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Some hormonal factors in the etiology of endometrial cancer

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ABSTRACT

Some hormonal factors in the etiology of endometrial cancer

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The main purpose of this dissertation was to study the impact of some hormone-related factors in the etiology of endometrial neoplasms, i.e. hormone replacement therapy, use of oral contraceptives, serum levels of 20 different organochlorine substances, and polymorphisms in the estrogen receptor α (ER) gene.

We conducted two population-based case-control studies among post-menopausal women. In the first one, 789 women with a reported diagnosis of primary endometrial cancer and 3368 age-frequencymatched control women were enrolled from allover Sweden. These women answered a questionnaire on use of oral contraceptives and hormone replacement therapy, among other questions. We used unconditional logistic regression to calculate odds ratios (OR) as estimates of relative risks. We found a duration-dependent increase in the relative risk of endometrial cancer both among women who used orally administered estriol, 1-2 mg/day (multivariate OR following 5 or more years of use: 3.0; 95% confidence interval [CI] 2.0-4.4) and medium-potency estrogens without addition of progestins (multivariate odds ratio following 5 or more years of use: 6.7; 95% CI 4.3-10.5), compared to women who never used these substances. Following combined estrogen-progestin use, the association was considerably weaker (multivariate odds ratio following 5 or more years of use: 1.6; 95% CI 1.1-2.4) than for estrogens without progestins, and the increased relative risk was confined to women using cyclic regimens (i.e. with less than 16 days of added progestins, mostly 10 days; multivariate OR for 5 or more years of use: 2.9,95% CI 1.8-4.6). Continuous addition of progestins reduced the relative risk (OR for 5 or more years of use 0.2, 95% CI 0.1-0.8), compared to women who never used these hormone replacement regimens. Women who used oral contraceptives had a 30% decreased relative risk for endometrial cancer compared to women who never used these compounds. The protective effect of combined oral contraceptives use remained for at least 20 years after cessation of use. Subsequent use of hormone replacement did not modify these protective effects.

In the second study we enrolled women - 154 with endometrial cancer and 205 age-frequency-matched controls - who never used hormone replacement, and who were residents in 12 selected counties of Sweden. We collected questionnaire information and blood samples, from which we separated serum for analysis of organochlorine compounds (pesticides and polychlorinated biphenyls), and extracted DNA for analyses of ER gene polymorph isms. We found no significant associations between endometrial cancer risk and serum levels of the 10 pesticides and 10 polychlorinated biphenyls
studied. We conclude that our data do not support the hypothesis that organochlorine exposures
studied increase risk for endometrial cancer. Results from analysis of the ER gene polymorphism showed a multivariate OR for the Xba I XX genotype of 0.51 (95% CI 0.20-1.27) compared to the xx genotype. The PP Pvu II genotype was also associated with a non-significantly decreased risk for endometrial cancer (multivariate OR 0.69, 95% CI 0.34-1.43) compared with the pp genotype. The multivariate OR for two short TA (< 19 repeats) alleles versus two long alleles was 1.50 (95% CI 0.72-3.17). We observed the same pattern of results in an expanded group of subjects, which included women who had used hormone replacement (in total 288 cases and 392 controls). These data suggest that variants of the ER gene may be associated with an altered risk of endometrial cancer.

KEY WORDS: **endometrial neoplasms, hormone replacement therapy, estrogens, progestins, contraceptive agents, organochlorines, pesticides, polychlorinated biphenyls, estrogen receptor alfa polymorphisms.**

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To all women who took part in these studies

ABSTRACT

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CONTENTS

LIST OF PAPERS

The thesis is based on the following papers, referred to in the text by their Roman numerals:

I) Weiderpass E, Baron JA, Adami HO, Magnusson C, Lindgren A, Bergström R, Correia N, Persson I. Low potency oestrogen and risk of endometrial cancer: a case-control study. Lancet 1999; 353:1824-1828.

II) Weiderpass E, Adami HO, Baron JA, Magnusson C, Bergström R, Lindgren A, Correia N, Persson I. Risk of Endometrial Cancer Following Estrogen Replacement With and Without Progestins. J Natl Cancer Inst 1999; 91:1131-1137.

III) Weiderpass E, Adami H-O, Baron JA, Magnusson C, Lindgren A, Persson I. Use of oral contraceptives and endometrial cancer risk (Sweden). Cancer Causes Control 1999; 10:277-284.

IV) Weiderpass E, Adami HO, Baron JA, Wicklund-Glynn A, Aune M,, Atuma S, Persson I. Organochlorines and Endometrial Cancer Risk. Cancer Epidemiol Biomarkers Prev 2000; 9:487-493.

V) Weiderpass E, Persson I, Melhus H, Wedren S, Kindmark A, Baron J. Estrogen receptor α gene polymorphisms and endometrial cancer risk. Carcinogenesis 2000; 21:623-627.

ABBREVIATIONS

BMI	Body mass index (kg/m^2)
CB	Chlorinated biphenyl
CI	Confidence intervals
COC	Combined oral contraceptives
DMPA	Depot medroxyprogesterone acetate
ER	Estrogen receptor-α
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HNPCC	Hereditary non-polyposis colorectal cancer
HRT	Hormone replacement therapy
IARC	International Agency for Research on Cancer
IDDM	Insulin dependent diabetes mellitus
IUD	Contraceptive intrauterine device
MPA	Medroxyprogesterone acetate
NIDDM	Non-insulin dependent diabetes mellitus
o,p'-DDE	1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethylene
o,p'-DDT	1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
OC	Oral contraceptives
OR	Odds ratio
p,p'-DDD	1,1- dichloro-2,2-bis(4-chlorophenyl)ethane
p,p'-DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene
p,p'-DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
PCB	Polychlorinated biphenyls
RFLP	Restriction fragment length polymorphism
RR	Risk ratio
SHBG	Sex hormone binding globulin

INTRODUCTION

Endometrial cancer is the 6th most common cancer among women worldwide, accounting for 3.8% of all incident cancers. Incidence rates are higher in North America and northern Europe than in Asia and Africa (Parkin et al., 1999). Socioeconomic gradients in incidence within populations are inconsistent. Increased risk seems to be related to late menopause, low parity, early age at last birth, obesity, some medical conditions, and possibly with intake of saturated animal fat. Smoking, intake of vegetables and fruits, and physical activity may be protective (Slattery et al., 1989; Shu et al., 1993a; Sturgeon et al., 1993; Zheng et al., 1993; Kelsey et al., 1994; Daly et al., 1995; Grady and Ernster, 1996; Faggiano et al., 1997; International Agency for Research on Cancer - IARC - 1990; World Cancer Research Fund and American Institute of Cancer Research, 1997).

Although menopausal estrogen therapy without progestins is established as a strong risk factor for endometrial cancer, important uncertainties remain regarding the persistence of the risk after discontinuation of treatment, the effect of use of low potency estrogens (i.e. estriol and dienoestrol) and the impact of combined hormone regimens. The addition of progestins to estrogen regimens is thought to counteract the increased risk conferred by the estrogens, but the available studies are few and 'the risk associated with different durations of progestins supplementation per cycle…remains unclear' (IARC, 1999).

Use of combined oral contraceptives (COCs), i.e. formulations with synthetic high-potency estrogens and progestins, has consistently been found to markedly reduce the risk for endometrial cancer (IARC, 1999). However, several aspects of this association remain to be clarified, including effects after long-term use, persistence of the protective association, and the impact of subsequent use of hormone replacement.

The role of other environmentally related factors such as persistent pollutants (e.g. DDT and polychlorinated biphenyls, reported to have hormone-like effects) on endometrial carcinogenesis is unclear. These substances have been hypothesized as potentially increasing risk for hormone related malignancies, such as breast and endometrial cancers.

Some data indicate that endometrial cancer may occur in genetically susceptible individuals (Goldgar et al. 1994, Gruber et al., 1996; Hemminki et al., 1998), but besides some rare syndromes and Hereditary non-polyposis colorectal cancer (HNPCC, an autosomal dominant condition) (Lynch, 1976), little is known about individual susceptibility to this disease. The role of polymorphisms in the estrogen receptor α (ER) gene on endometrial cancer susceptibility is unknown.

The aim of this work is to examine the associations between some of these possible hormone-related risk factors and endometrial cancer risk.

BACKGROUND

Histopathology

Cancers of the endometrium constitute the vast majority of the neoplasms of the uterine body, and the predominant histopathological type is endometrioid adenocarcinoma (prevalence among all histological types over 85%), followed by seropapillary adenocarcinoma (prevalence among all histological types between 1 and 10%), clear-cell adenocarcinoma (prevalence among all histological types between 1 and 5%), and endometrioid adenocarcinoma with squamous differentiation (adenoacanthoma, and adenosquamous carcinoma), which are extremely rare (Scully et al., 1994; Kurman et al., 1994). Some authors actually consider endometrial cancer as two diseases: Type I or endometrioid and Type II or nonendometrioid (including serous adenocarcinoma, clear-cell adenocarcinoma, squamous cell carcinoma). The endometrioid type would occur in association with hyperplasia, and would be responsive to unopposed estrogenic stimulation. The non-endometrioid type may develop *de novo* in the atrophic endometrium, and is *not* accompanied by endometrial hyperplasia. Estrogenic stimulation would not be likely to play a relevant role in Type II tumors (Koshiyama et al., 1998). However, most of the epidemiological data available on endometrial cancer, briefly resumed here, do not make distinctions between these postulated two different types, and we will denote the tumors as 'endometrial cancer' for simplicity. Other malignancies that may affect the uterus are anaplastic carcinomas, malignant mixed tumors (a mesenchymal tumor), and leiomyosarcomas (Kurman et al., 1994).

The diagnosis of endometrial cancer may not be straightforward. A continuum of histological appearances from normal, through hyperplasia, to frank cancer (Persson et al., 1986) may exist concomitantly. The distinction between hyperplasia and early carcinoma may be difficult. The association between estrogen use and endometrial cancer, especially when highly differentiated and with no or little invasiveness, could be influenced by diagnostic biases (if the reporting pathologist was aware of the hormone exposures); the strength of the relationship between putative risk factors and the disease might vary with the diagnostic criteria for the definition of endometrial cancer.

Clinical aspects of endometrial neoplasms

Endometrial cancer occurs mostly among post-menopausal women (80% or more of all cases) around the 6th decade of life. Vaginal bleeding, or among pre-menopausal women intermenstrual bleeding or menorrhagia, are the most common symptoms.

According to the International Federation of Gynecology and Obstetrics (FIGO) Cancer Committee, endometrial carcinomas are classifiable in four stages, according to surgicopathologic findings in the uterus, cervix, adnexa, and pelvic and/or periaortic nodes, and peritoneal cytologic findings (Mikuta, 1993). These stages are:

I) the cancer is confined to the uterine body;
II) the cancer has extended to the uterine cervix;
III) the cancer has spread outside the uterine body but is still limited to the pelvis;

IV) the cancer has invaded the bladder or large bowel, or metastasized outside the pelvis.

The adenocarcinomas of the endometrium in various stages can be subgrouped in 'grades', according to the proportion of undifferentiated (solid growth) as:

Grade 1 or *well differentiated carcinomas, with maximum 5%* solid areas*;*
Grade 2 or moderately differentiated, with 6 to 50% solid areas,
Grade 3 or *poorly differentiated*, with more than 50% solid areas or entirely undifferentiated (Photopulos, 1994).

The choice of the treatment depends on the tumor stage and differentiation, and commonly consists in hysterectomy with bilateral oophorectomy, lymphadenectomy, with external or vaginal radiation. The prognosis varies according to histopathological type and grade, stage, degree of myometrial invasion, and age at diagnosis.

Descriptive epidemiology – the burden of endometrial cancer

Data on both incidence and mortality are based on registry information. In registries usually the International Classification of Diseases (ICD) codes are used: ICD-9 (9th revision) code 182 or ICD-7 (7th revision) code 172 for *corpus uteri cancer* (which includes both the endometrium and the myometrium). Because most of the cancers of the corpus uteri are indeed endometrial cancers, these estimates are briefly presented.

Incidence

Endometrial cancer is the 6th most common cancer among women worldwide. It accounts for 3.8% of all incident cancers among women or 1.8% of all cancers, which corresponds to approximately 142,000 new cases in 1990 (Parkin et al., 1999). There is great variation in incidence in different populations. Incidence rates are higher in North America and other developed areas as northern Europe than in Asia and Africa (IARC, 1997). Time trends in incidence are not consistent worldwide, but in the Nordic countries there seems to be an increase in lifetime risk (IARC, 1993).

In Sweden, endometrial cancer is still the 3rd most frequent incident cancer among women (1133 cases in 1995, representing 2.7% of all cancers or 5.4% of cancers in women), being preceded in importance by cancers of the breast and colon. Time trends in Sweden show an increasing incidence. In 1961 the age-specific incidence was 16.7 per 100,000; in 1976, 21.6 per 100,000; in 1991, 21.8 in 1991; and in 1995 22.5 per 100,000 (Socialstyrelsen, 1998).

Mortality / Survival

Mortality rates start to increase at time of menopause and increases into high ages to reach maximum levels that are 3- to 6-fold lower than the incidence (Persson, 1990).

Worldwide, survival from endometrial cancer is about 74% in five years (IARC, 1993). Mortality trends are consistent with an overall decrease in Europe, USA and Canada.

In Sweden, the 1-year *crude survival* was 81% in 1961-63, and increased to 90% in 1987-89. The 5-year crude survival has increased slightly from 67% in 1961-63 to 71% in 1985-87. The 1-year *relative survival* rate has increased from 87% in 1961-63 to 92% in 1987-89. The 5-year *relative survival* increased from 76% in 1961-63 to 80% in 1985-87. The age group 55-74 had a 12% increase in the 5-year relative survival from 1961-63 (relative survival of 70%) to 1985-87 (relative survival 82%). The relative survival is lower for older patients, but the differences between women 0-54 and 55-74 are decreasing (Odlind et al., 1995).

Risk factors for endometrial cancer

Age

In Sweden, the incidence of endometrial cancer rises steadily from 5-10 years before menopause until age 65-70, and then declines (Persson et al., 1990). The mean age at diagnosis is around 66 years (Socialstyrelsen, 1998). Almost 95% of the cases are diagnosed in women after the age of 50, and the proportion of women who are 65 years or older at diagnosis increased from 37% in 1961 to 57% in 1989 (Odlind et al., 1995).

Social class

Studies of migrants indicate that there is a risk increase in populations who move to areas with westernized life-styles (IARC, 1990). Differences in rates between urban and rural populations are inconsistent (IARC, 1990). Socioeconomic patterns are inconsistent between countries. The excess seems to be concentrated in high social classes in Cali (Colombia), Sao Paulo (Brazil) and in the US (1969-1971), and in lower social classes in Canada and in Italy. No trends were observed for incidence in Istanbul (Turkey) and Turin (Italy) (Faggiano et al., 1997).

Studies on social class and endometrial cancer from the Nordic countries show the following patterns: in Finland the standardized incidence rates increase towards higher social classes (1971-1985), and differences between social classes seem to remain similar over time (Pukkala and Weiderpass, 1999). No trends in incidence were observed in Sweden and Denmark (Faggiano et al., 1997). Endometrial cancer mortality, however, seem higher in lower social classes in Finland (1969-1972) and Denmark (Faggiano et al., 1997).

Reproductive risk factors

Under the hypothesis that estrogens cause an increased incidence of endometrial cancer, all factors reflecting ovarian, placental or adrenal sex hormone production may influence the risk. Thus, aspects of menstruation and reproduction have been explored in several studies of risk factors for endometrial cancer.

The effects of childbearing on endometrial cancer risk have been examined in a large number of epidemiological studies (Grady and Ernster, 1996). Nulliparous women have been consistently found to be at two to threefold higher risk as compared with parous women (Parazzini et al., 1991; Kvåle et al. 1988; Lesko et al., 1991; Albrektsen et al., 1995; Shu et al., 1991; Brinton et al., 1992a; La Vecchia et al., 1984). Most studies have found an inverse relation between increasing parity and endometrial cancer risk (Pettersson et al., 1986; Kvåle et al., 1988; Shu et al., 1991; Brinton et al., 1992a; Henderson et al., 1983; Kelsey et al., 1982; Koumantaki et al., 1989; Parazzini et al., 1998), an association that has been attributed to the influence of progesterone levels during pregnancy (Preston-Martin et al., 1990).

While age at first birth appears to be unrelated to risk (Parazzini et al., 1991; Brinton et al., 1992a), several investigations have reported that a last birth occurring late in the reproductive life reduces endometrial cancer risk (Parazzini et al., 1991; Pettersson et al., 1986; Kvåle et al., 1988; Lesko et al., 1991; Kvåle et al., 1992; Albrektsen et al., 1995; McPherson et al., 1996; Parazzini et al., 1998). The biological mechanism behind this association is unknown. Some hormonal factors may play a role in explaining it: women capable of having children at an older age belong to a subgroup not experiencing anovulatory cycles. Pregnancies have short and long-term effects on endogenous hormone levels that may vary slightly according to both maternal age and birth order (Bernstein et al., 1985; Musey et al., 1987). It is possible that prolonged exposure to progesterone is particularly beneficial at older age; high levels of progesterone slow down the mitotic rate and stop the estrogen-induced proliferation of the epithelium, preventing hyperplasia and reducing the risk of malignant transformation. Progesterone may also make epithelial cells less susceptible to malignant change by promoting differentiation (Pitot, 1986). An attractive alternative explanation, but not mutually exclusive, is a non-hormonal effect. It has been suggested that giving birth at an older age may provide protection by mechanically clearing the uterine lining from cells that have undergone malignant transformation. This would happen due to the shedding of cells from the mucosa lining of the uterine cavity during delivery (Kvåle, 1989; Kvåle et al., 1992). Given that the risk of endometrial cancer increases with age, the risk-reducing effect of any pregnancy could be postulated to increase with age at delivery. Uterine changes during pregnancy include thinning, decomposition and strengthening of the uterine wall as well as increasing vascularization. At term the volume of the uterus is 500-1,000 times greater than in non-pregnant state, while the weight increases up to 20 times (Blackburn and Loper, 1992). Following delivery, the uterus goes back to its non-pregnant size and shape within 6 weeks. After the placenta separates, only the basal proportion of the decidua remains. The subsequent restoration of the endometrium varies by site, becoming functional again after about 2-3 weeks, except at insertion point of the placenta where the regeneration process is slower (Resnik, 1984). This hypothesis of a mechanical cleaning of the uterine lining is supported by observations that the risk of endometrial cancer appears to increase with time since the most recent pregnancy (Albrektsen et al., 1995). Also there is some epidemiological evidence of a long-lasting reduction in endometrial cancer risk among women who ever used a contraceptive intrauterine device (IUD) compared to non-users (Castellsauge et al., 1993; Parazzini et al., 1994; Hill et al., 1997; Sturgeon et al., 1997). The reason for this association is unclear, but may reflect a mechanical effect on the uterine lining.

The largest case-control study on these issues to date was recently carried out in Sweden (Lambe et al., 1999), and showed that childless women were at higher risk of endometrial cancer compared to uniparous women. This association was stronger in younger (< 50 years) than in older (50+years) women. At all ages at first birth, a delivery was associated with a reduced risk of endometrial cancer that slowly diminished with time. Among parous women, the risk decreased by almost 20% for each additional live birth. In an analysis limited to women with two or more births that compared the independent effects of age at first and last birth, only older age at last birth was associated with a lowered risk of endometrial cancer. The risk decreased at a rate of about 15% per five-year delay of last birth.

