FINAL

Report on Carcinogens Background Document for

Steroidal Estrogens

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Prepared for the:
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

U.S. Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

Steroidal Estrogens

Carcinogenicity

Steroidal estrogens are *known to be human carcinogens,* based on sufficient evidence from human epidemiology studies showing that use of estrogen replacement therapy in postmenopausal women is associated with a consistent increase in the risk of endometrial cancer and a less consistent increase in the risk of breast cancer. Higher risks of endometrial and breast cancer were associated with longer durations of exposure or higher doses of estrogens. Some evidence suggests that oral contraceptive use also may be associated with increased risk of breast cancer. The evidence in humans for the carcinogenicity of steroidal estrogens is supported by findings from experimental animal studies that have shown a variety of neoplasms including endometrial, cervical, and mammary tumors in mice, mammary and pituitary neoplasms in rats, and renal carcinomas in hamsters.

The carcinogenic effects of hormone replacement therapy used to relieve symptoms of menopause were evaluated by the International Agency for Research on Cancer (IARC) (1999). Most of the studies reviewed did not differentiate between the effects of estrogen-only and estrogen–progestin combination therapies. An increased risk of endometrial cancer was associated with increasing duration of therapy. A small increased risk of breast cancer also was found. One cohort and three large case-control studies not included in the IARC (1999) review reported an association of estrogen replacement therapy with endometrial cancer risk (Persson *et al*. 1999, Cushing *et al*. 1998, Shapiro *et al*. 1998, Weiderpass *et al*. 1999); the latter two studies both reported stronger positive associations between estrogen replacement therapy and endometrial cancer with increasing duration of estrogen use. Three recent cohort studies of the effects of hormone replacement therapy have shown an association with breast cancer (Schairer *et al.* 2000, Persson *et al.* 1999, Gapstur *et al.* 1999). Two of four recent case-control studies found that estrogen-only replacement therapy was associated with increased risk of breast cancer (Magnusson *et al.* 1999, Henrich *et al.* 1998), whereas Brinton *et al*. (1998) reported a slight protective effect of hormone replacement therapy (estrogen content not specified) on breast cancer risk, and Titus-Ernstoff *et al*. (1998) found no association with breast cancer risk. One recent study (Purdie *et al*. 1999) found that estrogen therapy was associated with increased risk for ovarian cancer. In general, the results of recent studies are consistent with previously reviewed studies of estrogen use (IARC 1999).

Numerous case-control and cohort studies have addressed the risks of various cancers associated with the use of oral contraceptives (IARC 1999). Most of these studies involved estrogen–progestin combinations. In general, oral contraceptive use was associated with a small increased risk of breast cancer. Three recent case-control studies (Titus-Ernstoff *et al*. 1998, Brinton *et al*. 1998, Rohan and Miller 1999) did not support the positive association between oral contraceptive use and breast cancer suggested in the earlier studies reviewed by the IARC (1999). However, an inverse association between oral contraceptive use and ovarian and endometrial cancer was recently reported

(Salazar-Martinez *et al.* 1999), confirming the IARC review. None of the recent studies specified the hormone content of the oral contraceptives used.

Studies in rats, mice, hamsters, and guinea pigs have been conducted with estrogens alone or in combination with known carcinogens. Estrogen had a carcinogenic effect in all species and by all routes of administration. Most studies showed induction of benign and malignant neoplasms, as well as preneoplastic lesions, in a variety of target organs, including the breast and female reproductive tract.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

Although there is no evidence suggesting genotoxic effects in nonmammalian systems (IARC 1999), steroidal estrogens can damage chromosomes and DNA in mammals. The most frequently reported effects include DNA adduct formation, cytogenic alterations (*e.g.*, chromosome and chromatid breaks, micronuclei, SCEs), aneuploidy, and cell transformation. Most of these effects have been demonstrated in various *in vitro* assays using cultured animal cells or cell-free systems. Fewer effects have been reported in whole-animal studies or in studies with human cells, and no human *in vivo* studies were identified.

Estrogen metabolism is essentially similar among mammalian species, with aromatic hydroxylation to catechol intermediates and glucuronidation, sulfonation, and *O*-methylation.

Although there is strong evidence that estrogen carcinogenesis is mediated by the estrogen receptor, there is evidence that this activity alone is insufficient to explain the carcinogenic effects of estrogens in all tissues. Although the molecular mechanisms responsible for estrogen carcinogenicity are not well understood, the evidence indicates that steroidal estrogen carcinogenesis is complex and may involve proliferative effects and direct and indirect genotoxic effects. The relative importance of each mechanism is likely a function of the specific estrogen, as well as the exposed tissue or cell type and its metabolic state (Yager and Liehr 1996).

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1 Introduction

Conjugated estrogens were listed in the Fourth Annual Report on Carcinogens (RoC) (1985) as *known to be human carcinogens.* A number of individual steroidal estrogens, including estradiol-17∞, estrone, ethinylestradiol, and mestranol, also were listed in that RoC as *reasonably anticipated to be human carcinogens*. In 1987, the International Agency for Research on Cancer (IARC) identified steroidal estrogens as *carcinogenic to humans* (Group 1), based on sufficient evidence of carcinogenicity in humans (IARC 1987). The IARC noted that its evaluation applied to the group of chemicals as a whole, and not necessarily to all individual chemicals within the group. Also in 1987, and again in 1999, the IARC identified postmenopausal estrogen therapy as *carcinogenic to humans* (Group 1), based on sufficient evidence of carcinogenicity in humans (IARC 1987, 1999). These IARC listings are based on a consistent, strongly positive association between exposure to a number of steroidal estrogenic substances and increased risks of endometrial and breast cancer in women. Steroidal estrogens (including postmenopausal estrogen therapy and oral contraceptives) were nominated for listing in the RoC by the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP) RoC Review Group (RG1), based on the IARC listing of steroidal estrogens as *carcinogenic to humans* (Group 1).

1.1 Chemical identification

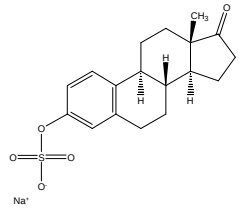
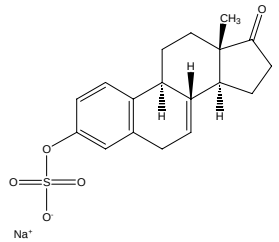
Estrogen is a steroid hormone occurring naturally in both females and males. Hormones are signaling molecules secreted into the bloodstream by endocrine cells; a hormone acts on target cells that possess receptors for that hormone. Steroid hormones are fat-soluble (lipophilic) hormones with a tetracyclic base structure, and are essential for the growth, differentiation, and function of many tissues in both humans and animals. “Estrogen” is a collective term for the female hormones, the most powerful of which is estradiol. These hormones control female secondary sexual characteristics and prepare and maintain the uterine lining. Estrogens affect the growth, differentiation, and function of peripheral tissues of the reproductive system, including the mammary gland, uterus, vagina, and ovary. Estrogens also play an important role in bone maintenance and exert cardioprotective effects. In the brain, estrogens modulate physiological parameters important for regulating procreation, including reproductive behavior, gonadotropin production and release from the pituitary, and mood. Both naturally occurring and synthetic estrogens are widely used medicinal drugs (IARC 1999). Although estrogen is best known for its critical role in influencing female secondary sexual characteristics, reproductive cycle, fertility, and maintenance of pregnancy, less well known are the important actions of estrogen in male tissues, such as the prostate, testis, and epididymis. In addition to their well-known role in female bone formation and maintenance, estrogens are essential for the normal development of bone tissue in males. Modification of the hormonal environment can increase or decrease the spontaneous occurrence or induction of tumors (IARC 1999).

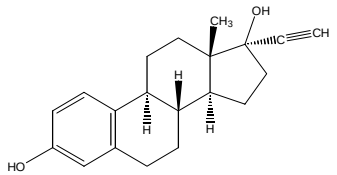
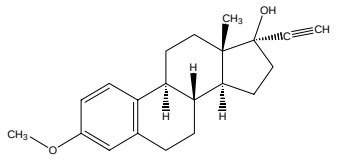
1.2 Physical-chemical properties

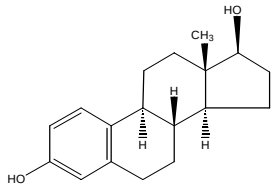
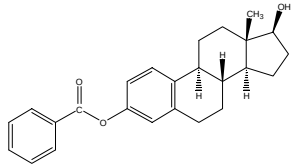
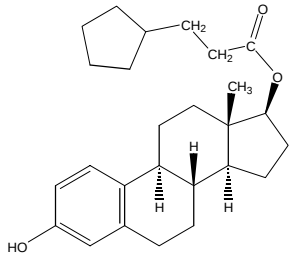
“Conjugated estrogens” (sulfate conjugates) refer to mixtures that contain any of at least eight different compounds, including sodium estrone sulfate and sodium equilin sulfate. These are

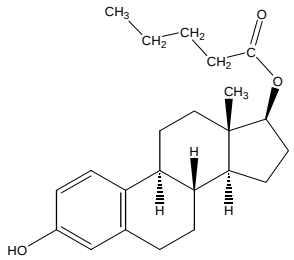
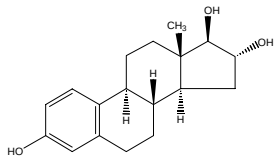
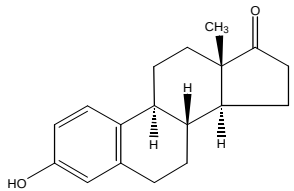
derived wholly or partly from equine urine or synthetically from estrone and equilin. The chemical structures and physical-chemical properties of conjugated estrogens and other commonly used steroidal estrogens are listed in Table 1-1.

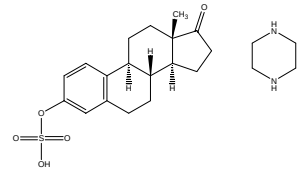
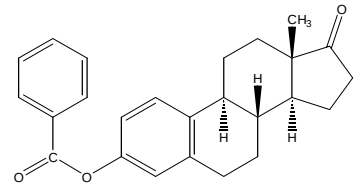
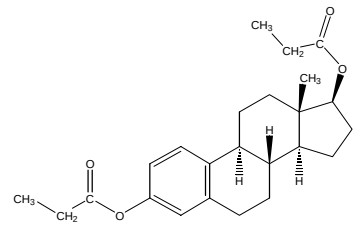
Table 1-1. Physical and chemical properties of estrogens

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Sodium estrone sulfate 438-67-5	3-(sulfooxy)-estra-1,3,5(10)-trien-17-one, sodium salt; estrone sodium sulfate; estrone, hydrogen sulfate sodium salt	$C_{18}H_{21}NaO_5S$ 372.41		buff-colored odorless powder soluble in water
Sodium equilin sulfate 16680-47-0	–	$C_{18}H_{19}NaO_5S$ 370.4		buff-colored odorless powder soluble in water

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Ethinylestradiol 57-63-6	17-ethynyl estradiol; 17∞-ethynyl-1,3,5(10)-estratriene-3,17∞-diol; estone; 19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol, (17∞)-; 19-nor-17∞-pregna-1,3,5(10)-trien-20-yne-3,17,diol; amenoron; Anovlar; chee-o-genf; 3,17∞-dihydroxy-17∞-ethynyl-1,3,5(10)-estratriene; diognat-e; diogyn-e; Dyloform; EE; Esteed; Estigyn; Estinyl; estoral (orion); estroals; estra-1,3,5(10)-triene-3,17∞-diol, 17∞-ethynyl-; Ethidol; ethinoral; 17∞-ethynyl-3,17-dihydroxy-∞(sup1,3,5)-estratriene; Primogyn; Primogyn c (or m); Progynon c; Eticyclin; eticyclol; etinestrol; etinestryl; ginestrene; inestra; Linoral; Lynoral; Menolyn; Neo-Estrone; Novestrol; oradiol; orestralyn; Palonyl; perovex; Feminone; roldiol; Spanestrin; ylestrol; 17∞-ethynyl-3-hydroxy-1,3,5(10)-estratrien-17∞-ol; ethinylestradiol; ethinyloestradiol	$C_{20}H_{24}O_2$ 296.41		fine white to creamy white odorless crystalline powder melting point, 182–184°C practically insoluble in water (< 0.1 g/100 mL at 21°C) soluble in acetone, ethanol, chloroform, dioxane, diethyl ether, and vegetable oils
Mestranol 72-33-3	ethynylestradiol 3-methyl ether; 3-methoxy-17∞-ethynyl-1,3,5(10)-estratriene-17∞-ol; 17∞ ethynyl-estradiol-3-methyl ether; 3-methoxy-19-nor-17∞-pregna-1,3,5-trien-20-yn-17-ol; compound 33355; ∞-MVE; 3-methylethynylestradiol; 17∞l-19-norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-; methoxy-19-nor-17∞-pregna-1,3,5(10)-trien-20-yn-17-ol; norpregna-1,3,5(10)-trien-20-yn-17-ol, 3-methoxy-	$C_{21}H_{26}O_2$ 310.44		white to creamy white odorless crystalline powder melting point, 150–151°C practically insoluble in water sparingly soluble in ethanol slightly soluble in methanol soluble in acetone, dioxane, and diethyl ether freely soluble in chloroform

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estradiol 50-28-2	∞-estradiol; dihydrofolliculin; dihydroxyestrin; 1,3,5(10)-estratriene-3,17∞-diol; 3,17-dihydroxy-∝(1,3,5-10)-estratriene; 3,17-epidihydroxyestratriene; estradiol-17∞; 17∞-estradiol; estra-1,3,5(10)-triene-3,17∞-diol	$C_{18}H_{24}O_2$ 272.38		white to creamy white odorless crystalline powder melting point, 173–179°C practically insoluble in water soluble in ethanol, chloroform, diethyl ether, acetone, and dioxane a natural hormone present in pure form in the urine of pregnant mares and in the ovaries of pigs
Estradiol benzoate 50-50-0	estradiol monobenzoate; estradiol benzoate; 17∞-estradiol benzoate; estradiol-3-benzoate; 17∞-estradiol-3-benzoate	$C_{25}H_{28}O_3$ 376.49		white crystalline powder melting point, 191–196°C practically insoluble in water slightly soluble in ethanol and diethyl ether sparingly soluble in acetone and vegetable oils
Estradiol cypionate 313-06-4	estradiol cyclopentylpropionate; ∞-estradiol-17-cyclopentanepropionate; 1,3,5(10)-estratriene-3,17∞-diol, 17-cyclopentapropionate; Depofemin	$C_{26}H_{36}O_3$ 396.57		white odorless crystalline powder melting point, 151–152°C practically insoluble in water soluble in ethanol, chloroform, diethyl ether, acetone, and dioxane

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estradiol valerate 979-32-8	estradiol-17-valerate; estradiol-17∞-valerate	$C_{23}H_{32}O_3$ 396.50		white odorless crystalline powder melting point, 144–145°C practically insoluble in water soluble in benzyl benzoate, dioxane, methanol, and castor oil sparingly soluble in arachis oil and sesame oil
Estriol 50-27-1	drihydroxyestrin; ∝(1,3,5-10)-estratriene-3,16-cis-17-trans-diol; 1,3,5(10)-estratriene-3,16∞,17∞-triol; estra-1,3,5(10)-triene-3,16∞,17∞-triol; estriol (R&D)	$C_{18}H_{24}O_3$ 288.39		white odorless crystalline powder melting point, 282°C practically insoluble in water sparingly soluble in ethanol soluble in acetone, dioxane, diethyl ether, and vegetable oils
Estrone 53-16-7	folliculin; ketohydroxyestrin; 1,3,5(10)-estratrien-3-ol-17-one; oestrone; ∞-estrone; estra-1,3,5(10)-trien-17-one, 3-hydroxy-; estrol; oestrin; 3∞-hydroxyestra-1,3,5(10)-trien-17-one; 3-hydroxy-1,3,5(10)-estratrien-17-one	$C_{18}H_{22}O_2$ 270.37		white to creamy white crystalline powder melting point, 254.5–256°C practically insoluble in water sparingly soluble in ethanol, chloroform, acetone, dioxane, and vegetable oils slightly soluble in diethy ether and solutions of alkali hydroxides

Name CASRN	Synonyms	Formula Mol. wt.	Structure	Properties
Estropipate 17280-37-7	piperazine estrone sulfate; 3-(sulfooxy)estra-1,3,5-(10)-trien-17-one compd. with piperazine (1:1); estrone, hydrogensulfate compd. with piperazine (1:1); Harmogen; Ogen; piperazine 17-oxo-estra-1,3,5(10)-trien-3-yl sulfate; sulestrex piperazine	$C_{22}H_{32}N_2O_5S$ 436.56		white to yellowish white odorless fine crystalline powder melting point, 190°C; solidifies on further heating and decomposes at 245°C very slightly soluble in water (0.08 g/100 mL), ethanol, chloroform, and diethyl ether soluble in warm water and warm ethanol
Polyestradiol phosphate 28014-46-2	(17b)-estra-1,3,5(10)-triene-3,17-diol polymer with phosphoric acid	NA	NA	melting point, 195 °C
Estrone benzoate 2393-53-5	3-(benzoyloxy)estra-1,3,5(10)-trien-17-one	$C_{25}H_{26}O_3$ 374.48		melting point, 220 °C
Estradiol dipropionate 113-38-2	alpha-estradiol dipropionate; 17∞-estradiol dipropionate; estral,3,5(10)-triene-3,17-diol(17∞)-dipropionate	$C_{24}H_{32}O_4$ 384.5144		melting point, 104 °C

Source: IARC 1987 and 1999, ChemFinder 2000

1.3 Identification of metabolites

Administered estrogens and their esters are handled within the body essentially in the same way as the endogenous hormones. Metabolic conversion of estrogens occurs in the liver and at local target tissues (FDA 1999). Although naturally occurring estrogens circulate in the blood largely bound to sex hormone–binding globulin and albumin, only unbound estrogens enter target-tissue cells. Section 6 provides more information on the metabolic pathways.

2 Human Exposure

2.1 Use

Steroidal estrogens comprise a group of structurally related hormones derived from the cholesterol molecule. They control sex and growth characteristics, are highly lipophilic, and elicit biological responses by binding to nuclear receptors that act as DNA transcription factors.

2.1.1 Hormone replacement therapy

Conjugated estrogens, estradiol, and synthetic esters of estradiol, especially estradiol valerate, are most commonly used for estrogen replacement therapy to treat symptoms of menopause, including menopause surgically induced by removal of the ovaries. They are used to prevent the sweating episodes called hot flashes and the shrinking and irritation that sometimes occur in the vulva, vagina, and urinary organs. Estrogens also can be used to prevent common post-menopausal conditions such as osteoporosis and ischemic heart disease. They also have been used to treat hypoestrogenism due to hypogonadism, castration, or primary ovarian failure. Estrogen replacement therapy can employ steroidal estrogens only or a combination of steroidal estrogens and progestogens (IARC 1999, FDA 1999, HSDB 2000). Steroidal estrogens used for hormone replacement therapy (HRT) are summarized in Table 2-1.

Table 2-1. Commonly prescribed estrogens used for hormone replacement therapy in the United States.

Estrogens	Brand	Strength (mg)	Manufacturer
Conjugated estrogens	Premarin	0.3, 0.625, 0.9, 1.25, 2.5	Wyeth-Ayerst
Vaginal cream	Premarin Vaginal Cream	625	Wyeth-Ayerst
Esterified estrogens	Estratab	0.3, 0.625, 1.25, 2.5	Solvay
	Menest	0.3, 0.625, 1.25, 2.5	SmithKline Beecham
17 ∝-Estradiol			
Transdermal patch	Estraderm	0.05, 0.10	Ciba-Geneva
Transdermal patch	Climara	0.05, 0.10	Berlex
Transdermal patch	Vivelle	0.0375, 0.05, 0.075, 0.10	Ciba-Geneva
Vaginal cream	Estrace	1000	Bristol-Myers Squibb
Estradiol, micronized	Estrace	0.5, 1.2	Bristol-Myers Squibb
Estropipate	Orgen	0.75, 1.5, 3	Upjohn
	Ortho-Est	0.75, 1.5	Ortho
Vaginal cream	Ogen	1000	Upjohn
Combination Therapy			
Conjugated estrogens + MPA, continuous combined regimen[a]	Prempo	0.625/2.5	Wyeth-Ayerst
Conjugated estrogens + MPA, cyclic regimen[b]	Premphase	0.625/5	Wyeth-Ayerst

Source: Klein and Berlin 1996
[a]Conjugated estrogens and medroxyprogesterone acetate (MPA) taken daily.
[b]Conjugated estrogens taken daily, MPA taken for last half of 28-day cycle.

2.1.2 Oral contraceptives

Steroidal estrogens, most commonly ethinylestradiol, also are used with various progestogens in combined oral contraceptive (OC) formulations. Estrogens have been used in oral contraceptives for over 30 years. During the 1960s and 1970s, research was done to attempt to reduce the estrogen content of oral contraceptives, because of the risks of thromboembolic disorders associated with the use of high doses of estrogens. Currently, many of the oral contraceptives used in the United States contain either 30 or 35 ∝g of ethinylestradiol, because this dose has contraceptive efficacy, good tolerability, and a low risk of adverse effects such as breakthrough bleeding (Schwend and Lippman 1996). Mestranol also is used in some formulations of oral contraceptives (IARC 1999). Combined oral contraceptives usually are administered as a pill taken daily for 20 to 22 days followed by a 7-day pill-free interval, where a withdrawal bleed is expected to occur.

Daily administration of a mixture containing ethinylestradiol for five consecutive days can prevent pregnancy if given within 72 hours after coital exposure (IARC 1999, HSDB 2000). Appendix A (Annex 2, Table 1) summarizes information relating to combinations of estrogens used in oral contraceptives.

2.1.3 Other uses

Steroidal estrogens are used to treat breast cancer (for palliation only) in selected women and men with metastatic disease. They also are used in palliative treatment of androgen-dependent carcinoma of the prostate. Use of estrogens to treat acne is not recommended, because of lack of evidence for efficacy. Veterinarians use estrogens to induce ovulation and estrus in animals. They also can be used to treat anal adenomata and prostatic hypertrophy in male dogs and mesalliance pseudopregnancy, vaginitis, and incontinence in female dogs. Mixed androgen–estrogen therapy is used in canine geriatrics. Steroidal estrogens also are used for biochemical research (Novartis 2000, HSDB 2000).

2.2 Production

Steroidal estrogens are produced from estrogens obtained from the urine of pregnant mares or synthetically. The principal estrogen present in conjugated estrogens is sodium estrone sulfate (between 52.5% and 61.5%). The estrogenic potency of the conjugated estrogens is expressed as the equivalent quantity of sodium estrone sulfate. Conjugated estrogens also contain sodium equilin sulfate (between 22.5% and 30.5%). Ethinylestradiol is formed by treatment of estrone with potassium acetylide in liquid ammonia. Mestranol is prepared by reaction of estrone with methyl sulfate to produce its 3-methoxy analogue (IARC 1999).

2.3 Analysis

Gas chromatography with flame ionization detection is used to identify steroidal estrogens, their components, and impurities. Infrared and ultraviolet absorption spectrophotometry and thin-layer chromatography are the most common methods used to identify ethinylestradiol, mestranol, estradiol, estriol, estrone, and estropipate. Liquid chromatography and high-pressure liquid chromatography usually are used to assay their purity. Thin-layer chromatography, liquid chromatography, ultraviolet absorption spectrophotometry, and potentiometric titration are used to determine purity and content of various steroidal estrogens in pharmaceutical preparations (IARC 1999).

2.4 Environmental occurrence

Steroidal estrogens are naturally occurring hormones that stimulate growth and development of the female sex organs in vertebrates. Under normal conditions, estrogens are synthesized in the ovaries in response to pituitary hormones. In a normally cycling adult woman, the ovarian follicle secretes 70 to 500 ∝g of estradiol per day, depending on the phase of the menstrual cycle. This estradiol is converted primarily to estrone and small amounts of estriol. After menopause, endogenous estrogen is produced by the conversion of androstenedione secreted by the adrenal cortex to estrone by peripheral tissues (FDA 1999).

Steroidal estrogens and nonsteroidal compounds with estrogenic activity also occur naturally in plants; over 360 plants have been identified as possessing estrogenic activity.

Estrogens have been found naturally in such plants as licorice, French bean, date palm, pomegranate, and apples. A few plants contain the principal mammalian estrogens, estradiol and estrone (Satchell 1985). Screens have been established to determine estrogen content in meat and milk. Estradiol equivalent concentrations in meat and milk were determined by a uterine estrogen receptor assay (a competitive protein binding assay). Meat, including chicken, pork, and beef, was shown to contain 57 ± 29.5 pg of estradiol equivalents (range 38 to 88 pg, n = 144), and milk to contain 53 ± 6.8 pg of estradiol equivalents (range 35 to 65 pg, n = 81) (Collins and Musey 1985). Veterinary use of steroidal estrogens (for growth promotion and therapeutic purposes) can increase tissue levels in food-producing animals above those resulting from endogenous estrogen production.

2.5 Environmental fate

Information about the environmental fate of steroidal estrogens was not identified in the current literature. The biological fate of estrogens is discussed in Section 2.8, below.

2.6 Environmental exposure

Estrogens are responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Although circulating estrogens exist in a dynamic equilibrium of metabolic interconversions, estradiol is the main naturally occurring estrogen. Estradiol is substantially more potent than its metabolites estrone and estriol at the receptor level. The primary source of estrogen in a normally cycling adult woman is the ovarian follicle, which secretes 70 to 500 ∝g of estradiol per day, depending upon the phase of the menstrual cycle. After menopause, most endogenous estrogen is produced in the peripheral tissues by the conversion of androstenedione to estrone. Androstenedione is secreted by the adrenal cortex. Thus, estrone, and its sulfate conjugated form, estrone sulfate, are the most abundant circulating estrogens in post-menopausal women (IARC 1999).

Exposure to estrogens in the United States occurs mostly when they are administered in oral contraceptives and to a lesser degree in post-menopausal estrogen therapy. In the United States, 15% of women in 1990 used oral contraceptives containing estrogens. Of the 35,800,000 women in the United States in 1990, about 5,191,000 used oral contraceptives. The use of post-menopausal estrogen therapy became widespread in the United States in the 1960s. Between 1962 and 1967, the number of women using this therapy increased by 240%. By 1967, approximately 13% of the women in the United States aged 45 to 64 used this type of therapy. Estrogen–androgen combinations accounted for an estimated 14% of noncontraceptive prescriptions in the United States in 1966, but by 1983, the percentage had fallen to < 2%. The number of estrogen-androgen prescriptions then began to rise again, from 0.1 million in 1982 to 0.8 million in 1992 (IARC 1999).

2.7 Occupational exposure

No information about occupational exposure to estrogens was found in the current literature.

2.8 Biological indices of exposure

Estrogens, like all steroid hormones, have a wide range of actions and affect almost all systems in the body, yet act in a tissue-specific manner. Although estrogen's mode of action has been studied extensively, the molecular mechanism of action still is unclear. Estrogen acts by binding with high affinity and high specificity to the protein receptors present in hormone-responsive tissues. When the hormone binds with the receptor, the receptor undergoes a conformational change and binds to specific DNA sequences. This transcription complex regulates the expression of specific genes within a cell (Edwards and Prendergast 1996). Circulating estradiol and other naturally occurring estrogens are bound mainly to the sex hormone binding globulin, and to a lesser degree to albumin (Novartis 2000).

Estrogens, whether exogenous or endogenous, circulate in the body, undergoing various metabolic interconversions. Estrogens undergo enterohepatic recirculation via sulfate and glucuronide conjugation in the liver (where most of the transformations take place), biliary secretion of conjugates into the intestine, and hydrolysis in the gut, followed by reabsorption. Estradiol, the most abundant endogenous estrogen, can be converted reversibly to estrone, and both can be converted to the major urinary metabolite, estriol. Estradiol, estrone, and estriol are excreted in the urine, along with glucuronide and sulfate conjugates (Mosby 2000).

When given orally, naturally occurring estrogens and their esters are extensively metabolized by the liver and circulate primarily as estrone sulfate, which limits the potency of orally administered estrogen. Synthetic estrogens, like ethinylestradiol, are degraded very slowly in the liver and other tissues, resulting in higher innate potency (Mosby 2000). Estradiol has a peak plasma level at 2 to 4 hours after administration, with a plasma half-life of 24 hours (Infomed-Verlags AG 1996).

2.9 Regulations

The U.S. Food and Drug Administration (FDA), through the Federal Food, Drug, and Cosmetic Act, regulates manufacturers, packers, and distributors to ensure proper labeling, certification, and usage requirements for any drug containing steroidal estrogens. The FDA also describes specifications and conditions of use for injectable or implantable formulations containing steroidal estrogens for animals, and sets estradiol tolerances in tissues of heifers, steers, calves, and lambs. FDA regulations are summarized in Table 2-2.

Table 2-2. FDA regulations

Regulatory action	Effect of regulation and other comments
21 CFR 201—PART 201—LABELING. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	The regulations govern the proper labeling procedures for a drug and drug product. For drugs containing estrogen and its derivatives, no new drugs may be released for interstate commerce without proper labeling.
21 CFR 201.301—Notice to manufacturers, packers, and distributors of estrogenic hormone preparations. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	Some drug preparations fabricated wholly or in part from estradiol and labeled as to potency in terms of international units or in terms of international units of estrone activity have been marketed. The declaration of the estradiol content of an estrogenic hormone preparation in terms of weight is considered appropriate.
21 CFR 201.313—Estradiol labeling. Promulgated: 40 FR 13998 03/27/75. U.S. Codes: 21 U.S.C. 321, 331, 352-53, 355-58, 360, 360b, 360gg-360ss, 371, 374, 379e.	"Estradiol" and that which is said to be "17-cis-beta estradiol" is the same substance formerly recognized in the United States Pharmacopeia under the designation "Alpha Estradiol." The substance should no longer be referred to in drug labeling as "Alpha Estradiol."
21 CFR 310—PART 310—NEW DRUGS. Promulgated: 39 FR 11680, 03/29/74. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e, 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	Regulations govern the administrative rulings and decisions on new drug status, new drugs exempted from prescription-dispensing requirements, records, reports, and requests for specific new drugs or devices.
21 CFR 310.515—Patient package inserts for estrogens. Promulgated: 55 FR 18723, 05/04/90. U.S. Codes: 21 U.S.C. 321, 331, 351, 352, 353, 355, 356, 357, 360b-360f, 360j, 361(a), 371, 374, 375, 379e, 42 U.S.C. 216, 241, 242(a), 262, 263b-263n.	The FDA concludes that the safe and effective use of drug products containing estrogens requires that patients be fully informed of the benefits and risks involved in the use of these drugs. Accordingly, each estrogen drug product restricted to prescription distribution, including products containing estrogens in fixed combinations with other drugs, shall be dispensed to patients with a patient package insert containing information concerning the drug's benefits and risks. An estrogen drug product that does not comply with the requirements of this section is misbranded under section 502(a) of the Federal Food, Drug, and Cosmetic Act.
21 CFR 522—PART 522—IMPLANTATION OR INJECTABLE DOSAGE FORM NEW ANIMAL DRUGS. Promulgated: 40 FR 13858 03/27/75. U.S. Codes: 21 U.S.C. 360b.	This part regulates specifications, indications, and conditions of use and limitations of animal drugs. The subpart affects estrogen injections.

Regulatory action	Effect of regulation and other comments
21 CFR 522.840—Estradiol. Promulgated: 57 FR 41861, 08/14/92. U.S. Codes: 21 U.S.C. 360b.	Estradiol is used for implantation in steers and heifers as follows: For increased rate of weight gain in suckling and pastured growing steers; for improved feed efficiency and increased rate of weight gain in confined steers and heifers. Each silicone rubber implant contains 25.7 or 43.9 mg of estradiol. Limitations: For subcutaneous ear implantation in steers and heifers only. A second implant may be used if desired. No additional effectiveness may be expected from reimplanting in less than 200 days for the 25.7-mg implant or 400 days for the 43.9-mg implant. Increased sexual activity (bulling, riding, and excitability) has been reported in implanted animals.
21 CFR 522.842—Estradiol benzoate and testosterone propionate in combination. Promulgated: 61 FR 5506, 02/13/96. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in steers and heifers for growth promotion and improved feed efficiency. Dosage includes 20 mg of estradiol benzoate. Limitations: For heifers weighing 400 lb or more; for subcutaneous ear implantation, one dose per animal; not for use in dairy or beef replacement heifers.
21 CFR 522.850—Estradiol valerate and norgestomet in combination. Promulgated: 54 FR 1165, 01/12/89. U.S. Codes: 21 U.S.C. 360b.	This combination is used for synchronization of estrus and ovulation in cycling beef cattle and non-lactating dairy heifers. An injectable solution (sesame oil) contains 3.0 mg of norgestomet and 5.0 mg of estradiol valerate per 2 mL. Limitations: Insert implant subcutaneously in the ear only; then immediately inject solution intramuscularly only. Counting the day of implantation as day 1, remove the implant on day 10. Collect all implants as they are removed and burn them. While animals are restrained for artificial insemination, avoid other treatments such as vaccinations, dipping, pour-on grub and louse prevention, spraying, etc. For insemination without estrus detection, the entire treated group should be started at 48 hours after the last implant has been removed and should be completed within 6 hours. Where estrus detection is preferred, insemination should be approximately 12 hours after first detection of estrus. Those that do not conceive can be re-bred when they return to estrus approximately 17 to 25 days after implant removal. Do not use in cows producing milk for human consumption.
21 CFR 522.1940—Progesterone and estradiol benzoate in combination. Promulgated: 62 FR 8372, 02/25/97. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in animals to increase rate of weight gain. Amounts used are 100 mg of progesterone and 10 mg of estradiol benzoate per dose. Limitations: For animals weighing 400 lb or more; for subcutaneous ear implantation, one dose per animal, and for additional improvement in rate of weight gain in steers fed in confinement for slaughter, reimplant at approximately day 70.

Regulatory action	Effect of regulation and other comments
21 CFR 522.2477—Trenbolone acetate and estradiol. Promulgated: 62 FR 28629, 05/27/97. U.S. Codes: 21 U.S.C. 360b.	This combination is used for implantation in animals to increase rate of weight gain and improve feed efficiency in feedlot steers. Amounts used include 120 mg of trenbolone acetate and 24 mg of estradiol (6 pellets, each pellet containing 20 mg of trenbolone acetate and 4 mg of estradiol) per animal. Limitations: Implant subcutaneously in ear only. Not for use in animals intended for subsequent breeding or in dairy animals.
21 CFR 522.2478—Trenbolone acetate and estradiol benzoate. Promulgated: 61 FR 29479, 06/11/96. U.S. Codes: 21 U.S.C. 360b.	This combination is used in implantation in animals for improved feed efficiency in steers fed in confinement for slaughter. Amounts used are 200 mg of trenbolone acetate and 28 mg of estradiol benzoate (one implant consisting of 8 pellets, each pellet containing 25 mg of trenbolone acetate and 3.5 mg of estradiol benzoate) per animal. Limitation: Implant subcutaneously in ear only.
21 CFR 556—PART 556--TOLERANCES FOR RESIDUES OF NEW ANIMAL DRUGS IN FOOD. Promulgated: 40 FR 13942 03/27/75. U.S. Codes: 21 U.S.C. 342, 360b, 371.	Tolerances are established based upon residues of the new drugs in the treated edible products of food-producing animals. All of these drugs have been shown to directly or indirectly (through metabolites) induce cancer when ingested by humans or animals.
21 CFR 556.240—Estradiol and related esters. Promulgated: 56 FR 67175, 12/30/91. U.S. Codes: 21 U.S.C. 342, 360b, 371.	No residues of estradiol, resulting from the use of estradiol or any of the related esters, are permitted in excess of the following increments above the concentrations of estradiol naturally present in untreated animals: (a) In uncooked edible tissues of heifers, steers, and calves: (1) 120 parts per trillion for muscle, (2) 480 parts per trillion for fat, (3) 360 parts per trillion for kidney, (4) 240 parts per trillion for liver. (b) In uncooked edible tissues of lambs: (1) 120 parts per trillion for muscle, (2) 600 parts per trillion for fat, kidney, and liver.

