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A REVIEW OF HUMAN CARCINOGENS

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 14-21 October 2008

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer
World Health Organization

COMBINED ESTROGEN–PROGESTOGEN MENOPAUSAL THERAPY

Combined estrogen–progestogen menopausal therapy was considered by previous IARC Working Groups in 1998 and 2005 (IARC, 1999, 2007). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

Combined estrogen–progestogen menopausal therapy involves the co-administration of an estrogen and a progestogen to peri- or menopausal women. The use of estrogens with progestogens has been recommended to prevent the estrogen-associated risk of endometrial cancer. Evidence from the Women's Health Initiative (WHI) of adverse effects from the use of a continuous combined estrogen–progestogen has affected prescribing. Patterns of exposure are also changing rapidly as the use of hormonal therapy declines, the indications are restricted, and the duration of the therapy is reduced (IARC, 2007).

1.1 Identification of the agents

1.1.1 Estrogens

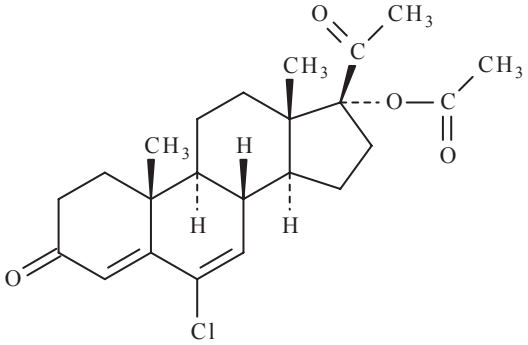
For Estrogens, see the *Monograph* on Estrogen-only Menopausal Therapy in this volume.

1.1.2 Progestogens

(a) Chlormadinone acetate

Chem. Abstr. Serv. Reg. No.: 302-22-7
Chem. Abstr. Name: 17-(Acetyloxy)-6-chloropregna-4,6-diene-3,20-dione
IUPAC Systematic Name: 6-Chloro-17-hydroxypregna-4,6-diene-3,20-dione, acetate
Synonyms: 17α-Acetoxy-6-chloro-4,6-pregnadiene-3,20-dione; 6-chloro-Δ^6-17-acetoxyprogesterone; 6-chloro-Δ^6-[17α] acetoxyprogesterone

Structural and molecular formulae, and relative molecular mass

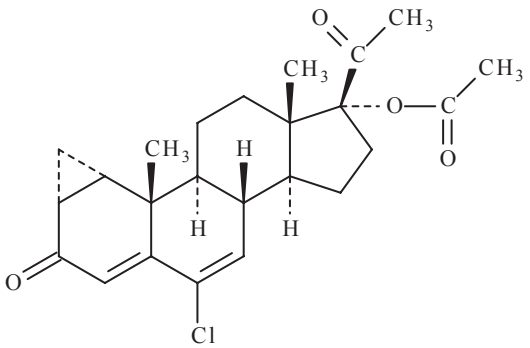


$C_{23}H_{29}ClO_4$
Relative molecular mass: 404.9

(b) Cyproterone acetate

Chem. Abstr. Serv. Reg. No.: 427-51-0
Chem. Abstr. Name: (1*β*,2*β*)-17-(Acetyloxy)-6-chloro-1,2-dihydro-3′*H*-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione
IUPAC Systematic Name: 6-Chloro-1*β*,2*β*-dihydro-17-hydroxy-3′H-cyclopropa[1,2]pregna-1,4,6-triene-3,20-dione acetate
Synonyms: Cyproterone 17-*O*-acetate; cyproterone 17*α*-acetate; 1,2*α*-methylene-6-chloro-17*α*-acetoxy-4,6-pregnadiene-3,20-dione; 1,2*α*-methylene-6-chloro-$\Delta^{4,6}$-pregnadien-17*α*-ol-3,20-dione acetate; 1,2*α*-methylene-6-chloro-pregna-4,6-diene-3,20-dione 17*α*-acetate; methylene-6-chloro-17-hydroxy-1*α*,2*α*-pregna-4,6-diene-3,20-dione acetate

Structural and molecular formulae, and relative molecular mass

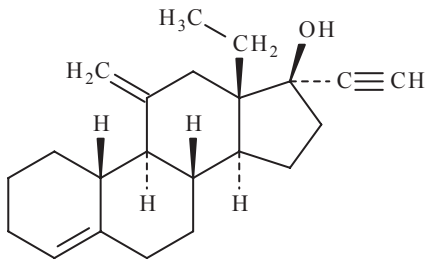


$C_{24}H_{29}ClO_4$
Relative molecular mass: 416.9

(c) Desogestrel

Chem. Abstr. Serv. Reg. No.: 54024-22-5
Chem. Abstr. Name: (17*α*)-13-Ethyl-11-methylene-18,19-dinorpregn-4-en-20-yn-17-ol
IUPAC Systematic Name: 13-Ethyl-11-methylene-18,19-dinor-17*α*-pregn-4-en-20-yn-17-ol
Synonyms: 13-Ethyl-11-methylene-18,19-dinor-17*α*-4-pregnen-20-yn-17-ol; 17*α*-ethynyl-18-methyl-11-methylene-Δ^4-oestren-17*β*-ol

Structural and molecular formulae, and relative molecular mass

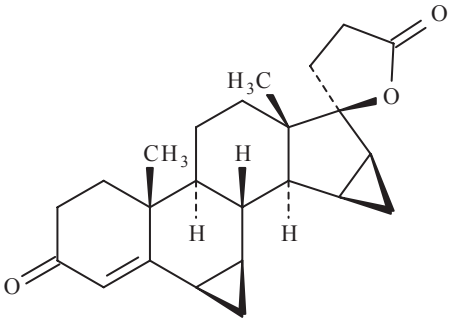


$C_{22}H_{30}O$
Relative molecular mass: 310.5

(d) Drospirenone

Chem. Abst. Services Reg. No.: 67392-87-4
Chem. Abstr. Name: (2′*S*,6*R*,7*R*,8*R*,9*S*,10*R*,-13*S*,14*S*,15*S*,16*S*)-1,3′,4′,6,7,8,9,10,11,-12,13,14,15,16,20,21-Hexadecahydro-10,13-dimethyl-spiro[17*H*-dicyclopropa[6,7:15,16]cyclopenta[*a*]phenanthrene-17,2′ (5′*H*)-furan]-3,5′ (2*H*)-dione
Synonyms: Dihydrospirorenone; 1,2-dihydrospirorenone; drospirenona; spiro[17*H*-dicyclopropa[6,7:15,16]cyclopenta[*a*]phenanthrene-17,2′(5′H)-furan]-3,5′(2H)-dione, 1,3′,4′,6,7,8,9,10,11,12,13,14,15,16,20,21-hexadecahydro-10,13-dimethyl-, [6*R*-(6*α*,7*α*,8*β*,9*α*,10*β*,13*β*,14*α*,15*α*,16*α*,17*β*)]-

Structural and molecular formulae, and relative molecular mass

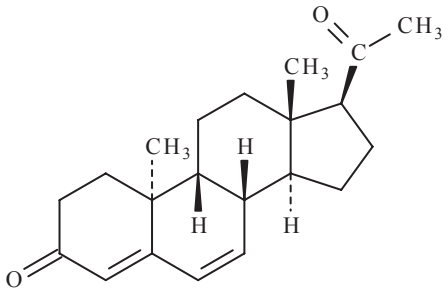


$C_{24}H_{30}O_3$
Relative molecular mass: 366.5

(e) Dydrogesterone

Chem. Abstr. Serv. Reg. No.: 152-62-5
Chem. Abstr. Name: (9*β*,10*α*)-Pregna-4,6-diene-3,20-dione
IUPAC Systematic Name: 10*α*-Pregna-4,6-diene-3,20-dione
Synonyms: 10*α*-Isopregnenone; dehydroretroprogesterone; dehydroprogesterone

Structural and molecular formulae, and relative molecular mass

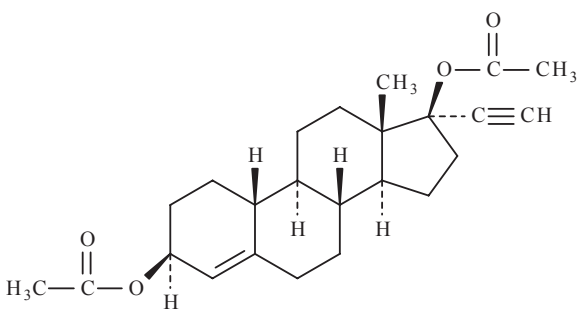


$C_{21}H_{28}O_2$
Relative molecular mass: 312.5

(f) Ethynodiol diacetate

Chem. Abstr. Serv. Reg. No.: 297-76-7
Chem. Abstr. Name: (3*β*,17*α*)-19-Norpregn-4-en-20-yne-3,17-diol, diacetate
IUPAC Systematic Name: 19-Nor-17*α*-pregn-4-en-20-yne-3*β*,17*β*-diol, diacetate
Synonyms: Ethinodiol diacetate; ethynodiol acetate; *β*-ethynodiol diacetate

Structural and molecular formulae, and relative molecular mass

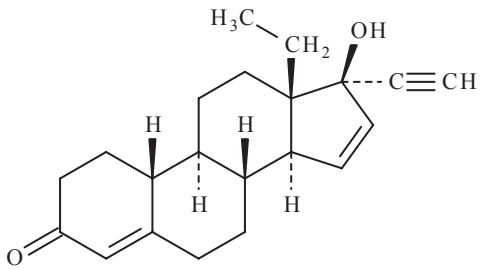


$C_{24}H_{32}O_4$
Relative molecular mass: 384.5

(g) Gestodene

Chem. Abstr. Serv. Reg. No.: 60282-87-3
Deleted CAS Reg. No.: 110541-55-4
Chem. Abstr. Name: (17*α*)-13-Ethyl-17-hydroxy-18,19-dinorpregna-4,15-dien-20-yn-3-one
IUPAC Systematic Name: 13-Ethyl-17-hydroxy-18,19-dinor-17*α*-pregna-4,15-dien-20-yn-3-one

Structural and molecular formulae, and relative molecular mass

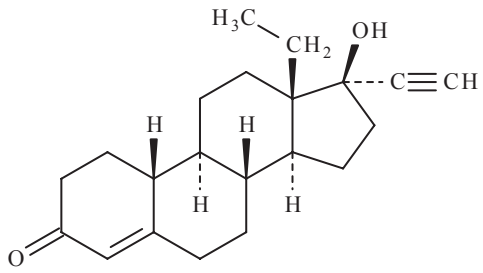


$C_{21}H_{26}O_2$
Relative molecular mass: 310.4

(h) Levonorgestrel

Chem. Abstr. Serv. Reg. No.: 797-63-7
Deleted CAS Reg. No.: 797-62-6; 4222-79-1; 121714-72-5
Chem. Abstr. Name: (17*α*)-13-Ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one
IUPAC Systematic Name: 13-Ethyl-17-hydroxy-18,19-dinor-17*α*-pregn-4-en-20-yn-3-one
Synonyms: 13-Ethyl-17-ethynyl-17*β*-hydroxy-4-gonen-3-one; 13-ethyl-17*α*-ethynyl-17-hydroxygon-4-en-3-one; 13-ethyl-17*α*-ethynylgon-4-en-17*β*-ol-3-one; 13*β*-ethyl-17*α*-ethynyl-17*β*-hydroxygon-4-en-3-one; 13-ethyl-17-hydroxy-18,19-dinor-17*α*-pregn-4-en-20-yn-3-one; 17-ethynyl-18-methyl-19-nortestosterone; 18-methylnorethindrone; L-norgestrel; DL-norgestrel; D-norgestrel

