13: Parabens analyses, example of composite chromatogram

Generally, the accuracy was better for the methyl, ethyl and n-propyl parabens than for the isobutyl, n-butyl and benzyl parabens. The calibration curves have been used to calculate the levels of the methyl, ethyl, n-propyl, isobutyl, n-butyl and benzyl-parabens in all samples. The detection limit for the method has been estimated as 0.25ng/ml. Some of the concentrations observed were beyond the calibration range used. The concentrations from the tissue samples have been corrected for the amount of tissue used in ng/g of tissue.

Recovery of Parabens

For those samples that had been spiked, the estimated recovery of parabens was calculated by comparing the measured amount with that spiked. The recoveries for benzyl paraben were low. However, it is possible that the recoveries may be different for the alkyl parabens. The calculated recoveries have been used to correct the measured ng/g values and these are presented in table 8 in ng/g of tissue (corrected minus blank values).

Statistical Methods

After the completion of the calculations the samples were unblinded to allow statistical analyses. The Parabens data followed a non-Normal distribution and therefore median and range summary statistics are presented. Comparisons between sites were carried

out using Friedman tests and Wilcoxon signed-rank tests. Differences between different patient groups were assessed using Mann-Whitney U-tests or Krukal-Wallis tests as appropriate. Associations with continuous outcomes (eg age) were assessed using Spearman correlations. All tests used the conventional 5% significance level and were carried out using SPSS version 15.0 statistical software.

5.4. Results - Parabens

Parabens data was available on n=40 patients and questionnaire data on n=35 patients. Data from the medical records and the study questionnaire are presented in table 6. The mean age of all patients was 65 years, ranging from 37 to 91 years. The mean age of the thirty-five patients that returned the study questionnaire was 64.6 years, ranging from 37 to 91 years at the time of the study. There was only one vegetarian and two left handed women thus not allowing any statistical evaluation of these parameters. 80% of those patients (n = 28) had used deodorants for a period of time in the lives (users). Twenty-three of the users were currently using deodorants at the time they completed the study questionnaire (current users), while the rest five had stopped using them at some point in their life. Most of the women used a combined type of deodorant antiperspirant. The other 20 % (n = 7) of the patients who returned the study questionnaire, had never used underarm deodorants or antiperspirants (non-users). This relatively small number of patients would allow us to use them as a control group against women who were exposed to the underarm cosmetics chemical cocktail. There were therefore in total 12 non-current users (i.e. 5 past-users + 7 non-users). The mean age of the patients that had never used deodorants was 75.7 ranging from 55 to 85 years. The mean age of all patients who had ever used deodorants was 62.7 years. This significant difference in age between users and non users is in accordance to cultural and lifestyle changes that occurred in the last decades. From those patients who are currently using deodorants, data on length of use was available for 20 patients. The median length of use was 44 years, ranging from 11 to 56 years! Estrogen receptor status was available for 37 patients. Out of those 73% (n=27) were ER positive which is concordant with the general breast cancer population statistics. In thirty-five patients pathology demonstrated a single breast tumour. There was one multifocal and three bifocal tumours, while the exact location of the tumor was unavailable for one patient.

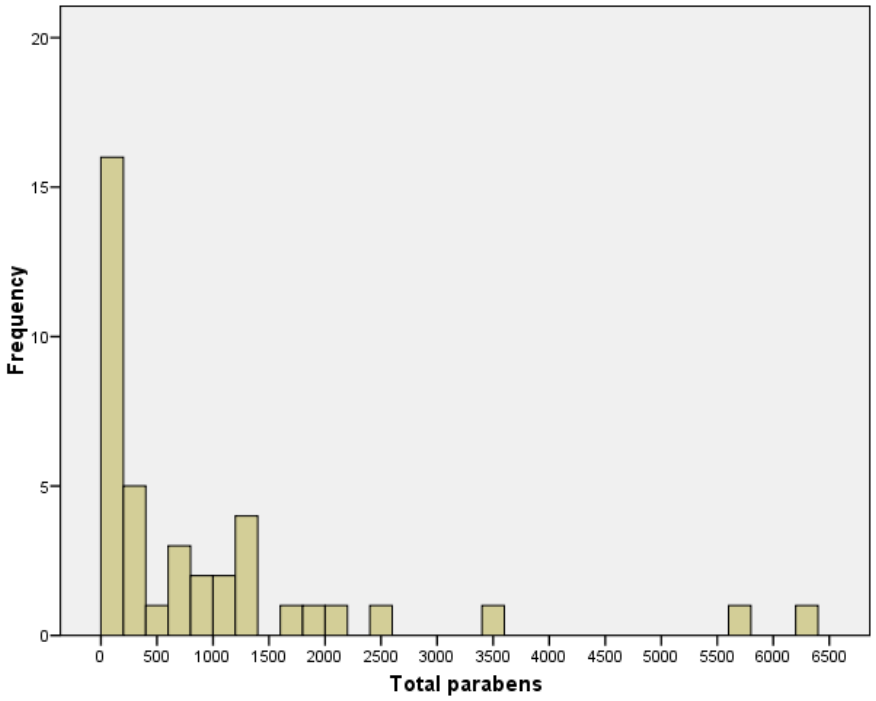
When related to the sample positions, the location of the tumour was lateral in n=16 patients, central (near the nipple-areolar complex) in n=14 patients and medial (near the sternum) in n=5 patients. There were no axillary tail tumors to correspond to the axillary samples.

	Age	Tumor location	ER	PR	Age @ Menarche	Parity Y/1 - N/0	Breast feeding	BF lenght	R/L Handed R/1-L/0	Vegeteria n Y/1- N/0	Urban (1)/ Rural (0)	User /1- NO/0	Age @ start Use	Current user Y/1-N/0	A/D/AD A/1- D/0-AD/2
1.	66	bifocal, LUIQ, LLOQ	+	+	12	1	1	4	1	0	1	1	16	1	2
2.	76	Multifocal	-	-											
3.	70	LEFT CENTRAL	+	+	15	1	0	0	1	0	1	1	20	1	0
4.	63	LEFT CENTRAL	-	-	13	1	1	2	1	0	0	1	18	1	2
5.	73	RIGHT CENTRAL	+	-	11	1	1	3	1	0	1	1	18	1	2
6.	45	LOML, LATERAL)	+	+	13	0	0	0	1	0	1	1		1	0
7.	52	LILQ, MEDIAL	+	+	12	1	0	0	1	0	1	1	15	1	2
8.	48	RLOQ, LATERAL	-	-	11	1	0	0	1	0	1	1	13	1	1
9.	53	RUOQ, LATERAL	+	+	15	1	0	0	1	0	1	1		1	0
10.	81	LUML, CENTRAL	+	+	13	0	0	0	1	0	1	1	14	0	
11.	79	BIF (L) LMED/LAT	+	+	14	1	1	3X9	1	0	1	0			
12.	63	LLIQ, MEDIAL	+	+											
13.	77	RUML, CENTRAL	+	+	12	0	0	0	1	0	1	1		1	2
14.	62	LUOQ, LATERAL	+	+	14	1	1	9+ 6	1	0	1	1	18	1	0
15.	85	LUOQ, LATERAL	-	-											
16.	52	RLOQ, LATERAL	-	-	16	0	0	0	1	0	1	11	14	1	2
17.	72	LUOQ, CENTRAL	+	+	12	1	1	5	1	0	1	1	16	1	2
18.	69	RLOQ, LATERAL	+	+											
19.	46	ROML, LATERAL	+	+											
20.	59	LUOQ	+	+	13	1	1	10	1	0	0	1	13	1	2
21.	85	LUOQ			10	1	1	3X8	1	0	1	0			
22.	60	RUOQ	+	-	10	1		6+ 3	0	1	1	1	16	1	2
23.	85	Paget Nipple R			14	1	0	0	1	0	0	0			
24.	74	RUOQ	+	+	18	0	0	0	0	0	1	1	40	0	
25.	69	MEDIAL UPPER R			15	1	1	2X9	1	0	1	1	15	1	2
26.	60	MEDIAL L	+	+	11	1	1	2+ 3	1	0	0	1	16	1	2
27.	68	CENTRAL L	+	+	14	1	0	0	1	0	0	1	16	1	2
28.	53	MEDIAL R	+	-	12	1	1	4	1	0	1	1	16	1	2
29.	55	LUOQ LATERAL	-	-	11	1	1	5	1	1	1	0			
30.	91	ROUQ LATERAL	-	-	11	1	0	0	1	0	1	1	14	0	
31.	79	CENTRAL L	-	-	15	1	1	11+5+7	1	0	1	0			
32.	72	MEDIAL	+	+	13	1	0	0	1	0	1	0			
33.	68	CENTRAL	-	-	14	1	0	0	1	0	0	1	57	0	2
34.	68	BIF (L) LMED/LAT	+	+	13	1	1	4+ 4	1	0	1	1	25	0	
35.	75	LUOQ LATERAL	+	+	14	1	0	0	1	0	0	0			
36.	38	LUOQ LATERAL	+	+	13	1	0	0	1	0	1	1	15	1	2
37.	52	BIF (L) LMED/LAT	+	-	11	1	0	0	1	0	0	1	15	1	2
38.	37	LUML CENTRAL	-	-	11	1	1	2	1	0	1	1	11	1	2
39.	71	L CENTRAL	+	+	16	1	1	2+ 2	1	0	0	1	60	1	2
40.	48	L LOWER BREAST	+	-	13	0	0	0	1	0	1	1	16	1	

Table 6 . Epidemiological, histological and lifestyle data obtained from patients notes and questionnaires. Highlighted are the patients that never used underarm cosmetics (n=7)

5.4.1. Total Parabens

The statistical analysis summarised the constituent parts using substituted zero values for the few negatives that occurred in the measurement of methyl-paraben, ethyl-paraben, etc. The following histogram demonstrates the distribution of total parabens values.



Graph 1: Distribution of total parabens values.

In table 7 we present the total paraben concentrations for the four different regions of the breast as well as the summaries of the inner and outer regions.

TOTAL PARABENS ng/grm tissue							
Pt	**Axilla**	**Lateral**	**Ax+Lat**	**Central**	**Medial**	**Cen+Med**	**In+out**
1	229.7	117.1	346.8	5134.6	214.6	5349.2	5696.0
2	48.6	47.3	95.9	60.4	48.8	109.2	205.1
3	86.1	51.8	137.9	1147.4	44.6	1192.0	1329.9
4	861.3	179.2	1040.5	125.8	172.4	298.2	1338.7
5	165.1	46.3	211.4	73.0	84.8	157.8	369.2
6	128.0	185.3	313.3	96.7	111.3	208.0	521.3
7	491.4	454.0	945.4	213.9	111.9	325.8	1271.2
8	99.8	1363.6	1463.4	106.0	141.3	247.3	1710.7
9	44.4	17.3	61.7	0	20.7	20.7	82.4
10	16.1	114.5	130.6	14.1	19.0	33.1	163.7
11	23.2	19.9	43.1	25.8	20.3	46.1	89.2
12	12.2	34.2	46.4	17.2	30.8	48.0	94.4
13	50.4	84.4	134.8	32.2	32.4	64.6	199.4
14	29.3	52.4	81.7	14.4	51.6	66.0	147.7
15	26.9	21.0	47.9	42.6	23.6	66.2	114.1
16	38.6	31.1	69.7	30.2	24.7	54.9	124.6
17	891.8	184.2	1076.0	43.6	52.0	95.6	1171.6
18	41.3	416.5	457.8	138.7	43.2	181.9	639.7
19	34.2	19.9	54.1	29.3	62.4	91.7	145.8
20	2322.3	1360.3	3682.6	1348.8	1280.9	2629.7	6312.3
21	1280.5	42.7	1323.2	810.6	485.7	1296.3	2619.5
22	33.8	35.7	69.5	58.5	12.9	71.4	140.9
23	20.3	13.4	33.7	353.1	491.0	844.1	877.8
24	126.8	51.5	178.3	126.4	62.9	189.3	367.6
25	49.8	60.6	110.4	57.7	28.7	86.4	196.8
26	933.5	71.2	1004.7	457.5	572.8	1030.3	2035.0
27	96.4	75.6	172.0	12.9	11.2	24.1	196.1
28	227.0	209.4	436.4	166.6	238.9	405.5	841.9
29	244.6	166.1	410.7	546.8	139.8	686.6	1097.3
30	462.4	255.3	717.7	2357.9	360.3	2718.2	3435.9
31	117.1	37.5	154.6	71.7	77.8	149.5	304.1
32	18.8	41.8	60.6	103.5	24.3	127.8	188.4
33	444.7	189.4	634.1	129.4	166.2	295.6	929.7
34	2.1	0	2.1	.3	5.1	5.4	7.5
35	117.6	102.5	220.1	106.9	124.0	230.9	451.0
36	204.8	302.1	506.9	464.1	1057.6	1521.7	2028.6
37	259.4	317.1	576.5	36.6	710.7	747.3	1323.8
38	16.3	13.6	29.9	7.3	2.7	10.0	39.9
39	98.7	122.0	220.7	118.0	120.1	238.1	458.8
40	139.8	187.2	327.0	142.3	177.2	319.5	646.5

Table 7: Total paraben ng/gm tissue. Patients who never used underarm cosmetics are highlighted

pt	METHYL				ETHYL-PARABEN				N-PROPYL-PARABEN				N-BUTYL-PARABEN				ISOBUTYL-PARABEN			
	A	L	C	M	A	L	C	M	A	L	C	M	A	L	C	M	A	L	C	M
1.	24.5	16.8	5102.9	44.0	3.4	2.4	3.7	4.6	195.2	17.5	24.6	153.9	6.5	27.0	3.1	9.9	0.1	53.4	0.3	2.2
2.	17.1	21.8	23.1	16.1	3.4	4.2	3.4	3.6	8.9	7.7	10.7	8.4	14.8	9.5	17.4	16.3	4.4	4.1	5.8	4.4
3.	31.0	19.9	1126.6	21.0	4.9	2.4	2.3	1.9	12.1	9.6	5.9	5.7	31.0	15.5	10.8	13.6	7.1	4.4	1.8	2.4
4.	62.5	63.5	58.3	54.8	157.8	12.3	7.8	9.4	46.7	40.8	35.8	57.0	0.0	52.1	18.4	37.1	594.3	10.5	5.5	14.1
5.	51.7	17.6	25.6	25.6	13.8	3.5	5.1	5.5	36.3	10.3	13.6	21.5	51.7	11.6	18.6	21.5	11.6	3.3	10.1	10.7
6.	25.4	37.7	32.0	25.0	9.4	11.2	12.0	10.4	85.5	120.7	46.0	68.9	6.0	12.7	5.9	5.3	1.7	3.0	0.8	1.7
7.	17.3	19.6	24.2	23.4	10.1	15.8	12.6	12.2	461.4	411.8	168.3	73.1	1.5	4.4	7.1	2.0	1.1	2.4	1.7	1.2
8.	55.3	819.1	48.4	60.7	2.8	499.7	8.4	30.9	16.4	12.9	18.3	23.8	20.8	27.4	25.9	22.4	4.5	4.5	5.0	3.5
9.	15.4	8.1	0	11.9	2.3	1.2	0	1.1	15.1	1.2	0	0	10.2	6.2	0	7.7	1.4	0.6	0	0
10.	8.5	13.5	7.1	11.3	1.6	2.6	1.5	1.6	3.0	5.3	0	0	3.0	92.6	5.5	6.1	0	0.5	0	0
11.	13.2	9.7	10.8	10.8	2.1	1.2	2.3	1.9	4.6	1.6	6.3	2.1	3.3	7.4	6.2	5.5	0	0	0.2	0
12.	9.8	18.2	9.9	13.8	2.3	5.2	2.8	3.2	0	9.4	4.5	13.6	0	0	0	0	0.1	1.4	0	0.2
13.	21.5	37.7	13.5	11.4	6.7	9.5	3.3	3.3	12.2	15.6	8.8	10.6	8.9	18.7	5.3	6.0	1.1	2.9	1.3	1.1
14.	15.9	23.8	11.0	24.0	1.7	2.6	1.6	3.6	5.8	4.0	1.8	15.4	5.3	8.6	0.0	7.3	0.6	13.4	0	1.3
15.	18.5	13.8	23.6	13.1	1.4	1.4	2.4	1.7	5.0	4.1	7.4	5.3	1.4	1.2	5.1	2.4	0.6	0.5	4.1	1.1
16.	19.4	16.0	15.1	11.5	5.9	4.5	2.7	1.8	8.8	6.2	2.7	8.3	3.2	3.7	8.0	3.0	1.3	0.7	1.7	0.1
17.	18.6	18.7	14.7	9.8	2.4	3.7	2.6	1.8	5.9	7.8	5.6	4.6	62.0	34.7	15.1	14.8	802.9	119.3	5.6	21.0
18.	16.6	24.1	49.5	16.5	5.2	7.9	16.3	4.7	16.2	372.3	49.1	16.1	2.1	9.8	20.6	4.1	1.2	2.4	3.2	1.8
19.	16.7	11.8	16.6	30.8	3.7	2.7	3.0	3.7	4.4	3.8	7.4	18.1	7.7	1.5	1.9	7.8	1.7	0.1	0.4	2.0
20.	161.8	6.7	9.0	6.6	4.1	3.0	4.4	1.0	2052.7	1255.0	1249.5	1217.2	95.4	86.8	79.7	51.4	8.3	8.8	6.2	4.7
21.	6.1	3.5	2.9	3.2	3.4	1.9	2.2	1.2	1199.0	0	760.7	456.7	64.1	35.5	41.2	23.9	7.9	1.8	3.6	0.7
22.	0	27.8	3.2	5.8	0	2.9	0.4	2.8	0	0	0	0	33.8	3.6	4.0	3.8	0	1.4	50.9	0.5
23.	0.7	0	0.2	4.5	3.1	1.2	1.2	4.3	0	0	328.1	0	4.6	1.1	18.5	0	11.9	11.1	5.1	482.2
24.	0	0	0	0	0	0	0	0	123.9	46.6	62.9	62.9	2.9	4.9	5.1	0	0	0	58.4	0
25.	15.0	4.1	10.4	3.5	3.3	0.6	6.9	0.8	27.7	55.6	37.2	23.3	1.8	0	0	0	2.0	0.3	3.2	1.1
26.	11.2	3.8	3.8	4.1	2.8	0.8	1.1	1.6	881.5	48.3	431.2	534.0	33.0	16.2	18.2	29.0	5.0	2.1	3.2	4.1
27.	3.3	4.8	0.7	8.7	1.1	0.6	0.0	0.7	91.1	68.3	8.0	0	0	0	1.6	0	0.9	1.9	2.6	1.8
28.	130.5	110.0	108.9	145.2	11.4	0	8.5	13.2	45.3	42.2	33.6	46.0	33.2	55.9	11.8	30.5	6.6	1.3	3.8	4.0
29.	132.2	115.9	98.6	74.5	61.6	12.8	11.7	10.8	46.0	29.5	35.2	36.7	0.0	0	59.2	10.7	4.8	7.9	342.1	7.1
30.	226.4	137.2	2232.9	179.3	16.5	12.1	8.7	12.3	122.4	68.1	65.4	92.1	80.3	27.9	39.8	62.3	16.8	10.0	11.1	14.3
31.	73.9	23.2	42.9	47.8	14.0	4.5	0	9.8	17.0	7.3	14.0	16.6	10.2	1.5	14.3	2.8	2.0	1.0	0.5	0.8
32.	14.3	13.2	16.4	17.1	0	5.7	5.3	5.4	4.0	19.4	12.2	1.8	0.5	3.5	69.6	0	0	0	0	0
33.	14.9	14.7	15.7	13.5	3.3	3.4	3.4	3.3	404.5	157.8	104.3	147.0	17.7	7.4	0	0	4.3	6.1	6.0	2.4
34.	0.7	0.0	0.3	2.9	1.4	0	0.0	2.2	0	0	0	0	0	0	0	0	0	0	0	0
35.	80.4	64.9	86.1	92.7	11.4	10.1	7.5	11.6	23.3	21.7	12.0	16.0	0	0	0	0	2.5	5.8	1.3	3.7
36.	102.1	157.5	241.6	132.5	8.3	12.5	20.1	11.6	80.1	99.6	174.1	846.6	5.8	14.2	0	54.3	8.5	18.3	28.3	12.6
37.	2.5	1.0	2.2	2.0	4.9	0.4	0.5	0.9	247.1	303.3	32.9	677.9	2.1	9.3	0	23.5	2.8	3.1	1.0	6.4
38.	7.7	9.0	5.6	1.9	2.8	3.1	0.7	0.3	0	0	0	0	3.3	0	0.2	0	2.5	1.5	0.8	0.5
39.	71.7	99.7	85.1	91.5	7.9	9.6	9.6	10.9	18.0	12.7	19.2	17.7	0	0	0	0	1.1	0	4.1	0
40.	85.6	106.2	86.5	114.5	0	10.1	6.6	7.0	46.0	60.6	42.8	47.9	0	0	0	0	8.2	10.3	6.4	7.8

