POST-MENOPAUSAL OESTROGEN THERAPY

1. Exposure

'Post-menopausal oestrogen therapy' refers to the use of oestrogen without progestogen for women in the period around the menopause, primarily for the treatment of menopausal symptoms but increasingly for the prevention of conditions that become more common in the post-menopausal period, such as osteoporosis and ischaemic heart disease. Currently, it is mainly given to women who have had a hysterectomy, as treatment with oestrogen alone in women with a uterus increases the risk for endometrial cancer. In the past, women with a uterus were often prescribed post-menopausal oestrogen therapy, although predominantly in the United States. Post-menopausal therapy with combined oestrogen and progestogen is discussed in another monograph in this volume.

Post-menopausal oestrogen therapy can be administered orally, transdermally (by patch or gel), by injection or by implant. Local, topical preparations are also available for relief of urogenital symptoms. Annex 2 (Table 3) gives a list of common brands of post-menopausal oestrogen therapy, with their constituents and doses and examples of countries in which they are available. Post-menopausal oestrogen therapy can also be administered in combination with an androgen or with an anxiolytic, and examples of such brands and formulations are given in Annex 2 (Table 4).

1.1 Historical overview

Whether menopause is natural or induced surgically, women in this condition have long been known to suffer from problems such as hot flushes (or 'flashes') and urogenital atrophy and to have increased rates of fracture and cardiovascular disease, in comparison with pre-menopausal women. These problems are particularly severe in women who have a premature menopause. In 1895, Marie Bra suggested that ovarian secretions could be used to treat ovarian failure (Bush & Barrett-Connor, 1985), and the first therapeutic investigations of the administration of ovarian tissue for the relief of climacteric symptoms were reported in 1896. Subsequently, researchers and clinicians investigated the use of various ovarian, placental and urine extracts, implantation of ovarian tissue and oral administration of dried ovarian tissue (Kopera & van Keep, 1991).

The identification of the ovarian hormones allowed a more specific understanding of the factors that might be responsible for climacteric symptoms. Oestrone, oestriol and progesterone were identified in 1929, and oestradiol was identified in 1936 (IARC, 1979). The first synthetic oestrogens, diethylstilboestrol and ethinyloestradiol, were isolated in 1938 (Bush & Barrett-Connor, 1985).

Clinical use of oestrogen for women with premature surgical or natural menopause began in the 1930s (Stadel & Weiss, 1975; Kopera & van Keep, 1991). Campbell and Collip (1930) demonstrated the clinical efficacy of extracts of human placenta in relieving menopausal symptoms and deviations from the normal menstrual cycle, like dysmenorrhoea. Although a product containing these extracts was introduced onto the market, it was impractical to produce on a large scale (Stern, 1982); most oestrogen was therefore administered by injection or subcutaneous implant. The earliest use of an implant was reported by Bishop (1938), who administered oestrogen to women after oophorectomy.

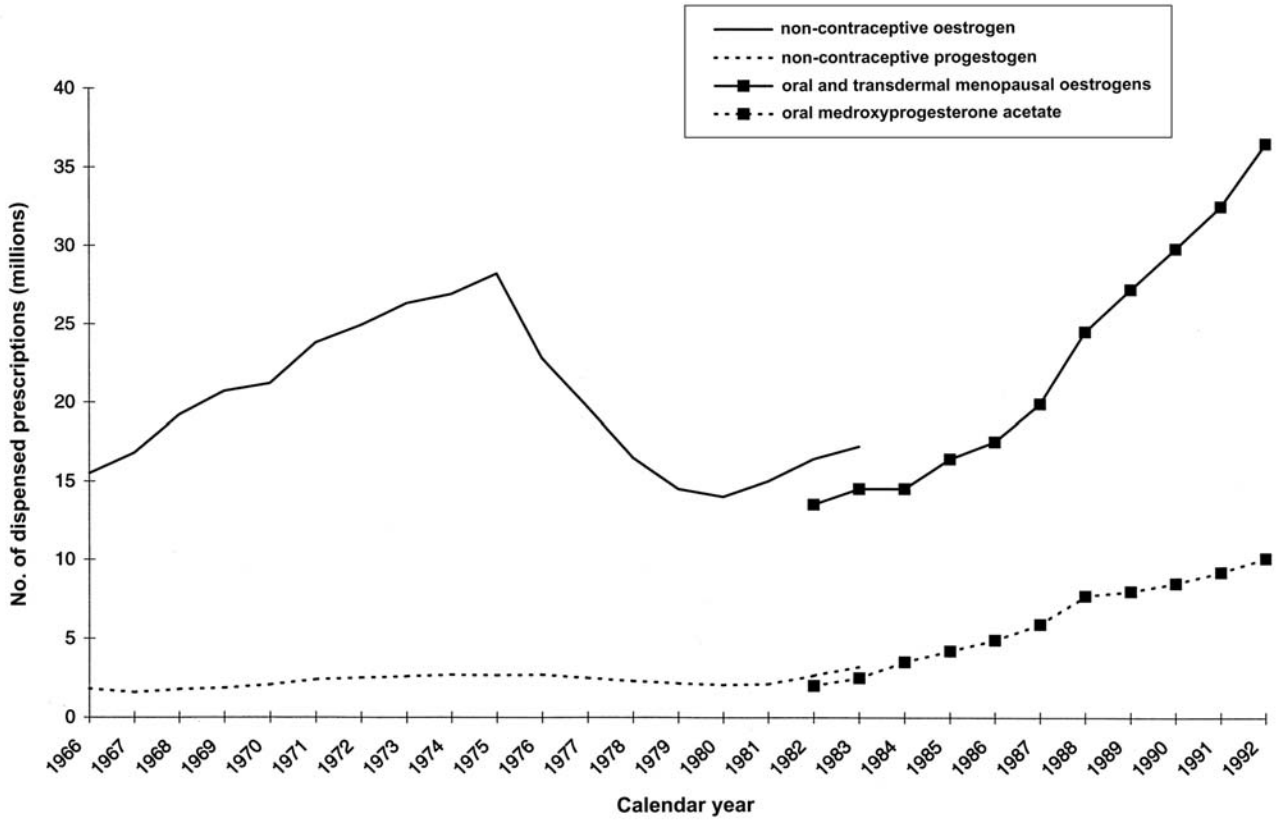
Clinical trials of conjugated equine oestrogens from the urine of pregnant mares were initiated in 1941 (Stern, 1982). In 1943, these preparations became available in the United States for use as oral post-menopausal oestrogen therapy and were introduced onto the market in the United Kingdom in 1956 (Godfree, 1994). Over the following years, post-menopausal oestrogen therapy began to be used less for women who had had a premature menopause than for women who had had menopause at a normal age, although women who had undergone a hysterectomy or an oophorectomy have been consistently more likely to receive post-menopausal oestrogen therapy than women who have had a natural menopause (Brett & Madans, 1997). The indications for use also widened, from short-term treatment for menopausal symptoms to longer-term treatment for the prevention of osteoporosis and cardiovascular disease; some clinicians advocated near-universal prescription for women after the menopause (Schleyer-Saunders, 1973).

Use of post-menopausal oestrogen therapy became widespread in the United States in the 1960s: the number of women using it was estimated to have increased by 240% between 1962 and 1967 (Bush & Barrett-Connor, 1985), such that approximately 13% of women in the United States aged 45–64 used post-menopausal oestrogen therapy (Stadel & Weiss, 1975). Figure 1 gives the estimated numbers of prescriptions for non-contraceptive oestrogens and progestogens from the National Prescription Audit in the United States (Kennedy *et al*., 1985; Wysowski *et al*., 1995) between 1966 and 1992. The dose of oral oestrogen prescribed decreased over the period 1975–83, as did the use of injectable post-menopausal oestrogen therapy (Kennedy *et al*., 1985). By 1992, transdermal oestradiol accounted for 15% of post-menopausal oestrogen therapy prescriptions in the United States (Jewelewicz, 1997).

In the United Kingdom, around 2% of English women aged 40–64 were using post-menopausal hormonal therapy during the period 1980–87, the prevalence rising rapidly to reach 22% by 1994. The majority of these prescriptions were for combined oestrogen–progestogen therapy (Townsend, 1998).