Structural and molecular formulae, and relative molecular mass

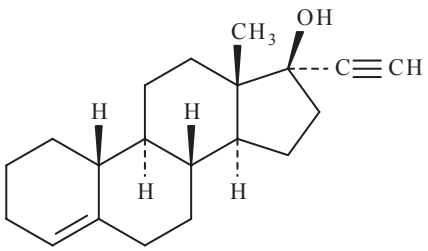


$C_{21}H_{28}O_2$
Relative molecular mass: 312.5

(i) Lynestrenol

Chem. Abstr. Serv. Reg. No.: 52-76-6
Deleted CAS Reg. No.: 60416-16-2
Chem. Abstr. Name: (17α)-19-Norpregn-4-en-20-yn-17-ol
IUPAC Systematic Name: 19-Nor-17α-pregn-4-en-20-yn-17-ol
Synonyms: 3-Desoxynorlutin; Δ^4-17α-ethinylestren-17β-ol; Δ^4-17α-ethinyloestren-17β-ol; ethynylestrenol; ethynyloestrenol; 17α-ethynylestrenol; 17α-ethynyloestrenol; 17α-ethynyl-17β-hydroxy-Δ^4-estrene; 17α-ethynyl-17β-hydroxy-Δ^4-oestrene

Structural and molecular formulae, and relative molecular mass

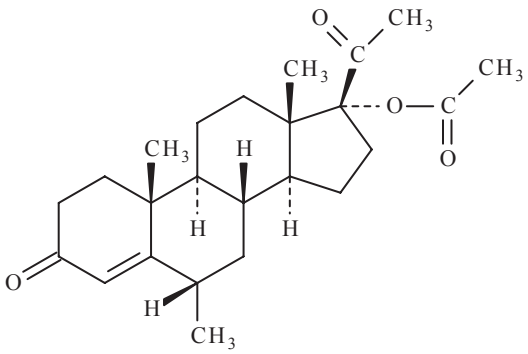


$C_{20}H_{28}O$
Relative molecular mass: 284.4

(j) Medroxyprogesterone acetate

Chem. Abstr. Serv. Reg. No.: 71-58-9
Chem. Abstr. Name: (6α)-17-(Acetyloxy)-6-methylpregn-4-ene-3,20-dione
IUPAC Systematic Name: 17-Hydroxy-6α-methylpregn-4-ene-3,20-dione, acetate
Synonyms: 17α-Acetoxy-6α-methylprogesterone; depomedroxyprogesterone acetate; depo-progestin; depot-medroxyprogesterone acetate; DMPA; 17-hydroxy-6α-methylprogesterone, acetate; 17α-hydroxy-6α-methylprogesterone acetate; MAP; MPA; medroxyprogesterone 17-acetate; 6α-methyl-17-acetoxyprogesterone; 6α-methyl-17α-hydroxyprogesterone acetate

Structural and molecular formulae, and relative molecular mass

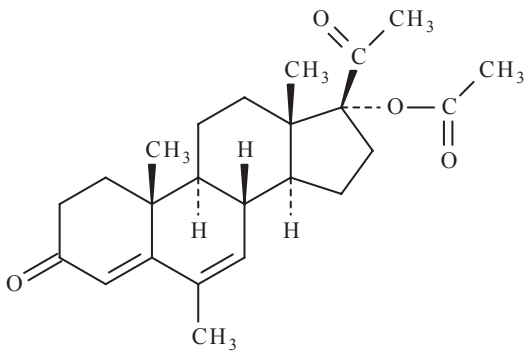


$C_{24}H_{34}O_4$
Relative molecular mass: 386.5

(k) Megestrol acetate

Chem. Abstr. Serv. Reg. No.: 595-33-5
Chem. Abstr. Name: 17-(Acetyloxy)-6-methylpregna-4,6-diene-3,20-dione
IUPAC Systematic Name: 17-Hydroxy-6-methylpregna-4,6-diene-3,20-dione, acetate
Synonyms: DMAP; megestryl acetate; MGA

Structural and molecular formulae, and relative molecular mass



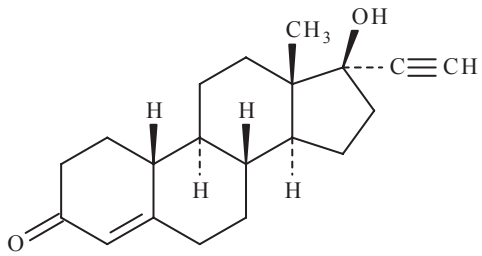
$C_{24}H_{32}O_4$

Relative molecular mass: 384.5

(l) Norethisterone

Chem. Abstr. Serv. Reg. No.: 68-22-4
Chem. Abstr. Name: (17α)-17-Hydroxy-19-norpregn-4-en-20-yn-3-one
IUPAC Systematic Name: 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one
Synonyms: Ethinylnortestosterone; 17α-ethinyl-19-nortestosterone; ethynylnortestosterone; 17-ethynyl-19-nortestosterone; 17α-ethynyl-19-nortestosterone; norethindrone; norethisteron; norethynodrone; 19-nor-17α-ethynyltestosterone; norpregneninolone

Structural and molecular formulae, and relative molecular mass



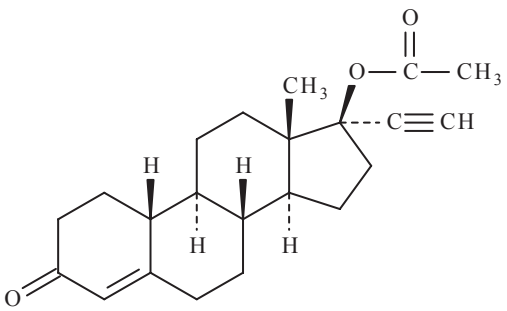
$C_{20}H_{26}O_2$

Relative molecular mass: 298.4

(m) Norethisterone acetate

Chem. Abstr. Serv. Reg. No.: 51-98-9
Chem. Abstr. Name: (17α)-17-(Acetyloxy)-19-norpregn-4-en-20-yn-3-one
IUPAC Systematic Name: 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one, acetate
Synonyms: 17α-Ethinyl-19-nortestosterone 17β-acetate; 17α-ethinyl-19-nortestosterone acetate; 17α-ethynyl-19-nortestosterone acetate; norethindrone acetate; norethindrone 17-acetate; norethisteron acetate; norethisterone 17-acetate; 19-norethisterone acetate; norethynyltestosterone acetate; 19-norethynyltestosterone acetate; norethysterone acetate

Structural and molecular formulae, and relative molecular mass



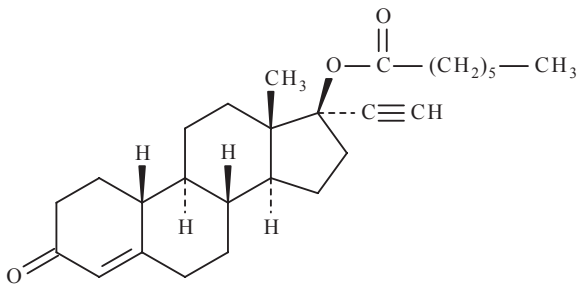
$C_{22}H_{28}O_3$

Relative molecular mass: 340.5

(n) Norethisterone enanthate

Chem. Abstr. Serv. Reg. No.: 3836-23-5
Chem. Abstr. Name: (17α)-17-(Heptanoyl)-19-norpregn-4-en-20-yn-3-one
IUPAC Systematic Name: 17-Hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one, heptanoate
Synonyms: Norethindrone enanthate; norethindrone oenanthate; norethisterone enanthate; norethisterone heptanoate; 17β-hydroxy-19-nor-17α-pregn-4-en-20-yn-3-one heptanoate

Structural and molecular formulae, and relative molecular mass

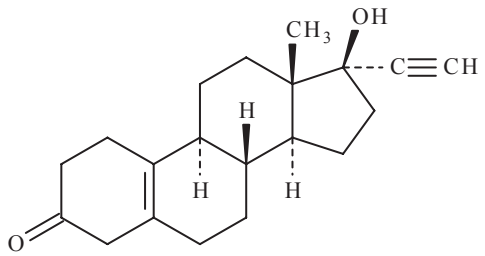


$C_{27}H_{38}O_3$
Relative molecular mass: 410.6

(o) Norethynodrel

Chem. Abstr. Serv. Reg. No.: 68-23-5
Chem. Abstr. Name: (17α)-17-Hydroxy-19-norpregn-5(10)-en-20-yn-3-one
IUPAC Systematic Name: 17-Hydroxy-19-nor-17α-pregn-5(10)-en-20-yn-3-one
Synonyms: Enidrel; noretynodrel

Structural and molecular formulae, and relative molecular mass

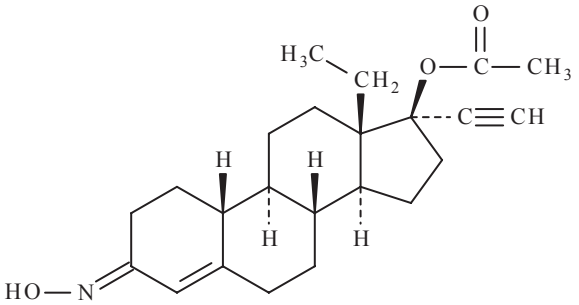


$C_{20}H_{26}O_2$
Relative molecular mass: 298.4

(p) Norgestimate

Chem. Abstr. Serv. Reg. No.: 35189-28-7
Chem. Abstr. Name: (17α)-17-(Acetyloxy)-13-ethyl-18,19-dinorpregn-4-en-20-yn-3-one, 3-oxime
IUPAC Systematic Name: 13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one oxime acetate (ester)
Synonyms: 17α-Acetoxy-13-ethyl-17-ethynylgon-4-en-3-one oxime; dexnorgestrel acetime

Structural and molecular formulae, and relative molecular mass

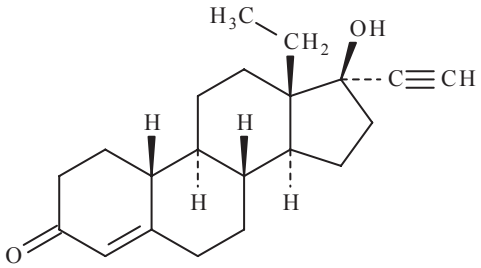


$C_{23}H_{31}NO_3$
Relative molecular mass: 369.5

(q) Norgestrel

Chem. Abstr. Serv. Reg. No.: 6533-00-2
Chem. Abstr. Name: (17α)-dl-13-Ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one
IUPAC Systematic Name: dl-13-Ethyl-17-hydroxy-18,19-dinor-17α-pregn-4-en-20-yn-3-one
Synonyms: (17α)-13-Ethyl-17-hydroxy-18,19-dinorpregn-4-en-20-yn-3-one; methylnorethindrone; α-norgestrel; dl-norgestrel; DL-norgestrel

Structural and molecular formulae, and relative molecular mass

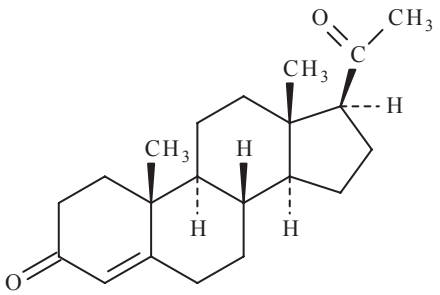


$C_{21}H_{28}O_2$
Relative molecular mass: 312.5

(r) Progesterone

Chem. Abst. Services Reg. No.: 57-83-0
Chem. Abstr. Name: Pregn-4-ene-3,20-dione
Synonyms: Corpus luteum hormone; luteal hormone; luteine; luteohormone; Δ^4-pregnene-3,20-dione

Structural and molecular formulae, and relative molecular mass



$C_{21}H_{30}O_2$

Relative molecular mass: 314.5

1.2 Use of the agents

Information for Section 1.2 is taken from IARC (2007), McEvoy (2007), and Sweetman (2008).