Infertility, defined as unsuccessful attempts to become pregnant during a three-year period, or seeking medical advice because of inability to become pregnant, is associated with an up to threefold increased risk of endometrial cancer (Brinton et al., 1992a; Henderson et al., 1983).

Late menopause has been shown to be a risk factor for endometrial cancer in most (Grady and Ernster, 1996), but not all (Brinton et al., 1992a), studies. Early age at menarche carries, if anything, a very modest increase in risk, but the evidence is inconsistent (Brinton et al., 1992a); and has been reported as limited to subgroups of premenopausal (La Vecchia et al., 1984) or obese women (Henderson et al., 1983). A history or irregular menstruations has been found more common in endometrial cancer cases (Henderson et al., 1983). These patterns suggest that factors pertaining to menstruation reflect aspects of ovarian function that may be important in endometrial carcinogenesis, either in excess of cycles without opposing progesterone (unovulatory cycles in fertile women or those with irregular periods) or a greater number of cycles without interrupting pregnancies (nulliparous, late menopause).

Cigarette smoking

A protective effect of cigarette smoking on endometrial cancer risk was initially suggested on the basis of data in a case-control study reported in 1980 (Weiss et al, 1980a). Since then, at least 12 additional case-control studies were reported, all but one confirming a reduced risk (Weiss, 1990; Grady and Ernster, 1996). In studies were results stratified by menopausal status are presented, the effect seemed confined to postmenopausal women (Koumantaki et al., 1989). Three of the studies found a relationship with the amount of cigarettes smoked, one with a positive finding only in heavy smokers, whereas three studies showed no evidence of dose-response. There seems to be no effect of former smoking; in a total of seven studies providing such information, five reported no risk reduction (Weiss, 1990; Grady and Ernster, 1996). Only one study provides data implying that cigarette smoking may reduce the excess risk of endometrial cancer after hormone replacement therapy (Weiss et al., 1980a). The adverse effect of obesity on endometrial cancer risk seems to be less pronounced among current smokers, but data are scarce (Elliot et al., 1990).

In summary, the preponderance of evidence suggests that current cigarette smoking confers protection against endometrial cancer. However, its possible modification of

HRT and obesity effects seems uncertain. Endometrial cancer mortality, however, seems not to be associated with smoking (Garfinkel., 1980; Cederlof et al., 1975).

The inverse association of cigarette smoking with endometrial cancer risk appears to be part of the general pattern in which female smokers behave as though they are relatively estrogen-deficient – the 'antiestrogenic' effect of smoking (Baron et al., 1990). Since cigarette smoking is related to an earlier age at menopause and an increased risk of osteoporotic fractures – both of which reflect less exposure to endogenous estrogens – it has been hypothesized that smoking acts by diminishing estrogenic effects, the suggested mechanisms being an up-regulation of 2-hydroxylation and a net reduction in estradiol levels (Michnovicz et al., 1988). However, several studies could not find a reduction in serum estrogen levels among smokers compared to non-smokers (Key et al., 1996; Baron, 1990; Barret-Connor, 1990). One study suggested that elevated androgen levels might be an explanation of a protective effect (Khaw et al., 1988). In a recent report comparing estrone, estradiol and estriol levels in urine samples, no difference in smokers versus non-smokers was noted in premenopausal women; in post-menopausal women, estriol excretion rates were about 20 percent lower in smokers (Key et al., 1996). However, among women who take oral estrogens, smokers appear to have lower circulating estrone and estradiol levels than non-smokers (Cassidenti et al., 1990; Jensen et al., 1985). Moreover, the impact of oral estrogens on bone density and serum lipids is attenuated in smokers, indicating that there is a clinical impact of the decreased circulating estrogen levels (Jensen et al., 1985; Jensen and Christiansen, 1988). These data suggest that cigarette smoking affects the absorption or metabolism of exogenous estrogens. Smoking is known to induce hepatic microsomal enzymes (Dawson and Vestal, 1982), and since all orally-administrated drugs must pass through the liver before reaching systemic circulation, an hepatic effect seems likely. Indeed, smoking is known to enhance the metabolism of other orally administered drugs such as theophylline; higher doses of this drug are required for smokers than for non-smokers (Dawson and Vestal., 1982).
There is some epidemiological support for this phenomenon. In one study, smoking had a substantial impact on hip fracture risk among women who had ever taken oral estrogens, while among those who had never taken estrogens, smoking had no effect (Kiel et al., 1992).

Alcohol

The published data regarding the relationship between alcohol intake and endometrial cancer risk are conflicting. There is a report of a positive association from a hospital-based case-control study, with an over fourfold increase in the risk for women taking more than 4 drinks per day compared with non-users (La Vecchia, 1986a). Other reports suggest no effect (Shu et al., 1991; Gapstur et al., 1993), a non-significant inverse association (Austin et al., 1993; Swanson et al, 1993a), or a frank protective influence against this cancer (Webster et al., 1989). Two studies that used 'other cancer' controls also reported reduced risks of endometrial cancer from alcohol, but these findings are hampered by possible biases introduced by their control groups (Williams and Horm, 1977; Kato et al., 1989). At large, the available data do not give evidence that alcohol consumption affects endometrial cancer risk.

An association with endometrial cancer could possibly be explained by hormonal effects of alcohol consumption. Cross-sectional studies of alcohol effects on levels of endogenous estrogens provided contradictory results: positive correlations with estradiol levels (Gavaler and Love, 1992), urinary estrogens (Katsouyanni et al., 1991), and plasma estrone sulfate levels (Hankinson et al., 1995) in postmenopausal women. In other studies, no association with estrone, estradiol (Cauley et al., 1989; London et al., 1991) or free (non-protein bound) estradiol (London et al., 1991) was observed.

Body size in different periods of life and some obesity related conditions

Adult obesity (generally defined as body mass index [BMI], i.e. or weight in kg divided by height in meters squared, over 30) has consistently been associated with an increased risk of endometrial cancer both in the pre- and postmenopausal periods (World Cancer Research Fund and American Institute of Cancer Research, 1997). This association has largely been ascribed to increased estrogen levels in obese women (Hankinson et al., 1995; Shoff and Newcomb, 1998) (see below). However, several aspects of the relationship between body size in different periods of life and endometrial cancer remain uncertain. Weight gain between adolescence and menopause has been reported to explain the associations between weight and post-menopausal breast cancer (Brinton and Swanson, 1992; Magnusson et al., 1998) and hip fracture (Cummings et al., 1995), two estrogen related conditions, but it is not clear if weight change has an independent relationship with endometrial cancer. Some studies suggested that weight gain would also explain the association between obesity and endometrial cancer risk (Le Marchand et al., 1991; Shu et al., 1992; Swanson et al., 1993b; Olson et al., 1995), but there are also contradictory data (Levi et al., 1992). Most of these analyses did not consider the association in detail, and, in particular, did not adjust for recent weight.

Both height and obesity during adolescence or early adulthood are determined by genetic constitution and energy intake during puberty (Stoll et al., 1994). Most of the evidence however, indicate that hormonal events during childhood and adolescence are unrelated to endometrial cancer risk (Le Marchand et al., 1991; Shu et al., 1992; Swanson et al., 1993b; Levi et al., 1992; Henderson et al., 1983). This pattern contrasts with findings for breast cancer, for which both height and adult weight gain seem to have important effects (Brinton and Swanson, 1992b; Magnusson et al., 1998; Huang et al., 1997; Tretli, 1989; Ziegler et al., 1996; De Stavola et al., 1993; Vatten et al., 1992).

The mechanisms that underlie the relationship between obesity and endometrial cancer are thought to be hormonal. Adult obesity may be associated with anovulatory ovarian cycles and diminished production of progesterone among premenopausal women (Pike et al., 1993). After menopause, obesity leads to augmented conversion of androstenedione to estrone and decreased levels of SHBG, and thus increased levels of bioavailable estrogens (Pike et al., 1993). Furthermore, adiposity may be part of a metabolic syndrome involving insulin resistance and compensatory hyperinsulinemia, which may increase endometrial cancer risk through estrogenic or growth factor pathways (Kaaks, 1996; Rutanen, 1998).

Findings regarding the association of endometrial cancer with non-insulin dependent diabetes mellitus (NIDDM) – a obesity-related disorder – indicate a probable association (Grady and Ernster, 1996). When BMI has been taken into account, diabetes seems to confer about a doubling of risk for endometrial cancer (Shoff and Newcomb, 1998; Brinton and Swanson, 1992a; Levi et al., 1992; Elwood et al., 1977; Hoogerland et al., 1978; Hulka et al., 1980; Kelsey et al., 1982; O'Mara et al., 1985; Adami et al., 1991; La Vecchia et al., 1994; Maatela et al., 1994). These data suggest that diabetes mellitus is associated with endometrial cancer risk independently of obesity. Studies on insulin dependent diabetes mellitus (IDDM) are scarce; their results indicate that probably women with IDDM have even higher risks than women with NIDDM (Weiderpass et al., 1997; Weiderpass et al., 1999). However, these results are based on small numbers, and another study reported an increased risk of endometrial cancer only among NIDDM (La Vecchia et al., 1994).
Several mechanisms could explain the increased endometrial cancer risk observed among women with NIDDM or IDDM. Insulin levels are commonly increased in NIDDM, at least in initial phases. In IDDM, use of exogenous insulin also leads to high circulating levels. Insulin can stimulate androgen synthesis in the ovarian stroma, decrease levels of SHBG and increase levels of free estradiol premenopausally and estrone postmenopausally (Kaaks, 1996), resulting ultimately in higher levels of bioavailable estrogens. It has also been hypothesized that insulin may enhance the effects of insulin-like growth factors, and consequently endometrial mitogenesis (Shoff and Newcomb, 1998; Rutanen, 1998).

The literature regarding the association between hypertension – in most cases a obesity related condition - and endometrial cancer is inconclusive. There are some reports of a positive association (Olson et al., 1995; Elwood et al., 1977; Hoogerland et al., 1978; Hulka et al., 1980; Gray et al., 1977; Weiss et al., 1980a; Jelovsek et al., 1980; La Vecchia et al., 1986a) and others of no association (Shu et al., 1992; Kelsey et al., 1982; Mack et al., 1976; Horwitz and Feinstein, 1978; Spengler et al., 1981; Ewertz et al., 1988), including one study where adjustment for BMI and use of hormone replacement as possible confounding factors were made (Brinton, 1992b).

Gallbladder diseases – another condition related to obesity – has been associated with endometrial cancer risk in some studies (Grady and Ernster, 1996). However, this assocation seems to be confounded by use of estrogens and obesity, and in at least one study that took into consideration these factors in the analysis no association was observed (Brinton et al., 1992b).

Dietary factors

According to the World Cancer Research Fund and American Institute of Cancer Research (1997) the effect of other dietary factors beside energy and related factors (leading to high body mass / obesity) is insufficiently studied to permit any conclusions. Intake of fruits and vegetables possibly decrease the risk of endometrial cancer (Barbone et al., 1993; Levi et al., 1993a; Shu et al., 1993b), and diets high in total fat and saturated or animal fat possibly increase the risk (Potischman et al., 1993; Shu et al., 1993b; Goodman et al., 1994; La Vecchia et al., 1986a, Levi et al., 1993a; Armstrong and Doll, 1975). These associations seem to be independent of the effect of obesity.

The World Cancer Research Fund and American Institute for Cancer Research, (1997) declared that, due to scarcity of data and contradictory results, no judgement was possible regarding the association between endometrial cancer risk and diets high in monounsaturated fats, or rich in polyunsaturated vegetable fat, cholesterol, animal protein, carotenoids, vitamine C, cereals and grains, fish, poultry and eggs. There is, however, some evidence that diets high in monounsaturated fats and (Potischman et al., 1993) cholesterol (Barbone et al., 1993; Potischman et al., 1993; Goodman et al., 1994) may increase the risk for endometrial cancer, and that diets high in carotenoids (Barbone et al., 1993; La Vecchia et al., 1986a; Levi et al., 1993a) may decrease the risk.

Physical activity

Increasing physical activity has been reported to decrease the risk for endometrial cancer in two recent prospective studies in Sweden (Moradi et al., 1998; Terry et al., 1999). Physical activity was also associated with decreased risk for endometrial cancer in several (Olson et al., 1997; Shu et al., 1993a; Zheng et al., 1993; Sturgeon et al., 1993; Hirose et al., 1996; Levi et al., 1993b), but not all (Dosemeci et al., 1993) case-control studies.

A proposed mechanism for the protective effect of exercise is lowered body fat (Shu et al., 1993a; Sturgeon et al., 1993), and consequently decreases the aromatization in adipose tissue of androstenedione to estrone, the major source of estrogens in post-menopausal women (Enriori and Reforzo-Membrives, 1984). However, some studies found that the protective effects of physical activity are independent of body mass (Shu et al., 1993a; Terry et al., 1999). Other proposed mechanisms, as intermediary steps in a possibly protective chain of events are late age at menarche, early onset of menopause and anovulation (Moradi et al, 1998; Frisch et al., 1980; Warren, 1980).

Tamoxifen

Tamoxifen is a non-steroidal compound that has estrogenic or anti-estrogenic effects, according to the target tissue. In 1996, the International Agency for Research on Cancer (IARC) classified tamoxifen as a human carcinogen. Since the 1970s tamoxifen has been prescribed for women with breast cancer – initially, for palliative treatment in advanced stages; later also as an adjuvant to primary surgery. Recently, long-term use has been considered for primary prevention in women at high risk of developing breast cancer (IARC, 1996).

Pathological abnormalities are frequently observed in the endometria of tamoxifen-treated women, as hyperlasias, endometrial polyps, endometrial polyp carcinomas, and invasive endometrial carcinomas (Koshiyama et al., 1998). The most convincing support for a carcinogenic effect of tamoxifen on the endometrium stems from randomized clinical trials in which adjuvant tamoxifen has been compared with placebo (Fisher et al., 1994) or observation (Fornander et al., 1991; Andersson et al, 1992; Ryden et al., 1992; Rutqvist et al., 1995). However, several, mostly

smaller, randomized studies showed no excess risk (IARC, 1996) while observational cohort (Robinson et al., 1995; Curtis et al., 1996) and case-control (Hardell, 1988; van Leeuwen et al., 1994; Cook et al., 1995; Sasco et al., 1996) studies produced contradictory results; of those considered adequate (IARC, 1996), one (Curtis et al., 1996) showed a statistically significant higher risk in breast cancer patients ever treated with tamoxifen. Pooled information from 55 trials that began before 1990 of adjuvant tamoxifen versus no tamoxifen before recurrence of breast cancer, comprising 37,000 women or 87% of the worldwide evidence was published recently (Early Breast Cancer Trialists' Collaborative Group, 1998). Their results show that the incidence of endometrial cancer was approximately doubled in trials of 1 or 2 years of tamoxifen and approximately quadrupled in trials of 5 years of tamoxifen.
There seems to be a higher proportion of unusual histopathologic subtypes of endometrial carcinomas among tamoxifen-associated invasive tumors, such as serous, clear-cell, or mucinous types (Dallenbach-Hellweg and Hahn, 1995). Malignant mixed mullerian tumors and adenocarcinomas have also been related to tamoxifen use (Clement et al., 1996). There is still controversy on the magnitude of the adverse effect on the endometrium, the impact of dose and duration and further the biological behavior of tamoxifen associated endometrial tumors (Rutqvist, 1998).

Estrogens and progestins

Effects on the human endometrium

Estrogens and progestins – used in contraceptives and hormone replacement – have different effects in human target tissues, as the endometrium, cervix, breast, colon, and ovary. These effects can be explained, at least partially (Duval et al., 1983), by estrogen and progestin receptor mechanisms (King, 1991).

On the endometrium, estrogens increase the cells mitotic rate with induction of DNA synthesis and of estrogen receptor protein (Desombre et al., 1987). Progestins oppose the effect inducing differentiation of endometrial cells and down-regulating estrogen receptor, exerting an anti-proliferative, differentiating influence (Gurpide, 1991; Key and Beral, 1992). Progestins also enhances estradiol metabolism, and promotes a shedding of the endometrium (Mattsson and Sporrong, 1998). On the breast, estrogen has adverse effects, but the role of progestins is unclear (IARC, 1999).

The effect of estrogens is observed during the follicular phase of the menstrual cycle when estradiol levels rise, as do cell division rates, followed by a quick decrease associated with increase in progesterone release during the luteal phase.

The strong influence of hormonal factors on endometrial cancer risk is a central theme of the current understanding of the causes of this cancer. Indeed, exposure to excessive estrogens of endogenous as well as exogenous origins, without the opposing effect of progesterone/progestins, entailing continued stimulation of the endometrium, is suggested to be the key mechanism in endometrial carcinogenesis, that is associated with most risk factors for endometrial cancer (Key and Beral, 1992).

Endogenous hormones

The naturally occurring estrogens, estradiol-17β, estriol and estrone, the androgen testosterone, and the progestin progesterone are present in all vertebrate species as secretions of the ovaries, testes and/or adrenal glands or as products of the mammalian placenta. They form part of the total endocrine environment, with complex interrelations and control (IARC, 1979).

Estrogens are responsible, together with other hormones, for the development and maintenance of the female sex organs and for the regulation of the menstrual cycle in primates and estrus cycle in other mammals (IARC, 1979).

Estradiol is the most important endogenous estrogen in women, but estriol may be significant in pregnancy and estrone in the postmenopausal period (Key and Beral., 1992). In premenopausal women the production of endogenous hormones is regulated by feedback mechanisms, and can be influenced by use of exogenous hormones.

Elevated endogenous estrogen levels - as observed in pathological conditions such as polycystic ovarian syndrome (PCO or Stein-Leventhal syndrome) and estrogen-secreting ovarian tumors (as granulosa-cell and theca-cell tumors) - have been reported to be associated with increased risks for endometrial cancer (Björkholm et al., 1980; Grady and Ernster, 1996). Women with PCO have chronically elevated luteinizing hormone (LH), which stimulates the ovaries resulting in increased androstenedione secretion, which is aromatized peripherally to estrone. The mean age at diagnosis of endometrial cancers in PCO patients is around their 40s, i.e. much below the age of non-PCO associated endometrial cancers (Grady and Ernster, 1996).

Besides the pathological conditions described above, there is still some controversy regarding the association of circulating levels of steroid hormone levels and endometrial cancer risk (Grady and Ernster, 1996). Some earlier studies reported no differences in levels of estradiol-17β and estrone in postmenopausal women with endometrial cancer cases compared with controls (Schenker et al., 1979; von Holst et al., 1981). In more recent case-control studies consistent associations were observed for high levels of free estrone, estradiol, and androstenedione, and with low levels of serum hormone binding globulin (SHBG) (Austin, 1993; Nyholm et al., 1993; Potischman, 1996; Sherman, 1997). These associations were independent of body size. Regarding premenopausal women, one of the studies showed that high androstenedione levels, but not estrogen levels, were associated with increased risk (Potischman, 1996).