The fall in the number of prescriptions of oestrogen in the United States corresponded to scientific reports and growing public awareness of the elevated risks for endometrial cancer of women using post-menopausal oestrogen therapy who had not had a hysterectomy (Smith *et al*., 1975; Ziel & Finkle, 1975). Thereafter, the prescription rates for such therapy in the United States began to rise but more frequently in combination with a progestogen (Hemminki *et al*., 1988; see the monograph on 'Post-menopausal oestrogen–progestogen therapy'). Administration of unopposed therapy to women with a uterus

Figure 1. Estimated numbers of dispensed prescriptions (in millions) of non-contraceptive oestrogens and progestogens in the United States, 1966–92



Adapted from Kennedy *et al.* (1985) and Wysowski *et al.* (1995)
The estimates for 1966–83 are for prescribed oestrogens and progestogens other than those that are part of an oral contraceptive, and the estimates for 1982–92 are for the more specific categories of oral and transdermal menopausal oestrogens and oral medroxyprogesterone acetate (the most commonly prescribed menopausal progestogen in the United States).

continued, predominantly in the United States, with the recommendation that the endometrium be monitored (e.g. American College of Physicians, 1992). A more substantial shift in consciousness in the United States occurred in 1995, with the publication of the results of a trial that showed the occurrence of adenomatous or atypical endometrial hyperplasia in 34% of women receiving 0.625 mg of unopposed conjugated equine oestrogens daily (Writing Group for the PEPI Trial, 1995). Subsequently, the protocol of the nationwide Women's Health Initiative trial of post-menopausal hormonal therapy was amended so that women with a uterus could be randomized to receive only combined oestrogen–progestogen therapy or placebo (Finnegan *et al*., 1995).

Oestrogen–androgen combinations accounted for an estimated 14% of non-contraceptive oestrogen prescriptions in the United States in 1966, but this had fallen to less than 2% in 1983 (Kennedy *et al*., 1985). The number of oestrogen–androgen prescriptions then began to increase again, from 0.1 million in 1982 to 0.8 million in 1992 (Wysowski *et al*., 1995). Oestrogen in combination with a tranquillizer represented an estimated 3% of non-contraceptive oestrogen prescriptions in the United States in 1975, falling to less than 1% of prescriptions in 1983 (Kennedy *et al*., 1985).

Outside the United States, use of post-menopausal oestrogen therapy was generally uncommon until the late 1970s and 1980s and was prescribed mainly to women who had had a hysterectomy (although very few data are available about international use before the 1980s). In Europe, women with an intact uterus have been treated with combined oestrogen–progestogen therapy since the early 1970s (Maddison, 1973).

Transdermal post-menopausal oestrogen therapy became available in the mid-1980s.

1.2 Post-menopausal oestrogen therapy preparations

The main oestrogens used in oral post-menopausal oestrogen therapy are conjugated equine oestrogens, oestradiol and oestradiol valerate, although esterified oestrogens, mestranol, oestriol and oestropipate, are also used. Oestriol is more commonly used in Scandinavia (Persson *et al*., 1983; Stadberg *et al*., 1997). The appropriate dose of oestrogen varies with the indication for therapy; for menopausal symptoms, it can be raised until a minimum effective dose is found. The usual oral dose of conjugated equine oestrogens is 0.625–1.25 mg daily, and the usual oral dose of oestradiol is 0.5–4.0 mg daily (British Medical Association, 1997). For prevention of osteoporosis, the United Kingdom guidelines stipulate minimal doses of 0.625 mg/day oral conjugated equine oestrogens, 2 mg/day oral oestradiol or 0.050 mg/day oestradiol by patch, and not all types of oestradiol patches are specifically licensed for this indication (Anon., 1996).

Oestradiol can also be administered transdermally via a gel or patch, which avoids the first-pass effect of the liver, where a substantial proportion of orally administered oestrogen is deactivated, thus providing more constant blood hormone concentrations than oral preparations. In addition, because it does not cause the peaks and troughs in hormone concentration characteristic of oral medication, a lower overall dose of oestrogen can be given by transdermal administration (Williams & Stancel, 1996). Transdermal doses of oestradiol range from 0.025 mg to 0.1 mg per 24 h (British Medical Association, 1997). The

patches available initially consisted of a central oestradiol reservoir and an external adhesive ring, while in those developed more recently oestradiol is distributed throughout the adherent part of the patch (Anon., 1996). The patches are applied to a clean, non-hairy area of the skin, generally below the waist, and are reapplied every three to four days at a different site.

The available implants consist of crystalline pellets of oestradiol which are inserted subcutaneously under local anaesthesia and provide continuous oestrogen therapy for several months (Anon., 1996). Implants containing 50 mg oestradiol provide effective concentrations for approximately six months, and those containing 100 mg may last for up to nine months (Studd, 1976).

Oestrogen combined with androgen is usually taken orally but can also be given by implant. The usual indication for this type of therapy is menopausal symptoms accompanied by loss of libido (British Medical Association, 1997; Reynolds, 1998). A combination of esterified oestrogen and the anxiolytic chlordiazepoxide (given as 5–10 mg daily) is available in the United States for menopausal symptoms accompanied by anxiety (Reynolds, 1998).

Local topical preparations are available in the form of creams, pessaries and vaginal tablets containing oestriol, oestradiol, conjugated oestrogens, dienoestrol or other oestrogens. They generally have low systemic absorption and are given for urogenital symptoms when systemic therapy is not required. They are not discussed further in this monograph.

1.2.1 *Patterns of use*

Menses cease normally around the age of 50, often preceded and accompanied by climacteric symptoms. Women who have had a hysterectomy often have earlier onset of symptoms, and those who have had pre-menopausal oophorectomy often develop symptoms soon after their operation. Treatment of climacteric symptoms with post-menopausal oestrogen therapy is generally begun around or before the age of menopause and continued until withdrawal of treatment does not lead to a return of the symptoms. Symptomatic treatment generally lasts for less than five years and often for one to two years. Longer-term treatment for the prevention of osteoporosis and other conditions may continue for 10 years or more.

Table 1 shows the prevalence of use of post-menopausal oestrogen therapy in selected international population-based studies. These studies are difficult to interpret and compare as they tended to involve regions within a country that are not necessarily representative of the nation as a whole, include different age groups and usually do not allow a distinction between post-menopausal oestrogen therapy and oestrogen–progestogen therapy. Use of post-menopausal oestrogen therapy varies enormously from country to country and may also show substantial variation within a particular country (Keating *et al*., 1997). This regional variation is particularly marked in the United States. No figures on prevalence of use are available for many countries. Use is generally considered to be low in most of Africa and Asia.

Table 1. Prevalence of use of post-menopausal hormonal therapy (HT) in selected studies, 1975–97

Country	Reference	Year(s)	Age group (years)	Current use (%)			Any use (%)		
				HT	Oestrogen alone	Oestrogen–progestogen	HT	Oestrogen alone	Oestrogen–progestogen
Australia	MacLennan *et al*. (1993)	1991	> 40	14			25		
Denmark	Pedersen & Jeune (1988)	1983	40–59	16			33	16	12
	Køster (1990)	1987	51	22			37	10	17[a]
	Oddens & Boulet (1997)	1994	45–65	18			31		
Finland	Topo *et al*. (1995)	1989	45–64	22					
France	Ringa *et al*. (1992)	1986–87	45–55[b]	8	3	3[a]			
Norway	Topo *et al*. (1995)	1981	45–55	9					
Sweden	Persson *et al*. (1983)	1980	50–54	9					
			55–59	6					
	Lindgren *et al*. (1993)	1988	55, 57, 59 and 65	10			20		
	Stadberg *et al*. (1997)	1992	46, 50, 54, 58 and 62	21			41		
	Hammar *et al*. (1996)	1995	55–56	35[c]			40[c]		
United Kingdom	Spector (1989)	Late 1980s	45–65				10		
	Sinclair *et al*. (1993)	1991	33–69[b]	9			16		
	Griffiths & Jones (1995)	1993	45–65	20			33		
	Lancaster *et al*. (1995)	1993	45–64	15					
	Banks *et al*. (1996)	1994–95	50–64	30			43		
	Porter *et al*. (1996)	NR	45–54	19					
	Kuh *et al*. (1997)	1993	47	18			25		
	Townsend (1998)	1987	40–64	3					

Table 1 (contd)

Country	Reference	Year(s)	Age group (years)	Current use (%)			Any use (%)		
				HT	Oestrogen alone	Oestrogen–progestogen	HT	Oestrogen alone	Oestrogen–progestogen
United States	Stadel & Weiss (1975)	1973–74	> 18[b]				51		
	Barrett-Connor *et al*. (1979)	1973–75	55–74	39					
	Egeland *et al*. (1988)	1983–84	40–52	6	4	1			
	Barrett-Connor *et al*. (1989)	1984–87	50–79	31					
	Harris *et al*. (1990)	1986–87	NR	32	26	6			
	Cauley *et al*. (1990)	1986–88	> 65	18	15	3			
	Derby *et al*. (1993)	1981–82	40–64	5					
	Derby *et al*. (1993)	1989–90	40–64	11					
	Nabulsi *et al*. (1993)	1986–89	45–64						
	Black			17	16	1	33		
	White			22	17	5	39		
	Johannes *et al*. (1994)	1981–87	45–61	12[d]					
	Handa *et al*. (1996)	1986–87	> 65	6			25		
	Salamone *et al*. (1996)	1991		17			45		
	Brett & Madans (1997)	1982–92	25–74[b]				45	31	14
	Keating *et al*. (1997)	1995	45–74[b]	38					