1.2.1 Indications

Estrogen–progestogen combinations are used for the treatment of moderate-to-severe vasomotor symptoms, vulvar and vaginal atrophy associated with menopause, and for the prevention and treatment of osteoporosis. Women with an intact uterus are prescribed a progestogen in addition to estrogen to reduce the increased risk of endometrial carcinoma.

1.2.2 Dosages and formulations

A variety of products are available for use in combined estrogen–progestogen menopausal therapy, either as individual estrogen and progestogen components that can be co-administered, or as a combined tablet.

Available products can be defined by their estrogen form, dose, and mode of delivery. The most common estrogens available for menopausal therapy are conjugated equine estrogen, conjugated plant-based estrogens (A and B; see the *Monograph* on Estrogen-only Menopausal Therapy in this volume), estradiol and ethinylestradiol. A range of 3–5 different doses are often available for each product, varying from low doses (0.3–0.5 mg orally) to higher doses (2.5–5 mg). The doses of estrogens used are generally lower than those used in combined oral contraceptives, and do not therefore provide contraception. They are available as oral tablets, intranasal sprays, transdermal skin patches and gels, subcutaneous implants, topical applications for vulvovaginal use, and intravaginal rings.

Generally, if prolonged therapy (for more than 2–4 weeks) with an estrogen by any route is envisaged in a woman with an intact uterus, a progestogen is given to prevent endometrial proliferation. A range of progestogens are available for use in combined therapy. Those most commonly used are medroxyprogesterone acetate, norethisterone, levonorgestrel, and micronized progestogens. Several doses of each progestogen are usually available. While oral forms predominate, progestogens are also available as a vaginal pessary, a systemically absorbed vaginal gel, a transdermal patch and an intrauterine device. The administration of progestogens may follow one of three types of schedule. In continuous combined therapy, the same dose of both estrogen and progestogen is administered each day. Cyclic or sequential therapy consists of estrogen-alone daily, followed by progestogen with estrogen for 7–20 days and then 5–7 days with no hormones. The duration of each phase can vary.

Combined oral products contain both estrogen and progestogen. The various preparations available differ in their estrogen component, their progestogen component, the dose of these components, and the schedule and mode of drug administration. Continuous exposure to both hormones (both estrogen and progestogen at fixed daily doses) is common, particularly in the United States of America, whereas cyclic dosing, in which progestogen is added periodically to daily estrogen, is prevalent in other

countries. Other scheduling strategies are also used occasionally.

When conjugated estrogens (A or B) are used with medroxyprogesterone acetate for the management of moderate-to-severe vasomotor symptoms associated with menopause or for the management of vulvar and vaginal atrophy, conjugated estrogens are administered in a continuous daily dosage regimen while medroxyprogesterone acetate is administered in a continuous daily dosage regimen or cyclically. When both drugs are administered in a continuous daily dosage regimen, conjugated estrogens are administered in a daily dosage of 0.3 mg in conjunction with oral medroxyprogesterone acetate in a daily dosage of 1.5 mg. Alternatively, conjugated estrogens are administered in a daily dosage of 0.45 mg in conjunction with medroxyprogesterone acetate in a daily dosage of 1.5 mg, or conjugated estrogens are administered in a daily dosage of 0.625 mg in conjunction with medroxyprogesterone acetate in a daily dosage of 2.5 or 5 mg. When conjugated estrogens are administered in a continuous daily dosage regimen and medroxyprogesterone acetate is administered cyclically, conjugated estrogens are administered in a daily dosage of 0.625 mg, while oral medroxyprogesterone acetate is administered in a daily dosage of 5 mg on Days 15–28 of the cycle.

1.2.3 Trends in use

When the addition of a progestogen to estrogens was introduced after 1975 as a strategy to reduce the risk of endometrial cancer, the use of the combination for menopausal therapy increased steadily in the 1980s, particularly in developed countries. Combined estrogen–progestogen menopausal therapy is now administered to women who have not undergone a hysterectomy.

Combined hormonal therapy is much more commonly used in developed countries than in developing countries. At the peak of use in 1999, approximately 20 million women in developed countries used combined hormonal therapy. Use has fallen by more than 50% since 2002, particularly for continuous combined hormonal therapy. Use in some developing countries has also declined modestly, although the data are more limited. Among peri- and postmenopausal women in developed countries, current users of combined hormonal therapy tend to be younger and more highly educated, to have a lower body mass, and to use health care more regularly than non-users (IARC, 2007).

2. Cancer in Humans

2.1 Cancer of the breast

At the time of the first *IARC Monograph* of hormones and breast cancer (IARC, 1999), almost all of the epidemiological evidence came from studies that evaluated estrogen prescribed without a progestogen. Data on breast cancer risk related to estrogen plus a progestogen were deemed insufficient to reach any firm conclusions about the carcinogenicity of combined hormone therapy.

The next *IARC Monograph* on this topic (IARC, 2007) reviewed two randomized trials, ten cohort studies, and seven case–control studies on combined postmenopausal hormone therapy and breast cancer published up to and including 2004. These studies consistently reported an increased risk for breast cancer in users of estrogen plus progestogen therapy compared with non-users. The risk increased with increasing duration of use, was largely confined to current or recent users, and decreased soon after hormone treatment was stopped. Although the previous IARC evaluation concluded that there is *sufficient* evidence in humans for the carcinogenicity of combined estrogen–progestogen menopausal therapy in the breast, it was not possible to evaluate whether

breast cancer risk varied according to the type of progestogen or its dose, or according to the number of days each month that the progestogen was taken.

The present review of studies published through August 2008 includes four new systematic reviews, additional analyses from two clinical trials, five cohort studies, and four case–control studies, as well as many studies of time trends, and two trials of hormone therapy in breast cancer survivors. Studies were included if the authors provided risk estimates (odds ratios [OR], hazard ratios [HR] or relative risks [RR]) and 95% confidence intervals (CI) comparing breast cancer risk in women who used combined (estrogen plus progestogen) hormone therapy with non-hormone users for at least 1 year, or if the authors specified that at least 80% of estrogen-taking women were likely to be using combined therapy. Evidence from many studies of varying breast cancer incidence during recent years of increasing and then decreasing prescription of combined hormone therapy is also reviewed.

2.1.1 *Systematic reviews*

Four systematic reviews published after 2004 comprise studies of combined postmenopausal hormone use and breast cancer risk (Campagnoli *et al.*, 2005; Collins *et al.*, 2005; Greiser *et al.*, 2005; Shah *et al.*, 2005). All support the conclusion that the use of estrogen plus progestogen increases the risk of breast cancer in women, although each review included a somewhat different set of studies, offered some distinct conclusions based on the different studies chosen, had different definitions of hormone exposure, and different subset analyses related to different hormone regimens, duration, and recency of use.

Campagnoli *et al.* (2005) reviewed several publications, mainly those reviewed in the previous *IARC Monograph*, and confirmed a significantly increased risk of breast cancer risk with combined hormone therapy. In a subset analysis of ten studies comparing continuous combined progestogens with sequential progestogens, half of the studies suggested a higher risk with continuous combined therapy, but most differences were small and had overlapping 95% confidence intervals (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-13-Table2.1.pdf).

Another meta-analysis of papers published between 1989–2004 included 21 case–control studies, 15 cohort studies, and six controlled clinical trials. Trials and cohort studies had more cancer cases than case–control studies, but provided separate stratified analyses of estrogen plus progestogen for only three ever-never use comparisons, and four duration-of-use analyses. Most of the relevant results are from the subset of ten case–control studies that reported combined hormone treatment separately. The summary statistics showed an increased cancer risk for estrogen plus progestogen, especially for data after 1992, when estrogen plus progestogen in a single tablet became more widely available (Greiser *et al.*, 2005).

Shah *et al.*, (2005) published a meta-analysis of eight studies of current hormone therapy and breast cancer excluding women with a history of oral contraceptive use. The summary analysis showed that estrogen plus progestogen use for less than 5 years significantly increased breast cancer risk (OR, 1.35; 95%CI: 1.16–1.57), and use for more than 5 years showed a somewhat greater risk (OR, 1.63; 95%CI: 1.22–2.18).

A systematic review by Collins *et al.* (2005) included published data on estrogen plus progestogen and breast cancer from four randomized trials, two of which – WHI and Heart and Estrogen/Progestin Replacement Study (HERS) – were included in the previous *IARC Monograph*, and two other small earlier trials that together added only five cancer cases (all in the placebo group), which did not change the overall risk estimates. This review includes

very useful pre-planned subset analyses with the following results:

- the relative risk for breast cancer was lower in the intent-to-treat analysis (OR, 1.24; 95%CI: 1.01–1.54) than in the adherent women analysis (OR, 1.49; 95%CI: 1.13–1.96), compatible with the high drop-out and crossover rate in the WHI-Estrogen-Progestogen Trial (EPT);
- the dominance of the Million Women Study (Beral *et al.*, 2003) (1934 estrogen user cases) did not explain the excess breast cancer risk associated with combined hormone therapy in published studies, in that the published summary risk in the seven epidemiological studies that included the Million Women Study (OR, 1.70; 95%CI: 1.36–2.13) was very similar to the summary risk for the other six studies that did not include the Million Women Study (OR, 1.67; 95%CI: 1.29–2.17);
- the analysis confirms the WHI-EPT data showing a significantly increased risk of breast cancer begins within 5 years of initiating combined therapy and increases with increasing years of use; the analysis highlights an important result from the Million Women Study, showing similar relative risks for equine estrogen (OR, 1.29; 95%CI: 1.16–1.43) and estradiol (OR, 1.24; 95%CI: 1.12–1.37); and,
- an analysis (based on pooled data from the Million Women Study and the Danish Nurses Cohort) showed essentially identical risk for C21 progestogens (medroxyprogesterone acetate) and C19 progestogens (norethisterone, levonorgestrel) (OR, 2.14 for each).

[The Working Group noted that more than 400 user cases for the estradiol versus equine estrogen comparison and the more than 1900 user cases for the progestogen comparisons make it less likely that lack of power concealed clinically meaningful differences.]

2.1.2 Studies of changing breast cancer incidence in the context of changing patterns of menopausal hormone therapy use

The widely publicized increased risk of breast cancer in the WHI-EPT trial published in 2002 (Rossouw *et al.*, 2002) was followed by rapid and substantial drops in the incidence of invasive breast cancer in the USA (Clarke *et al.*, 2006; Glass *et al.*, 2007; Jemal *et al.*, 2007; Kerlikowske *et al.*, 2007; Ravdin *et al.*, 2007; Robbins & Clarke, 2007), Germany (Katalinic & Rawal, 2008), France (Allemand *et al.*, 2008), and Switzerland (Bouchardy *et al.*, 2006). The most striking changes were observed in countries with the highest rates of postmenopausal estrogen use before the WHI-EPT trial results were known. Declines were not reported in Norway or Sweden (Zahl & Maehlen, 2007) or in African-Americans in the USA (Hausauer *et al.*, 2007). Most of the studies were based on representative regional or national cancer registries, with validated diagnoses, but had only self-reported data on individual hormone treatment or mammography.