Exogenous hormones

Basic concepts

Estrogens and progestins, from natural or synthetic origin, are among the most widely used drugs worldwide. Their use varies among countries and within countries, as do their indication, dosages, and regimens. Some common indications for their use include contraception, treatment of dysmenorrhoea, endometriosis, dysfunctional uterine bleeding, and as replacement therapy in patients with gonadal dysgenesis and in women with climacteric symptoms (IARC, 1979). In obstetric practice, progestins have been used in the management of threatened abortion and to prevent premature labor, and local estrogens have been used into the cervix for induction of labor (IARC, 1979).

In the USA, postmenopausal estrogen is the most commonly prescribed medication, and IARC estimates that oral contraceptives are now used by about 90 million women worldwide (IARC, 1999). In Scandinavian countries, combined estrogen-progestin hormone replacement were marketed during the 1970s, while in the USA this sort of treatment was introduced in the 1980s. In Sweden, the sales of replacement hormones has increased threefold in the 1990s.

Natural estrogens used in therapy include estradiol valerate, estriol, and conjugated equine estrogens. Synthetic estrogens include the steroidal products ethinylestradiol and mestranol. Progesterone is the most important natural progestin, but synthetic steroids with similar properties are extensively used. Progestins can be progesterone-derived (17-hydroxyprogesterone derivatives, e.g. medroxyprogesterone acetate) or testosterone derived (19-nor-testosterone derivatives, e.g. norethisterone, norethisterone acetate, levonorgestrel or lynestrenol). In combined oral contraceptives it is common to use 19-nor-testosterone derivatives, which have some androgenic activity. Medroxyprogesterone acetate is a 17α-acetoxyprogesterone derivative commonly used in hormone replacement.

Hormonal contraceptives

The dosages of sex steroid hormones used in combined estrogen-progestin contraception have changed over time. In the 1960s, 100-150 µg ethinylestradiol and about 1-5 mg of progestins were used (IARC, 1999). Doses of ethinylestradiol were successively lowered to 75 µg and 50 µg. More recently the most common contraceptives used in Sweden contain fixed dosages of 30 µg ethinylestradiol and 150 µg levonorgestrel or 150 µg desogestrel, given concurrently in a monthly cycle. Desogestrel, gestodene and norgestimate are new progestins increasingly used.

Progestin-only contraceptives were introduced in the 1970s, and are available as pills, injections, implants, and progestin-releasing intrauterine devices. Progestin-only contraceptives are mostly used in developing countries as intramuscular depot injections and subcutaneous implants. ‘Mini-pills’, or oral progestin-only pills, are used in North-America and Europe, but to a lesser extent than combined oral contraceptives or parentally administered progestin-only contraceptives.

The effect of different progestins in the endometrium seems to differ but the literature is confusing in this regard. In one study (King and Whitehead, 1986) 1 mg of norethisterone produced a similar endometrial secretory response as 125 µg of levonorgestrel or 11 mg or medroxyprogesterone when given for 7 days in women using conjugated estrogens. These dosages have generally been considered equipotent with regard to the endometrial anti-proliferative effects.

Besides regular contraceptive use, combination of estrogens and progestins or progestins-only can be used up to 3 days after intercourse as emergency contraceptives.

The use of hormonal contraceptives is relatively recent. Women exposed to oral contraceptives in young adulthood in the 1960s are only now reaching ages when incidence of malignancies arise. Thus, long-term health effects of use of oral contraceptives are still mostly unknown (IARC, 1999).

Combined hormonal contraceptives

The impact of use of combined hormonal contraceptives on endometrial cancer has been evaluated in at least three cohort (Trapido, 1983; Beral et al., 1988 and 1999; Vessey and Painter, 1995) and 16 case-control studies (Weiss and Sayvetz, 1980; Kaufman et al., 1980; Kelsey et al., 1982; Hulka et al., 1982; Henserson et al., 1983; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; La Vecchia et al., 1986b; Pettersson et al., 1986; WHO 1988 expanded by Rosenblatt et al., 1991; Koumantaki et al., 1989; Levi et al., 1991; Shu et al., 1991; Stanford et al., 1993; Jick et al., 1993; Voigt et al., 1994; Kalandidi et al., 1996).

These studies indicate that users of combined oral contraceptives have a risk reduction for endometrial cancer by about one-half, compared to non-users. The reduction in risk is evident after about two to five years of use, and is stronger the longer the duration of use. The protective effect persists for at least 10 years after cessation of treatment. Some studies report a greater reduction in risk with more recent use, but others report no difference (IARC, 1999).

The available studies do not allow drawing any conclusion about the joint effects of duration and recency of use or about the effects of different dosages of progestins (IARC, 1999).

Data on possible effect modification of the protective effects of oral contraceptives by body mass index are conflicting. No reduction in endometrial cancer risk was found among the highest categories of body weight in two studies (Henderson et al., 1983; Stanford et al., 1993), while two other studies (The Centers for Disease Controls and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi et al., 1991) found a similar reduction in risk for endometrial cancer regardless of body mass.

The protective effect of combined oral contraceptives on endometrial cancer was observed only among nulliparous women in one study (The Centers for Disease

Controls and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987), while other studies reported stronger risk reduction among parous women (Levi et al., 1991) or women with five or more births (WHO, 1988; Stanford et al., 1993).

Two studies reported no reduction in endometrial cancer risk among users of combined oral contraceptives who subsequently used estrogen replacement therapy (Stanford et al., 1993; Voigt et al., 1994). Four other studies did find a reduction in endometrial cancer risk despite subsequent use of estrogen replacement (Kaufman et al., 1980; Hulka et al., 1982; The Centers for Disease Controls and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi et al., 1991).

Up to now, data on combined oral contraceptives with low-dose formulations are very scarce.

Other effects of combined oral contraceptives

Combined oral contraceptives act through inhibition of ovulation. Besides preventing pregnancy, use of oral contraceptives seems to reduce risk of benign breast disease (McGonigle and Huggins, 1991), uterine myomas (Parazzini et al., 1992; Lumbagnon et al., 1996), iron deficiency and anemia, dismenorrhoea, ovarian cysts (Mehta, 1993), salpingitis (Mishelll, 1993; Burkman, 1994), and rheumatoid arthritis (Brennan et al., 1997). Pills containing desogestrel, gestodene or norgestimate seem to improve acne (Mango et al., 1996; Redmond et al., 1997). Evidence on cardiovascular safety has been recently reviewed (WHO Scientific Group on Cardiovascular Disease and Steroid Hormone Contraception, 1998). In summary, they concluded that use of combined oral contraceptives adds to the risk of myocardial infarction and stroke among women with cardiovascular risk factors (smoking, hypertension, diabetes mellitus). Among women without cardiovascular risk factors, there is no increased risk of myocardial infarction associated with low-dose oral contraceptive. Ischaemic stroke risk among non-smokers and non-hypertensive women who use low-dose oral contraceptives is increased 1.5-fold. The risk for haemorragic stroke is not increased before the age of 35 for women without other cardiovascular risk factors, but there is a twofold increase in risk after the age 35. Risk for venous thrombotic embolism is increased 3- to 6-fold among current users of oral contraceptives (regardless of other risk factors as smoking or hypertension); the risk persists elevated after discontinuation of treatment for at least one year. Combined oral contraceptives containing desogestrel or gestodene probably entails a greater risk of thromboembolic diseases than combined oral contraceptives containing levonorgestrel (WHO Scientific Group on Cardiovascular Disease and Steroid Hormone Contraception, 1998). Combined oral contraceptives, and probably low-dose combined oral contraceptives as well, entails a small increase (1.3-1.8 mm Hg in diastolic pressure) in blood pressure (Shen et al., 1994). Current use of combined oral contraceptives (Thijs and Knipschild, 1993) or low-dose oral contraceptives (Strom et al., 1986) increases risk for gallbladder disease.

Several studies on use of combined oral contraceptives and cancer risk in other sites than the endometrium have been recently reviewed (IARC, 1999). In summary, current or recent use of combined oral contraceptives probably entails a very small

increase in breast cancer risk. After 10 years of cessation of treatment, there is no difference in risk between women who used or did not use combined oral contraceptives. Cervical cancer risk seems to be slightly increased after long-term use of combined oral contraceptives. This association was also observed in studies with HPV infected individuals. Ovarian cancer risk and risk for borderline ovarian malignancies seem to be reduced by 50% with use of combined oral contraceptives; this reduction seems to be duration-of-use dependent, and seems to persist for at least 10-15 years after interruption of treatment. Risk for benign hepatocellular tumor and hepatocellular carcinoma are strongly associated with use of combined oral contraceptives. These associations were observed in women not infected with hepatitis B and C viruses, and without chronic liver diseases, and in populations where these conditions are of low prevalence. In populations with high prevalence of hepatitis B and C viruses, use of combined oral contraceptives does not seem to increase risk for hepatocellular carcinoma. There is too little information on use of combined oral contraceptives and risk of cholangiocarcinoma or cancer of the gall-bladder to permit any conclusion about a possible risk relationship. There seems to be no association between use of combined oral contraceptives and risk of colorectal cancer, cutaneous malignant melanoma, or thyroid cancer (IARC, 1999).

IARC (1999) evaluated that there is conclusive evidence that combined oral contraceptives have a protective effect against cancers of the ovary and endometrium. There is also *sufficient evidence* in humans for the carcinogenicity of combined oral contraceptives (Group 1), based on the increased risk of hepatocellular carcinoma in the absence of hepatitis viruses observed in studies of predominantly high-dose preparations.

Progestins-only contraceptives

The mechanism by which progestin-only pills prevent pregnancy is through alteration on the cervical mucus, direct endometrial effects and possibly inhibition of ovulation. There is little information about use of progestins without estrogens and endometrial cancer risk.

One multi-center case-control study in the US among women under 55 years of age, one case out of 433 women with endometrial cancer and six of the 3191 control women had used *progestin-only oral contraceptive* (OR 0.6, 95% CI 0.1-5.0) (The Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987).

One cohort study in the US reported one case of uterine cancer among women receiving *depot medroxyprogesterone acetate* (DMPA) injections, relative to 0.83 expected (RR 1.2; 95% CI 0.1-6.7) (Liang et al., 1983).

In a case-control study in Thailand, *depot medroxyprogesterone acetate* was associated with a 80% lower risk for endometrial cancer (OR 0.2, 95% CI 0.1-0.8) (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1991).

In summary, the available studies indicate that progestin only contraceptives possibly reduce risk for endometrial cancer, but the numbers are too small to reach firm conclusions (WHO Collaborative Study, 1991; IARC, 1999).

The IARC (1999) evaluated the carcinogenicity of progestins-only contraceptives as *inadequate* in humans. The overall evaluation was that progestins-only contraceptives are *possibly carcinogenic to humans* (Group 2B). This evaluation was based on studies on other cancer sites as the endometrium.

Sequential oral contraceptives

Oral contraceptive combinations with high dose unopposed estrogen, followed in sequence by five to seven days estrogen-progestin - sequential oral contraceptives - were removed from the consumer market in the 1970s. These contraceptives were reported to be associated with endometrial abnormalities as proliferation and severe atypical hyperplasia (Lyon and Frisch, 1976; Kaufman et al., 1976; Cohen and Deppe, 1977) and to increase risk for endometrial cancer (Henderson et al., 1983; Voigt and Weiss, 1989; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer Steroid Hormone Study, 1987; Weiss and Savitz, 1980).

Multiphasic oral contraceptives

Multiphasic oral contraceptives consist of varying dosages of estrogens and progestins given concurrently over one cycle. They have been available since the 1980s. There are no data available on long-term risk for endometrial cancer for this sort of contraceptives (IARC, 1999).

Hormone replacement therapy (HRT)

Compounds, regimens and indication

Hormone replacement therapy can be used in different regimens, that vary according to dose and number of days that estrogens and progestins are combined per month, and route of administration (oral, injections, percutaneous). Regimens may include only progestins, estrogens without progestins (‘unopposed estrogens’), cyclical estrogen-progestin combinations, sequential estrogen-progestin, continuous estrogen-progestin combinations, or ‘spacing out’ or ‘long cycle’, i.e. three months use of estrogens with supplementation of a progestins for a period of 14 days.

Combined hormone replacement therapy denotes a regimen with an estrogen combined in different ways with a progestin. Typically the unopposed estrogen is used for 18-24 days per month, and the combination for 7-12 days. In The United States, most of the estrogen used is conjugated equine estrogen, 0.625 mg, daily or with a 5-7-day estrogen-free period at the end of the month, and most of the progestin is medroxyprogesterone acetate (MPA), 5-10 mg day for 10-14 days of the month (Grady and Ernster, 1997). In Sweden the predominant practice today is to

use estradiol 17β or estradiol valerate combined with levonorgestrel or norethisterone acetate. Hormone replacement preparations available in Sweden in 1998 and their indicated mode of use are listed in Table 1.

Progestins only (without estrogens), besides being used in contraception, are also used in the perimenopause to treat bleeding disorders (Mattsson and Sporrong, 1998).

Indications for use of hormone replacement

The task force group on climacteric problems of the Swedish Society of Obstetrics and Gynecology in their most recent report (1998) concluded that:

- Women with early spontaneous menopause or early bilateral oophorectomy (before 40-45 years of age) should be recommended HRT.
- Women with vasomotor symptoms should be recommended therapy with systemic estrogens.
- All women with local vaginal discomfort should be offered therapy with local or systemic low-potency estrogens if they do not use HRT.
- In well-informed women seeking prophylactic HRT because of an increased risk of osteoporosis, therapy may be used…

Recommended choices of treatment regimens in different phases (Mattson and Sporrong, 1998) are:

Premenopause:
progestin (cyclically)
estrogen-progestin (cyclically or sequentially)

Perimenopause:
estrogen-progestin (cyclically or sequentially)

Postmenopause
estrogen-progestin (continuously)
estrogen-progestin (‘spacing out’)
low-potency estrogens (locally or orally).

Table 1. Compounds available on the Swedish market in August 1998, used in hormone replacement therapy as oral treatment, if not otherwise specified (adapted from Mattsson and Sporrong, 1998):

	Generic name	*Trade name*	*Dose*
Medium potency estrogens	*Estradiol*	*Climara*	*50-100 µg/24 h; 1 depot patch / week*
		Estraderm	*25-100 µg/24 h; 2 depot patch / week*
		Estraderm Matrix	*25-100 µg/24 h; 2 depot patch / week*
		Evorel	*25-100 µg/24 h; 2 depot patch / week*
		Menorest	*37,5-100 µg/24 h; 2 depot patch / week*
		FemSeven	*50 µg/24 h; 1 depot patch / week*
		Divigel	*0.5-1 mg/dose as a gel*
		Femanest	*1-2 mg day*
		Progynon	*1-2 mg day*
	Conjugated estrogens	*Premarina*	*0.3-1.25 mg day*
Low potency estrogens	*Estriol*	*Oestriol NM Pharma*	*1-2 mg/ day per os*
		Oestriol Rosemont	*1-2 mg/ day per os*
		Pharma	*1-2 mg/ day per os or*
		Ovesterin	*0.5-1 mg 2 times week, vaginal cream/pessary*
	Estradiol	*Oestring*	*7.5 µg/24 h; 1 vaginal ring every 3rd month*
		Vagifem	*25 µg; 1 vaginal tablet twice weekly*
	Dienestrol	*Dienoestrol*	*0.1-0.5 mg, 1-2 doses vaginal cream or pessary/week*
Progestins	*medroxyprogesterone acetate (MPA)*	*Gestapuran*	*5-10 mg /day*
		Provera	*5-10 mg /day*
	Norethisterone acetate	*Primolut-Nor*	*2.5-5 mg/day*
	Lynestrenol	*Orgametril*	*2.5-5 mg/day*
Combined preparations	*Estradiol 2 mg + Norethisterone acetate 1 mg*	*Kliogest Femanor*	*1 tablet/day, continuous treatment*
	Conjugated estrogens 0.625 mg + MPA 5 mg	*Prempac Premelle*	*1 tablet/day, continuous treatment*
	Estradiol valerate 2 mg (11 days/cycle)+	*Cyclabil*	*1 tablet/day, in 3 out of 4 weeks*

Estradiol valerate 2 mg & Levonorgestrel 250µg (10 days/cycle)		
Estradiol 2 mg (11 days/cycle) + Estradiol 2 mg & MPA 10 mg (10 days/cycle)	*Divina*	*1 tablet/day, in 3 out of 4 weeks*
Estradiol valerate 2 mg (16 days/cycle +) Estradiol valerate 2 mg & MPA 10 mg (12days /cycle)	*Divina plus*	*1 tablet/day*
Estradiol valerate 2 mg (70 days) + Estradiol valerate 2 mg & MPA 20 mg (14 days) + Lactose (7 days)	*Trivina*	*Estrogen only tablets for 10 weeks, then combination of estrogen and progestin for 2 weeks; followed by 1 week with lactose only*
Conjugated estrogens 0.625 mg (14 days/cycle)+ Conjugated estrogens 0.625 mg & MPA 10 mg (14 days/cycle)	*Prempac Sekvens*	*1 and 2 tablets daily, respectively*
Estradiol 2 mg (16 days/cycle) + Estradiol 2 mg & Norethisterone acetate 1 mg (12 days / cycle)	*Femasekvens*	*1 tablet/day*
Estradiol 2 mg (12 days/cycle +) Estradiol 2 mg & Norethisterone acetate 1 mg (10 days/cycle) + Estradiol 1 mg (6 days/cycle)	*Trisekvens*	*1 tablet/day*
Estradiol 1 mg + Norethisterone acetate 0.5 mg	*Activelle*	*1 tablet/day*
Estradiol 50 µg/24 h Nerethisterone acetate 250 µg	*Estracomb Estalis Estalis Sekvens*	*2 depot patches / week*

Estrogen replacement therapy

Menopausal estrogen therapy, unopposed by progestins, is a well established and strong risk factor for endometrial cancer that may explain incidence trends in the last decades in western countries (IARC, 1999). In the US, the incidence of endometrial cancer started rising among postmenopausal women in the 1960s, reached a peak in the mid-1970s and then declined until the 1990s. In 1976 the U.S. Food and drug Administration warned physicians about the possible endometrial side effects of unopposed estrogen replacement therapy. After this warning the use of unopposed estrogen replacement therapy decreased, being followed by an increase in estrogen-progestin therapy (IARC, 1999). Such clear patterns were not observed in the Nordic countries, with the possible exception of Denmark (Ewertz and Jensen., 1984).

The association between use of estrogens without progestins and endometrial cancer has been studied in eight cohort and over 30 case-control studies, and has recently been reviewed (IARC, 1999; Grady et al., 1995).

In summary, these studies show an association between use of unopposed estrogen replacement and increased risk for endometrial cancer. Duration of use is one of the strongest determinants of risk: there seems to be no perceptible increase in risk with use for less than 6 months, and at least a tenfold elevated risk after more than ten years of estrogen intake (IARC, 1999). In a meta-analysis of published results (Grady et al., 1995), the relative risk (RR) for less than one year of use was 1.4 (95% CI 1.0-1.8) and for more than 10 years of use 9.5 (95% CI 7.4-12.3).

In some studies, the risk for endometrial cancers seems to decrease with time since last use of unopposed estrogens, but remain higher than that of untreated women for at least 10 years (Shapiro et al., 1985; Levi et al., 1993c; Finkle et al., 1995; Green et al., 1996). Other studies did not confirm this pattern (Brinton et al., 1993; Finkle et al., 1995). Women who used unopposed estrogens for long periods seem to maintain their increased endometrial cancer risk after cessation of use (Rubin et al., 1990; Green et al., 1996). In the meta-analysis of published results, the summary RR for recent use, i.e., within 1 year of cessation, was 4.1 (95% CI 2.9-5.7) and for distant use, i.e. after 5 or more years of cessation, 2.3 (95% CI 1.8-3.1).