Table 8: Parabens distribution (ng/grm tissue) across the breast (A=axilla, L=lateral, C=central, M=medial). Patients who never used underarm cosmetics are highlighted

The total parabens values followed a non-Normal, positively skewed distribution. Hence we present the median values and range as summary statistics.

Total parabens	Median	Range
Axilla	99.2	2 to 2322
Lateral	73.4	0 to 1364
Central	100.1	0 to 5135
Medial	70.4	3 to 1281
Outer total	194.8	2 to 3683
Inner total	185.6	5 to 5349
All	454.9	8 to 6312

Table 9. Mean total parabens concentrations (ng/gm tissue) and range

There was no obvious gradient in the total parabens concentrations from the axilla to the sternum and no significant difference between the 4 positions (Friedman test; p=0.29). Also, there was no significant difference between the inner (medial + central) and outer (axilla + lateral) total parabens concentrations (Wilcoxon paired test; p=0.38) (table 9 & 11). Spearman correlations test demonstrated no significant relation between the concentration of parabens in any of the four positions and the patients' age (table 10).

Axilla:	rho= -0.12	p=0.44
Lateral:	rho= -0.27	p=0.10
Central:	rho= 0.06	p=0.72
Medial:	rho= -0.13	p=0.43
Outer:	rho = -0.15	p=0.36
Inner:	rho = -0.05	p=0.77
All:	rho= -0.04	p=0.79

Table 10. Total parabens correlation with age

Total parabens	Median	Range
Axilla	117.6	2 to 2322
Lateral	84.4	0 to 1364
Central	106.0	0 to 5135
Medial	111.3	3 to 1281
Outer (total)	220.1	2 to 3683
Inner (total)	230.9	5 to 5349
All	521.3	8 to 6312

Table 11. Total parabens in patients who returned questionnaires.

Total parabens	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Ever used (n=28)	Never used (n=7)	
Axilla	127.4 (2, 2322)	117.1 (19, 1280)	p=0.53
Lateral	115.8 (0, 1364)	41.8 (13, 166)	**p=0.039**
Central	101.4 (0, 5134)	106.9 (26, 811)	p=0.45
Medial	98.0 (3, 1281)	124.0 (20,491)	p=0.76
Outer (total)	267.0 (2,3682)	154.6 (34,1323)	p=0.30
Inner (total)	223.0 (5, 5349)	230.9 (46,1296)	p=0.70
All	583.9 (8, 6312)	451.0 (89,2619)	p=0.76

Table 12. Total parabens: users vs non users.

Patients who had used deodorants had significantly higher total parabens concentration in the lateral samples of the breast, comparing to non-users (table 12). When comparison was made between the current users (n=23) and the past users (n=5), there was no significant difference in the total parabens concentrartions in any area of the breast (table 13).

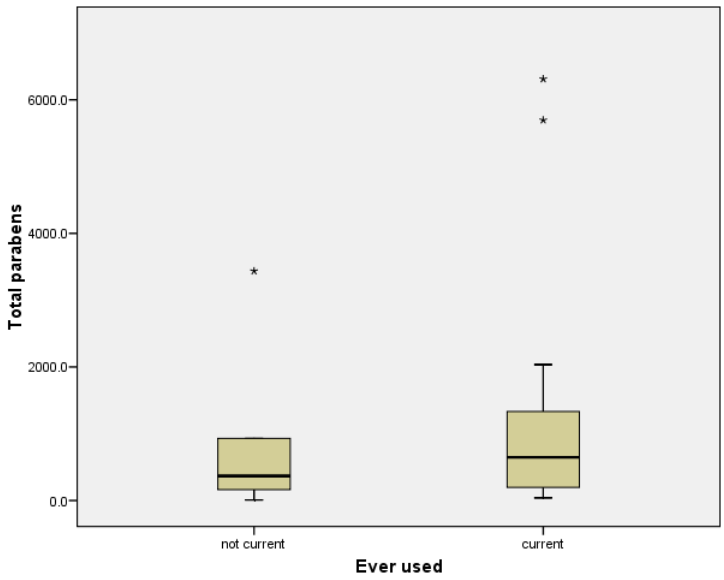
Total parabens	Median (range)		p-value (Mann-Whitney U-test)
	Current users (n=23)	Past users (n=5)	
Axilla	128 (16, 2322)	127 (2, 462)	p=0.56
Lateral	117 (14, 1364)	114 (0, 255)	p=0.73
Central	97 (0, 1281)	126 (0, 2358)	p=0.95
Medial	111 (3, 3683)	63 (5, 360)	p=0.60
Outer (total)	313 (30, 3683)	178 (2, 718)	p=0.64
Inner (total)	238 (10, 5349)	189 (5, 2718)	p=0.60
All	646 (40, 6312)	368 (8, 3436)	p=0.56

Table 13. Total parabens: current users vs past users

When comparison was made between the current users and the non-current users of deodorants (i.e. past users + never-users) there was no significant difference in the total concentration of parabens in all areas of the breast (table 14, graph 2). In both comparisons there is a positive trend towards the current users with an exception for the central breast samples but this trend does not reach statistical significance.

Total parabens	Median (range)		p-value (Mann-Whitney U-test)
	Current users (n=23)	Non-current users (n=12)	
Axilla	128.0 (16, 2322)	117.4 (2, 1280)	p=0.36
Lateral	117.1 (14, 1364)	47.1 (0, 253)	p=0.09
Central	96.7 (0, 5135)	116.6 (0, 2358)	p=0.62
Medial	111.3 (3, 1281)	100.9 (5, 491)	p=0.82
Outer (total)	313.3 (30, 3683)	166.4 (2, 1323)	p=0.26
Inner (total)	238.1 (10, 5349)	210.1 (5, 2718)	p=0.93
All	646.5 (0, 6312)	409.3 (8, 3436)	p=0.50

Table 14. Total parabens: current users vs non-current users



Graph 2: Total parabens, comparison between current and non-current users

For those 20 patients with data on length of usage, the Spearman correlations test did not demonstrate any significant relation between the length of usage and the concentration of parabens (table 15).

Axilla:	rho= 0.17	p=0.48
Lateral:	rho= -0.27	p=0.25
Central:	rho= -0.03	p=0.89
Medial:	rho= -0.25	p=0.28
Outer:	rho = 0.01	p=0.95
Inner:	rho = -0.07	p=0.77
All:	rho = 0.04	p=0.87

Table 15. Total parabens: correlation with length of usage

There was no significant difference of the parabens concentrations in all areas of the breast, between the ER+ve and the ER-ve groups.

Total parabens	**Median (range)**		**p-value (Mann-Whitney U-test)**
	ER +ve (n=27)	ER -ve (n=10)	
Axilla	98.7 (2, 2322)	108.4 (16, 861)	p=0.72
Lateral	84.4 (0, 1360)	106.7 (14, 1364)	p=0.85
Central	96.7 (0, 5135)	88.8 (7, 2358)	p=0.78
Medial	62.4 (5, 1281)	108.8 (3, 360)	p=0.83
Outer (total)	211.4 (2, 3683)	282.6 (30, 1463)	p=0.72
Inner (total)	181.9 (5, 5349)	198.4 (10, 2718)	p=0.91
All	451.0 (8, 6312)	616.9 (40, 3436)	p=0.88

Table 16. Total parabens: ER+ve vs ER-ve tumour group

When comparison was made between the three groups of different tumour locations (medial, central, lateral) there was no significant difference in the concentration of total parabens (table 17).

Total parabens	Median (range)			p-value (Kruskal-Wallis test)
	Central (n=14)	Lateral (n=16)	Medial (n=5)	
Axilla	98 (16,892)	109 (27,2322)	227 (12,933)	p=0.94
Lateral	80 (13,189)	77 (17,1364)	71 (34,454)	p=0.89
Central	72 (7,1147)	106 (0,2358)	167 (17,458)	p=0.52
Medial	65 (3,491)	87 (13,1281)	112 (24,573)	p=0.69
Outer (total)	163 (30,1076)	267 (48,3683)	436 (46,1005)	p=0.83
Inner (total)	154 (10,1192)	199 (21,2718)	326 (48,1030)	p=0.60
All	414 (40,1339)	486 (82,6312)	842 (94,2035)	p=0.97

Table 17. Total parabens: Medial vs Lateral vs Central tumour group

5.4.2. Methyl - paraben

Methyl-paraben data was available on n=40 patients and questionnaire data on n=35 patients. After subtracting the blanks the methyl-paraben values included a small number (n=7) of negative values: Axilla : (n=2): -3,1, -2.1. Lateral: (n=2): -3.7, -2.7. Central: (n=2):-5.9, -3.7. Medial (n=1): -3.1. These negative values were recoded to zeros. The methyl-paraben values followed a non-Normal, positively skewed distribution. Median values and range are presented as summary statistics.

Methyl	Median	Range
Axilla	17.2	0 to 226
Lateral	17.9	0 to 819
Central	16.0	0 to 5103
Medial	15.0	0 to 179
Outer total	37.1	0 to 874
Inner total	34.2	0 to 5147
All	75.3	0 to 5188

Table 18. Methyl-paraben concentrations

There was no obvious gradient in methyl-paraben concentration from the Axilla to Medial area and no significant difference between the 4 positions (Friedman test; p=0.63). Also there was no significant difference between inner (axilla + lateral) and outer (central + medial) total concentrations (Wilcoxon paired test; p=0.67) (table 18). Spearman correlations test did not demonstrate a relation of the methyl-paraben concentration in any of the four positions with the patient age (table 19 & 20).

Axilla:	rho=-0.14;	p=0.40
Lateral:	rho= -0.18;	p=0.27
Central:	rho= -0.09;	p=0.60
Medial:	rho= -0.14;	p=0.37
Outer:	rho = -0.18;	p=0.28
Inner:	rho = -0.10;	p=0.55
All:	rho =-0.15;	p=0.36

Table 19. Methyl-paraben correlation with age

Methyl-paraben	Median	Range
Axilla	18.6	0 to 226
Lateral	17.6	0 to 819
Central	15.1	0 to 5103
Medial	13.5	0 to 179
Outer (total)	37.3	0 to 874
Inner (total)	29.2	0 to 5146
All	74.7	0 to 5188

Table 20. Methyl-paraben concentration in patients that returned questionnaires

There was no significant difference in the concentrations of methyl-paraben between the users and non-users of deodorants (table 21) or between the current users and the non-current users of deodorants (table 22).

Methyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Ever used (n=28)	Never used (n=7)	
Axilla	19.0 (0, 226)	14.3 (1, 132)	p=0.92
Lateral	18.2 (0, 819)	13.2 (0, 116)	p=0.64
Central	14.9 (0, 5103)	16.4 (0. 99)	p=1.0
Medial	12.7 (0, 179)	17.1 (3, 93)	p=0.86
Outer (total)	38.5 (0, 874)	27.5 (1, 248)	p=0.70
Inner (total)	27.9 (0, 5147)	33.5 (5, 179)	p=0.89
All	79.4 (0, 5188)	61.0 (5. 421)	p=0.67

Table 21. Methyl-paraben concentration: Users vs Non users

Methyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Current use (n=23)	No current use (n=12)	
Axilla	21.5 (0, 162)	13.8 (0, 226)	p=0.29
Lateral	19.6 (1, 819)	13.4 (0, 137)	p=0.22
Central	15.1 (0, 5103)	13.2 (0, 2232)	p=0.48
Medial	21.0 (2, 145)	12.4 (0, 179)	p=0.67
Outer (total)	41.3 (3, 874)	25.2 (0, 364)	p=0.21
Inner (total)	35.0 (4, 5147)	25.4 (0, 2412)	p=0.38
All	84.5 (8, 5188)	51.6 (0, 2776)	p=0.20

Table 22. Methyl-paraben concentration: Current users vs Non current users

The Spearman test demonstrated a significant negative association between lateral methyl-paraben and length of use. Lower concentrations were related with longer use of deodorants (table 23).

Axilla:	rho= -0.23;	p=0.33
Lateral:	rho= -0.46;	**p=0.04**
Central:	rho= -0.19;	p=0.43
Medial:	rho= -0.33;	p=0.15
Outer:	rho = -0.32;	p=0.17
Inner:	rho = -0.12;	p=0.60
All:	rho = -0.18;	p=0.44

Table 23. Methyl-paraben concentration: correlation with length of use

There was no significant difference in the concentration of Methyl paraben between the ER+ve and the ER-ve tumours (table 24).

Methyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	ER +ve (n=27)	ER -ve (n=10)	
Axilla	16.7 (0, 162)	37.4 (8, 226)	p=0.16
Lateral	18.2 (0.158)	22.5 (9, 819)	P=0.18
Central	14.7 (0. 5103)	33.2 (6, 2233)	p=0.19
Medial	16.5 (0, 145)	32.0 (2, 179)	p=0.34
Outer (total)	37.3 (0, 260)	68.0 (17, 874)	p=0.16
Inner (total)	33.5 (0, 5147)	65.0 (7, 2412)	p=0.32
All	75.9 (0, 5188)	133.0 (24, 2776)	p=0.29

Table 24. Methyl-paraben ER+ve vs ER-ve group

5.4.3. Ethyl-paraben

After subtracting the blanks the Ethyl-paraben concentrations included a small number of negative values: Axilla : (n= 4):-3,1, -2.1, -1.7, -1.2 Lateral: (n=3);-2.7, -2.3, -1.9 Central: (n=3): -3.7, -1.7, -1.1 Medial (n=1): -3.1 These negative values were recoded to zeros. The Ethyl-paraben concentration values followed a non-Normal, positively skewed distribution. Hence, we present the median values and range as summary statistics.

There was no obvious gradient in ethyl-paraben concentration from Axilla to Medial and no significant difference between the 4 regions (Friedman test; p=0.45). Also there was no significant difference between inner (medial+ central) and outer (lateral + axilla) areas of the breast (Wilcoxon paired test; p=0.10) (table 25 & 27)

Ethyl-paraben	Median	Range
Axilla	3.4	0 to 158
Lateral	3.2	0 to 500
Central	3.2	0 to 20
Medial	3.4	0 to 31
Outer total	6.9	0 to 502
Inner total	6.6	0 to 39
All	13.4	0 to 542

Table 25. Ethyl-paraben concentration.

The Spearman correlation test did not demonstrate any significant relation of Ethyl – paraben concentration and patients' age (table 26).

Axilla:	rho= -0.06;	p=0.72
Lateral:	rho= -0.19;	p=0.23
Central:	rho= -0.21;	p=0.20
Medial:	rho= -0.10;	p=0.53
Outer:	rho = -0.21;	p=0.19
Inner:	rho = -0.17;	p=0.28
All:	rho=-0.17;	p=0.30

Table 26. Ethyl-paraben concentration, correlation with age

Ethyl-paraben	**Median**	**Range**
Axilla	3.4	0 to 158
Lateral	3.1	0 to 500
Central	3.3	0 to 20
Medial	3.3	0 to 31
Outer (total)	6.7	0 to 502
Inner (total)	6.6	0 to 39
All	13.4	0 to 542

Table 27. Ethyl-paraben concentrations in patients who returned the study questionnaire.

There was no significant difference in the concentrations of ethyl-paraben between the users and non-users of deodorants (table 28).

Ethyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Ever used (n=28)	**Never used (n=7)**	
Axilla	3.4 (0, 158)	3.4 (0, 62)	p=0.56
Lateral	3.0 (0, 500)	4.5 (1, 13)	p=0.70
Central	3.4 (0, 20)	2.3 (0, 12)	p=0.86
Medial	3.0 (0, 31)	5.4 (1, 12)	p=0.38
Outer (total)	6.9 (0, 502)	5.7 (3, 74)	p=0.82
Inner (total)	6.0 (0, 39)	9.8 (3, 22)	p=0.48
All	13.0 (0, 542)	16.4 (7, 97)	p=0.56

Table 28. Ethyl-paraben concentration users vs non users

There was no significant difference in the concentrations of ethyl-paraben between the current users and the non-current users of deodorants (table 29).

Ethyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	Current use (n=23)	No current use (n=12)	
Axilla	4.1 (0, 158)	3.2 (0, 62)	p=0.72
Lateral	3.1 (0, 500)	3.0 (0, 13)	p=0.67
Central	3.7 (0, 20)	2.2 (0, 12)	p=0.33
Medial	3.3 (0, 31)	3.8 (0, 12)	p=0.80
Outer (total)	7.3 (2, 502)	5.5 (0, 74)	p=0.46
Inner (total)	6.6 (1, 39)	6.1 (0, 22)	p=0.74
All	14.1 (2, 542)	11.6 (0, 97)	p=0.64

Table 29. Ethyl-paraben current users vs non-current users

The Spearman correlation test did not demonstrate any significant relation of Ethyl – paraben concentration with the length of use (table 30)

Axilla:	rho= -0.10	p=0.69
Lateral:	rho= -0.40	p=0.08
Central:	rho= -0.36	p=0.12
Medial:	rho= -0.41	p=0.08
Outer:	rho = -0.41	p=0.08
Inner:	rho = -0.36	p=0.12
All:	rho = -0.36	p=0.12

Table 30. Ethyl-paraben concentration, correlation with length of use

Lateral ethyl-paraben values and total outer (lateral + axillary) ethyl-paraben values were significantly lower for ER+ve patients (table 31)

Ethyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	ER +ve (n=27)	ER -ve (n=10)	
Axilla	3.4 (0, 14)	4.6 (1, 158)	P=0.10
Lateral	2.9 (0, 16)	4.5 (1, 500)	**P=0.05**
Central	3.0 (0, 20)	3.4 (0, 12)	P=0.67
Medial	3.3 (0, 13)	6.5 (0, 31)	P=0.39
Outer (total)	6.4 (0, 26)	14.4 (3, 502)	**P=0.04**
Inner (total)	6.0 (0, 32)	8.4 (1, 39)	P=0.34
All	13.1 (0, 52)	21.6 97, 542)	P=0.08

Table 31. Ethyl-paraben concentration: ER+ve vs ER-ve group

5.4.4. Propyl-paraben

After subtracting the blanks the propyl-paraben values included a number of negative values. Axilla: (n= 5): -89.6, -47.5, -25.0, -10.0, -7.1 Lateral: (n=5); -371.7, -115.5, -31.6, -29.8, -15.2 Central: (n=5): -50.2, -42.7, -27.1, -6.2, -0.4 Medial (n=7): -286.7, -80.2, -63.5, -22.0, -12.5, -1.0, -0.9. These negative values were recoded to zeros. The propyl-paraben values followed a non-Normal, positively skewed distribution. Hence we present median values and range as summary statistics.

There was no obvious gradient in propyl-paraben concentrations from Axilla to Medial but there is a significant difference between the 4 positions (Friedman test; p=0.007). However, there was no significant difference between inner (medial+ central) and outer (lateral + axilla) areas of the breast (Wilcoxon paired test; p=0.12) (table 32 & 34).

Propyl	**Median**	**Range**
Axilla	17.5	0 to 2053
Lateral	14.2	0 to 1255
Central	16.2	0 to 1250
Medial	17.2	0 to 1217
Outer total	37.8	0 to 3308
Inner total	36.0	0 to 2467
All	77.4	0 to 5774

Table 32. Propyl-paraben concentrations.

Spearman correlations test demonstrated no significant relation between the concentration of parabens in any of the four regions and the patients' age (Table 33).

Axilla:	rho= -0.14;	p=0.38
Lateral:	rho= -0.26;	p=0.10
Central:	rho= 0.00;	p=1.0
Medial:	rho= -0.27;	p=0.10
Outer:	rho = -0.15	p=0.36
Inner:	rho = -0.10;	p=0.52
All:	rho=-0.10;	p=0.53

Table 33. Propyl-paraben, correlation with age

Propyl-paraben	Median	Range
Axilla	27.7	0 to 2053
Lateral	17.5	0 to 1255
Central	19.2	0 to 1250
Medial	21.5	0 to 1217
Outer (total)	46.6	0 to 3308
Inner (total)	42.1	0 to 2467
All	143.8	0 to 5774

Table 34. Propyl-paraben concentrations in patients who returned the study questionnaire

There was no significant difference in the concentrations of propyl-paraben between the users and non-users of deodorants (table 35).

Propyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	Ever used (n=28)	Never used (n=7)	
Axilla	40.8 (0, 2053)	17.0 (0, 1199)	P=0.36
Lateral	29.2 (0, 1255)	7.3 (0, 30)	P=0.09
Central	21.9 (0, 1250)	14.0 (6, 761)	P=0.56
Medial	23.6 (0, 1217)	16.0 (0, 457)	P=0.38
Outer (total)	85.4 (0, 3308)	24.3 (0, 1199)	P=0.34
Inner (total)	51.3 (0, 2467)	30.6 (8, 1217)	P=0.86
All	155.4 (0, 5774)	73 (15, 2416)	P=0.95

Table 35. Propyl-paraben, users vs non-users

There was no significant difference in the concentrations of propyl-paraben between the current users and the non-current users of deodorants (table 36).

Propyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	Current use (n=23)	No current use (n=12)	
Axilla	36.3 (0, 2053)	20.2 (0, 1199)	P=0.50
Lateral	17.5 (0, 1255)	13.4 (0, 158)	P=0.22
Central	19.2 (0, 1250)	24.6 (0, 761)	P=0.62
Medial	23.3 (0, 1217)	16.3 (0, 457)	P=0.46
Outer (total)	83.3 (0, 3308)	34.6 (0, 1199)	P=0.46
Inner (total)	42.1 (0, 2467)	51.2 (0, 1217)	P=0.99
All	143.8 (0, 5774)	110.2 (0, 2416)	P=0.85

Table 36. Propyl-paraben, users vs non-current users

The Spearman correlation test did not demonstrate any significant relation of propyl – paraben concentration with the length of use (table 37)

Axilla:	rho= 0.03	p=0.89
Lateral:	rho= -0.08	p=0.73
Central:	rho= -0.14	p=0.79
Medial:	rho= -0.21	p=0.38
Outer:	rho = -0.01	p=0.97
Inner:	rho = -0.20	p=0.40
All:	rho = -0.06	p=0.57

Table 37. Propyl-paraben, correlation with length of use

There was no significant difference in the concentration of propyl-paraben between the ER+ve and the ER-ve groups (table 38).

Propyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	ER +ve (n=27)	**ER -ve (n=10)**	
Axilla	18.0 (0, 2053)	16.7 (0, 404)	P=0.96
Lateral	17.5 (0, 1255)	10.3 (0, 158)	P=0.51
Central	12.2 (0, 1250)	16.2 (0, 104)	P=0.80
Medial	16.1 (0, 1217)	20.2 (0, 147)	P=0.88
Outer (total)	45.0 (0, 2208)	26.8 (0, 562)	P=0.62
Inner (total)	28.0 (0, 2467)	36.4 (0, 251)	P=0.93
All	73.0 (0, 5774)	63.2 (0, 814)	P=0.65

Table 38. Propyl-paraben, ER+ve vs ER-ve

5.4.5. N-butyl-paraben

After subtracting the blanks the N-butyl values included a number of negative values: Axilla : (n= 7):-103.6, -56.9, -9.8, -6.5, -6.4, -2.6, -2.2, Lateral: (n=10);-102.5, -78.1, -19.1, -7.9, -6.9, -4.5, -2.5, -1.7, -1.4,-1.4 Central: (n=11): -130.4,-53.2, -9.3, -6.6, -6.5, -5.8, -4.8,-4.8,-4.6, -0.9, -0.6 Medial (n=13): -123.8,-42.3,-20.6,-14.4,-8.8,-4.7,-3.4,-3.3,-2.0,-1.7,-0.3, -0.3,-0.3 These negative values were recoded to zeros. The N-butyl-paraben values followed a non-Normal, positively skewed distribution. Hence we present median values and range as summary statistics.

There was no obvious gradient in N-butyl-paraben concentrarion from Axilla to Medial and no significant difference between the 4 regions (Friedman test; p=0.50). Also, there was no significant difference between inner and outer (Wilcoxon paired test; p=0.20) (table 39 & 41).

N-butyl-paraben	Median	Range
Axilla	5.0	0 to 95
Lateral	7.4	0 to 93
Central	5.7	0 to 80
Medial	5.8	0 to 62
Outer total	12.9	0 to 182
Inner total	11.6	0 to 131
All	32.4	0 to 313

Table 39. N-butyl-paraben concentrations

Spearman correlations test demonstrated no significant relation between the concentration of n-butyl-paraben in any of the four positions and the patients' age (Table 40).

Axilla:	rho= 0.08;	p=0.62
Lateral:	rho= 0.08;	p=0.61
Central:	rho= 0.27;	p=0.09
Medial:	rho= -0.11;	p=0.49
Outer:	rho = 0.12;	p=0.46
Inner:	rho = 0.08;	p=0.62
All:	rho = 0.12;	p=0.47

Table 40. N-butyl-paraben, correlation with age

N-butyl-paraben	Median	Range
Axilla	5.3	0 to 95
Lateral	7.4	0 to 93
Central	5.9	0 to 80
Medial	6.0	0 to 62
Outer (total)	16.4	0 to 182
Inner (total)	11.7	0 to 131
All	34.9	0 to 313

Table 41. N-butyl-paraben concentrations in patients who returned questionnaires

Non-users had significantly higher concentrations of n-butyl-paraben in the central breast samples (table 42).

N-butyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	Ever used (n=28)	Never used (n=7)	
Axilla	5.9 (0, 95)	3.3 (0, 64)	0.41
Lateral	10.4 (0, 93)	1.5 (0, 35)	0.11
Central	5.2 (0, 80)	18.5 (0, 70)	0.039
Medial	6.7 (0, 62)	2.8 (0, 24)	0.30
Outer (total)	22.6 (0, 182)	5.7 (0, 100)	0.14
Inner (total)	11.2 (0, 131)	18.5 (0, 70)	0.18
All	36.9 (0, 313)	28.8 (0,165)	0.92

Table 42. N-butyl-paraben, users vs non users

There was no significant difference in the concentrations of n-butyl-paraben between the current users and the non-current users of deodorants (table 43).

N-butyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	Current use (n=23)	No current use (n=12)	
Axilla	6.0 (0, 95)	3.2 (0, 80)	P=0.44
Lateral	11.6 (0, 87)	4.2 (0, 93)	P=0.34
Central	5.3 (0, 80)	10.2 (0, 70)	P=0.25
Medial	7.7 (0, 54)	1.4 (0, 62)	P=0.13
Outer (total)	20.0 (0, 182)	9.2 (0, 108)	P=0.40
Inner (total)	11.3 (0, 131)	14.4 (0, 102)	P=0.74
All	38.9 (0, 313)	27.0 (0, 210)	P=0.90

Table 43. N-butyl-paraben, current users vs non-current users

The Spearman correlation test did not demonstrate any significant relation of n–butyl-paraben concentration with the length of use (table 44)

Axilla:	rho= 0.36;	p=0.33
Lateral:	rho= 0.25;	p=0.28
Central:	rho= 0.38;	p=0.10
Medial:	rho= 0.06;	p=0.81
Outer:	rho = 0.40;	p=0.08
Inner:	rho = 0.14;	p=0.56
All:	rho = 0.33;	p=0.16

Table 44. N-butyl-paraben, correlation with length of use.

There was no significant difference in the concentration of n-butyl-paraben between the ER+ve and the ER-ve groups (table 45).

N-butyl-paraben	Median (range)		p-value (Mann-Whitney U-test)
	ER +ve (n=27)	ER -ve (n=10)	
Axilla	5.3 (0, 95)	6.8 (0, 80)	P=0.80
Lateral	8.6 (0, 93)	5.6 (0, 52)	P=0.75
Central	5.1 (0, 80)	15.8 (0, 59)	P=0.08
Medial	6.0 (0, 54)	6.8 (0, 62)	P=0.65
Outer (total)	13.9 (0, 182)	18.0 (0, 108)	P=0.93
Inner (total)	11.3 (0, 131)	25.4 (0, 102)	P=0.29
All	34.9 (0, 313)	43.4 (4, 210)	P=0.58

Table 45. N-butyl-paraben, ER+ve vs ER-ve groups

5.4.6. Iso-butyl-paraben

After subtracting the blanks the iso-butyl-paraben values included a number of negative values: Axilla : (n= 7): -42.4, -31.4, -3.2, -3.1, -2.2, -0.5, -0.1 Lateral: (n=6);-27.2, -25.5, -2.8, -1.6, -0.5, -0.4 Central: (n=7):-6.5, -2.1, -1.9, -0.8, -0.6, -0.5, -0.5 Medial (n=8):-31.6, -21.7, -3.3, -1.8, -1.6, -1.4, -0.4, -0.5 These negative values were recoded to zeros. The iso-butyl-paraben values followed a non-Normal, positively skewed distribution. Hence we present median values and range as summary statistics.

There was no obvious gradient in iso-butyl-paraben from Axilla to Medial and no significant difference between the 4 positions (Friedman test; p=0.50). Also there was no significant difference between inner and outer (Wilcoxon paired test; p=0.50) (table 46 & 48).

Iso-butyl-paraben	Median	Range
Axilla	2.0	0 to 803
Lateral	2.4	0 to 119
Central	3.2	0 to 342
Medial	1.8	0 to 482
Outer total	5.3	0 to 922
Inner total	4.7	0 to 487
All	13.6	0 to 949

Table 46. Iso-butyl-paraben concentrations

Spearman correlations test demonstrated no significant relation between the concentration of iso-butyl-paraben in any of the four positions and the patients' age (Table 47).

Axilla:	rho= -0.08;	p=0.61
Lateral:	rho= -0.14;	p=0.40
Central:	rho= 0.04;	p=0.81
Medial:	rho= -0.11;	p=0.51
Outer:	rho = -0.10;	p=0.53
Inner:	rho = 0.02;	p=0.90
All:	rho =-0.03;	p=0.87

Table 47. Iso-butyl-paraben, correlation with age

Iso-butyl-paraben	**Median**	**Range**
Axilla	2.5	0 to 803
Lateral	2.9	0 to 119
Central	3.2	0 to 342
Medial	1.8	0 to 482
Outer (total)	7.1	0 to 922
Inner (total)	4.4	0 to 487
All	14.4	0 to 949

Table 48. Iso-butyl-paraben concentrations in patients who returned the study questionnaire

There was no significant difference in the concentrations of iso-butyl-paraben between the users and the non users of deodorants (table 49).

Iso-butyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Ever used (n=28)	**Never used (n=7)**	
Axilla	2.2 (0, 803)	2.5 (0, 12)	P=0.95
Lateral	3.0 (0, 119)	1.8 (0, 11)	P=0.56
Central	3.2 (0, 58)	1.3 (0, 342)	P=0.48
Medial	2.0 (0, 21)	0.8 (0, 482)	P=0.73
Outer (total)	6.5 (0, 922)	8.3 (0, 23)	P=0.64
Inner (total)	5.8 (0.58)	4.3 (0, 487)	P=0.79
All	15.5 (0, 949)	13.3 (0, 510)	P=0.50

Table 49. Iso-butyl-paraben, users vs non-users

There was no significant difference in the concentrations of iso-butyl-paraben between the current users and the non-current users of deodorants (table 50).

Iso-butyl-paraben	**Median (range)**		**(Mann-Whitney U-test)**
	Current use (n=23)	**No current use (n=12)**	
Axilla	2.5 (0, 803)	2.2 (0, 17)	P=0.34
Lateral	3.0 (0, 119)	1.4 (0,11)	P=0.18
Central	3.2 (0,51)	2.4 (0, 342)	P=0.74
Medial	2.2 (0,51)	0.8 (0, 482)	P=0.25
Outer (total)	7.1 (1, 922)	5.5 (0, 27)	P=0.20
Inner (total)	4.4 (0, 51)	4.6 (0, 487)	P=0.82
All	15.3 (2, 949)	13.6 (0, 510)	P=0.46

Table 50. Iso-butyl-paraben, current users vs non-current users

The Spearman correlation test did not demonstrate any significant relation of iso–butyl-paraben concentration with the length of use (table 51).

Axilla:	rho= 0.13	p=0.58
Lateral:	rho= 0.21	p=0.37
Central:	rho= 0.02	p=0.95
Medial:	rho= 0.24	p=0.32
Outer:	rho = 0.26	p=0.26
Inner:	rho = 0.14	p=0.56
All:	rho = 0.32	p=0.17

Table 51. Iso-butyl-paraben, correlation with length of use

There was no significant difference in the concentration of n-butyl-paraben between the ER+ve and the ER-ve groups (table 52).

Iso-butyl-paraben	**Median (range)**		**p-value (Mann-Whitney U-test)**
	ER +ve (n=27)	**ER -ve (n=10)**	
Axilla	1.2 (0, 803)	4.4 (1, 594)	P=0.09
Lateral	2.4 (0, 803)	4.3 (1, 10)	P=0.41
Central	1.7 (0, 58)	5.2 (1, 342)	P=0.09
Medial	1.8 (0, 21)	3.0 (0, 14)	P=0.30
Outer (total)	4.0 (0, 922)	8.8 (1, 605)	P=0.29
Inner (total)	4.2 (0, 58)	8.4 (1, 349)	P=0.26
All	13.3 (0, 949)	18.1 (4, 624)	P=0.41

Table 52. Iso-butyl-paraben, ER+ve vs ER-ve groups

5.5. Results - Aluminium

The first group of samples (n=19 patients) had been analysed for the presence of aluminium at an earlier stage. The measurement was performed with the use of graphite furnace atomic absorption spectrometry (GFAAS). The aluminium concentrations of breast tissue and breast tissue fat were in the range 4–437 nmol/g dry wt. and 3–192 nmol/g oil, respectively. The aluminium content of breast tissue in the outer regions (axilla and lateral) was significantly higher (P = 0.033) than the inner regions (middle and medial) of the breast. The results suggested that further research would be needed toascertain whether there is a relation between the regional differences in the distribution of aluminium and the higher incidence of tumours in the outer upper quadrant of the breast[321].

The analysis of the second group of samples (n=21 patients) is presented. The data are expressed as nmol/g tissue dry weight. (x27 = value in ng Al). These data are for 'whole tissue', not defatted tissue as presented for the first group. The decision to analyse the whole tissue rather than fat content separately was based on the fact that it became apparent that the fat content of different samples was variable and the data would be less accurate and reliable if aluminium was disproportionately higher or lower in fat than non-fat tissue.

