POST-MENOPAUSAL OESTROGEN–PROGESTOGEN THERAPY

1. Exposure

Post-menopausal oestrogen–progestogen therapy involves administration of the oestrogens described in the monograph on 'Post-menopausal oestrogen therapy' accompanied by a progestogen or progesterone to women around the time of the menopause, primarily for the treatment of menopausal symptoms but also for the prevention of conditions that become more common after the menopause, such as osteoporosis and ischaemic heart disease. The progestogen can be administered orally or transdermally and either continuously or at various intervals. Intermittent progestogen administration causes withdrawal uterine bleeding, while continuous therapy generally does not. In 'peri-menopausal hormonal therapy', the components are not specified but are usually oestrogen with or without a progestogen. Annex 2 (Table 5) gives examples of brands of post-menopausal oestrogen–progestogen therapy. Progestogens that can be given in combination with the oestrogens are listed in Annex 1, with their constituents, doses, routes of administration and the names of some countries in which the brands are available; Annex 1 also gives the chemical formulae and some information on indications for use.

1.1 Historical overview

The earliest forms of hormones used for the treatment of ovarian failure or after oophorectomy were natural extracts of ovarian tissue, placenta and urine from pregnant women and thus contained both oestrogen and progestogen, as well as other substances. Crystalline progesterone was first identified in 1934, and shortly afterwards experimental treatment of women with injected oestrogen and progesterone began (Hirvonen, 1996). In the decades that followed, however, menopausal symptoms were treated mainly with oestrogen alone rather than with combined oestrogen–progestogen therapy.

Oral progesterone equivalents did not become readily available until the 1940s, when Russell Marker synthesized diosgenin from extracts of the Mexican yam. Further experimentation yielded the synthesis of norethisterone (norethindrone) by Carl Djerassi in 1950 and norethynodrel by Frank B. Colton in 1952. These compounds were named progestogens (or progestins) owing to their progesterone-like actions (Kleinman, 1990). They were ultimately used in combined oral contraceptives (see section 1 of the monograph on 'Oral contraceptives, combined' for details), developed in the late 1950s.

During the 1960s and early 1970s, most hormonal therapy was used in the United States (particularly in California) and took the form of post-menopausal oestrogen therapy,

without progestogen. At that time, some clinicians, especially those in Europe, prescribed oestrogen–progestogen therapy, primarily for better control of uterine bleeding during treatment, as post-menopausal oestrogen therapy sometimes causes irregular bleeding in women with a uterus (Maddison, 1973; Studd, 1976; Bush & Barrett-Connor, 1985). Figure 1 of the monograph on 'Post-menopausal oestrogen therapy' shows the estimated numbers of prescriptions of non-contraceptive progestogens and medroxyprogesterone acetate in the United States between 1966 and 1992.

Studies linking post-menopausal oestrogen therapy with increased rates of endometrial cancer were first published in 1975 (Ziel & Finkle, 1975). These led to a rapid decrease in prescription of such therapy in the United States and the recommendation by many clinicians and researchers that progestogen be added to oestrogen when treating post-menopausal women with an intact uterus, as this had been shown to attenuate the risk of endometrial cancer associated with the use of oestrogen alone (Bush & Barrett-Connor, 1985; Kennedy *et al*., 1985). In Europe, when post-menopausal hormonal therapy was indicated for women with an intact uterus, it became accepted practice to administer combined oestrogen–progestogen therapy; post-menopausal oestrogen therapy was still given to hysterectomized women. In the United States, some clinicians continued to prescribe post-menopausal oestrogen therapy to women with a uterus, following guidelines to monitor the endometrium (American College of Physicians, 1992), although increasing prescription of progestogens was noted after 1975 (see Figure 1 in the monograph on 'Post-menopausal oestrogen therapy'). In the United States in 1980, approximately 5% of the Premarin®, the commonest oestrogen sold, was accompanied by oral Provera®, the commonest progestogen, while in 1983 this figure had risen to 12% (Kennedy *et al*., 1985). In the United Kingdom, prescription of oestrogen–progestogen therapy increased throughout the late 1970s and early 1980s, until in 1984 almost equal amounts of oestrogen alone and oestrogen–progestogen therapy were used (Townsend, 1998).

The Women's Health Initiative trial of post-menopausal hormonal therapy was begun in the United States in 1992. In this trial, women with a uterus could be randomized to post-menopausal oestrogen therapy with monitoring of the endometrium, reflecting a proportion of clinical practice at the time (Finnegan *et al*., 1995). In 1995, the results of the Postmenopausal Estrogen/Progestin Interventions (PEPI) trial showed adenomatous or atypical endometrial hyperplasia in 34% of women receiving 0.625 mg unopposed conjugated equine oestrogens daily (Writing Group for the PEPI Trial, 1995). The protocol of the Women's Health Initiative trial was therefore amended so that women with a uterus could be randomized to receive only combined oestrogen–progestogen therapy or placebo (Finnegan *et al*., 1995). No figures on the prevalence of post-menopausal oestrogen–progestogen therapy after this publication are available, but it is expected that use will increase relative to that of post-menopausal oestrogen therapy.

1.2 Post-menopausal oestrogen–progestogen therapy preparations

The oestrogens used in post-menopausal oestrogen–progestogen therapy are described in the monograph on 'Post-menopausal oestrogen therapy' and in Annex 1; the proges-

togens used in oestrogen–progestogen therapy are derived from 17α-hydroxyprogesterone and 19-nortestosterone, although progesterone itself is sometimes used. Tibolone is a centrally acting compound with both oestrogenic and progestogenic actions. Of the 17α-hydroxyprogesterone derivatives, medroxyprogesterone acetate is the most widely used; dydrogesterone is also available. Of the 19-nortestosterone derivatives, norethisterone, norethisterone acetate, norgestrel and levonorgestrel are used in post-menopausal oestrogen–progestogen therapy. Progesterone is now administered orally in a micronized form but was given by injection in the past (British Medical Association, 1997).

In the commonest treatment regimen, the oestrogen component is taken daily orally or transdermally, usually at a constant dose, with a progestogen given for 10–14 days per month, causing withdrawal bleeding. A typical dose of progestogen is 5–10 mg medroxyprogesterone acetate orally, daily for 10–14 days. Preparations are also available in which the progestogen is given every three months, causing quarterly bleeding. Another widely used regimen is a constant dose of oestrogen taken daily continuously, accompanied by continuous progestogen. A typical continuous progestogen dose would be about 2.5 mg medroxyprogesterone acetate orally per day (British Medical Association, 1997). The continuous progestogen can also be given transdermally as 0.25 mg norethisterone acetate per 24 h; this regimen usually does not result in withdrawal bleeding (Cameron *et al*., 1997). Tibolone is given orally, continuously and does not usually result in bleeding, except if treatment is started within 12 months of the woman's last menstrual period (British Medical Association, 1997).

In some countries, primarily in Europe, a progestogen is sometimes given alone for the treatment of menopausal symptoms. A progestogen can also be given in the form of a levonorgestrel-releasing intrauterine device, accompanying oral or transdermal oestrogen, to deliver the progestogen directly to the endometrium (British Medical Association, 1997). This system is not widely licensed for use as post-menopausal oestrogen–progestogen therapy.

1.2.1 *Patterns of use*

Like post-menopausal oestrogen therapy, combined oestrogen–progestogen therapy is started around the time of the menopause and can be used for both short- and long-term treatment. Table 1 in the monograph on 'Post-menopausal oestrogen therapy' shows the prevalence of current and any use of post-menopausal hormonal therapy in selected studies internationally, with post-menopausal oestrogen therapy and oestrogen–progestogen therapy use shown in the studies in which they were reported separately; very few studies mentioned use of post-menopausal oestrogen–progestogen therapy. In the United States, post-menopausal oestrogen–progestogen therapy was being used currently by 1–5% of women aged 45–64 and had ever been used by 14% of a nationally representative sample of post-menopausal women aged 25–76 in the late 1980s. In Denmark, 12% of 40–59-year-old and 17% of 51-year-old women had ever used post-menopausal oestrogen–progestogen therapy in 1983 and 1987, respectively. In Sweden, combined oestradiol–progestogen use (usually with levonorgestrel or norethisterone acetate) became popular in the

early 1970s and is now standard practice. Thus, the increase in the sales of replacement hormones in Sweden since the early 1990s almost entirely entails progestogen-combined regimens. In England, prescription data showed that an estimated 1% of women aged 40–64 used post-menopausal oestrogen–progestogen therapy in 1989, compared with an estimated 11% of women in 1994. About 1% of women in this age group were using tibolone in 1994 (Townsend, 1998).