Source: The regulations in this table have been updated through the 1999 Code of Federal Regulations 21 CFR, 1 April 1999.

3 Human Cancer Studies

3.1 IARC evaluation

In 1999, the IARC critically reviewed numerous case-control and cohort studies that evaluated the relationship of oral contraceptives and hormone replacement therapy (HRT) to the risk of various cancers (see Appendix A). Breast, cervical and endometrial cancers were the most commonly evaluated cancers in relation to exogenous estrogen use. Most studies of oral contraceptive use or HRT, have, however, been unable to evaluate estrogen use specifically and have instead been limited to investigations of various estrogen-progestin combinations (IARC 1999).

The IARC (1999) concluded that the use of oral contraceptives was associated with a very small increased risk of breast cancer, independent of other gynecological risk factors. However, 10 or more years after cessation of oral contraceptive use, breast cancer risk appeared similar to that for women who had never used oral contraceptives. Oral contraceptive users also were shown to be at greater risk of cervical cancer; however, risk estimates were not adequately adjusted for other health and lifestyle factors in these studies.

Despite the increased risk reported for some cancers, hormone use may be protective for others. IARC (1999) indicated that oral contraceptive use nearly halved the risk of endometrial and ovarian cancers. For both cancers, the protective effect of oral contraceptives was greater for longer duration of use and persisted for at least 10 years after cessation of use.

Studies generally have reported no association between oral contraceptives and colorectal cancer, malignant melanoma, or thyroid cancer, although most of these studies have had few exposed cases and limited exposure information overall. Studies of liver cancer have produced mixed results: two studies reported a strong dose-response relationship between oral contraceptive use and benign hepatocellular tumors, while three others showed no association. These studies have generally evaluated the effects of estrogen-progestin combinations, lacking sufficient information to formally evaluate the effects of estrogen alone (IARC 1999).

The IARC (1999) also summarized studies that evaluated cancer risk associated with use of HRT to relieve symptoms of menopause. Some recent studies have evaluated separately the effects of estrogen-only and estrogen-progestin combination therapies; however, others have not had adequate information to do so. Thus, much of what is known from these studies applies to HRT generally, not estrogen therapy specifically.

The studies reviewed by the IARC (1999) generally reported a small increased risk of breast cancer associated with HRT, especially when used recently and for longer than five years. The studies that separately evaluated estrogen-only therapy and estrogen-progestin combinations reported similar risks associated with either type of therapy. However, the dose and type of hormones administered varied considerably and these factors have not been thoroughly evaluated.

Studies consistently reported an increased risk of endometrial cancer associated with increasing duration of estrogen therapy, which remained high 10 years after cessation of therapy. In contrast, studies of cervical, liver, and thyroid cancers, and malignant melanoma showed no association with estrogen replacement therapy. Studies of ovarian and colorectal cancer have produced mixed results. Most studies have shown no relation between estrogen therapy and either ovarian or colorectal cancer; however a few reports have associated estrogen replacement therapy with an increased risk of ovarian cancer and a slightly reduced risk of colorectal cancer.

Although the IARC's review was released only one year ago, several studies have since been published. For the most part, the newer studies, summarized in Tables 3-1 and 3-2, support the IARC's conclusions based on the studies it reviewed (summarized in Appendix A). Although many studies have been published on the relationship between hormone uses and various cancers, this review focuses on studies that were able to evaluate the effects of estrogens specifically and discusses the results for combination therapies only for studies in which estrogen-specific information was not available.

3.2 Hormone replacement therapy

The effects of HRT have been evaluated in several studies of cancer. Hormones used for replacement therapy can be estradiol, conjugated estrogens, other estrogen-only formulas, or combinations of estrogen and progestin. This review focuses primarily on studies of the effects of estrogen-only therapy, which are summarized in Table 3-1.

3.2.1 Breast cancer

Three cohort studies, one in Sweden and two in the United States, have evaluated the relationship between HRT and the risk of breast cancer. All of these studies adjusted for typical reproductive factors related to breast cancer. Schairer *et al.* (2000) identified 2,082 cases of breast cancer among 46,355 post-menopausal women followed in the Breast Cancer Detection Demonstration Project between 1979 and 1989. In general, the use of estrogen replacement therapy was not associated with breast cancer. However, among thinner women (body mass index [BMI] ± 24.4 kg/m^2), breast cancer risk increased moderately with the duration of estrogen use (see Section 6). Thinner women may be more susceptible to the effects of exogenous estrogen, because their endogenous estrogen levels are lower. The duration of estrogen use was not associated with specific tumor histology in this study.

Persson *et al.* (1999) evaluated the risk of breast cancer among 11,231 women prescribed HRT by comparing those who complied with the prescription with those who did not. Breast cancer risk (reported as relative risk, RR) was elevated among women who used estrogen-progestin combinations for more than six years (RR = 1.7, 95% CI = 1.1 to 2.6, n = 44) but not among women who used estrogen alone (RR = 1.1, 95% CI = 0.7-1.7, n = 35). The authors cautioned that women complying with HRT also may be more likely to be screened for breast cancer, potentially resulting in bias in breast cancer detection.

Gapstur *et al.* (1999) identified 1,520 cases of breast cancer among 37,105 post-menopausal women followed in Iowa. This study evaluated the relationship between HRT (estrogen content not specified) and breast cancers of differing prognostic histologies.

HRT was associated with increased risk of breast cancer of favorable histology, but not with risk of ductal *in situ* carcinoma or invasive ductal or lobular carcinoma. This study also evaluated the timing of exposure, but with little power to detect differences. Information on estrogen-only therapy was not available.

Four case-control studies also evaluated the risk of breast cancer associated with HRT. Brinton *et al.* (1998) reported a slight protective effect of HRT (estrogen content not specified) on breast cancer (reported as an odds ratio, OR) in women over 55 years of age (OR = 0.7, 95% CI = 0.5 to 0.9). This study also evaluated the combined effects from HRT and oral contraceptives. A three-fold increased risk of breast cancer among women with who had used oral contraceptives for more than three years and HRT for more than 10 years was observed. A large questionnaire-based case-control study in which the estrogen content of HRT was not specified was reported by Titus-Ernstoff *et al.* (1998). In this study, use of HRT was not associated with an increased risk of breast cancer, regardless of duration of use (< 3 years or >3 years). Both Magnusson *et al.* (1999) and Henrich *et al.* (1998) reported associations between estrogen-only replacement therapy and breast cancer. In a large Swedish case-control study, Magnusson *et al.* (1999) reported that the risk of breast cancer associated with estrogen replacement therapy increased with duration of estrogen use from OR = 1.7 for use < 2 years to OR = 2.7 for use > 10 years. In a smaller Connecticut study of post-menopausal women, Henrich *et al.* (1998) reported that the risk of breast cancer was twice as high among estrogen users than among controls (OR 2.2, 95% CI = 1.2 to 4.2). The effect estimates were slightly higher for non-conjugated than conjugated estrogens and slightly lower for breast cancer *in situ* than for invasive breast cancer. Although this study did not have information on other reproductive factors typically associated with breast cancer, the authors indicated that adjusting for these factors in other studies has not typically altered estimates of risk associated with estrogen use.

3.2.2 Endometrial cancer

Persson *et al.* (1999) evaluated risk of endometrial cancer in the cohort of Swedish women described above. They found a large elevation in risk in women using estrogen only HRT (RR = 4.2, 95% CI = 2.1-8.4, n=27) and a smaller elevation among those using estrogen-progestin combinations (RR = 1.4, 95% CI = 0.6-3.3, n = 11).

All three of the large case-control studies that evaluated the association between estrogen replacement therapy and endometrial cancer supported the positive associations found by the studies reviewed by the IARC (see Appendix A).

Cushing *et al.* (1998) interviewed 484 women with endometrial cancer from the Washington State cancer registry and 780 controls identified through random-digit telephone dialing about their estrogen use. Endometrial cancer was associated with use of conjugated estrogen (OR 5.4, 95% CI = 2.3 to 13.0), primarily among women who had used estrogen therapy within the previous two years. Slightly stronger associations were seen with estrogen doses higher than 1.25 mg. A sensitivity analysis indicated that even with 20% exposure misclassification, the risk of endometrial cancer among women who had had estrogen replacement therapy would be four times that of controls.

Two other large case-control studies, one from Washington State (Shapiro *et al.* 1998) and one from Sweden (Weiderpass *et al.* 1999), collected specific information about estrogen use through questionnaires. Shapiro *et al.* (1998) found the magnitude of the effect of estrogen therapy to be inversely related to tumor grade (OR = 7.8, 5.8, 2.9 for tumor grades I, II, III, respectively). Weiderpass *et al.* (1999) reported that endometrial cancer was strongly associated with use of conjugated estrogens (OR 4.0, 95% CI = 2.5 to 6.4) and estradiol (OR = 2.5, 95% CI = 1.7 to 3.6). Both studies reported stronger positive associations between estrogen replacement therapy and endometrial cancer with increased duration of estrogen use.

3.2.3 Other cancers

Only one recent study has evaluated the association between estrogen replacement therapy and ovarian cancer. Purdie *et al.* (1999) conducted a large interview study in Australia with 793 women who had ovarian cancer and 855 population-based controls. Although estrogen therapy was only modestly associated with all ovarian cancers, the risk of clear-cell epithelial ovarian tumors evaluated separately was significantly increased among estrogen users (OR = 2.6, 95%CI = 1.3 to 4.9). No trend for duration or recency of estrogen use was apparent.

Two recent studies of colon cancer have been conducted among members of California health maintenance organizations (HMOs). Paganini-Hill (1999) surveyed 249 women with and 7,452 women without colorectal cancer about their use of estrogen replacement therapy, and reported only slight inverse associations between estrogen use and colorectal cancer, adjusted for age. Jacobs *et al.* (1999) used pharmacy records to indicate use of estrogen therapy by 341 women with colon cancer and 1,679 controls. No association was found between estrogen therapy and colon cancer. These studies, like earlier ones (Appendix A)**,** do not provide strong evidence for any association between estrogen use and colon cancer.

3.3 Oral contraceptives

In general, the hormone content of oral contraceptives in cancer studies has not been known; however, the contraceptives most likely contained combinations of estrogen and progesterone. Three recent case-control studies in the United States evaluated the association between breast cancer and oral contraceptive use (summarized in Table 3-2).

Titus-Ernstoff *et al.* (1998) and Brinton *et al.* (1998) identified cases of breast cancer through regional cancer registries. Oral contraceptive use was compared between women with breast cancer and population-based controls. Effect estimates were adjusted for reproductive factors typically associated with breast cancer (e.g., age at menarche, parity, age). Titus-Ernstoff *et al.* (1998) evaluated both pre- and post-menopausal breast cancer and Brinton *et al.* (1998) evaluated dose, timing, and duration of use. Although both studies had reasonable power, neither reported marked associations between oral contraceptive use and breast cancer.

Using the National Breast Cancer Screening Cohort in Washington State, Rohan and Miller (1999) evaluated the effect of oral contraceptive use among 1,425 women with benign breast disease, 691 women with benign proliferative epithelial dysplasia, and 5,443

women without either disease. Oral contraceptive use generally was not associated with either proliferative or non-proliferative forms of breast disease. However, contraceptive use for more than seven years was associated with a slightly decreased risk of proliferative forms of breast disease (OR = 0.7, 95% CI = 0.5 to 0.9). A slight increase in the risk of breast disease with atypia also was associated with oral contraceptive use, but was based on a small number of cases.

In general the results of these three studies do not support the positive association between oral contraceptive use and breast cancer suggested in the earlier studies reviewed by the IARC (Appendix A).

Oral contraceptive use was evaluated in a small case-control study of ovarian and endometrial cancer in Mexico (Salazar-Martinez *et al.* 1999). As in previous studies, an inverse association was shown for both types of cancer, especially when oral contraceptives were used for longer than one year.

3.4 Summary

The results of these studies are generally consistent with previous studies of estrogen use (Appendix A). Although early studies were not always able to distinguish between the use of estrogen-only contraceptives or HRT from the use of estrogen-progestin combinations, recent studies are beginning to make this distinction while also considering how dose, duration, and the specific form of estrogen may affect the associated cancer risk. Results from studies of HRT are somewhat more consistent that those from studies of oral contraceptives. The weight of evidence suggests that estrogen use, as HRT by post-menopausal women, is associated with a slight increase in the risk of breast cancer and a stronger increase in the risk of endometrial cancer. Positive and negative associations between estrogens and various other cancers found in previous studies are less consistent.

Table 3-1. Studies of estrogen replacement therapy and cancer of the breast, endometrium, ovaries, and colon

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Schairer *et al*. 2000	breast US cohort 1979—1989	postmenopausal women Breast Cancer Detection Demonstration Project, screening sites throughout US. 2,082 cases 44,273 non-cases	estrogen questionnaire and interview	estrogen only, ever BMI ″ 24.4kg.m^2 use < 8 yr use 8 < 16 yr use ≥ 16 yr	805 80 82 72	1.1 (1.0—1.3) 1.0 (0.8—1.3) 1.5 (1.2—2.0) 1.6 (1.2—2.2)	Adj. for typical reproductive factors. No association in women with BMI > 24.4 kg/m^2. Duration not associated with extent of invasive disease or tumor histology.
Persson *et al*. 1999	breast and endometrial Sweden cohort 1987—1993	11,231 women prescribed HRT followed using national cancer registry 198 incident breast cancer cases, 66 incident endometrial cancer cases non-compliers and users for < 1 year used as reference group.	estrogen questionnaire	estrogen only, ever breast cancer use 1—6 yr use 6+ yr endometrial cancer use 1—6 yr use 6+ yr estrogen-progestin combination breast cancer use 1—6 yr use 6+ yr endometrial cancer use 1—6 yr use 6+ yr	 23 35 5 27 28 44 6 11	 1.0 (0.6—1.7) 1.1 (0.7—1.7) 0.9 (0.3—2.5) 4.2 (2.1—8.4) 1.4 (0.9—2.3) 1.7 (1.1—2.6) 1.1 (0.4—3.1)	Adj. for age, follow-up time, age at first full-term pregnancy, body mass index, education menopausal age/status. No effect of duration on breast cancer risk. Increased risks associated with combined HRT.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Gapstur *et al.*1999	breast Iowa cohort 1986—1996	women aged 55—69 1,520 cases 35,585 non-cases	HRT, unspec. questionnaire	favorable histol. HRT ″ 5yr HRT > 5yr past use ″ 5yr past use > 5yr current use ″ 5 yr current use > 5 yr	 28 15 - - - -	 1.7 (1.0—2.7) 2.2 (1.2—4.0) 1.4 (0.8—2.6) 2.7 (1.1—6.7) 4.4 (2.0—9.8) 2.6 (1.2—5.9)	Adj. for age, BMI, and other reproductive factors. No relation between HRT and DCIS or invasive cancer, only cancer with favorable histology. Type of hormone not specified.
Magnusson *et al*. 1999	breast Sweden case-control 1993—1995	women aged 50-74 3,345 cases, hospital registries 3,454 controls national registry	estrogen questionnaire and interview	estrogen only, ever use 1—24 mo use 25—60 mo use 61—-120 mo use 120+ mo	150/106 55/42 27/25 22/13 33/18	1.9 (1.5—2.6) 1.7 (1.1—2.6) 1.5 (0.9—2.6) 2.2 (1.1—4.5) 2.7 (1.5—5.0)	Adj. for typical reproductive factors. ORs for estrogen-progestin combinations similar.
Henrich *et al*. 1998	breast Connecticut case-control 1987—1992	postmenopausal women aged 45+ 109 cases of *in situ* or invasive cancer 545 controls screening from regional sites	estrogen questionnaire	invasive cancer estrogen only, ever conjugated nonconjugated	 19/51 12/44 9/23	 2.2 (1.2—4.2) 1.9 (0.9—4.1) 2.5 (1.0—5.9)	Not adjusted for typical reproductive factors. ORs slightly lower when *in situ* cases included.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Brinton *et al*. 1998	breast Atlanta, GA case-control 1990—1992	women aged < 55 1,031 cases, registry 919 controls random-digit dialing	estrogen and HRT, unspec. interview	estrogen only, ever HRT, unspec. + oral contraceptive use HRT, unspec. > 10 yr + oral contraceptive use > 3 yr	98/122 179/178 25/?	0.7 (0.5—0.9) 1.0 (0.7—1.4) 3.2 (1.4—7.4)	Evaluation of joint effects of OC and HRT indicated positive association when both used for a longer time, but no independent effects. Adj. for typical reproductive factors.
Titus-Ernstoff *et al*. 1998	breast Northeast, U.S. case-control 1988—1991	women aged 20—72 1,636 premenopausal and 4,992 postmenopausal cases, population-based registries 2,760 premenopausal and 6,391 postmenopausal controls driver's license and Medicare lists	HRT, unspec. questionnaire	postmenopausal cancer use ″ 3yr use > 3yr	 15/14 15/16	 1.1 (0.9—1.2) 0.9 (0.8—1.1)	Adj. for typical reproductive factors. Type of hormones not specified.
Shapiro *et al*.1998	endometrial Washington State. case-control 1985—1991	women aged 45—74 730 cases, state registry 1,002 controls random-digit dialing	estrogen questionnaire	estrogen only use < 3yr use ≥ 3yr tumor grade I tumor grade II tumor grade III	 21/85 93/96 115/96 104/96 28/96	 1.9 (1.1—3.3) 8.4 (5.7—12.4) 7.8 (5.4—11.4) 5.8 (4.0—8.3) 2.9 (1.7—4.8)	Adj. for age and BMI

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Cushing *et al.*1998	endometrial Washington State case-control 1985—1996	Women agee 45—54 484 cases, state registry 780 controls random-digit dialing	estrogen interview	conjugated estrogen dose 0.625 mg ″ 2 yr since use > 2 yr since use dose 1.25 mg ″ 2 yr since use > 2 yr since use	18/8 57/24 14/19 34/7 24/37	5.4 (2.3—13.0) 6.0 (3.6—10) 1.6 (0.8—3.3) 12.6 (5.4—29.2) 1.5 (0.8—2.6)	Adj. for typical reproductive factors. Recent users at any dose at higher risk than those > 2 yr since use. Unspec. HRT decreased risk, but unopposed estrogen increased risk.
Weiderpass *et al*. 1999	endometrial Sweden case-control 1994—1995	women aged 50—74. 789 cases, registry 3,368 controls, population	estrogen questionnaire	estrogen only, ever use 2—4 yr use 5—9 yr use 10—14 yr use 15+ yr conjugated estrogen estradiol	98/177 16/41 16/23 15/12 23/11 46/51 55/125	3.2 (2.4—4.4) 2.1 (1.1—4.0) 3.3 (1.6—6.6) 8.4 (3.7—19.2) 12.6 (5.8—27.2) 4.0 (2.5—6.4) 2.5 (1.7—3.6)	Increased effects with increasing dose and duration, but not recency of use. Effects slightly stronger for high doses, but trends for duration similar.
Purdie *et al*. 1999	ovarian Australia case-control 1990—1993	women aged 18—79 793 cases, clinic registry. 855 controls, population	estrogen interview	all ovarian cancer epithelial clear cell	68/662 18/132	1.3 (0.9—1.9) 2.6 (1.3—4.9)	Adj. for typical reproductive factors. No duration or recency trend.

Reference	Cancer type Study design Period	Study population	Hormone Information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95% CI)	Comments
Paganini-Hill	colorectal	women aged 44—98	estrogen	estrogen only, ever	129	0.8 (0.6—1.0)	Adj. only for age.
1999	California	249 cases		dose ≤ 0.625 mg	29		
	cohort	7,452 non-cases	questionnaire	dose ≥ 1.25 mg	42	0.6 (0.4—0.9)	
	1981—1985			last use ″ 15 yr	51	0.8 (0.5—1.1)	
				2—14 yr	43	1.0 (0.8—1.4)	
				0—1 yr	32	0.7 (0.5—1.0)	
						0.7 (0.4—1.0)	
Jacobs *et al*.1999	colon	women aged 55—79 through HMO	estrogen	estrogen			Adj. only for age.
	California	341 cases		1—749 tablets	21/17	0.9 (0.5—1.4)	
	case-control	1,679 controls	pharmacy records	≥ 750 tablets	28/129	1.1 (0.7—1.7)	
	1984—1993			conjugated estrogen			
				< 375 mg	18/112	0.8 (0.5—1.3)	
				> 375 mg	30/112	1.3 (0.7—2.0)	

Table 3-2. Studies of oral contraceptive use and cancer of the breast, endometrium, or colon

Reference	Cancer type study design and period	Study Population	Exposure information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95%CI)	Comments
Titus-Ernstoff *et al*. 1998	breast Northeast, U.S. case-control 1988—1991	women aged 20—72 1,636 premenopausal cases and 4,992 postmenopausal cases population-based registries. 2,760 premenopausal and 6,391 post-menopausal controls drivers license and Medicare lists.	oral contraceptive, unspecified interview	premenopausal use < 3 yr use > 3 yr postmenopausal use ″3 yr use > 3 yr	 44/45 32/30 11/12 7/7	 (0.9—1.3) (0.9—1.2) (0.9—1.2) (0.9—1.2)	Adj. for typical reproductive factors. Type of hormones not specified.
Brinton *et al*. 1998	breast Atlanta, GA case-control 1990—1992	women aged < 55 1,031 cases, registry. 919 random digit dialing controls	oral contraceptive, unspecified interview	Oral contraceptive ever use 5—9 yr use 10+ yr first use < 15 yr first use 15-19 yr first use 20+ yr	748/641 231/204 173/127 71/56 165/125 512/460	1.1 (0.9—1.4) 1.1 (0.9-1.4) 1.3 (0.9—1.7) 1.3 (0.8—2.1) 1.3 (0.9—1.8) 1.1 (0.9—1.4)	Hormones in oral contraceptive not specified. Adjusted for typical reproductive factors.
Rohan and Miller 1999	breast Washington case-cohort	women aged 40—49 National Breast Cancer Screening Study Cohort 1,425 benign breast disease cases, 691 benign proliferative epithelial disorder cases, 5,443 non-cases.	oral contraceptive, unspecified questionnaire	nonproliferative proliferative without Atypia with Atypia	877/548 424/267 229/359 19/50	1.0 (0.9—1.1) 0.9 (0.8—1.1) (0.8—1.1) 1.5 (0.9—2.7)	Inverse association for proliferative forms of benign breast disease, increased with duration of use. No relation between duration and benign proliferative epithelial disorder

Reference	Cancer type study design and period	Study Population	Exposure information source	Exposure/ disease subgroups	No. exposed or cases/ controls	OR (95%CI)	Comments
Salazar-Martinez *et al*. 1999	endometrial and ovarian Mexico case-control 1995—1997	women attending hospital clinic. 84 ovarian cancer, 85 endometrial cancer 668 clinic/age-matched controls	oral contraceptive, unspecified questionnaire	ovarian cancer use 1-12 mo use ≥13 mo endometrial cancer use 1-12 mo use ≥13 mo	 6/78 7/117 6/78 7/117	 0.6 (0.2—1.3) 0.4 (0.2—0.8) 0.5 (0.2—1.4) 0.4 (0.1—0.9)	Adj. for typical reproductive factors. Type of hormones not specified.

4 Studies of Cancer in Experimental Animals

The International Agency for Research on Cancer (IARC) reviewed carcinogenicity studies of estrogens (conjugated estrogens, estradiol, estriol, estrone, and synthetic estrogens) in experimental animals. These substances were tested via oral administration (diet and drinking water), subcutaneous injection, and implantation (IARC 1999, 1987, 1979; Appendices A, B and C). A summary of the results of these studies is presented in Table 4-1. An overview of the studies reviewed by IARC is presented in the following sections.

4.1 Conjugated estrogens

IARC concluded that there is limited evidence to evaluate the carcinogenicity of conjugated estrogens in animals (IARC 1979, 1987, 1999; Appendices A, B and C).

Groups of 20 male and female weanling Sprague-Dawley rats were fed diets containing conjugated estrogens (Premarin) at 0, 0.07, or 0.7 mg/kg body weight (b.w.) per day for two years (Gibson et al., 1967). Mammary, pituitary, and thyroid tumors were reported in treated and control animals. These data were considered insufficient to evaluate the carcinogenicity of conjugated estrogens (IARC 1979).

Subcutaneous administration studies were conducted in hamsters with equilin, *d*-equilenin, or deconjugated hormones (estrone, equilin and *d*-equilenin), and premarin. Microscopic renal carcinomas were detected in animals treated with equilin, estrone, equilin and d-equilenin, and premarin but not in those treated with d-equilenin alone (Li *et al.* 1983, 1995).

4.2 Estradiol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of estradiol-17∞in experimental animals (IARC 1979, 1987, 1999; Appendices A, B, and C).

Dietary administration of five ppm estradiol to female mice increased the incidences of endometrial preneoplastic lesions and adenocarcinomas, cervical adenocarcinoma, cranial osteosarcoma, adenoacanthoma of the uterus, and mammary adenocarcinoma in female mice (Niwa et al. 1991; Highman *et al.* 1977, 1980). Administration with drinking water doses of 0.5 mg/L estradiol-17∞to groups of female C3H/HeJ (MTV^+) mice resulted in a significantly increased incidence of mammary tumors and benign vaginal stromal polyps (Welsch *et al.* 1977, Sheehan *et al.* 1982).

Increased incidence of mammary tumors were observed in mice following subcutaneous implantation with one to five mg estradiol (Rudali 1975, Rudali *et al.* 1978).

In rats, subcutaneous doses of 5 mg estradiol caused increases in the incidence of pituitary tumors females while administration with subcutaneous doses of 27.5 mg induced

increased incidence of both pituitary and mammary gland tumors (Satoh *et al.* 1997, Shull *et al.* 1997). No increase in the incidence tumors were seen in rats given 0.1 mg subcutaneous estradiol-3-benzoate (Shellabarger and Soo, 1973). Subcutaneous implantaion of rats with 5 mg/mL estradiol also did not induce increased incidence of benign vaginal stromal polyps tumors (Sheehan *et al.* 1982).

In studies in which a limited number of animals were used, renal tumors were observed in castrated male and ovariectomized female Syrian hamsters administered 20 or 25 mg subcutaneous doses of estradiol (Kirkman 1959; Li *et al.* 1983; Liehr *et al.* 1986; Li and Li 1987; Goldfarb and Pugh 1990).

4.3 Estriol

IARC concluded that there is *limited evidence* for the carcinogenicity of estriol in animals (IARC 1979, 1987, 1999 Appendices A, B, and C).

Subcutaneous implantation of estriol was not carcinogenic in rats (IARC 1999, Appendix A). In mice, increased incidence of mammary tumors was seen in castrated males and females subcutaneously implanted with estriol (0.64-0.85 mg estrogen) (Rudali 1975). Increased incidence of renal tumors were seen in hamsters of heterogenous origin subcutaneously exposed to 20 mg pellets of estriol (Kirkman 1959).

4.4 Estrone

IARC concluded that there is *sufficient evidence* for the carcinogenicity of estrone in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

In rats, mammary gland tumors were seen following subcutaneous implantation with estrone (Dunning *et al.* 1953, Cutts 1966). Pituitary tumors and adrenal carcinomas were also seen in rats following subcutaneous doses of estrone (Geschickter and Byrnes 1942; Chamorro 1943; Noble *et al.* 1975).

In mice, drinking water doses of 125 or 2,000 ∝g/L estrone resulted in high incidences of mammary gland tumors (33/68 and 119/169, no control data given) (Boot and Muhlbock 1956). The incidence of mammary tumors was also observed to increase in castrated male mice given 6 ∝g/day dietary estrone (Rudali *et al.* 1978). Mammary tumors were found in male and female mice given subcutaneous doses of estrone (Bonser 1936; Shimkin and Grady 1940; Bittner 1941).

Intact and castrated male Syrian hamsters given subcutaneous implantations of estrone have been reported to develop significant numbers of renal tumors (Dontenwill 1958; Kirkman 1959; Li *et al.* 1983).

4.5 Synthetic estrogens

4.5.1 Ethinylestradiol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of ethinylestradiol in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

Oral administration of ethinylestradiol produced benign liver tumors in male and female rats and malignant liver tumors in female rats (Committee on Safety of Medicines 1972, Ogawa *et al.* 1995). Female Mead-Johnson rats fed 53 µg/day of ethinylestradiol did not develop any tumors (McKinney *et al.* 1968).

Groups of 120 CF-LP (MTV^+) mice were given ethinylestradiol at 2 to 400 times the human dose. Pituitary tumors were observed in 26 males and 38 females compares to two and eight tumors in control male and female mice, respectively (Committee on Safety of Medicines 1972). A small increase in the incidence of pituitary tumors (both sexes), mammary tumors (both sexes), cervical tumors, and benign gonadal tumors (males) was also reported in BDH-SPF mice (Committee on Safety of Medicines 1972).

Female dogs treated with a combination of ethinylestradiol and norgestrel at 10 to 25 times the human dose had and increased incidence of mammary nodules (Finkel and Berliner 1973).

4.5.2 Mestranol

IARC concluded that there is *sufficient evidence* for the carcinogenicity of mestranol in experimental animals (IARC 1979, 1987, 1999; Appendix A, B and C).

Unspecified doses of mestranol via an unspecified route induced increased incidence of mammary tumors in rats (Committee on Safety of Medicines 1972). Female Sprague-Dawley rats given 6 or 30 µg/kg b.w. of mestranol in the diet developed hepatic nodules and hepatocellular carcinomas (Yager *et al.* 1984).

Dietary mestranol given to castrated male mice at doses from 0.075 to 1 mg/kg b.w. per day developed mammary tumors (Rudali *et al.* 1971). Pituitary tumors were increased in mice of both sexes given 2 to 400 times the human dose in the diet (Committee on Safety of Medicines 1972)., Barrows et al. (1977) reported no increase in hepatocellular tumors in female Swiss Webster or CF-LP mice given 5, 30, 60, or 200 µg/kg b.w. per day.

Female dogs given mestranol at did not show an increased incidence of tumors (Geil and Lamar 1977, Giles *et al.* 1978, Kwapien *et al.* 1980).

A summary of the carcinogenicity studies of steroidal estrogens in experimental animals is presented in Table 4-1.

