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VOLUME 100 A
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

ESTROGEN-ONLY MENOPAUSAL THERAPY

Estrogen therapy was considered by previous IARC Working Groups in 1987 and 1998 (IARC, 1987, 1999). 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

1.1 Identification of the agents

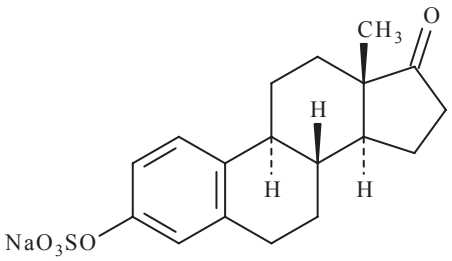
1.1.1 Conjugated estrogens

The term 'conjugated estrogens' refers to mixtures of at least eight compounds, including sodium estrone sulfate and sodium equilin sulfate, derived wholly or in part from equine urine or synthetically from estrone and equilin. Conjugated estrogens contain as concomitant components the sodium sulfate conjugates of 17α-dihydroequilin, 17β-dihydroequilin, and 17α-estradiol (United States Pharmacopeial Convention, 2007).

(a) Sodium estrone sulfate

Chem. Abstr. Serv. Reg. No.: 438-67-5
Chem. Abstr. Name: 3-(Sulfooxy)-estra-1,3,5(10)-trien-17-one, sodium salt
IUPAC Systematic Name: Sodium [(8*R*,9*S*,13*S*,14*S*)-13-methyl-17-oxo-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl] sulfate
Synonyms: Estrone sodium sulfate; estrone sulfate sodium; estrone sulfate sodium salt; oestrone sodium sulfate; oestrone sulfate sodium; oestrone sulfate sodium salt; sodium estrone sulfate; sodium estrone-3-sulfate; sodium oestrone-3-sulfate

(i) Structural and molecular formulae, and molecular mass



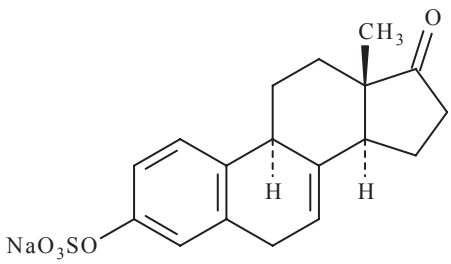
$C_{18}H_{21}O_5S.Na$
Relative molecular mass: 372.4

(b) Sodium equilin sulfate

Chem. Abstr. Serv. Reg. No.: 16680-47-0
Chem. Abstr. Name: 3-(Sulfooxy)-estra-1,3,5(10),7-tetraen-17-one, sodium salt
IUPAC Systematic Name: Sodium (13-methyl-17-oxo-9,11,12,14,15,16-hexahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl) sulfate
Synonyms: Equilin, sulfate, sodium salt; equilin sodium sulfate; sodium equilin 3-monosulfate
Description: buff-coloured amorphous powder, odourless or with a slight characteristic odour [when obtained from natural sources]; white to light-buff-coloured crystalline or amorphous powder, odourless or with a slight odour [synthetic form] (Sweetman,

2008)

(i) Structural and molecular formulae, and molecular mass

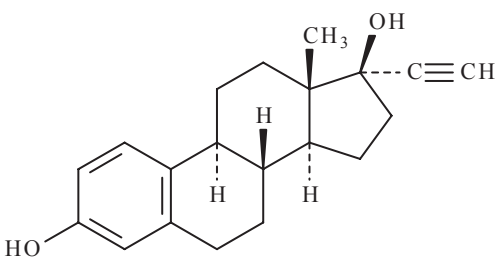


$C_{18}H_{19}O_5S.Na$
Relative molecular mass: 370.4

1.1.2 Ethinylestradiol

Chem. Abstr. Serv. Reg. No.: 57-63-6
Chem. Abstr. Name: (17α)-19-Norpregna-1,3,5(10)-trien-20-yne-3,17-diol
IUPAC Systematic Name: (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthrene-3,17-diol
Synonyms: 17-Ethinyl-3,17-estradiol; 17-ethinylestradiol; 17α-ethinyl-17β-estradiol; ethinylestradiol; 17α-ethinylestradiol
Description: White to creamy- or slightly yellowish-white, odourless, crystalline powder (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

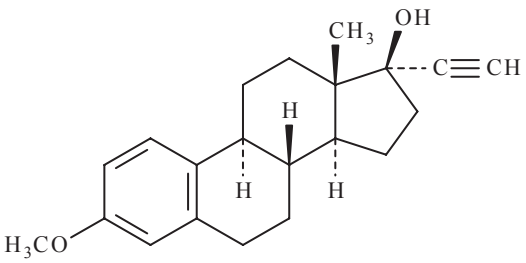


$C_{20}H_{24}O_2$
Relative molecular mass: 296.4

1.1.3 Mestranol

Chem. Abstr. Serv. Reg. No.: 72-33-3
Chem. Abstr. Name: (17α)-3-Methoxy-19-norpregna-1,3,5(10)-trien-20-yn-17-ol
IUPAC Systematic Name: (8*R*,9*S*,13*S*,14*S*,17*R*)-17-Ethynyl-3-methoxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-17-ol
Synonyms: Ethinylestradiol 3-methyl ether; 17α-ethinylestradiol 3-methyl ether; ethinyloestradiol 3-methyl ether; 17α-ethinyloestradiol 3-methyl ether; ethynylestradiol methyl ether; ethynylestradiol 3-methyl ether; 17-ethynylestradiol 3-methyl ether; 17α-ethynylestradiol 3-methyl ether; 17α-ethynylestradiol methyl ether; ethynyloestradiol methyl ether; ethynyloestradiol 3-methyl ether; 17-ethynyloestradiol 3-methyl ether; 17α-ethynyloestradiol 3-methyl ether; 17α-ethynyloestradiol methyl ether; 3-methoxy-17α-ethinylestradiol; 3-methoxy-17α-ethinyloestradiol; 3-methoxy-17α-ethynylestradiol; 3-methoxyethynylestradiol; 3-methoxy-17α-ethynyloestradiol; 3-methoxyethynyloestradiol; 3-methylethynylestradiol; 3-*O*-methylethynylestradiol; 3-methylethynyloestradiol; 3-*O*-methylethynyloestradiol; Δ-MVE
Description: White or almost white to creamy-white, odourless, crystalline powder (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

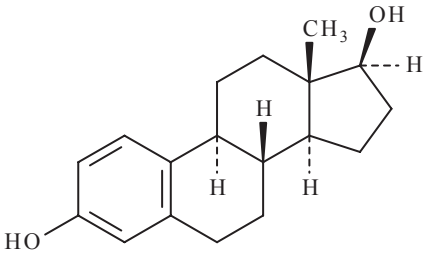


$C_{21}H_{26}O_2$
Relative molecular mass: 310.4

1.1.4 Estradiol

Chem. Abstr. Serv. Reg. No.: 50-28-2
Chem. Abstr. Name: (17*β*)-Estra-1,3,5(10)-triene-3,17-diol
IUPAC Systematic Name: (8*R*,9*S*,13*S*,14*S*,17*S*)-13-Methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,17-diol
Synonyms: Dihydrofollicular hormone; dihydrofolliculin; dihydromenformon; dihydrotheelin; dihydroxyestrin; 3,17*β*-dihydroxyestra-1,3,5(10)-triene; 3,17-epidihydroxyestratriene; *β*-estradiol; 17*β*-estradiol; 3,17*β*-estradiol; (*d*)-3,17*β*-estradiol; oestradiol-17*β*; 17*β*-oestradiol
Description: White or creamy-white, odourless, crystalline powder (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

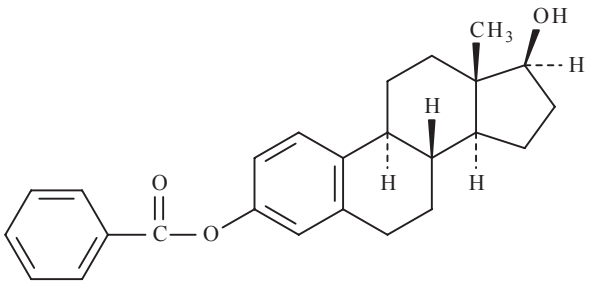


$C_{18}H_{24}O_2$
Relative molecular mass: 272.4

1.1.5 Estradiol benzoate

Chem. Abstr. Serv. Reg. No.: 50-50-0
Chem. Abstr. Name: Estra-1,3,5(10)-triene-3,17*β*-diol, 3-benzoate
IUPAC Systematic Name: [(8*R*,9*S*,13*S*,14*S*,17*S*)-17-Hydroxy-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthren-3-yl] benzoate
Synonyms: Estradiol benzoate; *β*-estradiol benzoate; *β*-estradiol 3-benzoate; 17*β*-estradiol benzoate; 17*β*-estradiol 3-benzoate; estradiol monobenzoate; 1,3,5(10)-estratriene-3,17*β*-diol 3-benzoate; *β*-oestradiol benzoate; *β*-oestradiol 3 benzoate; 17*β*-oestradiol benzoate; 17*β*-oestradiol 3-benzoate; oestradiol monobenzoate; 1,3,5(10)-oestratriene-3,17*β*-diol 3-benzoate
Description: Almost white crystalline powder or colourless crystal (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass



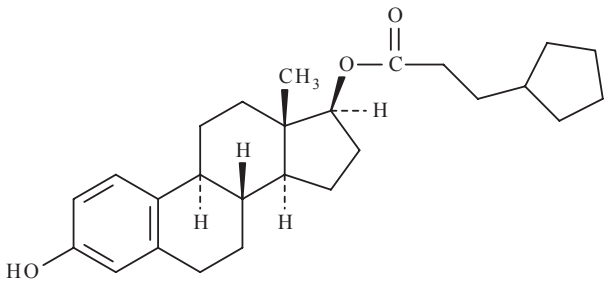
$C_{25}H_{28}O_3$
Relative molecular mass: 376.5

1.1.6 Estradiol cypionate

Chem. Abstr. Serv. Reg. No.: 313-06-4
Chem. Abstr. Name: (17*β*)-Estra-1,3,5(10)-triene-3,17-diol, 17-cyclopentanepropanoate
IUPAC Systematic Name: [(8*R*,9*S*,13*S*,14*S*,17*S*)-3-Hydroxy-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthren-17-yl] 3- cyclopentylpropanoate
Synonyms: Cyclopentanepropionic acid, 17-ester with oestradiol; cyclopentanepropionic acid, 3-hydroxyestra-1,3,5(10)-trien-17*β*-yl ester; depo-estradiol cyclopentylpropionate; depoestradiol cypionate; estradiol 17*β*-cyclopentanepropionate; estradiol cyclopentylpropionate; estradiol 17-cyclopentylpropionate; estradiol

17β-cyclopentylpropionate; 17β-estradiol 17-cyclopentyl-propionate; estradiol cypionate; estradiol 17-cypionate; estradiol 17β-cypionate
Description: White to practically white crystalline powder, odourless or with a slight odour (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

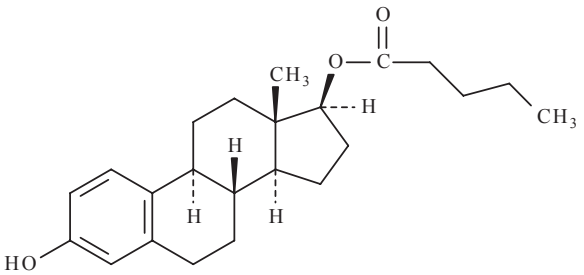


$C_{26}H_{36}O_3$
Relative molecular mass: 396.6

1.1.7 Estradiol valerate

Chem. Abstr. Serv. Reg. No.: 979-32-8
Chem. Abstr. Name: (17β)-Estra-1,3,5(10)-triene-3,17-diol, 17-pentanoate
IUPAC Systematic Name: [(8*R*,9*S*,13*S*,14*S*,17*S*)-3-Hydroxy-13-methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthren-17-yl] pentanoate
Synonyms: Oestradiol valerate; estradiol 17β-valerate; estradiol valerianate; estra-1,3,5(10)-triene-3,17β-diol 17-valerate; 3-hydroxy-17β-valeroyloxyestra-1,3,5(10)-triene
Description: White or almost white crystalline powder or colourless crystal, odourless or with a faint fatty odour (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

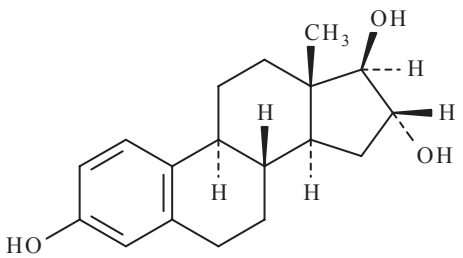


$C_{23}H_{32}O_3$
Relative molecular mass: 356.5

1.1.8 Estriol

Chem. Abstr. Serv. Reg. No.: 50-27-1
Chem. Abstr. Name: (16α,17β)-Estra-1,3,5(10)-triene-3,16,17-triol
IUPAC Systematic Name: (8*R*,9*S*,13*S*,14*S*,16*R*,17*R*)-13-Methyl-6,7,8,9,11,12,14,15,16,17-decahydrocyclopenta[*a*]phenanthrene-3,16,17-triol
Synonyms: Estra-1,3,5(10)-triene-3,16α,17β-triol; estratriol; 16α-estriol; 16α,17β-estriol; 3,16α,17β-estriol; follicular hormone hydrate; 16α-hydroxyestradiol; 3,16α,17β-trihydroxyestra-1,3,5(10)-triene; trihydroxyestrin
Description: White or practically white, odourless, crystalline powder (Sweetman, 2008)

(a) Structural and molecular formulae and relative molecular mass

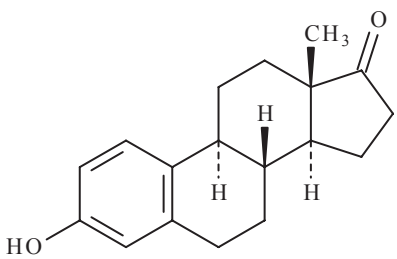


$C_{18}H_{24}O_3$
Relative molecular mass: 288.4

1.1.9 Estrone

Chem. Abstr. Serv. Reg. No.: 53-16-7
Chem. Abstr. Name: 3-Hydroxyestra-1,3,5(10)-trien-17-one
IUPAC Systematic Name: (8*R*,9*S*,13*S*,14*S*)-3-Hydroxy-13-methyl-7,8,9,11,12,14,15,16-octahydro-6*H*- cyclopenta[*a*]phenanthren-17-one
Synonyms: *d*-Estrone; *d*-oestrone
Description: Odourless, small white crystals or white to creamy-white crystalline powder (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass

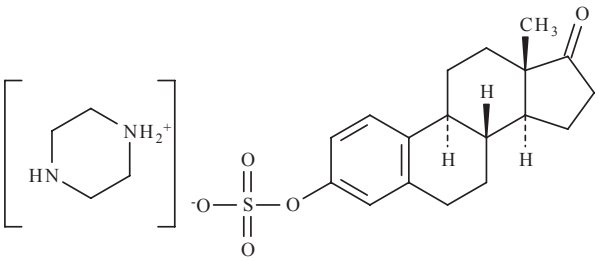


$C_{18}H_{22}O_2$
Relative molecular mass: 270.4

1.1.10 Estropipate

Chem. Abstr. Serv. Reg. No.: 7280-37-7
Chem. Abstr. Name: 3-(Sulfooxy)-estra-1,3,5(10)-trien-17-one, compd. with piperazine (1:1)
IUPAC Systematic Name: [(8*R*,9*S*,13*S*,14*S*)-13-Methyl-17-oxo-7,8,9,11,12,14,15,16-octahydro-6*H*-cyclopenta[*a*]phenanthren-3-yl] hydrogen sulfate: piperazine (1:1)
Synonyms: Piperazine estrone sulfate; piperazine oestrone sulfate; 3-sulfatoxyestra-1,3,5(10)-trien-17-one piperazine salt; 3-sulfatoxyoestra-1,3,5(10)-trien-17-one piperazine salt
Description: White or almost white to yellowish-white, fine crystalline powder, odourless or with a slight odour (Sweetman, 2008)

(a) Structural and molecular formulae, and relative molecular mass



$C_{22}H_{32}N_2O_5S$
Relative molecular mass: 436.6

1.2 Use of the agents

Information for Section 1.2 is taken from IARC (1999) and McEvoy (2007).

1.2.1 Indications

Menopausal estrogen therapy refers to the use of estrogen without a progestogen for women in the period around the menopause, primarily for the treatment of menopausal symptoms but also for the prevention of conditions that become more common in the postmenopausal period, such as osteoporosis and ischaemic heart disease. It is mainly given to women who have had a hysterectomy.

Conjugated estrogens, estradiol and its semi-synthetic esters (especially estradiol valerate), are the main estrogens used in the treatment of menopausal disorders. Their use has also been proposed in the prevention of cardiovascular diseases. Conjugated estrogens have been used extensively in the United Kingdom, Australia, Canada, and the United States of America for the treatment of climacteric [menopausal] symptoms. In Europe, micronised estradiol and estradiol valerate are used. Mestranol, estriol, and estropipate have also been used.

Estrogens may be used adjunctively with other therapeutic measures (e.g. diet, calcium, vitamin D, weight-bearing exercise, physical therapy) to

retard bone loss and the progression of osteoporosis in postmenopausal women, either orally (e.g. estradiol, estropipate, conjugated estrogens) or transdermally (e.g. estradiol).

Estrogens are also used in the treatment of a variety of other conditions associated with a deficiency of estrogenic hormones, including female hypogonadism, castration, and primary ovarian failure. In addition, estrogens may be used in the treatment of abnormal uterine bleeding caused by hormonal imbalance not associated with an organic pathology.