In a study of general medical practices in the United Kingdom in 1993, 96% of women with a hysterectomy who were taking post-menopausal hormonal therapy were taking oestrogen alone, and 96% of women who had not undergone hysterectomy were taking combinations of oestrogen and progestogen (Lancaster *et al*., 1995).

2. Studies of Cancer in Humans

2.1 Breast cancer

The progesterone present during natural cycles and the progestogens added in hormonal therapy may be important in cancer etiology (Stanford & Thomas, 1993). Some epidemiological data suggest that short menstrual cycles or having many regular cycles during a life-time, reflecting exposure to progesterone, may have an adverse effect on the risk for breast cancer (Kelsey *et al*., 1993). Several studies (Anderson *et al*., 1982; Longacre & Bartow, 1986; Going *et al*., 1988; Potten *et al*., 1988; Anderson *et al*., 1989), but not all (Vogel *et al*., 1981), indicate that progesterone in natural cycles and exogenous progestogens in the cycles of users of combined oral contraceptives augment proliferative activity in breast epithelial cells (Key & Pike, 1988). Furthermore, progesterone probably down-regulates oestrogen receptors but maintains the numbers of progesterone receptors in natural cycles (Söderqvist *et al*., 1993). As increased proliferation may cause neoplastic cell transformation (Preston-Martin *et al*., 1990), progestogens in treatment regimens may further enhance the risk for breast cancer.

The use of added progestogens to control menstrual bleeding and to prevent development of hyper- and neoplasia of the endometrium in women with an intact uterus has increased markedly since the 1970s, when reports of an increased risk for endometrial cancer after unopposed oestrogen therapy were first published. Both use and the number of progestogen compounds (progesterone- or testosterone-derived) and treatment schedules (cyclical, sequential, long cycle and continuous) have surged. For these reasons, the effects of progestogen combinations on the risk for breast cancer is an important topic in epidemiological research; however, epidemiological data on the effects of oestrogen plus progestogen treatment are rather scarce, especially for long-term use. Some data on the risks associated with combined use are available in nine cohort studies and five case–control studies. The Collaborative Group on Hormonal Factors in Breast Cancer (1997) pooled and re-analysed individual data on such use from some of these and other studies (see section 2.1.3).

2.1.1 *Cohort studies*

The cohort studies on use of post-menopausal oestrogen–progestogen therapy and breast cancer are summarized in Table 1.

Hunt *et al.* (1987) reported the results of the surveillance of a cohort of 4544 women recruited at 21 menopause clinics in Great Britain, all of whom had been placed on hormones and 43% with a variety of combined progestogens for an average of 67 months. The incidence of breast cancer was ascertained through several sources, including mailed questionnaires, morbidity registers and hospital notes. On the basis of about 20 000 person–years of observation and 50 observed cases, a standardized incidence ratio (SIR) of 1.6 (95% confidence interval [CI], 1.2–2.1) was calculated for any use. Analyses of use of oestrogens only or oestrogens plus progestogens, by classifying the different regimens, did not produce any interpretable results. A trend of increasing risk with increasing time since first use (SIR, 3.1; 95% CI, 1.5–5.6 after 10 years or longer) was found for all types of treatment.

In the cohort study of Bergkvist *et al.* (1989), over 23 000 women were recruited by analysing registered prescriptions for various types of hormonal treatment dispensed in six counties in central Sweden. These women were followed-up by record-linkage with the National Cancer Registry. Individual data on exposure and risk factors were obtained from the accumulated prescriptions and from questionnaires sent to a sample of the cohort and all 253 women with newly diagnosed cases of breast cancer. In cohort and nested case–control analyses, exposure to oestrogens alone for nine years or longer was associated with a relative risk of 1.8 (95% CI, 1.0–3.1); for exposure to oestradiol combined with levonorgestrel, the relative risk was 4.4 (based on 10 cases only) after use for more than six years. In women with mixed intakes of oestrogens only and oestrogens plus progestogens exceeding six years, the relative risk estimates varied between 1.2 and 7.2 (not significant).

This cohort was also followed-up for death from breast cancer by linkage to a population-based mortality registry (Yuen *et al.*, 1993). On the basis of prescription data collected during 1977–80 and corrected external mortality rates (calculated from newly diagnosed cases of breast cancer only), an overall standardized mortality ratio (SMR) of 0.8 (95% CI, 0.2–1.1) emerged. When only those women to whom an oestradiol–levonorgestrel combination had been prescribed were included, the SMR was similarly close to baseline, 0.8 (95% CI, 0.4–1.3). In the same study, Persson *et al.* (1996) conducted a 13-year record-linkage follow-up, yielding 634 new cases of breast cancer. Any use of an oestradiol–levonorgestrel combination conferred a slightly increased relative risk (1.3; 95% CI, 1.1–1.4), whereas women receiving oestradiol or conjugated oestrogens only had no alteration of their risk (RR, 0.9; 95% CI, 0.8–1.1).

The results of the Nurses' Health Study were reported on at least two occasions by Colditz *et al.* (1992, 1995). Data on exposure and risk factors were obtained from a baseline questionnaire in 1976 which was administered every two years for up-dates and ascertainment of breast cancer outcome. In the latest report (1995), the cohort of over 121 000 women had been followed-up for 16 years, resulting in over 700 000 person–years of observation and 1935 cases of breast cancer. The results were similar to those reported

Table 1. Summary of cohort studies on post-menopausal oestrogen-progestogen therapy and breast cancer

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Hunt *et al.* (1987)	Menopause clinics in the UK, 1978–82, counsel for menopausal symptoms	Cohort of 4 544 women who used hormones ≥ 1 year; 17 830 person–years; follow-up through contact letters and medical record data; exposure data through baseline interview	**Incidence**: 50 cases Any use of hormones: incidence: SIR, 1.6 (95% CI, 1.2–2.1) Time since first intake: trend for incidence ≥ 10 years: SIR, 3.1 (95% CI, 1.5–5.6) 43% of all treatment episodes combined with progestogens, analyses by subcategories not feasible	Possible selection bias in study of mortality Heterogeneous exposure regimens with regard to progestogens
Bergkvist *et al.* (1989)	Six counties in Sweden, 1977–83; women given hormonal therapy; age, ≥ 35 years	Population-based cohort; 23 244 women; 133 375 person–years; follow-up through record linkage with National Cancer Registry; exposure data from prescriptions; questionnaire data in a random sample; cohort, case–cohort and case–control analyses	**Incidence**: 253 cases Regimens, duration ≥ 9 years: oestrogens alone: RR, 1.8 (95% CI, 1.0–3.1) oestrogen and progestogens > 6 years: RR, 4.4 (95% CI, 0.9–22)	Low power to examine long-term progestogen combined regimens
Colditz *et al.* (1992)	Nurses' Health Cohort, USA, 1976–88, 30–55 years at entry	Cohort, 118 300 nurses at post-menopausal ages; 480 665 person–years; individual follow-up through questionnaires, 95% complete for incidence and 98% for deaths; internal comparisons; baseline questionnaire, 1976; up-dated questionnaires every 2 years	**Incidence**: 12 years' follow-up, 1 050 cases Post-menopausal hormones: any use: RR,1.1 (95% CI, 1.0–1.2) current use: RR, 1.3 (1.1–1.5) Oestrogen and progestogen, current intake: RR, 1.5 (95% CI, 1.0–2.4)	Main relationship with current intake
Yuen *et al.* (1993)	Uppsala health care region, Sweden, 1977–80; women given hormonal therapy	See Bergkvist *et al.* above; follow-up through record linkage with Causes of Death Registry; comparison with external, *corrected* mortality rates; exposure data from prescriptions	**Mortality**, 12 years' follow-up, 73 deaths Any use: oestradiol and progestogen: SMR, 0.8 (95% CI, 0.4–1.3)	Exposure only from prescription data (population rates corrected for prevalent cases)