Table 4-1. Carcinogenic effects of steroidal estrogens in experimental animals[a]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Conjugated estrogen							
Conjugated equine estrogens and equilin	s.c.	hamsters (castrated male), 8–9	20 mg pellet, 9 mo	renal tumors; 6/8	NA	NS	Li *et al.* 1983[b]
Deconjugated hormones (estrone, equilin, *d*-equilenin, Premarin)	s.c.	hamsters (castrated male), 6–8	111 ∝g/d, 9 mo	estrone, 15 renal tumors equilin + *d*-equilenin, 18 renal tumors Premarin, 16 renal tumors	NA	NS	Li *et al.* 1995[b]
Estradiol							
Estradiol	diet	ICR mice (female), 30–31	5 ppm, 20 wk	NA	endometrial preneoplastic lesions; 48% endometrial adenocarcinoma; 7/31	no endometrial tumors	Niwa *et al.* 1991[b]
Estradiol	diet	ICR mice (female), 41	5 ppm, 16 wk	NA	development of cystic glandular hyperplasia and adenomatous and atypical hyperplasia of the endometrium	NS	Niwa *et al.* 1991[b]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol	diet	C3H/HeJ (MTV^+) mice[d] (female), 200–227	100, 1,000, 5,000 ∝g/kg, 104 wk	NA	cervical adenosis; 1,000 ∝g/kg, 8/20 5,000 ∝g/kg, 3/6 uterine adenocarcinoma; 5,000 ∝g/kg, 5/207 mammary hyperplastic alveolar nodules; 5,000 ∝g/kg, 6/17 (wk 95–105) mammary adenocarcinoma; 5,000 ∝g/kg, 8/17 (wk 95–105)	cervical adenosis; NR uterine adenocarcinoma; 0/227 mammary hyperplastic alveolar nodules; 6/50 (wk 95–105) mammary adenocarcinoma; 19/50 (wk 95–105)	Highman *et al.* 1980[b]
Estradiol	diet	C3H/HeJ (MTV^+) mice[d] (female), 48	100, 1,000, 5,000 ∝g/kg, 24 mo or 104 wk	NA	mammary adenocarcinoma: 100 ∝g/kg, 0/35 1,000 ∝g/kg, 6/36 5,000 ∝g/kg, 8/48 100 ∝g/kg, 1 cervical adenocarcinoma, 1 cranial osteosarcoma 5,000 ∝g/kg, 2 uterine adenocarcinoma, 3 cervical adenocarcinoma 1 adenoacanthoma	mammary adenocarcinoma; 4/47	Highman *et al.* 1977
Estradiol	drinking water	C3H/HeJ (MTV^+) mice (female), 99	0.5 mg/L, 19 mo	NA	mammary tumors; 27/99	mammary tumors; 11/100	Welsch *et al.* 1977

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol dipropionate	s.c. injection	Fischer 344 rats (female), 2–16	5 mg, once every 2 wk for 13 wk	NA	pituitary adenoma; 11/12 (wk 7) carcinoma; 16/16 (wk 13)	0/10 tumors	Satoh *et al.* 1997[b]
Crystalline estradiol	s.c. implant	ACI rats (intact female, ovariectomized female), 21	27.5 mg, 197 d	NA	mammary carcinoma; intact, 21/21 pituitary tumors; similar incidence in intact and ovariectomized	0/3	Shull *et al.* 1997[b]
Estradiol	s.c. implant	Sprague-Dawley rats (ovariectomized female), 19	0.5 mg/L, 16 mo	NA	benign vaginal stromal polyps; 0/17	NS	Sheehan *et al.* 1982[b]
Estradiol-3-benzoate	s.c. injection	Sprague-Dawley rats (female)	0.1 mg	NA	no tumors	NS	Shellabarger and Soo 1973
Estradiol	s.c. implant	(C3H x RIII)F1 (MTV^+) (castrated male) mice	1, 2.5, 5, 10, 100 ∝g	mammary tumors; 1 ∝g, 11/31 2.5 ∝g, 23/27 5 ∝g, 24/27 10 ∝g, 27/27 100 ∝g, 24/24	NA	mammary tumors; 11/33	Rudali *et al.* 1978
Estradiol	s.c. injection	Syrian golden hamsters (castrated male), 5	20 mg, 5.3 mo	renal carcinoma	NA	no tumors	Goldfarb and Pugh 1990[b]

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estradiol	s.c. injection	Syrian golden hamsters (castrated male), 6	20 mg, 8.3 mo	renal carcinoma	NA	no tumors	Li *et al.* 1983[b]
Estradiol	s.c. implant	Syrian golden hamsters (castrated male)	25 mg, 6 mo or 9–10 mo	renal-cell carcinoma; 6 mo, 4/5 9 or 10 mo, 6/6	NA	NS	Liehr *et al.* 1986, Li and Li 1987[b]
Estriol							
Estriol	s.c. implant	(C3H x RIII)F1 (MTV^+) mice (castrated male, female)	0.64–0.85 mg	mammary tumors; 25/30	mammary tumors; 18/18	mammary tumors; males, 10/16 females, 28/34	Rudali 1975
Estriol	s.c. implant	hamsters (heterogenous)	20 mg, 318–601 d	renal tumors; 6/11	NA	NS	Kirkman 1959
Estrone							
Estrone	drinking water	C3H mice C3He (MTV^-) mice	125 or 2,000 ∝g/L	NA	mammary gland tumors; C3H mice, 33/68 (C3He) (MTV^-) mice, 119/169	NS	Boot and Muhlbock 1956[b] (and cited in IARC 1979)
Estrone	diet	(C3H x RIII)F1 (MTV^+) mice (castrated male)	0.66, 0.6, 6 ∝g/d	mammary tumors; 11/33 (0.66 ∝g/day), 15/30 (0.6 ∝g/day), 33/34 (6 ∝g/day)	NA	mammary tumors; 12/33	Rudali *et al.* 1978
Estrone	s.c. implant	Sprague-Dawley rats (female)	10% estrone, 370 d	NA	no tumors	no tumors	Lemon 1975
Estrone	s.c. implant	hooded rats (female)	NS	NA	adrenal cortical tumors; 20%	adrenal cortical tumors; 5%	Noble 1967

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estrone	s.c. implant	hooded rats (female)	10 mg 90% estrone, 10–53+ wk	NA	adrenal carcinoma, mammary carcinoma, pituitary tumors[e]	NS	Noble *et al.* 1975
Estrone	s.c. implant	Fischer 344 rats	10 mg	NA	mammary gland tumors; 12/74	NS	Cutts 1966
Estrone	s.c. implant	Wistar rats	10 mg	NA	mammary gland tumors; 12/50	NS	Cutts 1966
Estrone	s.c. implant	Lewis rats	10 mg	NA	mammary gland tumors; 17/44	NS	Cutts 1966
Estrone	s.c. implant	Sprague-Dawley rats	10 mg	NA	mammary gland tumors; 16/38	NS	Cutts 1966
Estrone	s.c. implant	hooded rats	10 mg	NA	mammary gland tumors; 182/212	NS	Cutts 1966
Estrone	s.c. implant	AxC rats (male, female)	8–12 mg	mammary gland tumors; 4/30	mammary gland tumors; 3/32	NS	Dunning *et al.* 1953
Estrone	s.c. implant	Fischer rats (male, female)	8–12 mg	mammary gland tumors; 2/29	mammary gland tumors; 3/29	NS	Dunning *et al.* 1953
Estrone	s.c. implant	August rats (male, female)	8–12 mg	mammary gland tumors; 9/25	mammary gland tumors; 5/12	NS	Dunning *et al.* 1953
Estrone benzoate	Subcutaneous injection	Rats (male, female)	50–100 ∝g, twice weekly for 20 mo	mammary gland tumors; 1/2 pituitary tumors; 100%	mammary gland tumors; 5/8 pituitary tumors; 100%	NS	Chamorro 1943
Estrone	s.c. injection	rats (castrated male, ovariectomized female)	50–200 ∝g/d, for total dose of 30-40 mg	mammary gland tumors; castrated males, 6/6 intact males, 2/6	mammary gland tumors; ovariectomized females, 4/5 intact females, 3/8	NS	Geschickter and Byrnes 1942

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Estrone	s.c. implant	A strain mice (male, female) C3H (MTV^+) mice (female)	2 mg	mammary tumors	mammary tumors	NS	Bittner 1941
Estrone	s.c. implant	Hybrid (A, C3H, C57, JK) mice	1 to 7 mg	lymphoid tumors; 19/105[f]	lymphoid tumors; 19/105[f]	lymphoid tumors; 21/391[g]	Gardner and Dougherty 1944
Estrone benzoate	s.c. injection	A strain (MTV^+) mice	30–50 ∝g weekly, 43 wk	mammary tumors; 3/21	NA	NS	Bonser 1936
Estrone benzoate	s.c. injection	C3H (MTV^+) mice (male)	50 ∝g weekly, 24 wk	mammary tumors; 2/10	NA	NS	Shimkin and Grady 1940
Estrone benzoate	s.c. injection	C3H mice (female)	50 ∝g weekly, 24 wk	NA	mammary tumors; 100%	mammary tumors; 100%	Shimkin and Grady 1940
Estrone	s.c. implant	Syrian hamster (intact and castrated male)	20 mg, 8.5 mo	renal carcinoma; 8/10	NA	NS	Li *et al.* 1983[b]
Estrone	s.c. implant	Syrian hamster (intact and castrated male)	20 mg	malignant renal tumors; intact, 7/8 castrated, 10/10	NA	malignant renal tumors; intact, 0/6 castrated, 0/60	Kirkman 1959[b]
Estrone	s.c. injection	Syrian golden hamsters (castrated male)	NS	malignant renal tumors; 60% pituitary adenoma; 25%	NA	NS	Dontenwill 1958

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Synthetic estrogens							
Ethinylestradiol	diet	Mead-Johnson rats, 30 (females)	53 µg/kg per day	NA	no increase in any type	NS	McKinney *et al.* 1968
Ethinylestradiol	NS	rats, 73–120	low, med, high (2–400 X human dose)	benign liver-cell tumors; 15%	benign liver-cell tumors; 23%	0 to 8% benign liver-cell tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	rats, 73–120	low, med, high (2–400 X human dose)	NA	malignant liver-cell tumors; 7.5%	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	gavage	Wistar rats, 23-26 (females)	75 or 750 µg	NA	hepatocellular carcinomas; 2 (low dose), 10 (high dose)	no tumors	Ogawa *et al.* 1995[b]
Ethinylestradiol	Diet	CF-LP (MTV^+) mice, 120	low, med, high (2–400 X human dose)	pituitary tumors, 26	pituitary tumors, 38	pituitary tumors; males, 2 females, 8	Committee on Safety of Medicines, 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	pituitary tumors; 4%	pituitary tumors; 10%	pituitary tumors; males, 2% females, 0%	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	benign gonadal tumors; 8 to 10%	NA	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	mammary tumors; 9%	mammary tumors; 32%	mammary tumors; males, 0% females, 3%	Committee on Safety of Medicines 1972

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Ethinylestradiol	NS	BDH-SPF mice, 71–87	NS	NA	uterine or cervical tumors; 4 to 11%	no tumors	Committee on Safety of Medicines 1972
Ethinylestradiol + norgestrel	NS	dogs, 12 (females)	10–25 X human dose	NA	mammary nodules; 8 (33.3%)	mammary nodules; 2 (16.7%)	Finkel and Berliner 1973
Mestranol	NS	rats 100 50 controls (females)	unspec.	NA	mammary tumors; 22%	mammary tumors; 5%	Committee on Safety of Medicines 1972
Mestranol	diet	Sprague-Dawley, 15-16 (females)	6 or 30 µg/kg per day	NA	hepatic nodules and carcinomas; 4 (25%)	none	Yager *et al.* 1984[b]
Mestranol	diet	RIII (MTV^+) mice (males), 13–19	0.1 mg/kg per day	mammary tumors; castrated, 11 (84.6%) intact, 8 (42.1%)	NA	NS	Rudali *et al.* 1971
Mestranol	diet	C3H x RIII) F_1 (MTV^+) mice (castrated), 26–41	1 mg/kg per day	mammary tumors; 24 (92.3%)	NA	mammary tumors; 7 (17.1%)	Rudali *et al.* 1971
Mestranol	diet	C3H x RIII) F1 (MTV^+) mice (castrated), 32–61	0.075 mg/kg per day	mammary tumors; 26 (81.3%)	NA	mammary tumors; 10 (16.4%)	Rudali *et al.* 1972
Mestranol	diet	CF-LP mice, 120–240	low, med, high (2–400 X human dose)	pituitary tumors; 12 (10%)	pituitary tumors; 17 (14.2%)	pituitary tumors; males, 4 (1.7%) females, 12 (5%)	Committee on Safety of Medicines 1972
Mestranol	diet	Swiss mice, 47–123	low, med, high (2–400 X human dose)	mammary tumors; 4%	mammary tumors; 4%	no tumors	Committee on Safety of Medicines 1972

Test substance	Route	Species (sex), no.	Exposure	Tumor type and incidence or total tumors			Reference
				Males	Females	Controls	
Mestranol	NS	Swiss Webster and CF-LP mice (females), unspec.	5, 30, 60, or 200 µg/kg per day	no increase in hepatocellular tumors	no increase in hepatocellular tumors	NS	Barrows *et al.* 1977
Mestranol	NS	dogs (females), 13-20	10–25 X human dose	NA	mammary adenoma; 1	benign mixed mammary tumors; 2	Geil and Lamar 1977, Giles *et al.* 1978
Mestranol	NS	beagle dogs (females), 15	0.02 or 0.05 mg/kg per day	NA	none	NS	Kwapien *et al.* 1980[b]
Mestranol	oral	monkeys (females), 16–20	2, 10, or 50 X human dose	mammary nodules; unspec. no. randomly distributed after 7 yr	mammary nodules; unspec. no. randomly distributed after 7 yr	mammary nodules; unspec. no. randomly distributed after 7 yr	Geil and Lamar 1977
Enovid (1.5% mestranol, 98.5% norethynodrel)	NS	Rhesus monkeys (females), 6	1 mg/day	NA	mammary adenocarcinoma; 1	NS	Kirchstein *et al.* 1972

Source: Cited in IARC 1979 unless otherwise noted

[a]NA = not applicable; NS = not specified; NR = not reported.

[b]Cited in IARC 1999.

[c]Although no control tumor incidence data were reported, a zero incidence has been estimated for the experimental conditions of this study (Liehr *et al.* 1986a).

[d]Strain with a high titer of antibodies to the mouse mammary tumor virus.

[e]The incidence of mammary adenomas was increased in treated males and females up to one year, but was lower than that of controls thereafter.

[f]Overall incidence.

[g]Value in corresponding controls.

4.6 Neonatal exposure to estrogens

4.6.1 Mice

Data from several studies on the effects of neonatal estrogen exposure on mouse vaginal tissue suggest that estrogens affect the fornical and cervical tissues of the genital tract, causing irreversible cornifications, downgrowths, adenosis, and adenocarcinomas (Kimura and Nandi 1967, Forsberg 1972, 1973, 1975, 1979, Takasugi 1976, 1979, Jones and Bern 1977, cited in IARC 1979). Increased mammary tumorigenesis has also been reported as a consequence of neonatal exposure of mice to estrogens (estradiol-17∞) (Bern *et al.* 1975, 1976, Mori 1968; Mori *et al.* 1967, 1976, Warner and Warner 1975, Jones and Bern 1977, all cited in IARC 1979).

4.7 Summary

Experimental animal studies in rats, mice, and hamsters have been conducted using estrogens alone or in combination with known carcinogens. Estrogen had a carcinogenic effect regardless of the animal model or route of administration. Most studies resulted in induction of benign and malignant neoplasms as well as preneoplastic lesions in a variety of target organs, including the breast and female reproductive tract.

Dietary estradiol and estradiol administered in drinking water were carcinogenic to mice, inducing increased incidence of mammary tumors in females. Increased incidence of mammary tumors was also evident in male mice administered subcutaneous doses of estradiol. In rats, subcutaneous implantations of estradiol increased the incidence of mammary and pituitary tumors in females. Renal carcinomas were observed in hamsters exposed to estradiol via the subcutaneous route.

Mice given subcutaneous implantations of estriol developed mammary tumors while male hamsters of heterogenous origin, similarly treated, developed renal tumors. Subcutaneous implantation of estriol did not induce any carcinogenic effect in rats.

Increased incidences of mammary tumors were observed in both sexes of mice following oral exposure to estrone and in both sexes of rats following subcutaneous exposure. Increases in the incidence of adrenal, lymphoid, pituitary tumors were also evident in rats following subcutaneous exposure to estrone. Hamsters exposed by subcutaneous administration to estrone developed renal tumors.

The synthetic estrogens have also been found to be carcinogenic in experimental animals. In poorly reported studies where routes of administration and/ or doses were not clearly identified, ethinylestradiol, caused mammary, cervical/uterine, and renal tumors in mice while mestranol caused increased incidence of mammary and pituitary tumors in mice.

5 Genotoxicity

The IARC reviewed the literature through 1999 regarding the genotoxicity of sex hormones, hormonal contraceptives, and post-menopausal hormone therapy (IARC 1987, 1999). The relevant genotoxicity information from the IARC (1999) monograph is summarized in Table 5-1. For a more complete review of these data, see Appendices A and B.

Table 5-1 includes results for the two synthetic estrogens, ethinylestradiol and mestranol, that are widely used in oral contraceptives, as well as for endogenous estrogens and metabolites. The most widely studied compounds are the synthetic hormones and estradiol. Results from studies that combined estrogens with other hormones or chemicals are not included in the table but are available for review in Appendix A. In general, estrogens combined with other chemicals did not show genotoxic effects that were not also seen with individual estrogens. One exception was the induction of reverse mutation in bacterial systems exposed to mestranol combined with 2-acetylaminofluorene, nitrosopiperidine, or a progestogen (IARC 1999).

There was no evidence of genotoxic effects in nonmammalian systems (IARC 1999). The most common findings in mammalian systems included DNA adduct formation in laboratory animals (*in vitro* and *in vivo*), transformation in animal cell lines, and aneuploidy in animal and human cell lines. *In vitro* studies with human cell lines, in addition to aneuploidy, gave some evidence of DNA strand breaks, micronucleus formation, and sister chromatid exchange (Table 5-1). No human *in vivo* data were available.

Sections 5.1 through 5.4 present results of genotoxicity studies that were not reviewed in IARC (1999).

5.1 Prokaryotic systems

5.1.1 Gene mutation in Salmonella typhimurium

Neither ethinylestradiol, cyclotriol, nor cyclodiol induced reverse mutation in the Ames assay, with or without S9 metabolic activation (Hundal *et al.* 1997). A modified host-mediated version of this assay also did not show significant mutagenic effects.

5.2 Plants

No information on the genotoxicity of estrogens in plants was found in the published literature.

5.3 Lower eukaryotic systems

No information on the genotoxicity of estrogens in lower eukaryotes was found in the published literature.

Table 5-1. Genetic toxicology and related effects of steroidal estrogens reviewed in IARC (1999)

	Test system and results[a]																			
	In vitro															*In vivo*				
	Animal cells[b]									Human cells[b]						Animals[b]				
Estrogen type	**A**	**C**	**D**	**G**	**I**	**M**	**S**	**T**		**A**	**C**	**D**	**M**	**S**		**A**	**C**	**D**	**M**	**S**
Synthetic																				
Ethinylestradiol	+[1]	–[1]		–	w			?			–[1]						+	+	–[1]	
Mestranol					w[1]						?			+[1]			?	–[1]	+[1]	+[1]
Endogenous																				
Estradiol	+	–	?	–		+	–	+		+	–		+[1]			?	+[1]	?		?
17∞-Estradiol																	–[1]			
4-OH-estradiol			+					+[1]										+		
2-OH-estradiol			?					+[1]										–[1]		
Estradiol-3,4-quinone			+[1]															+[1]		
Estrone			–[1]	–														–		
Estrone-3,4-quinone			w[1]									+[1]								
16∞-OH-estrone			+[1]					+[1]												
2-OH-estrone			–[1]																	
4-OH-estrone			+																	
Estriol	+[1]		–[1]					–[1]						w						

Source: Adapted from IARC 1999

[a]Blank cells, not tested or not reported; +, predominantly positive responses; +[1], positive response in a single study; w, weak positive responses; w[1], weak positive response in a single study; ?, both positive and negative responses; –, only negative responses; –[1], negative response in a single study

[b]A, aneuploidy; C, chromosomal aberrations; D, DNA damage; G, gene mutation; I, inhibition of intercellular communication; M, micronuclei; S, sister chromatid exchange; T, cell transformation

5.4 Mammalian systems

5.4.1 In vitro *assays*

5.4.1.1 Cytogenetic effects

Estrogen-induced aneuploidy and micronuclei have been reported in various animal and human cell types (Pfeiffer and Metzler 1992, Schnitzler and Metzler 1992, Schuler *et al.* 1996, Metzler *et al.* 1996, Sato and Aizu-Yokota 1996). Steroidal estrogens, with peroxidase-mediated oxidation, interfered with microtubule assembly in a cell-free system (Pfeiffer and Metzler 1992). Interaction with microtubular proteins was proposed as a possible mechanism for estrogen-induced aneuploidy. Schnitzler and Metzler (1992) reported that estradiol, 2-hydroxyestradiol, and 4-hydroxyestradiol induced micronuclei in Syrian hamster embryo fibroblasts and sheep seminal vesicles. Schuler *et al.* (1996) reported that estradiol induced micronuclei in human chorionic villus cells. Sato and Aizu-Yokota (1996) tested several natural estrogens and their catechol derivatives for their ability to disrupt the cellular microtubule network in Chinese hamster V79 cells. The effective concentration required to disrupt microtubules in 50% of the cells (EC_{50}) ranged from 2 mM for 2-methoxyestradiol to > 100 mM for estrone. The EC_{50} for the catechol derivatives of estrone ranged from 30 to 70 mM.

Cultured human lymphocytes were exposed to ethinylestradiol, cyclotriol, or cyclodiol at a concentration of 1, 10, or 100 ∝g/mL, with and without S9 metabolic activation, for 24, 48, or 72 hours (Hundal *et al.* 1997). All three of these oral contraceptive drugs significantly increased chromosomal aberrations without S9 metabolic activation. Ethinylestradiol was the most potent, inducing both chromosomal and chromatid-type aberrations at all doses and durations except at the lowest concentration for the shortest duration. Six-hour exposure in the presence of S9 significantly increased the frequency of chromosomal aberrations at the two highest concentrations.

5.4.1.2 Sister chromatid exchange

17∝-Estradiol at a concentration of 10^{-5} M increased the incidence of sister chromatid exchange (SCE) in epithelial cells from the cervix and vagina of neonatal NMRI mice (Hillbertz-Nilsson and Forsberg 1989). Human peripheral blood lymphocyte cultures were exposed to ethinylestradiol, cyclotriol, or cyclodiol at a concentration of 1, 10, or 100 ∝g/mL for 24 or 48 hours without metabolic activation (Hundal *et al.* 1997). All three estrogens significantly increased SCEs at all concentrations. In separate experiments, cultures were given 90-minute pulse exposures (with or without metabolic activation) at all three concentrations. Significant increases in SCEs were reported for most exposures.

5.4.1.3 DNA damage or repair

In a review article, Liehr *et al.* (1990) reported that the catechol estrogen metabolites were genotoxic *in vitro*, resulting in formation of quinone and DNA adducts. The comet assay (single-cell gel electrophoresis) was used to detect DNA breaks in human peripheral blood lymphocytes and sperm exposed to estradiol (Anderson *et al.* 1997). Exposure of peripheral blood lymphocytes to estradiol at concentrations ≥ 50 ∝M for 0.5 hours significantly increased DNA damage. Sperm samples were exposed for one hour; exposure to estradiol at concentrations ≥10 ∝M significantly increased DNA damage.

5.4.2 In vivo *assays*

5.4.2.1 Aneuploidy and micronucleus formation

The incidences of aneuploidy and micronuclei were increased by factors of 8.0 and 4.3, respectively, in estrogen-induced renal tumors in male Syrian hamsters. Endomitosis, chromatid and chromosome breaks, and telomeric associations also were increased in these tumors (Banerjee *et al.* 1992). Micronuclei were induced in bone marrow cells from Swiss albino mice exposed to ethinylestradiol, cyclotriol, or cyclodiol at 1, 5, or 10 mg/kg body weight (b.w.) via a single intraperitoneal (i.p.) injection (Hundal *et al.* 1997).

5.4.2.2 Sister chromatid exchange

Swiss albino mice were exposed to ethinylestradiol, cyclotriol, or cyclodiol at 1, 5, or 10 mg/kg b.w. via a single i.p. injection (Hundal *et al.* 1997). After 30 hours, the animals were sacrificed, and bone marrow cells were examined for SCEs. Each drug induced a dose-dependent increase in the frequency of SCEs.

5.4.2.3 DNA adduct formation

DiAugustine *et al.* (1992) observed multiple DNA adducts in kidneys from adult male Syrian golden hamsters in both control and estrogen-exposed groups. Chronic subcutaneous exposure to estrogens characterized as strongly carcinogenic (diethylstilbestrol, 17∞-estradiol), weakly carcinogenic (ethinylestradiol), or noncarcinogenic (17∞-estradiol, ∞-dienestrol, indanestrol) did not alter the DNA adduct profiles. These results call into question the significance of estrogen-induced DNA adducts in hormonal carcinogenesis.

5.5 Summary

Both synthetic and endogenous steroidal estrogens cause damage to chromosomes and DNA. The most frequently reported effects include formation of DNA adducts, cytogenetic alterations (e.g., chromosome and chromatid breaks, micronucleus formation, SCE), aneuploidy, and cell transformation. Most of these effects have been demonstrated in various *in vitro* assays with cultured animal cells or cell-free systems. Fewer effects have been reported in whole-animal studies or in studies with human cells, and no human *in vivo* data were available.

6 Other Relevant Data

Many tissues, particularly the uterus and mammary glands, contain estrogen receptors and respond to estrogen exposure. 17∞-Estradiol (estradiol) is the natural ligand for the estrogen receptor and is used as the standard for determining the estrogenicity of other compounds. Two high-affinity, low-capacity forms of the estrogen receptor (∞ and ∞) have been identified. The specific function of the ∞ receptor has not been determined; therefore, most of the data regarding binding affinity, receptor-ligand interactions and transcriptional regulation pertain to the ∞ receptor (IARC 1999). Although there is strong evidence that estrogen carcinogenesis is mediated through the estrogen receptor, there is evidence that estrogenic activity alone is insufficient to explain the carcinogenic effects of estrogens in all tissues. For example, the synthetic estrogen ethinylestradiol binds to the estrogen receptor with affinity equal to that of estradiol, but the former is a much weaker carcinogen. In other cases, the target cells do not contain estrogen receptors (Barrett and Tsutsui 1996).

This section summarizes current views on the probable mechanisms involved in estrogen carcinogenicity. Although the discussion focuses on estrogens, combined estrogen–progestin therapies have become more common in recent years. Combination therapies with progestins are used to lower the risk of endometrial cancer, but they do not reduce breast cancer risk (Colditz 1998, Henderson and Feigelson 2000). There is some evidence that taking estrogens in combination with progestins might increase the risk of breast cancer. A possible explanation is that progestin is a mitogen in mammary ductal epithelial cells but not in the uterus (Liehr 1997, Colditz 1998, Henderson and Feigelson 2000).

6.1 Estrogen metabolism

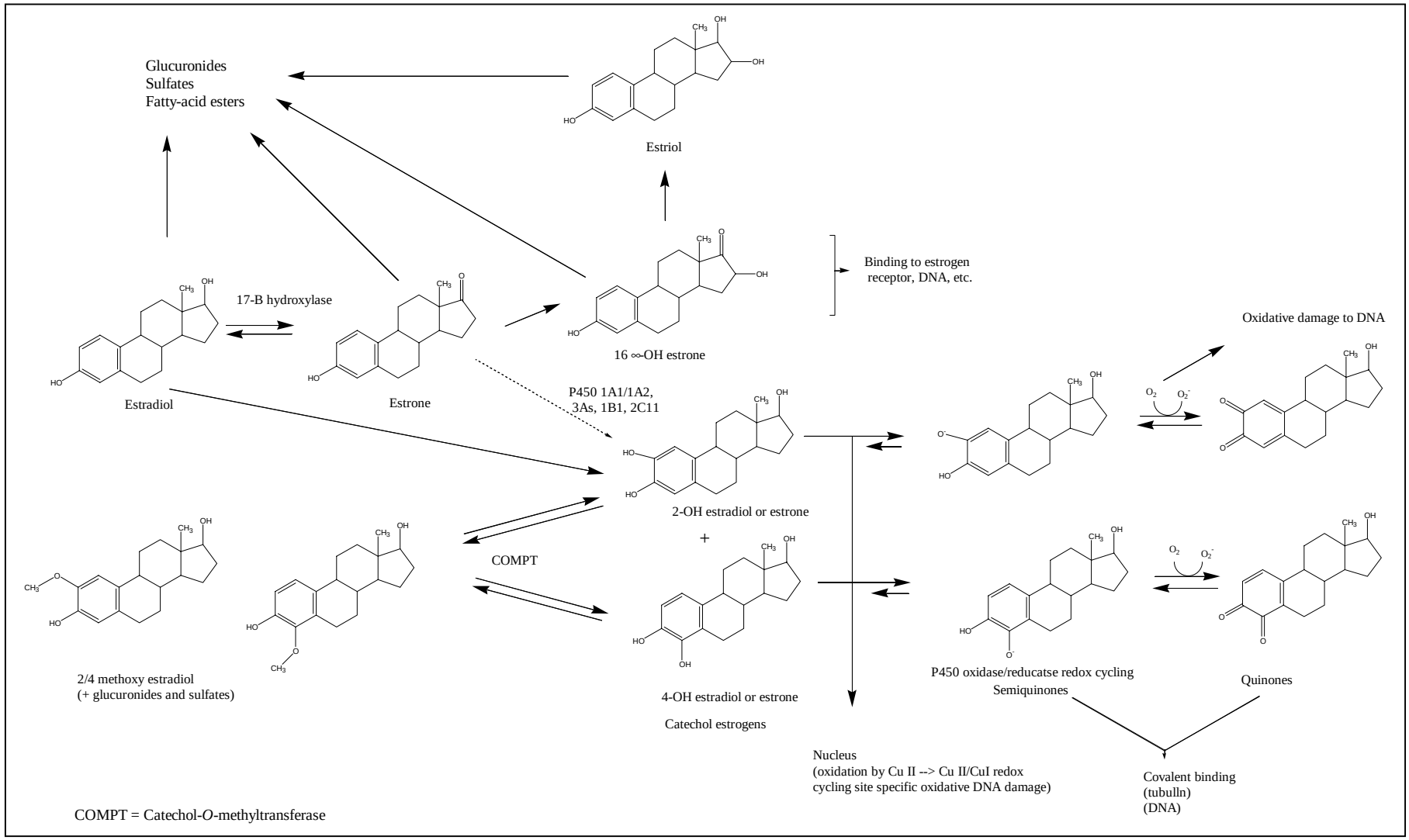
Many different formulations of synthetic and naturally produced estrogens are prescribed for use as oral contraceptives or in postmenopausal hormone replacement therapy. Ethinylestradiol and mestranol are synthetic estrogens commonly used in contraceptives. In the United States, conjugated estrogens are commonly used in postmenopausal estrogen therapy, while in Europe, various preparations of estradiol are preferred. Conjugated estrogens are a mixture of any of at least eight different compounds derived wholly or in part from equine urine or synthetically from estrone and equilin (IARC 1999).

Exogenous estrogens are well absorbed from the gastrointestinal tract and the skin of humans and laboratory animals; therefore, oral, sublingual, dermal, and transdermal preparations are available. The absorption rate, maximum and steady-state concentrations, half-life, and clearance rate depend on the particular estrogen preparation, route of administration, and dose. Estrogens are metabolized in the gastrointestinal tract, liver, and other tissues. It is difficult to make generalizations regarding the pharmacokinetics of estrogens; however, oral administration results in lower circulating levels and faster elimination than dermal or transdermal applications, because of the first-pass effect in the liver (IARC 1999).

In both humans and animals, estradiol, estrone, and estriol undergo similar phase I and phase II reactions. Aromatic hydroxylation reactions catalyzed by cytochrome P-450

enzymes are the primary phase I pathways. Sulfation, methylation, and glutathione conjugation are the major phase II pathways. The ratio of metabolic products depends on the target tissues, species, strain, sex, and experimental conditions (IARC 1999). The primary metabolic pathways for estrogens are illustrated in Figure 6-1 and are discussed in more detail below. The available data indicate that metabolism of conjugated equine estrogens is similar to that of estradiol and estrone; however, conjugated equine estrogens have not been as extensively studied (Bolton *et al.* 1998, IARC 1999).

Figure 6-1. Metabolic pathways for estradiol, estrone, and estriol as adapted from IARC 1999



The major phase I metabolic pathway for endogenous estrogens is aromatic hydroxylation to catechol intermediates. The catechol intermediates have binding affinities for the estrogen receptor similar to the binding affinity of estradiol and undergo cytochrome P-450–mediated redox cycling reactions (Yager and Liehr 1996, Bolton *et al.* 1998, IARC 1999). Phase II reactions include glucuronidation, sulfonation, and *O*-methylation (Figure 6-1). Estrone sulfate is found at the highest concentration in plasma. Sulfate conjugates bind to albumin and circulate in the blood; glucuronides are excreted in urine and bile and may undergo enterohepatic recirculation (IARC 1999).

Estrone and estradiol are biochemically interconvertible and yield the same metabolic products (Figure 6-1). Hydroxylation in the liver by various P-450 isozymes at the 2-position is favored over hydroxylation at the 4-position by a factor of 2 to 10 in all species tested and is greater in women than in men (Bolton *et al.* 1998). The catechol intermediates are further oxidized to semiquinones and quinones. Quinones are highly reactive and can covalently bind to DNA and tubulin (Yager and Liehr 1996, IARC 1999). The catechol intermediates may be detoxified by catechol *O*-methyltransferase (COMT). COMT is present in most tissues and converts catechols into their corresponding methyl ester metabolites. Recent data suggest that 2-methoxyestradiol may inhibit breast cancer (Zhu and Conney 1998). Furthermore, inhibition of COMT potentiates carcinogenicity in the hamster kidney; however, its role in steroid hormone–associated cancers in humans has not been studied (Yager and Liehr 1996).

Evidence links the metabolites of 4-hydroxyestrone (4-OHE) and carcinogenesis. In male Syrian golden hamsters, 4-OHE is carcinogenic, but 2-OHE is not. Furthermore, 4-OHE formation is favored, in all species tested, in tissues that are susceptible to tumor induction by estrogens (e.g., hamster kidney, mouse uterus, and rat pituitary). The liver, where formation of 2-OHE is favored, is more resistant to estrogen carcinogenesis (Bolton *et al.* 1998).

Estrone also may be hydroxylated at the 16∞-position to form 16∞-hydroxyestrone (Figure 6-1). Although this metabolite's binding affinity for the estrogen receptor is lower than that of the catechol estrogens, it initiates a strong response in growth-promoting genes (Yager and Liehr 1996, Bolton *et al.* 1998). 16∞-Hydroxyestrone also alkylates amino acid residues and binds DNA *in vitro* (Yager and Liehr 1996). There are conflicting data regarding the role of 16∞-hydroxyestrone in breast cancer in humans (Service 1998).

Conjugated equine estrogens are hydrolyzed to their free forms in the gastrointestinal tract and are absorbed and metabolized in the liver before entering the bloodstream. The dissolution rate affects where the active ingredients are released in the gastrointestinal tract and may ultimately affect the pattern of active and inactive metabolites. The metabolism of equilin and equilenin corresponds to the interrelation between estrone and estradiol (Figure 6-1) (IARC 1999). Although there have been few metabolism studies of equine estrogens, the available data indicate that the relative rates of 2- and 4-hydroxylation differ from those for estrone and estradiol. Studies with baboon, rat, and hamster microsomes show that 2-hydroxylation is the primary metabolic pathway for estrone, but 4-hydroxylation predominates with equilenin (Bolton *et al.* 1998).

6.2 Risk factors and endogenous estrogen

Epidemiological and animal studies have identified estrogen exposure as a risk factor for several cancers. Much of the evidence comes from the observation that cancer risk increases with increased exposure to endogenous estrogens (early menarche or late menopause) or exogenous estrogens (oral contraceptives or hormone replacement) (see Section 3), and a positive relationship between blood levels of estrogens and breast cancer risk (Bolton *et al.* 1998, Colditz 1998).

Obesity is associated with an increased risk of postmenopausal endometrial and breast cancer (Boyd 1996, Colditz 1998). This has been attributed to increased endogenous estrogen production by fat tissue, because fat cells can metabolize androgens to estrogens. Therefore, the relative contribution of estrogen replacement therapy to post-menopausal estrogen concentrations is likely to be greater in thin women than obese women (IARC 1999). Some studies have shown a greater effect of estrogens in obese women, and others have shown a greater effect in thin women (see Section 3). Smoking, for individuals who also are slow acetylators, and alcohol consumption may increase the breast cancer risk from postmenopausal estrogen therapy (Zumoff 1998). An Oxford University study reanalyzed the data from 51 epidemiological studies, which included over 52,000 women with breast cancer and over 100,000 women without breast cancer. This study indicated a positive association between duration of exogenous hormone use (primarily unopposed estrogens) and breast cancer (2.3% increase in risk for each year of use) (Colditz 1998). Other factors, including dosage, type of estrogen, regimen of use, route of administration, ovarian status, and family history, have not shown consistent risk patterns (Brinton and Schairer 1993, IARC 1999).

6.3 Molecular mechanisms

The molecular mechanisms responsible for estrogen carcinogenicity are not well understood. The most widely proposed mechanisms include mitogenesis in cells expressing estrogen receptors, direct genotoxic effects, and indirect effects (Barrett and Tsutsui 1996, Yager and Liehr 1996, Bolton *et al.* 1998). The evidence indicates that estrogen carcinogenesis is complex and involves proliferative effects as well as direct and indirect genotoxic effects. The relative importance of each mechanism is likely a function of the exposed tissue or cell type and its metabolic state (Yager and Liehr 1996).

6.3.1 Cell proliferation and promotion

The endometrium, breast, and liver possess estrogen receptors. Prolonged estrogen exposure induces DNA synthesis and cell proliferation in these tissues and appears to be responsible for tumor formation (Bolton *et al.* 1998). Cell proliferation can facilitate carcinogenesis by increasing the probability that mutations are fixed, thus allowing for clonal expansion of preneoplastic cells. Several lines of evidence support the role of cell proliferation in estrogen carcinogenesis: hormonal influence on the growth of transplanted tumors, estrogen promotion of carcinogen-initiated tumors, and evidence for late-stage effects in human breast cancer (Barrett and Tsutsui 1996). For example, epidemiological studies show an increased risk of breast cancer with current use of estrogen replacement therapy, whereas the risk of breast cancer in women who had stopped taking hormones for

at least five years was no greater than the risk among those who had never taken hormone treatments (Colditz 1998).

Nandi *et al.* (1996) hypothesized that the hormonal environment present during fetal development determines the proportion of mammary epithelial cells that later proliferate as a direct response to hormones. Two types of luminal mammary epithelial cells develop, some with estrogen receptors and some without. Hormones directly stimulate the cells with estrogen receptors to proliferate and to produce growth factors. These growth factors can stimulate proliferation of cells without estrogen receptors. The ratio of replicating cells with and without estrogen receptors at the time of carcinogen exposure determines the eventual frequencies of hormone-dependent and hormone-independent tumors.