NR, not reported
[a] 2% progestogen only
[b] Post-menopausal women only
[c] Oestriol users excluded
[d] Any use during study period

In the United States, the sales of non-contraceptive oestrogens increased from around 16 million per year in 1966 to around 36 million in 1992 (Kennedy *et al*., 1985; Wysowski *et al*., 1995). Use of post-menopausal hormonal therapy is more common in southern and western United States than in other regions (Keating *et al*., 1997). In England, a 10-fold increase in the estimated proportion of women using post-menopausal oestrogen therapy was seen in the period 1987–94, with less than 1% of women using oestradiol or conjugated oestrogens in 1987 and 10% in 1994 (Townsend, 1998). Table 1 shows that the prevalence of post-menopausal hormonal therapy in the mid-1990s was about 20% in the age group 45–64. In Scandinavia, use of post-menopausal hormonal therapy increased throughout the 1980s and early 1990s, Norway appearing to have consistently lower rates of use than Sweden, Finland and Denmark (Topo *et al.,* 1995). Sales of post-menopausal hormones (mainly oestradiol compounds cyclically or continuously combined with progestogens) have risen in Sweden since the 1970s, with a clear acceleration and doubling of use rates since the beginning of the 1990s (National Corporation of Pharmacies, 1997). Use of post-menopausal hormonal therapy in general appears to be also relatively common in Australia (MacLennan *et al.*, 1993).

Estimates based on sales data for 1991–92 show that enough post-menopausal hormonal therapy was sold to supply 20% of 45–70-year-old women in the United States (assuming only long-term use), 9–16% of women in Scandinavia and the United Kingdom, 7% in France, 5% in Belgium, 4% in the Netherlands, 3% in Austria and less than 1% of women in this age group in Italy and Spain (Jolleys & Olesen, 1996). Use of post-menopausal hormonal therapy is low in Japan (Nagata *et al.,* 1996).

The post-menopausal oestrogen therapy used in the United States is predominantly conjugated equine oestrogens, Premarin® being the single most commonly prescribed proprietary medicine overall in 1995 (Reynolds, 1998). The oestrogen composition of Premarin® is shown in Table 2. In Scandinavia and the United Kingdom, oestradiol is the oestrogen therapy most commonly prescribed for the menopause (Persson *et al*., 1997a; Townsend, 1998). Studies from Sweden show that 15–20% of current users of post-menopausal hormonal therapy take oestriol (Persson *et al*., 1983; Stadberg *et al*., 1997).

Women taking post-menopausal oestrogen therapy differ from women who do not take it in a number of ways; most notably, they are more likely to have had a hysterectomy and/or oophorectomy (Cauley *et al.*, 1990; Derby *et al.*, 1993; MacLennan *et al.*, 1993; Brett & Madans, 1997). Hysterectomy not only increases the likelihood that a woman will take post-menopausal hormonal therapy in general but also affects the type of therapy taken. Lancaster *et al.* (1995) reported from their general practice-based study in the United Kingdom that, of women taking post-menopausal hormonal therapy, 96% of those with a hysterectomy were taking oestrogen alone and 96% of women without a hysterectomy were taking combined oestrogen and progestogen. As post-menopausal oestrogen therapy is increasingly reserved for women who have had a hysterectomy, the relative use of oestrogen and combined oestrogen and progestogen therapy increasingly reflects the prevalence of hysterectomy in a population. Several studies have shown that, in comparison with non-users, post-menopausal oestrogen therapy users are more likely to have

Table 2. Oestrogen composition (as sulfates) of Premarin®

Oestrogen	% of total
Oestrone sulfate	42
8-Dehydrooestrone sulfate	18
Equilin sulfate	17
17α-Dehydroequilenin sulfate	10
Equilenin sulfate	4.3
17α-Dehydroequilin sulfate	3.4
17α-Oestradiol sulfate	2.4
17β-Oestradiol sulfate	1.5
17β-Dehydroequilin sulfate	0.7
17β-Dehydroequilenin sulfate	0.7

From Li *et al.* (1995)

taken the oral contraceptive pill in the past and to suffer from more severe menopausal symptoms (Sinclair *et al.*, 1993; Handa *et al.*, 1996; Persson *et al.*, 1997a). The relationship between post-menopausal oestrogen therapy and factors such as education, alcohol consumption, smoking and parity is not consistent from study to study.

2. Studies of Cancer in Humans

The use of oestrogen by post-menopausal women is referred to in the following text as 'oestrogen therapy' when oestrogen alone is specified or assumed and as 'oestrogen plus progestogen therapy' when the combination has been specified. The term 'hormone replacement therapy' is not used because none of the currently prescribed regimens offers physiological hormone replacement.

2.1 Breast cancer

Most of the established risk factors for breast cancer seem to operate through hormonal pathways. The fundamental importance of ovarian hormones in the etiology of breast cancer is evident from the established associations with early age at menarche, late age at first full-time pregnancy and late age at menopause (Kelsey *et al*., 1993). Of particular relevance for the assessment of risk after hormonal therapy is the effect of age when menopause or oestrogen deficiency occurs. Re-analyses of individual data from 51 epidemiological studies on breast cancer showed that the risk for breast cancer increased by about 3% per year the later a woman began menopause in the absence of hormonal therapy (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Because the serum concentrations of hormones among post-menopausal women taking oestrogen therapy are increased to those of pre-menopausal women, it is reasonable to hypothesize *a priori* that

such treatment can cause a similar increase in risk as delay of natural menopause (Colditz, 1996).

2.1.1 *Descriptive studies*

The sales of orally administered non-contraceptive hormones have surged during recent years in the United States (see section 1.2.1), and analyses of age-adjusted trends in breast cancer incidence in some areas of the United States (Devesa *et al*., 1987) have revealed an increase that might be compatible with increasing exposure to hormonal therapy. The trends in incidence, which were related mainly to birth cohorts (Holford *et al*., 1991), could, however, have other explanations, such as increasing intensity of mammography screening, changes in reproductive behaviour and life-style factors.

With the increased use of hormonal therapy in Sweden (see section 1.2.1), the nationwide age-standardized incidence of breast cancer has increased linearly, by an average of 1.3% since the 1960s up through the mid-1980s (Persson *et al.*, 1993). Thereafter, transient, period-related increases were seen in women aged 50–69 years, which are probably associated with the implementation of population-based mammography screening in Sweden from the late 1980s (Persson *et al.*, 1998). These ecological relationships between use of hormonal therapy and breast cancer incidence are, however, difficult to interpret.

2.1.2 *Analytical cohort and case–control studies*

Owing to concern in the 1970s that oestrogen therapy might increase the risk for breast cancer, numerous epidemiological studies have been conducted to evaluate possible relationships. Most explored the risk associated with intake of conjugated oestrogens, as predominantly practised in the United States; a few late studies also addressed the risk associated with oestradiol compounds, mainly prescribed in Europe. These studies are summarized in Tables 3a and 3b.

The Collaborative Group on Hormonal Factors in Breast Cancer (1997) reanalysed the original data from 51 out of 61 epidemiological studies with relevant data. Tables 3a and 3b give the relative risks found in those studies and those derived in this reanalysis. The study covered a total of 52 705 cases of breast cancer and 108 411 control subjects. The key results showed that use of hormonal therapy at the time the breast cancer was diagnosed or that had ceased within five years of the diagnosis was associated with a 2.3% (95% confidence interval [CI], 1.1–3.6%) increase in the relative risk for breast cancer for each year of intake (Figure 2). The relative risk reached 1.3 (95% CI, 1.2–1.5) after five to nine years and 1.6 (95% CI, 1.3–1.8) after 15 years of intake for current or recent users. No excess risk was seen five years after discontinuation of treatment. Further, the effect of current or recent long-term treatment was greater in lean than in overweight women (Figure 3) and seen chiefly for clinically less advanced tumours.