Table 1 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Schairer *et al*. (1994)	Populations in 27 cities, USA, breast cancer screening, 1980–89	Cohort of 49 017 participants; 313 902 person–years; follow-up through interviews and questionnaires; information on exposure and risk factors from questionnaires	**Incidence**, both in-situ and invasive tumours, 1 185 cases Conjugated oestrogens, combinations with progestogens All tumours: any use: oestrogens and progestogens: RR, 1.2 (95% CI, 1.0–1.6) In-situ tumours: oestrogens and progestogens: any use: RR, 2.3 (95% CI, 1.3–3.9) current use: RR, 2.4 (95% CI, 1.2–4.7) past use: RR, 2.3 (95% CI, 1.0–5.4)	Risk relationship limited to in-situ tumours Low power to assess long-term duration of oestrogen and progestogen regimens
Risch & Howe (1994)	Inhabitants of Saskatchewan, Canada, 43–49 years of age, 1976–91	Registry-based cohort; 33 003 women followed-up through linkage to cancer registry; 448 716 person–years; exposure data from prescription roster	Incidence: 15 years' follow-up, 742 cases Conjugated oestrogens, added progestogens Oestrogens and progestogens: no significant risk increase	Limited power to study long-term oestrogen–progestogen combined treatment
Colditz *et al*. (1995)	Nurses' Health Cohort, see Colditz *et al*. (1992) above	Cohort of 121 700 nurses; 725 550 person–years; baseline questionnaire in 1976, questionnaires every two years, up-dates on exposure and outcome (follow-up)	Incidence: 16 years' follow-up, 1 935 cases Conjugated oestrogens and added progestogens Current intake: conjugated oestrogen: RR, 1.3 (95% CI, 1.1–1.5) Any use: oestrogen and progestogen: RR, 1.4 (95% CI, 1.2–1.7)	No relationship with past use Detection bias unlikely First study to show an increased risk for death with hormonal therapy
Persson *et al*. (1996)	See Yuen *et al*. (1993) above, Swedish cohort	22 597 women with registered hormone prescriptions; record linkage follow-up of incidence and mortality; risk factor data in questionnaire survey	Incidence, 13 years' follow-up, 634 cases Prescriptions for various regimens any use: oestradiol/levonorgestrel: RR, 1.3 (95% CI, 1.1–1.4)	No data on duration

Table 1 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Persson *et al*. (1997)	Participants in mammography screening, Uppsala, Sweden, 1990–92, 40–74 years	Cohort of 30 982 women participating in two screening rounds; follow-up through screening and in diagnostic registry of pathology department; questionnaires at visits; nested case–control approach.	Follow-up through June 1995: 435 cases (87% invasive), 1 740 controls any use: all compounds: odds ratio, 1.1 (95% CI, 0.8–1.4) Duration ≥ 11 years: Oestradiol and progestogen: odds ratio, 2.4 (95% CI, 0.7–8.6) Oestradiol–conjugated oestrogen alone: odds ratio, 1.3 (95% CI, 0.5–3.7)	Low power in regimen subgroups

SIR, standardized incidence ratio; SMR, standardized mortality ratio

earlier, showing increased risks for current intake of conjugated oestrogens alone (relative risk, 1.3; 95% CI, 1.1–1.5) and for combinations with progestogens (chiefly medroxyprogesterone acetate; relative risk, 1.4; 95% CI, 1.2–1.7). Owing to the low statistical power, no data were presented on the risk for use of combinations with progestogens by categories of duration.

Schairer *et al.* (1994) followed a cohort of some 49 000 women participating in a breast cancer screening programme in several cities in the United States. To ascertain data on their exposure, risk factors and occurrence of breast cancer, the women were sent questionnaires or were interviewed. After a mean follow-up of 7.2 years, 1185 cases of in-situ or invasive breast cancer had occurred. For both types of tumour together, any use of oestrogens plus progestogens yielded a relative risk of 1.2 (95% CI, 1.0–1.6); for in-situ tumours only, a significant excess risk was noted (2.3; 95% CI, 1.3–3.9). The risk estimates were 2.4 (95% CI, 1.2–4.7) for current use and 2.3 (95% CI, 1.0–5.4) for past use.

Risch and Howe (1994) used a prescription database to establish a cohort of some 33 000 women in the province of Saskatchewan, Canada, who had received hormonal treatment. Through linkage with the population-based cancer registry, 742 newly diagnosed cases of breast cancer were ascertained during 15 years of follow-up. Use of oestrogens plus progestogens was not associated with a significant change in the risk for breast cancer. In this study, the power to show any effects of combined use was low, with only three exposed cases.

Persson *et al.* (1997) investigated breast cancer incidence in relation to hormonal treatment in a cohort of some 31 000 women who had participated in mammography screening on two regular visits. Data on their exposure to hormones and reproductive factors were collected through interviews at the visits. In all, 435 new cases of breast cancer were ascertained during five years of follow-up, chiefly through mammography screening but also through linkage to a local pathology register; 87% of the cases were invasive. In a nested case–control study, use of oestradiol plus a progestogen (usually norethisterone acetate) for 11 years or longer was associated with a relative risk of 2.4 (95% CI, 0.7–8.6), whereas use of oestradiol alone was associated with a relative risk of 1.3 (95% CI, 0.5–3.7). The risk for combined long-term use seemed to be greater than for other use, but the difference in risk estimates between the two treatment types was not statistically significant.

2.1.2 *Case–control studies*

The results of case–control studies on use of post-menopausal oestrogen–progestogen therapy and breast cancer are shown in Table 2.

In a case–control study in Denmark of 1486 cases and 1336 controls, Ewertz (1988) had the opportunity to examine the effects of various treatment regimens. Data were collected through mailed questionnaires, filled in by 88% of the cases and 78% of the controls, with details on reproductive factors and hormone use. Women who had ever used oestradiol–progestogen combined treatments had a non-significantly increased relative risk of 1.4 (95% CI, 0.9–2.1) compared with a relative risk of 1.0 (95% CI, 0.8–1.3) for use of oestrogen only. Analyses by duration were not possible owing to the small numbers.

Table 2. Summary of case–control studies of post-menopausal oestrogen-progestogen therapy and breast cancer

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Ewertz (1988)	Denmark, 1983–84, > 70 years	Population-based, national. 1 486 cases/1 336 controls (random); self-administered, mailed questionnaire	Oestradiol and oestradiol–progestogen combinations: Combination oestradiol–progestogens any use: RR, 1.4 (95% CI, 0.9–2.1)	First study to show similar risk with combined treatments
Kaufman *et al*. (1991)	Metropolitan areas, primarily eastern USA, 1980–86, post-menopausal women, 40–69 years	Hospital-based; 1 686 cases/ 2 077 controls; interviews	Oestrogens and progestogens, any use: RR, 1.7 (95% CI, 0.9–3.6)	Low power for long-term treatment with oestrogen-progestogen combinations
Yang *et al*. (1992)	British Columbia, Canada, 1988–89, post-menopausal women, < 75 years	Population-based; 699 cases/685 controls (random); mailed questionnaire	Mainly conjugated oestrogens: any use: oestrogens and progestogens: RR, 1.2 (95% CI, 0.6–2.2)	Low power in analyses of use of oestrogen plus progestogen
Stanford *et al*. (1995)	13 counties, Washington State, USA, 1998–90, cancer survey system, white women, 50–64 years	Population-based; 537 cases/492 controls (random-digit dialling); personal interviews	Conjugated oestrogens, added progestogens: Combined, any use: odds ratio, 0.9 (95% CI, 0.7–1.3) Duration ≥ 8 years: odds ratio, 0.4 (95% CI, 0.2–1.0)	Low power for long-term use
Newcomb *et al*. (1995)	Four states in northern/eastern USA, tumour registries, 1988–91, age < 75	Population-based; 3 130 cases/ 3 698 controls (from rosters); personal interviews	'Non-contraceptive hormones, oestrogens and progestogen combinations': any use of progestogen in combination: RR, 1.0 (95% CI, 0.8–1.3) Duration ≥ 15 years: RR, 1.1 (95% CI, 0.5–2.3) No trend with timing	Limited power for long-duration categories No effects in sub-groups

In a study by Kaufman *et al.* (1991), 1686 case and 2077 hospital-based control women were interviewed. Only 1% of the controls had used oestrogen–progestogen combinations. Women who had ever used such combined treatments showed an elevated relative risk, but with wide confidence limits (relative risk, 1.7; 95% CI, 0.9–3.6). Use of oestrogen only, at any time or for several years, was not associated with an excess risk.

Yang *et al.* (1992) examined the effects of conjugated oestrogens and combinations with progestogens in a population-based study of 699 cases and 685 controls. Data on exposure and risk factors were acquired from mailed questionnaires. Use of oestrogens plus progestogens, reported by 3% of the controls, was linked to a risk near the baseline (1.2; 95% CI, 0.6–2.2).

Stanford *et al.* (1995) conducted a population-based study on 537 cases of breast cancer and 492 controls in Washington State, United States, using random-digit dialling to recruit controls. Data from personal interviews revealed that 21% of the controls had used combined treatments. The relative risk was 0.9 (95% CI, 0.7–1.3) for any use and 0.4 (95% CI, 0.2–1.0) for use for eight years and more.