6.3.2 Direct genotoxic effects

In addition to the long-recognized mitogenic effects of estrogens, evidence is accumulating that some estrogen metabolites may be directly responsible for the initial genetic damage leading to tumors (Service 1998). 16∞-Hydroxyestrone, 4-hydroxyestradiol, and 4-hydroxyestrone are the primary estrogen metabolites that have been associated with direct genotoxic effects and carcinogenicity (Yager and Liehr 1996, Bolton *et al.* 1998, Service 1998, IARC 1999). The evidence for a role of these metabolites in carcinogenicity is reviewed below.

Cultured breast cells exposed to 16∞-hydroxyestrone have shown increased DNA repair rates, and this metabolite has been detected in and around breast tumors (Service 1998). In mouse mammary epithelial cells, 16∞-hydroxyestrone caused a small but significant increase in unscheduled DNA synthesis, hyperproliferation, and increased colony growth in soft agar, effects not observed with estradiol and estriol (Yager and Liehr 1996). In addition, covalent binding of 16∞-hydroxyestrone to DNA *in vitro* has been demonstrated (Yager and Liehr 1996, Service 1998). Increased levels of 16∞-hydroxyestrone may increase the risk of breast cancer by increasing both cell proliferation and direct DNA damage. However, the role of 16∞-hydroxyestrone in breast cancer is not certain. Some studies have reported that estrogen metabolism favoring formation of 16∞-hydroxyestrone over 2-OHE increases breast cancer risk, but other studies have not found this effect (Fishman *et al.* 1995, Yager and Liehr 1996, Bolton *et al.* 1998, Meilahn *et al*. 1998, Zumoff 1998, Ursin *et al.* 1999).

Liehr (1997) described mechanistic similarities between human breast cancer and estrogen-induced kidney cancer in hamsters, and identified metabolism to the 4-hydroxylated catechols as the primary pathway leading to tumor development. The 4-hydroxylated catechols may undergo subsequent redox cycling between semiquinone and quinone forms. The quinones may undergo nonenzymatic isomerization to quinone methides. The quinone and quinone methide intermediates are highly reactive and may form covalent DNA adducts; thus, these metabolites are candidates for the ultimate estrogen carcinogens (Bolton *et al.* 1998). Furthermore, redox cycling generates superoxide radicals that are capable of direct and indirect damage to DNA (see Section 6.3.3). Supporting evidence includes higher levels of urinary catechol estrogens in women at risk of breast cancer than in controls, predominance of 4-hydroxylation over 2-hydroxylation in breast cancer cells,

and induction of kidney and liver tumors in laboratory animals by 4-hydroxylated catechols (Liehr 1997, Service 1998).

6.3.3 Indirect effects

6.3.3.1 Reactive oxygen species

Excessive production of reactive oxygen species has been reported in breast cancer tissue, and free-radical toxicity (DNA single-strand breaks, lipid peroxidation, chromosomal abnormalities) has been reported in hamsters treated with estradiol (Bolton *et al.* 1998). Reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals, may be produced through redox cycling between the *o*-quinones and their semiquinone radicals (Figure 6-1). These reactive oxygen species can cause oxidative cleavage of the phosphate–sugar backbone and oxidation of the purine and pyrimidine residues of DNA. Incubation of 4-hydroxylated catechols with microsomes, NADPH, and DNA resulted in 8-hydroxylation of guanine bases (Yager and Liehr 1996). 8-Hydroxydeoxyguanosine is a biomarker for oxidative damage and is considered an important factor in carcinogenesis (Yager and Liehr 1996, Bolton *et al.* 1998). Hamsters given both estradiol and antioxidants had significantly fewer tumors than those receiving estradiol alone (Bolton *et al.* 1998).

Another possible mechanism for generation of reactive oxygen species is copper-mediated metabolism (Figure 6-1). Copper is present throughout the body and is particularly associated with guanine-rich DNA sequences. The divalent copper ion can oxidize the catechol estrogens, resulting in oxidative damage to DNA (Yager and Liehr 1996).

6.3.3.2 Protein binding

In addition to directly binding to DNA, reactive estrogen metabolites may form covalent bonds with proteins. Covalent binding of quinones to microtubular proteins is a possible mechanism for aneuploidy and cell transformation reported in animal *in vitro* studies. Covalent binding of estrogen quinone metabolites to tubulin has been demonstrated *in vitro* (Yager and Liehr 1996).

6.3.3.3 Protooncogene regulation and genetic susceptibility

Protooncogenes are involved in normal cell growth and development; however, overexpression can lead to cell transformation. Hyder *et al.* (1992) identified several uterine protooncogenes regulated by estradiol, including c-*fos*, c-*jun*, c-*myc*, N-*myc*, *ras*, and *erb* B. Chronic administration of estrogens to male Syrian hamsters resulted in 100% incidence of kidney tumors. The mRNA levels of c-*fos*, c-*jun*, and c-*myc* in the tumors were 14, 6, and 4 times higher than levels in the controls. However, these researchers also noted that protooncogene overexpression in target tissues could be due to estradiol; other physiological, pharmacological, or toxicological agents; or a combination of these agents and estradiol. Therefore, breast and endometrial cancer could result from *fos* overexpression even if the endocrine profile were normal. Estrogen-regulated events also occur throughout the cell cycle. Estrogens rapidly stimulate expression of protooncogenes associated with the G_0 to G_1 transition, but later stimulate expression of other genes that are associated with progression through G_1 to S phase. In hormonal carcinogenesis, the tumor phenotype would depend upon the affected estrogen-regulated event (Hyder *et al.* 1992).

Boyd (1996) reported some evidence for K-*ras* involvement in estrogen-related endometrial carcinomas. This *ras* mutation was observed in 10% to 30% of human endometrial carcinomas and appeared to be an early event. However, the data also indicated that hyperplastic lesions with the *ras* mutation were no more likely to progress to carcinoma than those without it.

The expression of c-*myc*, c-*fos*, c-*jun*, and c-*myb* in the uterus and mammary gland is altered rapidly in response to estrogens. Li *et al.* (1999) demonstrated that the expression of these genes increased in the Syrian hamster kidney and renal tumors after five to six months of continuous estrogen exposure. The c-*myc* gene in particular appears to play a critical role in abnormal cell proliferation, cell immortalization, and neoplastic development. Increased expression of this gene may be due in part to a gain in chromosome number. Chromosome 6qb, which contains the c-*myc* gene, had a high frequency of trisomies and tetrasomies after five months of estrogen exposure.

Serum estradiol level variations in part may be explained by genetic differences. For example, North American women have higher blood levels of estradiol and a higher incidence of breast cancer than Asian women. The specific genes involved in hormone-related cancers are unknown; however, candidate genes include those involved in the endocrine pathways, DNA repair, or tumor suppression, as well as oncogenes (Henderson and Feigelson 2000). Polygenic models of endometrial and breast cancer, developed to help define a high-risk profile for hormone-related cancers, identified several genes involved in estrogen biosynthesis, intracellular binding, and transport. These included genes for 17∞-hydroxysteroid dehydrogenase 1 (*HSD17B1*), cytochrome P-459c17∞ (*CYP17*), aromatase (*CYP19*), and the estrogen receptor alpha (*ER*). Although environmental factors do influence the lifetime hormone burden of an individual, endogenous hormone levels also have a genetic basis that can be an important risk factor for hormone-dependent tumors.

6.4 Summary

The presence of estrogen receptors within certain tissues and tumors and the association between duration of exposure to endogenous or exogenous estrogens and tumor probability indicate that estrogens influence tumor growth in these tissues. Prolonged estrogen exposure induces cell proliferation in estrogen-dependent target cells, affects cellular differentiation, and alters gene expression. However, there is increasing evidence for both direct and indirect genotoxic effects of estrogens. Endogenous and exogenous estrogens are metabolized to electrophilic metabolites capable of binding intracellular proteins and DNA. Furthermore, redox cycling pathways can generate reactive oxygen species, which may cause oxidative damage to DNA. Therefore, in some cases, estrogens may initiate as well as promote carcinogenesis.

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Appendix A: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Hormonal Contraception and Post-menopausal Hormonal Therapy. V 72. 1999 pp 288-294, 498-500, 556-558.

WORLD HEALTH ORGANIZATION

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER

IARC MONOGRAPHS

ON THE

EVALUATION OF CARCINOGENIC RISKS TO HUMANS

Hormonal Contraception and Post-menopausal Hormonal Therapy

VOLUME 72

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a contraceptive. [It is unclear whether this dose refers to (dl)- or levonorgestrel.] After 24, 48 or 72 h of culture, the pre-embryos were examined microscopically, and the number of cells counted up to the morula and late blastocyst stages. A similar experiment was carried out in which one-cell pre-embryos were harvested 24 h after injection of human chorionic gonadotrophin and exposed in culture to norgestrel at a concentration of 8, 80 or 800 ng/mL for up to 72 h. In neither study was any difference found in the number of pre-embryos at various cell stages or in the number of degenerating or abnormal pre-embryos (Logan *et al.*, 1989).

5. Summary of Data Reported and Evaluation

5.1 Exposure

Oral contraceptives have been used since the early 1960s and are now used by about 90 million women worldwide. 'The pill' is given as a combination of an oestrogen and a progestogen or as sequential therapy. Since the 1970s, progestogen-only pills have been available. Continuous development of the formulas and the development of new progestogens have allowed for lower dosages with fewer acute side-effects, while offering effective, convenient contraception.

The oestrogen component of combined oral contraceptives is either ethinyloestradiol or mestranol, and the progestogens used are cyproterone acetate, desogestrel, ethynodiol diacetate, gestodene, levonorgestrel, lynoestrenol, megestrol, norethisterone, norethisterone acetate, norethynodrel, norgestimate and norgestrel. Currently, the most commonly used oestrogen is ethinyloestradiol, and commonly used progestogens are levonorgestrel and norethisterone.

Large differences exist in the worldwide use of oral contraceptives. These products were already being used extensively in the 1960s in northern Europe (e.g. the Netherlands, Sweden and the United Kingdom) and the United States. Extensive use of oral contraceptives by adolescents was documented in Sweden and the United Kingdom as early as 1964. Very little use of oral contraceptives is reported in Japan, the countries of the former Soviet Union and most developing countries. Contraceptive use also differs in relation to religion, ethnicity, educational level, use before or after marriage and use before or after first pregnancy.

The type of oral contraceptives prescribed differs between countries, and both the type of oral contraceptive and the doses of oestrogens and progestogens have changed between and within countries over time.

Oral contraceptives may be used for emergency post-coital contraception, and the components of oral contraceptives are used to treat peri- and post-menopausal symptoms and a number of other conditions.

It is important to stress that use of oral contraceptives is a recent human activity, and the health benefits and adverse effects in women have not yet been followed over a complete generation, even though they are some of the most widely used drugs in the

world. Women who began using oral contraceptives before the age of 20 in the 1960s are only now reaching the ages (50–60 years) at which the incidences of most malignancies begin to increase.

Oestrogens and progestogens belonging to the same chemical groups may have different oestrogenic, androgenic and progestogenic effects. Little is known about the long-term health risks and potential protective effects of the individual components. The effects become increasingly complex as women grow older, as they may be exposed to different types and doses of hormones, starting with oral contraceptives and progressing to post-menopausal hormonal therapy.

5.2 Human carcinogenicity

Breast cancer

More than 10 cohort and 50 case–control studies have assessed the relationship between use of combined oral contraceptives and the risk for breast cancer. The studies included over 50 000 women with breast cancer. The weight of the evidence suggests a small increase in the relative risk for breast cancer among current and recent users, which is, however, unrelated to duration of use or type or dose of preparation. By 10 years after cessation of use, the risk of women who used oral contraceptives appears to be similar to that of women who never used them. Important known risk factors do not account for the association. The possibility that the association seen for current and recent users is due to detection bias has not been ruled out. Even if the association is causal, the excess risk for cancer associated with patterns of use that are typical today is very small.

Cervical cancer

Five cohort and 16 case–control studies of use of combined oral contraceptives and invasive cervical cancer have been published; these consistently show a small increase in relative risk associated with long duration of use. These associations were also seen in four studies in which some analyses were restricted to cases and controls who had human papillomavirus infections. Biases related to sexual behaviour, screening and other factors cannot be ruled out as possible explanations for the observed associations.

Endometrial cancer

Three cohort and 16 case–control studies addressed the relationship between use of combined oral contraceptives and the risk for endometrial cancer. The results of these studies consistently show that the risk for endometrial cancer of women who have taken these pills is approximately halved. The reduction in risk is generally stronger the longer the oral contraceptives are used and persists for at least 10 years after cessation of use. Few data are available on the more recent, low-dose formulations.

Use of sequential oral contraceptives which were removed from the consumer market in the 1970s was associated with an increased risk for endometrial cancer.

Ovarian cancer

Four cohort and 21 case–control studies addressed the relationship between ovarian cancer and use of combined oral contraceptives. Overall, these studies show a consistent reduction in the risk for ovarian cancer with increasing duration of use. The reduction is about 50% for women who have used the preparations for at least five years, and the reduction seems to persist for at least 10–15 years after use has ceased. Few data are available on the more recent, low-dose formulations. A reduction in risk for ovarian tumours of borderline malignancy is also observed.

Cancers of the liver and gall-bladder

Two case–control studies of benign hepatocellular tumours showed a strong relationship with duration of use of combined oral contraceptives. Three cohort studies showed no significant association between use of combined oral contraceptives and the incidence of or mortality from liver cancer, but the expected numbers of cases were very small, resulting in low statistical power. Long-term use of combined oral contraceptives was associated with an increase in risk for hepatocellular carcinoma in all nine case–control studies conducted in populations with low prevalences of hepatitis B and C viral infection and chronic liver disease, which are major causes of liver cancer, and in analyses in which women with these factors were excluded. Few data are available for the more recent, low-dose formulations. In the two case–control studies conducted in populations with a high prevalence of infection with hepatitis viruses, there was no increase in risk for hepatocellular carcinoma associated with use of combined oral contraceptives, but there was little information on long-term use.

Little information was available on the association between use of combined oral contraceptives and the risk for cholangiocarcinoma or cancer of the gall-bladder.

Colorectal cancer

Four cohort investigations and 10 case–control studies provided information on use of combined oral contraceptives and risk for colorectal cancer. None showed significantly elevated risks in women who used these preparations for any length of time. Relative risks lower than 1.0 were found in nine studies, and the risk was significantly reduced in two.

Cutaneous malignant melanoma

Four cohort investigations and 16 case–control studies provided information on use of combined oral contraceptives and the risk for cutaneous malignant melanoma. The relative risks were generally close to 1.0 and not related to duration of use.

Thyroid cancer

Ten case–control studies provided information on use of combined oral contraceptives and the risk for cancer of the thyroid gland. In general, there was no elevation in the risk associated with oral contraceptive use.

5.3 Carcinogenicity in experimental animals

Oestrogen–progestogen combinations

Several combinations of oral contraceptives have been tested alone and together with known carcinogens in mice, rats and monkeys. Consistent tumorigenic effects that are seen with various combinations which are important for classifying the degree of evidence for carcinogenicity of this class of compounds are as follows.

The incidences of pituitary adenoma in male and female mice were increased by administration of mestranol plus chlormadinone acetate, mestranol plus ethynodiol diacetate, ethinyloestradiol plus ethynodiol diacetate, mestranol plus norethisterone, ethinyloestradiol plus norethisterone (females only) and mestranol plus norethynodrel, which also increased the incidence of pituitary adenomas in female rats.

The incidence of benign mammary tumours was increased in mice by ethinyloestradiol plus chlormadinone acetate (in intact and castrated males) and by mestranol plus norethynodrel (only in castrated males). In rats, the incidence of benign mammary tumours was increased by administration of ethinyloestradiol plus norethisterone acetate. This combination did not cause tumour formation in any tissue in one study in monkeys.

The incidence of malignant mammary tumours was increased in male and female mice by ethinyloestradiol plus megestrol acetate and in rats by ethinyloestradiol plus ethynodiol diacetate (males and females), mestranol plus norethisterone (females) and mestranol plus norethynodrel (females).

In female mice, the incidence of malignant uterine tumours (non-epithelial) was increased by ethinyloestradiol plus ethynodiol diacetate and the incidence of vaginal or cervical tumours by norethynodrel plus mestranol. In mice treated with 3-methylcholanthrene to induce genital tumours, ethinyloestradiol plus lynoestrenol, ethinyloestradiol plus norgestrel and mestranol plus norethynodrel increased the incidence of uterine tumours; however, this occurred only at the highest doses of ethinyloestradiol plus lynoestrenol and ethinyloestradiol plus norgestrel that were tested. Lower doses inhibited tumorigenesis induced by 3-methylcholanthrene alone.

In rats, the incidence of benign liver tumours (adenomas) was increased by mestranol plus norethisterone (males) and by ethinyloestradiol plus norethisterone acetate (males); the latter combination also increased the incidence of hepatocellular carcinomas in females. Liver foci, which are putative preneoplastic lesions, were induced in rats by mestranol plus norethynodrel. In rats initiated for hepatocarcinogenesis with *N*-nitrosodiethylamine, mestranol plus norethynodrel increased the formation of altered hepatic foci.

Oestrogens

The synthetic oestrogens ethinyloestradiol and mestranol have been tested extensively alone and together with known carcinogens in mice, rats, hamsters, dogs and monkeys.

The incidence of pituitary adenomas was increased by ethinyloestradiol and mestranol in male and female mice and by ethinyloestradiol in female rats.

The incidences of malignant mammary tumours in male and female mice and female rats were increased by ethinyloestradiol and mestranol; however, mestranol did not increase the incidences of mammary tumours in dogs in a single study.

Ethinyloestradiol increased the incidence of cervical tumours in female mice.

In one mouse strain, ethinyloestradiol increased the incidences of hepatocellular adenomas. In female rats, ethinyloestradiol and mestranol increased the numbers of altered hepatic foci. Ethinyloestradiol increased the incidence of adenomas in males and females and of hepatocellular carcinomas in females, whereas mestranol increased the incidence of hepatic nodules and carcinomas combined in female rats.

The incidence of microscopic malignant kidney tumours was increased in hamsters exposed to ethinyloestradiol.

In mice initiated for liver carcinogenesis and exposed to unleaded gasoline, ethinyloestradiol increased the number of altered hepatic foci; however, when given alone after the liver carcinogen, it reduced the number of spontaneous foci.

In female rats initiated for liver carcinogenesis, ethinyloestradiol and mestranol increased the number of altered hepatic foci and the incidences of adenomas and carcinomas. Ethinyloestradiol also increased the incidences of kidney adenomas, renal-cell carcinomas and liver carcinomas in rats initiated with *N*-nitrosoethyl-*N*-hydroxyethylamine. In hamsters initiated with *N*-nitrosobis(2-oxopropyl)amine, ethinyloestradiol increased the incidence of renal tumours and the multiplicity of dysplasias.

Progestogens

Various progestogens have been tested alone and together with known carcinogens in mice, rats and dogs.

The incidence of pituitary adenomas was increased by norethisterone in female mice and by norethynodrel in male and female mice and male rats.

The incidence of malignant mammary tumours was increased in female mice by lynoestrenol, megestrol acetate and norethynodrel. In female rats, lynoestrenol and norethisterone slightly increased the incidence of malignant mammary tumours. Norethisterone also slightly increased the incidence of malignant mammary tumours in male rats, while norethynodrel increased the incidence of both benign and malignant mammary tumours in male rats. In dogs, chlormadinone acetate, lynoestrenol and megestrol acetate increased the incidence of benign and malignant mammary tumours; however, lynoestrenol had a protective effect at a low dose but enhanced tumour incidence at two higher doses. Levonorgestrel did not increase the incidence of mammary tumours in one study in dogs.

In female mice treated with 3-methylcholanthrene to induce uterine tumours, norethynodrel further increased the tumour incidence.

In male mice treated with chlormadinone acetate, ethynodiol diacetate, lynoestrenol, norethisterone or norethisterone acetate, the incidence of liver adenomas was increased. Megestrol acetate increased the incidence of adenomas in female mice. Cyproterone acetate increased the incidences of liver adenomas and hepatocellular carcinomas in male and female mice, but at doses exceeding the maximum tolerated dose. In rats, the inci-

dence of liver adenomas was increased by norethisterone acetate (males and females), norethisterone (males), norethynodrel and cyproterone acetate (males and females). The numbers of altered hepatic foci in female rats were also increased by norethisterone acetate and cyproterone acetate. In rats treated with *N*-nitrosodiethylamine to initiate hepatocarcinogenesis, norethynodrel increased the number of altered hepatic foci. Norethynodrel alone was shown to increase the incidence of hepatocarcinomas in male rats.

Levonorgestrel in combination with *N*-nitrosobis(2-oxopropyl)amine did not enhance the incidence of renal dysplastic lesions or tumours in hamsters.

5.4 Other relevant data

After single or multiple doses, oestrogens and progestogens in combined oral contraceptives are rapidly absorbed and reach maximal serum levels quickly. The proportion of the absorbed hormone that becomes biologically available depends on the extent of enterohepatic circulation and metabolic transformation of pro-drugs. Interactions between some of these hormones affect their disposition and that of the oestrogen or progestogen with which they are combined. Several progestogens also exhibit some oestrogenic activity and can thus modify the effects of the oestrogens. In three studies, women taking oestrogen–progestogen combinations had increased epithelial cell proliferation in the breast, and in one of these studies the effect was related to the dose of oestrogen in the presence of progestogen. The constituents of combined oral contraceptives may stimulate rat hepatocyte cell proliferation *in vitro* and *in vivo*, and this growth potentiation may be selectively effective in preneoplastic hepatocytes. In addition to the major routes of metabolism, a minor proportion of oestrogen may be metabolized to catechol intermediates, with significant potential for formation of reactive intermediates and damage to DNA. Some of the constituents of combined oral contraceptives can cause changes in DNA at the nuclear level in some experimental systems. Most, but not all, human studies show effects of this type, which occur at conventional therapeutic doses of combined oral contraceptives. When given during pregnancy, combined oral contraceptives can cause developmental abnormalities of the genital tract of offspring. There is evidence for other malformations, but this is controversial and not considered proven.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of combined oral contraceptives.

This classification is based on an increased risk for hepatocellular carcinoma in the absence of hepatitis viruses observed in studies of predominantly high-dose preparations.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethinyloestradiol plus ethynodiol diacetate and mestranol plus norethynodrel.

There is *limited evidence* in experimental animals for the carcinogenicity of ethinyloestradiol plus megestrol acetate, mestranol or ethinyloestradiol plus chlormadinone

acetate, mestranol plus ethynodiol diacetate, mestranol plus lynoestrenol, mestranol or ethinyloestradiol plus norethisterone and ethinyloestradiol plus norgestrel.

There is *sufficient evidence* in experimental animals for the carcinogenicity of ethinyloestradiol and mestranol.

There is *sufficient evidence* in experimental animals for the carcinogenicity of norethynodrel and lynoestrenol.

There is *limited evidence* in experimental animals for the carcinogenicity of chlormadinone acetate, cyproterone acetate, ethynodiol diacetate, megestrol acetate, norethisterone acetate and norethisterone.

There is *inadequate evidence* in experimental animals for the carcinogenicity of levonorgestrel and norgestrel.

Overall evaluation

Combined oral contraceptives are *carcinogenic to humans (Group 1)*.

There is also conclusive evidence that these agents have a protective effect against cancers of the ovary and endometrium.

6. References

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A combination of oestradiol and testosterone administered to male rats resulted in DNA binding in the dorsolateral prostate but not in the ventral or anterior prostate.

In a single study, the frequency of chromosomal aberrations was not increased in renal proximal convoluted tubules of male hamsters treated with 17α-oestradiol.

DNA strand breakage was demonstrated in kidney cells of male hamsters treated subcutaneously with 4-hydroxyoestradiol. In hamster cells *in vitro*, 4-hydroxyoestradiol caused cell transformation and formation of DNA adducts in the presence of exogenous metabolic activation. Induction of oxidative damage in male hamster liver DNA and binding to calf thymus DNA were seen after in-vitro treatment with 4-hydroxyoestradiol, and similar results were observed *in vivo* in mammary cells of rats treated with this compound.

DNA strand breakage was not demonstrated in kidney cells from male hamsters treated subcutaneously with 2-hydroxyoestradiol, and this compound did not bind to liver DNA of hamsters *in vitro* in one study. In hamster cells, 2-hydroxyoestradiol caused cell transformation and formation of DNA adducts in the presence of exogenous metabolic activation.

Oestradiol-3,4-quinone bound to DNA both *in vitro* and *in vivo* in rat mammary cells.

Oestrone did not cause gene mutation at various loci in hamster ovary cells. It did not induce oxidative damage in hamster liver DNA, nor did it bind to kidney or liver DNA of male hamsters or to liver DNA of rats treated *in vivo*.

In vitro, oestrone-3,4-quinone induced DNA strand breaks in human MCF-7 cells and bound weakly to calf thymus DNA.

In a mammary cell line derived from mice, DNA repair and cell transformation were induced by treatment with 16α-hydroxyoestrone.

No induction of oxidative DNA damage was seen in the presence of an exogenous metabolic activation system in male hamster liver cells treated *in vitro* with 2-hydroxyoestrone, but 4-hydroxyoestrone was active in this assay. Furthermore, the latter compound bound to calf thymus DNA under these conditions.

Neither DNA repair nor cell transformation was induced in mouse mammary epithelial cells treated with oestriol, whereas aneuploidy was induced in male hamster DON cells. Oestriol weakly induced sister chromatid exchange in human lymphocytes *in vitro*.

In one study, equilin and equilenin decreased the formation of single- and double-strand DNA breaks induced by hydrogen peroxide alone or with Cu^{2+} (Tang & Subbiah, 1996).

5. Summary of Data Reported and Evaluation

5.1 Exposure

The numbers of women who have used post-menopausal oestrogen therapy vary between countries and within regions of individual countries. The prevalence of use has been greater in the United States than in most other countries; use of oestrogen therapy after the menopause is rare in developing countries but is increasing. Conjugated equine

oestrogens are the most widely prescribed preparation for oestrogen therapy for women in the United States, but oestradiol and its esters have greater use in most of Europe. Oral administration is the most popular route, but percutaneous methods are becoming commoner; use of injections, the first form of post-menopausal oestrogen therapy, has been declining.

5.2 Human carcinogenicity

Breast cancer

Information on the relationship between post-menopausal oestrogen therapy and risk for breast cancer is available from many epidemiological studies. A pooled analysis of the original data from 51 of those studies and a review of data from 15 cohort and 23 case–control studies showed that in the majority of the studies there is a small increase in risk with longer duration of use (five years or more) in current and recent users. Although there is far less information about women who used post-menopausal oestrogen therapy and then ceased use, the increase in risk appears to cease several years after use has stopped. The increase in risk is predominantly for small localized carcinomas of the breast. There are insufficient data to determine whether the risk varies with type of compound or dose.

Endometrial cancer

Three cohort and more than 30 case–control studies consistently showed an association between use of post-menopausal oestrogen therapy and an increased risk for endometrial cancer. The risk increases with increasing duration of use. It decreases with time since last use but remains higher than that of untreated women for at least 10 years.

Cervical cancer

Only one cohort and two case–control studies were available on the relationship between use of post-menopausal oestrogen therapy and the risk for invasive cervical cancer; in none of them were the possible confounding effects of oncogenic human papillomaviruses considered. On balance, the limited evidence available suggests that post-menopausal oestrogen therapy is not associated with an increased risk for invasive cervical carcinoma. The results provide some suggestion that post-menopausal oestrogen 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 post-menopausal oestrogen therapy.

Ovarian cancer

The four cohort and 12 case–control studies that addressed the risk for ovarian cancer (largely epithelial) among women undergoing post-menopausal oestrogen therapy gave mixed results. One cohort study and one large case–control study showed a significant excess risk for ovarian cancer in women who used this therapy, but a pooled analysis of the individual data from case–control studies showed no excess risk. There is therefore no clear association between post-menopausal oestrogen therapy and the risk for ovarian cancer.

Cancers of the liver and gall-bladder

The two cohort and two case–control studies that addressed the association between use of post-menopausal oestrogen therapy and the risk for cancers of the liver or biliary tract showed no alteration in risk.

Colorectal cancer

Seven cohort and 12 case–control studies have provided information on use of post-menopausal oestrogen therapy and the risk for colorectal cancer. The risk was not increased and appeared to be reduced in one-half of the studies. The reduced risk tended to be observed among recent users and did not appear to be related to duration of use.

Cutaneous malignant melanoma

One cohort and nine case–control studies addressed the risk for cutaneous malignant melanoma in relation to use of post-menopausal oestrogen therapy. Most suggested no alteration in risk.

Thyroid cancer

Seven case–control studies that provided information on thyroid cancer and use of post-menopausal oestrogen therapy suggested no effect on risk.

5.3 Carcinogenicity in experimental animals

Conjugated oestrogens

Hydrolysed conjugated equine oestrogens, equilin and d-equilenin were tested in male hamsters by subcutaneous implantation. The hydrolysed oestrogens and equilin induced microscopic renal carcinomas, whereas d-equilenin was inactive.

Oestradiol

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

Oral administration of oestradiol to mice bearing murine mammary tumour virus increased the incidences of uterine (endometrial and cervical) adenocarcinomas and 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.

Invasive pituitary tumours were induced in rats treated with oestradiol dipropionate. In hamsters, a high incidence of malignant kidney tumours occurred in intact and castrated males and in ovariectomized females treated with oestradiol, but not in intact females. In guinea-pigs, diffuse fibromyomatous uterine and abdominal lesions were observed. Subcutaneous injections to neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life and an increased incidence of mammary tumours. The 4-hydroxy metabolite of oestradiol induced renal-cell carcinomas in castrated male hamsters.

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

4.3 Genetic and related effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

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

5. Summary of Data Reported and Evaluation

5.1 Exposure

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

5.2 Human carcinogenicity

Breast cancer

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

Endometrial cancer

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

Ovarian cancer

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

Liver cancer

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

Other cancers

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

5.3 Carcinogenicity in experimental animals

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

5.4 Other relevant data

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

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

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

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

5.5 Evaluation

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

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

Overall evaluation

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

6. References

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Appendix B: IARC Monographs on the Evaluation of the Carcinogenic Risks to Humans. Overall Evaluation of Carcinogenicity: An Updating of IARC Monographs. Suppl. 7. 1987.

[3]Liehr, J.G., Ballatore, A.M., Dague, B.B. & Ulubelen, A.A. (1985) Carcinogenicity and metabolic activation of hexestrol. *Chem.-biol Interactions, 55*, 157-176

[4]*IARC Monographs, Suppl 6*, 336-337, 1987

Chlorotrianisene

A. Evidence for carcinogenicity to animals (*inadequate*)

Chlorotrianisene was tested in only one experiment in rats by oral administration. The data were insufficient to evaluate the carcinogenicity of this compound[1].

B. Other relevant data

No data were available to the Working Group.

Reference

[1]*IARC Monographs, 21*, 139-146, 1979

STEROIDAL OESTROGENS (Group 1*)

Evidence for carcinogenicity to humans (*sufficient*)

Oestrogen replacement therapy (Group 1)

A. Evidence for carcinogenicity to humans (*sufficient*)

A number of studies, utilizing a variety of designs, have shown a consistent, strongly positive association between exposure to a number of oestrogenic substances and risk of endometrial cancer, with evidence of positive dose-response relationships both for strength of medication and duration of use[1]. Consistent findings have also been seen in more recent studies[2-16]. The rise and fall of incidence of endometrial cancer in several areas of the USA was compatible with trends in oestrogen use[1,15].

Of the 20 epidemiological studies of oestrogen replacement therapy and breast cancer risk[16-35], nine show a positive relation between oestrogen use and breast cancer[17-20,22-24,28,33]. The increased risks tend to be small; for example, a 50% increase was found with 20 years of menopausal oestrogen replacement therapy use[24]. All except one[33] of the positive studies involved use of population controls (eight of the nine studies with population controls gave positive results), and most showed increased risk after prolonged use or after ten or more years since initial exposure. One study showed a positive association with current oestrogen use[28].

* This evaluation applies to the group of chemicals as a whole and not necessarily to all individual chemicals within the group (see also Methods, p. 38).

One possible reason that studies with hospital controls gave negative results and those with population controls positive results is that oestrogen replacement therapy may be used more frequently in hospitalized women than in the general population. However, in two studies involving use of both hospital and population control groups, one giving positive[29] and the other largely negative[25] results, similar results were obtained when hospital and population controls were used to estimate the relative risk. Three of the studies with negative results[26,27,34] probably did not permit the authors to address satisfactorily the question of long-term use of oestrogen replacement therapy. The large hospital-based study that showed a positive finding used as controls subjects with a large spectrum of acute conditions unrelated to any of the known or suspected risk factors for breast cancer[33].

One cohort study of 1439 women initially treated for benign breast disease showed increased risk for women who took exogenous oestrogens after biopsy, but not for those who had taken them before biopsy. The increased risk in the former group appeared to be associated with epithelial hyperplasia or calcification in the initial lesion[35].

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Conjugated oestrogens

A. Evidence for carcinogenicity to animals (*limited*)

Conjugated oestrogens were tested inadequately in rats by oral administration in one study[1]. In male hamsters castrated as adults, equilin administered as a subcutaneously planted pellet produced renal tumours in 6/8 treated animals. In contrast, *d*-equilenin administered similarly did not induce renal tumours[2,3].

B. Other relevant data

No data were available on the genetic and related effects of conjugated oestrogens in humans.

A commercial preparation of conjugated oestrogens did not induce chromosomal aberrations in human lymphoblastoid cells *in vitro* or in Chinese hamster V79 cells exposed in diffusion chambers implanted into mice after oestrogen treatment. It was not mutagenic to bacteria[4].

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[1]*IARC Monographs, 21*, 147- 159, 1979

Appendix C: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Sex Hormones (II). Vol 21. 1979 pp 62-82, 155-159, 242-255, 264-278, 298-326, 335-341, 352-362.

chromatographic techniques, in conjunction with flame-ionization detection, electron capture detection or mass spectrometric detection of one or several characteristic ions. In view of the time-consuming nature of many of these clean-up procedures, increasing use is being made of radioimmunoassay, which requires little preliminary sample purification and is highly sensitive and specific. Methods similar to those employed for biological samples are used for environmental samples.

Analytical methods for the individual sex hormones are tabulated in the respective monographs.

HORMONES AND CARCINOGENICITY

The mechanism(s) by which hormones result in cancer development is not understood. Although many carcinogens show a mutagenic action, none of the hormones (including diethylstilboestrol) nor any of their metabolic products has so far been shown convincingly to be mutagenic; however, there have been reports of covalent binding of diethylstilboestrol metabolites to DNA and of results in short-term tests that indicate interactions with DNA (see the monograph on diethylstilboestrol, p. 173)

Hormones may be essential to carcinogenesis by preparing the background on which tumours may ultimately arise. Thus, mammary tumours arise from mammary tissue that is in an appropriate developmental state: hormones that directly stimulate mammary gland development provide the necessary substrate. In laboratory rodents, these hormones include those of the ovary, the adrenal cortex and the pituitary. The hormones of these endocrine glands can be viewed as essential factors for the development of mammary cancer, although this does not mean that they should be considered as having a direct carcinogenic action.