The results of selected, large, published studies are reviewed in more detail, and abstracted information from these 15 cohort and 23 case–control studies is given in Tables 4 and 5, respectively. The risk relationships with regard to any use, duration, recency, latency, dose, type of oestrogen, regimens and route of administration are reviewed for

Table 3a. Prospective studies of post-menopausal oestrogen therapy and breast cancer

Reference, country	Original data or reanalysis[a]	No. of cases	Any use	
			Relative risk	95% CI
Hoover *et al*. (1976), USA	Original	49	1.3	1.0–1.7
Hunt *et al*. (1987), UK	Original	50	1.6	1.2–2.1
Miller *et al*. (1992), Canada	Reanalysis	448	1.0	[0.78–1.2]
Schairer *et al*. (1994), USA	Original	1 185	1.0	0.9–1.2
Risch & Howe (1994), Canada	Original	742	NR	
Colditz *et al*. (1995), USA	Original	1 935	1.3	1.1–1.5
Schuurman *et al*. (1995), Netherlands	Original	471	0.99	0.68–1.4
Folsom *et al*. (1995), USA	Reanalysis	468	1.2	[0.94–1.5]
Thomas *et al*. (1982), USA; Hiatt *et al*. (1984), USA; Alexander *et al*. (1987), UK; Wang *et al*. (1987), UK; Kay & Hannaford (1988), UK; Bergkvist *et al*. (1989), Sweden; Mills *et al*. (1989), USA; Vessey *et al*. (1989), UK; Willis *et al*. (1996), USA; Goodman *et al*. (1997a), Japan	Grouped reanalysis	667	0.62	[0.24–1.0]
Persson *et al*. (1997b), Sweden	Original	435	1.1	0.8–1.4

[a] Reanalyses by the Collaborative Group on Hormonal Factors in Breast Cancer (1997)
NR, not reported

cohort and case–control studies together. Some comments on methods are given in the tables.

(*a*) *Use of any type of oestrogen for any length of time*

Most studies give risk estimates reflecting 'ever use', i.e. use of any type of compound for any amount of time. Most of the relative risks are close to 1.0, and only a few significantly exceed that value (La Vecchia *et al*., 1986; Hunt *et al*., 1987; Mills *et al*., 1989; Colditz *et al*., 1995; Lipworth *et al*., 1995). Comparisons of any use of hormones with no use are not, however, very informative, since most use has been short.

(*b*) *Duration of intake*

Long-term use was linked to an increased incidence of breast cancer in seven cohort studies and six case–control studies (Ross *et al*., 1980; Hoover *et al*., 1981; Brinton *et al*., 1986; La Vecchia *et al*., 1986; Hunt *et al*., 1987; Ewertz, 1988; Bergkvist *et al*., 1989;

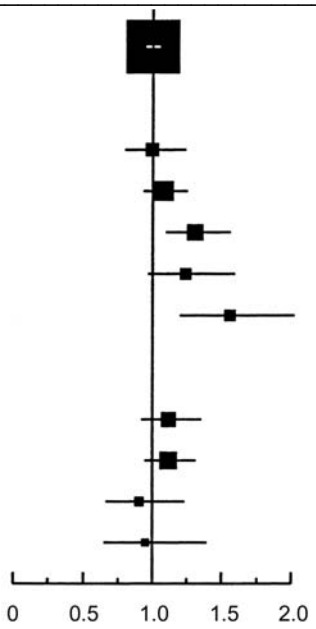
Table 3b. Case–control studies of post-menopausal oestrogen therapy and breast cancer

Reference, country	Original data or reanalysis[a]	No. of cases/ no. of controls	Any use	
			Relative risk	95% CI
Hoover *et al*. (1981), USA	Original	345/611	1.4	1.0–2.0
Kaufman *et al*. (1984), USA and Canada	Original	1 610/1 606	1.0	0.8–1.2
Brinton *et al*. (1986), USA	Original	1 960/2 258	1.0	0.9–1.2
Wingo *et al*. (1987), USA	Original	1 369/1 645	1.0	0.9–1.2
Hislop *et al*. (1986), Canada	Reanalysis	361/366	1.2	[0.67–1.6]
Siskind *et al*. (1989), Australia	Reanalysis	265/544	1.3	[0.64–2.0]
Ewertz (1988), Denmark	Original	1 486/1 336	1.3	0.96–1.7
Kaufman *et al*. (1991), USA	Original	1 686/2 077	1.2	1.0–1.6
Harris *et al*. (1992a), USA	Original	604/520	NR	
Weinstein *et al*. (1993), USA	Original	1 436/1 419	1.1	0.86–1.4
Newcomb *et al*. (1995), USA	Original	3 130/3 698	1.1	0.9–1.2
Yang *et al*. (1992), Canada	Original	699/685	1.0	0.8–1.3
Stanford *et al*. (1995), USA	Original	537/492	0.9	0.6–1.1
Ross *et al*. (1980), USA; Nomura *et al*. (1986), USA; Lee *et al*. (1987), Costa Rica; Rohan & McMichael (1988), Australia; Paul *et al*. (1990), New Zealand; Palmer *et al*. (1991), Canada; Ursin *et al*. (1992), USA; Rookus *et al*. (1994), Netherlands; White *et al*. (1994), USA; Brinton *et al*. (1995), USA	Grouped reanalysis	1 080/1 640 (with population controls)	0.96	[0.66–1.3]
Morabia *et al*. (1993), USA	Reanalysis	184/322	1.4	[0.67–2.2]
Vessey *et al*. (1983); McPherson *et al*. (1987), UK	Original	416/462	1.2	[0.53–1.9]
La Vecchia *et al*. (1992a), Italy	Reanalysis	1 615/1 450	1.7	[1.2–2.2]
Lipworth *et al*. (1995), Greece	Reanalysis	446/840	1.2	[0.55–1.8]
La Vecchia *et al*. (1995), Italy	Original	2 569/2 588	1.2	0.9–1.5
Talamini *et al*. (1985), Italy; Marubini *et al*. (1988), Italy; Ravnihar *et al*. (1988), Slovenia; Hulka *et al*. (1982), USA; WHO Collaborative Study (1990) (multinational); Ngelangel *et al*. (1994), Philippines; Levi *et al*. (1996), Italy	Grouped reanalysis	1 470/5 144 (with hospital controls)	1.0	[0.75–1.3]

[a] Reanalyses by the Collaborative Group on Hormonal Factors in Breast Cancer (1997)
NR, not reported

Figure 2. Relative risks (RR) for breast cancer by duration of use within categories of time since last use of post-menopausal hormonal therapy

Duration of use and time since last use	No. of cases/ no. of controls	RR (FSE)[a]	RR and 99% FCI[a]
No use	12 467/23 568	1.0 (0.021)	
Last use < 5 years before diagnosis (including current use)			
< 1 year	368/860	0.99 (0.085)	
1–4 years	891/2 037	1.1 (0.06)	
5–9 years	588/1 279	1.3 (0.079)	
10–14 years	304/633	1.26 (0.11)	
≥ 15 years	294/514	1.6 (0.13)	
Last use ≥ 5 years before diagnosis			
< 1 year	437/890	1.1 (0.079)	
1–4 years	566/1 256	1.1 (0.068)	
5–9 years	151/374	0.90 (0.12)	
≥ 10 years	93/233	0.95 (0.14)	



Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1997)
FSE, floated standard error; FCI, floated confidence interval
[a] Relative to no use, stratified by study, age at diagnosis, time since menopause, body-mass index, parity and age when first child was born

Mills *et al*., 1989; Yang *et al*., 1992; Risch & Howe, 1994; Schairer *et al*., 1994; Colditz *et al*., 1995; Persson *et al*., 1997b). At least six case–control studies (Hulka *et al*., 1982; Kaufman *et al*., 1984; Wingo *et al*., 1987; Kaufman *et al*., 1991; Newcomb *et al.*, 1995; Stanford *et al*., 1995) and one cohort study (Schuurman *et al*., 1995) found no statistically significant association with years of oestrogen intake. In two European studies (Ewertz, 1988; Bergkvist *et al*., 1989), the excess risks seemed to be higher with shorter periods of intake, the relative risk estimates exceeding 2.0 after six years of intake, than in studies in the United States (Brinton *et al*., 1986; Colditz *et al*., 1995). The American cohort studies gave increases in risk that are best explained by current intake rather than duration (Mills *et al*., 1989; Colditz *et al*., 1992, 1995), although there seemed to be duration-dependent effects in these data for current hormone takers (see Table 4).

Two recent, large population-based case–control studies in the United States (Newcomb *et al*., 1995; Stanford *et al*., 1995), the latter with considerable power to examine risk after long-term use, reported no effect of exposure of any duration.