Newcomb *et al.* (1995) presented data on combined use from the largest population-based study of breast cancer hitherto reported, 3130 cases and 3628 controls, from four states in northern and eastern United States. Data obtained at interview showed that about 4% of the healthy controls had used combined progestogen treatment, but few of these women had had long-term treatment. Any use of progestogen combinations was associated with a baseline risk (1.0; 95% CI, 0.8–1.3), whereas use for 15 years or longer (based on 15 cases and 15 controls) gave a relative risk of 1.1 (95% CI, 0.5–2.3). There was no indication of a trend with categories of duration of intake.

2.1.3 *Pooled analysis of individual data*

The Collaborative Group on Hormonal Factors in Breast Cancer (1997) compiled and re-analysed the original data from 51 studies, of which 22 provided data on the hormonal constituents of the preparations. Of the eligible women in the re-analysis, such data were available for 4640, 12% of whom had received combinations of oestrogens and progestogens. Current use or last use 1–4 years before diagnosis, with a duration of less than five years, was associated with a relative risk of 1.2 (95% CI, 0.8–1.5), and a duration of five years or longer with a relative risk of 1.5 (95% CI, 0.9–2.2). These estimates were not statistically different from those for the corresponding categories of oestrogen-only use (RR, 1.3).

These limited data do not provide a basis for firm conclusions on the effects of oestrogen–progestogen use on the risk for breast cancer. One major limitation is the small amount of information available on use for many years. Overall, there is little evidence to suggest that added progestogens confer a risk different from that associated with oestrogens alone.

2.2 Endometrial cancer

Women who use oestrogen–progestogen regimens have a lower occurrence of endometrial hyperplasia than those who use oestrogen-only therapy, especially when the progestogen is used for 10 or more days per month or continuously with oestrogen (Sturdee *et al.*, 1978; Thom *et al.*, 1979; Paterson *et al.*, 1980; Postmenopausal Estrogen/Progestin Interventions Trial, 1996; Speroff *et al.*, 1996). Depending on the type, dose and duration of progestogen supplementation, it may be given for 10–14 days once every three months as well, in a so-called 'long cycle' regimen. In two studies in which women were followed-up for one to two years while receiving a 14-day supplementation with 10 or 20 mg medroxyprogesterone acetate, less than 2% developed hyperplasia (Ettinger *et al.*, 1994; Hirvonen *et al*., 1995). In contrast, in a Scandinavian randomized controlled trial, a higher occurrence of hyperplasia was found in women who received a 10-day supplementation of 1 mg norethisterone (6%) than in women who received monthly progestogen supplementation (< 1%) (Cerin *et al*., 1996). Although information is available on endometrial hyperplasia, there is much less information on combined oestrogen–progestogen therapy and the risk for endometrial cancer.

2.2.1 *Randomized trial*

In a very small randomized trial in which 168 institutionalized women were randomized to receive post-menopausal oestrogen–progestogen therapy or placebo, no case of endometrial cancer occurred in the treated group and one occurred in those receiving placebo (Nachtigall *et al.*, 1979).

2.2.2 *Cohort studies*

Only three cohort studies have provided information on the risk for endometrial cancer among women who used combined therapy relative to women who did not use any post-menopausal hormonal therapy.

Hammond *et al.* (1979) (see Table 5 of the monograph on 'Post-menopausal oestrogen therapy') followed-up approximately 600 hyperoestrogenic women, roughly half of whom used either oestrogen-only or oestrogen–progestogen preparations and half of whom did not use hormones. No cases of endometrial cancer were observed among the 72 women who received oestrogen–progestogen therapy, whereas three cases were observed among the non-users.

In the cohort study of Gambrell (1986) (summarized in Table 5 of the monograph on 'Post-menopausal oestrogen therapy'), the incidence of endometrial cancer among women who used combined hormonal therapy (eight cases/16 327 woman–years) was lower than that among women who did not use any hormonal therapy (nine cases/4480 woman–years) [no age-adjusted results were reported].

A Swedish cohort study (Persson *et al.*, 1989) (see Table 5 of the monograph on 'Post-menopausal oestrogen therapy') of endometrial cancer occurrence among women using combination therapy identified through pharmacy records in comparison with the population rates in the Uppsala health care region, showed that women using oestrogen-only

therapy (predominantly oestradiol) had an increased risk (48 cases observed versus 34.3 expected; relative risk, 1.4; 95% CI, 1.1–1.9), whereas no increase in risk was found in women using combination therapy (seven cases observed versus 7.6 expected; relative risk, 0.9; 95% CI, 0.4–2.0).

2.2.3 *Case–control studies*

The case–control studies on post-menopausal oestrogen–progestogen therapy and endometrial cancer risk are summarized in Table 3.

Jick *et al.* (1993) studied women who were members of a large health maintenance organization in western Washington State, United States. Women with endometrial cancer were identified from the tumour registry of the organization, and the control women were other members; both groups included only those women who used the pharmacies of the organization, and who had previously completed a questionnaire sent to all female members for a study of mammography. Use of post-menopausal hormonal therapy was ascertained from the pharmacy database. Relative to women who had never or briefly (≤ six months) used menopausal hormones, those who had used any oestrogen–progestogen therapy within the previous year had a slightly increased risk (odds ratio, 1.9; 95% CI, 0.9–3.8), after adjustment for age, calendar year, age at menopause, body mass index and history of oral contraceptive use, although those with a longer duration of use (≥ three years) did not. Former users (last use ≥ one year earlier) had no increase in risk (odds ratio, 0.9; 95% CI, 0.3–3.4). Women with recent (within the past year) oestrogen-only use (32 cases and 26 controls) had a strongly elevated risk for endometrial cancer (odds ratio, 6.5; 95% CI, 3.1–13), but no increased risk was seen for past users of oestrogen alone (odds ratio, 1.0; 95% CI, 0.5–2.0).

A multicentre study was conducted with 300 menopausal women with endometrial cancer diagnosed at seven hospitals, in Chicago, Illinois; Hershey, Pennsylvania; Irvine and Long Beach, California; Minneapolis, Minnesota; and Winston-Salem, North Carolina, United States, and 207 age-, race- and residence-matched control women from the general population (Brinton *et al*., 1993). Any oestrogen–progestogen therapy for three months or longer was reported by 4% of the case women and 5% of the control women (odds ratio, 1.8; 95% CI, 0.3–0.7), after adjustment for age, parity, weight and years of oral contraceptive use. Use of oestrogens only was associated with a relative risk of 3.4 (95% CI, 1.8–6.3).

Pike *et al.* (1997) identified 833 women with endometrial cancer from a population-based cancer registry in Los Angeles County, United States, and matched them to control women of similar age and race (white) who lived in the same neighbourhood block as the matched case or to 791 randomly identifed women on the United States Health Care Financing Administration computer tapes. The risk for endometrial cancer was investigated among women who had used unopposed oestrogens, oestrogen–progestogen with progestogen added for fewer than 10 days per cycle, oestrogen–progestogen with progestogen added for 10 or more days per cycle and continuous combined therapy. An elevated risk was noted for women with longer use of oestrogen–progestogen if the progestogen

Table 3. Summary of case–control studies of post-menopausal oestrogen–progestogen therapy and endometrial cancer risk, by number of days progestogen was added per cycle and duration, when available

Reference	Location; period	Age (years)	Source of controls	Participation (%)		Type/measure of combined therapy	No. of subjects		Adjusted odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Jick *et al.* (1993)	Washington; USA; 1979–89	50–64	Members of health maintenance organization	NR	NR	No use, ≤ 6 months' use	97	606	Referent
						Any use within past year[a]	18	83	1.9 (0.9–3.8)
						Duration (years)			
						< 3	NR	NR	2.2 (0.7–7.3)
						≥ 3	NR	NR	1.3 (0.5–3.4)
						Any use ≥ 1 year previously	6	64	0.9 (0.3–3.4)
Brinton *et al.* (1993)	Five US areas; 1987–90	20–74	General population	86	66	No use	222	176	Referent
						Any use for ≥ 3 months[a]	11	9	1.8 (0.6–4.9)
Pike *et al.* (1997)	California, USA; 1987–93	50–74	General population (neighbours)	57	NR	*Any use, progestogen < 10 days/cycle*[b]			
						Duration (months)			
						0	759	744	Referent
						1–24	35	22	1.4 (NR)
						25–60	12	12	1.5 (NR)
						> 60	27	13	3.5 (NR)
						Any use, progestogen ≥ 10 days/cycle			
						Duration (months)			
						0	754	703	Referent
						1–24	37	30	1.0 (NR)
						25–60	19	25	0.7 (NR)
						> 60	23	33	1.1 (NR)
						Any use, progestogen all days/cycle			
						Duration (months)			
						0	739	710	Referent
						1–24	45	41	1.1 (NR)
						25–60	25	15	1.4 (NR)
						> 60	24	25	1.3 (NR)