Out of the twenty-one patients, six had never used deodorants and eleven were current users. Consequently, there were ten non-current users (i.e. four past-users and six never- users). Data on length of use was available for all 11 patients who currently used deodorants. The median length of use was 37 years, ranging from 11 to 54 years. In this group of patients data on hormone receptor ststus was available for n=18. 72% (n=13) were ER positive

The aluminium values followed a non-Normal, positively skewed distribution. Hence we present median values and range as summary statistics. There was no obvious gradient in aluminium concentrations from the Axilla to the Medial area of the breast and no significant difference between the 4 positions (Friedman test; p=0.62). Also,

there was no significant difference between inner (medial + central) and outer (axilla + lateral) areas of the breast (Wilcoxon paired test; p=0.34) (table 49 & 51)

Aluminium	Median	Range
Axilla	14	4 to 178
Lateral	14	6 to 123
Central	14	4 to 104
Medial	14	6 to 151
Outer total	29	10 to 301
Inner total	28	10 to 191
All	57	20 to 458

Table 49. Aluminium concentrations across the breast

The Spearman correlations test did not demonstrate any realtion between aluminium concentrations and age of the patients (table 50).

Axilla:	rho= -0.08;	p=074
Lateral:	rho= -0.12;	p=0.60
Central:	rho= -0.16;	p=0.49
Medial:	rho= -0.08;	p=0.74
Outer:	rho = -0.01;	p=0.90
Inner:	rho = -0.13;	p=0.58
All:	rho =-0.03;	p=0.96

Table 50. Aluminium concentration, correlation with age

There was no significant difference in the concentration of aluminium between users and non-users (table 51).

Aluminium	Median (range)		p-value (Mann-Whitney U-test)
	Ever used (n=15)	Never used (n=6)	
Axilla	14 (5, 158)	13 (4, 178)	P=0.73
Lateral	15 (7, 94)	13 (6, 123)	P=0.85
Central	14 (5, 104)	14 (4, 63)	P=0.68
Medial	13 (7, 37)	17 (6, 151)	P=0.42
Outer (total)	29 (13, 187)	30 (10, 301)	P=0.97
Inner (total)	27 (12,122)	32 (10,191)	P=0.52
All	57 (27, 231)	58 (20, 458)	P=0.68

Table 51. Aluminium concentration, users vs non-users

There was no significant difference in the concentration of aluminium between current users and non-current users (table 52).

Aluminium	**Median (range)**		**p-value (Mann-Whitney U-test)**
	Current use (n=11)	**No current use (n=10)**	
Axilla	14 (9, 52)	14 (4, 178)	P=0.86
Lateral	15 (7, 94)	12 (6, 123)	P=0.25
Central	18 (5, 104)	14 (4, 63)	P=0.35
Medial	16 (7, 37)	12 (6, 151)	P=0.76
Outer (total)	31 (16, 120)	28 (10, 301)	P=0.60
Inner (total)	35 (12, 122)	26 (10, 191)	P=0.43
All	64 (28, 231)	52 (20, 458)	P=0.65

Table 52. Aluminium conmcentration, current users vs non-current users

The Spearman correlations test did not demonstrate any realtion between aluminium concentrations and age of the patients (table 53)

Axilla:	rho= 0.00;	p=0.99
Lateral:	rho= 0.27;	p=0.43
Central:	rho= -0.07;	p=0.84
Medial:	rho= 0.32;	p=0.34
Outer:	rho = 0.32;	p=0.34
Inner:	rho = 0.15;	p=0.65
All:	rho = 0.22;	p=0.52

Table 53. Aluminium, correlation with length of deodoarant use.

There was no significant difference in the concentrations of aluminium between the ER+ve and the ER-ve tumour groups

Aluminium	**Median (range)**		**p-value (Mann-Whitney U-test)**
	ER +ve (n=13)	**ER -ve (n=5)**	
Axilla	12 (4, 158)	18 (5, 22)	P=0.21
Lateral	14 (6, 94)	12 (8, 53)	P=0.92
Central	14 (4, 104)	13 (6, 43)	P=0.92
Medial	13 (6, 37)	10 (8, 23)	P=0.63
Outer (total)	26 (10, 187)	29 (13, 75)	P=0.78
Inner (total)	27 (10, 122)	23 (14, 66)	P=0.70
All	53 (20, 231)	50 (27, 141)	P=0.78

Table 54. Aluminium concentrations, ER+ve vs ER-ve group

5.6. Discussion

The results that presented statistical significance are summarised below.

- The patients who had used deodorants or antiperspirants had significantly higher total parabens concentration in the lateral samples of the breast, than those who had not.
- A significant negative association was demonstrated between lateral methyl-paraben concentration and length of deodorant – antiperspirant use. Lower concentrations were related with longer use of deodorants.
- There was no obvious gradient in propyl-paraben concentrations from Axilla to Medial but there is a significant difference between the 4 positions (Friedman test; p=0.007)
- Estrogen receptor positive breast cancer patients were found to have significantly lower lateral ethyl-paraben values in comparison to the ER negative group.
- Patients who had never used deodorants or antiperspirants had significantly higher concentrations of n-butyl-paraben in the central breast samples.

The main weak points of our study are the small number of patients included, and the lack of a control group of healthy patients. The decision to recruit fourty patients and no healthy controls in this pilot study, was based on the lack of previous strong relevant evidence, the associated high cost and the great amount of time needed for the laboratory tests to be carried out. In view of the new evidence on the properties and presence of these chemicals from this and other studies, we suggest that control tissue samples for future research may be obtained from the following groups:

- Healthy individuals.
- Women in high risk for breast cancer (i.e. BRCA gene mutation carriers or strong Family History) who would undergo a risk reducing mastectomy.
- Individuals who who would undergo a diagnostic biopsy for benign breast disease
- Healthy individuals who would undergo breast reduction.
- Researchers may use control autologous tissue samples from other parts of the body (e.g abdominal subcutaneous tissue), as well as blood samples to correlate the concentrations accordingly.

Parabens are used in a wide range of cosmetic and food products as preservatives. They have been shown to have estrogenic properties in-vitro and in-vivo[304]. The authors of the CIR Expert Panel review in 2008, conclude that parabens are generally safe in the doses used, despite the reports of endocrine related effects[305]. On the other hand the Scientific Committee on Consumer Products (SCCP) of the European Union in 2005 concluded that methyl-paraben and ethyl-paraben can be used safely at concentrations up to 0.4% in cosmetics, but also that more data would be needed for propyl-paraben, isopropyl-paraben, butyl-paraben and isobutyl-paraben[280] The recommendation for further reliable reproduction toxicity data, with emphasis on the male reproductive system, remained unchanged until 2008[281, 282].

Parabens and aluminium were present in all of our patients. This is to our knowledge, the first study to demonstrate the presence of the widely used esters of para-hydroxybenzoic acid, in healthy breast tissue samples. Despite the small number of patients, it is very likely that these findings reflect the universal presence of parabens in the breast tissue of the general population, within the same geographical and cultural environment. Previous studies had demonstrated that parabens can be absorbed systemically by injestion, skin application and environmental exposure, they are excreted intact with urine, and they had also been traced in breast cancer tumour[307]. Our study's results offer evidence that parabens can be found intact in measurable concentrations within healthy breast tissue. During the last decade, invitro and animal studies demonstrated that parabens present weak estrogenic activity. Similarly, this was the first study to demonstrate the presence of aluminium in healthy breast tissue. The difference between the first report[321] and the second group is attributed to the improved and more reliable methodology used for the latter resulting in more robust results.

This study illustrated a pattern of homogenous distribution of parabens and aluminium across four different regions of the breast from the axilla to the sternum. The concentrations vary but are independent of the patient's age. These results may be used to contribute towards determining the chemical burden of the female breast.

Our patients presented similar high incidence of upper outer quadrant tumours as in the general population. However this disproportionate prevalence did not correlate with

the measured concentrations of parabens or aluminium. Due to the lack of a concentration gradient from the axilla to sternum, one cannot suggest a causative relationship between parabens or aluminium and breast cancer.

The lack of a gradient also suggests that the use of underarm deodorants as a source of these artificial chemicals cannot be accounted for as an independent risk factor for the development of breast cancer. Our study results show that underarm cosmetics (most women use combined deodorant/antiperspirant) are not the only and most likely not the most important source of parabens for the female breast. Flarend et al. demonstrated in two adults that only 0.012% of aluminium chlorohydrate can be absorbed following skin application. This would account for the 2.5% only of the total absorbed aluminium by the gut from food over the same period of time[322]. The daily application of underarm cosmetics is not an important contribution to the breast aluminium burden. This assumption is also based on the lack of significant concentration difference, between the users (n=28) and non-users (n=7) of underarm deodorants – antiperspirants in all four regions of the breast for the aluminium. The pattern and length of use does not affect the concentrations. We did not find any correlation between those who had used underarm cosmetics in the past but have now stopped using them. Finally, there was no correlation between the length of underarm cosmetic use and the measured concentrations.

The significance of the difference between deodorant users and non-users, in the lateral total paraben concentration, needs further evaluation by means of comparison with control non-cancer, user and non-user groups. This result may only reflect the small number of the non-user group patients.

The exposure of a subject to environmental chemicals and the accumulation of those in tissue may start as early as in prenatal life. During critical periods of susceptibility this may result in adverse health effects in infant or later life[323, 324, 325]. Most of the commercially available chemicals in food and cosmetic industry have not been tested for possible developmental toxicity to fetuses, infants, and children. One would expect that if there was a lifelong cumulative effect on the concentration of chemicals in human tissues, there would consequently exist a correlation between that concentration and the age of the subject. Our study demonstrates that the breast content for both

parabens and aluminium did not differ within a population cohort with an age ranging from 37 to 91 years. We therefore accept that there is a parallel pathway of metabolism and excretion of these chemicals, which retains their tissue deposit to a maximum limit. The wide age range of our study's cohort, suggests that this maximum limit of low concentrations may be easily achieved earlier than the clinical or pre-clinical (e.g. screening) detection of breast cancer. Early exposure of the developing breast to chemicals may increase the risk of breast cancer in later life. The current lifestyle trends favour unlimited usage of cosmetic and other care products in younger ages, even in infants[326]. There is a need for prospective clinical studies in younger individuals to determine the pattern of chemical burden in tissues or blood. In other words, there is a need to investigate the chemical body burden built-up in all stages of life. This will enable the establishment of accurate reference ranges that can be used to determine whether an individual or a group (e.g. minors, pregnant women) has an unusually high exposure to chemicals. It will also enable us to track, over time, trends in levels of exposure and effects on the population.

Studies in the previous decades had demonstrated low toxicity levels and lack of carcinogenicity for parabens and aluminium. However, the constant presence of them within the breast tissue may allow their properties to be expressed for a lifelong period of time. Their contribution to the changes that eventually initiate the development of a cancer tumour is unknown. In vitro[327, 328] and animal studies have shown that the combined effect of a mixture of individual substances may be higher than that noted under the influence of each independent congener. In view of the existing evidence on endocrine disrupters, some authors suggest prevention by limiting the exposure to ingredients such as butyl- and propyl paraben, phthalates, bisphenol and others[329]. It is important to determine other chemicals that are present in breast tissue and have similar estrogenic properties. The combined effect of such a coctail in human tissues should be subject to further research.

6. CONCLUSIONS

In this pilot study, we recruited fourty breast cancer patiens who would undergo mastectomy as a first stage of their treatment. We investigated the presence of five commonly used esters of para-hydroxybenzoic acid (parabens) and aluminium, in healthy breast tissue samples obtained from the mastectomy specimens. We also investigated the plausibility of a causative relationship theory between these artificial chemicals and breast cancer. Finally, we attempted to shed light into the link hypothesis between underarm cosmetics, deodorants and antiperspirants, and breast cancer developement. From the results of this study we conclude that:

- Parabens and aluminium are present and can be found intact in various concentrations, within healthy breast tissue.
- The distribution of parabens and aluminium in different regions across the breast, from the axilla to the sternum, is homogenous and independent of the patient's age.
- There is no correlation between parabens or aluminium concentrations and the higher cancer incidence in the upper outer quadrant of the breast.
- There is no correlation between parabens or aluminium concentrations and the hormone receptor status of the cancer tumours.
- There is no difference in the concentration of parabens and aluminium between women who had used underarm cosmetics and those who had not, or those who had used them in the past but have now stopped using them. There is no correlation between the length of underarm cosmetic use and the the concentration of parabens and aluminium in the breast. Underarm deodorants or antiperspirants do not constitute the main source of parabens and aluminium for the female breast.
- The use of underarm deodorants or antiperspirants does not constitute an independent risk factor for breast cancer.

This study adds new evidence on the presence of these substances in the female breast. In the light of the existing data from in vitro, animal and human studies on the

properties, presence and combined effect of several widely used chemical substances; there is an urge for determining the body burden of chemicals, developing biomonitoring techniques, and investigating mixture effects.in humans. Although a causative relationship for most of these chemicals and in particular endocrine disrupters is difficult to prove there is enough evidence to support the introduction of precautionary health policies and support further research and more extensive safety assessments.

7. BIBLIOGRAPHY

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. CA Cancer J Clin 2008;58(2):71-96.
2. Pisani P. Breast cancer: geographic variation and risk factors. J Environ Pathol Toxicol Oncol. 1992 Sep-Oct;11(5-6):313-6.
3. Gregorio DI, Kulldorff M, Barry L, Samociuk H. Geographic differences in invasive and in situ breast cancer incidence according to precise geographic coordinates, Connecticut, 1991-95. Int J Cancer. 2002;100(2):194-8.
4. Gregorio DI, Kulldorff M, Barry L, Samocuik H, Zarfos K. Geographical differences in primary therapy for early-stage breast cancer. Ann Surg Oncol. 2001;8(10):844-9.
5. Mullee MA, De Stavola B, Romanengo M, Coleman MP. Geographical variation in breast cancer survival rates for women diagnosed in England between 1992 and 1994. Br J Cancer. 2004;90(11):2153-6.
6. Bulbrook RD.Geographical variation in endocrine function and its relation to breast cancer incidence: some general considerations. Breast Cancer Res Treat. 1991;18 Suppl 1:S37-40.
7. Curtis M Global Breast Cancer Mortality Statistics CA Cancer J Clin 1999;49:138-144.
8. Parkin DM Cancers of the breast, endometrium and ovary: geographic correlations. Eur J Cancer Clin Oncol 1989;25(12):1917-25.
9. Nagata C, Kawakami N, Shimizu H. Trends in the incidence rate and risk factors for breast cancer in Japan. Breast Cancer Research and Treatment 1997;44:75–82.
10. Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA, Willett WC. Premenopausal fat intake and risk of breast cancer. J Natl Cancer Inst. 2003;95(14):1079-85.
11. Thiébaut AC, Kipnis V, Chang SC, Subar AF, Thompson FE, Rosenberg PS, Hollenbeck AR, Leitzmann M, Schatzkin A. Dietary fat and postmenopausal invasive breast cancer in the National Institutes of Health-AARP Diet and Health Study cohort. J Natl Cancer Inst. 2007;99(6):451-62.
12. Lipworth L Epidemiology of breast cancer. Eur J Cancer Prev. 1995;4(1):7-30.
13. Charles MJ, Schell MJ, Willman E, Gross HB, Lin Y, Sonnenberg S, and Graham ML. Organochlorines and 8-hydroxy-20 deoxyguanosine (8-OHdG) in cancerous and noncancerous breast tissue: Do the data support the hypothesis that oxidative DNA damage caused by organochlorines affects breast cancer? Arch Environ Contam Toxicol. 2001;41:386–395.
14. Woolcott CG, Aronson KJ, Hanna WM, SenGupta SK, McCready DR, Sterns EE, and Miller AB. Organochlorines and breast cancer risk by receptor status, tumor size, and grade (Canada). Cancer Causes Control 2001;12: 395–404.
15. WHO Fact sheet. The top ten causes of death by broad income group 2005. http://www.who.int/mediacentre/factsheets/fs310/en/index.html (accessed 25/09/10)
16. Incidence and Mortality rates in EU and Worldwide. www.cancer.gov
17. Coleman MP. Trends in breast cancer incidence, survival, and mortality. Correspondence Lancet. 2000;356(9229):590-1
18. Kawamura T, Sobue T. Comparison of Breast Cancer Mortality in Five Countries: France, Italy, Japan, the UK and the USA from the WHO Mortality Database (1960–2000). Jpn J Clin Oncol 2005;35(12)758–759.
19. Micheli A, Mugno E, Krogh V, Quinn M. J., M. Coleman, T. Hakulinen, Berrino F,. Capocaccia R, EUROPREVAL Working Group. Cancer prevalence in European registry areas Annals of Oncology 2002;13: 840–865,
20. Burke JP, Power C, Gorey TF, Flanagan F, Kerin MJ, Kell MR. A comparative study of risk factors and prognostic features between symptomatic and screen detected breast cancer. Eur J Surg Oncol. 2008;34(2):149-53.
21. Shen Y, Yang Y, Inoue LY, Munsell MF, Miller AB, Berry DA. Role of detection method in predicting breast cancer survival: analysis of randomized screening trials. J Natl Cancer Inst. 2005; 97(16):1195-203.
22. Immonen-Räihä P, Kauhava L, Parvinen I, Holli K, Kronqvist P, Pylkkänen L, Helenius H, Kaljonen A, Räsänen O, Klemi PJ. Mammographic screening reduces risk of breast carcinoma recurrence. Cancer. 2005;103(3):474-82.
23. Joensuu H, Lehtimäki T, Holli K, Elomaa L, Turpeenniemi-Hujanen T, Kataja V, Anttila A, Lundin M, Isola J, Lundin J. Risk for distant recurrence of breast cancer detected by mammography screening or other methods. JAMA. 2004;292(9):1064-73.