In laboratory mice, both oestrogen and prolactin increase the incidence of mammary tumours by their actions on the mammary gland. In virgin mice of a strain in which the milk-borne mammary tumour virus (MTV)[1] is unexpressed, continuous exposure to prolactin may cause tumours to appear. The role of oestrogen is even more complex, since it can also stimulate prolactin secretion. Oestrogen and prolactin, and probably progesterone, all contribute to the development of mammary tumours in carcinogen-exposed rats.

When investigating carcinogenesis with female animal models, it is important to take into account essential differences in reproductive physiology; animals with spontaneous oestrus cycles, with functional corpora lutea (dog) and without (mouse and rat), and with reflex ovulation (rabbit) may respond differently to exogenous hormones. The differences

[1] In this volume, MTV^+ indicates the presence of a virus able to express its biological activity, whereas MTV^- refers to a virus in an occult or biologically inactive form.

which occur in the basic endocrine relationships in a range of laboratory animal species as compared with those in man have been discussed at length by Neumann and Elger (1972). The importance of species differences in the metabolism of sex hormones is outlined on p. 60.

Another important consideration in interpreting the relevance of various animal models to the human experience is the epizoology of the cancer being investigated, compared with its corresponding epidemiology. For example, while pregnancy confers relative protection against breast cancer in humans and in rats, it increases the breast cancer risk in mice. Additionally, although the reproductive physiology of dogs is different from that of humans, the protective effects of early age at first pregnancy and of oophorectomy at a young age against the development of breast cancer appear to be the same in both species.

Hormones may stimulate carcinogenesis by providing a background—normal (permissive) or abnormal (teratogenic, see pp. 64–67)—for subsequent tumorigenesis by chemical, physical or viral agents or by promoting the growth and metastasis of tumours once they have been initiated, or in a variety of other ways. The following list of possible mechanisms is undoubtedly incomplete and in part speculative:

1. Hormones may increase the binding of chemical carcinogens to cellular constituents, e.g., by influencing metabolic activation systems.

2. Hormones may activate oncogenic virus production (e.g., mammary tumour virus in mice).

3. Hormones may be immunosuppressive and could thus influence tumour occurrence and growth.

4. Exposure to hormones may result in lesions (preneoplastic) which provide an environment for the survival of cells with abnormal growth potentials.

5. Hormones may influence the rate of progression of preneoplastic cells to neoplastic cells.

6. Hormones may preferentially stimulate proliferation of abnormal cell populations.

7. Hormones may stimulate the DNA synthesis and mitosis essential for fixation of the transformed state.

8. Hormones, by stimulating the proliferation of normal cells with a definite number of cell divisions, may exhaust the normal cell population and thus eliminate their inhibitory influence over the proliferation of abnormal cells (Nandi, 1978a,b).

9. As a result of hormonal imbalance in a target organ, proliferation may be favoured over functional differentiation; conversely, certain hormonal milieux in target organs may favour specific synthetic and secretory activities and hence reduce tumorigenic potential**.**

HORMONES AND MUTAGENICITY

The majority of mutagenicity studies on the sex hormones considered in this volume were carried out before the statistical concepts for mutagenicity testing were developed (Vollmar, 1977); therefore, many results, especially those from cytogenetic studies, are inconclusive.

The Working Group also noted the scarcity of mutagenicity studies on sex hormones, particularly of those using mammalian germ cells, such as cytogenetic studies on oocytes, spermatocytes and spermatogonia. Only two dominant lethal tests have been reported, one on a combination of mestranol and lynoestrenol and the other on norethisterone acetate; the authors considered these to be positive, although confirmation is required .

Before a definite statement about the possible mutagenicity of sex hormones can be made, therefore, additional studies are required.

HORMONES AND EMBRYOTOXICITY AND TERATOGENICITY

Many steroidal sex hormones cause antifertility, embryotoxicity and fetotoxicity in several species, and such effects are usually dose-related. Some oestrogens also produce teratogenic effects and impaired fertility in exposed offspring. Certain progestins, testosterone and testosterone derivatives have a virilizing effect on the fetus.

In humans, birth defects have been observed in fetuses after maternal ingestion of various drugs. However, only in a limited number of cases is it possible to ascribe a particular defect to a specific drug. If an adverse effect is to be produced, exposure must occur during the relevant susceptible period of embryogenesis; and in interpreting the results of a study, the influence of the drug must be distinguished from any effects of the condition for which the drug was administered.

Diethylstilboestrol

Non-neoplastic alterations in the genital tract are commonly found in female children born to women who received diethylstilboestrol during pregnancy. These changes include vaginal adenosis, cervical ectropion and transverse fibrous ridges on the cervix or in the

vagina. In addition, there is some evidence of alterations in the structure of the fundus of the uterus. Changes in the male genital tract include epididymal cysts, testicular abnormalities and alterations in the seminal fluid. Many of these changes appear to be related to disturbances in the development of the Mullerian tract in females and of the Wolffian duct in males. They are considered in greater detail in the monograph on diethylstilboestrol (see p. 173).

Neonatal mice provide a useful model for studying the long-term effects of prenatal exposure of humans to diethylstilboestrol and sex steroids (Bern, 1979; Bern *et al*., 1975, 1976; Forsberg, 1972, 1975, 1976; Kohrman, 1978). Both mice and rats are born with incompletely developed genital tracts, in a stage similar to that in the first trimester in humans. In mice, the first few days after birth constitute a critical period during which injection of sex steroids or diethylstilboestrol may induce irreversible changes in the genital tract. Although hormones injected into neonatal mice are not metabolized by the placenta (as may occur in humans exposed prenatally), the responses observed are similar to those that occur in mice exposed prenatally, either transplacentally or directly (Kimura, 1975; McLachlan, 1977; McLachlan *et al*., 1975). Some of these responses, such as vaginal adenosis (Forsberg, 1976, 1979) and epididymal cysts (McLachlan *et al*., 1975), resemble those seen in humans after transplacental exposure to diethylstilboestrol (Gill *et al*., 1976, 1977; Herbst, 1978; Herbst *et al*., 1975a,b). Even when the animal model system involves transplacental administration, however, it should be remembered that the placenta of different species may handle steroids and diethylstilboestrol in different ways.

Progestins and androgens

Masculinization of the external genitalia in female fetuses has been observed after their exposure *in utero* to large doses of progestational agents, especially 19-nor steroids, which also have some androgenic activity. The changes include clitoral hypertrophy, labio-scrotal fusion, and occasionally the occurrence of penile urethra. Similar changes have been reported after exposure to combinations of these compounds with oestrogens. Advancement of skeletal maturation has also been noted (Breibart *et al*., 1963). Milder degrees of masculinization have been reported with progestational compounds that have a lesser degree of androgenic activity, such as medroxyprogesterone acetate (Burstein & Wasserman, 1964). Androgens themselves produce similar masculinizing effects on the fetus, but drugs such as methyltestosterone, testosterone, 19-normethyltestosterone and methylandrostenediol, have apparently been less widely used than progestins during pregnancy (Grumbach & Ducharme, 1960).

Oestrogens, progestins and oral contraceptives

There is no evidence that children born to women who used oral contraceptives at times prior to pregnancy have an increased frequency of birth defects (Peterson, 1969; Robinson, 1971; Rothman & Louik, 1978; Royal College of General Practitioners, 1976).

Chromosomal abnormalities (principally triploidy and tetraploidy) in foetuses have been reported to be more common following the use of oral contraceptives by their mothers (Alberman *et al.*, 1976; Carr, 1970; Harlap *et al.*, 1979), but these findings have not been confirmed (Bishun, 1976; Klinger *et al.*, 1976; Lauritsen, 1975).

It has been suggested that more congenital abnormalities occur in infants born to women who became pregnant while actually taking oral contraceptives. The defects described include the VACTERL syndrome (a pattern of multiple anomalies: vertebral, anal, cardiac, tracheal, oesophageal, renal, limb) (Nora & Nora, 1973, 1975; Nora *et al.*, 1978), cardiovascular abnormalities (Heinonen *et al.*, 1977; Janerich *et al.*, 1977; Levy *et al.*, 1973; Rothman *et al.*, 1979) and limb reduction defects (Janerich *et al.*, 1974). A VACTERL syndrome was seen in a child of a mother given hormone therapy at the beginning of pregnancy (Kaufman, 1973). Other authors have found no evidence of such abnormalities (Mulvihill *et al.*, 1974; Vessey *et al.*, 1976).

The evidence relating the use of hormonal pregnancy tests to congenital malformations is stronger, but not conclusive (Gal et *al.*, 1967; Greenberg *et al.*, 1975; Janerich *et al.*, 1977; Laurence *et al.*, 1971; Oakley *et al.*, 1973). Studies that implicate hormones used to support pregnancy are difficult to interpret, since the indication for which the drug was given might itself be expected to be associated with an increased risk of birth defects.

Clomiphene and clomiphene citrate

Clomiphene citrate alone or in combination with gonadotrophic hormones stimulates the synthesis of sex hormones in the gonads and induces germ-cell maturation. It is thus used to induce ovulation. Higher risks of multiple births and abortions, among other side effects accompanying use of this agent, are well documented.

A slight increase in chromosomal anomalies has been reported in early embryos of mice and rabbits after superovulation (Fujimoto *et al.*, 1974; Maudlin & Fraser, 1977; Takagi & Sasaki, 1976); however, negative effects have also been reported (Fechheimer & Beatty, 1974). After superovulation induced by conjugated oestrogens and gonadotrophic hormones, an inheritable limb defect in connection with neural tube defects and a shifted sex ratio favouring females to males (2:1) can occur in the F_1 generation of Swiss albino mice, which is transmitted over several generations. The highly increased steroid hormone levels

may be the origin of these inheritable lesions (Elbling, 1973, 1975a,b,c). Smith and Chrisman (1975) could not confirm these effects in other mouse strains.

In humans, neural tube defects have been reported in the 11 children of 10 women given clomiphene to induce ovulation [Barrett & Hakim (1973) (1 case), Dyson & Kohler (1973) (2 cases), Field & Kerr (1974) (2 cases), Nevin & Harley (1976) (4 women, 5 children) and Santler (1973) (1 case)]. Other authors have reported no increase in congenital defects following clomiphene usage (Hack *et al*., 1972).

It has been suggested that subfertility in women may be associated with an increased prevalence of congenital defects (Ahlgren *et al*., 1976). Additionally, a high proportion of early spontaneous abortuses have been shown to be chromosomally abnormal (Boué & Boué, 1973; Dhadial *et al*., 1970); however, no information is available on the karyotypes of abortuses from subfertile women or of those from women given clomiphene to induce superovulation. Chromosome analyses may help to clarify the problem. At present, no definite association has been demonstrated between clomiphene administration and congenital defects in humans.

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3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Oral administration

Rat: Groups of 20 male and 20 female weanling Sprague-Dawley rats were fed diets containing conjugated oestrogens (Premarin®) at concentrations resulting in intakes of 0, 0.07 and 0.7 mg/kg bw per day for two years. Survivors at 24 months were nine male and 12 female controls, 13 males and 14 females given 0.07 mg/kg bw and five males and six females given 0.7 mg/kg bw. Mammary tumours, mainly fibroadenomas, occurred in 1/20 male rats treated with the low dose and in 3/20 treated with the high dose, compared with 0/20 male controls, and in 4/20 females treated with the low dose and in 7/20 treated with the high dose, compared with 8/20 female controls. The incidences of pituitary tumours in males were 2/20 in those given the low dose and 7/20 in those given the high dose, compared with 4/20 controls; in females, the respective incidences were 9/20, 7/20 and 4/20. Thyroid carcinomas occurred in two females that received the low dose and in one female that received the high dose; no such tumours occurred in controls (Gibson *et al.*, 1967).

3.2 Other relevant biological data

No data were available on sodium equilin sulfate, a principal component of conjugated oestrogens, or on piperazine oestrone sulfate. Oestrone sulfate is rapidly taken up by isolated rat liver cells and hydrolysed to the free oestrogen. The oestrone formed is further converted via the pathways used by natural oestrogens (see the monograph on oestradiol-17β, p. 279) (Höller *et al.*, 1977; Schwenk *et al.*, 1978).

Oestrone sulfate is a major oestrogen found in human plasma (Longcope, 1972; Ruder *et al.*, 1972). The metabolism of natural oestrogens in humans is discussed in the monograph on oestradiol-17β (p. 279).

No data on the toxicity, embryotoxicity or mutagenicity of conjugated oestrogens were available.

3.3 Case reports and epidemiological studies

See the section, ‘Oestrogens and Progestins in Relation to Human Cancer’, p. 83 and the monograph on diethylstilboestrol, p. 173.

4. Summary of Data Reported and Evaluation[1]

4.1 Experimental data

Conjugated oestrogens (Premarin®) were tested in only one experiment in rats by oral administration. The data were insufficient to evaluate the carcinogenicity of this compound.

4.2 Human data

Case reports and epidemiological studies on steroid hormones used in oestrogen treatment have been summarized in the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83. Because most of the studies which concerned endometrial carcinoma involved the use of conjugated oestrogens, the evidence in humans that administration of these agents is causally related to an increased risk of developing this cancer is particularly convincing.

4.3 Evaluation

The available experimental data are insufficient to evaluate the carcinogenicity of conjugated oestrogens in animals. Studies in humans strongly suggest that the administration specifically of conjugated oestrogens is causally related to an increased incidence of endometrial carcinoma.

[1] This section should be read in conjunction with pp. 62–64 in the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones', p. 131.

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3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Most studies have been made with combinations of ethinyloestradiol with progestins. These experimental data have therefore been summarized both in this monograph and in those on the other compounds used in such combinations. It is important to note that the effects reported may reflect the action either of an individual constituent or of the combination.

(a) Oral administration

Mouse: Groups of 24 virgin female C57L mice received 7 or 70 µg of a mixture of norethisterone:ethinyloestradiol (50:1) in oil by gavage, five times per week, commencing when the animals were 13 weeks of age. Pituitary tumours were found at autopsy after 84–89 weeks of treatment in 7/15 surviving mice given the lower dose and in 5/8 mice given the higher dose, compared with 2/15 controls. Hepatomas were found in 10/96 mice treated with norethisterone:ethinyloestradiol and in a concurrent experiment with norethynodrel plus mestranol, but the report does not specify in which group or groups they arose. No hepatomas occurred in 48 controls (Poel, 1966).

Ethinyloestradiol alone or in combination with ethynodiol diacetate, norethisterone acetate, norgestrel or megestrol acetate was incorporated into the diet of groups of 120 male and 120 female CF-LP (MTV^+)[1] or BDH (MTV^-) mice for 80 weeks. The doses were identified only as low (2–5 times the human contraceptive dose), medium (50–150 times) or high (200–400 times); the amounts administered were not specified. Ethinyloestradiol administered alone resulted in an increased incidence of pituitary tumours in both male (26 tumours) and female (38 tumours) CF-LP mice (MTV^+) in one of two experiments versus two and eight tumours in male and female control groups of 120 animals. Similar increases were found after administration of ethinyloestradiol in combination with ethynodiol diacetate or norethisterone acetate but not with norgestrel [The negative findings in one group given ethinyloestradiol alone and in one group administered ethinyloestradiol plus norgestrel may have been due to undetected differences in the conduct of the trial]. Malignant tumours of the connective tissue of the uterus [unspecified] were found in 6/120

[1] MTV^+: mammary tumour virus expressed; MTV^-: mammary tumour virus not expressed (see p. 62)

female mice fed ethinyloestradiol plus ethynodiol diacetate, compared with 0–1/120 controls. In groups of 71–87 mice of the BDH-SPF Carshalton stock, administration of ethinyloestradiol alone or in combination with megestrol acetate was associated with a small increase in the incidence of pituitary tumours in treated males and females (4–10% in treated groups compared with 2% and 0% in 57 male and 65 female controls); benign gonadal tumours [unspecified] were found in males (8–10% compared with 0% in controls); incidences of malignant mammary tumours were increased in both males and females (9–32% compared with 0% and 3% in controls); malignant tumours of the uterine fundus and of the cervix were found in 4–11% and in 4%, respectively, of treated females, compared with 0% in female controls (Committee on Safety of Medicines, 1972).

Intact female RIII, C3H and (C3H × RIII)F1 mice (MTV^+) were fed Lutestral (97.5% chlormadinone acetate and 2.5% ethinyloestradiol) in the diet at 8 mg/kg (daily intake, 20–30 μg/mouse); neither the mammary tumour incidence nor latent period were altered. In intact male (C3H × RIII)F1 mice, the mammary tumour incidence was increased from 0/76 to 10/32, and in castrated male (C3H × RIII)F1 mice it was increased from 10/61 to 23/28, with a decrease in the latent period (Rudali, 1975).

Rat: Groups of 30 female Mead-Johnson rats administered ethinyloestradiol in the diet, either alone at an average dose of 53 μg/kg bw per day or at the same dose level with megestrol acetate (average, 2.63 mg/kg bw per day), for 105 weeks had no increase in incidence of tumours in any tissue. When ethinyloestradiol (30 μg/kg bw per day) was given for 16 days followed by a mixture of ethinyloestradiol (30 μg/kg bw per day) plus megestrol acetate (1.5 mg/kg bw per day) for five days and then a period of no steroid treatment for seven days, for a total of 26 cycles (104 weeks), there was a significant reduction in the incidence of mammary tumours compared with that in controls (McKinney *et al*., 1968).

Groups of 73–120 rats were given ethinyloestradiol alone or in combination with ethynodiol diacetate, norethisterone acetate, norgestrel or megestrol acetate at low (2–5 times the human dose), medium (50–150 times) and high (200–400 times) doses for 104 weeks. Control groups consisted of 24–100 rats. Benign mammary tumours were found more frequently in males given the combination with norethisterone acetate (28% compared with 2% in controls), and malignant mammary tumours were found more frequently in males given the combination with ethynodiol diacetate (10% compared with 0% in controls). The incidence of benign liver-cell tumours was higher in males and females given ethinyloestradiol alone (15 and 23%) or in combination with megestrol acetate (11 and 14%) than in male (0) and female (81) controls. In females, the incidence of malignant liver-cell tumours in groups treated with ethinyloestradiol alone or in combinations ranged from 4% for ethinyloestradiol plus megestrol acetate (1:5) to 7.5% for ethinyloestradiol alone, a significant finding compared with the virtual absence of such lesions in 12 separate control groups of female rats (Committee on Safety of Medicines, 1972).

Dog: In a preliminary report, it was stated that groups of 12–16 female dogs were given a combination of ethinyloestradiol and norgestrel at dose levels of 0, 10 and 25 times the projected human dose levels. After five years, 2/12, 3/12 and 5/12 animals, respectively, showed mammary nodules (Finkel & Berliner, 1973).

Groups of 16 female beagles, 6–12 months of age at the start of the experiment, were given combinations of norethisterone and dimethisterone with ethinyloestradiol at dose levels of one, 10 and 25 times the projected human dose levels for seven years. Dogs were killed after two and four years. The combination with norethisterone resulted in a dose-related development of cystic endometrial hyperplasia, pyometra and alopecia. None of the controls showed mammary nodules, whereas one dog given the intermediate dose had a single nodule in the fifth year. Dimethisterone and ethinyloestradiol were given sequentially, as oestrogen alone, and as oestrogen combined with the progestin, followed by a steroid-free period. Again, alopecia, cystic endometrial hyperplasia and pyometra were noted. Many acne-like lesions were found in the group given the high dose and some in the intermediate dose group. Four hyperplastic mammary nodules were found by palpation in the group given the low dose, compared with two in controls (Weikel & Nelson, 1977) [The Working Group noted the lack of information concerning the number of tumour-bearing animals].

Monkey: In a preliminary report of a study in progress, a combination of 20:1 ethynodiol diacetate:ethinyloestradiol (Demulen) was administered orally at 1, 10 and 50 times the human contraceptive dose (0.021, 0.21 and 1.05 mg/kg bw) cyclically (3 weeks on and 1 week off) to three groups of 16 mature (three- to eight-year-old) female *Macaca mulatta* (rhesus) monkeys for five years. Clinical examination during 65 cycles revealed the presence and subsequent disappearance of a nodule in one control animal and in one monkey in the highest dose group (Drill & Golway, 1978).

(b) Subcutaneous and/or intramuscular administration

Rat: Groups of 10 female Wistar rats were injected subcutaneously with 5, 10 or 15 mg/kg bw of a mixture of ethinyloestradiol:megestrol acetate (1:8) in olive oil once every other day for 30 days. Mammary fibroadenomas occurred in 2/10, 4/8 and 2/9 survivors at between 29 and 59 weeks, compared with none in 10 surviving controls (Hisamatsu, 1972) [The Working Group noted the small numbers in each group].

3.2 Other relevant biological data

(a) Experimental systems

The relative activity of ethinyloestradiol in the Allen-Doisy test in mice is about 1.2 times greater than that of oestradiol-17β after subcutaneous administration and more than 10 times greater by oral administration (Emmeus & Martin, 1962). In the uterine-growth test (Rubin's test) in mice, it is about 1.2 times more active than oestradiol-17β (Brotherton 1976).

Toxic effects

The acute and chronic toxicity of this compound have been reviewed by Plotz and Haller (1971). The oral LD_{50}s in rats and mice are >5000 and >2500 mg/kg bw, respectively (Brotherton, 1976).

Embryotoxicity and teratogenicity

Oral administration of 0.1–3.0 mg/animal ethinyloestradiol on day 1 of gestation or 0.1–0.3 mg on days 2, 3 or 4 of gestation to Swiss albino mice terminated pregnancy in 100% of animals; administration of 0.003–0.03 mg terminated pregnancy in 20–60%. Four days after administration of 0.1-3.0 mg ethinyloestradiol on day 1 of gestation, 60% of embryos were found to have abnormalities, such as fragmental vacuolization. Simultaneous subcutaneous injections of progesterone (5 mg in a single dose or 20 mg in four injections) and ethinyloestradiol (0.1–0.3 mg on day 1 or on days 1, 2, 3 and 4 of gestation) did not prevent termination of all pregnancies induced by ethinyloestradiol (Yanagimachi & Sato, 1968).

Groups of 10 ICR/JCL mice were given 0.01 or 0.02 mg/kg bw ethinyloestradiol orally on days 11–17 of gestation. Female offspring were killed at 10–14 weeks of age; in 8/13 exposed *in utero* to the high dose and in 2/11 exposed to the low dose, cystic glandular hyperplasia was observed, suggested by the authors to be a precursor lesion of uterine cancer (Yasuda *et al.*, 1977).

Rats received 5–500 μg/kg bw ethinyloestradiol by gavage on the first two days of gestation; the highest dose terminated pregnancy in 50% of animals. Normally implanted foetuses were found on day 9; no abnormalities were reported (Blye, 1970).

Hamsters were fed ethinyloestradiol at various doses: a single dose of 2.5 mg on day 3 before mating decreased pregnancy rates by 50% without affecting oestrus. Treatment over three oestrus cycles with 0.21 mg per day on days 14–3 before mating delayed oestrus but did not reduce pregnancy rates. Treatment during one cycle with 0.63 mg per day on days 6–3 before mating impaired oestrus and pregnancy rates by 67% and induced 15.6% of foetuses to be resorbed (Davis *et al.*, 1972).

Absorption, distribution, excretion and metabolism

Studies on the metabolism of ethinyloestradiol have been carried out in rats, rabbits, guinea-pigs, dogs and monkeys. It is very rapidly and effectively absorbed from rat intestine; no appreciable metabolic transformation is reported to take place during the absorption process (Reed & Fotherby, 1976). The main metabolic pathway of ethinyloestradiol in rats is by aromatic 2-hydroxylation (Ball *et al.*, 1973; Bolt *et al.*, 1973); hydroxylations at ring B

(C-6/C-7) are of only minor importance. Rat liver forms 2-hydroxyethinyloestradiol and the methyl ethers thereof, 2-methoxyethinyloestradiol and 2-hydroxyethinyloestradiol-3-methyl ether, as its mayor metabolic products (see Fig. 1). This pathway is also important in humans (Bolt *et al.*, 1974a). Metabolites of ethinyloestradiol in rats are excreted almost exclusively in the faeces (Bolt & Remmer, 1972).

Rabbits excrete ethinyloestradiol metabolites mainly via the urine (Higashi, 1969). A peculiarity of the rabbit's metabolism of ethinyloestradiol is the large amount of 'ring-D-homoannulated' metabolites (D-homo-oestrone-17α and D-homo-oestradiol-17aα) that results from metabolic attack at the 17α-ethinyl group of ethinyloestradiol (Abdel-Aziz & Williams, 1969). This metabolic pathway is much less important in humans (Abdel-Aziz & Williams, 1970).

The pattern of urinary and faecal excretion of ethinyloestradiol metabolites in guinea-pigs (Reed & Fotherby, 1975) and in beagle dogs (Keeley *et al.*, 1975) is similar to that of humans, in that more than half of the ethinyloestradiol metabolites are excreted in the faeces.

In baboons, 2-hydroxylation and de-ethinylation of ethinyloestradiol to the 'natural' oestrogens, oestradiol, oestrone, etc., are important pathways (Helton *et al.*, 1977). This situation appears to be similar to that in humans (Williams *et al.*, 1975), although differences in quantitative metabolic patterns have been observed (Goldzieher & Kraemer, 1972).

Mutagenicity and other short-term tests

In a study reported as an abstract, no mutagenic activity was reported in *Salmonella typhimurium* G46 or *Escherichia coli* K12 in the presence of a liver microsomal system (Kraemer *et al.*, 1974).

No dominant lethal mutations were observed in female mice treated for three days with 8.5 μg ethinyloestradiol and 170 μg norethisterone (Badr & Badr, 1974).

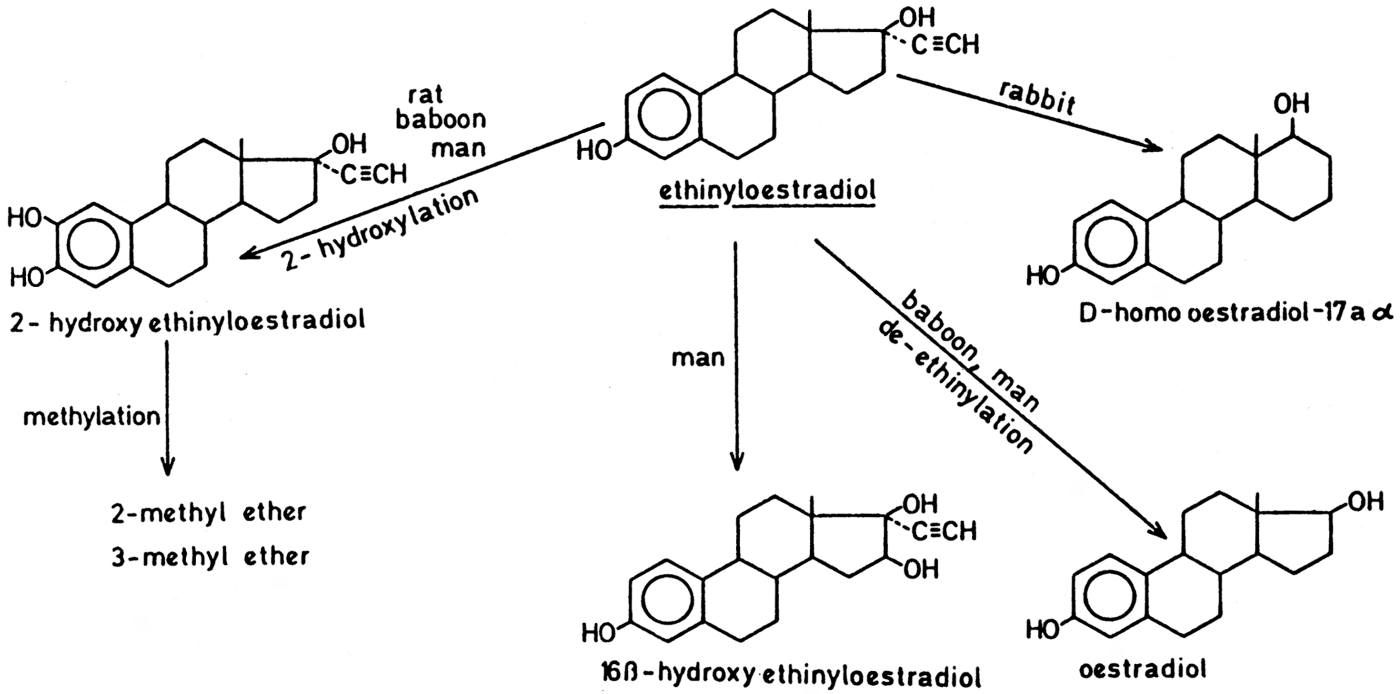
(b) Humans

In humans, ethinyloestradiol is about 40 times more active than oestradiol-17β when administered orally (Brotherton, 1976; Kupperman *et al.*, 1953).

The metabolic fate of ethinyloestradiol in humans has been reviewed extensively (Helton & Goldzieher, 1977a). Comparisons with its metabolism in mammals are described in section 3.2 (a). The major pathways are 2-hydroxylation (Bolt *et al.*, 1974a; Williams *et al.*, 1975) and 16β-hydroxylation (Williams *et al.*, 1975) (see Fig. 1); only trace amounts of D-

Figure 1

Major metabolic pathways of ethinyloestradiol in mammals[1]



[1]From Abdel-Aziz & Williams (1969) and Bolt (1979)

homoannulated metabolites are detected (Abdel-Aziz & Williams, 1970). It has been reported that the ethinyl group is removed by oxidative mechanisms, giving rise to the metabolites oestrone, oestradiol-17β, oestriol and 2-methoxy-oestradiol (Williams *et al.*, 1975). A major portion of ethinyloestradiol is conjugated directly with glucuronic acid and excreted (Fotherby, 1973).

Quantitative evaluation of the metabolic breakdown of ethinyloestradiol is difficult, due to substantial interindividual variation (Goldzieher, 1976; Helton & Goldzieher, 1977b). The extent of 2-hydroxylation in humans averages 29% of the ethinyloestradiol dose given (Bolt *et al.*, 1977), but in some individuals it may be as high as 64% (Bolt *et al.*, 1974b). Recently, remarkable geographic differences in the pharmacokinetics of ethinyloestradiol have been reported (Helton & Goldzieher, 1977b).

In contrast to the metabolites of natural oestrogens, a significant proportion of the metabolites of ethinyloestradiol in humans are excreted by the faecal route; ethinyloestradiol itself is excreted in urine and faeces in a ratio of about 4:6. About 90% of the metabolites of tritiated ethinyloestradiol are recovered in both faeces and urine (Speck *et al.*, 1976).

No chromosomal effects were observed when 0.1–100 μg/mL ethinyloestradiol were added to cultures of lymphocytes grown from the blood of healthy women (Stenchever *et al.*, 1969).

3.3 Case reports and epidemiological studies

See the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4. Summary of Data Reported and Evaluations[1]

4.1 Experimental data

Ethinyloestradiol was tested in mice, rats, dogs and monkeys by oral administration and in rats by subcutaneous injection; in most studies it was administered in combination with progestins.

[1] This section should be read in conjunction with pp. 62–64 in the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones', p. 131.

When administered alone to mice, it increased the incidence of pituitary tumours and malignant mammary tumours in both males and females and produced malignant tumours of the uterus and its cervix in females. In rats, it increased the incidence of benign liver-cell tumours in both males and females and produced malignant liver-cell tumours in females.

When ethinyloestradiol was given in combination with certain progestins, excess incidences of malignant tumours of the uterine fundus were observed in female mice and of benign and/or malignant mammary tumours in male rats; in female rats, the combinations reduced but did not prevent the incidence of malignant liver-cell tumours when compared with that produced by ethinyloestradiol alone. In dogs, no tumours that could be attributed to the treatment were found. The study in monkeys was still in progress at the time of reporting: no tumours had been found after five years of observation.

Mammary fibroadenomas were produced in female rats following subcutaneous injection of a combination of ethinyloestradiol with megestrol acetate.

Ethinyloestradiol is embryolethal for preimplantation embryos in some species.

4.2 Human data

No case reports or epidemiological studies on ethinyloestradiol alone were available to the Working Group. Epidemiological studies on steroid hormones used in oestrogen–progestin contraceptive preparations have been summarized in the section, ‘Oestrogens and Progestins in Relation to Human Cancer’, p. 83.

4.3 Evaluation

There is *sufficient evidence* for the carcinogenicity of ethinyloestradiol in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard ethinyloestradiol as if it presented a carcinogenic risk to humans. The use of oral contraceptives containing ethinyloestradiol in combination with progestins has been related causally to an increased incidence of benign liver adenomas and a decreased incidence of benign breast disease. Studies also strongly suggest that the administration of oestrogens is causally related to an increased incidence of endometrial carcinoma; there is no evidence that ethinyloestradiol is different from other oestrogens in this respect.

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Intact male (C3H × RIII)F1 mice (MTV^+) given 3 mg/kg (ppm) Ovulen (90% ethynodiol diacetate and 10% mestranol) mixed into the diet (intake, 7.5–10.0 μg/mouse per day) showed an increased incidence of mammary tumours, from 0/76 to 14/25; in castrated males, the incidence was increased from 10/61 to 21/28. The high spontaneous incidence (161/167) and short latent period of tumour induction (30–33 weeks) in intact females were not altered (37/38). In ovariectomized females, the tumour incidence was not altered by Ovulen (28/34 in controls as compared with 20/26), but the latent period was reduced in both ovariectomized females (from 49 to 26 weeks) and castrated males (from 82 to 43 weeks) (Rudali, 1975).

In C57BL females (MTV^-) given 20 mg/kg of diet Enovid for lifespan, chromophobe adenomas were seen in 36/49, compared with 15/51 controls. In BALB/c females (MTV^+) treated similarly, an increased incidence of non-metastasizing epithelial tumorous lesions of the cervix and vagina were reported in excess over that in controls (32/55 versus 18/55). The incidence of ovarian tumours in treated C3H (MTV^+) and C_3HfB (MTV^-) females was unchanged, and the incidence of mammary tumours in treated C3H females was decreased (39/53 versus 55/55) (Heston *et al*., 1973).

Twenty female BALB/c mice (MTV^-) were fed a liquid diet (Metrecal) containing an estimated dose of 10–12.5 μg Enovid/mouse per day for an average of 15 months. Among 16 mice that lived for 10 months or more, threedeveloped precancerous lesions and two, squamous-cell carcinomas of the cervix and/or vagina. Of a group of 40 mice treated with Enovid and with intravaginal inoculations of herpesvirus type 2, 31 survived 10 months or more; of these, one developed a precancerous lesion and six, squamous-cell carcinomas of the cervix and/or vagina. In 15/20 control mice that lived for 10 months or more, one precancerous lesion of the cervix was detected (Muñoz, 1973).

Administration of mestranol alone at a level of 0.1 mg/kg of diet, giving an estimated daily intake of 0.25 μg/mouse per day, resulted in an increased incidence of mammary tumours (to 11/13) in castrated male RIII mice (MTV^+) within eight months, compared with an incidence of 8/19 in intact male RIII (MTV^+) mice within 14 months. Of castrated male (C3H × RIII)F1 mice (MTV^+) fed 1 mg/kg of diet (2.5 μg/mouse per day), 24/26 developed mammary tumours within 28 weeks, compared with 7/41 controls within 69 weeks. No effects on the latent period or on the high spontaneous mammary tumour incidence were observed in females (Rudali *et al*., 1971).