Table 3 (contd)

Reference	Location; period	Age (years)	Source of controls	Participation (%)		Type/measure of combined therapy	No. of subjects		Adjusted odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Beresford *et al.* (1997)	Washington, USA; 1985–91	45–74	General population	72	73	No use, ≤ 6 months' use	337	685	Referent
						Any use[c]	67	134	1.4 (1.0–1.9)
						Progestogen ≤ 10 days/cycle			
						Duration (months)			
						6–35	12	14	2.1 (0.9–4.7)
						36–59	3	7	1.4 (0.3–5.4)
						≥ 60	15	12	3.7 (1.7–8.2)
						Progestogen > 10 days/cycle			
						Duration (months)			
						6–35	10	31	0.8 (0.4–1.8)
						36–59	5	23	0.6 (0.2–1.6)
						≥ 60	12	16	2.5 (1.1–5.5)
						Current use only			
						Progestogen ≤ 10 days/cycle			
						Duration (months)			
						6–59	11	13	2.2 (0.9–5.2)
						≥ 60	14	9	4.8 (2.0–11)
						Progestogen > 10 days/cycle			
						Duration (months)			
						6–59	12	48	0.7 (0.4–1.4)
						≥ 60	12	15	2.7 (1.2–6.0)

CI, confidence interval; NR, not reported

[a] Women with unopposed oestrogen use included

[b] Use of unopposed oestrogen and other combined therapy adjusted for in the analysis

[c] Women with unopposed oestrogen use excluded

was added for fewer than 10 days per cycle, but not if it was added for 10 or more days per cycle; the odds ratio was 1.9 (95% CI, 1.3–2.7) for each additional five years of use and 1.1 (95% CI, 0.8–1.4) after adjustment for age at menarche, time to regular cycles, parity, duration of incomplete pregnancies, weight, duration of breast-feeding, amenorrhoea, smoking, duration of oral contraceptive use, age at menopause and the other hormonal treatments. No increase in risk was noted for women who had had continuous combined therapy; the odds ratio for each additional five years of use was 1.1 (95% CI, 0.8–1.4). Each additional five years of unopposed oestrogen use was associated with a roughly twofold elevation in risk, with an odds ratio of 2.1 for each five-year period (95% CI, 1.9–2.5).

Beresford *et al.* (1997) expanded the study population originally investigated by Voigt *et al.* (1991) and evaluated the risk for endometrial cancer among women who had used only oestrogen–progestogen therapy. Women with endometrial cancer were identified from a population-based cancer registry and compared with control women from the general population in western Washington State, United States. After some exclusions, 394 cases and 788 controls were available for the analysis. Relative to women who had never or briefly (≤ six months) used menopausal hormones, women who had used only oestrogen–progestogen therapy had a slightly increased risk (odds ratio, 1.4; 95% CI, 1.0–1.9), after adjustment for age, body mass and county of residence. An elevated risk was noted among women with five or more years of exclusive oestrogen–progestogen use, regardless of the number of days a progestogen had been used. For women using oestrogen–progestogen therapy for ≤ 10 days/cycle, the odds ratio was 3.7 (95% CI, 1.7–8.2); the risk was similar for use on more than 10 days/cycle (odds ratio, 2.5; 95% CI, 1.1–5.5), and similar results were found when the analysis was restricted to current oestrogen–progestogen users. The small numbers of women (eight cases and 11 controls) who had used only oestrogen–progestogen therapy for five or more years, with the highest dose of medroxyprogesterone acetate (10 mg) added for more than 10 days/cycle, still had a significant increase in risk (odds ratio, 2.7; 95% CI, 1.0–6.8). Unopposed oestrogen therapy taken for at least six months was associated with a relative risk for endometrial cancer of 4.0 (95% CI, 3.1–5.1).

In summary, most of the small number of epidemiological studies conducted to date have shown no effect or a modest increase in the risk for endometrial cancer among women using combined hormonal therapy relative to women who had not used menopausal hormones. In all of the studies summarized above, a lower risk for endometrial cancer was associated with use of combined hormonal therapy than with oestrogen-only therapy. The two studies that were large enough to evaluate cyclic use of progestogen reported two- to fourfold increases in risk associated with use of oestrogen–progestogen if the progestogen was added for approximately 10 days or less per cycle (Beresford *et al*., 1997; Pike *et al*., 1997), but only one found an elevated risk if the progestogen was added for 10 days or more per cycle (Beresford *et al*., 1997). It is not clear whether the few cancers appearing in women taking oestrogens and progestogens represent failure of the progestogen to protect the endometrium or failure of the women to take the prescribed progestogen.

2.3 Ovarian cancer

2.3.1 *Cohort study*

In the cohort study of Hunt *et al*. (1987) described in section 2.1.1, six cases of ovarian cancer were observed up to 1986 among users of post-menopausal hormonal therapy versus 6.92 expected, corresponding to a nonsignificant SIR of 0.9 (95% CI, 0.3–1.9). In a follow-up until 1988 on mortality only (Hunt *et al.*, 1990), four more deaths from ovarian cancer were observed versus 6.33 expected (SMR, 0.63; 95% CI, 0.0–1.4).

2.3.2 *Case–control study*

In a multicentre case–control study of 377 cases of ovarian cancer and 2030 controls conducted between 1976 and 1985 in various areas of Canada, Israel and the United States (Kaufman *et al.*, 1989), only 1–2% of cases and controls had ever used combination post-menopausal therapy. The multivariate relative risk was 0.7 (95% CI, 0.2–1.8).

2.4 Liver cancer

Persson *et al.* (1996) studied cancer risks after post-menopausal hormonal therapy in a population-based cohort of 22 579 women aged 35 or more living in the Uppsala health care region in Sweden. Women who had ever received a prescription for post-menopausal hormonal therapy between 1977 and 1980 were identified and followed until 1991; information on hormone use was obtained from pharmacy records. The expected numbers of cases were calculated from national incidence rates. There was no information on smoking or alcohol consumption. The SIR for all cancers was 1.0 (95% CI, 0.9–1.0). There were 43 cancers of the hepatobiliary tract, comprising 14 hepatocellular carcinomas, five cholangiocarcinomas, 23 gall-bladder cancers and one unclassified. The expected number was 73.2, giving an SIR of 0.6 (95% CI, 0.4–0.8) for any type of post-menopausal hormonal therapy. The SIRs for treatment with oestradiol combined with levonorgestrel were 0.6 (95% CI, 0.1–2.3) for hepatocellular carcinoma and 0.7 (95% CI, 0.0–3.8) for cholangiocarcinoma. There was no information on infection with hepatitis viruses.

2.5 Colorectal cancer

In most of the studies on the influence of post-menopausal hormonal therapy on the risk for colorectal cancer, it was not possible to distinguish formulations. Only a few investigations provided separate information on combinations of oestrogens and progestogens, but in all instances the use of opposed hormonal therapy was very limited and was generally restricted to recent use. The available studies are summarized in Table 4.

2.5.1 *Cohort studies*

In a Canadian record linkage study described in detail in the monograph on ‘Post-menopausal oestrogen therapy’ (Risch & Howe, 1995), no case of colon or rectal cancer occurred in women who had used both oestrogens and progestogens and not oestrogens only; however, the number of such women (171) was small. One case of colon cancer

Table 4. Studies on use of post-menopausal oestrogen–progestogen therapy and colorectal cancer

Reference	Country	Population (follow-up) or cases/controls	Relative risk (RR) (95% confidence interval [CI]) (any versus no use)		Adjustment/comments
			Colon	Rectum	
Cohort					
Risch & Howe (1995)	Canada	33 003 (14 years) 230 cancers	–	–	Age No case of colorectal cancer among 171 hormone users
Persson *et al*. (1996)	Sweden	23 244 (13 years) 233 cancers, 62 deaths	0.6 (0.4–1.0)	0.8 (0.4–1.3)	Age No effect among 5 573 hormone users Refers to a fixed combined brand. RR for colon cancer mortality, 0.6 (95% CI, 0.2–1.1)
Troisi *et al*. (1997)	USA	33 779 (7.7 years) 313	1.4 (0.7–2.5)		Age (but unaltered by education, body mass index, parity or use of combined oral contraceptives)
Case–control					
Newcomb & Storer (1995)	Wisconsin, USA	694/1 622	Recent use: 0.54 (0.28–1.0)	1.1 (0.51–2.5)	Age, alcohol, body mass index, family history of cancer and sigmoidoscopy Combined post-menopausal hormonal therapy was used by 18% of users

occurred among 648 women who had used both oestrogen and progestogen and also oestrogens alone. [No estimates for relative risks were provided in the paper.]