24. Jatoi I, Miller AB. Why is breast-cancer mortality declining? Lancet Oncol. 2003 ;4(4):251-4.
25. Ernst MF, Voogd AC, Coebergh JW, Roukema JA. Breast carcinoma diagnosis, treatment, and prognosis before and after the introduction of mass mammographic screening. Cancer. 2004;100(7):1337-44.
26. Waller M, Moss Sue, Watson J, Moller H. The effect of mammographic screening and HRT use on breast cancer incidence in England and Wales. Cancer Epidemiol Biomarkers Prev 2007; 16: 2257-2261.
27. Svendsen AL, Olsen AH, Von Euler-Chelpin, Lynge E. Breast cancer incidence after the introduction of mammography screening : What should be expected? Cancer. 2006;106(9):1883-90.
28. Schouten LJ, de Rijke JM, Huveneers JA, Verbeek AL.Rising incidence of breast cancer after completion of the first prevalent round of the breast cancer screening programme. J Med Screen. 2002;9(3):120-4.
29. Feig SA. Ductal carcinoma in situ. Implications for screening mammography. Radiol Clin North Am, 2000. 38(4):653-68.
30. Evans AJ, Peter Se, Ellis IO, Wilson R. Screen detected ductal carcinoma in situ (DCIS): overdiagnosis or an obligate precursor of invasive disease? J Med Screen, 2001;8(3):149-51.
31. Evans AJ, Blanks RG. Should breast screening programmes limit their detection of ductal carcinoma in situ? Clin Radiol. 2002;57(12):1086-9.
32. Maxwell AJ, Hanson IM, Sutton CJ, Fitzgerald J, Pearson JM. A study of breast cancers detected in the incident round of the UK NHS Breast Screening Programme: the importance of early detection and treatment of ductal carcinoma in situ. Breast, 2001;10(5): 392-8.
33. NHSBSP, B., An audit of screen detected breast cancers for the year of screening April 2005-March 2006. Presented at BASO, 23 May 2007.
34. Breast Cancer Screening 1991: Evidence and Experience since the Forrest Report, Department of Health Advisory Committee, NHS Breast Screening Programme 1991
35. Effect of NHS Breast Cancer Screening Programme on Mortality from Breast Cancer in England and Wales, 1990-8: Comparison of Observed with Predicted Mortality. BMJ 2000:665-669.
36. 7th Handbook on Cancer Prevention, IARC, Lyons 2002
37. http://www.statistics.gov.uk
38. Rowan, S. Trends in cancer incidence by deprivation, England and Wales, 1999-2002 in Health Statistics Quarterly No.36; Winter 2007
39. Deapen D, Liu L, Perkins C, Bernstein L, Ross R. Rapidly rising breast cancer incidence rates among Asian-American women. Int J Cancer: 2002;99:747-750.
40. American Cancer Society, Cancer Research Center of Hawaii, Hawaii Department of Health. Hawaii cancer facts and figures 2003–2004. American Cancer Society, Honolulu, HI, 2004.
41. Gomez S, Le G, Undurraga D, Shema S, Stroup A, Clarke C, Keegan T, O'Malley C, Hu K, West D, Glaser SL. Cancer incidence among Asians in the Greater Bay Area, 1990–2002.
42. Fremont, CA, Northern California Cancer Center, 2005. www.ncc.org.
43. Pineda MD, White E, Kristal AR, Taylor V. Asian breast cancer survival in the US: a comparison between Asian immigrants US-born Asian-Americans and Caucasians. Int J Epidem 2001;30:976-982.
44. Ursin G, Wu AH, Hoover RN, West DW, Nomura AM, Kolonel LN, Pike MC, Ziegler RG. Breast cancer and oral contraceptive use in Asian-American women. Am J Epidemiol. 1999;150(6):561-7.
45. Ziegler RG, Hoover RN, Nomura AM, West DW, Wu AH, Pike MC, Lake AJ, Horn-Ross PL, Kolonel LN, Siiteri PK, Fraumeni JF Jr. Relative weight, weight change height and breast risk cancer risk in Asian-American women. J Natl Cancer Inst 1996;88(10):650-660.
46. Keegan THM, Gomez SL, Clarke CA, Chan JK , Glaser S. Recent trends in breast cancer incidence among 6 Asian groups in the Greater Bay Area of Northern California Int J Cancer: 2007;120:1324–1329.
47. Koifman S, Koifman RJ. Breast cancer mortality among Ashkenazi Jewish women in Sao Paulo and Porto Alegre, Brazil. Breast Cancer Res 2001;3:270-275.
48. McCracken M, Olsen M, Chen MS, Jemal A, Thun M, Cokkinides V, Deapen D, Ward E. Cancer incidence mortality and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean and Japanese Ethnicities. CA Cancer J Clin 2007;57:190-205.
49. Sheinfeld Gorin S, Heck JE, Cheng B, Smith SJ. Delays in Breast Cancer Diagnosis and Treatment by Racial/Ethnic Group. Arch Inter Med, 2006;Vol 166:2245-2252.
50. Curtis E, Quale C, Haggstrom D, Smith Bindam R. Racial and ethnic differences in breast

cancer survival Cancer. 2008;112(1):171-80.
51. Redaniel MT, Laudico A, Mirasol-Lumague MR, Gondos A, Uy GL, Mapua C, Brenner H. Breast cancer survival in different country settings: comparisons between a Filipino resident population, Filipino-Americans and Caucasians. Breast. 2010;19(2):109-14.
52. Statistical Information Team, Cancer Research UK, 2009.
53. Contractor KB, Kaur K, Rodrigues GS, Kulkarni DM, Singhal H. Male breast cancer: is the scenario changing World J Surg Oncol. 2008;6:58.
54. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tulinius H, Thorlacius S, Eerola H, Nevanlinna H, Syrjakoski K, Kallioniemi OP, Thompson D, Evans C, Peto J, Lalloo F, Evans DG, Easton DF. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 2003;72(5):1117-30.
55. Scully R, Puget N. BRCA1 and BRCA2 in hereditary breast cancer. Biochimie 2002;84(1): 95-102.
56. S.J. Boulton. Cellular functions of the BRCA tumour-suppressor proteins. Biochemical Society Transactions 2006;34(5):633-645.
57. Source American Cancer Society. http://www.cancer.org
58. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, Keller JJ, Westerman AM, Scott RJ, Lim W, Trimbath JD, Giardiello FM, Gruber SB, Offerhaus GJ, de Rooij FW, Wilson JH, Hansmann A, Mφslein G, Royer-Pokora B, Vogel T, Phillips RK, Spigelman AD, Houlston RS. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res. 2006;12(10):3209-15.
59. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, Lockwood GA, Tritchler DL, Yaffe MJ. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995;87:670–5.
60. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, Jong RA, Hislop G, Chiarelli A, Minkin S, Yaffe MJ Mammographic density and the risk and detection of breast cancer. N Engl J Med. 2007;356(3):227-36.
61. Boyd NF, Lockwood GA, Byng JW, Tritchler DL, Yaffe MJ. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
62. Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, Hopper JL. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347(12):886-94.
63. Ziv E, Shepherd J, Smith-Bindman R, Kerlikowske K. Mammographic breast density and family history of breast cancer. J Natl Cancer Inst 2003;95(7):556-8.
64. Boyd NF, Lockwood GA, Martin LJ, Byng JW, Yaffe MJ, Tritchler DL. Mammographic density as a marker of susceptibility to breast cancer: a hypothesis. IARC Sci Publ 2001; 154; 163-9.
65. Byrne C. Mammographic density: a breast cancer risk factor or diagnostic indicator? Acad Radiol 2002;9(3):253-5.
66. Barlow WE, White E, Ballard-Barbash R, Vacek P, Titus-Ernstoff L, Carney PA, Tice JA, Buist, DSM, Geller BM, Rosenberg R, Yankaskas BC, Kerlikowske K. Prospective Breast Cancer Risk Prediction Model for Women Undergoing Screening Mammography. J Natl Cancer Inst. 2006;98(17):1204-14.
67. Chen J, Pee D, Ayyagari R, Graubard B, Schairer C, Byrne C, Benichou J, Gail, MH. Projecting Absolute Invasive Breast Cancer Risk in White Women With Model That Includes Mammographic Density. Journal of the National Cancer Institute. 2006;98(17):1215-26.
68. Mascarineci G, Nagata C, Shimizu H, Kashiki Y. Comparison of mammographic densities and their determinants in women from Japan and Hawaii. Int. J. Cancer. 2002;102:29–33.
69. Bremnes Y, Ursin G, Bjurstam N, Rinaldi S, Kaaks R, Gram I. Endogenous sex hormones, prolactin, and mammagraphic density in postmenopausal Norwegian women. Int J Cancer. 2007;121:2506-2511.
70. Chiarelli A, Kirsh V, Klar N, Shumak R, Jong R, Fishell E, Yaffe MJ, Boyd N. Influence of Patterns of Hormone Replacement Therapy Use and Mammographic Density on Breast Cancer Detection. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1856-62.
71. Topal NB, Ayhan S, Topal U, Bilgin T.Effects of hormone replacement therapy regimens on mammographic breast density: the role of progestins. J Obstet Gynaecol Res. 2006;32(3):305-8.

72. Kavanagh AM, Cawson J, Byrnes GB, Giles GG, Marr G, Tong B, Gertig DM, Hopper JL.Hormone replacement therapy, percent mammographic density, and sensitivity of mammography. Cancer Epidemiol Biomarkers Prev. 2005;14(5):1060-4.
73. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: A meta-analysis Int. J. Cancer. 2007;121:856–862.
74. Sellers TA, Jensen LE, Vierkant RA, Fredericksen ZS, Brandt KR, Giuliano AR, Pankratz VS, Cerhan JR, Vachon CM. Association of diabetes with mammographic breast density and breast cancer in the Minnesota breast cancer family study.Cancer Causes Control. 2007;18(5):505-15.
75. Smigal C, Jemal A, Ward E, Cokkinides V, Smith R, Howe H, Thun M. Trends in breast cancer by race and ethnicity. CA Cancer J Clin 2005;56:168-183.
76. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D, Dolan N, Paskett ED, McTiernan A, Hubbell FA, Adams-Campbell LL, Prentice R. Ethnicity and Breast Cancer: Factors Infl uencing Differences in Incidence and Outcome J Natl Cancer Inst 2005;97:439–48.
77. Li C, Malone K, Daling J. Differences in Breast Cancer Hormone Receptor Status and Histology by Race and Ethnicity among Women 50 Years of Age and Older Cancer Epidemiology, Biomarkers & Prevention 2002;11(7):601–607.
78. Clavel-Chapelon, F. Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. Br J Cancer 2002;86(5):723-7.
79. Britt K, Ashworth A, Smalley M.Pregnancy and the risk of breast cancer. Endocr Relat Cancer. 2007;14(4):907-33.
80. Balogh GA, Russo J, Mailo DA, Heulings R, Russo PA, Morrison P, Sheriff F, Russo IH. The breast of parous women without cancer has a different genomic profile compared to those with cancer. Int J Oncol. 2007;31(5):1165-75.
81. Balogh GA, Heulings R, Mailo DA, Russo PA, Sheriff F, Russo IH, Moral R, Russo J.Genomic signature induced by pregnancy in the human breast. Int J Oncol. 2006;28(2):399-410.
82. Guo S, Russo IH, Russo J. Difference in gene expression profile in breast epithelial cells from women with different reproductive history Int J Oncol. 2003;23(4):933-41.
83. Collaborative Group on Hormonal Factors in Breast Cancer. Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet. 2002;360(9328):187-95.
84. Taylor T, Keyse L, Bryant A. (2006). Contraception and Sexual Health, 2005/06. London: Office for National Statistics. ISBN 1-85774-638-4.
85. Collaborative Group on Hormonal Factors in Breast Cancer Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Lancet 1996;347(9017):1713-27.
86. Beral V; Million Women Study Collaborators Breast cancer and hormone-replacement therapy in the Million Women Study. Lancet. 2003;362(9382):419-27.
87. Hankinson SE, Eliassen AH. Endogenous estrogen, testosterone and progesterone levels in relation to breast cancer risk.J Steroid Biochem Mol Biol. 2007;106(1-5):24-30.
88. Key T, Appleby P, Barnes I, Reeves G; Endogenous Hormones and Breast Cancer Collaborative Group. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst. 2002;94(8):606-16.
89. Tamimi RM, Byrne C, Colditz GA, Hankinson SE Endogenous hormone levels, mammographic density, and subsequent risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 2007;99(15):1178-87.
90. Johansson H, Gandini S, Bonanni B, Mariette F, Guerrieri-Gonzaga A, Serrano D, Cassano E, Ramazzotto F, Baglietto L, Sandri MT, Decensi A. Relationships between circulating hormone levels, mammographic percent density and breast cancer risk factors in postmenopausal women. Breast Cancer Res Treat. 2008;108(1):57-67.
91. Key TJ, Appleby PN, Reeves GK, Roddam A, Dorgan JF, Longcope C, Stanczyk FZ, Stephenson HE Jr, Falk RT, Miller R, Schatzkin A, Allen DS, Fentiman IS, Key TJ, Wang DY, Dowsett M, Thomas HV, Hankinson SE, Toniolo P, Akhmedkhanov A, Koenig K, Shore RE, Zeleniuch-Jacquotte A, Berrino F, Muti P, Micheli A, Krogh V, Sieri S, Pala V, Venturelli E, Secreto G, Barrett-Connor E, Laughlin GA, Kabuto M, Akiba S, Stevens RG, Neriishi K, Land CE, Cauley JA, Kuller LH, Cummings SR, Helzlsouer KJ, Alberg AJ, Bush TL, Comstock GW, Gordon GB, Miller SR, Longcope C; Endogenous Hormones Breast Cancer Collaborative

Group. Body mass index, serum sex hormones, and breast cancer risk in postmenopausal women. J Natl Cancer Inst. 2003;95(16):1218-26.
92. Brinton L. Long-term effects of ovulation-stimulating drugs on cancer risk. Reprod Biomed Online. 2007;15(1):38-44.
93. Kristiansson P, Bjφr O, Wramsby H. Tumour incidence in Swedish women who gave birth following IVF treatment. Hum Reprod. 2007;22(2):421-6.
94. Salhab M, Al Sarakbi W, Mokbel K. In vitro fertilization and breast cancer risk: a review. Int J Fertil Womens Med. 2005;50(6):259-66.
95. Dor J, Lerner-Geva L, Rabinovici J, Chetrit A, Levran D, Lunenfeld B, Mashiach S, Modan B.Cancer incidence in a cohort of infertile women who underwent in vitro fertilization. Fertil Steril. 2002;77(2):324-7.
96. Source ACS http://www.cancer.org
97. Dixon JM, McDonald C, Elton RA, Miller WR. Risk of breast cancer in women with palpable breast cysts: a prospective study. Edinburgh Breast Group. Lancet. 1999;353(9166):1742-5.
98. Lundqvist E, Kaprio J, Verkasalo PK, Pukkala E, Koskenvuo M, Sφderberg KC, Feychting M. Co-twin control and cohort analyses of body mass index and height in relation to breast, prostate, ovarian, corpus uteri, colon and rectal cancer among Swedish and Finnish twins. Int J Cancer. 2007;121(4):810-8.
99. Bruning PF, Bonfrer JMG, Hart AAM, Van Noord PAH, Van dek HoevenH, Collete HJA, Batterman JJ, De Jong-Baker M, Nooijen WJ, Waardz Z. Body measurements, estogen anvailability an the risk of human breast cancer: a case control study. Int. J. Cancer: 1992;51: 14-19.
100. Friedenreich CM. Review of anthropometric factors and breast cancer risk. Eur J Cancer Prev. 2001;10(1):15-32.
101. Lukanova A, Bjor O, Kaaks R, Lenner P, Linda B, Hallmans G, Stattin P. Body mass index and cancer. Results from the Northern Sweden Health and Disease Cohort Int. J. Cancer. 2006;118:458–466.
102. Ahn J, Schatzkin A, Lacey JV Jr, Albanes D, Ballard-Barbash R, Adams KF, Kipnis V, Mouw T, Hollenbeck AR, Leitzmann MF. Adiposity, adult weight change, and postmenopausal breast cancer risk Arch Intern Med. 2007;167(19):2091-102.
103. Eliassen AH, Colditz GA., Rosner B, Willett WC, Hankinson SE. Adult Weight Change and Risk of Postmenopausal Breast Cancer JAMA. 2006;296:193-201.
104. Kaaks R, Van Noord P, Tonkelaa I, Peeters PHM, Riboli E, Grobee DE. Breast cancer incidence in relation to height weight and body fat distribution in the dutch DOM cohort Int. J. Cancer. 1998;76:647–651.
105. Harvie M, Hooper L, Howell AH. Central obesity and breast cancer risk: a systematic review. Obes Rev. 2003;4(3):157-73.
106. Carmichael AR. Obesity and prognosis of breast cancer Obes Rev. 2006;7(4):333-40.
107. Cleveland RJ, Eng SM, Abrahamson PE, Britton JA, Teitelbaum SL, Neugut AI, Gammon MD Weight gain prior to diagnosis and survival from breast cancer. Cancer Epidemiol Biomarkers Prev. 2007;16(9):1803-11.
108. Lichtenstein P, Holm NV, Verkasalo PK . Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer-Analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343(2):78-85.
109. King MC, Marks JH and Mandell JB. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 2003;302:643-646.
110. Tryggvadottir L, Sigvaldason H, Olafsdottir GH, Jonasson JG, Jonsson T, Tulinius H, Eyfjörd JE. Population-based study of changing breast cancer risk in Icelandic BRCA2 mutation carriers, 1920-2000. J Natl Cancer Inst. 2006;98(2):116-22.
111. Taylor AJ, Winter DL, Stiller CA, Murphy M, Hawkins MM.Risk of breast cancer in female survivors of childhood Hodgkin's disease in Britain: a population-based study. Int J Cancer. 2007;120(2):384-91.
112. Sanna G, Lorizzo K, Rotmensz N, Bagnardi V, Cinieri S, Colleoni M, Nol F, Goldhirsch A. Breast cancer in Hodgkin's disease and non-Hodgkin's lymphoma survivors.Ann Oncol. 2007;18(2):288-92.
113. Aisenberg AC, Finkelstein DM, Doppke KP, Koerner FC, Boivin JF, Willett CG.High risk of breast carcinoma after irradiation of young women with Hodgkin's disease. Cancer. 1997;79(6):1203-10.
114. Faulkner K, Law J. Mammographic breast cancer screening for women previously treated with