In castrated male (C3H × RIII)F1 mice (MTV^+), administration of mestranol in the diet at an estimated intake of 75 μg/kg bw per day resulted in an increased incidence of mammary tumours (26/32), with an average latent period of 30 weeks, as compared with an incidence of 10/61 at 82 weeks in untreated control castrates (Rudali *et al*., 1972).

Mestranol alone or in combination with norethynodrel, ethynodiol diacetate, norethisterone, chlormadinone acetate or lynoestrenol was incorporated into the diet of CF-LP and Swiss mice for 80 weeks. The doses were identified only as low (2–5 times the human contraceptive dose), medium (50–150 times) and high (200–400 times); the amounts administered were not specified. Mestranol administered alone resulted in an increased incidence of pituitary tumours: 12 in 120 male and 17 in 120 female CF-LP mice, compared with four in 240 male and 12 in 240 female controls. Larger increases were found in both males and females given mestranol in combination with progestins, incidences ranging from 15 to 47 in males and from 27 to 42 in females per group of 120 animals. In groups of 47–123 Swiss mice administered mestranol alone, about 4% of malignant mammary tumours were found in males and females, compared with 0% in controls. When given in combination with lynoestrenol (1:33), the incidence in females increased to 6%, but no such tumours occurred in males (Committee on Safety of Medicines, 1972).

Administration of 5, 30, 60 and 200 μg/kg bw per day mestranol (2.5, 15, 30 and 100 times the human dose, respectively) to groups of 39–40 male and female Swiss-Webster mice and CF-LP mice did not influence the spontaneous incidence of hepatocellular tumours (Barrows *et al*., 1977).

Rat: In a group of 21 female Wistar rats given daily gastric instillations of 3 mg Enovid six times per week for 50 weeks, no mammary tumours were observed, compared with 1/54 in a group of untreated controls. In a further group of 47 female rats given a similar dose of Enovid together with 2–5 mg 3-methylcholanthrene six times per week for 52 weeks, the incidence of mammary tumours was neither increased nor decreased when compared with that produced by the administration of 3-methylcholanthrene alone (Gruenstein *et al*., 1964). Some inhibition of the induction of mammary tumours following a single dose of 15 or 20 mg 7,12-dimethylbenz[*a*]anthracence (DMBA)was observed after Enovid administration (Stern & Mickey, 1969; Weisburger *et al*., 1968).

A group of 100 female rats were given mestranol alone for 104 weeks. Twenty-two per cent of malignant mammary tumours were found in 100 treated animals and 5% in 50 controls. When mestranol was administered to male and female rats in combination with norethynodrel or norethisterone, the incidence of malignant mammary tumours in males was 12–20%, compared with 0% in controls, and 6–30% in females, compared with 5–7% in controls; the increase was significant. In male rats, the incidences of benign liver-cell tumours were 8-29% in groups of 120 rats administered mestranol with norethynodrel and 23% in 120 rats administered mestranol with norethisterone, compared with 2.5 and 4.0% in groups of 120 and 40 controls (Committee on Safety of Medicines, 1972) [The Working Group noted that the effective number of animals was not given].

Male and female Wistar rats were fed 0.01% fortwo2 years and 0.02% for one year of the oral contraceptive Sophia (norethisterone:mestranol, 100:1) in food pellets, resulting in daily doses of 1.53 mg norethisterone and 0.015 mg mestranol in the 0.01% group and in 3.74 mg and 0.037 mg for females and 4.28 and 0.43 mg for males in the 0.02% group. In those fed 0.01%, six mammary fibroadenomas and one pituitary tumour developed in 39 effective females, as compared with 0/6 female controls. No tumours were seen in 18 males treated with 0.01%, whereas 4/9 male controls developed mammary fibroadenomas. Considerable losses of animals occurred due to intercurrent deaths. Animals fed 0.02% lived for only one year. No tumours were observed within that time in animals of either group (Takahashi, 1974).

Three groups of female Wistar rats received 40 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) on three subsequent days. One group received an oral contraceptive (0.25 mg lynoestrenol + 0.075 mg mestranol) daily for 30 days before MNU treatment, whereas the other group received the contraceptive after the treatment. The third group served as a control. Tumour induction (especially of nephroblastomas) was changed only in the group that received the oral contraceptive before the carcinogen: a significant decrease was observed (Thomas *et al.*, 1972).

Dog: Groups of 13–20 female dogs were given mestranol alone (at 10 and 25 times the human dose, i.e., 0.02 and 0.05 mg/kg bw per day) or combinations of mestranol with chloroethinylnorgestrel or ethynerone (1:20) (at 2, 10 and 25 times the human dose, i.e., 0.084, 0.42 and 1.05 mg/kg bw per day) or mestranol:anagestone acetate (1:10) (at 10 and 25 times the human dose, i.e., 0.44 and 1.10 mg/kg bw per day). At the time of sacrifice, animals had received the combinations for 4.5–5 years and for 6.25 years. In the mestranol-treated dogs, one mammary adenoma was found in the highest dose group, whereas two benign mixed mammary tumours were found in controls. Dogs given progestin:mestranol developed more hyper- and neoplastic mammary nodules than did control dogs. Of animals given ethynerone plus mestranol, 2/16, 5/16 and 15/17 dogs, respectively, showed such nodules; whereas 7/16, 16/17 and 16/16 of the chloroethinylnorgestrel plus mestranol-treated dogs and 13/13 and 12/13 of the anagestone acetate plus mestranol-treated dogs showed nodules. Since pyometra occurred in some animals, all dogs, including the controls, were hysterectomized after two years of treatment (Geil & Lamar, 1977; Giles *et al.*, 1978).

Monkey: In a study still in progress at the time of reporting, an adenocarcinoma of the mammary gland was observed after 18 months in 1/6 female *Macaca mulatta* (rhesus) monkeys administered 1 mg Enovid per day. Widespread metastases were associated with the tumour (Kirchstein *et al.*, 1972) [The Working Group noted the incomplete reporting of this experiment].

In a preliminary report of a study in progress, oral administration of Enovid-E (2.5 mg norethynodrel and 0.1 mg mestranol per human dose) and Ovulen (1.0 mg ethynodiol diacetate and 0.1 mg mestranol) in dosages 1, 10 and 50 times the average human contraceptive dose to mature female rhesus monkeys (16 per group) resulted in no clinical evidence of mammary gland lesions or tumours after five years (Drill *et al*., 1974).

Groups of 16–20 female monkeys were given mestranol alone or in combination with chloroethinylnorgestrel, ethynerone or anagestone acetate orally in dosages of 2, 10 and 50 times the human dose level. At the time of publication, after seven years of observation, some palpable mammary nodules had been found distributed randomly in all groups, including the controls. Biopsies of mammary tissue revealed a slight ductal epithelial hyperplasia in some of the treated animals, also evenly distributed among the groups (Geil & Lamar, 1977).

(b) Subcutaneous and/or intramuscular administration

Mouse: Nulliparous female C3H/HeJ mice (MTV^+) were administered 0.1 mg Enovid subcutaneously twice weekly from one month of age for 21 months. A significantly increased incidence of mammary tumours was observed (30/100, as compared with 14/100 in controls) [$p < 0.01$] (Welsch *et al*., 1977).

Rat: Repeated subcutaneous injections of 10 or 100 μg Enovid per day into two groups of 25 female Sprague-Dawley rats for 40 days reduced the number of mammary tumours/rat produced by a single intravenous injection of 5 mg DMBA given on day 25 of treatment. The average numbers of tumours/rat were 10.9 in 37 controls given DMBA alone, compared with 7.6 and 3.9 in rats given 10 or 100 μg Enovid per day, respectively (Welsch & Meites, 1969).

Hamster: Thrice weekly subcutaneous injections into 46 male Syrian golden hamsters of 34 mg/kg bw Enovid in sesame oil, reduced to 17 mg/kg bw at 94 weeks and to 8.5 mg/kg bw at 104 weeks, did not affect the incidence of any tumour type (Sichuk *et al.*, 1967) [The Working Group noted that the average age of the animals at the start of the experiment was 76 weeks and that 50% of the animals had died by 103 weeks].

3.2 Other relevant biological data

(a) Experimental systems

In animals, as in humans, the oestrogenic activity of mestranol is equal to or slightly less than that of ethinyloestradiol, depending on species and route of administration (Haller, 1971). See also ‘General Remarks on Sex Hormones’, pp. 42, 43.

Toxic effects

The LD_{50} of mestranol by intraperitoneal administration in mice is 3500 mg/kg bw (Gosselin, 1968).

Geil and Lamar (1977) found a dose-dependent, non-progressive decrease in haemoglobin and haematocyte levels in dogs treated with mestranol or a combination of mestranol with ethynerone, chloroethinylnorgestrel or anagestone acetate. Diabetes mellitus developed in 10 dogs, all in groups that had received the medium or high dose of the chloroethinyl-norgestrel/mestranol or anagestone acetate/mestranol combinations. In addition, three monkeys (two anagestone acetate + mestranol-treated and one ethynerone + mestranol-treated) developed diabetes mellitus.

Embryotoxicity and teratogenicity

Daily oral administration of 0.05 or 0.2 mg/kg bw mestranol to NMRI × $ABAF_1$ hybrid mice daily from day 4 to 8 after mating inhibited implantation and increased the number of resorptions. Fetuses of mice of the NMRI strain had accessory ribs. Treatment from day 7 to 11 with doses of 0.1–0.2 mg/kg bw induced abortions but had no teratogenic effects (Heinecke & Klaus, 1975).

In rats, subcutaneous injection of 0.002–0.02 mg/kg bw mestranol five days before and 30 days after mating prevented implantation in a dose-dependent manner. Subcutaneous injection of 0.02 mg/kg bw or oral administration of 0.1 mg/kg bw on days 2–4 of gestation terminated pregnancy (Saunders & Elton, 1967).

Charles River rats received daily oral doses of 0.05–0.2 mg/kg bw Enovid (2.5 mg norethynodrel + 0.1 mg mestranol) or 0.01–0.1 mg/kg bw mestranol throughout pregnancy and for 21 days after parturition. The highest dose of mestranol terminated a significant percentage of pregnancies. No genital defects were observed in surviving male offspring, but female offspring showed an enlarged genital papilla and a prematurely open vagina, even with lower doses. In female offspring of rats treated with 0.1 mg Enovid, fertility was impaired by 55%. Higher doses of Enovid and a dose of 0.02 mg mestranol induced complete sterility in female offspring; examination of the ovary showed no corpora lutea or follicles of reduced size (Saunders, 1967).

Sixty female Wistar rats were given oral doses of 1 mg/kg bw Enidrel (0.075 mg mestranol + 9.2 mg norethynodrel) daily for two months, at which time they were mated. In 30 animals in which treatment was continued, complete fetal resorption occurred rapidly; however, after two weeks without treatment, fertility rates and litter sizes were normal. In the 30 animals in which treatment was discontinued, fertility and pre- and postnatal development of the offspring were also normal. No teratogenic effects were observed (Tuchmann-Duplessis & Mercier-Parot, 1972).

In rabbits, pregnancy was terminated by daily oral doses of more than 0.02 mg/kg bw mestranol from day 1 to 28 or 0.05 mg/kg bw from day 10 to 28 of pregnancy and by subcutaneous doses of 0.005 mg/kg bw from day 1 to 28 or more than 0.002 mg/kg bw from day 10 to 28. Doses that did not terminate pregnancy had no effects on litter size or weights of the offspring (Saunders & Elton, 1967).

In female Syrian golden hamsters that received a contraceptive steroid containing 18.7 µg mestranol and 0.6 mg lynoestrenol [route unspecified] daily for 4.5–8 months, fertility was found to be normal; no effects were seen on the sexual behaviour or fecundity of offspring of the following two generations (Cottinet *et al.*, 1974).

Adult female beagle dogs received 5 mg/kg bw mestranol orally on day 6 or 21 of pregnancy. Embryonic losses, based on corpora lutea counts, were 95.5 and 67.3%, respectively, as compared with 34.5% in controls. Surviving offspring appeared normal (Kennelly, 1969).

Absorption, distribution and excretion

Studies in rats indicate that enterohepatic circulation of metabolites of mestranol is an important factor in the excretion of this compound and may be impaired by administration of antibiotics such as neomycin (Brewster *et al.*, 1977).

Metabolism

Mestranol, the 3-methyl ether of ethinyloestradiol, is more lipophilic than ethinyloestradiol and has a greater affinity for adipose tissues, as shown by experiments in rats (Appelgren & Karlsson, 1971). Mestranol itself does not bind significantly to oestrogen receptors at the sites of their antifertility action; its hormonal effectiveness relies on transformation to ethinyloestradiol (Eisenfeld, 1974). About 35% of a mestranol dose is transformed into ethinyloestradiol in rats (Kappus *et al.*, 1972), 61% in mice (Bolt & Remmer, 1972), 56% in rabbits and 54% in man (Bolt & Bolt, 1974). The demethylated portion then follows the pathways for ethinyloestradiol that are typical for the particular species, e.g., 2-hydroxylation in rats (Ball *et al.*, 1973) and D-homoannulation in rabbits and guinea-pigs (Abdel-Aziz & Williams, 1969, 1974, 1975). Mestranol is also demethylated to ethinyloestradiol in non-human primates (Kulkarni *et al.*, 1977).

Mutagenicity and other short-term tests

Dominant lethal mutations were observed in female mice treated for three days prior to mating with doses of 12.5 µg mestranol and 420 µg lynoestrenol/kg bw (Badr & Badr, 1974) [For a consideration of the possible mutagenicity of this compound, see ‘General Remarks on Sex Hormones’, p. 64].

Female Holtzman rats treated orally for five days with 0.02 or 0.2 mg/kg bw mestranol were sacrificed on day 6. No damage to bone-marrow chromosomes, as compared with corn oil-treated controls, was observed (Edwards *et al.*, 1971).

(b) Humans

The metabolism of mestranol in humans is closely related to that of ethinyloestradiol (see monograph, p.233). Mestranol is transformed to ethinyloestradiol by demethylation (Warren & Fotherby, 1973): after intravenous administration of [^{14}C]mestranol to human volunteers, about 50% of the dose is demethylated to ethinyloestradiol (Bolt & Bolt, 1974). The main compound found in plasma is ethinyloestradiol-3-sulfate (Bird & Clark, 1973).

The excretion of metabolites in urine ranged from 10 to 27%; that of ethinyloestradiol metabolites ranged from 36 to 54% (Kulkarni & Goldzieher, 1970). When position 2 or 4 of the mestranol molecule is tritiated or marked with ^{14}C, between 14 and 45% of the radioactivity is released into the body water. Metabolites identified were ethinyloestradiol, 2-hydroxyethinyloestradiol, 2-methoxyethinyloestradiol and 2-hydroxyethinyloestradiol 3-methyl ether (Williams & Williams, 1975; Williams *et al.*, 1975).

No cytogenetic changes were detected in cultured human lymphocytes exposed *in vitro* to mestranol or derived from women exposed *in vivo* to mestranol in oestrogen/progestin contraceptive preparations (de Gutiérrez & Lisker, 1973; Shapiro *et al.*, 1972; Singh & Carr, 1970).

3.3 Case reports and epidemiological studies

See the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4. Summary of Data Reported and Evaluation[1]

4.1 Experimental data

Mestranol was tested in mice, rats, dogs and monkeys by oral administration; in most studies it was administered in combination with progestins. When administered alone, it increased the incidences of pituitary tumours in both sexes of one strain of mice and increased the incidence of malignant mammary tumours in castrated males of two further strains and in males and females of another strain. It also produced an increased incidence of malignant mammary tumours in female rats.

[1] This section should be read in conjunction with pp. 62–64 of the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones', p. 131.

Studies in dogs and monkeys are still in progress. Although no tumours have been observed in either species after seven years, no conclusive evaluation can yet be made.

In experiments in which mestranol was administered to female mice in combination with norethynodrel, pituitary tumours and vaginal and cervical squamous-cell carcinomas were produced; in male mice, an increased incidence of mammary tumours was observed following administration of mestranol in combination with norethynodrel or ethynodiol diacetate. Combinations with norethynodrel or norethisterone resulted in an excess of benign liver-cell tumours in male rats and increased the incidence of malignant mammary tumours in rats of both sexes.

In dogs, administration of combinations with various synthetic progestins led to the formation of mammary tumours. In monkeys given these combinations as well as combinations with norethynodrel or ethynodiol diacetate, no mammary nodules were observed after five and seven years of experimentation, respectively. These experiments are still in progress.

It was also tested in combination with norethynodrel by subcutaneous administration in mice, rats and hamsters; it produced an increased incidence of mammary tumours in female mice.

Mestranol is embryolethal for pre- and postimplantation embryos in some species.

4.2 Human data

No case reports or epidemiological studies on mestranol alone were available to the Working Group. Epidemiological studies on steroid hormones used in oestrogen-progestin contraceptive preparations have been summarized in the section, ‘Oestrogens and Progestins in Relation to Human Cancer’, p. 83.

4.3 Evaluation

There is *sufficient evidence* for the carcinogenicity of mestranol in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard mestranol as if it presented a carcinogenic risk to humans. The use of oral contraceptives containing mestranol in combination with progestins has been related causally to an increased incidence of benign liver adenomas and a decreased incidence of benign breast disease. Studies also strongly suggest that the administration of oestrogens is causally related to an increased incidence of endometrial carcinoma; there is no evidence that mestranol is different from other oestrogens in this respect.

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3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Mouse: Administration of 0.5 mg/L oestradiol-17β in the drinking-water for 19 months to C3H/HeJ (MTV^+)[1] female mice resulted in a significantly increased mammary tumour incidence (27/99, compared with 11/100 in controls); a combination of oestradiol-17β with a prolactin inhibitor, 2-bromo-α-ergocryptine (CB-154), prevented this increased tumour incidence (9/l00) (Welsch *et al*., 1977).

Groups of 48 C3H/HeJ (MTV^+) mice were fed 0, 100, 1000 and 5000 μg/kg of diet oestradiol-17β for 24 months starting at six weeks of age. Mammary adenocarcinomas were found in 4/47, 0/35, 6/36 and 8/48 animals in the respective groups after 52 weeks. Other malignant tumours occurred in those given 100 μg/kg: one adenocarcinoma of the cervix and one osteosarcoma of the cranium; in the 5000 μg/kg group, two adenocarcinomas of the uterus, three adenocarcinomas of the cervix and one adenoacanthoma of the uterus were seen. No such tumours were described in the controls (Highman *et al*., 1977).

(b) Subcutaneous and/or intramuscular administration

Mouse: Interstitial-cell tumours of the testis occurred in 10/24 male Strong A mice given weekly subcutaneous injections of 16.6 or 50 μg oestradiol 3-benzoate in sesame oil for six months (for the higher dose group) or until death. Concurrent administration of 1.25 mg testosterone propionate reduced the incidence of testicular tumours to 2/15 and increased the time of induction (Hooker & Pfeiffer, 1942).

Subcutaneous injections of 16.6 or 50 μg oestradiol 3-benzoate were given weekly for lifespan to 4–8-week-old hybrid mice derived from reciprocal matings of the C57 strain [MTV^-], which have a high incidence of oestrogen-induced pituitary tumours (Gardner & Strong, 1940), and CBA (MTV^+) mice. Pituitary tumours were found in 16/30 female and 19/23 male hybrids of C57 mothers and in 9/28 female and 18/24 male hybrids of CBA mothers. Mammary carcinomas (14/24 in males and 17/28 in females) occurred only in hybrid mice with CBA mothers and in 17/20 female and 0/4 male controls and were presumed to be dependent on the presence of the mammary tumour virus. The incidence of

[1] MTV^+: mammary tumour virus expressed; MTV^-: mammary tumour virus not expressed (see p. 62)

lymphoid tumours was higher in treated groups (7/52 males and females with CBA mothers and 5/53 males and females with C57 mothers) than in controls (2/24 and 2/48), but the differences were not statistically significant (Gardner, 1941).

Of 31 male RIII mice (MTV^+) given weekly subcutaneous injections of 50 μg oestradiol dipropionate, 13 were still alive after 20 weeks; mammary carcinomas were seen in nine animals between 20 and40 weeks. The incidence of mammary cancer in untreated females was about 63%. No mammary tumours occurred in treated CBA (MTV^-) males (Bonser & Robson, 1940).

Twice-weekly subcutaneous or intramuscular injections of 80 μg oestradiol-17β for six months (total dose, 3.3–4.2 mg) did not increase the incidence of mammary tumours in groups of 40 intact and 40 ovariectomized female Marsh-Buffalo mice [MTV^+] above that found in untreated controls. However, lymphosarcomas occurred earlier (between three and 10 months) and at a higher incidence (28% in intact, 47% in ovariectomized) than in controls (10%), the first tumour appearing at 12 months (Bischoff *et al*., 1942a). With discontinuous treatment in groups of 36–43 intact and castrated males of the same strain, lymphoid tumours occurred in 34% of castrates, compared with 8% in intact treated males and 5% in controls; the tumours developed much earlier in castrates (at 6–14 months) than in the other groups (Bischoff *et al*., 1942b).

Oestradiol-17β administered subcutaneously to mice of seven strains resulted in a greater incidence of lymphoid tumours than other oestrogens tested, the nature of which was not clearly defined. Oestradiol dipropionate given subcutaneously once a week for 10 weeks increased the incidence of lymphoid tumours in C3H mice [sex not stated] to 10/54 with 10-μg doses, to 4/18 with 25 μg and to 18/78 with 50 μg. The incidence of tumours in untreated mice was low (5/481 in C_3H mice; total, 11/822 in all strains tested) (Gardner *et al*., 1944).

BALB/c and CBA mice less than 57 weeks old were relatively resistant to the induction of lymphoid tumours by either X-rays, 3-methylcholanthrene or oestradiol dipropionate (5 μg in oil subcutaneously weekly for 14 weeks); however, in BALB/c mice, a combination of oestradiol dipropionate with 200-rad whole-body irradiation increased the incidence from 3/47 (with oestradiol dipropionate alone) to 16/71. Although 400 rads alone were no more effective than were 200 rads, the incidence of lymphomas was greater when 400-rad irradiation was combined with administration of 5 μg oestradiol dipropionate weekly for 14 weeks (12/30 by 43 weeks of age in BALB/c mice and 15/27 by 57 weeks in CBA mice). Thymectomy abolished the synergistic action of these two agents. The leukaemogenic action of 3-methylcholanthrene was not increased by combination with oestradiol-17β; however, in DBA mice, oestradiol dipropionate increased the leukaemogenic activity of both X-rays and 3-methylcholanthrene (Kirschbaum *et al*., 1953).

Invasive cervical lesions or carcinomas occurred in 4/10 female hybrid mice (C3H × PM strain) administered 16.6 μg oestradiol 3-benzoate subcutaneously, once weekly, starting at 4–9 weeks of age. In 25 female mice of the reciprocal hybrid (PM × C3H) strain receiving 16.6 or 25 μg oestradiol 3-benzoate, carcinomas or invasive epithelial lesions arose in the uterine cervix in eight mice and in the vagina in one mouse after 29 weeks or more of treatment. No carcinomas of the uterine corpus, cervix or vagina were found in 82 controls (Pan & Gardner, 1948).

Four female BC mice and one female CBA mouse that were injected subcutaneously with 16.6 μg oestradiol 3-benzoate in sesame oil once weekly commencing at ages ranging from 41–65 weeks had tumours of the uterine corpus at 78–89 weeks. Two of the BC mice also had cervical tumours (Gardner & Ferrigno, 1956) [The total incidence of these lesions in the treated mice cannot be assessed from the data, nor is the incidence of similar lesions in untreated mice known].

In an unspecified number of female mice of reciprocal crosses between C57 and CBA strains injected subcutaneously with weekly doses of 16.6 or 50 μg oestradiol 3-benzoate [vehicle not stated] commencing at 4–8 weeks of age, cervical lesions, ranging from invasion to gross tumours which invaded adjacent tissues, were seen in 15/24 mice with C57 mothers and in 10/20 with CBA mothers and surviving for more than 52 weeks. In the latter group there was a high incidence of mammary cancer, which reduced the lifespan. No lesions were seen before 59 weeks in either group. Of 10 mice in the two groups which survived more than 86 weeks, six had lesions at death. Although none of the tumours metastasized, it was concluded that the range of lesions seen probably represented various stages of carcinoma development. No cervical tumours occurred among an equal number of control mice (Allen & Gardner, 1941).

In a group of female CBA mice, three subcutaneous injections of 0.25 mg/animal polyoestradiol phosphate (Estradurin), seven days before and 30 and 60 days after an intraperitoneal injection of ^{90}Sr, led to an increased incidence of osteogenic tumours over that found with ^{90}Sr alone (from 36/50 to 44/49) and a shorter latent period (403 ± 9 versus 252 ± 4 days) (Nilsson & Broomé-Karlsson, 1976).

Rat: Female Sprague-Dawley rats were given 20 mg 7,12-dimethylbenz[α]anthracene (DMBA) in sesame oil by stomach tube at 50 days of age; 15 days later they were injected subcutaneously with oestradiol 3-benzoate and progesterone, either alone or in combination, at low incremental (0.01–1.6 μg), high incremental (1.0–7 μg) or constant (low, 0.1 μg; high, 1 μg) doses daily for 21 days. All rats were observed for more than five months. All DMBA-treated control rats developed mammary tumours (usually adenocarcinomas) within a short

latent period (average, 69 days). Administration of low or high incremental doses of oestradiol 3-benzoate alone increased the latent period and depressed both the number of tumour-bearing animals and the number of tumours per animal. High doses of the combination with progesterone resulted in an increased latent period and a decreased tumour incidence. The administration of incremental doses of progesterone alone or in combination with a constant dose of 0.1 μg oestradiol 3-benzoate stimulated the appearance of tumours (McCormick & Moon, 1973).

In a similar combination experiment, subcutaneous administration of 20 μg oestradiol 3-benzoate daily for 40 days, of 4 mg progesterone for 40 days or of a combination of 5 μg oestradiol 3-benzoate and 4 mg progesterone daily for 40 days significantly reduced the incidence of DMBA-induced mammary tumours in Sprague-Dawley rats (Kledzik *et al*., 1974).

Subcutaneous administration of 20 μg oestradiol 3-benzoate daily for three weeks to Sprague-Dawley rats 60 days after intravenous treatment with 5 mg DMBA at day 55, or of 20 μg oestradiol 3-benzoate with 2 mg/kg bw of a prolactin inhibitor, ergocornine methanesulfonate, led to a significant decrease in tumour size and in number of tumours per animal. The combination with the prolactin inhibitor was the most effective (Quadri *et al*., 1974).

Guinea-pig: In 22/24 ovariectomized guinea-pigs given subcutaneous injections three times weekly of 20–80 μg oestradiol 3-benzoate in olive oil (Progynon B), multiple tumours, described as fibromas or fibromyomas, arose in the uterus and mesentery at several locations. No tumours occurred in the thorax (Lipschütz & Iglesias, 1938; Lipschütz *et al*., 1938). Unesterified oestrogens that were tested concurrently were less active than the benzoate (Lipschütz & Vargas, 1939a).

Intact and castrated male guinea-pigs given 80 μg oestradiol 3-benzoate three times weekly were susceptible to induction of tumours in spleen, stomach and abdomen, but the size and extent of the abdominal lesions were less than in females (Koref *et al*., 1939). The tumours regressed after cessation of the treatment (Lipschütz *et al*., 1939). Histologically, these tumours had varying degrees of fibromyomatous and fibromatous proliferation, with some suggestion of transition to sarcoma. The tentative conclusion was that they were benign lesions peculiar to oestrogen-treated guinea-pigs (Lipschütz & Vargas, 1941).

(c) Subcutaneous implantation

Mouse: When castrated male (C3H × RIII)F_1 mice (MTV^+) were given a subcutaneous implant of a pellet containing 0.5–1 mg oestradiol-17β in paraffin wax at the age of 10 or 70 days, 15/16 and 18/18, respectively, developed mammary cancer. Such tumours also occurred in 7/41 castrated controls, within a mean latent period of 69 weeks, compared with 25–27 weeks in treated mice. In C3H and RIII strains, the incidences of mammary cancer in groups of castrated males treated at 10 days of age with oestradiol-17β paraffin pellets were

14/18 and 14/17, respectively. Mammary tumours occurred in only 3/19 male NLC mice and none of C57BL males (MTV^-) given similar implants (Rudali *et al*., 1971).

Lymphoid tumours, almost all thymic lymphosarcomas, were seen in 27/84 male BALB/c mice before 57 weeks of age after the subcutaneous implantation of a 1–2-mg pellet of oestradiol dipropionate at 18, 36 or 72 days of age. No lymphosarcomas were observed in 240 control mice before 57 weeks of age (Kirschbaum *et al*., 1953).

Of 20 BALB/c female mice treated every 3–4 months with 5-mg subcutaneous implants of an oestradiol-17β-cholesterol mixture for 20 months, 17 survived 10 months or more; two developed precancerous lesions and five developed squamous-cell carcinomas of the cervix and/or vagina. Of 40 mice treated with subcutaneous pellets of oestradiol-17β and with intravaginal inoculations of herpesvirus type 2, 36 survived 10 months or more; 10 developed precancerous lesions and seven developed squamous-cell carcinomas of the cervix and/or vagina. No cervical or vaginal lesions were seen in 16/20 control mice that lived for 10 months or more (Muñoz, 1973).

Paraffin pellets containing 10% oestradiol-17β or 10% oestriol (0.64–0.85 mg oestrogen) were implanted subcutaneously into male and female (C3H × RIII)F1 (MTV^+) mice, castrated on day 22–24 of life. Negative controls received a pellet containing only paraffin. Pellets recovered from mice at sacrifice contained 2–5% oestrogen; it was thus assumed that most mice absorbed at least several hundred μg of oestrogen. Mammary tumours [type not stated] occurred in 15/16 females and 15/16 males treated with oestradiol-17β, in 18/18 females and 25/30 males treated with oestriol and in 28/34 female and 10/61 male controls. The latent periods were 176 (± 31), 133 (± 10) and 446 (± 28) days, respectively, in females and 137 (± 15), 135 (± 44) and 576 (± 54) days, respectively, in males (Rudali *et al*., 1975).

In castrated male (C3H × RIII)F1 mice treated subcutaneously with paraffin pellets containing 0, 1, 2.5, 5, 10 or 100 μg oestradiol-17β, the incidences of mammary tumours were 11/33, 11/31, 23/27, 24/27, 27/27 and 23/24, with average latent periods of 515, 675, 270, 145, 185 and 175 days (Rudali *et al*., 1978).

Rat: Rats of the Wistar albino (Glaxo) strain (WAG), albino rats of the Royal Cancer Hospital (London) strain and hooded rats originally derived from the MRC (London) strain were given two pellets of 5–6 mg oestradiol-17β or oestradiol dipropionate, the initial implant at the age of four weeks and a further implant after 1–3 months. Pituitary enlargement due to chromophobe adenomas (320 mg; range, 29–606 mg) was common in all strains, occurring in 69/92 rats. Mammary cancers developed in 10/27 female WAG rats between 29 and 64 weeks of age; no such tumours were seen in five males that lived longer

than 64 weeks. Mammary cancers occurred in 2/38 female Cancer Hospital rats and in 6/19 female rats of the hooded MRC strain which lived longer than 29 weeks. The tumours were classified as adenocarcinomas, papillary carcinomas and anaplastic carcinomas. No carcinomas of the mammary gland occurred among equivalent numbers [not stated] of breeding or control rats of the strains used (Mackenzie, 1955).

Pituitary tumours occurred in adult Wistar albino rats [sex not stated] implanted with pellets of oestradiol 3-benzoate weighing 6–8 mg. The incidence of tumours is not clear from the data given, but an average pituitary weight of 217 mg was recorded in eight rats 14–21 weeks after the start of the experiment; this average was maintained or increased to 50 weeks in 73 other rats. Thrice weekly injections of 20 μg thyroxine accelerated pituitary hypertrophy in a smaller group treated with oestradiol 3-benzoate pellets. Treatment with oestradiol 3-benzoate did not induce pituitary hypertrophy in groups of rats fed a diet containing 0.5% thiouracil (Gillman & Gilbert, 1955).

Oestradiol-17β, oestriol and oestrone were administered subcutaneously to intact female Sprague-Dawley rats, 48 h before treatment with DMBA or procarbazine, as 1–20% pellets weighing 5–7 mg each. DMBA and procarbazine were given by gavage at amounts of 20 and 50–70 mg, respectively. No mammary carcinomas occurred up to 370 days in untreated controls or in oestrogen-treated female rats. Higher doses of oestradiol-17β had an inhibitory effect on carcinogen-induced tumour development (Lemon, 1975).

Hamster: Malignant renal tumours were found in 15/15 intact and 12/12 castrated male hamsters and in 10/16 ovariectomized female hamsters given one or more 20 mg pellets of oestradiol-17β subcutaneously every 21 weeks. The age at autopsy varied between 45 and 81 weeks for males and between 24 and 58 weeks for the ovariectomized females. No kidney tumours were found in six treated intact females, nor among 145 intact or 72 castrated controls of either sex (Kirkman, 1959).

Guinea-pig: Fibromyomas in the uterine corpus, mesentery and other abdominal sites were found in female guinea-pigs that had been ovariectomized three months before the subcutaneous implantation of a 20- or 50-mg pellet of oestradiol-17β. Fibromyomas were detected as early as 19 days after the start of treatment in all animals. The pellets lost up to 10 mg in weight over seven weeks (Lipschütz & Vargas, 1939b). Similar results were obtained by Woodruff (1941) with oestradiol 3-benzoate, and by Riesco (1947) with oestradiol dipropionate.

Monkey: Total doses of 575–825 mg oestradiol-17β were implanted subcutaneously at intervals of 5–6 weeks over a 24–28-month period in five female *Macaca mulatta* (rhesus) monkeys. Cystic hyperplasia of the mammary gland but no tumours were found (Engle *et al*., 1943).

Five female Capuchin monkeys *(Cebus apella)* were given subcutaneous implants of oestradiol dipropionate and/or oestradiol-17β. In some cases the pellet consisted of pure oestrogen and in others of a mixture of 40% oestrogen and 60% cholesterol. The amount of oestrogen absorbed per day was about 250–700 μg/animal, and the total duration of treatment ranged from 29 to 145 weeks. In addition, three animals received subcutaneous injections of other oestradiol esters (100–200 μg) twice weekly. A high degree of cystic and polypous glandular hyperplasia of the uterine mucosa developed in all animals. The only tumour observed (found in the longest survivor) was an adenocarcinoma or endothelioma of the pericardium, but it was considered not to be due to the treatment (Iglesias & Lipschütz, 1947).