In the Swedish cohort followed by Persson *et al*. (1996) (see section 2.4), 5573 women (i.e. about 25% of the total cohort) had received prescriptions for combined post-menopausal hormonal therapy consisting of 2 mg oestradiol and 250 mg levonorgestrol for 10–21 days. They had an age-adjusted relative risk of 0.6 (95% CI, 0.4–1.0) for colon cancer and 0.8 (95% CI, 0.4–1.3) for rectal cancer. The rate of mortality from colorectal cancer was marginally decreased (relative risk, 0.6; 95% CI, 0.2–1.1).

In the North American cohort described in detail in the monograph on 'Post-menopausal oestrogen therapy' (Troisi *et al*., 1997), the age-adjusted relative risk associated with use of combined oestrogen and progestogen therapy (i.e. 16% of woman–years of hormone therapy use) for colon cancer was 1.4 (95% CI, 0.7–2.5); there were insufficient numbers of exposed cases to evaluate the risk for rectal cancer or for cancers of the colon at sub-sites.

2.5.2 *Case–control study*

Only one case–control investigation (Newcomb & Storer, 1995), described in detail in the monograph on 'Post-menopausal oestrogen therapy', included detailed information on the influence of recent use of post-menopausal hormonal therapy on the risk for colon cancer. The relative risk, adjusted for age, alcohol, body mass index, family history of cancer and sigmoidoscopy, was 0.54 (95% CI, 0.28–1.0; 11 cases) and that for rectal cancer was 1.1 (95% CI, 0.51–2.5; 8 cases). Use of this formulation had been reported by 18% of hormone users, and the risk estimates were close to those for use of post-menopausal oestrogen therapy of any type.

2.6 Cutaneous malignant melanoma

2.6.1 *Cohort study*

In the study of Persson *et al*. (1996) (see section 2.4), an age-adjusted relative risk of 0.6 (95% CI, 0.3–1.1) was found for cutaneous malignant melanoma, with nine cases.

2.6.2 *Case–control study*

Østerlind *et al*. (1988) (see Table 11 of the monograph on 'Post-menopausal oestrogen therapy') reported a multivariate relative risk adjusted for age, naevi and sunbathing for any use of oestrogens and opposed progestogens of 1.5 (95% CI, 0.8–2.8), on the basis of 28 users among cases and 45 among controls.

3. Studies of Cancer in Experimental Animals

Oestrogen plus progestogen hormonal therapy is usually given in the form of conjugated oestrogens (Premarin®; for oestrogen composition, see the monograph on 'Post-menopausal oestrogen therapy', Table 2) plus medroxyprogesterone acetate or cyproterone

acetate. Studies of the carcinogenicity of conjugated oestrogens in experimental animals are described in the monograph on 'Post-menopausal oestrogen therapy', those on medroxyprogesterone acetate and implanted levonorgestrel are described in the monograph on 'Hormonal contraceptives, progestogens only', and those on cyproterone acetate and the 19-nortestosterone derivatives norethisterone, norethisterone acetate and lynoestrenol are summarized in the monograph on 'Oral contraceptives, combined'. No studies on micronized progesterone were available. For studies on progesterone, see IARC (1979).

Female Sprague-Dawley rats, 48 days of age, were given a single intravenous injection of 5 mg 7,12-dimethylbenz[*a*]anthracene (DMBA), separated into four groups of seven rats per group and given DMBA only, DMBA plus oophorectomy, DMBA plus oophorectomy plus Premarin® at a concentration of 1.875 mg/kg of diet or DMBA plus oophorectomy plus Premarin® plus medroxyprogesterone acetate at a concentration of 7.5 mg/kg of diet. The animals were observed for 285 days, at which time body and organ weights, tumour incidence, the plasma concentrations of prolactin, oestradiol and progesterone and bone density were determined in all rats. In two rats per group, the numbers of S-phase cells in mammary tumours were assessed by immunohistochemistry, after injection with bromodeoxyuridine (BdUr) 6 h before killing. Mammary tumours were found in 6/7 rats given DMBA, 0/7 given DMBA plus oophorectomy, 5/7 given DMBA plus oophorectomy plus Premarin® and 5/7 given the preceding treatment plus medroxyprogesterone acetate. The percentages of cells in S phase were (mean ± standard error) 7 ± 0.5 in tumours from rats given DMBA, 5.5 ± 0.8 in those from oophorectomized rats given DMBA plus Premarin® and 3.1 ± 0.5 in those from oophorectomized rats given DMBA, Premarin® and medroxyprogesterone acetate, the last value being significantly different from the percentage with DMBA alone ($p < 0.01$). Thus, oophorectomy completely inhibited mammary tumour development, and medroxyprogesterone acetate significantly decreased the percentage of S-phase cells in the tumours [number of tumours/rat not specified] (Sakamoto *et al*., 1997).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

The pharmacokinetics of the newer progestogens, desogestrel, norgestimate and gestodene, has been reviewed (Fotherby, 1996). There are only a few reports of studies on the disposition of these progestogens, mostly in combination with ethinyloestradiol, and these are discussed in detail in the monograph on 'Oral contraceptives, combined'.

After oral administration of crystalline progesterone, the progestogen undergoes a first-pass effect due to extensive metabolism in the gut and liver; it thus has minimal systemic bioavailability. Micronized progesterone is rapidly absorbed and provides an adequate

concentration in the blood (Whitehead *et al*., 1980; Ottoson *et al*., 1984; Kuhl, 1990; Simon *et al*., 1993). After oral intake of 100 or 200 mg micronized progesterone, maximal serum levels of 10–15 and 20 ng/mL, respectively, are achieved within 1–4 h; these decrease thereafter (Whitehead *et al*., 1980; Morville *et al*., 1982; Maxson & Hargrove, 1985). After oral intake, absorption is enhanced about twofold by the presence of food, but the bioavailability appears to be low, the integrated area under the curve of concentration versus time (area under the curve) after intramuscular injection of progesterone being about 10 times larger than after oral intake (Simon *et al*., 1993).

Both single and multiple treatments with progesterone or with most modified progesterone derivatives result in rapid absorption and maximum blood level within 1–2 h. Accumulation occurs in blood after multiple treatments as a result of binding to sex hormone-binding globulin until a steady-state concentration is reached. These progestogens are largely stored in fat tissues (Kuhl, 1990; Fotherby, 1996).

Gestodene, levonorgestrel, cyproterone acetate and chlormadinone acetate taken orally in combination with ethinyloestradiol do not undergo first-pass metabolism and consequently have a bioavailability of almost 100%, whereas norethisterone, desogestrel and norgestimate in combination with ethinyloestradiol are rapidly and extensively metabolized in the gastrointestinal tract, with a bioavailability of 50–75% (Kuhl, 1990; Fotherby, 1996). Specific examples of the disposition of some progestogens are given below. There are large interindividual variations in the pharmacokinetic parameters.

When gestodene is taken alone, a high serum concentration is found; the mean absorption time is 0.8–1.9 h. Subjects vary widely in the area under the curve for gestodene. When combined with ethinyloestradiol, daily treatment with gestodene or 3-keto-desogestrel results in accumulation of these progestogens in serum. Poor elimination as a result of binding to sex hormone-binding globulin and inactivation of metabolizing enzymes are considered to be a likely explanation for this effect (Fotherby, 1994).

Cyproterone acetate at 5 mg taken orally with 50 μg ethinyloestradiol is rapidly absorbed, and its bioavailability is 100%. The maximum serum concentration is reached within 1–2 h after both single and multiple doses. It is largely stored in fat tissue. An increase from 11 ng/mL after a single intake to 17 ng/mL within a week of multiple intakes suggests that long-term intake leads to accumulation. After multiple oral doses, the elimination half-life remains unchanged at 2.5 days (Schleusener *et al*., 1980; Kuhl, 1990).

In most of the pharmacokinetic studies of orally administered medroxyprogesterone acetate, high doses have been used. Absorption of orally administered compound is rapid, and the time to reach the maximum serum concentration is 1–3 h (Pannuti *et al*., 1982; Johansson *et al*., 1986).

Norethisterone is rapidly absorbed, and peak serum concentrations occur within 2–4 h. The bioavailability is about 60%, because of first-pass metabolism. Micronized norethisterone is quickly absorbed and results in a higher serum concentration within a shorter time (Shi *et al*., 1987; Kuhl, 1990; Fotherby, 1996).

4.1.2 *Experimental systems*

Most of the experimental data relate to the disposition of progesterone; very limited information is available on oestrogen and progestogen combinations.