high breast doses for diseases such as Hodgkin's. Radiat Prot Dosimetry. 2005;117(1-3):330-3.
115. Storm HH, Andersson M, Boice JD Jr, Blettner M, Stovall M, Mouridsen HT, Dombernowsky P, Rose C, Jacobsen A, Pedersen M. Adjuvant radiotherapy and risk of contralateral breast cancer.J Natl Cancer Inst. 1992;84(16):1245-50.
116. Thomas DB, Rosenblatt K, Jimenez LM, McTiernan A, Stalsberg H, Stemhagen A, Thompson WD, Curnen MG, Satariano W, Austin DF, Ionizing radiation and breast cancer in men (United States).Cancer Causes Control. 1994;5(1):9-14.
117. Ma H, Hill CK, Bernstein L, Ursin G. Low-dose medical radiation exposure and breast cancer risk in women under age 50 years overall and by estrogen and progesterone receptor status: results from a case-control and a case-case comparison. Breast Cancer Res Treat. 2008;109(1):77-90.
118. Broeks A, Braaf LM, Huseinovic A, Nooijen A, Urbanus J, Hogervorst FB, Schmidt MK, Klijn JG, Russell NS, Van Leeuwen FE, Van 't Veer LJ. Identification of women with an increased risk of developing radiation-induced breast cancer: a case only study. Breast Cancer Res. 2007;9(2):R26
119. Andrieu N, Easton DF, Chang-Claude J, Rookus MA, Brohet R, Cardis E, Antoniou AC, Wagner T, Simard J, Evans G, Peock S, Fricker JP, Nogues C, Van't Veer L, Van Leeuwen FE, Goldgar DE. Effect of chest X-rays on the risk of breast cancer among BRCA1/2 mutation carriers in the international BRCA1/2 carrier cohort study: a report from the EMBRACE, GENEPSO, GEO-HEBON, and IBCCS Collaborators' Group. J Clin Oncol 2006;24:3361-3366.
120. John EM, Phipps AI, Knight JA, Milne RL, Dite GS, Hopper JL, Andrulis IL, Southey M, Giles GG, West DW, Whittemore AS Medical radiation exposure and breast cancer risk: findings from the Breast Cancer Family Registry. Int J Cancer. 2007;121(2):386-94.
121. Cardis E, Hall J, Tavtigian SV. Identification of women with an increased risk of developing radiation-induced breast cancer. Breast Cancer Res. 2007;9(3):106.
122. Boice JD Jr, Harvey EB, Blettner M, Stovall M, Flannery JT. Cancer in the contralateral breast after radiotherapy for breast cancer. N Engl J Med. 1992;326(12):781-5.
123. Zucali-R, Luini A, Del Vecchio M, Sacchini V, Sverzellati E, Stucchi C, Banfi A, Veronesi U.Contralateral breast cancer after limited surgery plus radiotherapy of early mammary tumors. Eur J Surg Oncol. 1987;13(5):413-7.
124. Chobanova N, Vukov M, Yagova A. Cancer incidence among Bulgarian medical radiation workers: epidemiological study. J Buon. 2007;12(1):65-9.
125. Jartti P, Pukkala E, Uitti J, Auvinen A. Cancer incidence among physicians occupationally exposed to ionizing radiation in Finland. Scand J Work Environ Health. 2006;32(5):368-73.
126. Berrington A, Darby SC, Weiss HA, Doll R. 100 years of observation on British radiologists: mortality from cancer and other causes 1897-1997. Br J Radiol. 2001;74(882):507-19
127. Doody MM, Mandel JS, Lubin JH, Boice JD Jr. Mortality among United States radiologic technologists, 1926-90. Cancer Causes Control. 1998;9(1):67-75.
128. Rafnsson V, Tulinius H, Jσnasson JG, Hrafnkelsson J. Risk of breast cancer in female flight attendants: a population-based study (Iceland). Cancer Causes Control. 2001;12(2):95-101.
129. Rafnsson V, Tulinius H, Jσnasson JG, Hrafnkelsson J. Breast cancer risk in airline cabin Attendants: a nested case-control study in Iceland. Occup Environ Med. 2003;60(11):807-9.
130. Tokumaru O, Haruki K, Bacal K, Katagiri T, Yamamoto T, Sakurai Y Incidence of cancer among female flight attendants: a meta-analysis. J Travel Med. 2006;13(3):127-32.
131. Kojo K, Pukkala E, Auvinen A. Breast cancer risk among Finnish cabin attendants: a nested case-control study. Occup Environ Med. 2005;62(7):488-93.
132. Linnersjφ A, Hammar N, Dammstrφm BG, Johansson M, Eliasch H. Cancer incidence in airline cabin crew: experience from Sweden. Occup Environ Med. 2003;60(11):810-4.
133. Haldorsen T, Reitan JB, Tveten U. Cancer incidence among Norwegian airline cabin attendants. Int J Epidemiol. 2001;30(4):825-30.
134. Pukkala E, Kesminiene A, Poliakov S, Ryzhov A, Drozdovitch V, Kovgan L, Kyyrφnen P, Malakhova IV, Gulak L, Cardis E.Breast cancer in Belarus and Ukraine after the Chernobyl accident. Int J Cancer. 2006;119(3):651-8.
135. Sauvaget C, Nagano J, Hayashi M, Spencer E, Bauer S, Gusev BI, Pivina LM, Apsalikov KN, Grosche B.Radiation exposure due to local fallout from Soviet atmospheric nuclear weapons testing in Kazakhstan: solid cancer mortality in the Semipalatinsk historical cohort, 1960-1999. Radiat Res. 2005;164(4 Pt 1):409-19.
136. Land CE, Tokunaga M, Koyama K, Soda M, Preston DL, Nishimori I, Tokuoka S Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950-1990.

Radiat Res. 2003;160(6):707-17.
137. Ron E, Ikeda T, Preston DL, Tokuoka S. Male breast cancer incidence among atomic bomb survivors. J Natl Cancer Inst. 2005;97(8):603-5.
138. Shimizu Y, Allen N.Vegetables and fruit intake and cancer mortality in the Hiroshima/Nagasaki Life Span Study. Br J Cancer. 2003;88(5):689-94.
139. Harvey M. The Genesis breast cancer prevention diet. 2006, Rodale int, London national cancer institute.
140. Michels KB, Mohllajee AP, Roset-Bahmanyar E, Beehler GP, Moysich KB. Diet and breast cancer: a review of the prospective observational studies. Cancer. 2007;109(12 Suppl):2712-49.
141. Navarro Silvera SA., Jain M, Howe GR, Miller AB, Rohan TE Energy balance and breast cancer risk: a prospective cohort study Breast Cancer Res Treat. 2006;97: 97–106.
142. Travis RC, Allen NE, Appleby PN, Spencer EA, Roddam AW, Key TJ. A prospective study of vegetarianism and isoflavone intake in relation to breast cancer risk in British women. Int J Cancer. 2008;122(3):705-10.
143. Zhang SM, Lee IM, Manson JE, Cook NR, Willett WC, Buring JE. Alcohol consumption and breast cancer risk in the Women's Health Study. Am J Epidemiol. 2007;165(6):667-76.
144. Linos E, Willett WC. Diet and breast cancer risk reduction. J Natl Compr Canc Netw. 2007;5(8):711-718.
145. Ericson U, Sonestedt E, Gullberg B, Olsson H, Wirfδlt E. High folate intake is associated with lower breast cancer incidence in postmenopausal women in the Malmo Diet and Cancer cohort. Am J Clin Nutr. 2007;86(2):434-43.
146. Carruba G, Granata OM, Pala V, Campisi I, Agostara B, Cusimano R, Ravazzolo B, Traina A. A traditional Mediterranean diet decreases endogenous estrogens in healthy postmenopausal women. Nutr Cancer. 2006;56(2):253-9.
147. Cui X, Dai Q, Tseng M, Shu XO, Gao YT, Zheng W.Dietary patterns and breast cancer risk in the shanghai breast cancer study. Cancer Epidemiol Biomarkers Prev. 2007;16(7):1443-8.
148. Rogers IS, Northstone K, Dunger DB, Cooper AR, Ness AR, Emmett PM. Diet throughout childhood and age at menarche in a contemporary cohort of British girls. Public Health Nutr. 2010;June 8:1-12.
149. Touillaud MS, Thiιbaut AC, Fournier A, Niravong M, Boutron-Ruault MC, Clavel-Chapelon F.Dietary lignan intake and postmenopausal breast cancer risk by estrogen and progesterone receptor status. J Natl Cancer Inst. 2007;99(6):475-86.
150. Buck K, Zaineddin AK, Vrieling A, Linseisen J, Chang-Claude J. Meta-analyses of lignans and enterolignans in relation to breast cancer risk. Am J Clin Nutr. 2010;92(1):141-53.
151. Thiιbaut AC, Kipnis V, Chang SC, Subar AF, Thompson FE, Rosenberg PS, Hollenbeck AR, Leitzmann M, Schatzkin A. Dietary fat and postmenopausal invasive breast cancer in the National Institutes of Health-AARP Diet and Health Study cohort. J Natl Cancer Inst. 2007;99(6):451-62.
152. Sieri S, Pala V, Brighenti F, Pellegrini N, Muti P, Micheli A, Evangelista A, Grioni S, Contiero P, Berrino F, Krogh V. Dietary glycemic index, glycemic load, and the risk of breast cancer in an Italian prospective cohort study.Am J Clin Nutr. 2007;86(4):1160-6.
153. Lajous M, Willett W, Lazcano-Ponce E, Sanchez-Zamorano LM, Hernandez-Avila M, Romieu I. Glycemic load, glycemic index, and the risk of breast cancer among Mexican women. Cancer Causes Control. 2005;16(10):1165-9.
154. Navarro Silvera SA, Jain M, Howe GR, Miller A, Rohan TE. Dietary carbohydrates and breast cancer risk: A prospective study of the roles of overall glycemic index and glycemic load. International Journal of Cancer 2005;114(4):653 – 658.
155. Sant M, Allemani C, Sieri S, Krogh V, Menard S, Tagliabue E, Nardini E, Micheli A, Crosignani P, Muti P Berrino F. Salad vegetables dietary pattern protects against HER-2-positive breast cancer: A prospective Italian study Int. J. Cancer. 2007;121:911–914.
156. Do MH, Lee SS, Jung PJ, Lee MH. Intake of fruits, vegetables, and soy foods in relation to breast cancer risk in Korean women: a case-control study. Nutr Cancer. 2007;57(1):20-7.
157. Do MH, Lee SS, Kim JY, Jung PJ, Lee MH. Fruits, vegetables, soy foods and breast cancer in pre- and postmenopausal Korean women: a case-control study. Int J Vitam Nutr Res. 2007;77(2):130-41.
158. Suzuki R, Rylander-Rudqvist T, Ye W, Saji S, Adlercreutz H, Wolk A. Dietary fiber intake and risk of postmenopausal breast cancer defined by estrogen and progesterone receptor status. A prospective cohort study among Swedish women Int. J. Cancer. 2008;122:403–412.
159. Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK, Nemoto T, Graham S. Premenopausal breast cancer risk and intake of vegetables, fruits, and related

nutrients.J Natl Cancer Inst. 1996;88(6):340-8.
160. Nelson ZC, Ray RM, Wu C, Stalsberg H, Porter P, Lampe JW, Shannon J, Horner N, Li W, Wang W, Hu Y, Gao D, Thomas DB. Fruit and vegetable intakes are associated with lower risk of breast fibroadenomas in chinese women. J Nutr. 2010;140(7):1294-301.
161. Kesse-Guyot E, Bertrais S, Duperray B, Arnault N, Bar-Hen A, Galan P, Hercberg S. Dairy products, calcium and the risk of breast cancer: results of the French SU.VI.MAX prospective study. Ann Nutr Metab. 2007;51(2):139-45.
162. Robien K, Cutler GJ, Lazovich D.Vitamin D intake and breast cancer risk in postmenopausal women: the Iowa Women's Health Study. Cancer Causes Control. 2007;18(7):775-82.
163. Lin J, Manson JE, Lee IM, Cook NR, Buring JE, Zhang SM. Intakes of calcium and vitamin D and breast cancer risk in women. Arch Intern Med. 2007;167(10):1050-9.
164. O'Leary ES, Schoenfeld ER, Stevens RG, Kabat GC, Henderson K, Grimson R, Gammon MD, Leske MC; Electromagnetic Fields and Breast Cancer on Long Island Study Group.Shift work, light at night, and breast cancer on Long Island, New York. Am J Epidemiol. 2006;164(4):358-66.
165. Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Colditz GA. Rotating night shifts and risk of breast cancer in women participating in the nurses' health study. J Natl Cancer Inst. 2001;93(20):1563-8.
166. Blask DE, Brainard GC, Dauchy RT, Hanifin JP, Davidson LK, Krause JA, Sauer LA, Rivera-Bermudez MA, Dubocovich ML, Jasser SA, Lynch DT, Rollag MD, Zalatan F. Melatonin-depleted blood from premenopausal women exposed to light at night stimulates growth of human breast cancer xenografts in nude rats. Cancer Res. 2005;65(23):11174-84.
167. Schernhammer ES, Kroenke CH, Laden F, Hankinson SE. Night work and risk of breast cancer. Epidemiology. 2006;17(1):108-11.
168. Hansen J. Risk of breast cancer after night- and shift work: current evidence and ongoing studies in Denmark. Cancer Causes Control. 2006;17(4):531-7.
169. Jung B, Ahmad N.Melatonin in cancer management: progress and promise. Cancer Res. 2006;66(20):9789-93
170. Kumar V, Chakraborty A, Viswanath G, Roy P. Androgenic endocrine disruptors in wastewater treatment plant effluents in India: Their influence on reproductive processes and systemic toxicity in male rats. Toxicol Appl Pharmacol. 2008;226(1):60-73.
171. Schulte-Oehlmann U, Albanis T, Allera A, Bachmann J, Berntsson P, Beresford N, Carnevali DC, Ciceri F, Dagnac T, Falandysz J, Galassi S, Hala D, Janer G, Jeannot R, Jobling S, King I, Klingmüller D, Kloas W, Kusk KO, Levada R, Lo S, Lutz I, Oehlmann J, Oredsson S, Porte C, Rand-Weaver M, Sakkas V, Sugni M, Tyler C, van Aerle R, van Ballegoy C, Wollenberger L. COMPRENDO: Focus and approach. Environ Health Perspect. 2006;114 Suppl 1:98-100.
172. Rudel RA, Attfield KR, Schifano JN, Brody JG. Chemicals Causing Mammary Gland Tumors in Animals Signal New Directions for Epidemiology, Chemical Testing, and Risk Assessment for Breast Cancer Prevention," Environmental Factors in Breast Cancer, Cancer. 2007;109(12 Suppl):2635-66
173. Thompson LU, Boucher BA, Liu Z, Cotterchio M, Kreiger N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. Nutr Cancer. 2006;54(2):184-201.
174. Trock BJ, Hilakivi-Clarke L, Clarke R. Meta-analysis of soy intake and breast cancer risk. J Natl Cancer Inst. 2006;98(7):459-71.
175. Yamamoto S, Sobue T, Kobayashi M, Sasaki S, Tsugane S. Japan Public Health Center-Based Prospective Study on Cancer Cardiovascular Diseases Group. Soy, isoflavones, and breast cancer risk in Japan.J Natl Cancer Inst. 2003;95(12):906-13.
176. Nishio K, Niwa Y, Toyoshima H, Tamakoshi K, Kondo T, Yatsuya H, Yamamoto A, Suzuki S, Tokudome S, Lin Y, Wakai K, Hamajima N, Tamakoshi Consumption of soy foods and the risk of breast cancer: findings from the Japan Collaborative Cohort (JACC) Study. Cancer Causes Control. 2007;18(8):801-8.
177. Hirose K, Imaeda N, Tokudome Y, Goto C, Wakai K, Matsuo K, Ito H, Toyama T, Iwata H, Tokudome S, Tajima K. Soybean products and reduction of breast cancer risk: a case-control study in Japan. Br J Cancer. 2005;93(1):15-22.
178. Shu XO, Jin F, Dai Q, Wen W, Potter JD, Kushi LH, Ruan Z, Gao Y, Zheng W. Soyfood Intake during Adolescence and Subsequent Risk of Breast Cancer among Chinese Women. Cancer Epidem Biomark Prev. 2001;10:483–488.
179. Peterson J, Lagiou P, Samoli E, Lagiou A, Katsouyanni K, La Vecchia C, Dwyer J, Trichopoulos D. Flavonoid intake and breast cancer risk: a case-control study in Greece British

Journal of Cancer. 2003;89,1255-1259.
180. Sakla MS, Shenouda NS, Ansell PJ, Macdonald RS, Lubahn DB. Genistein affects HER2 protein concentration, activation, and promoter regulation in BT-474 human breast cancer cells Endocrine. 2007;32(1):69-78.
181. Hedelin M, Lof M, Olsson M, Adlercreutz H, Sandin S, Weiderpass E. Dietary Phytoestrogens Are Not Associated with Risk of Overall Breast Cancer But Diets Rich in Coumestrol Are Inversely Associated with Risk of Estrogen Receptor and Progesterone Receptor Negative Breast Tumors in Swedish Women. J Nutr. 2008;138:938-945.
182. Wang Hong Xu, Wei Zheng, Yong Bing Xiang, Zhi Xian Ruan, Jia Rong Cheng, Qi Dai, Yu Tang Gao, Xiao Ou Shu, Soya food intake and risk of endometrial cancer among Chinese women in Shanghai: population based case-control study BMJ 2004;328:1285.
183. West MC, Anderson L, McClure N, Lewis SE. Dietary oestrogens and male fertility potential. Hum Fertil (Camb). 2005;8(3):197-207.
184. Dillingham BL, McVeigh BL, Lampe JW, Duncan AM. Soy protein isolates of varying isoflavone content exert minor effects on serum reproductive hormones in healthy young men. J Nutr. 2005;135(3):584-91.
185. Heald CL, Ritchie MR, Bolton-Smith C, Morton MS, Alexander FE. Phyto-oestrogens and risk of prostate cancer in Scottish men. Br J Nutr. 2007;98(2):388-96.
186. Messina M, McCaskill-Stevens W, Lampe JW. Addressing the Soy and Breast Cancer Relationship: Review, Commentary, and Workshop Proceedings. J Natl Cancer Inst. 2006;98(18):1275-84.
187. Qin LQ, Xu JY, Tezuka H, Wang PY, Hoshi K. Commercial soy milk enhances the development of 7,12-dimethylbenz(a)anthracene-induced mammary tumors in rats. In Vivo. 2007;21(4):667-71.
188. Duffy C, Perez K, Partridge A. Implications of phytoestrogen intake for breast cancer. CA Cancer J Clin. 2007;57(5):260-77.
189. Singer S, Grismaijer S. Dressed to Kill: The Link Between Breast Cancer and Bras, 1995 Avery, ISBN-10: 0895296640 ISBN-13: 978-0895296641
190. Ashizawa K, Sugane A, Gunji T. Breast Form Changes Resulting From A Certain Brassiere Journal of Hum. Ergol.(Tokyo) 1990;19(1):53-62.
191. Hsieh CC, Trichopoulos D. Breast size, handedness and breast cancer risk. Department of Epidemology, Harvard School of Public Health, Boston, Massachusetts Eur J Cancer. 1991;27(2):131-5.
192. Lee YA, Hyun KJ, Tokura H. The effects of skin pressure by clothing on circadian rhythms of core temperature and salivary melatonin. Chronobiol Int. 2000;17(6):783-93.
193. Caufriez A. Hormonal replacement therapy (HRT) in postmenopause: a reappraisal. Ann Endocrinol. 2007;68(4):241-50.
194. Travis RC, and Key TJ. Oestrogen exposure and breast cancer risk. Breast Cancer Res 2003;5:239-247.
195. Lawlor DA, Smith GD & Ebrahim S. The hormone replacement - coronary heart disease conundrum: is this the death of observational epidemiology? International Journal of Epidemiology. 2004;33:464-467
196. Renard F, Vankrunkelsven P, Van Eycken L, Henau K, Boniol M, Autier P. Decline in breast cancer incidence in the Flemish region of Belgium after a decline in hormonal replacement therapy. Ann Oncol. 2010 May 3. [Epub ahead of print]
197. Fontenoy AM, Leux C, Delacour-Billon S, Allioux C, Frenel JS, Campone M, Molinié F. Recent trends in breast cancer incidence rates in the Loire-Atlantique, France: a decline since 2003. Cancer Epidemiol. 2010;34(3):238-43.
198. Printz C. Declines in ductal hyperplasia may be linked to hormone therapy reduction. Cancer. 2010;116(8):1843.
199. Vilela ML, Willingham E, Buckley J, Liu BC, Agras K, Shiroyanagi Y, Baskin LS Endocrine disruptors and hypospadias: role of genistein and the fungicide vinclozolin. Urology. 2007;70(3):618-21.
200. McLachlan JA, Newbold RR, Burow ME, Li SF. From malformations to molecular mechanisms in the male: three decades of research on endocrine disrupters. APMIS. 2001;109(4):263-72.
201. Della Seta D, Farabollini F, Dessì-Fulgheri F, Fusani L. Environmental-like exposure to low levels of estrogen affects sexual behavior and physiology of female rats. Endocrinology. 2008;149(11):5592-8.
202. Warner M, Eskenazi B, Mocarelli P, Gerthoux PM, Samuels S, Needham L, Patterson D,