The increased risk for endometrial cancer was observed with all usual hormone replacement dosages of conjugated estrogens. In the meta-anaysis of published results (Grady et al., 1995), summary RR for low dose (0.3 mg), intermediate dose (0.625 mg) and high dose (1.25 mg or more) did not differ significantly from each other. Ever use of conjugated equine estrogens seems to entail a higher risk for endometrial cancer (summary RR of published results 2.5; 95% CI 2.1-2.9) than ever use of synthetic estrogens, e.g. estradiol (summary RR 1.3; 95% CI 1.1-1.6) (Grady et al., 1995).

Endometrial cancers found after estrogen treatment are predominantly early stage (in situ and stage I), highly differentiated cancers (McDonald et al., 1977; Antunes et al., 1979; Buring et al., 1986; Rubin et al., 1990). However, the association with menopausal estrogen treatment has been noted for both early-stage non-invasive

cancers and for later-stage invasive tumors. In the meta-analysis of published results (Grady et al., 1995) summary RR for early stages (4.2, 95% CI 3.1-5.7) was higher than for higher stages (RR 1.4, 95% CI 0.8-2.4).

In the meta-analysis of published results, there were no significant differences in the summary RR estimates for continuous (RR 2.9; 95% CI 2.2-3.8) or intermittent regimens (RR 3.0, 95% CI 2.4-3.8) (Grady et al., 1995).

The possible modification of the effect of unopposed estrogens by other factors has been addressed by examining subgroups of women. Some studies suggest no interaction with obesity and use of unopposed estrogens (Kelsey et al., 1982; La Vecchia et al., 1982; La Vecchia et al., 1984; Ewertz et al., 1988; Levi et al., 1993c); in one study estrogen replacement's deleterious effect seemed confined to lean women (La Vecchia et al., 1982).

In three studies (Franks et al., 1987; Levi et al., 1987; Koumantaki et al., 1989) the effect of unopposed estrogen replacement was reported to be modified by smoking, i.e. postmenopausal non-smokers using unopposed estrogens had higher relative risks of developing endometrial cancer than smokers using HRT.

With regard to interactions with parity, hypertension, diabetes, and use of combined oral contraceptives, the findings are inconclusive.

IARC (1999) evaluated that there is *sufficient evidence* in humans for the carcinogenicity of postmenopausal estrogen therapy. Postmenopausal estrogen therapy was therefore classified as *carcinogenic to humans* (Group 1).

Estrogen-progestin replacement therapy

Estrogen-progestin replacement therapy came into use in the USA relatively late (at the end of 1980s), while in Scandinavian countries combined compounds were marketed already during the 1970s.

The addition of progestins to estrogens in hormone replacement aims to reduce the risk of endometrial hyperplasia associated with use of estrogens alone (without progestins).

There are only scant epidemiological data regarding combined HRT and endometrial cancer risk: one small clinical trial, three cohort and four case-control studies. These studies started to be published in the late 1970s and early 1980s.

Early studies of combined hormone replacement regimens

In a Swedish cohort investigation (Persson et al., 1989), the relative risk (RR) for women using cyclic progestin-combined treatment – mainly with estradiol 2 mg and levonorgestrel 250 µg during 10 days of the cycle – was 0.9 (95% confidence interval, CI, 0.4-2.0), a risk estimate based on only seven exposed cases. The RR for women who had taken estrogens without progestins was 1.4 (95% CI 1.1-1.9). A later follow-up of the same Swedish cohort showed that women who used more than 6

years combined regimens had a non-significant 40% increased risk for endometrial cancer, compared to women with short term exposure or no exposure (Persson et al., 1999).

In three case-control studies from the USA (Voigt et al., 1991; Jick et al., 1993; Brinton et al., 1993), reporting mainly on treatments with conjugated estrogens and addition of medroxyprogesterone acetate, some evidence of a protective effect by the added progestins emerged. Voigt et al. (1991) found that a cyclic addition of duration less than ten days conferred a doubled risk, while such addition ten days or more per cycle was not associated with a risk increase. Jick et al., (1993) and Brinton et al. (1993) reported elevated relative risk estimates, however non significant, for women with a combined estrogen-progestin treatment. Estimates were of a lower magnitudes than those for women using estrogens only. These early studies had small numbers and lacked detailed data for subgroups with regard to treatment duration. Overall, the patterns of results indicated that there could be an increase of endometrial cancer risk, even after addition of progestins.

Two recent case-control studies

There are two recent studies (Pike et al., 1997; Beresford et al., 1997) which were large enough to evaluate cyclic progestin use in detail. Both reported increased endometrial cancer risk among users of HRT regimens with added progestins for 10 days of less per cycle.

Pike et al. (1997) reported results from a population-based case-control study of 833 women with an invasive endometrial cancer – verified through review – and 791 control women, aged 50-74. The women who had been exposed to estrogens alone (conjugated estrogens) were found to have an increased risk with an average odds ratio (OR) of 2.2 for each five-year period of medication. The women who had taken a sequentially combined regimen with less than 10 days of addition per treatment cycle had an increased risk, OR 1.9 (95% CI 1.3-2.7) per five-year of treatment; if the addition was of 10 days or longer, no risk increase was noted (OR 1.1, 95% CI 0.8-1.4). Women who had received a continuous combined regimen, 94 cases and 90 controls – with conjugated estrogen and medroxyprogesterone acetate (probably dosages less than 10 mg) – had a risk similar to that of non-exposed women, OR 1.1 (0.8-1.4), per five-year of treatment. The excess risk in connection with exposure to estrogens alone or progestin addition less than 10 days was the highest for tumors localized to the endometrium or limited to the inner part of the myometrium; the risk increase persisted for these women more than 10 years after ending the treatment. The authors reason that more than 10 days of addition is necessary for an adequate shedding of the endometrium and prevention of endometrial neoplasia. In these data, continuous combined regimens did not confer a protective effect greater than that of cyclic addition.
In the second case-control study from the west-coast of the USA, by Beresford et al. (1997), 832 women with a verified invasive endometrial cancer were interviewed together with 1,154 population-based control subjects. Replacement therapy, reported by 14.5% of the cases and 13.1% of the controls, comprised conjugated estrogens (the doses were not given) and combinations with medroxyprogesterone acetate. Treatment with estrogens alone was associated with a fourfold risk increase (OR 4.0; 95% CI 3.1-5.1); if treatment had been combined with progestins less than

10 days per cycle, the risk was threefold increased (OR 3.1; 95% CI 1.1-5.7), when 10 days or longer there was no significant risk increase (OR 1.3; 95% CI 0.8-2.2). Among women who used combined treatment for five years or longer, the risk in association with progestin addition less than ten days per cycle was increased (OR 3.7; 95% CI 1.7-8.2), as well as when added longer (however, at lower level) (OR 2.5; 95% CI 1.1-5.5). A similar risk pattern emerged when the analyses were restricted to women who had an ongoing or recent treatment at time of diagnosis; the OR for women with combined treatments that had been terminated five years or longer ago was 2.7 (95% CI 1.2-6.0). The authors conclude that addition of progestins for 10-12 days (the dose of medroxyprogesterone acetate addition was not given) can not fully eliminate the risk increase caused by intake of estrogens for many years.

In summary these recent studies reported an increased risk for endometrial cancer associated with use of cyclic regimens with 10 or less days of progestin addition and no increased endometrial cancer risk if progestins were added for 10-12 or more days per cycle, and no elevation in risk among users of continuous combined therapy.

Data on ‘spacing out’ or ‘long cycle’ randomized controlled trial reported a higher (6%) occurrence of hyperplasia in ‘long cycle’ group than in women receiving monthly progestin (< 1%) (Cerin et al., 1996).

IARC (1999), concluded that ‘compared to non-users of hormone replacement, combined HRT users presented no excess risk, or a slight increased risk for endometrial cancer. The increase in risk was smaller than observed with use of estrogens without progestins.’

According to IARC (1999) there is a reduction in endometrial hyperplasia observed with 10 or more days of addition of progestins per cycle; postmenopausal estrogen-progestin therapy is possibly carcinogenic to humans (Group 2B), although the evidence of carcinogenicity was considered limited (IARC, 1999).

Low-potency estrogen formulations used in hormone replacement

Data from other epidemiological studies regarding low-potency estrogen formulations and endometrial cancer are scarce. In a hospital based case-control study in Finland, a 60% decrease in the relative risk of endometrial cancer was found among women exposed to oral estriol; however, dosages and duration of the treatment were not given (Salmi, 1980). Is this study (Salmi, 1980) no increased risk was associated with use of unopposed estrogens. In a population-based prospective cohort study in Sweden, women prescribed low potency estrogen formulations (oral estriol) showed no overall increase in the risk of endometrial cancer; however, data on duration and recency of intake were not available (Schairer et al., 1997). Kelsey and colleagues (1982) reported an increased risk of endometrial cancer after vaginal hormone use. However, this study had no information regarding formulations used or duration of therapy.

Some evidence suggests that oral estriol has systemic effects. Englund et al. (1980) showed that in postmenopausal women treated with oral estriol 6 mg daily for 3 months, more than half had menstrual bleeding after addition of a progestin. In a recent Swedish study postmenopausal women referred for undue vaginal bleeding were examined with ultrasound measurement of endometrial thickness before biopsy (Granberg et al., 1997). Among users of oral estriol and medium potency estrogens, the average endometrial thickness was greater and endometrial atypical hyperplasia more frequent when compared with unexposed women. A trial in Japanese postmenopausal women treated with oral estriol 2 mg daily for a year showed both prevention of bone loss and alleviation of climacteric symptoms (Minaguchi et al., 1996).

Due to its low affinity, estriol binds to the estrogen receptor in vitro for a relatively short time compared to medium potency estrogens (Esposito, 1991; Botella et al., 1995). Estriol administered orally is conjugated efficiently in the liver (Schiff et al., 1980), but because estriol does not bind strongly to proteins most of the serum estriol is biologically active (Vermeulen and Verdonck, 1968). When estriol is taken continuously a prolonged increase in serum levels can lead to long-standing proliferation of endometrial cells as medium potency estrogens (Clark et al., 1977; Korach et al., 1980).

In mice, estriol increases the incidence of N-methyl-N-nitrosourea induced endometrial adenocarcinoma. Estriol binds to the estrogen receptors in rat and human uterine tissue and Ishikawa human endometrial cancer cells (Botella et al., 1995), but with lower affinity - about 5% - than of estradiol-17β (Botella et al., 1995). IARC (1999) stated that ‘ there is limited evidence in experimental animals for the carcinogenicity of conjugated equine estrogens, equilin and estriol’. There is inter-conversion between estradiol-17β and estrone in vivo in humans, and the latter is converted to estriol. ‘Intake of 8 mg estriol orally resulted in a maximum plasma level of 75 pg/mL estriol after 2 h. Plasma concentrations increased over four weeks of estriol therapy to levels up to 130 pg/mL, although the serum concentrations of conjugated estriol remained unaltered’ (IARC, 1999; Schiff et al., 1978). In another study is has been shown that when taken orally, estriol is almost completely conjugated in the intestine to glucuronides (80-90%) and sulfates (10-20%). Only 1-2% of the parent steroid reaches the circulation (Kuhl, 1990). There is much less metabolism of estriol after vaginal application than after oral ingestion and 20% of the dose appears as unconjugated steroid in the blood. At a dose of 0.5 mg, peak levels of 100-150 pg/mL were observed within 2 hours. The maximal levels of estriol after vaginal application of 0.5 mg were similar to those obtained after oral intake of 8-12 mg estriol (Kuhl, 1990). There was no significant difference between the 24-hour systemic availability after vaginal administration of 1 mg estriol and the AUC after oral administration of 10 mg estriol (Heimer, 1987). Treatment with low dose estriol by the vaginal route may therefore induce systemic effects comparable to those achieved with high oral doses. Accumulation and storage of estradiol-17β, estrone and estriol after exogenous administration has not been thoroughly examined. In addition to distribution of estradiol-17β, estrone and estriol to various target and non-target organs through systemic circulation. There are few data related to local production and accumulation of these estrogens in target tissues particularly rich in fat.

Organochlorines

Organochlorine compounds have been widely used as insecticides, fungicides and industrial chemicals. Because of their widespread application and environmental stability, some degree of environmental contamination was common in the 1960s and 1970s. Although use of polychlorinated biphenyls (PCBs) and technical mixtures of DDT have been restricted or banned in most Western countries since the early 1970s, for example DDT is still used in many developing countries. Environmental burdens have generally declined, but measurable amounts remain, and the potential adverse health effects have become a contentious public issue. Contamination of the food-chain is likely to still exist, most importantly through fat-rich foods of animal origin. Because fat fish may accumulate high levels of organochlorines, populations with high consumption of such fish may be the most suitable to study the effects of exposure to environmental organochlorines. The concern that environmental organochlorine pollutants in food may cause cancer in humans is widespread (Safe, 1997). Although some organochlorine compounds are thought to be directly carcinogenic to some organs, their potential hormonal, notably estrogenic action, has been the main concern. For example, experimental data suggest that *o,p'*-DDT is an estrogen agonist, which increases uterine weight, vaginal epithelial cornification, DNA synthesis and cell proliferation in the endometrium, and precipitates precocious puberty in female animals. Other organochlorines - like chlordecone, demethylated metabolites of methoxychlor, some PCB congeners and PCB mixtures - may also show estrogenic-activity.

The theory that such effects would arise from weakly estrogenic effects of some organochlorines has been tested almost exclusively in epidemiological studies of breast cancer. While some relatively small studies were supportive (Wassermann et al., 1976; Mussalo-Rauhamaa et al., 1990; Falck et al., 1992; Wolff et al., 1993; Dewailly et al., 1994; Guttes et al., 1998), two larger carefully conducted prospective studies (Hunter et al., 1997; Krieger et al., 1994) and at least four recently published case-control studies (Lopez-Carillo et al., 1997; van't Veer et al., 1997; Moysich et al., 1998; Dorgan et al., 1999;) showed no convincing association between exposure to organochlorines (mainly DDT or PCBs) and breast cancer risk. To our knowledge, to date only one study has addressed the association between organochlorine pesticides or PCB's and the risk of endometrial cancer, and the results were negative (Sturgeon et al., 1998).

Despite accumulating reassuring evidence, public concern continues, as does scientific investigation, focused largely on breast cancer. However, if certain organochlorines do affect cancer risk through estrogenic mechanisms, their effects should be more easily detected in the endometrium. While breast cancer risk is affected to only a relatively small extent by oral estrogens (Collaborative Group on Hormonal Factors in Breast Cancer, 1997), risk for endometrial cancer increases markedly after just a few years of use (Grady et al., 1995).

Genetic and molecular epidemiology

Inherited susceptibility

Some data indicate that endometrial cancer may occur in genetically susceptible individuals (Goldgar et al. 1994, Gruber et al., 1996; Hemminki et al., 1998). Data on familiar aggregation of endometrial cancer, reflecting clustering of either sporadic or hereditary cases, are scarce. According to a recent Italian study, five percent of the cases in a hospital-based study had a history of endometrial cancer in first-degree relatives (Fornasarig, 1998). The association of endometrial cancer with a history of ovarian cancer (Kelsey, 1982) and with an increased risk for secondary cancers in the colon, ovary and breast (Nelson, 1993) also indicates that some endometrial cancers may occur in genetically susceptible individuals, but the possibility that these individuals are exposed to shared risk factors for these diseases cannot be ruled out.

An increased risk of endometrial cancer has been found in women with some rare syndromes and hereditary non-polyposis colorectal cancer syndrome (HNPCC), also known as the cancer family syndrome of Lynch Syndrome Type II (Lynch, 1976), an autosomal dominant condition determined by cancer susceptibility genes. Some authors propose that endometrial carcinomas associated with HNPCC should be considered separate from other endometrial carcinomas: the Type III endometrial carcinomas (Bandera and Boyd, 1997). Germline mutations of DNA mismatch repair MSH2 gene on chromosome 2p have been found in 60% of HNPCC families, and mutations of MLH1, another DNA repair gene on chromosome 3p, in 30%. Endometrial carcinoma is the second most common tumor type in HNPCC families, affecting 23% of the women (Mecklin et al., 1986). Affected women are younger than women with sporadic endometrial cancer, and their prognosis favorable (Koshiyama et al., 1998).

The associations between structural variations (polymorphisms) on key genes with potential effects in hormonal carcinogenesis may provide clues of susceptibility to endometrial cancer. Given the fundamental role of sex hormones in endometrial carcinogenesis, studies of genetic susceptibility have focused on the influence of polymorphisms in genes putatively involved in estrogenic effects. Most of the available data, however, emanate from breast cancer studies.

Microsatellite polymorphisms in the CYP19 gene, expressing the aromatase enzyme catalyzing conversion of androgen precursors to estrone has possible importance; a large number of TTA repeats was associated with a twofold increase in the risk of breast cancer (Kristensen et al., 1998). A restriction fragment length polymorphism (RFLP) of the CYP17 gene – regulating early steroidogenic conversion steps – the MspA1 allele – was linked to breast cancer risk in one study (Feigelson, 1997), but not in three subsequent studies (Weston et al., 1998; Helzlsouer et al., 1998; Dunning et al., 1998). A low–activity allele of the gene for the COMT enzyme – responsible for deactivation through methylation and clearence of estradiol metabolites – was associated with an increased risk of breast cancer, with a fourfold elevated risk in obese post-menopausal women (Lavigne et al.,1997). Another case-control study also reported an excess breast cancer risk, however, only in premenopausal women (Thompson et al., 1998). To our knowledge, the possible impacts of these polymorphisms have not yet been tested for endometrial cancer.

One small case-control study of endometrial cancer (80 cases and 60 controls) examined the effect of an aminoacid exchange polymorphism of methylenetetrahydrofolate reductase (MTHFR) – hypothetically altering DNA methylation – and of CYP1A1 – catalyzing 2-hydroxylation of estrogens (Esteller et al., 1997). The mutated alleles were associated with significant, 3- and 6-fold, respectively, increased risks, suggesting an effect on the susceptibility of endometrial cancer risk.

Because of its close relation to the effects of even low levels of estrogens, endometrial cancer would seem to be a useful model for further studies of genetic susceptibility to hormonal carcinogenesis, due to polymorphisms of these and other candidate genes. To obtain meaningful results, studies need to be carefully designed, importantly with adequate power for analysis of main effects as well as of effect modification, and have a proper definition of the study base.