Figure 3. Relative risks for breast cancer according to use of post-menopausal hormonal therapy by women with various characteristics

Last use < 5 years before diagnosis and duration of use < 5 years			
Characteristic	No. of cases/ no. of controls	RR (SE)	RR and 99% CI
Age at diagnosis			
< 60 years	1 042/2 341	1.1 (0.065)	
≥ 60 years	217/556	0.94 (0.11)	
Family history			
No	1 029/2 530	1.1 (0.061)	
Yes	163/230	0.9 (0.21)	
Ethnic group			
White	954/2 311	0.98 (0.06)	
Other	105/317	1.3 (0.29)	
Education			
< 13 years	593/1 237	1.0 (0.088)	
≥ 13 years	636/1 608	1.1 (0.078)	
Height			
< 165 cm	602/1 360	1.1 (0.086)	
≥ 165 cm	545/1 091	1.1 (0.093)	
Weight			
< 65 kg	676/1 416	1.1 (0.080)	
≥ 65 kg	444/993	1.0 (0.099)	
Body-mass index			
< 25.0 kg/m^2	786/1 626	1.1 (0.071)	
≥ 25.0 kg/m^2	331/774	1.0 (0.10)	
Age at menarche			
< 13 years	512/1 125	0.99 (0.092)	
≥ 13 years	721/1 702	1.1 (0.076)	
Parity			
Nulliparous	196/321	1.1 (0.16)	
Parous	1 058/2 568	1.0 (0.057)	
Age at first birth			
< 25 years	566/1 490	1.1 (0.084)	
≥ 25 years	466/1 042	1.0 (0.088)	
Use of COC in past 10 years			
No	948/2 129	1.0 (0.060)	
Yes	153/342	1.0 (0.26)	
Alcohol			
< 50 g/week	609/1 296	1.1 (0.082)	
≥ 50 g/week	284/457	1.1 (0.14)	
Smoking history			
Never	494/992	1.1 (0.097)	
Ever	566/1 187	1.1 (0.089)	
Type of menopause			
Natural	829/1 923	1.0 (0.062)	
Bilateral oophorectomy	430/974	0.92 (0.14)	

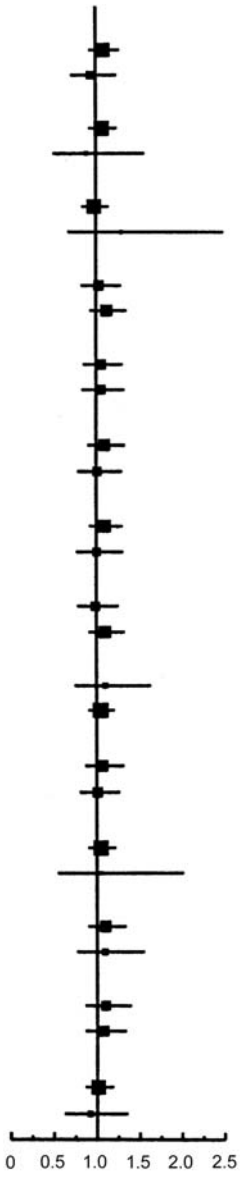


Figure 3 (contd)

Last use < 5 years before diagnosis and duration of use ≥ 5 years			
Characteristic	No. of cases/ no. of controls	RR (SE)	RR and 99% CI
Age at diagnosis			
< 60 years	612/1 390	1.3 (0.088)	
≥ 60 years	574/1 036	1.4 (0.085)	
Family history			
No	902/2 058	1.4 (0.070)	
Yes	188/246	1.1 (0.20)	
Ethnic group			
White	947/2 013	1.2 (0.067)	
Other	60/220	1.2 (0.30)	
Education			
< 13 years	522/960	1.2 (0.097)	
≥ 13 years	622/1 398	1.5 (0.092)	
Height			
< 165 cm	601/1 231	1.4 (0.094)	
≥ 165 cm	519/893	1.5 (0.11)	
Weight			
< 65 kg	721/1 228	1.6 (0.095)	
≥ 65 kg	362/839	1.1 (0.10)	
Body-mass index			
< 25.0 kg/m^2	797/1 412	1.5 (0.083)	
≥ 25.0 kg/m^2	280/651	1.0 (0.11)	
Age at menarche			
< 13 years	486/1 003	1.2 (0.10)	
≥ 13 years	682/1 408	1.4 (0.088)	
Parity			
Nulliparous	219/364	1.4 (0.16)	
Parous	962/2 043	1.3 (0.066)	
Age at first birth			
< 25 years	519/1 149	1.4 (0.10)	
≥ 25 years	434/877	1.3 (0.099)	
Use of COC in past 10 years			
No	996/2 023	1.4 (0.066)	
Yes	63/128	1.4 (0.40)	
Alcohol			
< 50 g/week	575/1 049	1.4 (0.096)	
≥ 50 g/week	288/371	1.7 (0.19)	
Smoking history			
Never	458/890	1.3 (0.11)	
Ever	561/962	1.6 (0.11)	
Type of menopause			
Natural	480/873	1.3 (0.090)	
Bilateral oophorectomy	706/1 553	1.3 (0.13)	

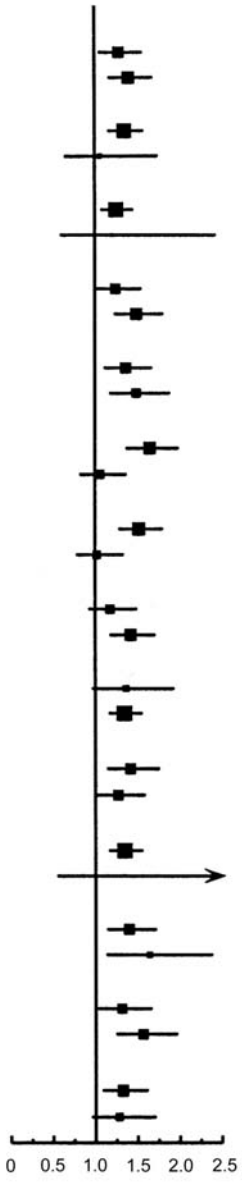
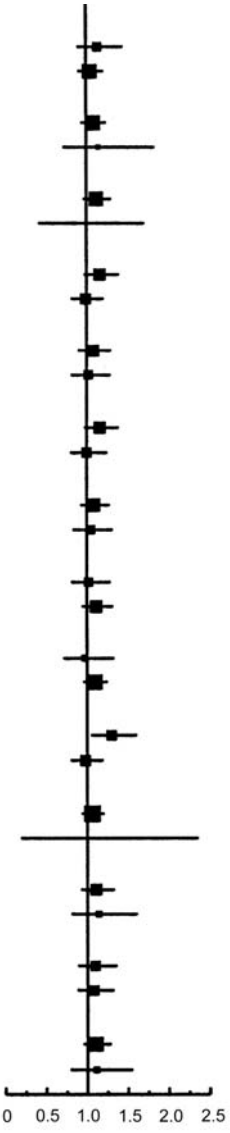


Figure 3 (contd)

Last use ≥ 5 years before diagnosis			
Characteristic	No. of cases/ no. of controls	RR (SE)	RR and 99% CI
Age at diagnosis			
< 60 years	342/829	1.1 (0.10)	
≥ 60 years	905/1 824	1.05 (0.059)	
Family history			
No	973/2 340	1.1 (0.057)	
Yes	215/315	1.2 (0.20)	
Ethnic group			
White	911/2 005	1.1 (0.06)	
Other	64/262	0.8 (0.25)	
Education			
< 13 years	726/1 318	1.16 (0.076)	
≥ 13 years	501/1 378	0.99 (0.077)	
Height			
< 165 cm	696/1 435	1.1 (0.074)	
≥ 165 cm	475/1 001	1.0 (0.092)	
Weight			
< 65 kg	666/1 352	1.2 (0.075)	
≥ 65 kg	480/1 056	1.0 (0.087)	
Body-mass index			
< 25.0 kg/m^2	737/1 538	1.09 (0.066)	
≥ 25.0 kg/m^2	403/863	1.0 (0.092)	
Age at menarche			
< 13 years	464/1 083	1.0 (0.091)	
≥ 13 years	771/1 644	1.1 (0.069)	
Parity			
Nulliparous	202/429	0.97 (0.12)	
Parous	1 042/2 313	1.1 (0.055)	
Age at first birth			
< 25 years	503/1 167	1.3 (0.094)	
≥ 25 years	530/1 135	0.98 (0.076)	
Use of COC in past 10 years			
No	1168/2 585	1.1 (0.052)	
Yes	16/60	0.67 (0.40)	
Alcohol			
< 50 g/week	642/1 284	1.1 (0.075)	
≥ 50 g/week	294/422	1.1 (0.14)	
Smoking history			
Never	519/964	1.1 (0.087)	
Ever	569/1 138	1.1 (0.083)	
Type of menopause			
Natural	827/1 721	1.1 (0.062)	
Bilateral oophorectomy	420/1 032	1.1 (0.14)	



Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1997)
RR, relative risk; CI, confidence interval; SE, standard error
Family history, mother or sister with breast cancer; COC, combined oral contraceptives
[a] Relative to no use, stratified by study, age at diagnosis, time since menopause, body-mass index, parity and age when her first child was born.