Brambilla P. Serum dioxin concentrations and breast cancer risk in the Seveso Women's Health Study. Environ Health Perspect. 2002;110(7):625-8
203. Matthiessen P, Johnson I. Implications of research on endocrine disruption for the environmental risk assessment, regulation and monitoring of chemicals in the European Union. Environ Pollut. 2007;146(1):9-18.
204. Palmer JR, Wise LA, Hatch EE, Troisi R, Titus-Ernstoff L, Strohsnitter W, Kaufman R, Herbst AL, Noller KL, Hyer M, Hoover RN. Prenatal diethylstilbestrol exposure and risk of breast cancer. Cancer Epidemiol Biomarkers Prev. 2006;15(8):1509-14.
205. Soto AM, Maffini MV, Sonnenschein C Neoplasia as development gone awry: the role of endocrine disruptors.Int J Androl. 2008;31(2):288-93.
206. Newbold RR, Padilla-Banks E, Snyder RJ, Phillips TM, Jefferson WN. Developmental exposure to endocrine disruptors and the obesity epidemic. Reprod Toxicol. 2007;23(3):290-6.
207. Community strategy for endocrine disrupters. WHO the international program on chemical safety, IPCS, Global assessment of the state of the science of endocrine disruptors. Chapter 6
208. Brody JG, Rudel RA, Michels KB, Moysich KB, Bernstein L, Attfield KR, Gray S. Environmental pollutants, diet, physical activity, body size, and breast cancer: where do we stand in research to identify opportunities for prevention? Cancer. 2007;109(12 Suppl):2627-34
209. Ibarluzea Jm J, Fernαndez MF, Santa-Marina L, Olea-Serrano MF, Rivas AM, Aurrekoetxea JJ, Expσsito J, Lorenzo M, Tornι P, Villalobos M, Pedraza V, Sasco AJ, Olea N. Breast cancer risk and the combined effect of environmental estrogens. Cancer Causes Control. 2004;15(6):591-600.
210. Petreas M, Smith D, Hurley S, Jeffrey SS, Gilliss D, Reynolds P. Distribution of persistent, lipid-soluble chemicals in breast and abdominal adipose tissues: lessons learned from a breast cancer study. Cancer Epidemiol Biomarkers Prev. 2004;13(3):416-24.
211. Klein PM, Lawrence JA. Lavage and nipple aspiration of breast ductal fluids: a source of biomarkers for environmental mutagenesis. Environ Mol Mutagen. 2002;39(2-3):127-33.
212. Martin FL, Carmichael PL, Crofton-Sleigh C, Venitt S, Phillips DH, Grover PL. Genotoxicity of human mammary lipid. Cancer Res. 1996;56(23):5342-6.
213. Hu YF, Russo IH, Zalipsky U, Lynch H, Russo J. Environmental chemical carcinogens induce transformation of breast epithelial cells from women withf amilial history of breast cancer. In Vitro Cell Dev Biol Anim 1997;33:495–498.
214. Rusiecki JA, Matthews A, Sturgeon S, Sinha R, Pellizzari E, Zheng T, Baris D. A correlation study of organochlorine levels in serum, breast adipose tissue, and gluteal adipose tissue among breast cancer cases in India. Cancer Epidemiol Biomarkers Prev. 2005;14(5):1113-24.
215. Guttes S, Failing K, Neumann J, Kleinstein J, Georgii S, Brunn H. Chlororganic pesticides and polychlorinated biphenyls in breast tissue of women withbe nign and malignant breast disease. Arch Environ Contam Toxicol 1998;35:140–147.
216. Archibeque-Engle SL, Tessari JD, Winn DT, Keefe TJ, Nett TM, Zheng T. Comparison of organochlorine pesticide and polychlorinated biphenyl residues in human breast adipose tissue and serum. J Toxicol Environ Health. 1997;52(4):285-93.
217. Rusiecki J.A., Holford T.R., Zahm S.H., and Zheng T. Polychlorinated biphenyls and breast cancer risk by combined estrogen and progesterone receptor status. Eur J Epidemiol 2004;19: 793–801.
218. Muñoz-de-Toro M, Beldoménico HR, García SR, Stoker C, De Jesús JJ, Beldoménico PM, Ramos JG, Luque EH.Organochlorine levels in adipose tissue of women from a littoral region of Argentina. Environ Res. 2006;102(1):107-12.
219. Stellman SD, Djordjevic, Britton J, Muscat JE, Citron ML, Kemeny M, Busch E, Gong L, breast cancer risk in relation to adipose concentrations of Organochlorine Pesticides and Polychlorinated Biphenyls in Long Island New York . Cancer epidemiol. Biomark. Prev. 2000;9:1241-1249
220. López-Cervantes M, Torres-Sánchez L, Tobías A, López-Carrillo L. DDE Burden and Breast Cancer Risk: A Meta-Analysis of the Epidemiologic Evidence. EHP. 2004;112(2):207-214.
221. Cohn BA, Wolff MS. DDT and Breast Cancer in Young Women: New Data on the Significance of Age at Exposure, Environ. Health Perspect. 2007;115:1406–1414.
222. Nakamura T, Nakai K, Matsumura T, Suzuki S, Saito Y, Satoh H. Determination of dioxins and polychlorinated biphenyls in breast milk, maternal blood and cord blood from residents of Tohoku, Japan. Sci Total Environ. 2008;394(1):39-51.
223. Wassermann M, Nogueira DP, Tomatis L, Mirra AP, Shibata H, Arie G, Cucos S, and Wassermann D. Organochlorine compounds in neoplastic and adjacent apparently normal breast tissue. Bull Environ Contam Toxicol 1976;15:478–484.

224. Falck F, Ricci A, Wolff MS, Godbold J, and Deckers P. Pesticides and polychlorinated biphenyl residues in human breast lipid and their relation to breast cancer. Arch Env Health. 1992;47:143–146.
225. Mussalo-Rauhamaa H, Pantzar P. Selenium and DDE in breast fat of breast cancer patients: their relationship to hormone receptors in breast tissue (letter). J Natl Cancer Inst. 1993;85:1964–1965.
226. Aronson KJ, Miller A, Woolcott C, Sterns E, McCready DR, Lickley LA, Fish EB, Hiraki GY, Holloway C, Ross T, Hanna WM, SenGupta SK, Weber J-P. Breast adipose tissue concentrations of polychlorinated biphenyls and other organochlorines and breast cancer risk. Cancer Epidem Bio Prev. 2000;9:55–63.
227. Ibarluzea Jm J, Fernández MF, Santa-Marina L, Olea-Serrano MF, Rivas AM, Aurrekoetxea JJ, Expósito J, Lorenzo M, Torné P, Villalobos M, Pedraza V, Sasco AJ, Olea N. Breast cancer risk and the combined effect of environmental estrogens. Cancer Causes Control. 2004;15(6):591-600.
228. Waliszewski SM, Bermudez MT, Infanzon RM, Silva CS, Carvajal O, Trujillo P, Gomez Arroyo S, Villalobos Pietrini R, Saldaña VA, Melo G, Esquivel S, Castro F, Ocampo H, Torres J, Hayward-Jones PM. Persistent organochlorine pesticide levels in breast adipose tissue in women with malignant and benign breast tumors. Bull Environ Contam Toxicol. 2005;75(4):752-9.
229. Raaschou-Nielsen O, Pavuk M, Leblanc A, Dumas P, Philippe Weber J, Olsen A, Tjønneland A, Overvad K, Olsen JH. Adipose organochlorine concentrations and risk of breast cancer among postmenopausal Danish women. Danish Cancer Society, Institute of Cancer Cancer Epidemiol Biomarkers Prev. 2005;14(1):67-74
230. Unger M, Kiær H, Blichert-Toft M, Olsen J, and Clausen J. Organochlorine compounds in human breast fat from deceased with and without breast cancer and in a biopsy material from newly diagnosed patients undergoing breast surgery. Environ Res 1984;34:24–28.
231. Zheng T, Holford TR, Mayne ST, Ward B, Carter D, Owens PH, Dubrow R, Zahm SH, Boyle P, Archibeque S, Tessari J. DDE and DDT in breast adipose tissue and risk of female breast cancer. Am J Epidemiol. 1999a;150:453–458.
232. Bagga D, Anders KH, Wang H-J, Roberts E, and Glaspy JA. Organochlorine pesticide content of breast adipose tissue from women with breast cancer and control subjects. J Natl Canc Inst 2000;92:750–753.
233. Negri E, Bosetti C, Fattore E, La Vecchia C. Environmental exposure to polychlorinated biphenyls (PCBs) and breast cancer: a systematic review of the epidemiological evidence. Eur J Crit Rev Toxicol. 2009;39(4):299-331.
234. Golden R, Kimbrough R. Weight of evidence evaluation of potential human cancer risks from exposure to polychlorinated biphenyls: an update based on studies published since 2003. Cancer Prev. 2003;12(6):509-16.
235. Nelson HD, Tyne K, Naik A, Bougatsos C, Chan B, Nygren P, Humphrey L. Screening for Breast Cancer: Systematic Evidence Review Update for the US Preventive Services Task Force. Rockville (MD): Agency for Healthcare Research and Quality (US); 2009 Nov. Report No.: 10-05142-EF-1. U.S. Preventive Services Task Force Evidence Syntheses, formerly Systematic Evidence Reviews.
236. Kalager M, Haldorsen T, Bretthauer M, Hoff G, Thoresen SO, Adami HO. Improved breast cancer survival following introduction of an organized mammography screening program among both screened and unscreened women: a population-based cohort study. Breast Cancer Res. 2009;11(4):R44.
237. Schopper D, de Wolf C. How effective are breast cancer screening programmes by mammography? Review of the current evidence. Eur J Cancer. 2009;45(11):1916-23.
238. Komenaka IK, Martinez ME, Pennington RE Jr, Hsu CH, Clare SE, Thompson PA, Murphy C, Zork NM, Goulet RJ Jr. Race and ethnicity and breast cancer outcomes in an underinsured population. J Natl Cancer Inst. 2010;102(15):1178-87.
239. Booth CM, Li G, Zhang-Salomons J, Mackillop WJ. The impact of socioeconomic status on stage of cancer at diagnosis and survival: a population-based study in Ontario, Canada. Cancer. 2010;116(17):4160-7.
240. Amir E, Freedman OC, Seruga B, Evans DG. Assessing women at high risk of breast cancer: a review of risk assessment models. J Natl Cancer Inst. 2010;102(10):680-91.
241. Kortenkamp A. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. Environ Health Perspect. 2007;115(Suppl 1):98-105.

242. E Silva, N. Rajapakse, A. Kortenkamp. Something from "Nothing" - Eight Weak Estrogenic Chemicals Combined at Concentrations below NOECs Produce Significant Mixture Effects Environ. Sci. Technol. 2002;36(8):1751 -1756.
243. Shen Y, Costantino JP, Qin J. Tamoxifen chemoprevention treatment and time to first diagnosis of estrogen receptor-negative breast cancer. J Natl Cancer Inst. 2008;100(20):1448-53.
244. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst. 2009;101(2):80-7.
245. Skytte AB, Gerdes AM, Andersen MK, Sunde L, Brøndum-Nielsen K, Waldstrøm M, Kølvraa S, Crüger D. Risk-reducing mastectomy and salpingo-oophorectomy in unaffected BRCA mutation carriers: uptake and timing. Clin Genet. 2010;77(4):342-9.
246. Evans DG, Lalloo F, Ashcroft L, Shenton A, Clancy T, Baildam AD, Brain A, Hopwood P, Howell A. Uptake of risk-reducing surgery in unaffected women at high risk of breast and ovarian cancer is risk, age, and time dependent. Cancer Epidemiol Biomarkers Prev. 2009;18(8):2318-24.
247. http://cancercode.org/index.html (accessed 20/09/2010)
248. Castrellon AB, Glück S. Chemoprevention of breast cancer. Expert Rev Anticancer Ther. 2008;8(3):443-52.
249. Russo IH, Russo J. Role of hormones in mammary cancer initiation and progression J Mammary Gland Biol Neoplasia. 1998;3(1):49-61.
250. Laden K, Felger CB. Antipersirants and deodorants. Marcel Dekker Inc 1988 ISBN-10: 0824778936
251. Krewski D, Yokel RA, Nieboer E, Borchelt D, Cohen J, Harry J, Kacew S, Lindsay J, Mahfouz AM, Rondeau V. Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. J Toxicol Environ Health B Crit Rev. 2007;10 Suppl 1:1-269.
252. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Supplement 7 (1987)
253. Munoz DG, Feldman H. Causes of Alzheimer's disease. CMAJ. 2000;162(1):65-72.
254. Kumar V, Gill KD. Aluminium neurotoxicity: neurobehavioural and oxidative aspects. Arch Toxicol. 2009;83(11):965-78.
255. Darbre PD. Aluminum, antiperspirants and breast cancer. J. Inorgan Biochem. 2005;99:1912-1919.
256. Mannello F, Tonti GA, Darbre PD. Concentration of aluminium in breast cyst fluids collected from women affected by gross cystic breast disease. J Appl Toxicol. 2009;29(1):1-6.
257. DeSalva SJ, Kong BM, Lin YJ. Triclosan: a safety profile. Am J Dent. 1989;2:185-96.
258. Bhargava HN, Leonard PA. Triclosan: applications and safety. Am J Infect Control. 1996;24(3):209-18.
259. Foran CM, Bennett ER, Benson WH. Developmental evaluation of a potential non-steroidal estrogen: triclosan. Mar Environ Res. 2000;50(1-5):153-6.
260. Ishibashi H, Matsumura N, Hirano M, Matsuoka M, Shiratsuchi H, Ishibashi Y, Takao Y, Arizono K. Effects of triclosan on the early life stages and reproduction of medaka Oryzias latipes and induction of hepatic vitellogenin. Aquat Toxicol. 2004;67(2):167-79.
261. Houtman CJ, Booij P, van der Valk KM, van Bodegom PM, van den Ende F, Gerritsen AA, Lamoree MH, Legler J, Brouwer A. Biomonitoring of estrogenic exposure and identification of responsible compounds in bream from Dutch surface waters. Environ Toxicol Chem. 2007;26(5):898-907.
262. Nik Veldhoen, Rachel C. Skirrow, Heather Osachoff, Heidi Wigmore, David J. Clapson, Mark P. Gunderson, Graham Van Aggelen and Caren C. Helbing. The bactericidal agent triclosan modulates thyroid hormone-associated gene expression and disrupts postembryonic anuran development. Aquatic Toxicology 2006;80(3):217–227.