A recent review of 21 papers published from 1987–2007 on the incidence of breast cancer in the USA in the 1980s (Krieger, 2008) documented the rise in breast cancer in women aged 50 years and older (9.9% per year in the 1980s) and the decrease (by 50%) since 2002 in the context of changing frequency of hormone therapy. In a French study (Allemand *et al.*, 2008), the age-adjusted breast cancer incidence increased 2.1% per year during 2000–04; decreased 4.3% during 2004–05, and 5.3% during 2005–06, a decrease observed only in women aged 50 years and older. This decrease was not likely to be explained by decreased mammography after cessation of hormones because the use of mammography increased by 335% during 2000–06.

The only study of time trends in breast cancer incidence that provides data on cancer incidence by cancer stage and estrogen receptor positive (ER+) status, mammography use, and hormone prescription rates in the same cohort comes from a large pre-paid health care plan in the USA, which included screening mammography, pharmacy dispensing of hormone therapy, and a tumour registry (Glass *et al.*, 2007). The age-adjusted incidence rates of breast cancer increased by 25% from the early 1980s to 1992–93, then increased an additional 15% through 2000–01, and dropped by 18% in 2003–04. Increases were mainly for ER+ breast cancers in women aged 45 years and older. During 1993–2006, 75–79% of women older than 45 years of age were screened within the previous 2 years. Postmenopausal hormone prescriptions, primarily estrogen plus progestogen, increased during 1988–2002, and dropped by approximately 75% after 2002, coincident with the publication of the WHI-EPT (estrogen plus progestogen) clinical trial results that showed that combined hormone therapy increased the risk of heart disease and breast cancer (Rossouw *et al.*, 2002).

Overall, the studies of time trends in ER+ breast cancers in women aged 45 or older are compatible with a substantial increase in breast cancer risk associated with increasing menopausal hormone use, with a remarkable decrease concurrent with the release of the WHI-EPT results. Although the increase in breast cancer could be partly explained by the increasing use of mammography in women concordant with increasing hormone therapy, the decrease in breast cancer concurrent with reduced hormone was not explained by a decrease in mammography.

2.1.3 Cohort studies

All five cohort studies (Stahlberg *et al.*, 2004; Fournier *et al.*, 2005; Lee *et al.*, 2006; Rosenberg *et al.*, 2006a; Corrao *et al.*, 2008) reported an increased risk of breast cancer in postmenopausal women using estrogen plus progestogen (see Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-13-Table2.2.pdf).

In a cohort study using data from a previously published Danish Nurses Study, Stahlberg *et al.* (2004) reported that current estrogen plus cyclical progesterone-like progestogen, cyclical testosterone-like progestogen, or continuous testosterone-like progestogen were each significantly associated with an increased risk of ductal carcinoma (OR, 3.10; 95%CI: 1.69–5.67; OR, 2.15; 95%CI: 1.31–3.54; and OR, 4.10; 95%CI: 2.29–7.30, respectively).

Fournier *et al.* (2005) reported the risk of breast cancer in 54548 postmenopausal French women who were followed an average of 2.8 years postmenopause, during which time 948 new invasive breast cancers were diagnosed. There was a similar increased risk of breast cancer with transdermal/percutaneous (RR, 1.4; 95%CI: 1.2–1.7) and oral estrogens (RR, 1.5; 95%CI: 1.1–1.9) when combined with a synthetic progestogen, compared to the risk in non-hormone users. There was no increased risk when estrogen was combined with micronized progesterone (RR, 0.9; 95%CI: 0.7–1.2) (*P* test for heterogeneity < 0.001).

Lee *et al.* (2006) reported a prospective study of combined hormone therapy and breast cancer in 55371 African-American, Native Hawaiian, Japanese-American, Latina, and Caucasian postmenopausal women from the US Multiethnic Study. The authors provide no information about the type of estrogen or progestogen used, but combined equine estrogen and medroxyprogesterone acetate accounted for more than 80% of combined hormone prescriptions in the USA during the mid-and late 1990s. Current use of estrogen plus progestogen was associated with a significantly increased risk of breast cancer within the first 5 years of use (adjusted RR, 1.43; 95%CI: 1.06–1.93), and this risk increased with duration of use. The increased risk was

associated with both ductal and lobular cancer, more advanced cancer (based on standard pathological criteria), and ER+/Progesterone Receptor positive (PR+), ER+/PR-, and ER-/PR- tumours. It was statistically significant in all ethnic groups, and persisted after adjusting for the lower frequency of mammograms in women not taking hormones.

Rosenberg *et al.* (2006a) reported an 8-year follow-up study of 32559 African-American women. Compared to non-hormone users, the incidence rate ratio associated with 10 or more years of estrogen plus progestogen was 1.45 (95%CI: 0.94–2.23). Shorter durations of use were not associated with estrogen plus progestogen therapy in these African-American women.

Corrao *et al.* (2008) followed 73505 Italian women in Lombardia who had received at least one prescription for postmenopausal hormone therapy in 1998–2000, and were followed until 2005. More than 88% began treatment using transdermal estradiol; and combined hormone use was assumed because few Italian women have had a hysterectomy. Breast cancer risk increased with duration of therapy and was greater with oral than with transdermal estradiol. The odds ratio for at least 25 months of transdermal estradiol was 1.27 (95%CI: 1.07–1.51), compared to 2.14 (95%CI: 1.43–3.21) for oral estradiol (P for heterogeneity < 0.01). This difference is consistent with and somewhat larger than that reported in the initial report from the Million Women Study (Beral *et al.*, 2003), with a similar mean follow-up of 2.6 years.

A new analysis from Prentice *et al.* (2008) combined data from the WHI-EPT trial (n = 16608) and the subset of women from the WHI observational study who were either not taking hormones at baseline (n = 32084) or were users at enrollment of the same hormone regimen used in the WHI-EPT trial (n = 25328). Women included from the observational study were also required to have had a mammogram in the past two years, to parallel the protocol in the trial. Hazard ratios for estrogen plus progestogen users compared to non-users were close to 2 (95%CI: 1.86–2.20) in the trial and the observational study groups, except for the clinical trial participants without prior hormone use who had a much smaller risk ratio (1.13). Women who initiated hormone therapy within 5 years of the menopause had a significantly higher risk of breast cancer than those who initiated hormone therapy later; and the risk increased with duration of use (HR, 1.85; 95%CI: 1.03–3.34) for 2–5 years of use and (HR, 2.75; 95%CI: 1.73–4.39) for more than 5 years of use.

A new analysis of the Million Women Study by Reeves *et al.* (2006) assessed the relative risk of current hormone therapy for different histological types of breast cancer. In analyses of current combined hormone use versus never use, the risk was significantly increased for all types of cancers for which there were more than 50 cases, including lobular cancer (n = 503, RR, 2.80; 95%CI: 2.46–3.18), tubular cancer (n = 186, RR, 3.51; 95%CI: 2.80–4.41), and ductal cancer (n = 2241, RR, 2.0; 95%CI: 1.89–2.12). The risk for these three most common types of breast cancer increased significantly with increasing duration of therapy.

2.1.4 Case–control studies

Four case–control studies also showed an increased risk of breast cancer in women using estrogen plus progestogen (Li *et al.*, 2006; Rosenberg *et al.*, 2006b, c; Wu *et al.*, 2007; see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-13-Table2.3.pdf).

In a Swedish study (Rosenberg *et al.*, 2006c), women who had used a medium-potency estrogen (i.e. not estriol) plus a progestogen for 5 or more years had a significantly increased risk of lobular cancer (OR, 5.6; 95%CI: 3.2–9.7), tubular cancer (OR, 6.5, 95%CI: 2.8–14.9), and ductal cancer (OR, 2.3; 95%CI: 1.6–3.3). In another report

from the same study (Rosenberg *et al.*, 2006b), estrogen (mainly estradiol) plus a progestogen (mainly levonorgestrel or norethisterone) for at least 5 years significantly increased the risk of ER+/PR+ cancers (OR, 3.0; 95%CI: 2.1–4.1) but not ER-/PR- tumours (OR, 1.3; 95%CI: 0.7–2.5).

A study of Asians (Chinese, Japanese or Filipino) living in the USA (Wu *et al.*, 2007) found a 26% increased risk of breast cancer among current users of estrogen plus progestogen for each 5 years of use (OR, 1.26; 95%CI: 1.04–1.52).

Li *et al.* (2006) reported breast cancer histology in a multicentre case–control study in the USA. Compared to never use, current use of estrogen plus progestogen was associated with an increased risk of all types of breast cancer; the excess risk was statistically significant for ductal-lobular (OR, 2.9; 95%CI: 1.7–4.9) and tubular (OR, 3.2; 95%CI: 1.3–7.5) cancers.

2.1.5 Postmenopausal breast cancer risk with prior oral contraceptive use, hormone therapy use, or both

Dumeaux *et al.* (2005) reported breast cancer risk in current hormone users according to prior use of hormones in a French cohort of 68670 postmenopausal women. The most widely used postmenopausal hormone regimen was transdermal estrogen in combination with either micronized progesterone or a progesterone derivative. In women currently using hormone therapy, a history of prior oral contraceptive use did not further increase the risk (OR, 0.91; 95%CI: 0.81–1.03), but women with a history of postmenopausal hormone use did have an increased risk (OR, 1.41; 95%CI: 1.26–1.59). Results were similar for women who had used both oral contraceptives and postmenopausal hormones (OR, 1.43; 95%CI: 1.25–1.64).

In contrast, in a Norwegian cohort study of 30118 postmenopausal women, reported by Lund *et al.* (2007), current users of hormone therapy sustained a significantly greater risk of breast cancer if they were former oral contraceptives users (RR, 2.45; 95%CI: 1.92–3.12) than if they had never used oral contraceptives (RR, 1.67; 95%CI: 1.32–2.12) (P = 0.002). The odds ratios in current hormone users who were past oral contraceptives users were very similar in women currently using estrogen (primarily estradiol) alone (OR, 2.63; 95%CI: 1.65–4.20) or estrogen plus a progestogen (primarily a testosterone derivative) (OR, 2.55; 95%CI: 1.94–3.35).

2.1.6 New WHI-EPT clinical trial analyses

Anderson *et al.* (2006) compared breast cancer rates in women in the WHI-EPT trial according to their history of menopausal hormone use before beginning the trial. Despite controlling for an extensive list of potential confounders, there was a significantly greater risk of invasive breast cancer in women who reported pre-WHI postmenopausal hormone use (HR, 1.96; 95%CI: 1.17–3.27) compared to the women without such a history (HR, 1.02; 95%CI: 0.77–1.36). These women also had a significantly higher risk of a larger tumour, higher number of positive nodes, and more regional/metastatic disease, but the number of women in these subsets was small and the 95% confidence intervals were large. The authors note that they did not adjust for multiple comparisons and that there were significant differences between the women who did or did not use hormones before entering the WHI-EPT trial, not all of which could be characterized well enough to exclude residual confounding.

Heiss *et al.* (2008) examined the effect of stopping combined hormone treatment 2–3 years after the WHI-EPT trial was stopped. More invasive breast cancers occurred in women assigned to continuous estrogen and progestogen than women assigned to placebo (HR, 1.27; 95%CI: 0.91–1.78), with a risk similar to that observed for combined therapy during the trial (HR, 1.26; 95%CI: 1.02–1.55). These data are the strongest evidence for a continued excess risk 2–3 years

after abrupt cessation of hormone therapy. [The Working Group noted that one limitation of this study is that women who abruptly stopped hormone therapy at the end of the trial may have restarted within a year when menopause symptoms recurred.]