Table 4. Summary of cohort studies on post-menopausal oestrogen therapy and breast cancer

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Hoover *et al*. (1976)	Records of private practice, Kentucky, USA, 1969–72	Retrospective cohort, 1 891 women; follow-up through individual contacts and medical records for 12 years; 22 717 person–years; comparison with external incidence rates	**Incidence**: 49 cases observed Conjugated oestrogens: Any use: RR, 1.3 (95% CI, 1.0–1.7) Time since inclusion: increasing trend, ≥ 15 years, RR, 2.0 (95% CI, 1.1–3.4) Increasing risk with increasing dose and number of prescriptions Similar risk relationships for age at start and ovarian status	Confounding difficult to assess Proxy variables of dose–response relationship
Buring *et al*. (1987)	Nurses in 11 US states, 1976–80, 30–55 years	Cohort, 33 335 women; follow-up questionnaires sent after 4 years	**Incidence**: 221 new cases Any use: RR, 1.1 (95% CI, 0.8–1.4) Current: RR, 1.0 (95% CI, 0.7–1.4) Past: RR, 1.3 (95% CI, 0.9–1.8) Duration: < 1 year: RR, 1.0 (95% CI, 0.6–1.7) 1–< 3 years: RR, 1.0 (95% CI, 0.7–1.6) 3–5 years: RR, 1.0 (95% CI, 0.6–1.6) > 5 years: RR, 1.3 (95% CI, 0.9–2.1)	Only exposure at baseline Short follow-up
Hunt *et al*. (1987)	Menopause clinics in UK, 1978–82, counsel for menopausal symptoms	Cohort of 4 544 women who used hormones ≥ 1 year; 17 830 person–years; follow-up through contact letters and medical records; exposure data from baseline interview	**Incidence**: 50 cases. **Mortality**: 12 deaths Various kinds of hormones Any use - incidence: RR, 1.6 (95% CI, 1.2–2.1) - mortality: SMR, 0.6 (95% CI, 0.3–1.0) Time since first intake: trend for incidence ≥ 10 years: RR, 3.1 (95% CI, 1.5–5.6)	Possible selection bias in study of mortality Heterogeneous exposure regimens

Table 4 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Bergkvist *et al*. (1989)	Six counties in Sweden 1977–83, women prescribed hormones	Population-based cohort; 23 244 ≥ 35 years; 133 375 person–years; follow-up through record linkage with national cancer registry; exposure data from prescriptions; questionnaire data in a random sample; cohort, case–cohort and case–control analyses	**Incidence**: 253 cases Hormone regimens prevalent in Sweden: Any use: RR, 1.1 (95% CI, 1.0–1.3) Duration of intake: - all compounds; ≥ 9 years: RR, 1.7 (95% CI, 1.1–2.7) - oestradiol: trend, ≥ 9 years: RR, 1.8 (95% CI, 0.7–4.6) - conjugated oestrogens: no trend, ≥ 6 years: RR, 1.3 (95% CI, 0.6–2.9) Regimens, duration ≥ 9 years - oestrogens alone: RR, 1.8 (95% CI, 1.0–3.1)	Adjustment for confounders led to higher risk estimates Low power to examine conjugated oestrogens
Mills *et al*. (1989)	Seventh-day Adventists, California, USA, 1976–82, Caucasians	Cohort of 20 341 women; internal comparisons; 115 619 person-years; individual follow-up through registry linkage; baseline questionnaire in 1976	**Incidence**: 6 years follow-up, 215 cases Conjugated oestrogens: Any use: RR, 1.7 (95% CI, 1.2–2.4) - current: RR, 2.5 (95% CI, 1.6–4.0) - former: RR, 1.4 (95% CI, 1.0–2.2) Duration: - significant trend, after 6–10 years: RR, 2.8 (95% CI, 1.7–4.6) Effect modification: - previous COC intake: RR, 1.4 (95% CI, 0.7–2.9)	Data on exposure duration incomplete (only baseline)

Table 4 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Colditz *et al*. (1992)	Nurses' Health Cohort, USA, 1976–88, 30–55 years at entry	Cohort, 118 300 nurses at post-menopausal ages; 480 665 person-years; individual follow-up through questionnaires, 95% complete for incidence and 98% for deaths; internal comparisons; baseline questionnaire 1976; up-date questionnaires every 2 years	**Incidence**: 12 years' follow-up, 1015 cases Conjugated oestrogens, combinations with progestogens: - any use: RR, 0.9 (95% CI, 0.8–1.1) - current use: RR, 1.3 (95% CI, 1.1–1.6) - previous use: RR, 0.9 (95% CI, 0.8–1.0) Duration, current intake 5–< 10 years: RR, 1.6 (95% CI, 1.3–2.1) Effect modification: - none with other risk factors - previous COC-intake: RR, 1.5	Mainly relationship with current intake, possibly also duration effect: current use: 2–< 5 years: RR, 1.3 (95% CI, 1.0–1.7) 5–10 years: RR, 1.6 (95% CI, 1.25–2.06) > 10 years: RR, 1.5 (95% CI, 1.1–2.0)
Yuen *et al*. (1993)	Uppsala health care region, Sweden, 1977–80; women prescribed hormonal therapy	See Bergkvist *et al*. above. Follow-up through record-linkages with causes of death registry; comparison with external, *corrected* mortality rates; exposure data from prescriptions	**Mortality**, 12 years' follow-up, 73 deaths Various hormone regimens Any use: SMR, 0.8 (95% CI, 0.6–1.0) Prescribed compounds: - oestradiol and/or conjugated oestrogens: SMR, 0.8 (95% CI, 0.2–1.1) - other oestrogens: SMR, 0.9 (95% CI, 0.5-1.3)	Exposure data only from prescriptions Correction for possible bias due to healthy drug use (population rates corrected for prevalent cases)
Schairer *et al*. (1994)	Populations in 27 cities in the USA, breast cancer screening programme, 1980–89	Cohort of 49 017 participants; 313 902 person–years; follow-up through interviews and questionnaires; information on exposure and risk factors through questionnaires	**Incidence**, both in-situ and invasive tumours, 1 185 cases Conjugated oestrogens, combinations with progestogens Any use: - oestrogens only: RR, 1.0 (95% CI, 0.9–1.2) In-situ tumours: - oestrogens only: RR, 1.4 (95% CI, 1.0–2.0) Duration: 10–14 years, oestrogens only/ in-situ tumours: RR, 2.1 (95% CI, 1.2–3.7)	Detection bias minimized Risk relationship limited to in-situ tumours Low power to assess long-term duration of oestrogen plus progestogen regimens

Table 4 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Risch & Howe (1994)	Inhabitants of Saskatchewan, Canada, 43–49 years, start in 1976	Registry-based cohort, 32 790 women followed-up through linkage to cancer registry; 448 716 person–years; exposure data from prescription roster	**Incidence**: 742 cases Conjugated oestrogens, added progestogens Oestrogens only: increasing RR 7% per year (RR, 1.07; 95% CI, 1.02–1.13)	Limited power to look at long-term treatment
Colditz *et al*. (1995)	Nurses' Health Cohort (see above)	Cohort of 121 700 nurses; 725 550 person-years; baseline questionnaire in 1976, biannual questionnaires, updates on exposure and outcome (follow-up)	**Incidence**: 16 years' follow-up, 1 935 cases Conjugated oestrogens and added progestogens Current intake: - oestrogens alone: RR, 1.3 (95% CI, 1.1–1.5) - 5–9 years' oestrogens: RR, 1.5 (95% CI, 1.2–1.7) - oestrogen plus progestogen: RR, 1.4 (95% CI, 1.2–1.7) By age at diagnosis/≥ 5 years' current hormones: - 50–54 years: RR, 1.5 (95% CI, 0.91–2.3) - 55–59 years: RR, 1.5 (95% CI, 1.2–2.0) - 60–64 years: RR, 1.7 (95% CI, 1.3–2.2) **Mortality**: 359 deaths: Current use, ≥ 5 years: RR, 1.4 (95% CI, 1.0–2.1)	No relationship with past use Detection bias unlikely as explanation First study to show an increased risk of death with post-menopausal oestrogen therapy
Schuurman *et al*. (1995)	The Netherlands, random selection from 204 municipal population registries, age 55–69 years	62 573 women responding to mailed questionnaire; record linkage follow-up; case–cohort approach (subcohort, 1 812 women)	**Incidence**, 3.3 years' follow-up, 471 cases 'Replacement hormones': Any use: RR, 1.0 (95% CI, 0.7–1.4) Duration: no trend Any use of COC and post-menopausal oestrogen therapy: RR, 1.0 (95% CI, 0.51–1.9)	Low power in duration categories No data on compounds or regimens Short latency