Oral conjugated estrogens and 'synthetic conjugated estrogens A' ['synthetic conjugated estrogens A' are a mixture of nine derivatives of estrone, equilin, estradiol, and equilenin] are used for the management of moderate-to-severe vasomotor symptoms associated with menopause, and for the management of vulvar and vaginal atrophy (atrophic vaginitis); for the latter, topical vaginal preparations are used.

'Synthetic conjugated estrogens B' [a mixture of ten derivatives of estrone, equilin, estradiol, and equilenin] are used for the management of moderate-to-severe vasomotor symptoms associated with menopause. Oral conjugated estrogens are also used for the management of female hypoestrogenism secondary to hypogonadism, castration, or primary ovarian failure.

Estradiol is the most active of the naturally occurring estrogens. Estradiol and its semisynthetic esters are used primarily as menopausal therapy. Estradiol may also be used for female hypogonadism or primary ovarian failure.

Although ethinylestradiol is used most extensively in oral contraceptives in combination with a progestogen, other indications include perimenopausal symptoms, hormonal therapy for hypogonadal women, treatment of postpartum breast engorgement, dysfunctional uterine bleeding, and therapy for carcinoma of the breast and prostate.

1.2.2 Dosages and preparations

Menopausal estrogen therapy is administered in a continuous daily dosage regimen or, alternatively, in a cyclic regimen. When estrogens are administered cyclically, the drugs are usually given once daily for 3 weeks followed by a 1 week washout period, or once daily for 25 days followed by 5 days washout, repeated as necessary.

Menopausal estrogen therapy is available as oral tablets, intranasal sprays, subcutaneous implants, topical applications for vulvovaginal use, intravaginal rings, and transdermal skin patches and gels.

(a) Conjugated estrogens

For the treatment of climacteric symptoms, conjugated estrogens are usually administered orally in a dose of 0.3–1.25 mg daily. Conjugated estrogens may also be administered intravaginally or by deep intramuscular or slow intravenous injection. When parenteral administration of conjugated estrogens is required, slow intravenous injection is preferred because of the more rapid response obtained following this route of administration compared to intramuscular injection. Topical vaginal therapy may be used specifically for menopausal atrophic vaginitis: 0.5–2 g of a 0.0625% cream may be used daily for 3 weeks of a 4-week cycle.

For the management of moderate-to-severe vasomotor symptoms associated with menopause, the usual oral dosage of 'synthetic conjugated estrogens A' is 0.45–1.25 mg daily, usually starting with 0.45 mg daily, and with any subsequent dosage adjustments dependent on the patient's response. For the management of vulvar and vaginal atrophy, the usual oral dosage of 'synthetic conjugated estrogens A' is 0.3 mg daily. The usual initial oral dosage of 'synthetic conjugated estrogens B' for the management of moderate-to-severe vasomotor symptoms associated with menopause is 0.3 mg daily, with any

subsequent dosage adjustment dependent on the patient's response.

For the prevention of osteoporosis, the usual initial oral dosage of conjugated estrogens is 0.3 mg once daily. Subsequent dosage should be adjusted based on the patient's clinical and bone mineral density responses. The drug may be administered in a continuous daily regimen or in a cyclic regimen (25 days on drug, followed by a 5-day washout period, repeated as necessary).

For therapy in female hypoestrogenism, the usual oral dosage of conjugated estrogens is 0.3–0.625 mg daily in a cyclic regimen (3 weeks on drug, followed by a 1-week washout period). For the management of female castration or primary ovarian failure, the usual initial oral dosage of conjugated estrogens is 1.25 mg daily in a cyclic regimen.

For the palliative treatment of prostatic carcinoma, an oral dose of 1.25–2.5 mg conjugated estrogens three times daily has been used. A dose of 10 mg three times daily for at least 3 months has been used for the palliative treatment of breast carcinoma in men and in postmenopausal women. Abnormal uterine bleeding has been treated acutely by giving 25 mg of conjugated estrogens by slow intravenous injection, repeated after 6–12 hours if required; the intramuscular route has also been used.

(b) Single estrogens

Ethinylestradiol has been used for menopausal therapy at doses of 10–20 µg daily.

Mestranol is rapidly metabolized to ethinylestradiol, therefore, acts in a similar fashion to that of estradiol. It has been used as the estrogen component of some preparations for menopausal therapy. It was usually given in a sequential regimen with doses ranging from 12.5–50 µg daily.

Estradiol may be used topically as transdermal skin patches that release between 14–100 µg of estradiol every 24 hours to provide a systemic effect. A low-dose patch supplying 14 µg daily is also available. Topical gel preparations can be applied to also provide a systemic effect; the usual dose is 0.5–1.5 mg of estradiol daily. A topical emulsion of estradiol is also available as the hemihydrate with a daily dose of 8.7 mg. This is also available as a nasal spray, delivering 150 µg of estradiol hemihydrate per spray. The usual initial dose is 150 µg daily. After two or three cycles the dose may be adjusted according to the response; the usual maintenance dose is 300 µg daily but may range from 150 µg once daily up to 450–600 µg daily in two divided doses.

Subcutaneous implants of estradiol may also be used in doses of 25–100 mg with a new implant being given after about 4–8 months, depending on whether therapeutic concentrations of estrogens are detected in the plasma.

Estradiol may be used locally either as 25 µg vaginal tablets, at an initial dose of one tablet daily for 2 weeks, followed by a maintenance dose of one tablet twice a week, or as a 0.01% vaginal cream in initial amounts of 2–4 g of cream daily for 1–2 weeks followed by half the initial dose for a similar period, then a maintenance dose of 1 g up to three times weekly. Also available for the relief of both local and systemic postmenopausal symptoms are local delivery systems using 3-month vaginal rings containing 2 mg of estradiol hemihydrate that release about 7.5 µg of estradiol daily or estradiol acetate that release either 50 or 100 µg of estradiol daily.

Intramuscular injections of estradiol benzoate or valerate esters have been used as oily depot solutions, given once every 3–4 weeks. The cypionate, dipropionate, enantate, hexahydrobenzoate, phenylpropionate, and undecylate esters have been used similarly. The enantate and cypionate esters are used as the estrogen component of combined injectable contraceptives (estradiol and other estrogens have sometimes been used at higher doses for the palliative treatment of prostate cancer and breast cancer in men, and breast cancer in postmenopausal women.)

Estriol is a naturally occurring estrogen with actions and uses that are similar to those described for estradiol valerate. For short-term treatment, oral doses of estriol are 0.5–3 mg daily given for 1 month, followed by 0.5–1 mg daily. Estriol has also been given with other natural estrogens, such as estradiol and estrone, with usual doses of estriol ranging from about 0.25–2 mg daily. Estriol is given intravaginally for the short-term treatment of menopausal atrophic vaginitis as a 0.01% or 0.1% cream or as pessaries containing 500 µg. It has also been given orally for infertility in doses of 0.25–1 mg daily on Days 6 to 15 of the menstrual cycle. Estriol succinate has also been given orally for menopausal disorders. The sodium succinate salt has been used parenterally in the treatment of haemorrhage and thrombocytopenia.

Estrone has been given in oral doses of 1.4–2.8 mg daily in a cyclic or continuous regimen for menopausal symptoms, and as a combination product with estradiol and estriol. Estrone has also been given by intramuscular injection in formulations with oily solutions or aqueous suspensions. When used for menopausal atrophic vaginitis, estrone has been given vaginally.

Estropipate is used for the short-term treatment of menopausal symptoms; suggested doses range from 0.75–3 mg daily, given cyclically or continuously orally; doses of up to 6 mg daily have also been given cyclically. When used long-term for the prevention of postmenopausal osteoporosis, a daily dose of 0.75–1.5 mg is given cyclically or continuously. Estropipate has also been used short-term for menopausal atrophic vaginitis as a 0.15% vaginal cream; 2–4 g of cream is applied daily. It can be given orally in the treatment of female hypogonadism, castration, and primary ovarian failure in doses of 1.5–3 mg daily, in a cyclic regimen; higher doses of up to 9 mg daily given cyclically have also been used.

1.2.3 Formulations

Conjugated estrogens are available as 0.3–1.25 mg tablets for oral administration, as a 25 mg solution for parenteral administration, and as a 0.0625% cream for topical administration. Synthetic conjugated estrogens A and B are available as 0.3–1.25 mg film-coated tablets for oral administration. Estradiol (alone) is available as a 0.06% gel and as a transdermal patch with doses ranging from 14–100 µg/24 hours for topical administration, as a 0.01% cream, and as a 2 mg/ring for vaginal administration. Estradiol (hemihydrate) is available as a 0.25% emulsion for topical administration and as 25 µg (of estradiol) film-coated tablets for vaginal administration. Estradiol (micronized) is available as 0.5–2 mg tablets for oral administration. Estradiol acetate is available as 0.45–1.8 mg tablets for oral administration, and as a 12.4–24.8 mg/ring (0.05–0.1 mg estradiol/24 hours) for vaginal administration. Estradiol cypionate is available as a 5 mg/mL injection solution in oil for parenteral administration. Estradiol valerate is available as a 10–40 mg/mL injection solution in oil for parenteral administration. Estropipate is available as 0.75–3 mg tablets for oral administration.