In conclusion, many new studies confirm the earlier studies reporting that estrogen plus progestogen increases the risk of breast cancer in postmenopausal women. The more recent studies provide additional evidence that the increased risk begins within 5 years of initiating combined therapy and increases with increasing duration of therapy. The data are insufficient to determine whether the risk differs by estrogen type, dose, or route of administration or the progestogen type (progesterone or progesterone-derived versus testosterone derived) or regimen (continuous versus cyclic) progestogen. The data provide no consistent evidence that any histological type of cancer is more often associated with combined hormone therapy in postmenopausal women.

2.1.7 *Postmenopausal hormone therapy after breast cancer*

Col *et al.* (2005) published a meta-analysis of two uncontrolled and unblinded randomized trials and eight observational studies of breast cancer survivors, of whom 1316 reported unspecified hormone therapy and 2839 did not. The trials showed that hormone therapy increased breast cancer recurrence (RR, 3.41; 95%CI: 1.59–7.33), while the observational studies suggested it reduced risk (RR, 0.64; 95%CI: 0.50–0.82). The apparently protective associations in observational studies were attributed by the authors to probable selection for hormone treatment of younger, more often node-negative women.

Holmberg & Anderson (2004) reported preliminary results from HABITS, a randomized clinical trial of hormone therapy for menopause symptoms in Scandinavian patients aged 40–70 years old who had a history of breast cancer (up to Stage II), were free of recurrence an average of 3 years after cancer treatment, had no other serious disease, and had severe menopause symptoms. Women were randomly assigned to hormones or to the best available symptom treatment without hormones. Hormone treatment in women without a hysterectomy included either cyclic or continuous estrogen and progestogen. At the time the trial was stopped early for harm, after an average of 2 years with at least one follow-up visit, the hazard ratio for women assigned to any estrogen regimen with or without a progestogen was 3.5 (95%CI: 1.5–8.1). The risk was not changed after adjusting for prior use of hormone replacement therapy, tamoxifen, and ER positivity.

In a follow-up study, Holmberg *et al.* (2008) showed persistence of harm after an extended 4-year follow-up of 442 of the 447 women in the HABITS trial; 39 of the 221 women in the hormone-treated group and 17 of the 221 women in the untreated group had experienced recurrent breast cancer (HR, 2.4; 95%CI: 1.3–4.2), i.e. 19 additional cases since the trial had been stopped. Compared to the 100 women taking estrogen plus continuous progestogen, the hazard ratio for recurrence was increased in women taking different types of hormone therapy (HR, 1.4 for the 150 women taking estrogen plus a continuous progestogen; 1.4 for the 100 taking sequential progestogen, and 1.4 for the 53 women taking progestogen, primarily norethisterone acetate), but numbers in each group were small and confidence intervals were wide.

Concurrent with the HABITS trial, the Stockholm randomized clinical trial also enrolled Swedish women with a history of breast cancer (von Schoultz & Rutqvist, 2005). The authors reported that the 77% of women who had had a hysterectomy were treated with estradiol valerate, and 23% with estradiol plus cyclic or spaced low-dose medroxyprogesterone acetate. After a median follow up of 4.1 years, there were 11 breast cancer recurrences in

the hormone-treatment group and 13 in the control group (HR, 0.82; 95%CI: 0.35–1.9). The authors speculated that the absence of harm in the Stockholm trial could reflect the low dose of medroxyprogesterone instead of the norethisterone acetate progestogen used in HABITS, but they also report that the women in this trial had less node-positive cancer, and were more likely to have had prior treatment with tamoxifen than women in the HABITS trial.

In summary, there is consistent evidence that combined estrogen–progestogen menopausal therapy increases the risk of breast cancer. There is evidence for an increasing risk with increasing duration of use among current users. However, determining whether all current formulations and treatment regimens are equally carcinogenic is not possible on the available data.

2.2 Cancer of the endometrium

The previous *IARC Monograph* (IARC, 2007) concluded that there is *sufficient evidence* that estrogen plus progestogen for at least 14 days can prevent estrogen-induced endometrial cancer. The most compelling evidence was from the Million Women Study (Beral *et al.*, 2005), and from the WHI-EPT trial (Anderson *et al.*, 2003), where endometrial cancer rates were low and were not increased by 5 years of continuous combined estrogen plus progestogen in a single tablet. The Million Women Study (1320 endometrial cancer cases) showed that the protective effect of a progestogen added to daily estrogen increased with increasing number of days/month that progestogen was used, while the WHI-EPT results (58 endometrial cancer cases) related only to daily (continuous) combined hormone therapy and only to one progestogen, medroxyprogesterone acetate.

Since the last *IARC Monograph*, two new case–control studies and two cohort studies have been published on the association of estrogen plus progestogen with endometrial cancer. All are from the USA, where more than 80% of combined estrogen regimens were conjugated equine estrogen plus medroxyprogesterone acetate. The newer studies provide contradictory evidence about the minimum progestogen required to reduce the estrogen-induced risk of endometrial cancer.

In a population-based case–control study in Pennsylvania, Strom *et al.* (2006) compared 511 endometrial cancer cases detected by active surveillance of regional hospitals with 1412 controls of similar age identified mainly from random-digit dialling. The history of hormone use was determined mainly by telephone, using a structured questionnaire and memory aids mailed in advance. Use of combined hormones of any duration was not associated with an increased risk of endometrial cancer (OR, 0.8; 95%CI: 0.6–1.1) (see Table 2.4 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-13-Table2.4.pdf). Numbers were too small for useful comparisons of continuous and sequential progestogen therapy.

Using data from three population-based case–control studies in different counties of Washington state, Weiss *et al.* (2006) examined the aggressiveness of endometrial cancer (based on pathology review) in 1304 cases from the state cancer registry, and 1779 controls of similar age who were recruited by random-digit dialling. Combined hormone therapy was not significantly associated with aggressive endometrial cancer (OR, 1.6; 95%CI: 0.4–7.2). The risk for the least aggressive endometrial cancer was increased with combined therapy when the progestogen was used for less than 10 days a month for at least 4 years (OR, 6.2; 95%CI: 3.2–12.0) and for 10–24 days/month for at least 4 years (OR, 2.9; 95%CI: 1.6–5.0).

In a cohort study of 30379 women from the US National Cancer Institute Breast Cancer Detection Demonstration Project, started in 1979 with a 13-year follow-up, combined hormone use was first queried in 1987–89, and

periodically thereafter by mail and telephone (Lacey *et al.*, 2005). Cancer was identified from self-reports, medical records, cancer registries, and death certificates. Endometrial cancer risk was increased with exclusive use of estrogen plus progestogen for less than 15 days/month (RR, 3.0; 95%CI: 2.0–4.6, 32 cases) and for more than 15 days/month (RR, 2.3; 95%CI: 1.3–4.0, 15 cases) (see Table 2.5 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-13-Table2.5.pdf). Risk increased with increasing duration of use for both regimens.

A cohort study of 73211 women from the National Institutes of Health-AARP Diet and Health Study included 51312 women who either never used estrogen or only used estrogen plus progestogen for at least 10 days/cycle (Lacey *et al.*, 2007). Hormone use was self-reported by mail, and cases were ascertained from state cancer registries and death indices. Compared to non-hormone users, neither estrogen plus progestogen for 10–14 days/cycle (RR, 0.74; 95%CI: 0.39–1.40, based on 11 cases) or for at least 20 days/cycle (RR, 0.80; 95%CI: 0.55–1.15, based on 35 cases) was associated with an increased risk of endometrial cancer. Similar results were seen in analyses restricted to women who had used combined therapy with either regimen for at least 3 years.

In conclusion, three of the four new US studies do not show consistent prevention of estrogen-associated endometrial cancer risk in women taking estrogen plus sequential progestogen (mainly medroxyprogesterone acetate) for at least 15 days a month, and contradict findings reported in many earlier studies (Anderson *et al.*, 2003; Beral *et al.*, 2005). [The Working Group noted that the reason for these differences is not clear, but poor hormone regimen recall or adherence cannot be excluded.]

There is consistent evidence that the risk of endometrial cancer is increased in women taking unopposed estrogen, and the increased risk remains evident when the opposing progestogen is taken for less than 15 days per month. The risk of endometrial cancer is inversely associated with the number of days per month that progestogens are allied to the regimen. It is not known whether continuous use (or daily use) reduces the risk of endometrial cancer compared to baseline.

2.3 Cancer of the colorectum

At the time of the previous *IARC Monograph* (IARC, 2007), data from two randomized trials, four cohort studies, and three case–control studies showed no elevated risks of colorectal cancer in women taking combined postmenopausal hormones. In fact, all but one study showed a relative risk estimate less than one (with a statistically significant reduction in two of these) suggesting protection. The reduced risk was mainly in recent users and unrelated to duration of use. Since the last review, there have been two new case–control studies and one cohort study that included analyses largely restricted to (or almost entirely restricted to) estrogen plus progestogen regimens.

A US population-based case–control study conducted in Washington state of 578 cancer cases and 590 controls, in which a history of hormone use and covariates was based on a 60-minute telephone interview, showed a 40% reduced risk of colorectal cancer (OR, 0.60; 95%CI: 0.05–0.09) in women who used combined hormone therapy exclusively for at least 5 years compared to non-users (Newcomb *et al.*, 2007) (see Table 2.6 available at http://monographs.iarc.fr/ ENG/Monographs/vol100A/100A-13-Table2.6.pdf). The reduced risk was restricted to current users. There was no association with cancer stage at diagnosis.

A German case–control study of 354 cases and 1422 age-matched controls (Dinger *et al.*, 2007) showed no significant associations with colorectal cancer by progestogen type (medroxyprogesterone use was rare), duration of progestogen use, or sequential versus continuous progestogen

use. The number of cancer cases in each subset analysis was small.

A cohort study from Lund, Sweden (Nazeri *et al.*, 2006) included 2452 women who reported estrogen plus progestin therapy, primarily a fixed-dose preparation containing 2 mg of estradiol and 2 mg of norethisterone, and 3600 women not taking postmenopausal hormones. There was a reduced risk of colorectal cancer in women taking combined hormones (OR, 0.18; 95%CI: 0.04–0.84) based on only 16 cases in the no-hormone group and two cases in the women reporting mostly combined hormone therapy (see Table 2.7 available at http://monographs.iarc.fr/ ENG/Monographs/vol100A/100A-13-Table2.7.pdf). Information about the type of estrogen or progestogen was not provided.

[The Working Group noted that one of two case–control studies and a single cohort study found that combined hormone therapy reduced the risk of colorectal cancer, which is compatible with suggestive evidence in the previous *IARC Monograph*. One of these three studies found that reduced risk was seen only with current hormone use of combined hormone therapy. These results are suggestive but insufficient to conclude that estrogen plus progestogen therapy protects against colorectal cancer, or to conclude that this putative effect varies by type of estrogen or progestogen used.]

2.4 Cancer of the ovary

In the previous *IARC Monograph* (IARC, 2007), results from two randomized trials, four cohort studies, and three case–control studies were inadequate to establish an association between ovarian cancer and combined estrogen–progestogen therapy. The largest clinical trial (WHI), in which 16608 women were assigned to conjugated equine estrogen and continuous medroxyprogesterone acetate or placebo, did not show a significantly increased risk of ovarian cancer (RR, 1.58; 95%CI: 0.77–3.24) (Anderson *et al.*, 2003).

Since the previous *IARC Monograph*, there have been two new case–control studies, three new cohort studies, and one new meta-analysis on estrogen plus progestogen therapy and risk of ovarian cancer.