Table 4 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Folsom *et al*. (1995)	Iowa 'Women's Health Study', USA, 55–69 years, 1986–92	41 070 post-menopausal women; 129 149 person–years; record linkage for incident cases	**Incidence**, 6 years' follow-up: 468 cases 'Replacement hormones': Former use: RR, 0.96 (95% CI, 0.81–1.1) Current use: RR, 1.2 (95% CI, 0.99–1.6) ≤ 5 years: RR, 1.4 (95% CI, 1.0–2.1) > 5 years: RR, 1.2 (95% CI, 0.92–1.6)	General cancer mortality: RR, 1.1 (95% CI, 0.81–1.5) Insufficient power for cause-specific mortality No data on duration of compounds or regimens
Willis *et al*. (1996)	Women volunteers in 50 states in the USA, 1982–91	422 373 post-menopausal women; follow-up through record linkage for cause-specific deaths; data in baseline questionnaires; fatal breast cancers	**Mortality**, 9 years' follow-up: 1469 breast cancer deaths Oestrogens: Any use: RR, 0.84 (95% CI, 0.75–0.94) Recent use: RR, 0.90 (95% CI, 0.75–1.1) Duration: ≥ 11 years: RR, 0.93 (95% CI, 0.75–1.2) (no trend)	Exposure data only at baseline No specific data on exposure Mortality only
Persson *et al*. (1996)	See Yuen *et al*. above	22 597 women with registered hormone prescriptions; record linkage follow-up of incidence and mortality; risk factors from questionnaire survey	**Incidence**, 13 years' follow-up, 634 cases Prescriptions for various regimens Any post-menopausal oestrogen therapy: RR, 1.0 (95% CI, 0.9–1.1) Oestradiol or conjugated oestrogens: RR, 0.9 (95% CI, 0.8–1.1) **Mortality**: 102 deaths Any post-menopausal oestrogen therapy: RR, 0.5 (95% CI, 0.4–0.6)	No direct adjustment for covariates 'Healthy drug user' bias in mortality analyses

Table 4 (contd)

Reference	Study base	Design: cohort, follow-up, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Persson *et al*. (1997b)	Participants in mammography screening, Uppsala, Sweden, 1990–95, 46–74 years	Cohort of 30 982 women participating in two screening rounds; follow-up through screening and in diagnostic registry of pathology department; questionnaires at visits; nested case–control approach	Five-year follow-up: 435 cases (87% invasive), 1 740 controls Any post-menopausal oestrogen therapy: odds ratio, 1.1 (95% CI, 0.8–1.4) Duration ≥ 11 years: odds ratio, 2.1 (95% CI, 1.1–4.0) Oestradiol or conjugated oestrogen ≥ 11 years: odds ratio, 1.3 (95% CI, 0.5–3.7)	Duration of intake significantly associated Low power in regimen subgroups

SMR, standardized mortality ratio; COC, combined oral contraceptives

Table 5. Summary of case–control studies of post-menopausal oestrogen therapy and breast cancer

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Ross *et al*. (1980)	Los Angeles, USA, retirement community, 1971–77, Caucasians, age 50–74	Population-based; 131 cases, 262 controls; data from interviews, pharmacy and medical records	Conjugated oestrogens: Any use: RR, 1.1 (95% CI, 0.8–1.9) Cumulative dose > 1 500 mg: RR, 1.9 (95% CI, 1.0–3.3) Ovaries retained: RR, 2.5 (95% CI, 1.2–5.6)	Dose and duration could not be separated
Hoover *et al*. (1981)	Portland, USA, members of insurance programme (prepaid health plan), 1969–75	Population-based; 345 cases, 611 controls; data from medical records, e.g. number of prescriptions	Conjugated oestrogens: Any use: RR, 1.4 (95% CI, 1.0–2.0) ovaries retained: RR, 1.3 ovaries removed: RR, 1.5 No. of prescriptions: trend, highest RR, 1.8 Dose: trend, highest RR, 1.8 Duration: trend, highest RR, 1.7 Effect modification: higher risk if family history	Only medical record data Adjustment only for type of menopause
Brinton *et al*. (1981)	Multicentre screening programme, 29 centres in the USA, age 35–74 years	Population-based; 881 cases, 863 controls; interviews	Conjugated oestrogens: Any use: RR, 1.2 (95% CI, 1.0–1.5) Oophorectomy: Any use: RR, 1.5 (95% CI, 0.9–2.8) ≥ 10 years: RR, 1.7 (NS)	Higher risks with higher-dose compounds Possible interaction with nulliparity, family history and benign breast disease
Hulka *et al*. (1982)	North Carolina, USA, city hospitals, 1977–78, post-menopausal women	Population- and hospital-based; 199 cases, 451 and 852 controls; data from interviews	Oestrogens: Any use: RR, 1.2 (NS) - natural menopause: RR, 1.8 (95% CI, 1.2–2.7) - surgical menopause: RR, 1.3 (NS) No relationship to duration or timing of exposure	Two control groups Speculations on higher risk with injectable oestrogens

Table 5 (contd)

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Hiatt *et al*. (1984)	Kaiser Foundation Health Plan, oophorecto-mized women, 1971–79	Population-based; 119 cases, 119 controls (matched); medical records	Oestrogens: Any use: RR, 0.7 (95% CI, 0.3–1.6) ≥ 5 notations: RR, 2.1 (95 % CI, 1.2–3.6) (significant trend)	No effect modification No trend with years of use
Kaufman *et al*. (1984)	Large cities, USA and Canada, 1976–81, < 70 years	Hospital-based; 1 610 cases, 1 606 controls; data from interviews	Conjugated oestrogens: Any use: RR, 1.0 (95% CI, 0.8–1.2) Duration: ≥ 10 years: RR, 1.3 (0.6–2.8) (no trend) Subgroup analysis: no risk relationships	Discussion on selection bias due to hospital controls
La Vecchia *et al*. (1986)	Northern Italy, 1983–85, age 26–74	Hospital-based; 1 108 cases, 1 281 controls; data from interviews	Non-contraceptive oestrogens: Any use: RR, 1.8 (95% CI, 1.3–2.7) Duration - ≤ 2 years: RR, 1.7 (95% CI, 1.1–2.6) - > 2 years : RR, 2.0 (95% CI, 1.0–4.1) Recency, latency: no relationship	Low prevalence of exposure in population
Brinton *et al*. (1986)	Nationwide screening programme, USA, 1977–80, Caucasians, post-menopausal; extension of study reported by Brinton *et al*. (1981), see above	Population-based; 1 960 cases, 2 258 controls (random); interviews	Conjugated oestrogens: Any use: RR, 1.0 (95% CI, 0.9–1.2) Duration: significant trend of increase; ≥ 20 years: RR, 1.5 (95% CI, 0.9–2.3) By menopause types: similar relationships Effect modification: benign breast disease, RR, 3.0 (95% CI, 1.6–5.5) for hormone use of ≥ 10 years Tumour stage: highest risk for in-situ and small tumours	Large study Diagnostic bias unlikely

Table 5 (contd)