1.2.4 Trends in use

Early treatment regimens of menopausal symptoms included estrogen-only therapy. After a substantial increase in use in the 1960s and early 1970s, the use of these regimens declined after 1975 when a strong association with the development of endometrial cancer was found. Estrogen-only menopausal treatment is still prescribed for hysterectomized women.

2. Cancer in Humans

Studies were included in this review if they provided information regarding estrogen use (unopposed/alone).

2.1 Cancer of the breast

The previous *IARC Monograph* (IARC, 1999) on postmenopausal estrogen therapy and breast cancer considered the pooled analysis of original data from 51 studies, and also reviewed data from 15 cohort and 23 case–control studies. The majority of studies showed a small increased risk in current users who had been using estrogen for at least 5 years. Several of the studies reviewed included any hormone therapy and were not restricted to estrogen alone. Data were insufficient to determine whether the risk varied with type or dose of estrogen.

The present review of postmenopausal estrogen therapy taken without a progestogen (unopposed estrogen) and breast cancer includes papers published from 1996 to August 2008. It includes one systematic review, over 20 cohort and case–control studies combined, and one randomized trial. Studies were included if they reported estimated relative risks (RRs), hazard ratios (HRs) or odds ratios (ORs) and 95% confidence intervals (CI), and if they compared women who used unopposed estrogen for at least 1 year with women who used no estrogen.

2.1.1 Systematic review

Greiser *et al.* (2005) conducted a meta-analysis of unopposed estrogen therapy and breast cancer in postmenopausal women, which included 12 case–control studies, five cohort studies, and three clinical trials published in 1989–2004.

The summary relative risk for studies published before 1992 showed no increased breast cancer risk for case–control studies (OR, 1.02; 95%CI: 0.93–1.11). The summary estimate for case–control studies published later (OR, 1.18; 95%CI: 1.08–1.30) was similar to that of cohort studies published earlier (OR, 1.19; 95%CI: 1.10–1.28). The summary risks from cohort studies published in 1992 or later were largely driven by the Million Women Study results (OR, 1.30), which showed increased risks only in current hormone users.

2.1.2 Cohort studies

Of the ten cohort studies reported since 1999, four found increased risk of breast cancer from ever use of estrogen alone (Colditz & Rosner, 2000; Beral *et al.*, 2003; Bakken *et al.*, 2004; Ewertz *et al.*, 2005). The largest published cohort study is the United Kingdom Million Women Study (Beral *et al.*, 2003) that included 1084110 women aged 50–64, which found increased risk of breast cancer by duration of use, and type of preparation used (see Table 2.1 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.1.pdf). The increased risk was already evident for women with less than 5 years' estrogen therapy (OR, 1.21; 95%CI: 1.07–1.37). Several other cohort studies found increased risks of breast cancer with longer durations of use of estrogen-alone postmenopausal therapy: Schairer *et al.* (2000) from 1–2 years of use but not longer, Olsson *et al.* (2003) from 48 months or more of use of estriol, Lee *et al.* (2006) from 5 or more years of current use (with a dose–response relationship), and Rosenberg *et al.* (2008) from 10 or more years of use in those with a body mass index (BMI) of less than 25. In two of the cohort studies, there was no evidence of an increased risk of breast cancer from estrogen-alone postmenopausal therapy (Porch *et al.*, 2002; Fournier *et al.*, 2005).

2.1.3 Case–control studies

Of the 16 case–control studies, two studies found increased risk of breast cancer for ever use of estrogen-alone postmenopausal therapy (Newcomb *et al.*, 2002; Rosenberg *et al.*, 2008). In the study of Newcomb *et al.* (2002), the risk increase was restricted to those with 5 or more years of estrogen-alone menopausal therapy, and in current users with use within the previous 5 years. Some studies found increased risks only in subgroups: one for those diagnosed with invasive breast cancer (Henrich *et al.*, 1998), one for users of estrogen-alone postmenopausal therapy for 60 months or more (principally in those diagnosed with a lobular breast cancer) (Chen *et al.*, 2002), one in those who weighed 61.3 kg or more (Wu *et al.*, 2007), one in those with an in-situ breast cancer after 5 or more years of use of estrogen-alone postmenopausal therapy (Ross *et al.*, 2000), and one in those diagnosed with comedo carcinomas (Li *et al.*, 2006). Increased risks in those diagnosed with lobular cancer of the breast was not confirmed in the studies of Daling *et al.* (2002), Li *et al.* (2002), nor in that of Li *et al.* (2003). Kirsh & Kreiger (2002) found borderline increased risks for those with a duration of use of estrogen alone of 10 or more years, while Weiss *et al.* (2002) and Sprague *et al.* (2008) found no increases in risk.

See Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.2.pdf.

2.1.4 Clinical trials

The Women's Health Initiative estrogen only trial (WHI-ET) is the only large clinical trial of unopposed estrogen use (Anderson *et al.*, 2004). Women were required to have an annual mammogram to receive study medication (see Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.3.pdf). At baseline, almost half of the subjects reported prior postmenopausal hormone therapy before randomization. The trial was closed after an average 6.8 years follow-up, at which point 218 incident cases of invasive breast cancer were identified. There was no evidence that oral conjugated equine estrogen (0.625 mg daily) increased the risk of breast cancer. [The Working Group noted that the number of women who continued taking their assigned medication was low, which could have weakened any effects of estrogen.]

2.2 Cancer of the endometrium

The previous *IARC Monograph* summarized data from hree cohort studies and over 30 case–control studies. These consistently showed an increased risk of endometrial cancer in women who received menopausal estrogen therapy. Risk increased with duration of use, and decreased with time since last use, but the risk remained elevated for at least 10 years after cessation of treatment.

2.2.1 Cohort studies

Results from four cohort studies reported since the previous *IARC Monograph* are summarized in Table 2.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.4.pdf), and each found an increased risk of endometrial cancer from the use of unopposed estrogen therapy. In the Million Women Study (Beral *et al.*, 2005), the risk was somewhat lower in women with a BMI of < 25 kg/m^2 than in women with a BMI of 25 kg/m^2 or more. In two of the cohort studies (Lacey *et al.*, 2005, 2007), the risk of endometrial cancer increased with duration of use, and decreased with time since last use. The risk remained elevated over that of non-users after 5 or more years since cessation of use in one study (Lacey *et al.*, 2007), and 10 or more in the other (Lacey *et al.*, 2005).

2.2.2 Case–control studies

Results from five case–control studies reported since the previous *IARC Monograph* are summarized in Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.5.pdf), and each found an increased risk of endometrial cancer in users of unopposed estrogen of 6 months or more, with risks increasing with duration of use. In one study (Shields *et al.*, 1999) that reported additional analyses of data from a prior study, the trend in risk was observed in women with and without other risk factors for endometrial cancer (low parity, hypertension, and BMI), and in women with and without protective factors (oral contraceptive use and history of smoking). A total of 85% of the estrogen users reported using conjugated estrogens. In a population-based study in Sweden (Weiderpass *et al.*, 1999), the primary estrogen in use was estradiol. This study also included women with atypical endometrial hyperplasia. Risk of this condition was also increased in estrogen users, and the risk increased with duration of use. In a third study (Beard *et al.*, 2000), the risk of endometrial cancer was increased for users of both conjugated estrogens and non-conjugated steroidal estrogens. [The Working Group noted that steroidal estrogens were not further defined in the published report.] In one study (Weiss *et al.*, 2006), the aggressiveness of endometrial cancers that had been the subject of a series of population-based cased–control studies was categorized, based on both tumour grade and extent of disease. The risk of tumours of all three levels of aggressiveness increased with duration of use of estrogen for menopausal therapy. The increase in risk was greatest for the cancers with low tumour aggressiveness.

2.3 Cancer of the colorectum

In the previous *IARC Monograph* (IARC, 1999), 12 case–control and seven cohort studies provided information on the use of estrogen therapy and the risk of colorectal cancer. The risk was not increased and appeared to be reduced in half of the studies, though the reduced risk was observed among recent users, and was not related to duration of use.

2.3.1 Case–control studies

Results from the two case–control studies reported since the previous *IARC Monograph* are summarized in Table 2.6 (available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.6.pdf). In one study, no association with colon cancer was found following recent use of estrogen therapy for a 5- or 10-year period (Jacobs *et al.*, 1999). In the second study, the odds ratio of colon cancer for women who had estrogen therapy alone was 0.5 (95%CI: 0.2–0.9) both for those who had used estrogen within the last year, and for those who had used estrogen for 5 or more years (Prihartono *et al.*, 2000).

2.4 Cancer of the ovary

In the previous *IARC Monograph* (IARC, 1999), 12 case–control and four cohort studies that addressed the risk for ovarian cancer were considered. No clear association between estrogen therapy and the risk of ovarian cancer was found. Since then, there have been seven case–control and seven cohort studies investigating estrogen-alone exposure and the risk of ovarian cancer (see Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.7.pdf and Table 2.8 at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.8.pdf).