The estrogen receptor α

The role of polymorphims in the estrogen receptor α (ER) gene on endometrial cancer susceptibility is unknown. The estrogen receptor α (ER) is an important mediator of the hormonal response in estrogen-sensitive tissues such as the endometrium, breast and bone. Since ER functioning is reflected in the proliferation of these tissues, it is plausible that variation in the function of the receptor could have clinically significant effects. Polymorphisms in the ER gene, namely the restriction fragment length polymorphisms *Xba I* and *Pvu II* (Kobayashi et al., 1996; Yaich et al., 1992; McGuire et al., 1992; Castagnoli et al., 1987) and an upstream microsatellite polymorphism - TA repeat (Sano et al., 1995; Del Senno et al., 1992), which are hypothetically related to biological function, have inconsistently been associated with bone density (Sano et al., 1995; Kobayashi et al., 1996; Mizunuma et al., 1997; Han et al., 1997; Ongphiphadhanakul et al., 1998; Willing et al., 1998; Gennari et al., 1998) but seem not to be related to breast cancer risk (Parl et al., 1989; Yaich et al., 1992; McGuire et al., 1992; Andersen et al., 1994; Roodi et al., 1995). Polymorphisms in the ER gene may be associated with endometrial cancer – probably the most ‘estrogen sensitive’ malignancy in women - through regulation of estrogenic effects on the cellular level.

Somatic events

Carcinogenesis in the endometrium for most tumors is believed to be a process with morphological transition from normal to hyperplastic, atypically hyperplastic and to neoplastic endometrial cells. This would be characteristic for Type I tumors, believed to be related primarily to risk factors reflecting an influence of estrogens (Deligdisch and Holinka, 1987). Estrogens, without an opposing antiestrogenic effect of progesterone or progestins, enhance proliferation of endometrial cells (see above), leading to a more frequent occurrence of spontaneous mutations, decreased time for mis-match repair, increased sensitivity to genotoxic agents and expansion of transformed clones (Preston-Martin et al., 1990). The resulting molecular alterations may for Type I cancers involve mutations activating the K-ras oncogene, downregulation of mis-match repair genes and over-expression of genes producing

cell-adhesion molecules like E-cadherin, and a general genome instability; further, the dysregulated expression of growths factors like TGFα in autocrine and EGF1 and TGFβ paracrine pathways (Boyd, 1996).

For the so called Type II endometrial cancers, assumed not to be related to estrogens, less is known about pathogenic events; overexpression of the erB-2 oncogen, and inactivation of the P53 tumor supressor gene are believed to be important steps late in the process (Boyd, 1996).

AIMS OF THE STUDIES

To examine the association in epidemiological case-control studies between endometrial cancer risk and:

Some exogenous hormones:

1. Use of low-potency estrogens, as postmenopausal estrogen replacement;
2. Use of medium potency estrogens without or with addition of progestins, as peri- or postmenopausal hormone replacement;
3. Use of combined oral contraceptives;

Some substances present in the environment that may act as xenoestrogens:

4. Serum levels of 20 organochlorine compounds present in the environment in Sweden, namely polychlorinated biphenyls (PCBs) congeners 28, 52, 101, 105, 118, 138, 153, 156, 167, 180 and Hexachlorobenzene (HCB), *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDT, α- Hexachlorocyclohexane (HCH), ß-HCH, γ-HCH, trans-nonachlor, and oxychlordane.

Polymorphisms that may be markers of susceptibility to hormonal carcinogenesis:

5. Some polymorphisms in the estrogen receptor α gene, namely the restriction fragment length polymorphisms *Xba I* and *Pvu II* and an upstream microsatellite polymorphism - TA repeat.

SUBJECTS AND METHODS

Two epidemiological studies were conducted, using case-control methodology. Papers I to III derived from the first study, and papers IV to V from the second one.

Study 1 - Papers I-III

I. Low potency estrogens and risk for endometrial cancer.
II. Use of oral contraceptives and endometrial cancer risk.
III. Use of oral contraceptives and endometrial cancer risk

Design

This was a population-based case-control study conducted among women aged 50-74 years, born in Sweden and resident there between January 1, 1994 and December 31, 1995. We restricted our study to post-menopausal women with an intact uterus and no previous breast cancer diagnosis. Eligible as cases were women with a newly diagnosed, histopathologically confirmed endometrial cancer. They were identified through the six regional cancer registries in Sweden, which comprise a virtually complete cancer registration system (Socialstyrelsen, 1998). Case women were approached after approval from their physicians.

Control women were randomly selected from a continuously updated population register including all residents in Sweden. To co-ordinate use of resources, most of the control women (n=2633) were also subjects in a concomitant breast cancer case-control study that used the same questionnaire (Magnusson et al., 1998); the remaining (n=735) control women were separately sampled after completion of the breast cancer study (March 1995), in order to have the same recruitment period for endometrial cancer cases and control women. Thus, control women were frequency matched either to the expected age distribution of breast or endometrial cancer cases.

Participation rates were 75% among cases (789 of 1055 eligible) and 80% among controls (3368 of 4216 eligible). Non-participation was due to refusal in 171 cases (16%) and 811 controls (19%) and to death or poor health in 37 controls (1%). The patients' physicians refused permission to contact an additional 95 (9%) cases, mostly because of poor patient health.

Data collection

Data and informed consent were obtained through mailed questionnaires requesting detailed information on use of replacement hormones, including brand, dosage, and date of first and last use of each treatment episode. Recall was aided by a picture chart of all brands commercially available in Sweden during the years 1950-1995. The questionnaire also covered reproductive and medical histories, anthropometric measures, and life style, e.g. smoking, drinking and dietary habits. Among case women, the mean interval from diagnosis to questionnaire response was 8.4 months (standard deviation 4.6 months).

Age at menopause was defined as the age of the last menstrual period or age at bilateral oophorectomy, if one year or more prior to data collection. If later, women were considered pre-menopausal and excluded.

Women with menses due to hormone replacement therapy or with missing information on age at menopause were considered post-menopausal if they had reached the 90th percentile of age at natural menopause of study subjects (current smokers: 55 years for case and control women; non-smokers: 56 years for case women and 55 years for control women). Subjects thus classified as post-menopausal (44 cases and 206 controls) were assigned an age at menopause according to their current smoking status and the mean ages at natural menopause in our data (current smokers: 51 years for cases, 50 years for controls; non-smokers: 52 years for cases, 50 years for controls). Women with missing information on age at menopause who had not reached the 90th percentile of age at natural menopause of the study subjects were excluded from the analyses (1 case and 51 controls).

Among participating controls, 491 (15 %) failed to return the mailed questionnaire but agreed to a telephone interview including most questionnaire items. Cases were not approached by phone since all who had consented returned the questionnaire. Approximately 50% of all cases and controls were contacted by telephone for essential missing information in their mailed questionnaires, mainly details of hormone use. Interviewers were blinded to the hypothesis of the study, but some subjects disclosed their case/control status to the interviewers.

Histopathological classification

Histological specimens for the case women were retrieved from all 35 pathology departments in Sweden. These specimens were reviewed by the study pathologist (Dr. Anders Lindgren) who was blinded to hormone use and other exposures, and reclassified them as: endometroid adenocarcinoma (n=648), seropapillary carcinoma (n=36), clear cell carcinoma (n=10), adenoacanthoma (n=3), adenosquamous carcinoma (n=12) or endometrial atypical hyperplasia (n=80), defined as adenomatous hyperplasia with slight, moderate or severely pronounced atypia. Cases with anaplastic carcinoma (n=13), malignant mixed Mullerian tumors (n=6), with cancer diagnoses other than endometrial (n=4) and those whose histopathological slides were missing (n=5) were excluded from the study. Endometroid adenocarcinomas (n=648) were further classified as well (Grade 1, n=241 or 37%), moderately (Grade 2, n=286 or 44%),or poorly (Grade 3, n=121 or 19%) differentiated. Endometrial cancers, in total 709, and atypical hyperplasias were analysed separately, and most analyses included only cases with invasive cancer (the few analysis done with atypical hyperplasia cases are presented separately in some of the tables in the papers). The hyperplasias were classified as severe in 44 % of the cases. Hysterectomy specimens were available from 542 (76%) of cases. Myometrial invasion was classified as none or less than 50% in 362 subjects (67% of those with myometrial slides), 50% or more of the myometrial thickness or through the serosa in 180 subjects (33%).

Hormone classification

We classified each reported hormone replacement treatment episode into one of the following four categories (von Schoultz, 1988):

- medium potency estrogens (i.e. conjugated estrogen, estradiol, and other synthetic estrogens), without added progestins. Low and high dose, respectively were defined as: oral estradiol: < or ≥ 2.0 mg / day; conjugated estrogens: < or ≥0.625 mg / day; ethinyl estradiol: < or ≥10 μg / day; transdermal estradiol: < or ≥50 μg / day.

- medium potency estrogens, cyclically combined with a progestin (< 16 days / cycle, most commonly 10 days) or continuously (19 or more days/cycle, most commonly 28 days with norethisterone acetate 1 mg). We also considered combined treatment with 19-nor-testosterone derivatives, (e.g. norethisterone, norethisterone acetate, levonorgestrel or lynestrenol) or progesterone-like progestins (17-hydroxy-progesterone derivatives, e.g. medroxyprogesterone acetate).

- low potency estrogens: oral estriol (1-2 mg / day) or vaginal dienoestrol (0.1-0.5 mg), estriol (0.5-1 mg), estradiol (25 μg): the usual prespricition of vaginal treatments consisted of daily applications in the first 2-3 weeks followed by applications twice a week.

- progestins without estrogen.

We censored all exposure after an index date: in case patients, six months before diagnosis; in control women, the date of the questionnaire arrival minus the mean time from diagnosis to questionnaire arrival in cases minus an additional six months.

Oral contraceptives

A complementary questionnaire was sent to subjects who reported any oral contraceptive (OC) use, requesting detailed information for each treatment episode: brand, dosage, and date of first and last use. Recall was aided by a picture chart of all brands commercially available in Sweden during the years 1960-1995.

We classified reported OC use into one of the following categories:

- Combined oral contraceptives (COCs): estrogen plus progestin (most commonly desogestrel, levonorgestrel and norethisterone) pills;

    - low dose: containing less than 0.04 mg of ethinylestradiol;

    - medium dose: containing 0.05 to 0.075 mg of ethinylestradiol or 0.05 to 0.08 mg mestranol;

    - high dose: containing 0.1mg (or more) of ethinylestradiol or 0.1 mg of mestranol;

- Progestin-only ('mini-pill'): norethisterone 0.3 mg, levonorgestrel 0.03 mg, or lynesterol 0.5 mg;

- Injectable intra-muscular progestin-only contraceptives: depot-medroxyprogesterone acetate, 150 mg.

No use of sequential oral contraceptives was reported in this population.

Statistical methods

As measures of relative risk, odds ratios (OR) were computed from unconditional logistic regression models fit by the maximum likelihood method (Breslow and Day, 1980). All tests of statistical significance (p values) were two-sided. Trends in ORs were tested on the basis of models with the explanatory variable in continuous form or through introduction of 'semi-continuous' variables obtained by assigning consecutive integers to levels of categorized variables. Tests of interaction were conducted with the log likelihood ratio test comparing models with and without interaction terms.

A substantial proportion of all women had used several different hormone regimens, and we employed two strategies to assess their independent effects. The first strategy used all subjects, adjusting for hormone replacement regimens other than the one under study in the modeling. The second strategy included women who had used only the studied compounds or no hormone replacement at all. Since the two approaches yielded very similar results, we present estimates obtained by the first strategy to preserve statistical power. We compared women who 'never' used a specific hormone regimen, e.g. oral estriol, to women who 'ever' used them. The 'ever' use groups were then subdivided according to duration of use (e.g. up to 5 years / 5 or more years; and per each single year of use) and recency of use, defined as the interval elapsed between cessation of treatment and index date (e.g. less than 1 year / 1 or more years; and per single year after cessation of treatment).
We estimated ORs firstly in age-adjusted models, and subsequently in multivariate models.

We included in the multivariate models covariates previously described in the literature as associated with endometrial cancer risk that did change relative risk estimates in our data. These covariates were: age (as a continuous variable), smoking (ever or never smoked regularly), parity (nulliparous, 1-3 children, 4 or more children), age at last birth (nulliparous, < 27 years, 27-29 years, 30-33 years, 34 or more years), age at menopause (before 45 years, 45-49 years, 50-51 years, 52-54 years, more than 54 years), body mass index (BMI, [kg]/height[m]^2, according to quartiles among controls*)*. For the analysis of hormone replacement we also adjusted for use of oral contraceptives (never, ever), and in the analysis of oral contraceptives we adjusted for duration of use of different hormone replacement regimens (separate variables for years of use of estrogens without progestins, estrogens with progestins added cyclically; estrogens with progestins added continuously, or oral estriol). In the analyses of each different hormone replacement regimen we adjusted for duration of use of other types of hormone replacement regimens. A description of the variables

included in the model is presented in the footnotes of each table (in the annexed papers).

In the second strategy we analyzed the effect of 'exclusive use' of each hormone replacement regimen, i.e. we excluded from the dataset women who had used any other kind of hormone replacement, and compared 'users' of each specific hormone replacement regimen with 'never users of any hormone replacement'. In the analysis of 'exclusive use' we included all covariates described above in the logistic regression models (except for the hormone replacement variables).

Estimates of increment in relative risk per year of use of hormones include unexposed women, to whom we attributed zero as duration of treatment. Because age-adjusted results were generally similar to results from the chosen multivariate models, we focused the presentation of results mostly in the latter.

We also considered the potential confounding effects of other covariates, i.e. education, history of diabetes mellitus and hypertension, family history of endometrial cancer, age at menarche, age at first birth, duration of breast feeding. However, they were not included in the final models because they did not substantially affect relative risk estimates or significantly improve goodness of fit (assessed through likelihood ratio tests) (Greenland, 1998). Estimates of increment in relative risk per year of hormone replacement or use of oral contraceptives were calculated including unexposed women.

We analyzed the influence of COCs treatment according to duration of use, and - to assess the persistence of effects - recency, the interval between cessation of treatment and menopause, and age at enrolment into the study.

We also repeated most of the analyses above described in two other ways: excluding women with an assigned age at menopause and excluding control women who responded only to the telephone interview. Results of these were virtually identical to those presented, and will not be presented.

Study 2- Papers IV-V (to be described separately).

Paper IV. Organochlorines and endometrial cancer risk.

Study population

Our study focused on women aged 50-74 years, resident in 12 Swedish counties on the coasts of the Gulf of Bothnia, the Baltic Sea or the largest Swedish lakes between February 1996 and November 1997. We assumed that intake of organochlorine compounds through intake of possibly contaminated fish would be higher in these counties than elsewhere in Sweden. Women were eligible if they were born in Sweden, had no prior hysterectomy and had never used hormone replacement therapy (except vaginal estriol, dienoestrol or estradiol), because we assumed that the use of such compounds could mask an effect of substances with less-potent hormone-like effects, such as organochlorine compounds.

Women with incident histopathologically confirmed endometrial cancer diagnosed between February 1996 and November 1997 were identified through a network of personnel at the departments of gynecology / gynecological oncology in the study area. (One of the 26 departments did not collaborate.) They reported 396 cases (approximately 95% of the expected number) (Socialstyrelsen, 1998). Of these, 288 (73%) volunteered to donate blood samples and complete the study questionnaire; 41 patients refused to participate, and 67 cases were not approached (due to failure of the medical staff to collect a blood sample before surgery). Subsequently, 134 case women were excluded because they had used hormone replacement therapy, leaving 154 in the study.

Population controls, randomly selected from a continuously up-dated population register, were frequency matched to cases by 5-year age groups. The period of control recruitment coincided with that of cases, since we sampled and enrolled controls in 4 phases: spring 1996; the fall of 1996; the spring 1997 and the fall 1997. Of 742 control women selected, 559 (75.3%) responded to the study questionnaire, and 492 (66.3%) agreed as well to donate blood samples. After exclusion of 287 women because of prior hysterectomy (46) or use of hormone replacement therapy (241), 205 control women were included in the study.

The self-administrated study questionnaire requested information on weight, height, reproductive history, diet, hormone use, smoking, physical activity, and medical history, among others. Missing information was supplemented by a telephone interview in approximately 50% of cases and controls.

Blood sampling

Blood samples from fasting case women were drawn at the hospital departments before any cancer treatment, and from controls at a primary health care unit or at home. Serum was separated within 2 hours of collection, and frozen at -20ºC until shipment to the Swedish National Food Administration laboratory for analysis.

Analysis of organochlorine compounds in human serum

We analyzed the lipid portion of serum samples for the ten organochlorine pesticides and ten PCB congeners (CBs 28, 52, 101, 105, 118, 138, 153, 156, 167, 180) chosen a priori because of their likelihood of being present in the food chain in Sweden. Serum samples (4 g) were mixed with methanol and extracted three times with n-hexane/diethyl ether (1:1). After evaporation of solvents, the fat content was determined gravimetrically. All samples were fortified with a mixture of internal standards to correct for analytical losses and ensure quality control. The fat was redissolved in n-hexane and treated with concentrated sulphuric acid. The PCB congeners were separated from the bulk of the chlorinated pesticides by elution through a silica gel column (4.5 g of 3% water-deactivated silica gel). The first fraction, containing the PCB congeners, HCB and *p,p'*-DDE, was eluted with about 30 ml of n-hexane and the second fraction, containing mainly chlorinated pesticides, was eluted with 40 ml of a n-hexane/diethyl ether mixture (3:1). Analysis of the two fractions was performed on a gas chromatograph with dual capillary columns and electron capture detectors (^{63}Ni). The columns were of different polarity to ease

identification of analytes, which was based on retention times relative to internal standards. Quantification was performed using multi-level calibration curves obtained by injection of standard solutions of at least three different concentrations.

Quantification limits were set at levels corresponding to the lowest standard concentration used: 10 pg/g serum for the PCB congeners, HCH-isomers and chlordanes, 20 pg/g for *p,p'*-DDD, *p,p'*-DDT, and *o,p'*-DDT, 50 pg/g for HCB and 200 pg/g for *p,p'*-DDE. The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample, included among the analytical batches during the course of the study. The coefficients of variations (CV) were less than 13% for most of the compounds, except the PCB congeners CB 28 (22%) and CB 105 (20%). The CV for fat content was 4%. The recovery qualification criteria were set at 70-120% depending on the substances. The average recoveries of the different PCB congeners in spiked serum samples were 98 ± 12% and 94 ± 8% for 0.1 and 0.8 ppb levels, respectively. The recoveries for the chlorinated pesticides varied from 78 to 118%. The results reported were not corrected for recovery.

The study laboratory participated in the fourth round of "World Health Organization's interlaboratory quality assessment study on human milk and blood" which included analyses of PCBs in blood plasma. The results obtained were in good agreement with the consensus values.

The study laboratory analysts were blinded to the case-control status of the samples. Since concentrations of compounds are dependent on the amount of lipid in serum at the time of sampling, we expressed results in ng/g lipid in the serum, without further adjustment for lipid contents in the statistical analyses (Hunter et al., 1998). When concentrations were below the quantification limit they were set to 50% of that limit in all statistical analysis.

Grouping of organochlorines

In addition to analyses of individual compounds, we considered groups of substances according to their possible hormonal activity. The grouping was based on a literature review by an outside expert (Dr. K Moysich), blinded to any study findings. In grouping compounds, we added molar concentrations to compensate for differences in molecular weight (unit of measurement = nanomol/g lipid).