Moorman *et al.* (2005) reported a North Carolina (USA) population-based case–control study of ovarian cancer with 364 cases and 370 controls in postmenopausal women (see Table 2.8 available at http://monographs.iarc.fr/ ENG/Monographs/vol100A/100A-13-Table2.8.pdf). Exposure to hormone therapy and covariates was obtained by a 90-minute interview. Sources of cancer cases included a rapid case ascertainment system and a state-wide cancer registry (only 70 cases and 87 controls used combined estrogen and progestogen exclusively). The only significant association was in women who had used combined hormones, but not exclusively, for > 119 months (22 cases, 14 controls); these women had an odds ratio of 2.4 (95%CI: 1.1–5.3). Types of cancer did not differ by hormone use history.

Rossing *et al.* (2007) reported on a US case–control study of 1054 women (440 cases, 614 controls) in western Washington state. Hormone exposure was determined by interview, aided by photographs to identify pills. Cancer data were from the regional cancer registry. No increased risk was reported among current users who used only estrogen plus progestogen, regardless of duration of use (OR, 1.1; 95%CI: 0.8–1.5).

The Million Women Study of postmenopausal women is the most compelling new cohort study based on its size, prospective design, large number of ovarian cancer cases, data on histological subtypes, high proportion of women taking hormone therapy (30%), and data for the most important covariates including hysterectomy (Beral *et al.*, 2007). In analyses limited to estrogen plus progestogen use (different stratified analyses included 69263 cancer cases), the

risk of ovarian cancer was significantly increased only in women who had taken combined hormones for at least 5 years (RR, 1.53; 95%CI: 1.27–1.84), and was similar for continuous or combined regimens. The risk was significantly increased for estrogen plus norethisterone, non-significantly increased for norgestrel, and not increased for medroxyprogesterone acetate. The overall increased risk of hormone therapy was primarily for epithelial serous tumours; histological data were not shown stratified by combined hormone therapy (see Table 2.9 available at http://monographs.iarc.fr/ ENG/Monographs/vol100A/100A-13-Table2.9.pdf).

A second cohort study from the NIH-AARP Diet and Health Study (Lacey *et al.*, 2006), which included 97638 postmenopausal women (of whom 73483 did not undergo a hysterectomy, and 51698 had used no hormone therapy or only estrogen plus progestogen), reported a significantly increased risk associated with 5 or more years of estrogen plus progestogen use either sequentially (RR, 3.09; 95%CI: 1.68–5.68) or continuously (RR, 1.82; 95%CI: 1.03–3.23).

The US Nurses Health Study followed 7394 postmenopausal women who had at some point reported current use of combined estrogen plus progestogen and 20853 never users (Danforth *et al.*, 2007) (the cohort was followed from 1976–2002, but few women were using estrogen plus progestin until the 1980s, and the duration of relevant follow-up and the number of cases for different analyses are not clear). No significant association between estrogen plus progestogen use and epithelial ovarian cancer was observed (RR, 1.04; 95%CI: 0.82–1.32, based on 82 cases), or by duration or recency of use, but there were only ten ovarian cancer cases among women using estrogen plus progestogen exclusively. There was also no association with serous (RR, 1.12; 95%CI: 0.84–1.51, based on 49 cases) or endometrioid tumours (RR, 1.04; 95%CI: 0.53–2.03, based on 15 cases).

A meta-analysis of 42 studies of hormone therapy and ovarian cancer published from 1966–2006 included 30 case–control studies, seven cohort studies, one randomized clinical trial and four cancer registry studies, with 12238 ovarian cancer cases. The summary risk for ever versus never estrogen plus progestin use, based on 31 data sets but no explicitly stated number of cancer cases, was 1.11 (95%CI: 1.02–1.21). There was no evidence for a significantly increased risk per year of use (22 data sets). Risks were slightly greater for European women (OR, 1.06; 95%CI: 1.03–1.09, based on 14 data sets) than for North American women (OR, 1.00; 95%CI: 0.96–1.04, based on seven data sets). Within the estrogen plus progestogen data sets, there were no differences by histological subtypes of ovarian cancer. Funnel plots suggest publication bias (not publishing small studies showing increased cancer risk) (Greiser *et al.*, 2007).

The overall risk estimate in a recent meta-analysis was 1.1 for ever versus never use. The two new case–control studies and three new cohort studies of ovarian cancer and combined hormone therapy suggest only very small increased ovarian cancer risk after use for 5 or more years; however, the evidence is not consistent across studies.

2.5 Cancer of the skin

Since the previous *IARC Monograph* (IARC, 2007), an updated analysis of data from a hospital-based case–control study in San Francisco, USA (Lea *et al.*, 2007), and a report on a hospital-based case-control study in Italy (Naldi *et al.*, 2005) have been published. These were based on 318 cases and 395 frequency-matched controls, and 316 cases and 308 controls, respectively. Neither studies showed any association between menopausal therapy and risk of cutaneous melanoma.

2.6 Cancer of the thyroid

Since the previous *IARC Monograph* (IARC, 2007), results from a population-based case–control study in New Caledonia, France, an area with an unusually high incidence of thyroid cancer, have been published (Truong *et al.*, 2005). Answers to in-person interviews of 293 cases and 354 controls selected from electoral rolls were compared. The odds ratio in women who ever took menopausal therapy at age 45 years or above was 0.9 (95%CI: 0.4–2.2), and no trend in risk with duration of use to 5 years or more was observed.

2.7 Lymphomas and leukaemias

Since the previous *IARC Monograph* IARC (2007), a Danish population-based cohort study of menopausal therapy and risk of non-Hodgkin lymphoma was published and was based on 157024 women aged 40 years or more of whom 23708 were users of menopausal therapy, followed for 13 years with linkage to the health service to determine menopausal therapy prescriptions and to the Danish Cancer Registry to identify cases (40 among users and 310 among non-users) (Nørgaard *et al.*, 2006). The odds ratio for ever use of menopausal therapy was 0.99 (95%CI: 0.71–1.39), no trend in risk with duration of use to 20 or more years was observed.

Ross *et al.* (2005) evaluated the effect of menopausal therapy on risk of leukaemia in a cohort of 37172 postmenopausal women (aged 55–69 years at entry) in Iowa, USA. A total of 201 cases of leukaemia were identified over 16 years of follow-up of which 71 had ever used menopausal therapy. The relative risk for ever use of menopausal therapy was 0.87 (95%CI: 0.65–1.16), with little difference according to type of leukaemia, and no trend in risk with duration of use of 5 or more years was observed.

2.8 Cancers of the central nervous system

In the cohort study based on the Canadian National Breast Screening study (Silvera *et al.*, 2006), 59 incident glioma cases occurred during an average 16.4 years of follow-up in postmenopausal women. Based on answers to a self-administered questionnaire at recruitment into the cohort, the hazard ratio for gliomas was 0.92 (95%CI: 0.54–1.55) in women who ever used menopausal therapy, and no trend in risk with duration of over 3 years of use was observed.

In a population-based case–control study of 45 postmenopausal women with gliomas and 182 controls in Sweden (Wigertz *et al.*, 2006), the odds ratio in women who ever used menopausal therapy was 0.9 (95%CI: 0.4–1.7), and risk did not vary appreciably with duration of use. Among 108 cases of meningioma compared to 185 controls, the odds ratio for ever use of menopausal therapy was 1.7 (95%CI: 1.0–2.8) but no consistent duration–response relationship was observed, the risk being highest among those who had used menopausal therapy for less than a year.

In a cohort study based upon records from the Mayo clinic of 335318 women aged 26–86 years, 18037 were ever users of menopausal therapy (Blitshteyn *et al.*, 2008). Among 1390 women with meningioma, 156 were ever users of menopausal therapy. The odds ratio for ever use of menopausal therapy was 2.2 (95%CI: 1.9–2.6, adjusted for age), with little or no variation by age over 55 years. No analysis of risk by duration of use was reported.

2.9 Cancer of the urinary tract

In a Canadian cohort study of women enrolled in a breast cancer screening trial (Kabat *et al.*, 2007), the hazard ratio for renal cell cancers in women who ever used menopausal therapy was

0.98 (95%CI: 0.69–1.41), and no trend in risk with duration of use was observed.

Two cohort studies in the USA showed no increased risks of cancers of the urinary bladder in users of menopausal therapy. During approximately 26 years of follow-up of 116598 women enrolled in the Nurse's Health Study (McGrath *et al.*, 2006), 22540 were postmenopausal, among which 307 cases of bladder cancer were diagnosed. The use of hormones was ascertained periodically during the follow-up period by mailed questionnaire. The relative risk in postmenopausal women with current use of menopausal therapy (defined in the study as estrogen plus progestogen) was 0.75 (95%CI: 0.44–1.26, based on 18 cases). No analysis of risk by duration of use was reported.

During an average follow-up of 15.3 years, 167 cases of bladder cancer developed in a cohort of 54308 women who were enrolled in the Breast Cancer Detection Demonstration Project (Cantwell. *et al.*, 2006). Menopausal therapy use was based on answers to telephone interviews at the time of recruitment. The relative risk of bladder cancer was 0.98 (95%CI: 0.71–1.37) in women who ever used menopausal therapy. No analysis of risk by duration of use was reported.

2.10 Cancer of the lung

La Vecchia (2006) reviewed the studies relating to menopausal therapy and lung cancer, largely comprising the data considered in the previous *IARC Monograph* (IARC, 2007), and concluded that there was no consistent association between menopausal therapy and risk for lung cancer.

A case–control study nested in the Royal College oral contraceptive study (Elliott & Hannaford, 2006) found no increased risk of lung cancer in ever users of menopausal therapy. No analysis of risk by duration of use was reported. The odds ratio for current users (at the time of diagnosis) was 1.2 (95%CI: 0.2–6.4).

In the Canadian cohort study of women enrolled in a breast cancer screening trial (Kabat *et al.*, 2007), the hazard ratio for lung cancer in women who ever used menopausal therapy was 1.05 (95%CI: 0.85–1.32). No analysis of risk by duration of use was reported.

Liu *et al.* (2005) reported on a population-based cohort study of 44677 middle-aged never-smoking Japanese women, followed for 8–12 years, with 153 lung cancers diagnosed. For women with a natural menopause, the relative risk from hormone use [not further characterized] was 1.19 (95%CI: 0.61–2.30), but for women with an induced menopause, it was 2.40 (95%CI: 1.07–5.40). No analysis of risk by duration of use was reported. [The Working Group considered that the majority of women with an induced menopause were likely to have received estrogen alone.]

Chen *et al.* (2007) conducted a case–control study of 826 women with lung cancer and 531 healthy controls in Taiwan, China. The odds ratio for ever use of menopausal therapy was 0.70 (95%CI: 0.53–0.94). Although no findings by duration of use were presented, many stratified analyses were conducted (e.g. by smoking status, age, exposure to cooking fumes, and family history of lung cancer), all showed similar inverse associations though several of the upper confidence intervals were > 1.0.

2.11 Cancer of the pancreas

In a Canadian cohort study of women enrolled in a breast cancer screening trial (Navarro Silvera *et al.*, 2005), the hazard ratio for pancreatic cancer in women who formerly used menopausal therapy was 0.86 (95%CI: 0.55–1.35), and 0.76 (95%CI: 0.47–1.24) among current users. However, no trend in risk with duration of use was observed.