In five of the case–control studies, an increased risk of ovarian cancer was found with estrogen alone, though in one, this was only significant for 5 or more years of use (Rossing *et al.*, 2007), and in two after 10 or more years of use of estrogen for all types of ovarian cancer combined (Riman *et al.*, 2002; Moorman *et al.*, 2005). Of the remaining two case–control studies, one study (Pike *et al.*, 2004) found a relative risk for 5 years of use of 1.16 (95%CI: 0.92–1.48), and the other did not find an overall elevation in risk for unopposed estrogen use for 5 or more years (Sit *et al.*, 2002). In the three case–control studies that evaluated risks for different histological types of ovarian cancer, the findings were not consistent. Thus, there was a suggestion that risk was elevated for the endometroid type of ovarian cancer and for all types of ovarian cancer in women who had not had a hysterectomy or tubal ligation in one study (Purdie *et al.*, 1999); in the study in Sweden, risk was increased for serous type borderline ovarian cancer (Riman *et al.*, 2001) and for invasive mucinous epithelial ovarian cancer (Riman *et al.*, 2002), while in the study in the USA, risk was increased only for serous epithelial cancer (Moorman *et al.*, 2005), but the numbers were often very small for the different types. In one of the case–control studies (Rossing *et al.*, 2007), the risk among those women who had ceased estrogen use 3 of more years ago was also evaluated. No increases in risk of ovarian cancer were found, even among those who had taken estrogen for 5 or more years (see Table 2.7 online).

In one of the cohort studies, there were only two cases of ovarian cancer, and the relative risk was not elevated (Bakken *et al.*, 2004). In the remaining six cohort studies, the effect of duration of exposure was evaluated (see Table 2.8 online). In one study (Rodriguez *et al.*, 2001), an elevated risk of death from ovarian cancer was seen after 10 years of use of estrogen, in another for incident cases after 10 years of use (Lacey *et al.*, 2006), in the other four (of incident cases), after 5 years of use (Lacey *et al.*, 2002; Folsom *et al.*, 2004; Beral *et al.*, 2007; Danforth *et al.*, 2007). In the study of Lacey *et al.* (2002), risk was documented as continuing to increase with increasing duration of use, the maximum after 20 or more years (RR, 3.4; 95%CI: 1.6–7.5, based on 14 cases). In another (Rodriguez *et al.*, 2001), risk of death from ovarian cancer did not seem to further increase after 5 years of use of estrogen therapy. In the one cohort study that reported risk by histological type, risk was significantly increased for the serous type and the mixed/other/not-otherwise-specified grouping (Beral *et al.*, 2007).

In a meta-analysis that included data from 13 case–control studies, three cohort studies and the WHI-ET trial, the relative risks for ever use were 1.28 (95%CI: 1.18–1.40), and per year of estrogen therapy, 1.07 (95%CI: 1.06–1.08) (Greiser *et al.*, 2007). In another meta-analysis of 13 population-based case–control and cohort studies, but not the WHI-ET trial data, [The Working Group noted that these studies include three recent studies not included in the Greiser *et al.* (2007) meta-analysis] the relative risk per 5 years of use of estrogen use was 1.22 (95%CI: 1.18–1.27) (Pearce *et al.*, 2009). In an additional meta-analysis, reviewing essentially the same data, Zhou *et al.* (2008) computed odds ratios from the case–control studies of 1.19 (95%CI: 1.01–1.40), and 1.51 (95%CI: 1.21–1.88) from the cohort studies for ovarian cancer from ever use of estrogen therapy alone.

2.5 Cancer of the urinary bladder

Since the previous *IARC Monograph* (IARC, 1999), three cohort studies from the USA provide data on cancer of the urinary bladder in users of estrogen therapy (Cantwell. *et al.*, 2006; McGrath *et al.*, 2006; Prizment *et al.* 2007; see Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100A/100A-12-Table2.9.pdf). No associations were found between the use of estrogen therapy and the risk of bladder cancer.

Risk by trend of duration of use was only reported in one study, and there was no trend in risk with increasing duration of use.

2.6 Cancer of the pancreas

A cohort of 387981 postmenopausal women in the USA, the Cancer Prevention Study (CPS)-II (Teras *et al.*, 2005), found no significant positive trends in pancreatic cancer mortality rates with years of estrogen therapy use, both for current and former users.

2.7 Exogenous estrogen use and melanoma risk

A review of the published literature, including a pooled analysis, concluded that neither oral contraceptives nor hormone therapy are associated with melanoma risk (Lens & Bataille, 2008). Two previous pooled analyses, based on 18 and 10 case–control studies, showed summary odds ratios of 2.0 (95%CI: 1.2–3.4) and 0.86 (95%CI: 0.74–1.01), respectively (Feskanich *et al.*, 1999; Karagas *et al.*, 2002).

2.8 Cancer of the cervix

Only two cohort studies and one case–control study investigated the relationship between the use of post-menopausal estrogen therapy and the risk for invasive cervical cancer. On balance, the limited evidence available suggests that postmenopausal estrogen therapy is not associated with an increased risk for invasive cervical carcinoma. The results provide some suggestion that postmenopausal estrogen therapy is associated with a reduced risk for cervical cancer, but the finding could be due to more active screening for pre-invasive disease among women who have received postmenopausal estrogen therapy (IARC, 1999).

2.9 Cancer of the thyroid

Seven case–control studies reporting on thyroid cancer and use of postmenopausal estrogen therapy did not show an effect on risk (IARC, 1999).

2.10 Synthesis

Estrogen-only menopausal therapy causes cancer of the endometrium, and of the ovary. Also, a positive association was observed between exposure to estrogen-only menopausal therapy and cancer of the breast. The Working Group also noted that an inverse relationship is established between exposure to estrogen-only menopausal therapy and cancer of the colorectum.

In addition, for cancer of the endometrium, the risk is increased in users of exogenous estrogen, and increases with duration of use. The excess in risk declines with time after use, but persists for over 10 years after exposure. The risk is also increased for atypical endometrial hyperplasia, a presumed precursor of endometrial cancer.

In addition, for cancer of the breast, a minority of case–control studies show an association between ever use or current use of estrogen therapy and breast cancer risk. Although the evidence is less consistent with regard to duration of estrogen therapy use, a few studies point to an increased risk of breast cancer with longer term use. The evidence is also inconsistent for the possibility that estrogen therapy increases the risk of lobular breast carcinoma. Although at the time of writing the evidence remains scant, estrogen therapy does not appear to be related differently to in-situ versus invasive breast cancer, to tumour stage, or to hormone receptor status.

The Working Group also concluded that the use of estrogen-only menopausal therapy is unlikely to alter the risk of cancer of the thyroid, bladder, pancreas, cervix, and of melanoma.

3. Cancer in Experimental Animals

3.1 Summary of the previous *IARC Monograph*

3.1.1 Conjugated estrogens

(a) Subcutaneous implantation

(i) Hamster

Hydrolysed conjugated equine estrogens, equilin and *d*-equilenin were tested in male hamsters by subcutaneous implantation. The hydrolysed estrogens and equilin induced microscopic renal carcinomas, whereas *d*-equilenin was inactive (Li *et al.*, 1983).

3.1.2 Estradiol

Estradiol and its esters were tested in neonatal mice, mice, rats, hamsters, guinea-pigs, and monkeys by subcutaneous injection or implantation, and in mice by oral administration.

(a) Subcutaneous injection or implantation

(i) Mouse

Subcutaneous injections of estradiol to neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life, and an increased incidence of mammary tumours. Its subcutaneous administration to mice resulted in increased incidences of mammary, pituitary, uterine, cervical, vaginal and lymphoid tumours, and interstitial cell tumours of the testis (IARC, 1999).

(ii) Rat

Invasive pituitary tumours were induced in rats treated subcutaneously with estradiol dipropionate (Satoh *et al.*, 1997).

(iii) Hamster

In hamsters, a high incidence of malignant kidney tumours occurred in intact and castrated males, and in ovariectomized females treated with estradiol, but not in intact females (Li *et al.*, 1983; Goldfarb & Pugh, 1990).

The 4-hydroxy metabolite of estradiol induced renal cell carcinomas in castrated male hamsters (Liehr *et al.* 1986; Li & Li, 1987).

(iv) Guinea-pig

In guinea-pigs, diffuse fibromyomatous uterine and abdominal lesions were observed (Lipschutz & Vargas, 1941).

(b) Oral administration

(i) Mouse

Oral administration of estradiol to mice bearing the murine mammary tumour virus increased the incidences of uterine (endometrial and cervical) adenocarcinomas and mammary tumours (Highman *et al.*, 1980).

(c) Administration with known carcinogens

Estradiol was tested in two-stage carcinogenesis models in mice with the known carcinogens *N*-methyl-*N*-nitrosourea (MNU), *N*-ethyl-*N*-nitrosourea or 3-methylcholanthrene, and in two-stage carcinogenesis models in rats with MNU, 2-acetylaminofluorene, *N*-nitrosodiethylamine, 7,12-dimethylbenz[*a*]anthracene (DMBA) or *N*-butyl-*N*-nitrosourea.