Thus, we considered the following compounds to be:

a) estrogenic: *p,p'*-DDD, *o,p'*-DDT, *p,p'*-DDT, ß-HCH, γ-HCH, trans-nonachlor, oxychlordane, and CBs 28, 52 ,101, and 153 (Bulger and Kupfer, 1983; Ahlborg et al., 1995; Shekar et al., 1997; Steinmetz et al, 1997; Loeber and van Helsen, 1984; Bigsby et al., 1997; Coosen and van Helsen, 1989; Cooper et al., 1989; Laws et al., 1994; Rawlings et al., 1998; Wedig and Vernon, 1973; Cassidy et al., 1994; Vonier et al., 1996; Ecobichon and MacKenzie, 1974; Gellert, 1978; Jansen et al., 1993; Soontornchat et al., 1994; Waller et al., 1995; Wolff et al., 1997; Patnode and Curtis, 1994; Gierthy et al., 1997);

b) antiestrogenic: CBs 105, 118, 156, 167 (Wolff et al., 1997; Krishnan and Safe, 1993);

c) no known estrogenic effect: α-HCH, HCB, *p,p'*-DDE, CBs 138, 180 (Ahlborg et al., 1995; . Wolff et al., 1997; Foster et al., 1992a; Foster et al, 1992b; Foster et al, 1995; Muller et al., 1978).

The hormonal activity of PCB-congeners 101, 110, 138, 153 and 180 has barely been studied. However, CB 153 seems to be weakly estrogenic (Ecobichon and MacKenzie, 1974; Soontornchat et al., 1994; Waller et al., 1995; Patnode and Curtis, 1994). The high concentration of CB 153 has a large influence on the results of the estrogenic group. Therefore we also analyzed the estrogenic group after exclusion of CB153. Because *p,p'*-DDE has been reported to have anti-androgenic effects (Kelce et al., 1995; Haake et al., 1987) we also analyzed the group of compounds with no known hormonal effect excluding this substance. Finally, we also considered all PCB congeners (total PCB).

Statistical Analysis

As many variables were strongly skewed, we used the non-parametric two-sample Wilcoxon test for unpaired data to conduct unadjusted comparisons of serum organochlorine concentrations in case and control women. Background variables were compared by t-tests or chi-square tests for homogeneity. In the main analyses odds ratios (ORs) and 95% confidence intervals (CI) were calculated using unconditional logistic regression models, fit by maximum likelihood (Breslow and Day, 1980). We considered the confounding effects of the following variables: age, menopausal status, ages at menarche, menopause, first and last births, parity, breast-feeding, height, body mass index (BMI, i.e. weight in kg/height squared), use of oral contraceptives or topic estriol, dienoestrol or estradiol, family history of endometrial cancer, smoking, clinical history of diabetes mellitus and hypertension. Only control for age and BMI affected risk estimates meaningfully and so were included in the final models. Tests for trend over categories were performed by the introduction of 'semi-continuous' variables obtained by assigning consecutive integers to levels of categorized variables.

The organochlorine pesticides and PCB congeners were analyzed both in untransformed form (presented in the results) and in logarithmically transformed form, with similar results. For most compounds, subjects were also grouped into quartiles according to the distribution among controls. For CBs 28, 52 and 101, a substantial number of subjects (32% of CB 28, 63% of CB 52, and 74% of CB 101) had values below the quantification limit. Therefore we categorized these variables into 3 groups: women with values below the quantification limit as a referent, and those above quantification limits divided into two equal sized groups among controls. Over 90% of women had values below the quantification limit for α-HCH, γ-HCH, *p,p'*-DDD, and *o,p'*-DDT; therefore we subdivided these as below (referent) and above the quantification limit. Because all subjects had *o,p'*-DDE concentrations below the quantification limit of 4 ng/g lipid in the serum, we could not include this variable in any analysis. We also considered variables in continuous form in the analysis of substances grouped according to possible hormonal effects.

Paper V. Estrogen receptor gene polymorphisms and endometrial cancer risk.

Study population

This study is based on the study IV presented above. To increase sample size for the molecular analysis, the study population was enlarged 5 months after the start of enrollment to include women who had used hormone replacement. Because blood samples were collected from all cases before answering the questionnaire, we were able to include the 288 case patients described above (134 who had used hormone replacement and 154 who had not; see above Paper IV, study population). Of the 241 controls who used hormone replacement, we collected blood samples from 187, but we missed 54 who belonged to the first group of controls (enrolled during the first 5 months of the study; controls were firstly asked to answer the questionnaire and afterward had blood samples collected). In total 392 control women were included in the study (187 who had used hormone replacement and 205 who had not).

For this paper, we performed all analysis separately for women who never used hormone replacement, the main focus of our analysis, and for all women, i.e. users and non-users of hormone replacement.

Molecular analyses

Leucocyte genomic DNA was extracted from whole blood (EDTA) according to standard procedures. The Pvu II and Xba I restriction fragment length polymosphisms (RFLP's) are located in intron 1, only 50bp apart, 400 bp upstream of exon 2 of the estrogen receptor gene (Kobayashi et al., 1996; Yaich et al., 1992; McGuire et al., 1992; Castagnoli et al., 1987). They were analyzed as described (Kobayashi et al., 1996) with the following exceptions: 50 pmol of each oligonucleotide primer was used in the PCR which was performed through 30 cycles at 94° C for 30 s, 62°C for 20 s, 72°C for 90 s, and a final extension for 5 min at 72°C using GeneAmp PCR 9600 (Perkin Elmer, Norwalk, CT, USA).

The TA-repeat at -1174-base pairs upstream of exon 1 of the human ER gene was analyzed by PCR amplification using the oligonuclotide primer sequences described (Sano et al., 1995), but with a forward primer that was labeled with a fluorescent dye, TET (Perkin-Elmer) (Holgersson et al., 1994). Genomic DNA (100 ng) was amplified with 10 pmol of each primer, in a total volume 50 µl containing 10 x PCR buffer, 2.5 U Taq DNA polymerase, 2.5 mM MgCl2 and 0.2 mM dNTPs. There was an initial denaturation at 94° C followed by 25 cycles of 30s at 94° C, 30 s at 55° C, and 30 s at 72° C. Fluorescence-labeled PCR products were separated on a 6% polyacrylamide gel using an ABI 373 A automatic DNA sequenator (Perkin Elmer). A fluorescence-labeled size marker, GS-350 TAMRA (Perkin-Elmer), was used as an internal-lane standard. GENESCAN 672 kit (Perkin Elmer) was used to quantitate fluorescence-labeled PCR products in base pairs and by amount of fluorescence. DNA fragment sizes were confirmed by automated DNA sequencing using a DNA sequencing kit, ABI Prism dye terminator cycle sequencing ready reaction kit, with Amplitaq DNA polymerase FS (Perkin-Elmer) exactly according to the manufacturer's instructions.

Statistical analysis

Using standard chi-square statistics, we tested if the allele frequencies deviated from the Hardy-Weinberg equilibrium. Considering the polymorphic genotypes as 'exposures', we calculated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression models, fit by maximum likelihood (Breslow and Day, 1980). Genotype frequencies for the Pvu II and Xba I polymorphisms were compared using subjects homozygous for the most common allele as the reference. For the TA repeats in cases and controls, the distribution of allele sizes from 6 to 30 was bimodal. Therefore we categorized genotypes to avoid making strong assumptions about the functional form of the relationship between allele size and disease status. Categories were chosen to correspond to the naturally occurring short (< 19 TA repeats) and long (19 or more TA repeats) alleles in our study population (in both cases and controls). We computed ORs using subjects homozygous for long TA alleles as the reference. We estimated unadjusted (univariate) ORs for various genotypes and subsequently included the following variables in the logistic regression models: age (continuous variable), menopausal status (pre- or postmenopausal), age at menopause (less than 50 years, 50-52 years, 53 or more years), age at last birth (less than 30 years, 30 or more years), nulliparity, number of births (1, 2, 3 or more), body mass index (BMI, i.e. weight in kg / height in meters squared, as a continuous variable), use of oral contraceptives (ever or never), use of different hormone replacement therapy regimens (HRT, classified according to ever or never exposed to the following compounds: estrogens without progestins, estrogens with cyclic addition of progestins, estrogens with continuous addition of progestins, progestins without estrogens, oral estriol, and vaginal use of estriol, dienoestrol or estradiol), smoking (ever or never smoked regularly), clinical history of diabetes mellitus and hypertension (self reported). Adjustment for age did not substantially affect the crude risk estimates. However, inclusion of other covariates in the models did change risk estimates modestly, and both age adjusted and multivariate models are presented. We computed tests for trend by the introduction of 'semi-continuous' variables obtained by assigning consecutive integers to levels of the categorized genotype variables.

RESULTS

Papers I to III

The differences between case patients and control women regarding age at menopause, parity, use of oral contraceptives, and smoking reflected established epidemiological associations (see Table 1 on the annexed Paper I).

Among women in the study, some 20% had taken estrogens, three-fourths of them estradiol compounds and one-fourth conjugated estrogens. About three-fourths of the control women and one-half of the cases reported use of added progestins, whereof 65% had used progestins cyclically (81% with an addition for 10 days of each cycle) and 47% continuously; 81% of the additions contained testosterone-derived compounds, norethisterone acetate 1 mg and levonorgestrel 250 µg, and 30% a progesterone-derived compound, medroxyprogesterone acetate 5-10 mg.

Low potency estrogens

Data in tabular format are presented in the annexed Paper I.

Use of oral estriol was reported by 142 endometrial cancer case subjects (20.1%) and 361 control women (10.8%). Among women who used oral estriol, 58% had used 1 mg, 31% 2 mg, and 11% could not remember if they used 1 or 2 mg. Ever use was associated with a twofold increased relative risk of endometrial cancer compared to those who never used oral estriol. Women exposed for less than 5 years had an odds ratio of 1.7 and those exposed for 5 or more years 3.0. When duration of use of oral estriol was analyzed as a continuous variable, relative risk increased by 8 percent per year ($p < 0.0001$).

Most women had recent exposure. Women who stopped exposure more than 1 year before the index date (six months before diagnosis) had no discernible increase in relative risk compared with never users. Because of small numbers, no stratified analyses were performed for duration within recency categories.

Exclusive use of orally administered estriol, without prior or subsequent use of any other hormone replacement formulations, was observed among 77 case women (9.6%) and 226 control women (6.3%). The odds ratios (multivariate analysis) for those who used exclusively oral estriol to those who did not use any treatment was 2.1 (95% 1.6-2.9) and the increment in relative risk per year of use was 1.09 (95% 1.05-1.13); these estimates were similar to those which included women with mixed exposures.

In a further subgroup analysis by histological grade, the association with oral estriol use was markedly stronger for well differentiated (grade 1) than for less differentiated (grades 2 and 3) cancers; following 5 or more years of use, relative risks were increased about fivefold and twofold, respectively. Similarly, the excess relative risk following oral estriol was higher for tumors with no or limited infiltration than for tumors with 50 percent or more infiltration.

Among the 80 case women who were reclassified after histopathologic review as having atypical hyperplasias rather than invasive cancer, 27 (34%) had used oral estriol. In a multivariate analysis, the odds ratio for ever use was 3.7, with an eightfold elevation in relative risk after 5 or more years of use. The increment in relative risk per year of use was 12 percent. As for invasive cancer, the excess relative risk for atypical hyperplasia vanished rapidly after cessation of treatment.

Vaginal low potency estrogen formulations

Data in tabular format are presented in the annexed Paper I.

Use of vaginally administered low potency estrogen formulations was reported by 104 endometrial cancer patients (14.7%) and 376 control women (11.3%). After multivariate adjustment, ever use conferred an OR of 1.2 (95% CI 1.0-1.6), and the OR per year of use was 1.02 (95% CI 0.97-1.06). Exclusive use of vaginal low potency estrogen formulations was reported by 56 case women (6.9%) and 241 control women (6.8%), yielding an OR for ever use of 1.4 (95% CI 1.0-2.0). We found no evidence of a differential effect of vaginal use of low potency estrogen formulations on tumor grade or myometrial invasiveness. We also examined those 15 women who had their tumor reclassified as atypical hyperplasias, and who had used vaginal low potency estrogen formulations. The odds ratio for ever use was 1.5 (95% CI 0.8-3.0) and the increment in relative risk per year of use was 1.07 (95% CI 1.00-1.14).

Of all vaginal treatment episodes, 49% consisted of estriol (0.5 mg), 44% dienoestrol (0.5 mg), and 7% estradiol (25 µg). Because of the possibility that vaginal use of estriol and dienoestrol could have different effects on the endometrium, we also analyzed these hormones separately. (We did not have enough subjects who used vaginal estradiol to perform a meaningful analysis in that subgroup). In the multivariate analysis, use of vaginal estriol (used by 49 cases and 195 controls) entailed an OR of 1.1 (95% CI 0.8-1.6), and vaginal dienoestrol (used by 46 cases and 188 controls) an OR of 1.0 (95% CI 0.7-1.5) as compared to never use of these formulations.

Estrogens without progestins

Data in tabular format are presented in the annexed Paper II.

Intake of estrogens without progestins, reported by 98 case subjects (14.3 %) and 177 control women (5.4 %), conferred a threefold increased relative risk of endometrial cancer. Relative risk increased by 17 percent per year of use (OR 1.17; 95% CI 1.12-1.21) to an OR of about ten after 10 years. The excess relative risk persisted even 5 or more years after cessation of treatment, when the OR for long term users was 6.3 (95% CI 3.4-11.8).

Among women who used estrogens without progestins, 94 (34.2%) had used conjugated estrogens, 180 (65.4%) estradiol, and 20 (7.3 %) other synthetic compounds. Only 19 subjects (7%) reported use of more than one type of estrogen. Conjugated estrogens and estradiol carried similar patterns of risk. Five or more

years of treatment entailed about a sixfold excess relative risk for both drugs (for conjugated estrogens: OR 6.6; 95 % confidence interval 3.6-12.0; for estradiol OR 6.2, 95% confidence interval 3.1-12.6) while 10 or more years use were associated with odds ratios of 9.2 (95% 4.4-19.3) and 7.5 (95% CI 3.0-18.9), respectively. The corresponding increments in relative risk per year of use were 15 percent (OR 1.15, 95% CI 1.10-1.20) for conjugated estrogens and 17 percent (OR 1.17, 95% CI 1.10-1.23) for estradiol.

Relative risk for endometrial cancer was strongly related to the daily dose of estrogens. After 5 or more years of use, women prescribed lowest dose regimens had a fourfold increased relative risk, and those with higher doses an eightfold increase. The corresponding increments in relative risk per year of use were 12 percent (95% CI 4-20 percent) and 18 percent (95% CI 13-24 percent), respectively.

Estrogens with progestins

Data in tabular format are presented in the annexed Paper II.

Intake of estrogens with progestins was reported by 119 case subjects (17.2 %) and 477 control women (14.6 %), and conferred an overall 30% increased relative risk of endometrial cancer. However, the excess relative risk was statistically significant only after 10 or more years of use. The estimated increment in relative risk per year of use was 6% (OR 1.06; 95% CI 1.02-1.10). In contrast to the findings reported for unopposed estrogen use, no substantial elevation of the relative risk remained 5 or more years after cessation of use.

Three hundred-and-ninety women (65.4 %) had used cyclic progestins with estrogens, 278 (46.6 %) continuous estrogen-progestins, and 72 (12%) a mixture of both regimens. Among the 390 users of a cyclic regimen, 17 (4%) added progestins for less than 10 days of the cycle, 368 (94.3%) for 10-14 days (being 315 or 81% for 10 days), while 5 (1%) subjects reported a mixture ranging from 5 to 14 days. Thus, our analyses of cyclic regimens pertained largely to 10 days of progestin per cycle. Among the 278 women reporting continuous combined regimens, 276 (99%) had daily progestins, and 2 (1%) progestins during 21 days of each cycle.

The increase in relative risk for endometrial cancer associated with combined regimens was confined to women exposed to cyclic addition of progestins, while continuous addition of progestins actually reduced relative risk (p for trend over duration of use 0.02). Odds ratios per year of use were 1.10 (95% CI 1.06-1.15) for cyclical regimens and 0.86 (95% CI 0.77-0.97) for continuous combined treatment.

There was no evidence of interaction between use of estrogens without progestins or with progestins added cyclically or continuously, since the differences in the OR in logistic regression models including or excluding the interaction terms were not statistically significant (two-sided p=0.69, likelihood ratio test for interaction).

We also analyzed subgroups of progestins classified according to their derivation. Among the 596 women exposed, 177 women (30%) used combinations with a progesterone-derived progestin, 483 (81%) a 19-nor-testosterone-derived progestin, and 64 (11%) both types. Regimens with progesterone-derived progestins were

associated with an increase in relative risk for endometrial cancer (OR per year of use 1.12, 95% CI 1.06-1.18), while no association was found with testosterone-derived progestins (OR per year of use 1.00, 95% CI 0.95-1.06).

We used further stratified analyses to separate the effects of pattern of use and progestin derivation. Progesterone derived progestins seemed to lead to higher relative risks of endometrial cancer than testosterone derived progestins, regardless of type of regimen. Continuous addition of testosterone-derived progestins entailed significant protection. The number of women who used continuous regimen with progesterone derived progestins was too small for analysis.

Tumor grade and invasiveness

Analyses of the association of hormone replacement with tumors having different histopathological characteristics were hampered by small numbers. However, some interesting patterns emerged. The ORs for use of estrogens without progestins became increasingly elevated with increasing differentiation of the endometrial neoplasia. Following 5 or more years of use, relative risks increased about ninefold for atypical hyperplasias and well differentiated tumors, fivefold for moderately differentiated tumors, and fourfold for poorly differentiated tumors. The excess relative risk following replacement with estrogens without progestin was slightly higher for tumors with no or limited myometrial infiltration than for tumors with 50% or more infiltration. Among users of combined regimens a similar pattern appeared, but was confined to cyclic treatment.

Use of oral contraceptives and endometrial cancer risk

Data in tabular format are presented in the annexed Paper III.

Use of some kind of OC, chiefly medium or high dose combined estrogen/progestin regimens, was reported by 22% (n=157) of the case patients and 33% (n=1107) of control women.

Overall, ever use of OCs entailed a 30 percent lower risk of endometrial cancer as compared to never use. This association was unchanged after multivariate adjustment. The pattern of risk reduction appeared similar for different types and regimens of OCs, and risk estimates were lowest for use of progestins only. Lack of statistical power precluded more detailed analysis of combined regimens subdivided by dosages, progestin-only pills and depot-medroxyprogesterone acetate injections and endometrial cancer risk. Further analysis presented here will be restricted to use of COCs, regardless of dose.

We found a strong trend of decreasing endometrial cancer risk with increasing duration of COC use. There was no association with use for less than 3 years (multivariate OR 1.0, 95% CI 0.7-1.3). However 3 or more years of use conferred a multivariate OR of 0.5 (95% CI 0.3-0.7), and for 10 or more years 0.2 (95% CI 0.1-0.4). On average, risk for endometrial cancer decreased by approximately 10 percent per each year of use of COCs.

In this population of postmenopausal women, subjects who had ever used COCs tended to have ceased use in the distant past. The median recency (time since last use) was 25 years for cases and 22 years for controls. The median age at cessation of use was 36 and 38 years, respectively. Regular menstrual cycling after COC use did not negate the protective effect, and the (negative) association between duration of COC use and endometrial cancer risk waned gradually with time since last use, to become essentially absent 30 years after cessation. Compared to use in the 20 years before the study, COC use 20-29 years previously conferred a multivariate duration-adjusted OR of 1.7 (95% CI 0.8-3.4); for use 30 or more years previously, the OR was 1.9 (95% CI 0.7-5.1). Small numbers prevented investigation of the persistence of the COC effect among women who had used them for prolonged periods.