(d) Neonatal exposure

Mouse: Data on vaginal lesions seen in female mice administered oestrogens neonatally have been summarized by Takasugi *et al.* (1970). Evidence that oestrogens given neonatally to mice 'select' special cell populations in the vagina and uterine cervix, which may give rise to abnormal lesions, has been presented by Forsberg (1972, 1973, 1975, 1979) and by Takasugi and Kamishima (1973). See also Takasugi (1976, 1979). It is apparent now that at least two different responses may occur in the genital tract of female mice given oestrogens neonatally: (1) retention of Müllerian-derived epithelium in the upper vagina (especially the fornix) and its proliferation in the fornical and cervical areas to give rise to adenosis (Forsberg, 1975, 1979); and (2) retention of a population of oestrogen-independent, cornifying cells in the vagina which proliferate, replace the original epithelium and give rise to irreversible, persistent vaginal cornification, hyperplastic downgrowths and squamous-cell carcinomas (Jones & Bern, 1977; Takasugi, 1976, 1979). The relation of adenosis to cervicovaginal adenocarcinomas is at present unknown (Scully *et al*., 1978).

Increased mammary tumorigenesis has been reported in MTV^+ mice treated neonatally with oestradiol-17β (Bern *et al*., 1975, 1976; Mori, 1968a,b); and hyperplastic nodules or metaplastic lesions have also been found in various accessory sex organs, including prostatic lobes, in male C3H/MS mice given oestrogens neonatally (Mori, 1967).

Female mice of four strains that received 5 μg oestradiol-17β daily for the first five days after birth showed hyperplastic and epidermoid vaginal lesions at 32–63 weeks of age: 16/23 A/Crgl strain, 6/14 BALB/cCrgl, 4/16 C57BL/Crgl and 3/15 RIII/Crgl. No lesions were found in six treated C3H/Crgl mice at 44 weeks of age, and one lesion occurred in five C57BL/Crgl controls, although most of these mice showed persistent vaginal cornification. Vaginal concretions ('stones') were found in almost all mice with vaginal lesions (Takasugi & Bern, 1964).

Female BALB/cCrgl mice were injected with 25, 5 or 0.1 μg oestradiol-17β as an aqueous suspension daily for the first five days of life. Approximately half the animals in each group were ovariectomized at 16–17 weeks of age. All mice that received the two higher doses and 37/42 mice that received the lower dose developed persistent vaginal cornification; this cornification was maintained after ovariectomy in all mice that had received 25 or 5 μg oestradiol-17β but not in those that had received 0.1 μg. The mice were killed between 64 and 73 weeks of age. Vaginal epithelial downgrowths were found in all of 16 and 11 intact mice that had been given 25 and 5 μg and in 16/19 intact mice that had received 0.1 μg. In the corresponding ovariectomized groups, the incidences of downgrowths were reduced to 15/16, 5/10 and 0/9, respectively. Hyperplastic vaginal lesions resembling epidermoid carcinoma were found at termination of the experiment in 19/27 intact mice and in 8/26 ovariectomized mice given 25 or 5 μg oestradiol-17β, but in only 3/19 intact mice and 0/9 ovariectomized mice that had been given 0.1 μg. The mean ovarian weights of all the intact oestradiol-17β-treated mice were more than twice those of the controls. Epithelial downgrowth was found in the vaginas of 5/10 intact controls, but no hyperplastic lesions were seen. Four ovariectomized controls had no vaginal dysplasias (Kimura & Nandi, 1967).

Groups of 2–19 newborn BALB/cfC3H (MTV^+), BALB/c (MTV^-) and C57BL (MTV^-) female mice were treated for five days with 5 or 20 μg/day oestradiol-17β and with 5 or 20 μg/day testosterone alone or in combination with 5 or 20 μg/day prolactin. In two identical experiments, both steroids resulted in higher mammary tumour incidences in BALB/cfC3H mice, as compared with those in untreated (0/40) and prolactin-treated (9/43) mice. Testosterone induced higher incidences (42/49 and 22/35) than oestradiol-17β (8/35 and 32/64). The combination with prolactin did not influence tumour incidence significantly. No mammary tumours occurred in the MTV^- strains (Mori *et al*., 1976).

Neonatal subcutaneous administration to newborn BALB/cfC3H mice (MTV^+) of 5 or 20 μg oestradiol-17β, alone or in combination with 100 μg progesterone, daily for five days resulted in an increased incidence of mammary adenocarcinomas at an earlier age, except in those given the high dose of oestradiol-17β. The number of animals with mammary adenocarcinomas was 17/19 in the low-oestradiol-17β, 20/32 in the low-oestradiol-17β-plus-progesterone, 4/11 in the high-oestradiol-17β, 33/44 in the high-oestradiol-17β-plus-progesterone and 5/17 in the vehicle control groups (Jones & Bern, 1977).

Female BALB/c mice (MTV^-) given subcutaneous injections of 40 μg oestradiol-17β daily for the first five days of life showed more and different kinds of mammary dysplasias than untreated controls when given DMBA by gavage later in life (Warner & Warner, 1975).

Rat: Single subcutaneous injections of 0.1 mg oestradiol 3-benzoate in 0.05 mL sesame oil to five-day-old female Sprague-Dawley rats reduced the incidence of DMBA-induced mammary tumours at 190 days of age (10/31 versus 20/33, $p < 0.05$). Treatment decreased mammary adenocarcinoma response to DMBA (4/28 total tumours versus 27/30) and increased fibroadenoma response to DMBA (24/28 total tumours versus 3/30, $p < 0.01$). No tumours occurred in rats given oestradiol 3-benzoate only (Shellabarger & Soo, 1973).

In female Sprague-Dawley rats given subcutaneous injections of increasing doses of oestradiol-17β (10–40 μg) for the first 30 days of life, DMBA-induced mammary tumorigenesis was completely inhibited 120 days after DMBA administration at 60 days of age (none versus 15/27 in DMBA controls). The degree of normal mammary development was no different among treated and untreated groups at 60 days of age; however, at 160 days after DMBA administration, mammary glands in oestrogenized rats had regressed completely (Nagasawa *et at*., 1974).

Neonatal treatment of Sprague-Dawley rats with 100 μg oestradiol-17β did not influence the incidence of ear-duct tumours induced by 20 mg DMBA given by stomach tube (Yoshida & Fukunishi, 1977).

Female Sprague-Dawley rats were given a single subcutaneous injection of 0.1 mg oestradiol-17β at two days of age and 20 mg DMBA by gavage at 50 days of age. The induction of mammary dysplasia was significantly accelerated in rats given oestradiol-17β, when compared with DMBA controls (37/44 versus 26/40); mammary carcinoma incidence was significantly reduced (24/44 versus 34/40) after 300 days of observation (Yoshida & Fukunishi, 1978).

3.2 Other relevant biological data

Oestradiol-17β is the most potent naturally occurring steroidal oestrogen (see also 'General Remarks on Sex Hormones', pp. 42, 43).

(a) Experimental systems

No data were available on its toxic effects.

Embryotoxicity and teratogenicity

Swiss-Webster mice were injected subcutaneously with oestradiol 3-benzoate between days 11 and16 of gestation at alternating doses of 0.1 and 0.2 mg. A significant incidence of cleft palate occurred in offspring (13.6% versus 1.1% in controls) (Nishihara, 1958).

ICR mouse fetuses from the 15th or 17th day of gestation were injected subcutaneously with 50 μg oestradiol-17β. Irreversible cornification or stratification of the vaginal epithelium was seen at birth in 85% of those treated on day 17 and in 66% of those treated on day 15. When examined at the age of three months, corpora lutea were absent in 4/12 treated on day 17 and in 5/6 mice treated on day 15. Administration of 50 μg oestradiol-17β on the day of birth or three days later to ICR mice resulted in absence of corpora lutea. The changes in their vaginal epithelium were less marked (Kimura, 1975).

Administration of 500 μg oestradiol-17β on day 15 of gestation to BALB/cfC3H/Crgl and C3H/Tw mice resulted in abnormal oestrus cycles in female offspring late in life and abnormalities of the cervicovaginal epithelium. Administration on day 12 had no such effects (Mori *et al*., 1978).

Pregnant rats were given subcutaneous injections of 0.8–35 mg oestradiol-17β daily from day 12–13 until day 18–21 of gestation, or 0.375–100 mg oestradiol dipropionate every second or third day from day 12 or 13 of gestation until day 17–19; in some animals, the latter compound was given as a single dose on day 12 or 13. Only 12/28 oestradiol-17β-treated rats and 94/164 oestradiol dipropionate-treated rats carried to term; litter size was reduced and post-partum mortality was 66 and 81%. In female offspring (106 newborn, 11 adult) of the two groups combined, abnormalities of the reproductive tract and absence of corpora lutes were observed. In male offspring (98 newborn, 22 adult), 3–6 pairs of well developed nipples, undescended testes and impairment of Wolffian-derived tissues occurred (Greene *et al*., 1940).

A single subcutaneous injection of 10 mg oestradiol dipropionate to rats on day 14 of gestation induced malformations of the mammary glands (missing mammary glands and hypertrophic nipples) in offspring of both sexes (Delost *et al*., 1962, 1963).

Rabbits were injected intramuscularly with 15 or 30 μg oestradiol-17β for 3–5 consecutive days during different periods of gestation, starting on the fifth day. Administration of 15 μg terminated pregnancy when given before day 21 of gestation; later, 30 μg were required to terminate pregnancy (Schofield, 1962).

Metabolism[1]

The metabolism of oestradiol-17β and oestrone is similar in rats and in humans, in that both species transform these steroids mainly by (aromatic) 2-hydroxylation (Bartke *et al*.,

[1] The metabolism of oestradiol-17β, of oestrone and of oestriol are considered together, since there is interconversion between oestradiol-17β and oestrone, and the latter is converted to oestriol.

1971; Keith & Williams, 1970), and also by 16α-hydroxylation (Bolt, 1979). Glucuronides of the various metabolites are excreted in the bile (Fig. 1). Differences in the metabolism of oestrogens by humans and rats lie mostly in the type of conjugation (Williams, 1970). A relatively large proportion of administered oestrone, oestradiol-17β and oestriol is transformed in rats to metabolites oxygenated both at C-2 and C-16 (Honma & Nambara, 1974; Menzies & Watanabe, 1976; Watanabe & Menzies, 1973). When oestriol is administered to rats, glucuronides and, to a lesser extent, sulfates of 16-ketooestradiol and of 2- and 3-methyl ethers of 2-hydroxyoestriol and 2-hydroxy-16-ketooestradiol are excreted in the bile (Nambara & Kawarada, 1975; Nambara *et al*., 1974). In contrast, hydroxylations at C-6 or C-7 of ring B of oestradiol-17β and oestrone are a minor pathway in rats (Lehmann & Breuer, 1969). 2-Hydroxyoestrogens ('catechol oestrogens') are further transformed by various routes (see Gelbke *et* al., 1978), including covalent binding to proteins.

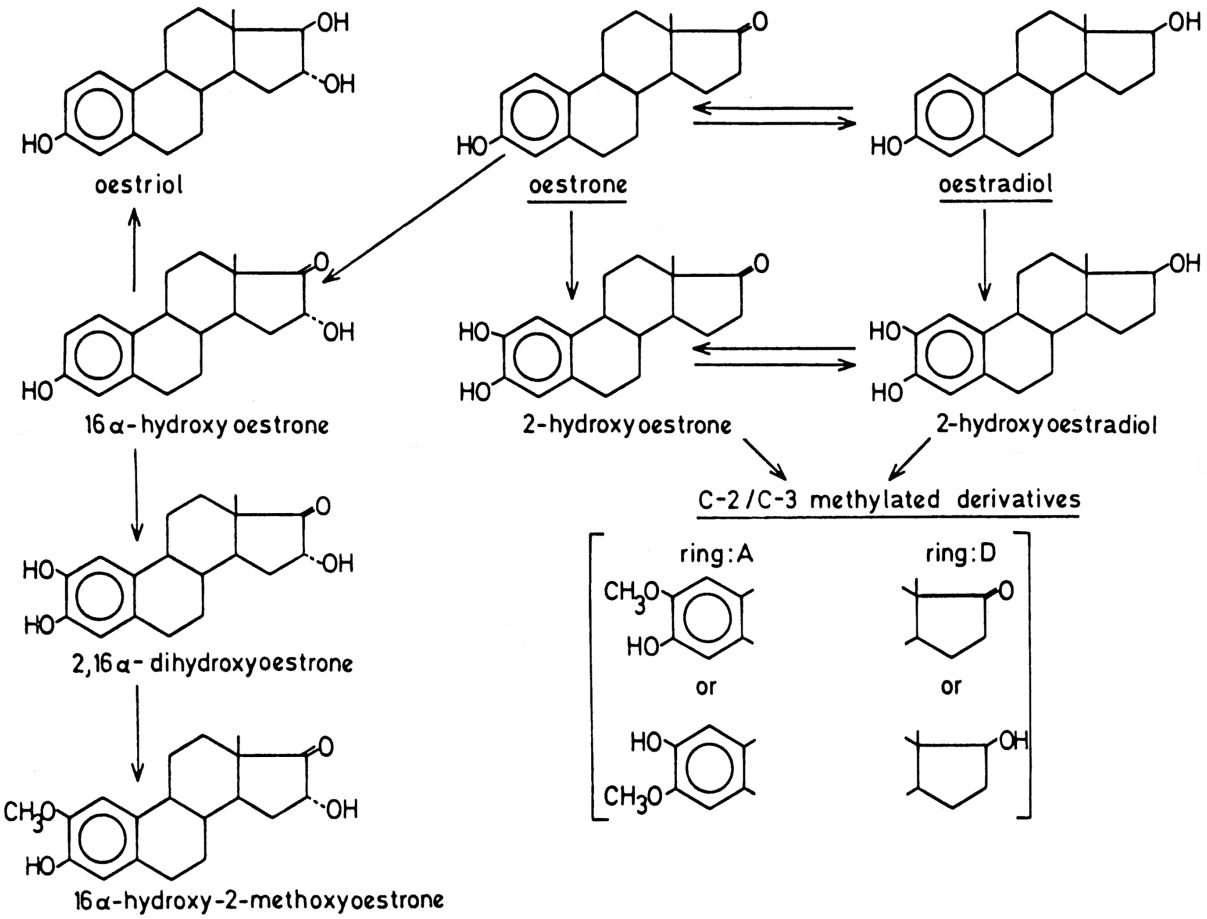
Rabbit liver can form 2-hydroxylated oestriol (King, 1961). However, the presence of two enzymes unique to this organ leads to an oestrogen metabolite pattern that cannot be compared with the human situation: (1) in addition to glucuronyltransferase, rabbit liver microsomes also contain glucosyl and *N*-acetylglucosaminyl transferases, which transfer glucose from UDP-glucose to the 3-hydroxy group of oestrone and oestradiol or N-acetylglucosamine from the UDP form to the 17α-hydroxy group of oestradiol-17α (Collins *et al*., 1970); (2) in addition to microsomal and cytoplasmic 17β-hydroxysteroid dehydrogenase, a 17α-hydroxysteroid dehydrogenase is found in rabbit liver cytosol (Breuer & Knuppen, 1968). Hence, the major metabolite of oestrone or oestradiol-17β in rabbits is oestradiol-17*a*, which is mainly conjugated to oestradiol-17α-*N*-acetylglucosaminide (Breuer & Knuppen, 1968) or to oestradiol-3-glucuronide-17α-*N*-acetylglucosaminide (Quamme *et al*., 1972). These metabolic pathways do not occur in man.

Sulfates are the major conjugates of oestradiol-17β and oestrone in guinea-pigs, and metabolism by 16α-hydroxylation is thought to take place on the oestrone 3-sulfate (Hobkirk *et al*., 1977). Glucuronidation, if any, plays only a minor role, in contrast to the human situation; 2-hydroxylation of oestrone also occurs. Only small amounts of labelled oestrone are converted to oestradiol in guinea-pigs (Yoshizawa *et al*., 1977).

Canine metabolism of oestrogens differs greatly from that in humans. Only small amounts of labelled oestradiol-17β are converted to oestriol; the bulk of the radioactive dose is excreted in urine as conjugates of oestradiol-17β and oestrone (Bering *et al*., 1975). After administration of labelled oestriol to dogs, a unique pattern of conjugates was found in bile and urine, probably including polyglucuronides (Kirdani & Sandberg, 1974). Furthermore, no significant enterohepatic circulation occurs, in contrast to the distribution of oestrogenic hormones in most other species.

Figure 1

Major pathways in metabolism of natural oestrogens in rats (conjugation reactions are not shown)[1]



[1]From Bolt (1979)

Oestradiol-17α is also a major oestrogenic metabolite in some other species, including domestic fowl (Chan & Common, 1974; Common & Robinson, 1976), bulls (Leung *et al*., 1975) and sheep (Challis *et al*., 1973). Liver tissues of pigs contain significant amounts of 17β-hydroxysteroid dehydrogenase and glucuronyl transferase (Rao *et al*., 1974); in minipigs, oxidoreduction at C-17 is the predominant reaction; whereas, in contrast to humans, hydroxylation of oestrogens plays only a minor role (Beckmann & Breuer, 1975). The metabolism of oestrogens in these species thus differs markedly from that in humans.

On the basis of the urinary and faecal excretion of oestrogens, non-human primates resemble humans much more closely than do rodents or dogs (Goldzieher & Kraemer, 1972). The conjugates of oestrogens are similar in *Macaca mulatta* (rhesus) monkeys and in humans (Musey *et al*., 1977). Baboons also show some similarities to humans in oestrogen metabolism (Musey *et al*., 1973).

No data on oestradiol-17β-valerate were available, but it is known that similar oestradiol esters, such as the benzoate (Kaltenbach *et al*., 1976), are converted in the organism to the corresponding active oestrogens. The derivatives as such are almost devoid of oestrogenic activity (in terms of affinity for oestrogen receptors); oestrogenicity relies on metabolic transformation to the parent compounds. The extent and pharmacokinetics of this metabolic step are very much dependent on the particular compound in question.

Mutagenicity and other short-term tests

Twice weekly injections to male Wistar rats of 1 mg/animal oestradiol 3-benzoate significantly lowered the mitotic index of bone marrow but induced no detectable chromosomal aberrations (Málková *et al*., 1977).

A twofold increase in the number of chromosomal aberrations was observed in cultures of human embryonic fibroblasts and renal epithelial cells treated with 1 μg/mL oestradiol-17β (Serova & Kerkis, 1974).

When cultures of human diploid synovial cells were exposed to 2.5×10^{-6}–10^{-9} M oestradiol-17β, high concentrations significantly increased the number of aneuploid cells in cultures from some individuals (Lycette *et al*., 1970).

No obvious chromosomal effects were observed when 0.1–100 μg/mL oestradiol-17β were added to cultures of lymphocytes grown from the blood of healthy women (Stenchever *et al*., 1969).

(b) Humans

The metabolic pathways of oestradiol-17β in humans have been reviewed extensively (Breuer *et al*., 1968; Diczfalusy & Lauritzen, 1961). Comparisons with its metabolism in mammals are described in section 3.2(*a*). 16-Oxygenation is a major step in the conversion of natural oestrogens. After injection of [16α-^{3}H]oestradiol, 55% of the tritium is liberated into the body water, although only 8% of the dose is excreted as urinary oestriol (Fishman *et al*., 1966); this indicates that 16*a*-hydroxylation is a quantitatively important pathway and that it may be followed by secondary routes of conversion. Another major pathway for the conversion of natural oestrogens in humans is 2-hydroxylation, since about 33% of the tritium of [2-^{3}H]oestradiol is released into the body water (Fishman *et al*., 1970). The product of 2-hydroxylation, 2-hydroxyoestrone, is found in human plasma (Yoshizawa & Fishman, 1971).

The two main metabolic routes, 2- and 16α-hydroxylation, are competitive. With high levels of thyroid hormone, 2-hydroxylation is increased and 16α-hydroxylation decreased (Fishman *et al*., 1965); whereas in liver cirrhosis, 2-hydroxylation is decreased and 16α-hydroxylation of oestrone enhanced (Zumoff *et al*., 1968). Oestriol also undergoes 2-hydroxylation, since 2-hydroxyoestriol has been identified in late-pregnancy urine (Gelbke & Knuppen, 1974). The catechol oestrogens, 2-hydroxyoestrone, 2-hydroxyoestradiol and 2-hydroxyoestriol, undergo further metabolic alteration; this has been reviewed extensively by Gelbke *et al*., (1978).

Oestrogens may be conjugated at the phenolic hydroxyl group of ring A, either by sulfuric or glucuronic acid. Moreover, conjugation with glucuronic acid has been reported to occur at hydroxy group of ring D (Hobkirk & Nilsen, 1971). The predominant conjugate in human plasma is oestrogen-3-sulfate: levels exceed those of oestradiol-17β by about 10 times (Longcope, 1972; Ruder *et al*., 1972). Oestrone sulphate may be reconverted to oestrone and oestradiol in extrahepatic organs like the uterus (Trolp *et al*., 1977).

3.3 Case reports and epidemiological studies

See the section ‘Oestrogens and Progestins in Relation to Human Cancer’, p. 83.

4. Summary of Data Reported and Evaluation[1]

4.1 Experimental data

Oestradiol-17β and its esters were tested in mice, rats, hamsters, guinea-pigs and monkeys by subcutaneous injection or implantation and in mice by oral administration. Its subcutaneous administration resulted in increased incidences of mammary, pituitary, uterine, cervical, vaginal and lymphoid tumours and interstitial-cell tumours of the testis in mice. In rats, there was an increased incidence of mammary and/or pituitary tumours. In hamsters, a high incidence of malignant kidney tumours occurred in intact and castrated males and in ovariectomized females, but not in intact females. In guinea-pigs, diffuse fibromyomatous uterine and abdominal lesions were observed. Oral administration of oestradiol-17β in mice led to an increased mammary tumour incidence. Subcutaneous injections in neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life and an increased incidence of mammary tumours.

Oestradiol-17β has teratogenic actions on the genital tract and possibly on other organs and impairs fertility.

4.2 Human data

No case reports or epidemiological studies on oestradiol-17β alone were available to the Working Group. Case reports and epidemiological studies on steroid hormones used in oestrogen treatment have been summarized in the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4.3 Evaluation

There is *sufficient evidence* for the carcinogenicity of oestradiol-17β in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard oestradiol-17β as if it presented a carcinogenic risk to humans. Studies in humans strongly suggest that the administration of oestrogens is causally related to an increased incidence of endometrial carcinoma; there is no evidence that oestradlol-17β is different from other oestrogens in this respect.

[1] This section should be read in conjunction with pp. 62–64 in the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones', p. 131.

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3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

Subcutaneous implantation

Mouse: Paraffin pellets containing 10% oestradiol-17β or 10% oestriol (0.64–0.85 mg oestrogen) were implanted subcutaneously into castrated male and female (C3H × RIII)F1 (MTV^+)[1] mice and castrated male RIII (MTV^+) mice on day 22–24 of life. Controls received a pellet containing only paraffin. The RIII males received only oestriol or control pellets. Pellets recovered from mice at sacrifice 4–5 months after implantation contained 2-5% oestrogen; it was thus assumed that most mice absorbed at least several hundred μg of oestrogen. Mammary tumours [type not stated] occurred in 15/16 female and 15/16 male F1 hybrids treated with oestradiol-17β, in 18/18 female and 25/30 male F1 hybrids treated with oestriol and in 28/34 female and 10/61 male control hybrids. The latent periods were 176 (± 31), 133 (± 10) and 446 (± 28) days, respectively, in females and 137 (± 15), 135 (± 44) and 567 (± 54) days, respectively, in males. All castrated RIII males treated with oestriol developed mammary tumours in 137 ± 6 days, compared with 0/22 controls (Rudali *et al*., 1975).

Rat: Oestradiol-17β, oestriol and oestrone were administered subcutaneously to intact female Sprague-Dawley rats 48 has before treatment with 7,12-dimethylbenz[*a*]anthracence (DMBA) or procarbazine as 1–20% pellets weighing 5–7 mg each. DMBA and procarbazine were given by gavage at amounts of 20 and 50–70 mg, respectively. No mammary cancers occurred up to 370 days in untreated controls or in oestrogen-treated female rats. Higher doses of oestriol (0.53–0.65 mg/pellet) had an inhibitory effect on carcinogen-induced tumour development (Lemon, 1975).

Hamster: Hamsters of heterogeneous origin were given subcutaneous implants of 20-mg pellets of oestriol, reimplanted every 150 days to ensure constant absorption, for 318–601 days. After latent periods of 396–593 days, 6/11 animals developed tumours in one or both kidneys. No data on controls were reported (Kirkman, 1959).

[1] MTV^+: mammary tumour virus expressed (see p. 62)

3.2 Other relevant biological data

Oestriol has about 1/10 the activity of oestradiol-17β or oestrone after subcutaneous administration (Diczfalusy & Lauritzen, 1961).

Administration of 0.1 μmol oestriol to Wistar rats from day 16 to 19 of gestation induced partial feminization of male fetuses (Brunt *et al.*, 1975).

Treatment of female rats with 10 μg oestriol seven days before mating led to a reduction of one-third of the litter size. Treatment on day of mating had only a slight effect on litter size; treatment during the early period of gestation (first 5–6 days) decreased litter size by two-thirds; and treatment on day of mating and on day 4 reduced litter size by one-third (Velardo *et al.*, 1956).

Oestriol is a common metabolite of oestrone and oestradiol-17β (see monograph on oestradiol-17β, p. 307) in animals and in humans. Oestriol is excreted in humans as conjugated and unconjugated 2-hydroxyoestriol after 2-hydroxylation (Gelbke & Knuppen, 1974).

No data on the mutagenicity of oestriol were available.

3.3 Case reports and epidemiological studies

See the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4. Summary of Data Reported and Evaluation[1]

4.1 Experimental data

Oestriol was tested by subcutaneous implantation in castrated mice and in rats and hamsters. It increased the incidence and accelerated the appearance of mammary tumours in both male and female mice and produced kidney tumours in hamsters.

Oestriol is embryolethal, especially for preimplantation embryos, in some species.

[1] This section should be read in conjunction with the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones'.

4.2 Human data

No case reports or epidemiological studies on oestriol alone were available to the Working Group. Case reports and epidemiological studies on steroid hormones used in oestrogen treatment have been summarized in the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4.3 Evaluation

There is *limited evidence* for the carcinogenicty of oestriol in experimental animals. Studies in humans strongly suggest that the administration of oestrogens is causally related to an increased incidence of endometrial carcinoma; there is no evidence that oestriol is different from other oestrogens in this respect.

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3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Mouse: Administration of 125 µg/L oestrone in the drinking-water resulted in the appearance of mammary tumours in 33/68 gonadectomized male C3H and C3He mice (MTV^-)[1]. With a concentration of 2000 µg/L, the incidence of these tumours increased to 119/169. No data were given on controls (Boot & Muhlbock, 1956).

In castrated male (C3H x RIII)F1 mice (MTV^+)[1] fed 0, 0.06, 0.6 or 6 µg oestrone per day in the diet, the incidences of mammary tumours were 12/33, 11/33, 15/30 and 33/34, respectively, with average latent periods of 540, 535, 475 and 190 days (Rudali *et al.*, 1978).

(b) Skin application

Mouse: Mammary tumours were observed in 5/5 male RIII mice (MTV^+) after skin application of oestrone as a 0.01% solution in chloroform twice weekly for 16 or more weeks. Female RIII mice had no mammary tumours after receiving the same treatment for more than six months, although the incidence in untreated females was 60–70%. Three pituitary tumours were observed among 12 male and female mice treated with oestrone. In a mixed strain of mice with a low incidence of mammary tumours, none were induced by oestrone treatment after 44 weeks (Cramer & Horning, 1936).

(c) Subcutaneous and/or intramuscular administration

Mouse: Lacassagne (1932) first reported the induction of mammary tumours in 3/3 male RIII mice (MTV^+) after weekly injections [not specified] of 0.6 mg oestrone benzoate for more than five months. Lacassagne (1939) also demonstrated the role of the maternal influence (i.e. transmission of the milk-borne MTV) by crossing RIII strain mice, which have a high incidence in untreated females, with strain 39 mice, which have no spontaneous mammary tumour incidence. Injection [not specified] of 50 µg oestrone benzoate weekly induced tumours of the mammary gland only in male hybrids whose mothers were of the high mammary tumour incidence strain (RIII). Males with a strain 39 mother and an RIII father had no mammary tumours with this treatment.

[1] MTV^-: mammary tumour virus not expressed; MTV^+: mammary tumour virus expressed (see p. 62)

Bonser (1936) found mammary tumours in 3/21 male A strain mice (MTV^+) but in none of 40 CBA males (MTV^-) after weekly subcutaneous injections of 30–50 μg oestrone benzoate in oil for 43 weeks or more. Shimkin and Grady (1940) induced mammary tumours in 2/10 male C3H mice (MTV^+) by the subcutaneous injection of 50 μg oestrone in oil weekly for 24 weeks. Similar treatment of female C3H mice reduced the average age at the appearance of mammary cancer from 46 weeks in untreated controls to 30 weeks in the treated animals. In both groups of females, the incidence of tumours was 100%.

Rat: Daily injection of 50–200 μg oestrone in oil (total dose, 30–40 mg) resulted in mammary cancers in 6/6 castrated male rats and in 4/5 ovariectomized females. A lower incidence was found in intact males (2/6) and in intact females (3/8). The average induction time ranged from 83 weeks with the lowest dose to 31 weeks with the highest (Geschickter & Byrnes, 1942). Mammary cancers were found in 1/2 male and 5/8 female rats given twice weekly subcutaneous injections of 50–100 μg oestrone benzoate in oil for 20 months. Pituitary tumours (140–271 mg in weight) were present in all rats (Chamorro, 1943).

Hamster: Malignant kidney tumours of different structures were found after eight months in about 60% of 86 castrated male golden hamsters injected with oestrone [dose not stated]. Pituitary adenomas also occurred in about 25% of the animals (Dontenwill, 1958).

(d) Subcutaneous implantation

Mouse: Pellets of oestrone (2 mg) were implanted subcutaneously in A and C3H mice (MTV^+) when the animals were 4–6 weeks of age. Mammary tumours arose in treated A males and in C3H and A females, but not in mice fostered by C57BL females nor in MTV^- strains (Bittner, 1941).

Mice of various hybrids between the A, C3H, C57 and JK strains, which were implanted with 1–7-mg pellets of oestrone, had an overall incidence of lymphoid tumours of 19/105, compared with 21/391 in corresponding control mice (Gardner & Dougherty, 1944).

Rat: Mammary tumours were observed in A×C rats (3/32 females, 4/30 males), Fischer rats (3/29 females, 2/29 males) and August rats (5/12 females, 9/25 males) implanted with a single pellet of 8–12 mg oestrone; average latent periods ranged from 50 to 97 weeks. Mammary cancer incidence was doubled in A×C rats by the implantation of two such pellets, and the latent period was reduced by about 50%. Adrenal cortical tumours were found in small numbers of rats with one pellet, but the incidence was greatly reduced with two pellets. No mammary tumours were seen in either sex of rats of the Copenhagen strain, but 6/10 males and 1/11 female had bladder cancer associated with bladder stones. The

amount of oestrone absorbed was calculated to be between 3.2 and 9.6 mg per rat (Dunning *et al*., 1953) [There was no control group. The occurrence of bladder cancer may have been related to the presence of stones].

Adrenal cortical tumours were found in 20% of female hooded rats implanted with pellets of oestrone [dose not stated]. The tumours frequently metastasized and were transplantable, but they regressed if the oestrone treatment was withdrawn. Adrenal tumours occurred in about 5% of untreated rats in that colony (Noble, 1967).

Cutts (1966) summarized extensive experiments in rats involving subcutaneous implantation of pellets of oestrone (average, 10 mg). The numbers of females of different strains with mammary tumours after 43–57 weeks of treatment were: Fischer, 12/74; Wistar, 12/50; Lewis, 17/44; Sprague-Dawley, 16/38; and hooded, 182/212. The estimated absorption of oestrone was 6–7 μg/day. There was no control group.

Black hooded rats (Nb strain) were given subcutaneously implanted pellets containing 90% oestrone and 10% cholesterol, weighing approximately 10 mg each. Pellets were implanted for 10–53 weeks or more in small groups of animals 3, 8, 12 or 38 weeks of age. An increased incidence of tumours was observed in male and female treated rats, as compared with 32 controls; the incidences of adrenal carcinomas, mammary carcinomas and pituitary tumours were increased. The incidence of mammary adenomas was increased in treated males and females up to one year but was lower than that in the controls thereafter. The duration of the experiment was not stated. Most of the tumours arising in the oestrogen-treated rats proved to be hormone-dependent upon transplantation into syngeneic hosts (Noble *et al*., 1975).

Testosterone propionate (TPP) and oestrone were administered subcutaneously to intact male Nb rats as 10 mg pellets containing 90% hormone and 10% cholesterol, either alone or in combination; two or three pellets were implanted and replaced every 6–8 weeks. Adenocarcinomas of the prostate developed in 5/30 and 11/55 TPP-treated rats that received 18 and 27 mg, respectively, as compared with 2/409 in untreated historical controls. Oestrone shortened the latent period of induction of these tumours; however, alone, it did not result in the development of carcinomas of the prostate (Noble, 1977).

Oestradiol-17β, oestriol and oestrone were administered subcutaneously to intact female Sprague-Dawley rats 48 h before treatment with 7,12-dimethylbenz[*a*]anthracene (DMBA) or procarbazine as 1–20% pellets weighing 3–7 mg each. DMBA and procarbazine were given by gavage at amounts of 20 and 50–70 mg, respectively. Mammary tumours were observed in the groups given DMBA, procarbazine or a combination of DMBA and oestrogen or procarbazine and oestrogen; no such tumours occurred in untreated controls or in oestrogen-treated female rats. Pellets containing 10% oestrone had an inhibitory effect on carcinogen-induced tumour development. The total length of the study was 370 days (Lemon, 1975).

Hamster: Implantation of 20 mg pellets of oestrone resulted in malignant kidney tumours [not specified] in 7/8 intact male Syrian hamsters and in 10/10 castrates. No kidney tumours were seen in 61 intact or in 60 castrated untreated males (Kirkman, 1959).

3.2 Other relevant biological data

No data on the toxicity of oestrone or oestrone benzoate were available.

Embryotoxicity and teratogenicity

When mice were injected subcutaneously with oestrone between days 11 and 16 of gestation at alternating doses of 0.1 and 0.2 mg, 12.4% of the offspring had cleft palates, compared with 1.1% in controls (Nishihara, 1958).

Injection of 1 μg oestrone to rats on day 3 of gestation had no embryotoxic effects (Dickmann, 1973).

Single doses of 0.02 mg/kg bw oestrone injected into rats during the early phase of gestation (time of tubal transport) terminated pregnancy. Injection of a single dose of 0.4 mg/kg bw oestrone on day 8–11 of gestation resulted in a marked reduction in the number of surviving fetuses and delayed delivery but had no effect on surviving fetuses (Dreisbach, 1959).

Single subcutaneous injections of 1–140 μg/rat were given at different times of gestation between days 1 and 10. Termination of pregnancy (100%) was achieved by administration of 20 μg on days 1 and 2, by 40 μg on day 3, by 80 μg on day 4 and by 50 μg on day 5. An injection of 140 μg oestrone did not terminate pregnancy when given between days 6 and 10 but induced a decreased number of implantations, an increased number of dead fetuses and abnormal growth and spacing of the fetuses (Haddad & Ketchel, 1969).

Subcutaneous injection of 0.002–0.05 mg/kg bw oestrone to rats on days 2–4 of gestation induced a dose-dependent reduction in fertility; significant effects were obtained with doses of 0.02 and 0.05 mg/kg bw (Saunders, 1965).