(i) Mouse

In mice, estradiol enhanced the incidences of endometrial adenomatous hyperplasia, atypical hyperplasia and adenocarcinomas induced by MNU and *N*-ethyl-*N*-nitrosourea (Niwa *et al.* 1991, 1996). A continuously high serum concentration of estradiol and a low concentration of progesterone appeared to be important for the development of endometrial adenocarcinomas in mice (Takahashi *et al.*, 1996). Estradiol decreased the development of uterine cervical carcinomas induced by 3-methylcholanthrene (Das *et al.*, 1988).

(ii) Rat

In rats, large doses of estradiol alone or estradiol with progesterone suppressed the development of mammary carcinomas induced by MNU (Grubbs *et al.*, 1983). Combined treatment of ovariectomized rats with estradiol and MNU induced vaginal polyps (Sheehan *et al.*, 1982). In a two-stage model of liver carcinogenesis in rats, estradiol showed no initiating activity (Sumi *et al.*, 1984). It did not show promoting effects in the liver of rats initiated with *N*-nitrosodiethylamine (Yager *et al.*, 1984). In one study, pretreatment with estradiol increased the number of liver foci positive for γ-glutamyl transferase induced by *N*-nitrosodiethylamine (Wotiz *et al.*, 1984). Estradiol did not affect mammary tumour development in intact or ovariectomized female rats treated with DMBA. Estradiol benzoate enhanced the incidence of mammary tumours in rats treated with γ-rays (Inano *et al.*, 1995).

3.1.3 Estriol

(a) Subcutaneous implantation

(i) Mouse

In castrated mice, estriol increased the incidence and accelerated the appearance of mammary tumours in both male and female mice (Rudali *et al.*, 1975).

(ii) Hamster

In hamsters, estriol produced kidney tumours (Kirkman, 1959a).

(b) Administration with known carcinogens

(i) Mouse

In female mice, estriol slightly increased the incidence of MNU-induced endometrial adenocarcinomas (Niwa *et al.*, 1993).

(ii) Rat

In several studies in female rats, estriol inhibited the induction of mammary tumours by DMBA when administered before the carcinogen; continuous treatment with estriol resulted in a decreased incidence of mammary tumours (Wotiz *et al.*, 1984). In one study in female rats, estriol inhibited the induction of mammary carcinomas when administered 13–15 days after irradiation with γ-rays (Lemon *et al.*, 1989).

3.1.4 Estrone

(a) Oral administration

(i) Mouse

Estrone was tested for carcinogenicity by oral administration in one study in castrated male mice. The incidence of mammary tumours was increased (Rudali *et al.*, 1978).

(b) Subcutaneous and/or intramuscular administration

(i) Mouse

Mammary tumours were induced in male mice, and the average age at the time of appearance of mammary tumours in female mice was reduced (Shimkin & Grady, 1940). In two studies of subcutaneous or intramuscular administration, estrone benzoate induced mammary tumours in male mice (Bonser, 1936; Shimkin & Grady, 1940).

(ii) Rat

In castrated male and female rats, subcutaneous injection of estrone resulted in mammary tumours (Geschickter & Byrnes, 1942). In one study in rats, subcutaneous injection of estrone benzoate induced mammary and pituitary tumours in animals of each sex (Chamorro, 1943).

(c) Subcutaneous implantation

(i) Mouse

In several studies involving subcutaneous implantation of estrone, the incidences of mammary and lymphoid tumours were increased in mice (Bittner, 1941; Gardner & Dougherty, 1944).

(ii) Hamster

In intact and castrated male hamsters, implantation of estrone resulted in malignant kidney tumours (Kirkman, 1959b). The estrone metabolite, 4-hydroxyestrone, induced renal tumours at a low incidence in castrated male hamsters (Li & Li, 1987).

(d) Administration with known carcinogens

(i) Mouse

The incidence of endometrial adenocarcinomas induced by MNU in the uterine corpus of mice was significantly increased in those receiving an estrone-containing diet; furthermore, the incidences of pre-neoplastic endometrial lesions in the MNU-treated and untreated uterine corpora were significantly increased in mice receiving the estrone-containing diet (Niwa *et al.*, 1993).

(ii) Toad

In one study in female toads, subcutaneous administration of estrone enhanced the incidence of hepatocellular carcinomas induced by subcutaneous injection of *N*-nitrosodimethylamine (Sakr *et al.*, 1989).

3.2 Studies published since the previous *IARC Monograph*

See Table 3.1

3.2.1 Estradiol

(a) Rat

In a study the objective of which was to characterize some of the genetic bases of estrogen-induced tumorigenesis in the rat, Schaffer *et al.* (2006) used young adult female ACI, BN, (BN x ACI)F1 (F1), and (BN x ACI)F2 (F2) rats that were treated with estradiol. Whereas nearly 100% of the ACI rats developed mammary cancer when treated continuously with estradiol, BN rats did not develop palpable mammary cancer during the 196-day course of estradiol treatment. Susceptibility to estradiol-induced mammary cancer segregated as a dominant or incompletely dominant trait in a cross between BN females and ACI males.

3.2.2 Administration with known carcinogens or other modifying agents

(a) Rat

Ting *et al.* (2007) used three mammary cancer carcinogen models. DMBA, MNU and estradiol were combined with local ovarian DMBA administration to induce progression to mammary and ovarian cancer concurrently in rats. Mammary hyperplasia was observed in DMBA/DMBA- (mammary carcinogen/ovarian carcinogen) and MNU/DMBA-treated rats; however, ovarian pre-neoplastic changes were seldom observed after these treatments. All estradiol/DMBA-treated rats had mammary hyperplasia, atypia, ductal carcinoma *in situ* and/or invasive adenocarcinoma, and half also developed pre-neoplastic changes in the ovary (ovarian epithelial and stromal hyperplasia and inclusion cyst formation).

Aiyer *et al.* (2008) fed female ACI rats with either AIN-93M standard diet or diets supplemented with either powdered blueberry or black raspberry or ellagic acid. They received estradiol implants and were killed after 24 weeks. A high incidence of mammary carcinomas was observed in the AIN-93M group. No differences were found in tumour incidence at 24 weeks; however, tumour volume and multiplicity were reduced significantly in the ellagic acid and black raspberry groups. The blueberry group showed a reduction (40%) only in tumour volume.

Callejo *et al.* (2005) evaluated the influence of different hormonal environments on the induction of breast cancer in the DMBA-induced mammary cancer model in rats. Breast cancer was induced by using a single intragastric dose

Table 3.1 Studies of cancer in experimental animals exposed to estradiol with known carcinogens or other modifying agents

Species, strain (sex) Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Female F344 (F) Ting *et al.* (2007)	Group 1: Controls Group 2: DMBA (10 mg/kg bw, p.o.)/ DMBA[a] Group 3: MNU (50 mg/kg bw, i.p.)/ DMBA[a] Group 4: E_2, 3.0 mg, pellet implant/ DMBA[a] 8; 6 controls/group 3 and 6 mo groups	Mammary carcinoma multiplicity at 3 and 6 mo, respectively: 0; 0 0; 1.00 ± 0.78 0.67 ± 0.21; 0.80 ± 0.20 2.40 ± 0.68; 3.50 ± 0.50 Ovarian carcinoma multiplicity at 3 and 6 mo, respectively: 0.83 ± 0.48; 0.75 ± 0.11 3.58 ± 0.69; 4.40 ± 1.20 3.42 ± 0.49; 4.00 ± 0.82 7.40 ± 0.25; 9.50 ± 0.88	 NS; NS NS; $P < 0.05$ $P < 0.05$; $P < 0.05$ $P < 0.05$[a]; $P < 0.05$[a] –; – $P < 0.05$; $P < 0.05$ $P < 0.05$; $P < 0.05$ $P < 0.05$[b]; $P < 0.05$[b]	Age at start NR; weight, 50–55 g
Rat, ACI (F) Aiyer *et al.* (2008)	AIN-93M diet for 2 wk; then silastic implant of E_2 (0 or 27 mg over 24 wk) Group 1: diet Group 2: diet + 2.5% powdered blueberry Group 3: diet + 2.5% black raspberry Group 4: diet + 400 ppm ellagic acid; 24 wk 19–25; 6 controls/group	Mammary carcinomas 100% in all E_2-treated animals at 24 wk	Reduction of tumour volume; reduction of tumour multiplicity –; – $P < 0.0001$; NS $P < 0.003$; NS $P < 0.001$; $P < 0.027$	Control incidence not specified but assumed to be 0%
Rat, ACI, BN, (BN x ACI)F1 (F1), and (BN x ACI)F2 (F2) (F) Schaffer *et al.* (2006)	E_2: 27.5 mg, implanted ACI: 23 rats BN: 13 rats F1: 22 rats F2: 257 rats	Mammary carcinomas (animals at risk): 94% 0% 86% 58%	Significance relative to ACI strain – – $P < 0.05$ $P < 0.05$	

Table 3.1 (continued)