In ovariectomized rats, the distribution and elimination half-lives of progesterone after a single intravenous administration of 500 μg/kg bw were 0.13 and 1.21 h, respectively. Progesterone was eliminated rapidly, with a total clearance of 2.75 L/h per kg bw (Gangrade *et al*., 1992).

Intravenous administration of ^{3}H-progesterone to cynomolgus monkeys (*Macaca fascicularis*) resulted in the total disappearance of the hormone from the circulation within 3 h; 0.5–1.75 h later, about 5% of the initial maximal concentration of the hormone reappeared, perhaps as a result of delayed release from tissue stores (Kowalski *et al*., 1996). In female cynomolgus monkeys (*Macaca fascicularis*), progesterone has a volume of distribution of 1.75 L/kg bw and a plasma clearance of 0.06 L/kg bw per min. In comparison with humans, plasma progesterone binding is greater and progesterone clearance is slower in cynomolgus monkeys (Braasch *et al*., 1988). In baboons (*Papio anubis*), the bioavailability of chlormadinone acetate was 100%, and the peak serum concentration was reached within 1–2 h (Honjo *et al*., 1976).

4.2 Receptor-mediated effects

4.2.1 *Humans*

A group of 14 post-menopausal women was given ethinyloestradiol for one month at a daily dose of 50 μg, followed by a dose escalation of 50 μg per day over four days to a final dose of 200 μg; half of the women received 5 mg per day chlormadinone acetate during the four-day period. Endometrial biopsy samples were taken at the end of the first month and at the end of the four-day dose escalation period. The addition of chlormadinone decreased the twofold increase in uterine progesterone receptor concentration induced by the ethinyloestradiol dose escalation to that observed after the first month of ethinyloestradiol treatment (approximately 2150 fmol/mg DNA). The uterine oestrogen receptor concentration was not affected by the chlormadinone treatment (Kreitmann *et al*., 1979).

Post-menopausal women continuously receiving either 2 mg/day oestradiol valerate, 1.5 mg/day oestropipate, 0.625 or 1.25 mg/day conjugated equine oestrogens (Premarin®), 50 mg oestradiol implants or 5 g/day of a skin cream which contained 3 mg oestradiol received an oral progestogen during the last 7–10 days of each month. In endometrial biopsy samples, the soluble progesterone receptor content was found to be elevated by approximately 40% as compared with proliferative-phase endometrium from 12 unexposed women; this increase occurred only in the nine women receiving oestradiol implants and the seven women receiving oestrone sulfate. The nuclear content of oestrogen receptor was increased (by about 30%) only in the endometrial samples from the 12–15 women who had received the high dose of Premarin® before progestogen as compared with proliferative-phase endometrium from 16 unexposed women. Progestogen treatment reduced the oestrogen receptor concentration to that found in secretory-phase endometrium within six days, regardless of the type of progestogen or dose. The percentage of endometrial glandular cells

from five women receiving 1.25 mg/day Premarin® that incorporated tritiated thymidine *in vitro* was similar to that of proliferative-phase endometrium of 12 unexposed women. After the start of progestogen treatment, the labelling index decreased to the very low levels found in secretory-phase endometrium within six days. This effect of progestogens was also found with norethisterone at doses of 1, 2.5 and 5 mg/day and with norgestrel at doses of 150 and 500 μg/day. At a dose of 10 mg/day, norethisterone showed less inhibition of cell proliferation than at lower doses (Whitehead *et al.*, 1981). Similar effects of norgestrel and norethisterone were observed in a separate study of the same design, confirming the absence of a dose–response relationship at the doses tested. Medroxyprogesterone (at 2.5, 5 and 10 mg/day), dydrogesterone (5, 10 and 20 mg/day) and progesterone (at 100, 200 and 300 μg/day) also inhibited the oestrogen-induced increase in labelling index, but these effects were dose-related, reaching a maximal effect at doses of 10 mg, 20 mg and 200 μg, respectively. All of these progestogens decreased the nuclear oestrogen receptor content and had clear progestational effects on endometrial morphology (King & Whitehead, 1986).

In groups of four to six post-menopausal women given Premarin® alone at 1.25 mg/day or Premarin® and norgestrel at a dose of 150 or 500 μg/day or norethisterone at a dose of 1, 2.5, 5 or 10 mg/day for three months, the progestogens reduced the high tritiated thymidine labelling index found in the epithelial cells (approximately 9%) and stromal cells (approximately 13%) in endometrial biopsy samples from Premarin®-treated women to values found in secretory-phase endometrium from unexposed women. The highest dose of norethisterone (10 mg/day) was less effective than the lower doses; the nuclear oestrogen receptor content of the endometrium was also reduced by more than 65% (Siddle *et al.*, 1982). Identical findings were reported from a study in groups of 6–12 post-menopausal women given dydrogesterone as the progestogen. Reduction of the endometrial labelling index and oestrogen receptor content was maximal at a dose of 10 mg/day of progestogen. With dydrogesterone at a dose of 20 mg/day, the labelling index was suppressed to a lesser extent than at 10 mg/day. An apparently positive relation was observed between the dose of Premarin® and induction of the endometrial enzymes oestradiol and isocitrate dehydrogenase (Lane *et al.*, 1986).

Oestradiol was given transdermally at a dose of 50 μg/day throughout the cycle and norethisterone acetate at a transdermal dose of 170 or 350 μg/day either for the last 14 days of each cycle or continuously. A reference group received the same transdermal dose of oestrogen, but norethisterone acetate (1 mg/day) or dydrogesterone (20 mg/day) was given orally for the last 14 days of each cycle. Each group consisted of at least 150 women who were followed for at least one year. Atrophy, presumably induced by the progestogens, was more frequent in the group receiving the progestogen transdermally in a continuous regimen (66 and 84% for high and low dose, respectively) than in the group given the progestogen orally or transdermally in a sequential regimen (32–38%). No hyperplastic changes occurred in women in any group (Johannison *et al.*, 1997).

Post-menopausal women received norethisterone at 5 mg/day, ethinyloestradiol at 50 μg/day or their combination orally, each for one month. Cervical biopsy samples were

taken at baseline and at the end of each treatment period. Multivariate analysis indicated that the oestrogen increased the percentage of cells in S-phase (by flow cytometry) and the endometrial content of both oestrogen and progesterone receptors. There were no significant effects of progestogen, and there was no interaction between the progestogen and oestrogen treatment on these three parameters (Bhattacharya *et al.*, 1997).

Dydrogesterone was given at 10 mg/day in combination with conjugated equine oestrogens at 0.625 mg/day orally to 12 post-menopausal women. The serum concentrations of sex hormone-binding globulin more than doubled, whereas the circulating concentrations of insulin-like growth factor-I decreased by approximately 20%. When the therapy of the women was changed after six months to an oral regimen of norethisterone at 6 mg/day and the oestrogens for three months, the increase in sex hormone-binding globulin was largely abolished and the decrease in insulin-like growth factor-I disappeared. In another six women given oestradiol at 0.05 mg/day transdermally, the combination with dydrogesterone and norethisterone did not alter these parameters, except for a small decrease in sex hormone-binding globulin concentration (Campagnoli *et al.*, 1994).

Six post-menopausal women were given 20 µg/day ethinyloestradiol, 1.25 mg/day conjugated equine oestrogens (Premarin®) or 2 mg/day oestradiol valerate for subsequent periods of four weeks; during the last 12 days of each treatment cycle, the women also received 10 mg/day medroxyprogesterone acetate. The serum concentrations of insulin-like growth factor-I were decreased by approximately 15–25% with all three treatments when compared with the pretreatment period, while the serum concentrations of growth hormone and growth hormone-binding protein were increased by two- to threefold when compared with the pretreatment period (Kelly *et al.*, 1993).

Women receiving transdermal oestradiol developed histological signs of progestational endometrial effects when given levonorgestrel in an intrauterine device releasing 20 µg/day of the progestogen; such effects were not seen in women receiving progesterone orally at 100 mg/day or vaginally at 100–200 mg/day (Suvanto-Luukkonen *et al.*, 1995). Insulin-like growth factor binding protein-I was also induced by intrauterine exposure to levonorgestrel but not by the other routes of exposure. The observations for the binding protein-I were confirmed in a similar comparison of intrauterine and subcutaneous treatment with levonorgestrel (Suhonen *et al.*, 1996).