The protective effect of use of COCs was observed for atypical hyperplasias, as well as for various degrees of tumor differentiation and invasiveness. The minimum duration of treatment that entailed a measurable protective effect for any degree of differentiation and invasiveness was 3 years.

We also explored whether the protective effect of COC use was modified by subsequent treatment with various hormone replacement regimens after menopause. The protective effect of COCs appeared, however, independent of subsequent use of any type of hormone replacement.

Finally we stratified the data according to parity (nulliparous or parous). The multivariate OR for 3 or more years of COC use among nulliparous women (6 cases and 29 controls) was 0.6 (95% CI 0.2-1.9) and for parous women 0.4 (95% CI 0.3-0.6). Similarly, we found a reduced risk following COC use regardless of body mass index (multivariate OR per year of use of COCs: 0.90, 95% CI 0.81-0.99 for quartile 1; 0.90, 95% CI 0.82-0.97 for quartile 2; 0.86,95% CI 0.77-0.97 for quartile 3, and 0.88, 95% CI 0.81-0.96 for quartile 4) or smoking history (multivariate OR per year of use of COCs: 0.89, 95% CI 0.84-0.95 for never smokers, 0.88, 95% CI 0.80-0.96 for current smokers, and 0.88, 95% CI 0.82-0.95 for former smokers – i.e. women who stopped smoking more than 1 year before enrolment into the study).

Paper IV. Organochlorines and endometrial cancer risk.

Data in tabular format are presented in the annexed Paper IV.

Case-control differences in this study largely reflected known epidemiological associations. There was no difference between cases and controls regarding fish consumption patterns, but controls reported more breast-feeding (a major excretory route for organochlorine compounds) than cases. We compared lipid adjusted concentrations of pesticides and PCBs between fasting and 15 non-fasting control women, and between users and non-users of topical estriol; in both comparisons, no meaningful differences were observed and we will present all results including these women in the analyses.

Among DDT compounds, *p,p'*-DDE (the principal metabolite of *p,p'*-DDT*)*, had the highest mean concentrations (600-700 ng/g lipid). The average concentrations of the other pesticides were usually lower by a factor 10 or more. For all compounds the range of exposure was substantial both among case patients and among control women.

In unadjusted analyses, median concentrations of *p,p'*-DDT*, p,p'*-DDE, β-HCH and oxychlordane were higher among case patients than among controls. However, after adjustment for age and BMI in logistic regression, odds ratios were close to unity and there was no evidence of any trends in risk over quartiles of exposure. Similarly, there were no substantial differences in risk between women with values above and below the quantification limit for *o,p'*-DDT (OR adjusted for age and BMI 1.4, 95% CI 0.6-3.5), *p,p'*-DDD (OR 0.9, 95% CI 0.5-1.7), α-HCH (OR 1.2, 95% CI 0.4-3.7), or γ-HCH (OR 1.5, 95% CI 0.8-2.8). In the analyses of organochlorines as continuous variables, we found no associations between risk for endometrial cancer and any of the ten pesticides evaluated.

Among the ten PCB congeners, CB 153 had the highest concentrations (mean approx. 236 ng/g lipid), whereas CB 28, CB 52 and CB 101 often had concentrations below the quantification limit (2 ng/g lipid). As for the pesticides, we found a wide range of serum concentrations of PCBs both among case patients and control women.

Unadjusted mean concentrations of CB 28 and CB 118, were higher among case patients than among control women. However, after adjustment there was no substantial increase in risk associated with high concentrations of any of the congeners evaluated, and there were no significant trends in risk. Likewise, no differences were seen for PCB congeners CB 28, CB 52, or CB 101, which we considered in two categories using undetectable levels as reference. Finally, in the analyses of the ten different PCB measurements in continuous form there was no significant association between any of the congeners and endometrial cancer risk.

No significant associations or trends were observed when we compared quartiles of exposure for different groups of compounds. In analyses stratified by BMI (above or below mean value among controls, i.e. 25.39), breast-feeding history (ever Vs never), parity (nulliparous Vs parous), use of topical estriol (ever Vs never), and menopausal status (pre- versus post-menopausal), there were no indications of associations or

dose-response relationships with endometrial cancer, and risk estimates in all sub-groups were non-significant.

Paper V. Estrogen receptor gene polymorphisms and endometrial cancer risk.

Data in tabular format are presented in the annexed Paper V.

Compared to controls, cases were slightly older, had a higher age at menopause, a lower parity, and a greater body mass index. Proportionally more cases than controls reported being nulliparous, having never smoked or used oral contraceptives, and having a history of diabetes mellitus or hypertension. Thus, these case-control differences largely reflected known epidemiological associations.

From the 288 enrolled cases, 261 were genotyped for Pvu II, Xba I and TA repeats. Of the 392 controls enrolled, 380 were successfully genotyped for Pvu II and Xba I, and 372 for TA repeats. None of the genotype frequency distributions deviated significantly from the Hardy-Weinberg equilibrium among cases or among controls.

Pvu II polymorphisms

The age-adjusted relative risks for the genotypes Pp and PP were not substantially different from that of the reference group pp. However, after multiple-adjustment, the PP genotype was associated with a non-significantly decreased risk: OR 0.69 (95% CI 0.34-1.43). A similar pattern of ORs was found in the expanded subject group. A trend of decreasing risks with increasing number of P alleles was present, but it was not statistically significant (multivariate p = 0.43 in women unexposed to hormone replacement, multivariate p = 0.23 in the expanded study group).

The Xba I polymorphisms

The X- allele appeared to confer a reduced risk of endometrial cancer; the ORs for genotypes XX and Xx were less than 1.0, and risk decreased with increasing numbers of X alleles (p for trend for the multivariate ORs = 0.07). For the XX genotype the multivariate OR was 0.51 (95% CI 0.20-1.27). With the expanded dataset, this was 0.59 (95% CI 0.32-1.05, p for trend =0.05).

Dinucleotide repeats

Among women who never used hormone replacement, there was a tendency toward a possible gene-dose effect. The multivariate OR for heterozygous long-short was 1.29 (95% CI 0.66-2.56) and for short-short 1.50 (95% CI 0.72-3.17) when compared to the long-long genotype (p for trend = 0.29). A similar pattern of results was observed in the analysis including all women (p for trend=0.08).

GENERAL DISCUSSION

General methodological comments on papers I to III – Study 1

Strengths of our study include the population-based design, large size, detailed assessment of exposure to hormone regimens and possible covariates, and uniform histopathologic review and classification of all cases.

The strong, consistent and statistically significant associations indicate that chance is unlikely to explain our findings, nor was there evidence that confounding could explain our results. Bias is a more serious concern, although the population-based design and relatively high participation rates reduce the potential for selection bias. However, data collection through telephone interviews in 15% of the controls could have introduced information bias, although the possible enhancement of recall in these controls would, if anything, lead to an underestimation of relative risks.

We think that information bias was unlikely since we found clear and differential patterns of relative risk with types of administration and with histopathological features of the tumour. Differential diagnostic classification with regard to hormone use can be ruled out since we used a blinded histopathological review. A more intense surveillance among women receiving low potency estrogen formulations is unlikely, since estriol has not been reported as affecting the endometrial cancer risk. This is not the case with other hormone replacement regimens, since estrogens are well known to enhance risk for endometrial cancer. However, in the Swedish health care system with readily available service for all citizens, post-menopausal vaginal bleeding would lead to an endometrial biopsy with short delay regardless of such exposure.

We reduced the possibility of a reverse causality association (because of treatment of symptoms) by considering only exposure up to half a year or longer before diagnosis.

Specific comments on papers I to III – Study 1

Paper I. Low potency estrogens and risk for endometrial cancer.

We found convincing evidence of an increased relative risk of endometrial cancer in postmenopausal women who had used oral estriol. The relative risk increased with duration of use and was highest for well differentiated and least invasive tumors. An even greater excess increase in relative risk was noted for endometrial atypical hyperplasia, a pre-malignant or early malignant lesion which may be difficult to distinguish unambiguously from invasive cancer as revealed by the histopathology review in this and previous investigations (Persson et al., 1986). In contrast to oral estriol, vaginally administered low potency estrogen formulations were not clearly associated with a substantial increase in relative risk for endometrial cancer or atypical hyperplasia.

Data from other epidemiological studies regarding low potency estrogen formulations and endometrial cancer are scarce. In a hospital based case-control study in Finland, a 60% decrease in the relative risk of endometrial cancer was found among women exposed orally to estriol; however, dosages and duration of the treatment were not

given (Salmi et al., 1980). In a population-based prospective cohort study in Sweden women prescribed low potency estrogen formulations (oral estriol) showed no overall increase in the risk of endometrial cancer; however, data on duration and recency of intake were not available (Schairer et al., 1997). Kelsey and colleagues (1982) reported an increased risk of endometrial cancer after vaginal hormone use. However, the study had no information regarding formulations used or duration of therapy.

Some evidence suggests that oral estriol may have systemic effects. Englund et al. (1980) showed that in postmenopausal women treated with oral estriol 6 mg daily for 3 months, more than half had menstrual bleeding after addition of a progestin. In a recent Swedish study postmenopausal women referred for undue vaginal bleeding were examined with ultrasound measurement of endometrial thickness before biopsy (Granberg et al., 1997). Among users of oral estriol and medium potency estrogens, the average endometrial thickness was greater and endometrial atypical hyperplasia more frequent when compared with unexposed women. A trial in Japanese postmenopausal women treated with oral estriol 2 mg daily for a year showed both prevention of bone loss and alleviation of climacteric symptoms (Minaguchi et al., 1996).

Due to its low affinity, estriol binds to the estrogen receptor in vitro for a relatively short time compared to medium or high potency estrogens (Esposito, 1991; Botella et al., 1995). Estriol administered orally is conjugated efficiently in the liver (Schiff et al., 1980), but because estriol does not bind strongly to proteins most of the serum estriol is biologically active (Vermeulen and Verdonck, 1968). When estriol is taken continuously a prolonged increase in serum levels can lead to long-standing proliferation of endometrial cells as other estrogens (Clarck et al., 1977; Korach et al., 1980).

Our observations that oral estriol intake but not vaginal estrogen use confers an excess relative risk of endometrial cancer is novel. Heimer and Englund (1984) found similar serum levels of unbound estriol twenty-four hours after vaginal administration of 1 mg of estriol as for oral intake of 10 mg of the compound, suggesting that vaginal absorption is indeed more effective than oral administration. However, extrapolation of these data to long-term effects is not straightforward, since oral estriol is usually given in dosages of 1 or 2 mg daily, and vaginal administration in a dose of 0.5 mg twice a week. Further, vaginal resorption and thereby serum levels decrease as the vaginal epithelium matures 1-2 weeks after start of vaginal treatment, as demonstrated both for vaginal estriol and estradiol (Nilsson and Heimer, 1992; Heimer and Englund, 1984).

Our findings that long-term intake of oral low potency estrogen formulations in routine dosages may increase the relative risk of endometrial cancer has important implications for medical practice. First, there is a need to monitor the endometrium during such treatment, and the addition of progestins during treatment should be considered. Secondly, if the indication for treatment is atrophy only, vaginal application of low potency estrogen formulations is to be preferred. Thirdly, early symptoms of a possible endometrial lesion, i.e. uterine bleeding, should lead to a prompt diagnostic procedure.

In some women there may be a rationale for using medium potency estrogens or an estrogen-progestin regimen instead in order to gain other benefits such as reduced risk of osteoporosis and the possibility of some reduction in the risk of coronary heart disease (Toozs-Hobson and Cardozo, 1996; Grodstein et al., 1997; Hulley et al., 1998; Petitti et al., 1998).

Paper II. Risk for endometrial cancer following estrogen replacement with and without progestins.

We found convincing evidence that treatment with conjugated estrogens or estradiol without progestins confers a marked, and rather persistent, duration- and dose-dependent increase in relative risk for endometrial cancer. The increased relative risk was slightly more marked for atypical hyperplasias, well and moderately differentiated tumors. Combination regimens with progestins added for less than 16 days (predominantly 10 days) also conferred an increased relative risk, though much less pronounced than for estrogens alone. Addition of progestins all days of the cycle appeared to reduce relative risk below levels of unexposed women.

An association between use of estrogens without progestins and risk for endometrial cancer has been consistently found (Grady and Ernster, 1996). Less well known is the persistence of the effect even after 5 years of cessation. This pattern clearly emerged in our data and can also be discerned in some prior reports (Rubin et al., 1990; Green et al., 1996).

Our findings regarding cyclically added progestins, mainly for 10 days of each cycle, agree with those recently published (Beresford et al., 1997), showing that 5 years or more of cyclic use yielded a threefold excess risk. In contrast, a smaller study found no increase in risk for cyclic regimens with 10 or more days of progestin per cycle (Pike et al., 1997). As for treatment with estrogens alone, the excess risk seemed to persist beyond 5 years of cessation. In some other case-control studies from the US, reporting mainly treatments with conjugated estrogens and addition of medroxyprogesterone acetate (MPA), some evidence of a protective effect by the added progestins emerged. Voigt et al. (1991) found that a cyclic addition of progestins of duration less than ten days conferred a doubled risk, while such addition ten days or longer was not associated with a risk increase. Jick et al. (1993) and Brinton et al. (1993) reported elevated relative risk estimates, however non significant, for women with a combined estrogen-progestin treatment, but their estimates were of lower magnitudes than for women with estrogens only. Summary data from previous case-control studies on estrogen-progestin hormone replacement and from our study are displayed in Table 2.

In an earlier Swedish cohort investigation (Persson et al., 1989), the relative risk (RR) for women using cyclic progestin-combined treatment – mainly with estradiol 2 mg and levonorgestrel 250 µg during 10 days of the cycle – was 0.9 (95% CI 0.3-2.0), a risk based on only seven exposed cases, to be compared with RR 1.4 (95% CI 1.1-1.9) for those who had taken estrogens alone. At a later follow-up of the same Swedish cohort of women, who had answered a detailed questionnaire, those with more than six years of progestin combined treatment had a non significant 40%

increased risk of endometrial cancer in relation to women with short term or no exposure (Persson et al., 1999).

The only published data regarding continuously combined treatment suggested an absence of an association with endometrial cancer (Pike et al., 1997), in contrast to the significantly reduced risk we observed. The predominant regimen used by our subjects - estradiol 2 mg plus norethisterone acetate 1 mg - may have more potent progestational effects (Hirvonen, 1996) than the compounds studied by Pike and colleagues (1997), i.e., conjugated estrogens (0,625 or 1,25 mg in 79% of users) and medroxyprogesterone acetate (used in 88% of those with combined regimens). Although never previously documented, a protective effect of continuous combined regimens is plausible, since combined oral contraceptives – providing a continuous combination of, synthetic and high dose, estrogens and progestins – clearly reduce endometrial cancer risk in premenopausal women (Grady and Ernster, 1996).

A gradient in the effects of estrogens without progestins on risk according to tumor grade has been previously reported (Grady et al., 1995; Brinton et al., 1993; Shapiro et al., 1998), and we now extend those observations to tumors after combined estrogen-progestin treatment. Together these results suggest that exogenous hormones predominantly cause less aggressive tumors. Higher risks for conjugated estrogens than for synthetic estrogens (Grady et al., 1995) were, however, not confirmed in our data.

Estrogenic stimulation promotes endometrial proliferation and can eventually cause hyperplasia, atypia and neoplasia (Key and Beral, 1992). Added progestins counteract estrogenic effects through several mechanisms including down-regulation of estrogen receptor levels, enhancement of estradiol metabolism by estradiol –17β dehydrogenase, regulation of several growth factors, decreased DNA synthesis and endometrial shedding (Key and Beral., 1992; Gurpide, 1991; Hsueh et al., 1975; Tseng and Gurpide, 1975; Graham and Clarke, 1997).

Our finding, based on small number of exposed subjects, that addition of progesterone-derived progestins seemed to confer a somewhat higher endometrial cancer risk than testosterone-derived progestins clearly needs confirmation. Separate effects might be due to differences in hormonal mechanisms, or to variation in the progestin potency of the two types of compounds, or to both. In one previous study 1 mg of norethisterone produced a similar endometrial secretory response as 125 µg of levonorgestrel or 11 mg of medroxyprogesterone when given for 7 days in women using conjugated estrogens (King and Whitehead, 1986). Among women in our study using cyclic regimens, the predominant dosages of testosterone-derived compounds were 1 mg for norethisterone acetate (17%) and 250 µg for levonorgestrel (48%), whereas dosages of medroxyprogesterone acetate were on the average less than 10 mg (66% with intake of 5 mg). Thus, the testosterone-derived progestins were prescribed in relatively higher dosages than the progesterone-derived compounds, possibly explaining in part our observation of differential effects on endometrial cancer risk.

Hormone replacement is becoming increasingly common in many countries, both for alleviation of menopausal symptoms and for prevention of osteoporosis and

cardiovascular diseases. Continuous addition of a progestin appears to be the safest regimen for women with a uterus, but a more complete evaluation of the risk-benefit balance is complex. There is no clear evidence that adding progestins throughout the cycle rather than, say 10 days – or not at all – would be more harmful to the breast and cardiovascular system. These issues are incompletely studied, however, and deserve further investigation.

Paper III. Use of oral contraceptives and endometrial cancer risk.

We found evidence of a protective effect of COCs after 3 years of use. The protective effect increased with duration of use, and persisted for at least 20 years after discontinuation of treatment. The protective effect was observed for all grades of invasive tumors as well as for atypical hyperplasias, without evidence of effect modification by BMI, smoking, or subsequent use of hormone replacement therapy.

We were able to observe the effect of COC use on endometrial cancer risk at more advanced ages (70-74 years) and a longer time after cessation of treatment (30 or more years) than in previous studies. Sample size limitations prevented us from evaluating in detail the effects of progestin- only contraceptives, or from studying subgroups using different doses of COCs. Similarly, we could not separate the effects of high and medium estrogen dose preparations, or different types or doses of progestins, because a substantial proportion of subjects could not recall the particular brand of COCs used.

Our results are in agreement with previous studies demonstrating that risk for endometrial cancer among ever users of COCs is approximately halved compared to never users (Gray et al., 1977; Weiss and Sayvetz, 1980; Kaufman et al., 1980; Hulka et al., 1982; Kelsey et al., 1982; La Vecchia et al., 1984; La Vecchia et al., 1986; Pettersson et al., 1986; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steoid Contraceptives, 1988; Koumantaki et al., 1989; Levi et al., 1991; Rosenblatt et al., 1991; Shu et al., 1991; Jick et al., 1993; Stanford et al., 1993; Voigt et al., 1994; Vessey and Painter, 1995) even after careful adjustment for potential confounding variables (Weiss and Sayvetz., 1980; Kaufman et al., 1980; Hulka et al., 1982; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988; Rosenblatt et al., 1991; Shu et al., 1991) the protective effect became apparent within a few years of initiation of treatment (Kaufman et al., 1980; Hulka et al., 1982; Pettersson et al., 1986; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; Levi et al., 1991; Shu et al., 1991; Jick et al., 1993; Stanford et al., 1993; Voigt et al., 1994; Henderson et al., 1983) and increased with the duration of use (Kaufman et al., 1980; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; Levi et al., 1991; Stanford et al., 1993; Henderson et al., 1983).