2.12 Cancer of the stomach

A population-based case–control study in ten Canadian provinces (Frise *et al.*, 2006) compared answers to a self-administered questionnaire by 326 women with gastric adenocarcinoma to answers from an equal number of age-matched controls. The odds ratio in women who ever used menopausal therapy was 0.72 (95%CI: 0.37–1.40). A slight trend in reduction in risk with increasing duration of use was observed, with an odds ratio for 15 or more years of use of 0.42 (95%CI: 0.15–1.16).

2.13 Cancer of the cervix

No further evidence has been published that alters the conclusions reached since the previous *IARC Monograph* (IARC, 2007). There is little evidence to suggest that combined estrogen–progestogen therapy alters the risk for cervical cancer.

2.14 Cancer of the liver

No further evidence has been published that alters the conclusions reached since the previous *IARC Monograph* (IARC, 2007). The data for liver cancer remains too sparse for evaluation.

2.15 Synthesis

A large body of evidence was evaluated for several organ sites, among which the Working Group concluded combined estrogen–progestogen menopausal therapy causes cancer of the breast, and of the endometrium. The increased risk for estrogen-induced endometrial cancer decreases with the number of days per month that progestogens are added to the regimen.

For cancer of the colorectum, the Working Group concluded that it is unlikely that the use of combined estrogen–progestogen menopausal therapy increases the risk of cancers of the colon or rectum. The Working Group concluded that the use of combined estrogen–progestogen menopausal therapy is unlikely to alter the risk of cancer of the thyroid, lung, stomach, liver, urinary tract, pancreas, ovary, cervix, or the risk of lymphoma and leukaemia, cutaneous melanoma, and tumours of the central nervous system.

3. Cancer in Experimental Animals

3.1 Summary of the previous *IARC Monograph*

Oral administration of combined hormonal therapy in mice that are prone to develop mammary tumours resulted in similar incidences of mammary tumours in controls, and in animals treated with conjugated equine estrogens alone or with conjugated equine estrogens plus medroxyprogesterone acetate. However, tumour latency was reduced in animals treated with conjugated equine estrogens plus medroxyprogesterone acetate. Conjugated equine estrogens plus medroxyprogesterone acetate suppressed the development of uterine adenomyosis (Sakamoto *et al.*, 1997a; IARC, 2007).

Oral administration of conjugated equine estrogens alone or with medroxyprogesterone acetate to ovariectomized rats pretreated with the carcinogen 7,12-dimethylbenz[*a*]anthracene increased the incidence of mammary tumours with equal frequency, and to a level equal to that in non-ovariectomized controls (Sakamoto *et al.*, 1997b; IARC, 2007).

3.2 Studies published since the previous *IARC Monograph*

3.2.1 Estradiol and progesterone

(a) Mouse

Medina *et al.* (2007) and Rajkumar *et al.* (2007) transplanted mammary ducts from the glands of *p53* null BALB/c female mice into the cleared mammary fat pads of *p53* wild-type female mice, and also used the activated Her-2/neu transgenic mouse (FVB) model to show in transplanted mice and FVB mice that short-term exposure (2 and 3 weeks) to estradiol and progesterone significantly decreases mammary carcinogenesis in pre-pubertal and mature mice.

(b) Rat

Blank *et al.* (2008) used intact and ovariectomized ACI rats to study the role of progesterone in mammary carcinogenesis. The animals were subcutaneously implanted with low- or high-dose estradiol, progesterone alone, low-dose estradiol plus progesterone, and ovariectomized ACI rats with high-dose estradiol plus progesterone. Also, ovariectomized ACI rats were treated with high-dose estradiol plus progesterone plus testosterone propionate to determine the role of the androgen in hormonal mammary carcinogenesis. In intact but not in ovariectomized rats, continuous exposure to high concentrations of estradiol alone induced mammary carcinogenesis. In ovariectomized ACI rats, mammary carcinogenesis require continuous exposure to high concentrations of estradiol and progesterone. Testosterone had no effect on tumour incidence.

3.2.2 Administration with a known carcinogen

(a) Rat

Yuri *et al.* (2006) examined the effects of different durations of exposure to estradiol and progesterone pregnancy levels on mammary carcinogenesis risk in Lewis rats. Mammary carcinomas were induced with *N*-methyl-*N*-nitrosourea at 28 days of age. One group was left untreated (control group), and one was subcutaneously implanted with estradiol and progesterone pellets. Rats that received long- or short-term estradiol plus progesterone treatment had a decreased incidence of any mammary carcinomas or of mammary carcinomas with a diameter greater or equal to 1 cm, compared to control rats. Long-term (but not short-term) estradiol plus progesterone treatment increased the incidence of fibroadenomas.

Tsukamoto *et al.* (2007) used short-term treatment with estradiol and progesterone to mimic pregnancy in aged female Lewis rats treated with *N*-methyl-*N*-nitrosourea to show promotion of mammary carcinogenesis. Development of *N*-methyl-*N*-nitrosourea-induced mammary carcinomas was accelerated after short-term estradiol plus progesterone treatment, compared with estradiol plus progesterone-untreated rats: the incidence of ≥ 1-cm mammary carcinomas increased (60 versus 44%), latency was shorter (28.7 versus 34.6 weeks), and cancer multiplicity increased significantly (number of all-sized carcinomas per rat; 1.8 versus 0.8).

The effects of hormones on mammary tumorigenesis were studied by Thordarson *et al.* (2004) in growth-hormone-deficient spontaneous dwarf rats. The rats were divided into several groups treated with: bovine growth hormone; estradiol plus progesterone; bovine growth hormone plus estradiol plus progesterone; and a control group. After 1 week, all animals were injected intraperitoneally with the carcinogen *N*-methyl-*N*-nitrosourea. Growth hormone treatment alone increased mammary tumour incidence from 4.8% in controls to 100%. Estradiol plus progesterone treatment did not significantly alter tumorigenesis (0% tumour incidence); estradiol plus progesterone and growth-hormone obliterated the growth hormone-stimulated increase in tumour development (16.7% tumour incidence).

See Table 3.1.

Table 3.1 Studies of cancer in experimental animals exposed to estrogen–progestogen combinations

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, BALB/c (F) 45–58 wk Rajkumar *et al.* (2007) Medina *et al.* (2007)	s.c. implant 50 µg of E_2 and 20 mg of P Initial number: NR	Mammary carcinomas (tumours/transplants):		BALB/c mice used in this experiment were transplanted with mammary ducts from *p53* null BALB/c mice
	Untreated	16/66 (24%)		
	E_2/P (5–7)[a]	2/66 (3%)	$P < 0.05$	
	Untreated	10/20 (50%)		
	E_2/P (5–7)[a]	3/20 (15%)	$P < 0.05$	
	E_2/P (23–25)[a]	4/20 (20%)	$P < 0.05$	
	Untreated, trans. (3)[a]	0/20 (0%)		
	E_2/P (5–7)[a], trans. (3)[a]	3/15 (20%)	$P < 0.05$	
	Untreated, trans. (11)[a]	9/20 (45%)		
	E_2/P (5–7)[a], trans. (11)[a]	4/20 (20%)	$P < 0.05$	
Mouse, FVB (F) 24–32 wk	s.c. implant 100 µg of E_2 and 15 mg of P Initial number: NR			
	Untreated	15/15 (100%)		
	E_2/P (for 3 wk)	6/15 (37%)	$P < 0.05$	
	E_2 (for 3 wk)	5/15 (33%)	$P < 0.05$	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, ACI (F) 6 wk 39 wk Blank *et al.* (2008)	s.c. implant – intact rats Initial number: NR	Mammary carcinomas:		
	Untreated control	0/10		
	Low E_2 (10 µg)	0/15	NS	
	High E_2 (30→3 mg)	5/7	$P < 0.007$	
	30 mg P	0/15	NS	
	Low E_2 plus 30 mg of P	0/15	NS	
	s.c. implant – ovariectomized rats			
	Untreated control	0/10		
	Low E_2	0/15	NS	
	High E_2	0/8	NS	
	30 mg P	0/15	NS	
	Low E_2 plus 30 mg of P	0/14	NS	
	High E_2 plus 30 mg of P	11/11	$P < 0.0001$	
	High E_2 plus 30 mg of P plus 30 mg of TP	14/14	$P < 0.0001$	
Rat, Lewis (F) Dependent on tumour volume (≥ 1 cm) or 29 wk Yuri *et al.* (2006)	MNU: 50 mg/kg bw i.p. E_2/P s.c. implant: 0.5 mg/32.5 mg Initial number: NR	Mammary carcinomas ≥ 1 cm in diameter, any carcinoma:		The E_2/P pellet was replaced every 3–4 wk (long-term) or implanted only once (short-term); 5/14 rats of The MNU + long-term E_2/P group developed fibroadenomas vs 0/20 MNU-treated rats ($P < 0.01$)
	MNU	19/20 (95%), 20/20 (100%)		
	MNU + Long-term E_2/P	5/14 (36%), 9/14 (64%)	$P < 0.01$, $P < 0.01$	
	MNU + Short-term E_2/P	4/19 (21%), 11/19 (58%)	$P < 0.01$, $P < 0.01$ MNU + E_2/P vs MNU	

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Lewis (F) 48 wk Tsukamoto *et al.* (2007)	MNU, 20 mg/kg bw (i.p.) at 7 wk of age MNU, 20 mg/kg bw (i.p.) at 7 wk of age + E_2 (0.5 mg)/P (32.5 mg), one pellet s.c. implanted at 24 wk 29 animals/group	Mammary carcinomas: 11/25 (44%) vs 9/15 (60%) Tumour multiplicity: 0.8 ± 0.2 vs 1.8 ± 0.5 Latency was shorter (28.7 vs 34.6 wk, $P < 0.05$)	$P < 0.05$	
Rat, SDR (F) 20 wk Thordarson *et al.* (2004)	bGH, 40–50 mg/kg bw in 50 µl weekly s.c. injections; MNU, 50 mg/kg bw (i.p.), 1 wk after hormone treatment	Mammary carcinomas:		Spontaneous dwarf rats are growth-hormone-deficient
	Control (MNU only)	1/21 (4.8%)		
	bGH	8/8 (100%)		
	E_2 (30 µg) + P (30 mg/2 mo) (s.c.)	0/11 (0%)		
	bGH and E_2 + P Initial number NR	1/6 (16.7%)	[$P < 0.003$] (bGH and E_2 + P vs bGH)	

[a] from week x to week y or at week x

bGH, bovine growth hormone; bw, body weight; E_2, estradiol; F, female; MNU, *N*-methyl-*N*-nitrosourea; mo, month or months; NR, not reported; NS, not significant; P, progesterone; TP, testosterone propionate; trans., transplanted; i.p., intraperitoneal; s.c., subcutaneously; vs, versus; wk, week or weeks

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Various combinations of estrogens and progestogens are used for hormonal menopausal therapy. Because steroids penetrate normal skin easily, a variety of systems have been developed that deliver estrogens and progestogens parenterally (e.g. transdermal patches), thus bypassing the liver.

While the mechanisms of absorption and distribution of estrogens and progestogens have been known for several years, only recently has an understanding of the genes that encode the enzymes which control the enzymatic steps involved in steroid metabolism been acquired. This applies especially to the oxidative metabolism of estrogens. The phase I enzymes cytochrome P450 1A1 and 1B1 catalyse the production of catechol estrogens further oxidized to estrogen quinones that can induce the formation of DNA adducts. This is counteracted by the phase II enzymes, catechol-*O*-methyltransferase and glutathione *S*-transferase P1, which reduce the levels of catechol and quinones by forming methoxyestrogens and glutathione conjugates. Polymorphic variants of these and other enzymes occur frequently in the population and several are associated with altered enzyme function (IARC, 2007).