Species, strain (sex) Reference	Route Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Rat, Sprague Dawley (F) Callejo *et al.* (2005)	Single dose of DMBA 20 mg, dissolved in 0.5 to 1 mL corn oil; estradiol valerate, one daily dose, 5 mg/kg bw i.m. starting at 11 wk	Mammary carcinomas (mean tumours/group):	Differences with controls	
	Group 1: control lpt + DMBA	4	–	
	Group 2: lpt + ovariectomy + DMBA	0.1	$P < 0.001$	
	Group 3: lpt + ovariectomy + DMBA + tibolone	0.3	$P < 0.001$	
	Group 4: lpt + ovariectomy + DMBA + raloxifene	0.1	$P < 0.001$	
	Group 5: lpt + ovariectomy + DMBA + E_2	0.1	$P < 0.001$	
	10 animals/group			
Rat, Sprague Dawley (F) Kang *et al.* (2004)	DMBA, 10 mg in sesame oil; MNU, 50 mg/kg bw in saline; E_3B 30 or 300 µg, implanted	Mammary carcinomas (incidence; multiplicity):		E_3B tends to decrease the multiplicity of DMBA or MNU-induced mammary gland tumours
	DMBA alone	11/12 (92%); 4.17 ± 0.96	–; –	
	DMBA + E_3B 30	6/12 (50.0%); 3.08 ± 1.17	$P < 0.05$; NS	
	DMBA + E_3B 300	8/12 (67%); 2.5 ± 1.52	NS; NS	
	MNU alone	7/12 (58%); 1.67 ± 0.57	–; –	
	MNU + E_3B 30	4/12 (33%); 0.58 ± 0.30	NS; NS	
	MNU + E_3B 300	7/12 (58%); 1.17 ± 0.46	NS; NS	
	16 animals/group			
	4 animals/group killed at 5 wk			
	12 animals/group killed at 21 wk			

[a] in the ovary

[b] differs significantly from Groups 1 and 3

bw, bodyweight; d, day or days; F, female; DMBA, 7,12-dimethylbenz[*a*]anthracene; E_2, 17β-estradiol; E_3B, estradiol-3-benzoate; i.m., intramuscular; lpt, laparotomy; MNU: *N*-methyl-*N*-nitrosourea; mo, month or months; NR, not reported; NS, not significant; wk, week or weeks

of 20 mg of DMBA in pre-pubertal Sprague-Dawley rats randomized into five groups: Group 1 (control); Group 2 (castrated pre-pubertal animals); and Groups 3, 4, and 5 (castration of pre-pubertal animals followed by hormonal treatment starting at puberty [11 weeks] with tibolone, raloxifene, and estradiol, respectively). For Group 5 (estradiol valerate), a single daily dose of 5 mg/kg from a suspension of 1.5 mg/mL was administered orally. Absence of ovarian activity was observed in Groups 2, 3, 4, and 5, as well as the expected variations in hormone levels in all groups. Breast cancers were induced in 100% of the animals in the control group, with an average of four (2–7) tumours per animal in this group. Only one cancer appeared in Groups 2, 3, and 4, and none appeared in Group 5.

Kang *et al.* (2004) used the DMBA and MNU mammary carcinogenesis models to evaluate the effects of estradiol-3-benzoate. The hormone decreased the multiplicity of DMBA- or MNU-induced mammary gland tumours. There was also increased branching of the mammary gland, and a decrease of estrogen receptor-α (ERα) and estrogen receptor-β (ERβ). The inhibitory effect on mammary carcinogenesis may be associated with the differentiation of mammary gland and modulation of ERα and ERβ.

3.2.3 Metabolites

Possible cancer suppressor effects of the physiological metabolite of estradiol, 2-methoxyestradiol were evaluated by Cicek *et al.* (2007) using a murine metastatic breast cancer cell line injected to BALB/C mice. They found that 2-methoxyestradiol inhibited tumour growth in soft tissue, metastasis to bone, osteolysis, and tumour growth in bone. Tumour-induced osteolysis was also significantly reduced in mice receiving 2-methoxyestradiol.

3.3 Synthesis

Estradiol causes malignant mammary tumours in mice and malignant kidney tumours in hamsters. Estrone causes malignant mammary tumours in mice.

4. Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

The absorption, distribution, metabolism and excretion of estrogens have been extensively reviewed previously in the *IARC Monograph* on combined estrogen-progestogen menopausal therapy (IARC 1999, 2007). In summary, cytochrome P450 1A1 and 1B1 catalyse the production of catechol estrogens that are further oxidized to estrogen *o*-quinones that can induce the formation of DNA damage. This is counteracted by the detoxification enzymes, catechol-*O*-methyltransferase, sulfotransferase, and uridine 5′-diphosphate(UDP)-glucuronosyl transferase which reduce the levels of catechols by forming methoxyestrogens, sulfates, and glucuronide conjugates, respectively (IARC 1999, 2007). As far as detoxification of the *o*-quinones are concerned, there have been some reports that the quinones can be detoxified through reduction by quinone reductase and/or conjugation with glutathione (GSH) catalysed by glutathione *S*-transferases (GSTs) (Hachey *et al.*, 2003; Zahid *et al.*, 2008), although the non-enzymatic reduction by nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and Michael addition with GSH are very facile reactions, enhanced enzymatic catalysis may be of questionable importance. A large body of epidemiological data has failed to identify a consistent association between exposure to estrogenic hormones and risk for cancer with any single enzyme variant of these phase I and phase II enzymes (Saintot *et al.*, 2003; Boyapati *et al.*,

2005; Cheng *et al.*, 2005; Rebbeck *et al.*, 2006; Hirata *et al.*, 2008; Justenhoven *et al.*, 2008; Van Emburgh *et al.*, 2008), but possible interactions between these genes remain to be examined in more detail.

4.2 Genetic and related effects

The genetic effects of endogenous estrogens, estradiol, and equine estrogens have been reviewed previously (IARC, 1999, 2007). New data that have appeared since are summarized below.

4.2.1 Direct genotoxicity

(a) DNA adducts

Estrogen quinoids can directly damage cellular DNA (see diethylstilbestrol, this volume; Liehr, 2000; Bolton *et al.*, 2004; Russo & Russo, 2004; Prokai-Tatrai & Prokai, 2005; Cavalieri *et al.*, 2006; IARC, 2007; Bolton & Thatcher, 2008; Gaikwad *et al.*, 2008). The major DNA adducts produced from 4-hydroxyoestradiol-*o*-quinone are depurinating N^7-guanine and N^3-adenine adducts resulting from 1,4-Michael addition both *in vitro* and *in vivo* (Stack *et al.*, 1996; Cavalieri *et al.*, 2000, 2006; Li *et al.*, 2004; Zahid *et al.*, 2006; Saeed *et al.*, 2007; Gaikwad *et al.*, 2008). Interestingly, only the N^3-adenine adduct may induce mutations because this adduct depurinates extremely rapidly, whereas the half-life of the N^7-guanine adduct is 6–7 hours (Saeed *et al.*, 2005; Zahid *et al.*, 2006). In contrast, the considerably more rapid isomerization of the 2-hydroxyestradiol-*o*-quinone to the corresponding quinone methides results in 1,6-Michael addition products with the exocyclic amino groups of adenine and guanine (Stack *et al.*, 1996; Debrauwer *et al.*, 2003). In contrast to the N^3- and N^7-purine DNA adducts, these adducts are stable which may affect their repair and mutagenicity *in vivo*. A depurinating N^3-adenine adduct of 2-hydroxyestradiol quinone methide has recently been reported in reactions with adenine and DNA (Zahid *et al.*, 2006). The levels of this adduct in DNA were considerably lower than corresponding depurinating adducts observed in similar experiments with 4-hydroxyestradiol-*o*-quinone. This is consistent with the suggestion that 2-hydroxylation does not lead to cancer, whereas 4-hydroxylation results in carcinogenesis. This same study (Zahid *et al.*, 2006) provided evidence to suggest that depurinating DNA adducts of estrogen quinoids were formed in much greater abundance than stable adducts. [The Working Group noted that this implies a causal role for the depurinating adducts in estrogen carcinogenesis; but these adducts were analysed by methods (high-perfomance liquid chromatography with electrochemical detection) that differed from those used to detect the stable adducts (^{32}P-postlabelling/thin-layer chromatography), making direct quantitative comparisons somewhat problematic.]

It is important to mention that stable DNA adducts at extracyclic aminogroups have been detected by ^{32}P-postlabelling in DNA from Syrian hamster embryo cells treated with estradiol and its catechol metabolites. The rank order of DNA adduct formation was 4-hydroxyestradiol > 2-hydroxyestradiol > estradiol. The adduct formation correlated with cellular transformation (Hayashi *et al.*, 1996). [The Working Group noted that these data do not clarify the relative importance of depurinating adducts versus stable DNA adducts in catechol estrogen carcinogenesis.]