Subcutaneous injection of 0.0175 mg/kg per day oestrone into rats during days 1–7 of gestation terminated 50% of pregnancies (Saunders & Elton, 1967).

Subcutaneous administration of 0.5 μg–10 mg oestrone in combination with 1–4 mg progesterone to rats from day 3 after mating until term resulted in 100% resorptions (Cheng, 1959).

Metabolism

The 17β-hydroxy steroid dehydrogenase transforms oestrone to oestradiol reversibly. This enzyme occurred in all tissues of all species examined and is linked to either the cytosolic or microsomal subcellular compartment. In human liver, a NAD-linked 17β-hydroxy steroid 3-hydrogenase occurs in cytosol and in microsomes, and a further NADP-linked enzyme has been found in cytosol (Littmann *et al*., 1971). Hence, oestrone and oestradiol are largely biologically equivalent; they are also metabolized via the same pathways (see the monograph on oestradiol-17β, p. 307).

No data on the mutagenicity of oestrone or oestrone benzoate were available.

3.3 Case reports and epidemiological studies

See the section 'Oestrogens and Progestins in Relation to Human Cancer', p. 83.

4. Summary of Data Reported and Evaluation[1]

4.1 Experimental data

Oestrone was tested in mice by oral administration; in mice, rats and hamsters by subcutaneous injection and implantation; and in mice by skin painting. Its administration resulted in an increased incidence of mammary tumours in mice; in pituitary, adrenal and mammary tumours, as well as bladder tumours in association with stones, in rats; and in renal tumours in both castrated and intact male hamsters.

Oestrone benzoate increased the incidence of mammary tumours in mice following its subcutaneous injection.

Oestrone is embryolethal for preimplantation embryos in some species.

4.2 Human data

No case reports or epidemiological studies on oestrone alone were available to the Working Group. Case reports and epidemiological studies on steroid hormones used in oestrogen treatment have been summarized in the section 'Oestrogens and Progestins in Relation to Human Cancer'', p. 83.

[1] This section should be read in conjunction with the 'General Remarks on Sex Hormones' and with the 'General Conclusions on Sex Hormones', p. 131.

4.3 Evaluation

There is *sufficient evidence* for the carcinogenicity of oestrone in experimental animals. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard oestrone as if it presented a carcinogenic risk to humans. Studies in humans strongly suggest that the administration of oestrogens is causally related to an increased incidence of endometrial carcinoma; there is no evidence that oestrone is different from other oestrogens in this respect.

5. References

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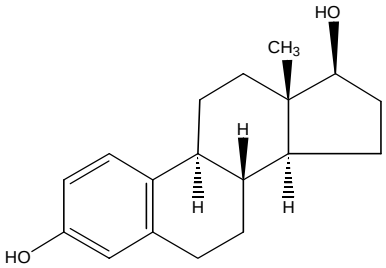
Appendix D: Report on Carcinogens (RoC), 9th Edition, Profile for Estrogens.

ESTROGENS (NOT CONJUGATED)

ESTRADIOL-17β
CAS No. 50-28-2

First Listed in the *Fourth Annual Report on Carcinogens*



CARCINOGENICITY

Estradiol-17β is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC V.6 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). When administered orally, the compound induced increased incidences of adenocarcinomas of the mammary gland, cervix, and uterus, adenoacanthoma of the uterus, and osteosarcoma of the cranium in female mice. Subcutaneous or intramuscular injection induced increased incidences of lymphosarcomas in mice of both sexes. Subcutaneous implants of estradiol-17β induced mammary tumors, including adenocarcinomas, papillary carcinomas, and anaplastic carcinomas in adult and newborn male and female mice and in female rats; pituitary chromophobe adenomas in male rats; fibromyomas of the uterus, mesentery, and abdomen in female guinea pigs; and malignant renal tumors in hamsters of both sexes (IARC V.6, 1974; IARC V.21, 1979).

There is inadequate evidence for the carcinogenicity of estradiol-17β in humans (IARC S.4, 1982). There is sufficient evidence for the carcinogenicity of steroidal estrogens in humans (IARC S.7, 1987). Studies of humans given estradiol-17β alone are not available to IARC Working Groups (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). However, studies strongly suggest that administration of estrogens is associated with an increased incidence of endometrial carcinoma in humans, and there is no evidence that estradiol-17β is different from other estrogens in this respect. An IARC Working Group concluded that in the absence of adequate data on humans, it is reasonable to regard estradiol-17β as if it presented a carcinogenic risk to humans (IARC V.21, 1979).

PROPERTIES

Estradiol-17β occurs as white or creamy-white prisms at room temperature. It is practically insoluble in water and soluble in ethanol, acetone, chloroform, diethyl ether, dioxane, and solutions of alkaline hydroxides. It is sparingly soluble in fixed oils. Estradiol-17β is unstable in light and air. The compound is available in the United States as a grade containing 97%-103% active ingredient on a dried basis. When heated to decomposition, it emits acrid smoke and fumes.

USE

Estradiol-17β is the most active naturally occurring estrogenic hormone. It is secreted by the ovaries in normal cycling adult females and by the placenta in pregnant females. It is essential for the growth and normal maintenance of the uterine lining, for the development of the accessory and secondary female sex characters, and for support of pregnancy (Prosser, 1973). It is used in human medicine for the treatment of symptoms of the climacteric, particularly for vasomotor and psychological disturbances (IARC V.21, 1979). It is also used for local treatment of atrophic vaginitis, for the chemotherapy of advanced prostatic carcinoma, and for the prevention of postpartum breast engorgement. Estradiol-17β is also used in the treatment of primary amenorrhea, delayed onset of puberty, and chemotherapy of breast neoplasms in postmenopausal women. It is believed to be a component of hormones derived from pregnant mares' urine used in cosmetic skin preparations. Estradiol-17β is used in veterinary medicine for estrogenic hormone therapy, as well as in food-producing animals as a growth promoter (IARC V.21, 1979).

PRODUCTION

Estradiol-17β is a naturally occurring steroid hormone produced endogenously by all mammalian species. The production rate in humans ranges between 6 µg/24 hr in prepubescent boys and 945 µg/24 hr in normal adult cycling females. The 1998 Chemical Buyers Directory lists two U.S. suppliers of estradiol, and Chemcyclopedia 98 names three suppliers (Tilton, 1997; Rodnan, 1997). In 1983, U.S. imports of estradiol-17β totaled 44 lb (USITCa, 1984). U.S. firms also imported 156 lb of the 3-benzoate form in 1983, compared to 379 lb in 1976 and 6 lb in 1975 (IARC V.21, 1979). Commercial production of estradiol-17β in the United States was first reported in 1939 by the U.S. Tariff Commission (IARC S.4, 1982).

EXPOSURE

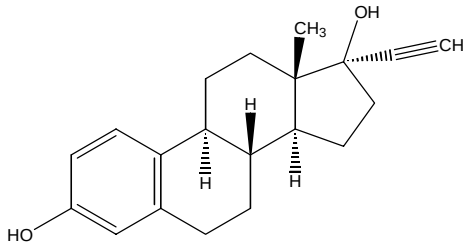
The primary routes of potential human exposure to estradiol-17β are ingestion, injection, inhalation, and dermal contact. Humans are potentially exposed to exogenous amounts of estradiol-17β through the consumption of meat from treated livestock. However, this is an insignificant amount (2.4 ng/157 g of meat) when compared to normal human production of the chemical. FDA reported that estradiol-17β also is ingested in minute levels through the consumption of milk from untreated dairy cows (about 18 ng in one pint of milk). It has also been found in certain drinking water samples at levels of 0.12-0.42 ng/L. When used as a medication, estradiol-17β is given in doses of up to 1.5 mg two or three times weekly by intramuscular injection, or daily by mouth. Currently, other estrogenic hormones are preferred for oral administration (IARC V.21, 1979). There is some potential for occupational exposure to estradiol-17β through dermal contact and inhalation, for workers involved in the formulation, manufacture, packaging, and administration of pharmaceuticals containing it. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 2,770 workers were potentially exposed to estradiol-17β in the workplace in 1970 (NIOSH, 1976).

REGULATIONS

Because estradiol-17β is used as a pharmaceutical and in low quantities relative to other chemicals, it is not regulated by EPA. However, there may be a small pollution problem relative to hospital wastes. FDA regulates estradiol-17β esters for use as implants in cattle, lambs, and chickens. Estradiol-17β is regulated as a prescription drug for human use under the Food, Drug, and Cosmetic Act (FD&CA). FDA has ruled that estrogens for general use must carry patient and physician warning labels concerning use, risks, and contraindications. OSHA also regulates estradiol-17β under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations are summarized in Volume II, Table B-130.

ESTROGENS (NOT CONJUGATED)

ETHINYLESTRADIOL
CAS No. 57-63-6
First Listed in the *Fourth Annual Report on Carcinogens*



CARCINOGENICITY

Ethinylestradiol is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). When administered in the diet, ethinylestradiol increased the incidence of pituitary tumors and malignant mammary tumors in mice of both sexes; malignant tumors of the uterus and cervix in female mice; and benign gonadal tumors in male mice. Oral administration of ethinylestradiol to rats increased the incidence of liver neoplastic nodules and pituitary chromophobe adenomas in both sexes, mammary tumors in males, and malignant liver tumors in females (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). When implanted as a pellet, ethinylestradiol induced mammary adenocarcinomas in 90% of rats given 1 mg; concomitant exposure to X-rays synergistically increased the number of tumors per rat and shortened the latency period of the tumors (IARC S.4, 1982; IARC S.7, 1987).

In other studies, ethinylestradiol administered orally in combination with certain progestins induced increased incidences of malignant tumors of the uterus, pituitary tumors, and hepatomas in female mice, and benign and/or malignant mammary tumors in male rats (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). Subcutaneous injection of an ethinylestradiol mixture induced mammary fibroadenomas in female rats (IARC V.21, 1979).

There is inadequate evidence for the carcinogenicity of ethinylestradiol in humans (IARC S.4, 1982). There is sufficient evidence for the carcinogenicity of steroidal estrogens in humans. Case reports and epidemiological studies of humans given ethinylestradiol alone were not available to IARC Working Groups (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). However, the use of oral contraceptives containing ethinylestradiol in combination with progestins is associated with an increased incidence of benign liver adenomas and a decreased incidence of benign breast disease, endometrial cancer, and ovarian cancer. Epidemiologic studies also suggest that the administration of estrogens alone is strongly associated with an increased incidence of endometrial carcinoma in humans, and there is no evidence that ethinylestradiol is different from other estrogens in this respect. An IARC Working Group concluded that in the absence of adequate data on humans, it is reasonable to regard ethinylestradiol as if it presented a carcinogenic risk to humans (IARC V.21, 1979).

PROPERTIES

Ethinylestradiol occurs as fine white needles. It is practically insoluble in water, and soluble in ethanol, diethyl ether, acetone, dioxane, chloroform, vegetable oils, and solutions of fixed alkaline hydroxides. Ethinylestradiol is available in the United States as a grade containing 97%-102% active ingredient on a dried basis.

USE

The most widespread use of ethinylestradiol is in oral contraceptives. Ethinylestradiol is one of the most active steroidal estrogens known when administered orally (IARC V.21, 1979). It not only is used as the estrogen component in progestin-estrogen combination therapy and progestin-estrogen sequential therapy but also is used in estrogen treatment alone (IARC V.6, 1974). Additionally, ethinylestradiol is used in human medicine to treat conditions such as amenorrhea, breast carcinoma, hypogonadism, menopausal disorders, postpartum breast engorgement, and prostatic carcinoma; in such applications, it sometimes is used in combination with androgens or progestins (IARC V.6, 1974).

Ethinylestradiol is not used as a growth promoter in animals. It is used in veterinary medicine for estrogenic hormone therapy (IARC V.6, 1974).

PRODUCTION

The USITC does not identify any producers for ethinylestradiol. The 1998 Chemical Buyers Directory, however, lists three U.S. suppliers of the compound (Tilton, 1997). The 1984 Chem Sources Directory identified two domestic companies as manufacturers (Chem Sources, 1984). In 1983, U.S. imports of ethinylestradiol totaled 82 lb (USITCa, 1984). The 1979 TSCA Inventory reported one U.S. importer of ethinylestradiol in 1977, but no volume of imports (TSCA, 1979). Total U.S. sales of ethinylestradiol for use in human medicine in the mid-1970s were estimated to be less than 110 lb annually (IARC V.6, 1974). Commercial production of the compound in the United States was first reported in 1945 (IARC V.21, 1979).

EXPOSURE

The primary routes of potential human exposure to ethinylestradiol are ingestion, inhalation, and dermal contact. In 1977, estimates indicated that more than 80 million women were exposed to ethinylestradiol through the regular use of oral contraceptives. In 1972 estimates indicated that only 41 to 48 million women were exposed similarly to the compound (IARC V.21, 1979). Potential occupational exposure to ethinylestradiol may occur through inhalation and dermal contact. A joint investigation of an oral contraceptive plant, conducted by NIOSH and CDC, found evidence of hyperestrogenism among male and female workers. Blood tests showed 60% higher elevations of estrogens among employees who handled the powdered product; air samples of estrogen and progesterone varied widely (Drug Cosmet. Indust., 1977). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 2,770 people were potentially exposed to ethinylestradiol in the workplace in 1970, and 1,230 workers were potentially exposed in 1974. These estimates were based only on observations of the actual use of the compound and tradename products containing the compound (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) estimated a total of 97 workers, including 62 women, potentially occupationally exposed to ethinylestradiol

(NIOSH, 1984). Another source of potential human exposure is the residue of ethinylestradiol found in foliage, soil, water samples, and some drinking water (IARC, V.21, 1979).

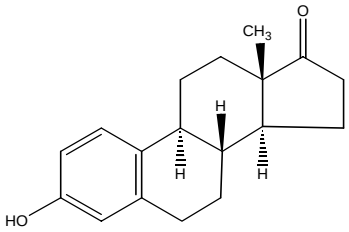
REGULATIONS

Because ethinylestradiol is used as a pharmaceutical and in low quantities relative to other chemicals, it is not regulated by EPA. However, there may be a small pollution problem relative to hospital wastes. FDA regulates ethinylestradiol under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug approved for human use. FDA has ruled that estrogens for general use must carry patient and physician warning labels concerning use, risks, and contraindications (has been extended to all oral contraceptives). OSHA regulates ethinylestradiol under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations are summarized in Volume II, Table B-132.

ESTROGENS (NOT CONJUGATED)

ESTRONE
CAS No. 53-16-7

First Listed in the *Fourth Annual Report on Carcinogens*



CARCINOGENICITY

Estrone is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). When administered orally, topically, subcutaneously, or by implantation, estrone induced an increased incidence of mammary tumors in mice. In rats, subcutaneous injection or implantation of estrone induced pituitary, adrenal, and mammary tumors, as well as bladder tumors in association with bladder stones. When administered subcutaneously, estrone caused kidney tumors in both castrated and intact male hamsters, and pituitary adenomas in castrated male hamsters.

There is inadequate evidence for the carcinogenicity of estrone in humans (IARC V.6, 1974). There is sufficient evidence for the carcinogenicity of steroidal estrogens in humans. Studies of humans given estrone alone were not available to IARC Working Groups (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). However, studies strongly suggest that administration of estrogens is associated with an increased incidence of endometrial carcinoma in humans, and there is no evidence that estrone is different from other estrogens in this respect. An IARC Working Group concluded that in the absence of adequate data for humans, it is reasonable to regard estrone as if it presented a carcinogenic risk to humans (IARC V.21, 1979).

PROPERTIES

Estrone is an odorless white crystalline solid. It is insoluble in water; slightly soluble in absolute ethanol, ether, and vegetable oils; and soluble in acetone, fixed oils, dioxane, pyridine, fixed alkaline hydroxide solutions, and chloroform. Estrone is available in the United States as a grade containing 97%-103% active ingredient. When heated to decomposition, it emits acrid smoke and fumes.

USE

Estrone is a metabolite of the most potent naturally occurring estrogen, estradiol-17β (IARC V.21, 1979). It is secreted by the ovaries in normal adult cycling females and by the placenta in pregnant females. It is essential for the growth and normal maintenance of the uterine lining, for the development of the accessory and secondary female sex characters, and for support of pregnancy (Prosser, 1973). Estrone, in its various forms, is used in human medicine to treat conditions such as amenorrhea, breast carcinoma, hypogenitalism, menopausal syndrome, postmenopausal osteoporosis, postpartum breast engorgement, prostatic carcinoma, and senile vaginitis. In such applications, it is frequently combined with other hormones or medicinals such as barbiturates and tranquilizers (IARC V.6, 1974). Additionally, estrone has been used in hormonal skin preparations for cosmetic use at levels of < 0.1% (IARC V.21, 1979). Therapeutically, it can serve as an oral contraceptive in combination with progestins, prevent threatened or habitual abortion, and treat dwarfism and acne at the early pubescent stage (HSDB, 1998).

PRODUCTION

Current production and import and export volumes were not available. Chemcyclopedia 98 lists two U.S. suppliers of estrone, and the 1998 Chemical Buyers Directory names three suppliers of estrone and salts or esters (Rodnan, 1997; Tilton, 1997). Currently, the USITC does not identify manufacturers for individual estrogens (USITC, 1988-1991, 1993-1995). It did identify one company that produced an unspecified amount of estrone from 1983 through 1985 (USITC, 1984-1986). The 1984 Chem Sources USA directory listed two other companies as manufacturers (Chem Sources, 1984). In 1983, U.S. imports of estrone totaled 55 lb (USITCa, 1984). The 1979 TSCA Inventory reported that a single company imported 500 lb of estrone in 1977 (TSCA, 1979). Commercial production of estrone in the United States was first reported in 1941 by the U.S. Tariff Commission (IARC V.21, 1979).

EXPOSURE

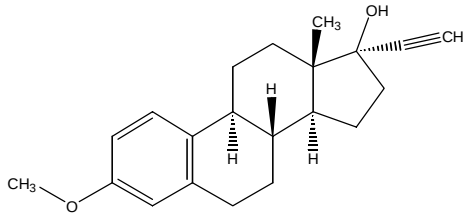
The primary routes of potential exogenous human exposure to estrone are injection of pharmaceuticals containing the compound, dermal contact, and inhalation. Injection dosages range from 0.1 mg/week up to 5 mg/day, depending on symptoms. For treatment of atrophic vaginitis, estrone may be administered by vaginal suppository (IARC V.6, 1974; IARC V.21, 1979). Estrone has also been used in hormonal skin preparations for cosmetic use at concentrations of < 0.1%. Unspecified estrogen and estrogenic hormones, which are believed to consist mainly of estrone, have been used in hormonal skin preparations (< 0.1%-5%), moisturizing lotions (1%-5%), wrinkle-smoothing creams, hair conditioners, hair straighteners, shampoos, and grooming aid tonics (< 0.1%) (IARC V.21, 1979). Potential occupational exposure may occur through inhalation or dermal contact during the production, formulation, packaging, or administration of estrone. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 2,770 workers were potentially exposed to estrone in the workplace in 1970 (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) estimated that 4,444 total workers, including 3,848 women, potentially were exposed to estrone (NIOSH, 1984). Estrone is found in the urine of pregnant women, mares, bulls, and stallions; in the follicular liquor of many animals; in human placentas; and in palm kernel oil. It has also been found in plant material, such as the roots of moghat and in the pollen grains of the date palm (IARC V.21, 1979).

REGULATIONS

Because this chemical is used as a pharmaceutical and in low quantities relative to other chemicals, estrone is not regulated by EPA. There may be a small pollution problem relative to hospital wastes. FDA regulates estrone under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug approved for human use. FDA has ruled that estrogens for general use must carry patient and physician warning labels concerning use, risks, and contraindications. OSHA regulates estrone under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations are summarized in Volume II, Table B-131.

ESTROGENS (NOT CONJUGATED)

MESTRANOL
CAS No. 72-33-3
First Listed in the *Fifth Annual Report on Carcinogens*



CARCINOGENICITY

Mestranol is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982; IARC S.7, 1987). When administered alone orally to mice, mestranol increased the incidences of pituitary and malignant mammary tumors. Mestranol also induced an increased incidence of malignant mammary tumors in female rats when administered orally (IARC V.6, 1974).

There are a number of studies involving the oral administration of mestranol in combination with progestins. In these studies, mice developed pituitary tumors, vaginal and cervical squamous cell carcinomas, and mammary tumors. Rats with similar mixed exposure developed benign liver tumors and malignant mammary tumors. Dogs developed mammary cancers after mixed exposure to progestins (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). Subcutaneous injection of a combination of mestranol and progestins induced cervical cancers and pituitary tumors in mice (IARC S.4, 1982).

There is inadequate evidence for the carcinogenicity of mestranol in humans (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). There is sufficient evidence for the carcinogenicity of steroidal estrogens in humans (IARC S.7, 1987). Case reports and epidemiological studies of humans given mestranol alone were not available. However, the use of oral contraceptives containing mestranol in combination with progestins is associated with an increased incidence of benign liver adenomas and a decreased incidence of benign breast disease, endometrial cancer, and ovarian cancer. Epidemiologic studies also strongly suggest that the administration of estrogens alone is associated with an increased incidence of endometrial carcinoma, and there is no evidence that mestranol is different from other estrogens in this respect. An IARC Working Group concluded that in the absence of adequate data in humans, it is reasonable to regard mestranol as if it presents a carcinogenic risk to humans (IARC V.21, 1979).

PROPERTIES

Mestranol is a white crystalline solid. It is practically insoluble in water; slightly soluble in methanol; and soluble in ethanol, acetone, diethyl ether, chloroform, and dioxane. Mestranol is available in the United States as a USP-grade containing 97-102% mestranol on a dried basis.

USE

The most widespread use of mestranol is in oral contraceptives where it is used as the estrogen in combination therapy, sequential therapy, or the estrogen tablet alone (IARC V.6, 1974). It also is used in combination with a progestin to treat such conditions as endometriosis and amenorrhea (IARC V.21, 1979). Mestranol is not known to be used in veterinary medicine (IARC V.6, 1974).

PRODUCTION

The USITC and the 1979 TSCA Inventory do not identify any producers or production volumes for mestranol. The 1998 Chemical Buyers Directory names one supplier of the compound (Tilton, 1997). The 1984 Chem Sources USA directory listed one producer and seven suppliers of mestranol (Chem Sources, 1984). In 1983, imports of mestranol totaled 22 lb (USITCa, 1984). IARC reported in 1979 that no commercial production of mestranol existed in the United States (IARC V.21, 1979). In 1974, total U.S. sales of mestranol for use in human medicine were estimated to be < 220 lb annually (IARC V.6, 1974).

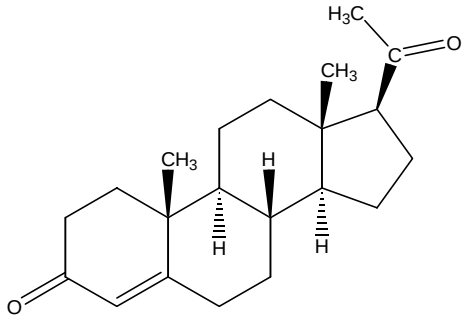
EXPOSURE

The primary routes of potential human exposure to mestranol are ingestion, dermal contact, and inhalation. Potential consumer exposure may occur through ingestion of pharmaceuticals containing mestranol. Up to 1% mestranol has been detected in norethynodrel (as normally manufactured). Potential occupational exposure to mestranol may occur through inhalation and dermal contact. In a study carried out in a plant producing oral contraceptives, mestranol was found in various sectors of the working environment at levels ranging from 0.06 to 8.61 $\mu g/m^3$, and on wipe samples at levels of 0.003 to 2.05 $\mu g/cm^2$ (IARC V.21, 1979). A joint investigation of an oral contraceptive plant, conducted by NIOSH and CDC, found evidence of hyperestrogenism among male and female workers. Blood tests showed 60% higher elevations of estrogens among employees who handled the powdered product; air samples of estrogen and progesterone varied widely (Drug Cosmet. Ind., 1977). Another source of potential human exposure to mestranol is the residue in foliage, soil, and water samples.

REGULATIONS

EPA has proposed regulating mestranol as a hazardous constituent of waste under the Resource Conservation and Recovery Act (RCRA). FDA regulates mestranol under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug approved for human use. FDA has ruled that estrogens for general use must carry patient and physician warning labels concerning use, risks, and contraindications. This ruling on warning labels has been extended to all oral contraceptives. OSHA regulates mestranol under the Hazard Communication Standard and as a chemical hazard in laboratories. Regulations are summarized in Volume II, Table B-133.

PROGESTERONE
CAS No. 57-83-0
First Listed in the *Fourth Annual Report on Carcinogens*



CARCINOGENICITY

Progesterone is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC S.4, 1982). When progesterone was implanted subcutaneously, mammary carcinomas were induced at a significantly earlier age and at a higher incidence in female mice. Long-term subcutaneous implants induced ovarian granulosa cell tumors or endometrial stromal sarcomas in female mice (IARC V.6, 1974; IARC V.21, 1979). Subcutaneous injections of progesterone induced increased incidences of mammary tumors in adult female mice and lesions of the vaginal or cervical epithelia and genital tract lesions in newborn female mice. Hyperplastic alveolar-like nodules and other dysplasias were also induced in female neonatal mice (IARC V.21, 1979). Long-term subcutaneous injections in female dogs induced endometrial hyperplasia, inhibition of ovarian development, marked mammary hyperplasia, and some fibroadenomatous nodules of the mammary gland (IARC V.21, 1979; IARC S.4, 1982).

Female mice injected subcutaneously with progesterone showed decreased latent periods for the induction of mammary tumors by 3-methylcholanthrene. Ovariectomized female mice receiving injections of progesterone developed sarcomas of the uterine horn when given an intrauterine implant of 3-methylcholanthrene and developed increased incidences of squamous cell carcinomas of the cervix or vagina when treated intravaginally with 7,12-dimethylbenz[*a*]anthracene (IARC V.6, 1974; IARC V.21, 1979). Local applications of 3-methylcholanthrene and subcutaneous implantations of progesterone induced increased incidences of vaginal-cervical invasive squamous cell carcinomas in female mice (IARC V.21, 1979). Rats receiving subcutaneous or intramuscular injections of progesterone had decreased latent periods and/or increased incidences of mammary tumors induced by oral administration of 3-methylcholanthrene or 7,12-dimethylbenz[*a*]anthracene, but only when the known carcinogens were administered first. An increased incidence of mammary tumors was induced in female rats fed 2-acetylaminofluorene in the diet and injected intramuscularly with progesterone. Newborn female rats receiving a subcutaneous injection of progesterone and a subsequent intragastric instillation of 7,12-dimethylbenz[*a*]anthracene developed increased incidences of mammary adenocarcinomas (IARC V.21, 1979).

There are no data available to evaluate the carcinogenicity of progesterone in humans (IARC S.4, 1982; IARC V.21, 1979; IARC V.6, 1974).

PROPERTIES

Progesterone is a crystalline solid at room temperature. It occurs in two forms that are readily interconvertible: white orthorhombic prisms and white orthorhombic needles. It is practically insoluble in water; sparingly soluble in vegetable oils; and soluble in ethanol, arachis oil, chloroform, diethyl ether, ethyl oleate, light petroleum, acetone, dioxane, and concentrated sulfuric acid. It is commercially available as a grade containing 98%-102% active ingredient on a dried basis, with $\leq$ 3% foreign steroids and other impurities. It is sensitive to light.

USE

Progesterone is a naturally occurring steroidal hormone found in a wide variety of tissues and biological fluids. It is secreted by the ovary in normal adult cycling females, by the placenta in pregnant females, and by the adrenal cortex. It is essential for the normal functioning of the uterine lining, for the development of mammary glands, and support of pregnancy through parturition (Prosser, 1973). Progesterone is used in medicine to treat secondary amenorrhea and dysfunctional uterine bleeding. It has also been used to treat female hypogonadism, dysmenorrhea and premenstrual tension, habitual and threatened abortion, preeclampsia and toxemia of pregnancy, mastodynia, uterine fibroma, and neoplasms of the breast and endometrium. Progesterone embedded in an intrauterine device is used for contraception. In veterinary medicine, progesterone is used to control habitual abortion and to delay estrus and ovulation in cattle, swine, and dogs (IARC V.21, 1979).

PRODUCTION

Progesterone is a naturally occurring steroid hormone produced endogenously by all mammalian species. The production rate in humans ranges from 0.15 mg/24 hr in prepubertal boys to 19.58 mg/24 hr in normal adult cycling females (Tagatz & Gurpide, 1973). The USITC identified one producer of progesterone for 1988, but no production data were reported (USITC, 1989). Chem Sources International identified two domestic suppliers of progesterone for 1988 and 1989 (Chem Sources, International, 1988). In 1986, one U.S. company produced an undisclosed volume of progesterone (USITC, 1987). The 1979 TSCA Inventory identified one importer of progesterone in 1977, but data on the amount of U.S. imports and exports of progesterone were not available (TSCA, 1979). In 1975, U.S. production of 13 estrogen and progestin substances, including progesterone, amounted to 23,100 lb. Before U.S. governmental restrictions in 1973, total U.S. sales of progesterone for use in human medicine were estimated to have been < 110 lb annually (IARC V.6, 1974).

EXPOSURE

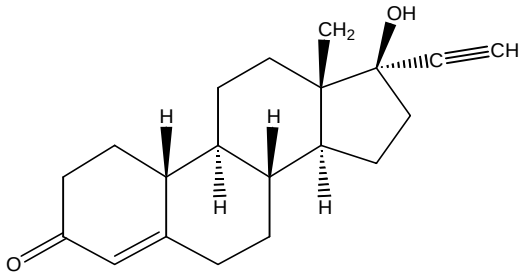
The primary routes of potential exogenous human exposure to progesterone are ingestion, injection of medications containing the compound, implantation, dermal contact, and inhalation. Injection dosages range from 2 to 50 mg, either in single or multiple administrations. Progesterone embedded in an intrauterine contraceptive device is a potential route of exposure to a limited population. Human placental extracts, of which progesterone is believed to be the main constituent, have been used in preparations for cosmetic use (at levels of 0.1%-1.0%), hair conditioners, shampoos, and grooming aid tonics (< 0.1%) (IARC V.21, 1979). Potential consumer exposure through dermal contact could occur from use of these cosmetics. FDA reported that progesterone has been detected in cow's milk at concentrations of 1-30 ng/ml and in milk products at up to 300 μg/kg (in butter). It has also been found to occur naturally in certain

plant species (IARC V.21, 1979). Animal meat may contain an average of 0.33 mg progesterone/kg if the animal was treated with a progesterone implant. Consumers could potentially be exposed to progesterone by ingesting these food products. Potential occupational exposure to progesterone may occur through inhalation and dermal contact during its production or formulation into pharmaceuticals. A joint investigation of an oral contraceptive plant, conducted by NIOSH and CDC, found evidence of hyperestrogenism in both male and female workers and wide variations in air sample concentrations of estrogen and progesterone (Drug Cosmet. Ind., 1977). The National Occupational Exposure Survey (1981-1983) indicated that 287 workers, including 54 women, potentially were exposed to progesterone (NIOSH, 1984). This estimate was derived from observations of the actual use of the compound. The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 22,963 workers were potentially exposed to progesterone in the workplace in 1970 (NIOSH, 1976).

REGULATIONS

Progesterone is not regulated by EPA because it is used as a pharmaceutical and in low quantities relative to other chemicals. However, there may be a small pollution problem relative to hospital wastes. FDA regulates progesterone under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug approved for human use. FDA has ruled that progesterone must carry a warning label for patients and physicians concerning use, risks, and contraindications. FDA also requires that no residues of progesterone be found in the uncooked edible tissues of lamb and steer. OSHA regulates progesterone as a chemical hazard in laboratories and under the Hazard Communication Standard. Regulations are summarized in Volume II, Table B-145.

NORETHISTERONE
CAS No. 68-22-4
First Listed in the *Fourth Annual Report on Carcinogens*



CARCINOGENICITY

Norethisterone is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in experimental animals (IARC V.6, 1974; IARC V.21, 1979; IARC S.4, 1982). When administered in the diet, norethisterone increased the incidences of benign liver cell tumors in male mice and male rats and pituitary tumors in female mice and induced benign and malignant mammary tumors in male rats. When administered subcutaneously, the compound induced granulosa cell tumors in ovaries of mice.

There are no data available to evaluate the carcinogenicity of norethisterone in humans (IARC S.4, 1982).

PROPERTIES

Norethisterone occurs as a white, odorless, crystalline powder with a slightly bitter taste. It is practically insoluble in water and nonvolatile oils, slightly soluble in diethyl ether, and soluble in ethanol, acetone, chloroform, dioxane, and pyridine. It is unstable in the presence of air and light. When heated to decomposition, it emits acrid smoke and fumes. Norethisterone is available in the United States as a grade containing 97%-102% active ingredient on an anhydrous basis.

USE

Norethisterone, an orally active progestin, has been used in small amounts in human medicine since 1957 to treat conditions such as amenorrhea, dysfunctional uterine bleeding, endometriosis, premenstrual tension, and dysmenorrhea. Since 1962, the most common use in the United States has been as the progestin in progestin-estrogen combination oral contraceptives. Norethisterone has been used in the treatment of inoperable malignant neoplasms of the breast or as an adjunct to surgery or radiotherapy (IARC V.21, 1979). Norethisterone is also used as an intermediate in the commercial synthesis of norethisterone acetate and possibly in the synthesis of ethynodiol diacetate (IARC V.6, 1974).

PRODUCTION

Chem Sources International indicated that one domestic firm supplies norethisterone (Chem Sources International, 1988). Norethisterone is not produced in the United States. Data on imports were not available. Total U.S. sales for human medicine containing norethisterone have been estimated to have been < 4,400 lb/year prior to 1972 (IARC V.6, 1974).

EXPOSURE

The primary routes of potential human exposure to norethisterone are ingestion, dermal contact, and inhalation. When used as an oral contraceptive, it is usually given in a dose of 0.5-2.0 mg daily in combination with mestranol or ethinylestradiol. It is also used continuously at a daily dose of 0.35 mg in the so-called contraceptive "mini-pill." In its other medicinal uses, norethisterone is given in daily doses ranging from 10 to 30 mg (IARC V.21, 1979). Potential occupational exposure may occur through inhalation or dermal contact for workers involved in the manufacture, formulation, packaging, or administration of norethisterone. In a study carried out in a factory producing oral contraceptives, norethisterone was found in various sectors of the working environment at concentrations ranging from 0.30 to 59.56 $\mu g/m^3$ and in wipe samples from 0.019 to 14.7 $\mu g/cm^3$ (IARC V.21, 1979).

REGULATIONS

Because this chemical is used as a pharmaceutical and in low quantities relative to other chemicals, it is not regulated by EPA. However, there may be a small pollution problem relative to hospital wastes. FDA regulates norethisterone under the Food, Drug, and Cosmetic Act (FD&CA) as a prescription drug approved for human use. FDA has ruled that oral contraceptives for general use must carry patient and physician warning labels concerning use, risks, and contraindications. OSHA regulates norethisterone under the Hazard Communication Standard and as a chemical hazard in laboratories Regulations are summarized in Volume II, Table B-137.