One of the particular areas of research on genetic variations concerns the capacity to metabolize hormones. Two common polymorphisms in the cytochrome P450 1A1 (CYP1A1) gene were examined in Chinese women with or without breast cancer. Homozygosity for both alleles was associated with a reduction of risk of borderline significance and the reduction was greater in slender women or those with a long history of menstrual cycles. Use of hormone replacement therapy (HRT) was not considered in this study (Boyapati *et al.*, 2005). In a case–control study of American women, sequence variations in the genotypes of the progesterone receptor and eight enzymes involved in the metabolism of estrogen were assessed in relation to endometrial cancer risk. Women with a particular polymorphism in the *SULT1A1* gene were found to have a significantly elevated risk of endometrial cancer if they received HRT (Rebbeck *et al.*, 2006). In another study of American women with breast cancer and their case–controls, polymorphisms in the genes for progesterone receptor and CYP3A4 were assessed in relation to breast cancer. It was reported that there was an elevated risk of ductal breast cancer or PR+ breast cancers among women with PGR331A alleles and greater than 3 years of combined HRT use. Women with at least one CYP3A4*1B allele who did not have a history of HRT use had a higher risk of ER- breast cancers (Rebbeck *et al.*, 2007).

4.2 Genetic and related effects

4.2.1 Direct genotoxicity

Data on the genetic effects of estrogens and their derivatives indicate that these compounds give rise to reactive metabolites and reactive oxygen species that can induce DNA damage. In recent years, it has been reported that metabolites of estrogen can form adducts on DNA and, based on this, it has been suggested that these lesions could induce the genetic alterations found in cancers (IARC, 2007; see also *Monograph* on Estrogen-only Menopausal Therapy in this volume). The evidence reported since the previous evaluation further substantiates the premise that these mechanisms could contribute to the induction of cancer by estrogens. Since the previous *IARC Monograph* (IARC, 2007), it has been shown that DNA adducts derived from equine estrogens can interfere with DNA synthesis. Using an in-vitro DNA primer extension assay past adducts with bypass polymerases

kappa and eta, it has been shown that 4-hydroxyequilenin adducts are highly mutagenic giving rise to both A→T transversions and A→G transitions. This finding offers a plausible mechanism for the contribution of estrogen adducts to the induction of cancer (Yasui *et al.*, 2006). [The Working Group noted that although these new findings increase the plausibility of these pathways as mechanisms of estrogen-related carcinogenesis, they do not prove that these are the major pathways to estrogen-related cancers. The way in which progestogens might influence the genotoxicity of estrogens is not known.]

Targeted studies have explored how sequence variations in specific genes influence cancer risk, including their effects on hormone receptor function or metabolism of hormones. One of these recently published studies showed that certain genetic variants in the *ERα* gene or progesterone receptor genes were linked to increased mammographic density following HRT (van Duijnhoven *et al.*, 2006). In another study, a specific mutation in the ERα was shown to be linked to a higher risk of breast cancer (Conway *et al.*, 2007). In contrast, in a study of Hispanic and non-Hispanic women in the South western USA, particular genetic variants in the *ERα* or androgen receptor genes were not found to be related to breast cancer risk (Slattery *et al.*, 2007a). In another study by the same group and the same study population, genetic variants in the genes of the insulin-related pathways were assessed with regard to breast cancer risk. In this study, some of the variants examined were related to breast cancer and to an involvement with HRT, with positive or negative relationships observed for particular variants (Slattery *et al.*, 2007b). [The Working Group noted that the study of single-nucleotide polymorphisms (SNPs) and other sequence variations as factors affecting the risk of breast cancer and increased breast density in patients treated with estrogen plus progestogen menopausal therapy are at an early stage of development. Some intriguing findings have been made in individual studies but their interpretation awaits repetition of findings particularly in other laboratories and other study populations.]

Other studies have considered the relationships between various genetic variants and the risk of colon cancer. In a study considering polymorphisms in the genes for IGF-1 and the IGF-binding protein 3 (IGF-BP3), women with the GG genotype of IGF-BP3 who received HRT had a reduced incidence of colon cancer compared with women who did not receive HRT (Morimoto *et al.*, 2005). Genetic variants of ERα, ERβ, and the androgen receptor were studied in relationship to colorectal cancer. It was found that women having an R allele (absence of an Rsa1 restriction site) at 1,082G > A in ERβ had reduced risk of colorectal cancer if they received HRT (Slattery *et al.*, 2005). In another study, SNPs were evaluated in ten estrogen-metabolism-related genes. None of the studied SNPs were associated with an elevated risk of colon cancer. There were no significant differences between women receiving HRT and those who did not (Huber *et al.*, 2005). In a study reflecting the expression levels of DNA mismatch repair genes and measuring the frequencies of mismatch repair defects detected in single- and di-nucleotide repeats, use of estrogen or estrogen–progestogen combinations were evaluated for their effect on colorectal cancer incidences. For all women taking combinations of estrogen and progestogen, there was a reduced risk of colorectal cancer. For those women with little or no evidence of mismatch repair defects, the colon cancer risk reduction was greater (40%). Neither of these effects was observed in women treated with estrogen alone (Newcomb *et al.*, 2007).

4.2.2 Receptor-mediated effects

The literature on the receptor-mediated effects of estrogen–progestogen menopausal therapy was reviewed in the previous *IARC Monograph* (IARC, 2007).

In more recently reported studies, colon cancer cells were transfected to transiently overexpress ERα and cultured with or without estrogen. Gene expression for hTNF-α and DNA fragmentation were evaluated as measures of apoptosis and β-catenin signalling was evaluated as a measure of cell proliferation. ERα overexpression, with or without estrogen treatment, activated DNA fragmentation. ERα overexpression combined with estrogen treatment increased both expression of hTNF-α and DNA fragmentation, and treatment of cells with antibodies to hTNF-α reduced the DNA fragmentation. ERα overexpression combined with estrogen treatment increased expression of proliferation inhibitory molecules p21 and p27 and decreased expression of β-catenin and its downstream pro-proliferation target genes, cyclin D1 and Rb (Hsu *et al.*, 2006). [The Working Group noted that this would be consistent with an estrogen-mediated inhibition of cell proliferation in the colon.]

The expression of steroid receptor coactivator AIB1 was examined in endometrial specimens from patients with endometrial cancer, comparing areas with cancer to normal areas or to those with complex atypical hyperplasia. Expression of AIB1 was assessed by immunohistochemistry in comparison to ERα and progesterone receptors. AIB1 was most highly expressed in endometrial cancers. There were no differences detected between the morphological groups for the expression of other co-regulators tested. It was suggested that when AIB1 and ER are expressed together, ER activity is enhanced, contributing to hyperplasia and malignancy (Balmer, *et al.*, 2006).

Transcriptional activation in vivo by ERα, ERβ and the androgen receptor were compared for estradiol and various equine estrogens with unsaturated B-rings found in conjugated equine estrogens used for HRT. Differences in binding of these estrogens to the ligand-binding domain of ERα were determined by crystallography. In comparison to binding by estradiol, decreased ligand flexibility and hydrophobicity was found for the equine estrogens with unsaturated B-rings (Hsieh *et al.*, 2008).

The effects of synthetic progestogens (progestins) on androgen receptors were considered in a review article. Evidence was reviewed indicating that synthetic hormones that act like progestins may exert their effect in part through their ability to bind to androgen receptors and, through linked pathways, act to suppress estrogen-induced functions. The activity of these progestins occurs independently of their effects through the progesterone receptors. The authors propose that some of the reported excess of breast cancer associated with synthetic progestins, such as medroxyprogesterone acetate, may occur because of their endocrine receptor effects on the androgen receptor (Birrell *et al.*, 2007).

The relationship between radiological breast density and breast histology was evaluated in a study of American breast cancer patients treated with HRT as compared to an equal number of patients not being treated with HRT. Studies focused on areas of the breast not involved with cancer and evaluated radiological breast density and histological fibrous stroma, ducts and lobules, and evidence of cell proliferation rates. The higher breast density in patients receiving HRT was correlated significantly with the presence of fibrous stroma and type I lobules, as well as increased proliferation in ducts and lobules. Estrogen receptor and progesterone receptor levels in the breast tissue did not correlate with HRT therapy or breast density. Because the increase in breast stroma and type I lobules was not related to hormone receptor levels, it is speculated that this effect may be mediated by paracrine factors (Harvey *et al.*, 2008).

In an in-vitro study, medium conditioned by progesterone-treated human breast cancer cells was shown to produce paracrine factors that induced the proliferation of endothelial cells and epithelial breast tumour cells through VEGF receptors. Inhibition of VEGF receptor 1 by antibody and VEGF receptor 2 using SU-1498 blocked the induced proliferation of the epithelial breast tumour cells and endothelial cells, as did anti-progestin mifepristone (RU486) treatment of the tumour cells that conditioned the medium. These results were interpreted as implicating VEGF and possibly other paracrine factors in the progestin-induced proliferation of endothelial cells surrounding breast tumours (Liang & Hyder, 2005).

In a study of ER+ and ER- breast cell lines, both malignant and non-malignant, it was shown that both estradiol and iron (as ferrous sulfate) increased cell proliferation as measured by Ki-67 and proliferating cell nuclear antigen levels and that the combination of estradiol and iron caused even greater increases (Dai *et al.*, 2008).

Norwegian women on systemic HRT had elevated plasma estrogen levels that were comparable to those in premenopausal women. Among women not on HRT, plasma levels of estradiol and serum hormone-binding globulin were influenced by the women's basal metabolic index (Waaseth *et al.*, 2008). In a study of Swedish ER+ breast cancer patients, HRT caused altered expression of 276 genes as compared to their expression in a larger control group of ER+ tumours from patients with unknown HRT usage. It was concluded that patients with postmenopausal HRT use had lower ER protein levels, a distinct gene expression profile, and better disease-free survival (Hall *et al.*, 2006).

4.3 Synthesis

Current knowledge indicates that hormone-receptor-mediated responses are a plausible and probably necessary mechanism for hormonal carcinogenesis by combined estrogen progestogen menopausal therapy . There is also support for the potential involvement of genotoxic effects of combined estrogen–progestogen menopausal therapy estrogenic hormones or their associated metabolic by-products including the formation of DNA adducts, and reactive oxygen species that damage DNA. Recent data suggests that these adducts slow down or block DNA replication and invoke bypass replication, which is prone to mutagenesis.

The predominant effects of combined estrogen–progestogen menopausal therapy associated with hormonal carcinogenesis are likely to be the result of one or more receptor-mediated processes. Progestogens including those used for combined estrogen–progestogen menopausal therapy appear to have the capacity to stimulate cell proliferation in the breast while they inhibit proliferation in the uterus. The magnitude of these effects vary for different synthetic progestogens, with a suggestion that medroxyprogesterone acetate is very active.

Cessation of hormonal treatment may reduce some receptor-mediated effects. The hormone-induced genotoxic effects may be persistent.

Use of combined estrogen–progestogen menopausal therapy was linked to increases in breast density, which is an established risk factor for breast cancer.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of combined estrogen–progestogen menopausal therapy. Combined estrogen–progestogen menopausal therapy causes cancer of the breast, and of the endometrium. The increased risk for estrogen-induced endometrial cancer decreases with the number of days per month that progestogens are added to the regimen.

There is *limited evidence* in experimental animals for the carcinogenicity of conjugated equine estrogens plus medroxyprogesterone acetate.

There is *limited evidence* in experimental animals for the carcinogenicity of estradiol plus progesterone.

Combined estrogen–progestogen menopausal therapy is *carcinogenic to humans (Group 1).*

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