263. Dayan AD. Risk assessment of triclosan [Irgasan] in human breast milk. Food Chem Toxicol. 2007;45(1):125-9.
264. Allmyr M, Adolfsson-Erici M, McLachlan MS, Sandborgh-Englund G. Triclosan in plasma and milk from Swedish nursing mothers and their exposure via personal care products. Sci Total Environ. 2006;372(1):87-93.
265. Gee RH, Charles A, Taylor N, Darbre PD. Oestrogenic and androgenic activity of triclosan in breast cancer cells. J Appl Toxicol. 2008;28(1):78-91.
266. Soni MG, Carabin IG, Burdock GA. Safety assessment of esters of *p*-hydroxybenzoic acid (parabens). Food Chem Toxicol. 2005;43:985–1015.
267. Elder RL. Final report on the safety assessment of methylparaben, ethylparaben, propylparaben and butylparaben. J Am Coll Toxicol. 1984;3:147–209.
268. Jackson EM. Moisturizers of today. J Toxicol-Cutan Ocul Toxicol. 1992;11:173–184.
269. Charnock C, Finsrud T.Combining esters of para-hydroxy benzoic acid (parabens) to achieve increased antimicrobial activity. J Clin Pharm Ther. 2007;32(6):567-72
270. Golden R, Gandy J, Vollmer G. A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol. 2005;35(5):435-58.
271. FDA CFSAN/Office of Cosmetics and Colors March 20, 2006.
272. Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. Parabens as urinary biomarkers of exposure in humans. Environ Health Perspect. 2006;114(12):1843-6.
273. Kiwada H, Awazu S, Hanano M. Study on the biological fate of paraben at the dose of practical usage in rat.1. Metabolism and excretion of ethyl para-hydroxybenzoate (ethyl paraben) and para-hydroxybenzoic acid. J Pharmacobio-Dynam. 1979;2:356–364.
274. Hindmarsh KW, John E, Asali LA, French JN, Williams GL, Mcbride WG. Urinary-excretion of methylparaben and its metabolites in preterm infants. J Pharm Sci. 1983;72:1039–1041.
275. Daston GP. Reply: comment on developmental toxicity evaluation of butylparaben in Sprague-Dawley rats. Birth Defects Res Part B-Develop Reprod. Toxicol. 2005;74:116–117.
276. Lobemeier C, Tschoetschel C, Westie S, Heymann E. Hydrolysis of parabenes by extracts from differing layers of human skin. Biol Chem. 1996;377:647–651.
277. Soni MG, Burdock GA, Taylor SL, Greenberg NA.Safety assessment of propyl paraben: a review of the published literature. Food Chem Toxicol. 2001;39(6):513-32.
278. Masten S. Butylparaben. Final review of toxicological literature [CAS No 94-26-8] June 2004
279. The Cosmetic Products (Safety) Regulations 2004, ISBN 0110497821 http://www.opsi.gov.uk/SI/si2004/20042152.htm (accessed 25/09/10).
280. European Commission – Health and consumer protection directorate – Public health and risk assessment – Scientific Committee on Consumer Products (SCCP) – Opinion on Parabens 2005
281. European Commission – Health and consumer protection directorate – Public health and risk assessment – Scientific Committee on Consumer Products (SCCP) – Opinion on Parabens 2006
282. European Commission – Health and consumer protection directorate – Public health and risk assessment – Scientific Committee on Consumer Products (SCCP) – Opinion on Parabens 2008
283. Bergfeld WE, Belsito DV, Marks JG, Andersen FA. Safety of ingredients used in cosmetics. J Am Acad Dermatol. 2005;52:125–132.
284. http://www.cfsan.fda.gov/~dms/cos-206.html (accessed 25/09/10)
285. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some Alkyl Hydroxy Benzoate Preservatives (Parabens) Are Estrogenic. Toxicology and Applied Pharmacology. 1998;153(1):12-19.
286. Pugazhendhi D, Sadler AJ, Darbre PD. Comparison of the global gene expression profiles produced by methylparaben, n-butylparaben and 17beta-oestradiol in MCF7 human breast cancer cells. J Appl Toxicol. 2007;27(1):67-77.
287. Pugazhendhi D, Pope GS, Darbre PD. Oestrogenic activity of p-hydroxybenzoic (common metabolite of paraben esters) and methylparaben in human breast cancer cell lines. J Appl Toxicol, 2005;25:301-309.
288. Darbre PD, Byford JR, Shaw LE, Hall S, Coldham NG, Pope GS, Sauer MJ. Oestrogenic activity of benzylparaben. J Appl Toxicol. 2003;23(1):43-51.
289. Lemini C, Hernández A, Jaimez R, Franco Y, Avila ME, Castell A. Morphometric analysis of mice uteri treated with the preservatives methyl, ethyl, propyl, and butylparaben. Toxicol Ind Health. 2004;20(6-10):123-32.
290. Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. Oestrogenic activity of parabens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol. 2002;80(1):49-60.

291. Okubo T, Yokoyama Y, Kano K, Kano I. ER-dependent estrogenic activity of parabens assessed by proliferation of human breast cancer MCF-7 cells and expression of ER-alpha and PR. Food Chem Toxicol. 2001;39(12):1225-32.
292. Cashman AL, Warshaw EM. Parabens: a review of epidemiology, structure, allergenicity, and hormonal properties. Dermatitis. 2005;16(2):57-66
293. Pedersen KL, Pedersen SN, Christiansen LB, Korsgaard B, Bjerregaard P.The preservatives ethyl-, propyl- and butylparaben are oestrogenic in an in vivo fish assay. Pharmacol Toxicol. 2000;86(3):110-3
294. Harvey PW. Parabens, oestrogenicity, underarm cosmetics and breast cancer: a perspective on a hypothesis. J Appl. Toxicol. 2003;23:285-288.
295. Fernandez SV, Russo IH, Russo J. Estradiol and its metabolites 4- hydroxyestradiol and 2-hydroxyestradiol induce mutations in human breast epithelial cells. Int J Cancer. 2006;118(8):1862-8.
296. Golden R, Gandy J, Vollmer G. A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol. 2005;35(5):435-58.
297. El Hussein S, Muret P, Berard M, Makki S, Humbert P. Assessment of principal parabens used in cosmetics after their passage through human epidermis-dermis layers (ex-vivo study). Exp Dermatol. 2007;16(10):830-6.
298. Pedersen S, Marra F, Nicoli S, Santi P. In vitro skin permeation and retention of parabens from cosmetic formulations. International Journal of Cosmetic Science 2007;29(5):361–367.
299. Janjua NR, Mortensen GK, Andersson AM, Kongshoj B, Skakkebaek NE, Wulf HC. Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. Environ Sci Technol. 2007;41(15):5564-70.
300. Lee E, An S, Choi D, Moon S, Chang I. Comparison of objective and sensory skin irritations of several cosmetic preservatives. Contact Dermatitis. 2007;56(3):131-6
301. Prusakiewicz JJ, Harville HM, Zhang Y, Ackermann C, Voorman RL. Parabens inhibit human skin estrogen sulfotransferase activity: possible link to paraben estrogenic effects. Toxicology. 2007;232(3):248-56.
302. Darbre PD. Environmental oestrogens, cosmetics and breast cancer. Best Pract Res Clin Endocrinol Metab. 2006;20(1):121-43.
303. Nakagawa Y, Moldéus P. Mechanism of p-hydroxybenzoate ester-induced mitochondrial dysfunction and cytotoxicity in isolated rat hepatocytes. Biochem Pharmacol. 1998;55(11):1907-14.
304. Chen-Jiangang, Ahn-Ki-Chang, Gee-Nancy-A, Gee-Shirley-J, Hammock-Bruce-D, Lasley-Bill-L.Antiandrogenic properties of parabens and other phenolic containing small molecules in personal care products. Toxicol-Appl-Pharmacol. 2007;221(3):278-84.
305. Final amended report on the safety assessment of methylparaben, ethylparaben, propylparaben, isopropylparaben, butylparaben, isobutylparaben,and benzylparaben as used in cosmetic products. Int J Toxicol 2008;27(Suppl. 4):1–82.
306. Darbre PD, Harvey PW. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. J Appl Toxicol 2008;28(5):561–78.
307. Darbre PD, Aljarrah A, Miller WR, Coldham NG, Sauer MJ, Pope GS. Concentrations of parabens in human breast tumours. J Appl. Toxicol. 2004;24:5-13.
308. http://www.unilever.com/, http://www.lonza.com/group/en.html, http://www.avea.co.uk/, http://www.purist.com/, http://www.aubrey-organics.com/
309. Mirick D.K, Davis S, Thomas D.B. Antiperspirant use and the risk of breast cancer. J Natl Cancer Inst 2002;94:1578-80.
310. McGrath KG. An earlier age of breast cancer diagnosis related to more frequent use of antiperspirants / deodorants and underarm shaving. Eur J Cancer Prev 2003;12(6):479-485.
311. Harvey PW, Everett DJ. Significance of the detection of Esters of p-Hydrobenzoic acid (parabens) in human breast tumors. J Appl. Toxicol. 2004;24:1-4.
312. Sharpe RM, Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? BMJ. 2004;328(7437):447-51.
313. Ellsworth DL, Ellsworth RE, Love B, Deyarmin B, Lubert SM, Mittal V, Hooke J, Shriver CD. Outer breast quadrants demonstrate increased levels of genomic instability. Ann Surg Oncol. 2004;11(9):861-8.

314. Darbre PD. Recorded quadrant incidence of female breast cancer in Great Britain suggests a disproportionate increase in the upper outer quadrant of the breast. Anticancer Res 2005;25:2543-2550.
315. Kjaerstad MB, Taxvig C, Andersen HR, Nelleman C. Mixture effects of endocrine compounds in vitro. Int J Androl 2010;33(2):425-33.
316. Lynch HT, Silva E, Snyder C, Lynch JF. Hereditary breast cancer: part I. Diagnosing hereditary breast cancer syndromes. Breast J. 2008;14(1):3-13.
317. O'Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis. 2010;31(6):961-7.
318. Eccles D, Tapper W. The influence of common polymorphisms on breast cancer. Cancer Treat Res. 2010;155:15-32.
319. Nathanson KL, Weber BL."Other" breast cancer susceptibility genes: searching for more holy grail. Hum Mol Genet. 2001;10(7):715-20.
320. Taioli E, Attong-Rogers A, Layne P, Roach V, Ragin C. Breast cancer survival in women of African descent living in the US and in the Caribbean: effect of place of birth. Breast Cancer Res Treat. 2010;122(2):515-20.
321. Exley C, Charles LM, Barr L, Martin C, Polwart A, Darbre PD. Aluminium in human breast tissue. J Inorg Biochem. 2007;101(9):1344-6.
322. Flarend R, Bin T, Elmore D, Hem SL.A preliminary study of the dermal absorption of aluminium from antiperspirants using aluminium-26. Food Chem Toxicol. 2001;39(2):163-8.
323. Washino N, Saijo Y, Sasaki S, Kato S, Ban S, Konishi K, Ito R, Nakata A, Iwasaki Y, Saito K, Nakazawa H, Kishi R. Correlations between prenatal exposure to perfluorinated chemicals and reduced fetal growth. Environ Health Perspect. 2009;117(4):660-7.
324. Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, Sparks A, Weiss B. Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl. 2010;33(2):259-69.
325. Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, Yoo HJ, Cho IH, Kim HW. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect. 2010;118(7):1027-32.
326. Sathyanarayana S, Karr CJ, Lozano P, Brown E, Calafat AM, Liu F, Swan SH. Baby care products: possible sources of infant phthalate exposure. Pediatrics. 2008;121(2):e260-8.
327. Karpeta A, Gregoraszczuk E. Mixture of dominant PBDE congeners (BDE-47, -99, -100 and -209) at levels noted in human blood dramatically enhances progesterone secretion by ovarian follicles. Endocr Regul. 2010;44(2):49-55.
328. Gregoraszczuk EL, Ptak A, Karniewska M, Ropstad E. Action of defined mixtures of PCBs, p,p'-DDT and its metabolite p,p'-DDE, on co-culture of porcine theca and granulosa cells: steroid secretion, cell proliferation and apoptosis. Reprod Toxicol. 2008;26(2):170-4.
329. Kortenkamp A, Faust M. Combined exposures to anti-androgenic chemicals: steps towards cumulative risk assessment. Int J Androl. 2010;33(2):463-74.

8. ADDENDUM

STUDY TO INVESTIGATE THE PRESENCE OF ARTIFICIAL SUBSTANCES IN HUMAN BREAST TISSUE

CONSENT FORM

Study Number: ……..
Patient Identification Number for this trial: ……
Name of Researcher: ……

Principle Investigator: ……..

Please initial box

1. I confirm that I have read and understand the information sheet dated……(version…….) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that sections of my medical notes will be looked at by members of the research team or regulatory authorities where it is relevant to my taking part n the research. I give my permission for these individuals to have access to my notes. ☐

4. I agree to take part in the above study

   a) I agree to the collecting of information about my treatment and follow up. ☐

   b) I agree to the research team keeping small samples of the breast tissue (mastectomy) specimen for further analysis after my operation. ☐

   c) I understand there will be no information from the research analysis of the tissue samples available directly to me. ☐

Name of Patient	Date	Signature
……		
Name of person taking consent	Date	Signature
……		
Researcher	Date	Signature
……		

1 for patient; 1 for researcher; 1 to be kept with hospital notes

CONCENTRATION OF PARABENS IN HUMAN BREAST TISSUE STUDY

INFORMATION FOR GPS AND CONSULTANTS

A patient under your care has agreed to participate in a study at the Nightingale Centre, Withington Hospital, Manchester in collaboration with the University of Reading Division of Cell and Molecular Biology.

This study involves taking samples of breast tissue from the mastectomy specimen of women undergoing surgery for breast cancer and subjecting them to laboratory analysis. The primary outcome measure is to determine the percentage of women who have detectable levels of parabens in the normal breast tissue. Parabens are preservatives found in many cosmetics and food stuffs and there has been controversy recently surrounding a possible link between these compounds and breast cancer. Previous studies have shown these compounds to be present in breast cancer cells but it is not known if they are absorbed systemically or peripherally. If parabens are found in our study of healthy breast tissue, we would like to see if a concentration gradient exists across the breast from the underarm area.

Rationale for the study

The incidence of breast cancer has been increasing worldwide over the past thirty years, with less affluent countries catching up with incidence rates found in Western countries. Specific causes of Breast cancer have not yet been found, though epidemiological studies show that 90% are environmental in origin. Changes in human breast throughout the lifetime are in part due to fluctuations in the hormone oestrogen. It is known that variations in lifetime exposure to oestrogen, through in menarche, menopause, childbirth, use of OCP and HRT does exert a small but definitely increased risk of developing breast cancer.

One theory amongst many for this observed trend, could be that the upper outer quadrant is the area closest to the axilla, and hence to where underarm cosmetics are applied. Underarm cosmetics contain a cocktail of diverse chemicals what are applied frequently to the body, without question of toxicity and are placed on the area directly adjacent to the breast. They are not rinsed off each time, thus allowing for local accumulation to occur and may penetrate continuously through the skin without invoking any major physiological carrier, such as blood or lymphatics.

The main active ingredients of these cosmetics are antiperspirant agents, deodorants and preservatives. These are a wide range of chemicals known to exert a variety of toxic effects. Alkyl esters of *p*-hydroxybenzoic acid (parabens) are widely used as preservatives owing to their high antimicrobial acivity and these are known to possess oestrogen-mimicking properties in human breast cancer cells. The Aluminium-zirconium salts and aluminium chlorhydrate are the main antiperspirant components, their mechanism of action thought to involve the formation of a physical plug at the top of the sweat duct, which then prevents the escape of sweat onto the body surface.

Any carcinogenic action by the constituent chemicals would require a minimal combination of chemicals capable of binding to DNA and agents capable of promoting growth of damaged breast cells. The diversity in usage of these cosmetics and the range of different products available provides ample possibility for breast cancer to arise through issues of quantity used, through pattern of usage or through individual susceptibility to specific product formulations.

We are keen to stress that this is a pilot study in response to several recently published articles in scientific journals calling for research into this particular area especially as very little data has been collected to date.

Contact information: Principle Investigator, ……

CONCENTRATIONS OF PARABENS IN HUMAN BREAST TISSUE STUDY

PARTICIPANT QUESTIONNAIRE

Thank you for agreeing to participate in our study. To help us gather as much information as possible, we would be grateful if you could spend a few moments filling in this questionnaire. All information supplied will be treated in the strictest confidence.

Please delete as appropriate

1. What is your age? ……
2. What is your occupation? ……
3. How old were you when you started having periods? ……
4. Have you had children? **YES / NO**
5. If yes, did you breast feed your children and for how many months?
   ……..
   ……..
6. Are you right or left-handed? **RIGHT / LEFT**
7. Are you vegetarian? **YES / NO**
8. Do you live in an urban or rural environment? **URBAN / RURAL**
9. Have you ever used underarm Deodorant or Antiperspirants? **YES / NO**
10. At what age did you first start using underarm deodorants?
    ……..
11. Do you currently use deodorants or Antiperspirants? **YES / NO**

    If yes, do you regularly use:

    a) Antiperspirant only **YES / NO**

    b) Deodorant only **YES / NO**

    c) Antiperspirant and Deodorant combined **YES / NO**

    If you are not sure which type, please could you write down the brand of the deodorant you use most frequently?

    ……..

Contacts for further information:

Please discuss any questions you may have with your doctor or members of the breast care team.

Your specialist is…….

Contact telephone no…….

Your research nurse is

Contact telephone no **Thank you for your help with this study**

CONCENTRATION OF PARABENS IN HUMAN BREAST TISSUE STUDY

PARTICIPANT CASE REPORT FORM PART 1

Eligibility criteria:

1. Subjects requiring single or bilateral mastectomy to treat their primary breast cancer.
2. Subjects who are able to give voluntary, written, informed consent to participate in this study and from whom consent has been obtained.
3. Genetic female subjects aged 18 years or above.

Two copies of this form to be made and kept:

1. Inside the participant's notes for the duration of the study.
2. In the study folder

Patient's name……

Date of Birth……

Address……
……
……
……

Place Identification sticker here if available

Action	**Stage at which action is to be taken**	**Date done** **(please also initial)**
Issue Subject information sheet	Out-Patient appointment after giving diagnosis	
Consent form completed	Pre-operative assessment clinic or on admission to hospital	
Participant questionnaire issued	Pre-operative assessment clinic or on admission to hospital	
Completed participant questionnaire received	Pre-operative assessment clinic or on admission to hospital	
Check Investigator available to harvest samples intra-operatively	Pre-operative assessment clinic or on admission to hospital	
Randomized labeling codes issued to Investigator harvesting samples	On day of surgery	
Harvesting, labeling and freezing of samples	Intra-operatively	

CONCENTRATION OF PARABENS IN HUMAN BREAST TISSUE STUDY

PARTICIPANT CASE REPORT FORM PART 2

To be read by the investigator harvesting the tissue samples prior to performing the procedure.

You will need to take a sugar-cubed sample of breast tissue (approx 2g) from 4 areas of the mastectomy specimen at the end of the operation. Obviously please avoid taking tissue from the area around the actual tumour.

Each sample must be carefully labeled NOT with its orientation in the breast but with the codes relating to its orientation. These will have been provided to you pre-operatively.

Each sample must be promptly frozen with liquid nitrogen and stored in the -70°c fridge in the transplant centre at Wythenshawe hospital. Instructions on how to do this will have been provided to you when you were given the orientation codes.

To be completed by the investigator harvesting the tissue samples:

Orientation of tissue samples:

Enter codes (given to you pre-operatively) i.e. a1, b3, c4 etc

Site within breast specimen	Code
Axillary fat	
Lateral Breast Tissue	
Mid-specimen Breast Tissue	
Medial Breast Tissue	

Adverse Event Report

An adverse event is defined as 'any untoward medical occurrence in a subject'

If you have reason to believe that participation in this study has resulted in the occurrence of an adverse event please record the date and nature of this below.

……
……
……
……
Signature of Investigator……Name print)……..