In the Postmenopausal Estrogen/Progestin Interventions Trial (1996) 875 post-menopausal women were assigned randomly to placebo, conjugated equine oestrogens (0.625 mg/day), conjugated equine oestrogens plus cyclic medroxyprogesterone acetate (10 mg/day for 12 days per month) or conjugated equine oestrogens plus cyclic micronized progestogen (200 mg/day for 12 days per month). During the three-year study, the women assigned to oestrogen were more likely to develop simple (cystic), complex, adenomatous or atypical hyperplasia than those given placebo (27.7 versus 0.8%, 22.7 versus 0.8% and 11.8 versus 0%, respectively). The rates of hyperplasia were similar in all groups, and the occurrence of hyperplasia was distributed across the three-year trial.

4.2.2 *Experimental systems*

The relevant effects in experimental systems of combinations of progestogens and oestrogens used in post-menopausal hormonal therapy are summarized in detail in the monographs on 'Oral contraceptives, combined', section 4.2, and 'Hormonal contraceptives, oestrogens only', section 4.2. These effects are briefly mentioned for each of the progestogens covered in this monograph, but the effects of oestrogen–progestogen combinations *in vivo* at doses similar to those used for humans are described in detail.

In some but not all of the studies, cyproterone acetate inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture; it was not oestrogenic. Desogestrel, gestodene, norethisterone and levonorgestrel have oestrogenic properties but also inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture. In some but not all of the studies, medroxyprogesterone acetate inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture; it was not oestrogenic.

Medroxyprogesterone acetate at 2 μg/rat per day decreased the hyperplastic effects of conjugated equine oestrogens at 50 μg/rat per day on the endometrium of ovariectomized rats, whereas dydrogesterone and cyproterone acetate at the same dose appeared to enhance these oestrogen-induced hyperplastic effects slightly (Kumasaka *et al.*, 1994).

Subcutaneous administration of medroxyprogesterone acetate at 1–1.5 mg/rat twice daily over 18 days inhibited stimulation by oestrone (1 μg/rat, subcutaneously twice daily) of the growth of mammary gland carcinomas induced by DMBA in female Sprague-Dawley rats which were ovariectomized after tumours had developed. The reductive activity of 17β-hydroxysteroid oxidoreductase [dehydrogenase] on mammary tumour tissue was altered by medroxyprogesterone acetate in such a way that the formation of oestradiol in tumours of these oestrone-treated animals was reduced by more than 50%. In the uterus, however, medroxyprogesterone acetate decreased the activity of this enzyme by less than 20% (Luo *et al.*, 1997).

The effects of a combination of dietary administration of conjugated equine oestrogens (Premarin®) and medroxyprogesterone acetate on the mammary glands of 25 adult female cynomolgus monkeys (*Macaca fascicularis*) were studied in comparison with 22 monkeys receiving control diet; all of the animals had been ovariectomized before the experiment. The daily dose of Premarin® was approximately 7.2 μg per animal for the first eight months of the experiment and 166 μg per animal for the subsequent duration of the 30-month study. The latter dose was stated by the authors to be equivalent to a human dose of 0.625 mg/day. The dose of medroxyprogesterone acetate was 650 μg/day throughout the experiment; this dose was stated by the authors to be equivalent to a human dose of 2.5 mg/day. The combined oestrogen–progestogen treatment increased the concentration of circulating oestradiol from 5 to 161 pg/mL; the concentration of medroxyprogesterone acetate in the blood was 116 pg/mL. Exposure to the hormones increased the thickness of the mammary tissue by 70% and significantly enlarged the estimated surface areas of lobular tissue and epithelial tissue; it also induced mammary gland hyperplasia in 18/21 animals, as compared with 41% of animals given Premarin® alone and none in the control group. The mean percentage of epithelial breast cells that

stained for Ki-67 MIB-1 antibody (a marker of cell proliferation) was increased from 2.5 to 8.0% in alveoli, from 0.6 to 1.9% in terminal ducts and from 1.2 to 5.5% in major mammary ducts. These effects on labelling were not different from those in monkeys given Premarin® alone (see the monograph on 'Post-menopausal oestrogen therapy', section 4.2). The mean percentage of epithelial breast cells that stained for progesterone receptor was not changed in these mammary structures, but the percentage of cells that stained for oestrogen receptor was decreased by approximately 65% in alveoli, by 40% in terminal ducts and by more than 90% in major mammary ducts (Cline *et al.*, 1996).

4.3 Genetic and related effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Relevant data are contained in section 4.3.2 of the monographs on 'Oral contraceptives, combined' and 'Post-menopausal oestrogen therapy'.

5. Summary of Data Reported and Evaluation

5.1 Exposure

Use of regimens in which a progestogen is added to post-menopausal oestrogen therapy has been increasing in order to reduce the increased risk for endometrial cancer observed with oestrogens alone. Regimens vary with respect to dose and timing of oestrogen and progestogen administration and in the number of days on which the progestogen is given per month. Several routes of administration are used, including oral (as tablets), injection, implantation, percutaneous application and intrauterine administration. The frequency and type of hormonal supplementation used vary widely within and between countries.

5.2 Human carcinogenicity

Breast cancer

Separate information on the effects of use of post-menopausal oestrogen–progestogen therapy was provided in only a minority of the studies on the risk for breast cancer. The results of nine cohort and five case–control studies that did include such information and the findings of a pooled analysis of the original data from these and other studies indicate that the increased relative risk observed with long-term use of post-menopausal oestrogen–progestogen therapy is not materially different from that for long-term use of oestrogens alone. The available information on long-term use of the combination is, however, limited. The data are insufficient to assess the effects of past use and of different progestogen compounds, doses and treatment schedules.

Endometrial cancer

The relationship between use of post-menopausal oestrogen–progestogen therapy and the risk for endometrial cancer was addressed in four follow-up and four case–control studies. In comparison with women who did not use hormonal therapy, the risk of women who did was no different or modestly increased, but the increase was smaller than that for women who used oestrogens alone. In the two studies that were recent and large enough to evaluate different durations of progestogen supplementation during each cycle, an increase in risk was found relative to non-users when the progestogen was added to the cycle for 10 days or fewer. The risk for endometrial cancer associated with different monthly durations of progestogen supplementation per cycle and different doses of progestogen supplementation remains unclear.

Ovarian cancer

One cohort and one case–control study are available on the possible relationship between use of post-menopausal oestrogen–progestogen therapy and the risk for ovarian cancer. The limited data suggest no association.

Liver cancer

One cohort study suggested that there is no association between use of post-menopausal oestrogen–progestogen therapy and the risk for liver cancer.

Other cancers

Very few studies were available of the risks for colorectal cancer, cutaneous malignant melanoma or thyroid cancer that allowed a distinction between use of post-menopausal oestrogen–progestogen and oestrogen therapy. They do not suggest an increased risk, but all included few exposed subjects.

5.3 Carcinogenicity in experimental animals

Only one study was available on combined oestrogen and progestogen therapy, in which conjugated equine oestrogens were tested with medroxyprogesterone acetate. Oral administration of this combination or of the conjugated oestrogens alone in the diet of ovariectomized female rats which had been given 7,12-dimethylbenz[*a*]anthracene, a known mammary carcinogen, increased the incidence of mammary tumours to a level equal to that in non-ovariectomized controls treated with the carcinogen.

5.4 Other relevant data

Combinations of oestrogens and progestogens are absorbed rapidly and reach maximal serum concentrations quickly. The proportion of absorbed hormones that becomes biologically available depends on the extent of enterohepatic circulation and metabolic transformation of pro-drugs. Oestrogens and progestogens may affect each other's disposition. Many progestogens have oestrogenic activity and can modify the effects of oestrogens. The addition of progestogens to therapy may decrease cell proliferation in human endometrium

over that with oestrogen alone. The extent of the cell proliferation response depends on the doses of oestrogen and progestogen, increasing with higher doses of oestrogen and decreasing with more progestogen, as compared with oestrogen alone.

In ovariectomized cynomolgus monkeys, the conjugated oestrogen–progestogen combination caused a higher incidence of mammary gland hyperplasia than did conjugated equine oestrogens alone. No information was available on whether the effect of oestrogen–progestogen combinations on the mammary gland is modified by sequential exposure to progestogens, by body weight or by the recency or duration of exposure in experimental animals. Similarly, no information was available on the possible relationship between exposure to oestrogen–progestogen combinations and the degree of malignancy of breast tumours.

No information was available on the genotoxic effects of formulations similar to those used in post-menopausal oestrogen–progestogen therapy.

5.5 Evaluation

There is *limited evidence* in humans for the carcinogenicity of post-menopausal oestrogen–progestogen therapy.

There is *inadequate evidence* in experimental animals for the carcinogenicity of conjugated equine oestrogens plus progestogen.

Overall evaluation

Post-menopausal oestrogen–progestogen therapy is *possibly carcinogenic to humans (Group 2B).*

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