Of particular interest is our finding that the protective effect of COCs waned slowly with time after cessation of use. This finding agrees with at least 5 previous studies

Table 2. Summary of case-control studies evaluating hormone replacement therapy with estrogen-progestin combinations and endometrial cancer risk, by number of days of addition of progestins per cycle, if available (adapted and complemented from IARC, 1999).

Study	**Local / Period / Ages**	**Design Cases/controls, Exposed cases/controls**	**Type / measure of combined hormonal therapy**	**OR and 95% CI [a]**
Jick et al. (1993)	Washington state, USA, / 1979-89, / 50-64 years	Members of HMO[b] 172/1720; 29/147 with E+P use	* nonuser, ≤ 6 months use: *Any E+P [c], <past year [d]: - duration <3 years: ≥3 years: *Any E+P, ≥1 year prior:	1.0 (referent) 1.9 (0.9-3.8) 2.2 (0.7-7.3) 1.3 (0.5-3.4) 0.9 (0.3-3.4)
Brinton et al. (1993)	Five US areas, / 1987-90, / 20-74 years	General population, 300/207; 11/9 exposed to E+P	* nonuser: *Any E+P use, ≥3 months [d]:	1.0 (referent) 1.8 (0.6-4.9)

Table 2, cont'd

Study	Local / Period / Ages	Design, number of cases and controls, data	Type / measure of combined hormonal therapy	OR and 95% CI [a]
Pike et al (1997)	California, USA, / 1987-93, / 50-74 years	General population 833/791; 247/216 with E+P	*Any E+P, progestin <10 days/cycle [e];	
			- duration (months): 0:	1.0 (referent)
			- duration: 1-24:	1.4 (NP)[f]
			25-60:	1.5 (NP)
			≥60:	3.5 (NP)
			Per 5 years:	1.87 (1.32-2.65)
			*Any E+P, progestin ≥10 days/cycle;	
			- duration (months): 0:	1.0 (referent)
			- duration: 1-24:	1.0 (NP)
			25-60:	0.7 (NP)
			≥60:	1.1 (NP)
			Per 5 years:	1.07 (0.82-1.40)
			*Any E+P, progestin all days/cycle [g];	
			- duration (months): 0:	1.0 (referent)
			- duration: 1-24:	1.1 (NP)
			25-60:	1.4 (NP)
			≥60:	1.3 (NP)
			Per 5 years:	1.07 (0.80-1.42)

Table 2, cont'd

Study	Local / Period / Ages	Design, number of cases and controls, data	Type / measure of combined hormonal therapy	OR and 95% CI [a]
Beresford et al (1997)	Washington state, USA, / 1985-91, / 45-74 years	General population 832/1154; 67/134 reported E+P use	* never use, ≤ 6 months use:	1.0(referent)
			*only used of E+P:	1.4 (1.0-1.9)
			*Progestins ≤10 days/cycle;	
			- duration: 6-35 months:	2.1 (0.9-4.7)
			36-59 months:	1.4 (0.3-5.4)
			≥60 months:	3.7 (1.7-8.2)
			*Progestins >10 days/cycle:	
			6-35 months:	0.8 (0.4-1.8)
			36-59 months:	0.6 (0.2-1.6)
			≥60 months:	2.5 (1.1-5.5)
			*Current only use E+P:	
			- Progestins ≤10 days / cycle:	
			- duration 6-59 months:	2.2 (0.9-5.2)
			≥60 months:	4.8 (2.0-11.4)
			- Progestins >10 days / cycle:	
			- duration 6-59 months:	0.7 (0.4-1.4)
			≥60 months:	2.7 (1.2-6.0)

Table 2, cont'd

Study	Local / Period / Ages	Design, number of cases and controls, data	Type / measure of combined hormonal therapy	OR and 95% CI [a]
Weiderpass et al. (1999)	Country of Sweden, / 1993-95, / 50-74 years	General population 709/3368; 119/447 with E+P use	*Cyclic E+P (< 16 days/cycle) [h]:	
			- never:	1.0 (referent)
			- ever:	2.0 (1.4-2.7)
			- duration <5 years:	0.8 (0.5-1.3)
			≥5 years:	2.9 (1.8-4.6)
			- per year of use:	1.10 (1.06-1.15)
			*Continuous (28 days/cycle):	
			- never:	1.0 (referent)
			- ever:	0.7 (0.4-1.0)
			- duration <5 years:	0.8 (0.5-1.3)
			≥5 years:	0.2 (0.1-0.8)
			- per year of use:	0.86 (0.77-0.97)
			*By progestin type/ cyclic regimens, per year of use;	
			- 19 nor testostenone derived:	1.09 (1.02-1.17)
			- progesterone derived:	1.12 (1.05-1.20)
			*Continuous, per yr of use;	
			- 19 nor testostenone derived:	0.85 (0.73-0.98)
			- progesterone derived:	1.07 (0.86-1.33)

Table 2, cont'd

Study	Local / Period / Ages	Design, number of cases and controls, data	Type / measure of combined hormonal therapy	OR and 95% CI [a]
Weiderpass et al. (1999) (continuation)			*By recency of use/duration:	
			- E+P <5 years ago;	
			- never:	1.0 (referent)
			duration <5 years:	1.3 (0.9-1.9)
			≥5 years:	1.6 (1.0-2.4)
			- E+P ≥5 years ago;	
			duration <5 years:	0.8 (0.4-1.6)
			≥5 years:	1.7 (0.6-4.9)

a: OR = odds ratio, CI = confidence interval
b: HMO = health maintenance organization
c: E+P = estrogen plus progestin
d: women with unopposed estrogen use included
e: unopposed estrogen use, as well as other regimens of combined therapy included, adjusted for in the analysis.
f: NP=not provided
g: i.e. continuous combined therapy
h: 81 % of users of cyclic regimens had 10 days of addition of progestins per cycle

(Kaufman et al., 1980; Centers for Disease Control and The National Institute of Child Health and Human development, The Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988; Rosenblatt and Thomas, 1991; Henderson et al., 1983), but contrasts with several others (Levi et al., 1991; Lick et al., 1993; Stanford et al., 1993). When we evaluated duration and recency of use of COCs jointly, duration was clearly dominant. Data on recency effects of COCs with only crude adjustment for duration of use (e.g. less or more than 3 years of use), as done in previous studies, may be biased because of incomplete control in the analysis for the confounding effect of duration of use (residual confounding). Thus, our results give further support for a remarkably persistent protection against endometrial cancer following only a few years of COC intake.

Our findings did not corroborate differential effects of COCs in users of hormone replacement, as previously suggested (Stanford et al., 1993; Voigt et al., 1994). These discrepancies might be due to incomplete control for duration of use of COCs in the other studies. When we analysed our data categorized as ‘never’ versus ‘ever’ or ‘users for 3 or more years’ of COCs there was no evidence of protection from COCs among ever users of estrogen replacement. However, a reduced risk was evident regardless of type of subsequent hormone replacement when duration of COC use was introduced in the logistic regression models as a continuous variable.

The absence of effect modification by BMI levels is corroborated by three studies (Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; Levi et al., 1991; Stanford et al., 1993) but contradict another (Henderson et al., 1983). We found similar effects in smokers and non-smokers, in agreement with other investigators (Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; Levi et al., 1991).

The small number of nulliparous women in our study prevents us of drawing firm conclusions about the effect of COCs in this subgroup.

The pronounced protective effect of COCs against endometrial cancer is biologically plausible in view of the composition of these preparations: progestins continuously combined with estrogen for three weeks followed by one week without estrogens or progestins (usually accompanied by withdrawal bleeding). Progestins reduce or eliminate proliferation of the glandular cells of the endometrium by downregulating estrogen receptors, enhancing metabolic inactivation of estradiol and reducing DNA synthesis (Key and Beral, 1992; Gurpide, 1991; Hsueh et al., 1975; Tseng and Gurpide, 1975; Graham and Clarke, 1997). Treatment regimens with unopposed replacement estrogens, or with progestin addition for 10 days or less per cycle, increase the risk of endometrial cancer in postmenopausal women (Grady et al., 1995; Beresford et al., 1997), sequential oral contraceptives with 5-7 days of added progestins also enhance the risks in young women (Weiss and Sayvetz, 1980; Kaufman et al., 1980; Centers for Disease Control and The National Institute of Child Health and Human Development, The Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988; Henderson et al., 1983). Interestingly, a continuous combined replacement regimen, with progestins added to estradiol during the entire cycle, seemed in recent analyses from the same study to reduce endometrial cancer compared to women who never used

replacement hormones. Our observation that after as short an exposure as three years the protective effect seemed to persist for decades regardless of subsequent hormone replacement, implies that the combined contraceptive hormones strongly reduces the sensitivity of the endometrium to malignant transformation. Hypothetically, such an effect could result if progestins added in these combined contraceptive regimens induce apoptosis of premalignant cell clones. However, since cancer development is a slow, stepwise process, the prevalence of early transformed cells should increase with age. Hence, additional mechanisms must be involved to explain why the protective effect of COCs is not more marked when the use continues into late premenopausal ages than when it ceases early. Moreover, whilst estrogen replacement mainly increases the incidence of well differentiated tumors, no such differential protective effect was discernible for COCs. Therefore, COCs and hormone replacement after menopause may exert their different effects on the endometrium, through at least in part different biologic mechanisms.

Given the widespread use of COCs worldwide their impact to reduce endometrial cancer incidence is of great potential importance. However, whether this beneficial effect holds also for modern low-dose COCs or whether it remains in nulliparous or women taking long-term hormone replacement are issues for further research.

General comments on papers IV and V

Paper IV. Organochlorines and endometrial cancer risk.

In this case-control study, we found no association between endometrial cancer risk and serum concentrations of organochlorine pesticides, pesticide metabolites or PCB congeners. The negative findings were found both in analysis of individual compounds and groups of substances with different putative hormonal activity, and the lack of association persisted in all subgroups analyzed. According to our *a priori* hypothesis, substances with an estrogen-like effect would be expected to increase the risk of endometrial cancer in a fashion similar to unopposed estrogens used as hormone replacement. Similarly, those classified as antiestrogenic would be expected to lower the risk.

Our study has several strengths, including its population-based design, relatively large sample-size, exclusion of women who ever used hormone replacement therapy (which could mask possible hormone like effects of organochlorine compounds), and the availability of detailed questionnaire information - which allowed us to adjust for potential confounding effects. Furthermore we conducted analyses of 20 specific compounds rather than of *p,p'*-DDE and/or total PCB only as in most previous studies. Laboratory analyses were carried out under strict quality control. Samples from cases were collected immediately after diagnosis and before surgery, when organochlorine concentrations could not possibly have been influenced by the treatment or by the disease itself. In Sweden most endometrial cancers are diagnosed in early stages when most women are readily cured by local treatment (Stenbeck et al., 1995).

Selection bias would have occurred only if non-participation was related differently to organochlorine concentrations among eligible cases and controls. Among cases, the

main reason for non-participation was the failure of the hospital staff to collect blood samples before surgery. Therefore, non-participation probably reflects mostly characteristics of the medical personnel, and not patients' characteristics. Differential participation according to organochlorine concentration among controls also seems unlikely.

We measured both original organochlorine products and major metabolites such as *p,p'*-DDE and oxychlordane. However, it is possible that we overlooked other meaningful exposures. Some non-persistent estrogenic DDT and PCB compounds cannot be detected in humans decades after exposure (vom Saal et al., 1998) and we did not measure a variety of other persistent compounds such as hydroxylated PCBs, polychlorinated dibenzo-para-dioxins, polychlorinated dibenzofurans, and non-ortho PCBs. Also, our grouping of substances is uncertain since there are relatively little animal or human data on hormonal activity of organochlorines. It is difficult to compare our findings with results from previous studies, because of differences in analytical procedures. However, after standardizing for lipid content in different tissues, the mean concentrations of organochlorines in our study were in the lower range of those previously reported for controls in North-American and European breast cancer studies, where sampling occurred in the late 1980s and early 1990s (average concentrations: 1020-2200 ng p,p'-DDE/g lipid, 350-1300 ng total PCB/g lipid) (3-6, 11-12, 17). The somewhat lower average exposure in our study (sampling 1996-97) is at least partially a reflection of the continuous decline in exposure in Europe and North America after the banning of the use of these compounds (Ahlborg et al., 1995; Lunden and Noren, 1998; Atuma et al., 1998; Hovinga et al., 1992; Ekbom et al., 1996).

It could be that exposure in our population was too low to cause biological effects, although endometrial cancer is known to be the most estrogen-sensitive malignancy in women. Also all compounds analyzed in our study had a substantial range of variation - one to two orders of magnitude. Yet no evidence of trend emerged in categorized or continuous analyses. It is reassuring that the previously published study on organochlorines and endometrial cancer (Sturgeon et al., 1998), also did not reveal any associations, even though median concentrations were higher than ours.

We studied women with no substantial use of menopause hormones among whom the effects of weakly estrogenic substances should be most apparent. Since we found no associations we conclude that the studied environmental contaminants do not cause endometrial cancer at the concentrations found in our population. These reassuring results are likely generalizable to other populations where similar levels of these contaminants are present in the environment.

Paper V. Estrogen receptor gene polymorphisms and endometrial cancer risk.

In the study reported here we found suggestive evidence of an association between ER gene polymorphisms and endometrial cancer risk. The P, X and long alleles seemed to confer decreased relative risks, especially when trends over the number of alleles were considered.

Our study has several strengths, including its population-based design, the relative ethnic homogeneity of the Swedish population, and the availability of detailed questionnaire information which allowed us to consider potential confounding factors.

One concern is that selection bias could have occurred if non-participation was related differently to genotype among eligible cases and controls. Among cases, the principal reason for non-participation was the failure of the hospital staff to collect blood samples, a reflection of the characteristics of the medical personnel, not of the patients. However, in our expanded study population, participation rates were higher among controls who had never used hormone replacement than among controls who used such hormones. This could have influenced our findings if ER polymorphisms were associated with use of hormone replacement. Therefore we analyzed our data in two ways – with and without subjects who used hormone replacement. We observed similar patterns of results in the two groups and conclude that a substantial distortion from selection bias is unlikely.

Misclassification of cancer cases may have occurred. In another recently-completed study in Sweden, we found that about 10% of cases reported as endometrial cancer to the cancer register were reclassified as severe atypical hyperplasia after blinded histopathological review. Atypical hyperplasia is considered a pre-malignant lesion, and its association with hormonal exposures are stronger than the associations observed for invasive endometrial lesions (Weiderpass et al., 1999). Therefore the possible inclusion of atypical hyperplasias in our study could have biased results towards stronger associations, if the polymorphisms are linked to increased sensitivity to estrogens. Our focus on women who never used hormone replacement therapy reduces this potential problem. Misclassification of genotypes is unlikely, since we obtained allele frequencies among controls which are similar to that reported by others (Del Senno et al., 1992).

The confidence limits for the ORs in most analysis of our data included unity and so the findings are consistent with chance. However, two of the trends in risk over alleles were statistically significant or nearly so. Clearly our suggestive finding of weak associations need confirmation by a study with greater statistical power to detect weak associations.

Estrogen mediates cellular growth and differentiation in tissues such as the endometrium, mammary gland, bone, cardiovascular system, brain, and urogenital tract in men and women (Kan et al., 1978; Clark et al., 1992; Turner et al., 1994; Farhat et al., 1996), with the intracellular ER functioning as a hormone-dependent transcriptional regulator (Beato et al., 1995). Polymorphisms in the ER have been studied mostly in relation to bone mass and mammary cancer. To our knowledge, no studies have previously been published on the relationship between ER Pvu II, Xba I, or TA repeat polymorphisms and endometrial cancer.

Reports on associations between the studied polymorphisms and bone mineral density are inconsistent. While in some studies no association between Pvu II or Xba I ER genotypes and body mass density were reported (Han et al., 1997; Gennari et al., 1998), other studies suggest an association between xx (Mizunuma et al., 1997) and PP genotypes (Ongphiphadhanakul et al., 1998) and low bone mass density. Since estrogen levels and bone mineral density are related and a high bone

mineral density is associated with an increased risk of breast cancer (Cauley et al., 1996; Zhang et al., 1997), we believe that any estrogenic effect of ER genotypes would also be detectable on the endometrium; i.e., through changes in the risk of endometrial cancer.

The Xba I x allele has been associated with an increased risk of breast cancer in a study from Norway (Anderson et al., 1994). However, the Pvu II restriction site polymorphism is apparently not associated with expression of ER in breast cancer (Parl et al., 1989; Yaich et al., 1992; McGuire et al., 1992), and was not related to breast cancer risk in two analyses that did not consider covariates such as hormone replacement therapy and reproductive history (Yaich et al., 1992; Andersen et al., 1994). The study by Parl et al. (1989) found pp genotype related to a younger age at breast cancer diagnosis.

The ER gene loci that we are studying are not in the coding domains of the gene. However these polymorphisms may be markers of altered cellular function in several other ways. Receptor function could be affected through differential splicing of mRNA (Dotzlaw et al., 1992; Fuqua et al., 1992), or alteration of transcriptional elements within introns (Roodi et al., 1995). Further, it is possible that some of these polymorphisms serve as markers by being in linkage disequilibrium with other, as yet undetected, sequence alterations of functional significance for the gene (Kan and Dozy, 1978).

In conclusion, our data provided suggestions that some women carry variants of the ER gene that may be associated with increased susceptibility to the disease.

CONCLUSIONS

- Postmenopausal women who used orally administered estriol present an increase in risk for developing endometrial cancer, compared to women who did not use this hormone regimen;

- Postmenopausal women who used conjugated estrogens or estradiol without addition of progestins have a marked, and rather persistent, duration- and dose-dependent increase in risk for developing endometrial cancer, compared to women who did not use these hormone regimens;

- Postmenopausal women who used combined hormone replacement regimens with added progestins for less than 16 days per cycle (predominantly 10 days) have an increased risk for developing endometrial cancer, compared to women who did not use these hormone regimens;

- Postmenopausal women who use estrogens combined with progestins during all days of the cycle have a reduced risk of developing endometrial cancer, compared to women who did not use these hormone regimens;

- Women who use combined oral contraceptives for at least 3 years have a reduced risk of developing endometrial cancer, compared to women who never used oral contraceptives. This protective effect increases with duration of use, and seems to persist for at least 20 years.

- Among women who were not exposed substantially to hormone replacement therapy there is no association between serum levels (at concentrations found presently in Sweden) of the organochlorine pesticides, pesticide metabolites or PCB congeners analyzed in our study (*p,p'*-DDT, *o,p'*-DDT, *p,p'*-DDE, *p,p'*-DDD, hexachlorobenzene, hexachlorocyclohexanes α, β, γ, oxychlordane, trans-nonachlor, and polychlorinated biphenyls CB 28, 52, 101, 105, 118, 138, 153, 156, 167, 180) and endometrial cancer risk, neither for individual compounds nor for groups of substances with different putative hormonal activity.

- There is suggestive evidence of a weak association between some of the estrogen receptor α gene polymorphisms and endometrial cancer risk. The absence of restriction sites for the enzymes Pvu II and Xba I in intron 1, and long alleles of a TA repeat upstream of the gene seem to confer decreased relative risks for endometrial cancer. However, the confidence limits for the relative risk estimates in most of our analyses included unity, and so the findings are consistent with chance.

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