For the major equine estrogens (equilin, equilenin, and 17β-ol derivatives) the data strongly suggests that the majority of DNA damage also results from reactions of 4-hydroxyequilenin-*o*-quinone through a combination of oxidative damage (ie. single-strand cleavage and oxidation of DNA bases) and through generation of apurinic sites as well as stable bulky cyclic adducts (Bolton & Thatcher, 2008). For example, a depurinating guanine adduct was

detected in in-vivo experiments with rats treated with 4-hydroxyequilenin, following liquid chromatography-tandem mass spectrometry (LC/MS-MS) analysis of extracted mammary tissue (Zhang *et al.*, 2001). However, analysis of this rat mammary tissue DNA by LC/MS-MS also showed the formation of stable cyclic deoxyguanosine and deoxyadenosine adducts as well as the aforementioned oxidized bases. Single-strand breaks were also detected (Ding *et al.*, 2003; Kolbanovskiy *et al.*, 2005; Yasui *et al.*, 2006; Ding *et al.*, 2007). [The Working Group noted that, interestingly, the ratio of the diasteriomeric adducts detected *in vivo* differs from in-vitro experiments. This suggests differential repair of these stereoisomeric lesions.] Using highly sensitive nano LC/MS-MS techniques to analyse DNA in five human breast tumours and five adjacent tissue samples, including samples from donors with a known history of Premarin-based hormone replacement therapy, cyclic 4-hydroxyequilenin-dC, -dG, and -dA stable adducts were detected for the first time in 4/10 samples (Embrechts *et al.*, 2003). [The Working Group noted that although the sample size in this study was small, and the history of the patients is not fully known, these results suggest that the equilin metabolite 4-hydroxyequilenin has the potential to form a variety of DNA lesions in humans.]

(b) Oxidative damage to DNA

As indicated earlier, these estrogens are oxidized to *o*-quinones which are electrophiles as well as potent redox active compounds (Bolton *et al.*, 2000). They can undergo redox cycling with the semiquinone radical generating superoxide radicals mediated through cytochrome P450/P450 reductase (Bolton *et al.*, 2000; IARC, 2007; see diethylstilbestrol, this volume) which gives rise to hydroxyl radicals. In support of this mechanism, various free radical effects have been reported in animals treated with estradiol (IARC, 2007), including DNA single-strand breaks (Nutter *et al.*, 1991; Roy & Liehr, 1999), 8-hydroxydeoxyguanosine formation (Cavalieri *et al.*, 2000; Lavigne *et al.*, 2001; Rajapakse *et al.*, 2005), and chromosomal abnormalities (Li *et al.*, 1993; Banerjee *et al.*, 1994; Russo & Russo, 2006). The estradiol catechol metabolite 4-hydroxyestradiol also induces oxidative stress and apoptosis in human mammary epithelial cells (MCF-10A) (Chen *et al.*, 2005). [The high concentrations used in this study (> 10 μM) have questionable physiological relevance.] It has been further shown that the equilenin catechol, 4-hydroxyequilenin, is also capable of causing DNA single-strand breaks and oxidative damage to DNA bases both *in vitro* and *in vivo* (IARC, 2007; Okamoto *et al.*, 2008). These and older data provide evidence for the generation of reactive oxygen species by redox cycling of estrogen metabolites – reactive oxygen species that are known to damage DNA. This is a proposed mechanism of estrogen-induced tumour initiation/promotion (Cavalieri *et al.*, 2000; Bolton & Thatcher, 2008).

(c) Genetic effects in women

Two studies have identified catechol estrogen adducts in breast tissue of women with breast cancer (see IARC, 2007), and the excessive production of reactive oxygen species in breast cancer tissue has been linked to metastasis in women with breast cancer (Malins *et al.*, 1996, 2006; Karihtala & Soini, 2007). [However, the Working Group was not aware of studies on markers of oxidative stress in women on estrogen therapy.]

(d) Genetic effects in animals

There is some in-vivo evidence for estradiol inducing DNA strand breaks, sister chromatid exchange, chromosomal aberrations, and aneuploidy, but not micronuclei or covalent binding of estrogen metabolites to DNA (IARC, 1999, 2007).

Injection of 4-hydroxyequilenin into the mammary fat pads of Sprague-Dawley rats

resulted in a dose-dependent increase in single-strand breaks and oxidized bases as analysed by the comet assay, and the formation of 8-hydroxydeoxyguanosine and 8-hydroxydeoxyadenine (Zhang *et al.*, 2001). In mice treated with equilenin, the levels of 8-hydroxydeoxyguanosine were increased 1.5-fold in the uterus (Okamoto *et al.*, 2008).

(e) Genetic effects in human and animal cells

There is some evidence for estradiol inducing DNA strand breaks, sister chromatid exchange, and chromosomal aberrations in various human cells, including MCF-7 breast cancer cells, although the evidence for induction of aneuploidy in human cells is equivocal (IARC, 1999, 2007).

Repeated treatment at physiological (70 nM) and at much lower (0.007 nM) doses of estradiol of MCF-10F immortalized human breast epithelial cells, which are ERα-negative, increased colony formation in a soft agar assay (Russo *et al.*, 2001, 2003). When grown in collagen, these cells changed from differentiated ductular growth to solid-spherical masses with the same dose–response relationship, and invasive growth in a Matrigel assay was increased (Russo *et al.*, 2002, 2006). Following estradiol exposure at a concentration of 70 nM of MCF-10F cells for four alternating 24-hour periods, several passages of the cells and subsequent selection, four clones grew in Matrigel, two of which formed tumours in nude mice (Russo *et al.*, 2006). Both clones had loss of chromosomal region 9p11–3 and all other subclones had variable other chromosomal losses, deletions, and gains. [The Working Group noted that this study established that estradiol is capable of inducing malignant transformation in cultured immortalized human breast cells that are ER-negative.]

The same types of genetic damage induced *in vivo* are also induced in cells in culture by estradiol, estrone, and their catechol metabolites as well as cell transformation, but mutations have not consistently been observed (IARC, 1999, 2007). The mutagenic properties of the aforementioned stable DNA adducts derived from 2-hydroxyestrogen-quinone-methide have been evaluated using oligonucleotides containing site-specific adducts transfected into simian kidney (COS-7) cells where G→T and A→T mutations were observed (Terashima *et al.*, 2001).

4.2.2 Indirect effects related to genotoxicity

(a) Estrogen-receptor-mediated effects

Receptor-mediated mechanisms by which estrogens may cause or contribute to carcinogenesis have previously been reviewed extensively (IARC, 1999, 2007). Estrogen therapy in the menopause increases the rate of cell proliferation in the postmenopausal human breast (IARC, 1999, 2007). This effect is mediated through nuclear ER-mediated signalling pathways, and this results in an increased risk of genomic mutations during DNA replication (Nandi *et al.*, 1995; Feigelson & Henderson, 1996; Henderson & Feigelson, 2000; Flötotto *et al.*, 2001; Yager & Davidson, 2006). [The Working Group noted that direct evidence for this mechanism in relevant experimental models of target tissues is, however, not available at present.] Similarly acting non-genomic pathways, potentially involving newly discovered membrane-associated estrogen receptors, appear to regulate extranuclear estrogen signalling pathways that can affect cell proliferation (Björnström & Sjöberg, 2005; Revankar *et al.*, 2005; Song *et al.*, 2006; Yager & Davidson, 2006; Hammes & Levin, 2007; Pietras & Márquez-Garbán, 2007; Levin & Pietras, 2008). Recent studies have shown the presence of ERα and ERβ in the mitochondria of various cells and tissues, which may be involved in deregulation of mitochondrial bioenergetics, and conceivably contribute to estrogen-exposure-related cancers (Yager & Davidson, 2006; Chen *et al.*, 2008). Cross-talk between these genomic and non-genomic second-messenger pathways may have

an important role in estrogenic control of cell proliferation, inhibition of apoptosis, and induction of DNA damage (Yager & Davidson, 2006).

4.3 Synthesis

Receptor-mediated responses to hormones are a plausible and probably necessary mechanism for estrogen carcinogenesis. In addition, there is support for a genotoxic effect of estrogenic hormones or their associated by-products such as reactive oxygen species. The types of DNA damage found in cells and tissues exposed to estrogens are consistent with such genotoxic effects. Current knowledge does not allow a conclusion as to whether either of these mechanisms is the major determinant of estrogen-induced cancer. It is entirely possible that both mechanisms contribute to and are necessary for estrogen carcinogenesis. Although there appears to be a direct link between exposure to estrogens, metabolism of estrogens, and increased risk of breast cancer, the factors that affect the formation, reactivity, and cellular targets of estrogen quinoids remain to be fully explored.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of estrogen-only menopausal therapy. Estrogen-only menopausal therapy causes cancer of the endometrium and of the ovary. Also, a positive association has been observed between exposure to estrogen-only menopausal therapy and cancer of the breast.

For cancer of the colorectum, there is *evidence suggesting lack of carcinogenicity*. An inverse relationship has been established between exposure to estrogen-only menopausal therapy and cancer of the colorectum.

There is *sufficient evidence* in experimental animals for the carcinogenicity of some estrogens used in estrogen-only menopausal therapy.

Estrogen-only menopausal therapy is *carcinogenic to humans (Group 1*).

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