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Nomura *et al*. (1986)	Patients in Hawaiian hospitals, 1975–80, age 45–74	Hospital and neighbourhood controls; 183/183 Japanese; 161/161 Caucasians	Oestrogen use: Caucasians/Japanese analysed separately: Any use: - Caucasians: RR, 0.9 (95% CI, 0.5–1.3) - Japanese, RR, 1.1 (95% CI, 0.7–1.6) Duration: no trend	Low response rates among cases Low power in subgroup analyses
Wingo *et al*. (1987)	Large cities, USA, 1980–82, age 25–54	Population-based; 1 369 cases, 1 645 controls; random-digit dialling; interviews	Conjugated oestrogens: Any use: RR, 1.0 (95% CI, 0.9–1.2) - natural menopause: RR, 0.8 (95% CI, 0.6–1.1) - 'surgical menopause': RR, 1.3 (95% CI, 0.9–1.9) Duration, latency, recency: no pattern	Limited to early post-menopausal women Low power for long-term treatment
Rohan & McMichael (1988)	Adelaide, Australia, 1982–84, post-menopausal women, < 74 years	281 cases, 288 controls; interviews	Exogenous oestrogens: Any use: RR, 1.0 (95% CI, 0.6–1.7) Duration, latency, recency: no pattern	Small numbers in duration categories
Ewertz (1988)	Denmark, nationwide, 1983–84, > 70 years	Population-based; 1 486 cases, 1 336 controls (random); self-administered, mailed questionnaire	Oestradiol and oestradiol-progestogen combinations: Any use: RR 1.0–1.3 (NS) depending on menopause status Duration: trend with increasing years of intake: RR, 0.9–2.3 after 3–13 years ($p > 0.002$)	Chiefly exposure to oestradiol compounds

Table 5 (contd)

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Kaufman *et al*. (1991)	Large cities, east coast, USA, 1980–86, post-menopausal women, 40–69 years	Hospital-based; 1 686 cases, 2 077 controls; interviews	Mostly conjugated oestrogens: Any use: RR, 1.2 (95% CI, 1.0–1.6) Duration: > 15 years RR, 0.9 (95% CI, 0.4–2.1) - conjugated oestrogens, current intake: RR, 1.2 (95% CI, 0.8–1.8) Dose: no pattern	Low power for long-term treatment
Palmer *et al*. (1991)	Toronto, Canada, one cancer hospital, 1982–86, < 70 years	Population-based; 607 cases, 1 214 controls; interviews	Conjugated oestrogens: Any use: RR, 0.9 (95% CI, 0.6–1.2) Duration ≥ 15 years: RR, 1.5 (95% CI, 0.6–3.8) (no significant trend) Current use and ≥ 5 years use: RR, 0.9 (95% CI, 0.4–1.9)	Low power in long duration categories Response rate of controls, 65%
Yang *et al*. (1992)	British Columbia, Canada, 1988–89, post-menopausal women < 75 years	Population-based; 699 cases, 685 controls; mailed questionnaire	Mainly conjugated oestrogens: Any use: RR, 1.0 (95% CI, 0.8–1.3) Current intake: RR, 1.4 (95% CI, 1.0–2.0) Duration ≥ 10 years: RR, 1.6 (95% CI, 1.1–2.5) Effect modification: highest risk after oophorectomy: RR, 1.9 (95% CI, 0.8–5.3)	Low response rate
Harris *et al*. (1992a)	New York city area, USA, 1987–89	Hospital-based; 604 cases, 520 controls; interviews in hospitals	'Use of oestrogens': lean, post-menopausal women: odds ratio, 2.0 (95% CI, 1.1–3.5) (body mass index, < 22) - < 5 years: odds ratio, 2.0 (95% CI, 1.0–3.8) - ≥ 5 years: odds ratio, 2.2 (95% CI, 0.8–5.6)	Risk increase with high body mass index and weight gain Few subjects with long duration of hormone therapy

Table 5 (contd)

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Weinstein *et al*. (1993)	Long Island, New York, USA, 1984–86	Population-based; 1 436 cases, 1 419 controls; telephone interviews	'Use of oestrogens': Any use: RR = 1.1 (0.86–1.4) No trend with duration. Current use: RR = 1.3 (0.76–2.3) Former use: no trend	Interaction with body mass index
La Vecchia *et al*. (1995)	Six areas in northern Italy, 1991–94, ≤ 74 years	Hospital-based; 2 569 cases, 2 588 controls; structured questionnaire at interview	'Conjugated and other oestrogens': Trend with duration: odds ratios, 1.0, 1.3 and 1.5 for < 1 year, 1–4 and > 5 years of intake (NS) Recency < 10 years: odds ratio, 2.0 (95% CI, 1.3–2.9)	Low power in long-duration categories
Stanford *et al*. (1995)	13 counties, Washington State, USA, 1998–90, cancer survey system, Caucasian, 50–64 years	Population-based; 537 cases, 492 controls (random-digit dialling); personal interviews	Any use: Oestrogen alone: RR, 0.9 (95% CI, 0.6–1.1) Oestrogen with progestogen: RR, 0.9 (95% CI, 0.7–1.3) Duration, recency: no association	Response rate 81% for cases and 73% for controls Low power for long-term use
Newcomb *et al*. (1995)	Four states in northern and eastern USA, tumour registries, 1988–91, age 65–74	Population-based; 3 130 cases, 3 698 controls; personal interviews	'Non-contraceptive hormones, oestrogens and progestogen combinations': Any use: RR, 1.1 (95% CI, 0.9–1.2) Duration: > 15 years: RR, 1.1 (95% CI, 0.9–1.4)	Response rates 81% for cases and 84% for controls Reasonable power for long-duration categories No effects in subgroups

Table 5 (contd)

Reference	Study base	Design: number of cases and controls, data	Risk relationships: relative risk (RR) and 95% confidence intervals (95% CI)	Comments
Levi *et al*. (1996)	Vaud, Switzerland, 1990–95, < 75 years	Hospital-based; 230 cases, 507 controls; interviews in hospitals	'Hormonal therapy': Any use: odds ratio, 1.2 (95% CI, 0.8–1.8) Recency < 10 years: odds ratio, 1.7 (95% CI, 1.1–2.9) Duration ≥ 10 years: odds ratio, 1.0 (95% CI, 0.4–2.4)	No information on participation rates Power limitations
Tavani *et al*. (1997)	Greater Milan area 1983–91, six areas in northern Italy, 1991–94, age 15–74	Two hospital-based studies; 5 984 cases, 5 504 controls; interviews	'Hormonal therapy': Any use: odds ratio, 1.2 (95% CI, 1.0–1.4) Duration > 5 years: odds ratio, 1.3 (95% CI, 0.8–2.0) Significant trend with duration Any use, age at diagnosis: trend of increasing risk with increasing age	Pooled data Low prevalence of any use of hormones Low power for long-duration categories
Lipworth *et al*. (1995)	Residents of greater Athens area, Greece, 4 major hospitals, 1989–91, all ages	Hospital-based; 820 cases, 795 orthopaedic patients; 753 healthy visitors; data from interviews in hospital	'Menopausal oestrogens': Any use: RR, 1.5 (95% CI, 1.2–2.3) Duration: - ≤ 11 months: RR, 1.8 (95% CI, 1.0–3.0) -12–35 months: RR, 1.3 (95% CI, 0.6–2.5) - ≥ 36 months: RR, 1.4 (95% CI, 0.6–3.3) (no trend)	No information on details of exposure, oestrogen–progestogen use rare Low prevalence of hormone use

NS, not significant

The pooled analysis of individual data showed a relationship of increasing risk with increasing duration only for women with current use or use ended within the previous four years. There was no significant variation in results across the individual studies (Collaborative Group on Hormonal Factors in Breast Cancer, 1997).

(*c*) *Recency of intake*

Several of the studies suggest that recency of exposure is the most important determinant of risk (Mills *et al*., 1989; Colditz *et al*., 1992, 1995; Folsom *et al*., 1995; La Vecchia *et al*., 1995; Levi *et al*., 1996). The investigators in the Nurses' Health Study in particular reported that their finding of a 50% increase in risk is best explained in this way (Colditz *et al*., 1995); however, numerous other studies found no relationship between excess risk and current or recent intake (Hulka *et al*. 1982; Kaufman *et al*., 1984; Wingo *et al*., 1987; Ewertz, 1988; Rohan & McMichael, 1988; Kaufman *et al*., 1991; Palmer *et al*., 1991; Stanford *et al*., 1995). Several of the studies did not include an analysis by recency of intake. The main results of the pooled analysis of individual data was an excess risk related to current use or use terminated within five years

(*d*) *Latency of intake*

Most studies showed no independent association with the number of years since first use (Hulka *et al*., 1982; Hiatt *et al*., 1984; Kaufman *et al*., 1984; Nomura *et al*., 1986; Wingo *et al*., 1987; Rohan & McMichael, 1988; Palmer *et al*., 1991; La Vecchia *et al*., 1995; Stanford *et al*., 1995; Brinton, 1997).

(*e*) *Compound, dose and route of administration*

Studies in the United States reflect use almost exclusively of conjugated oestrogens, while studies in Europe give results of exposure mainly to the other oestrogens, such as oestradiol valerate, oestradiol and, to a minor extent, the synthetic oestrogen ethinyloestradiol. In the European studies (Hunt *et al*., 1987; Ewertz, 1988; Bergkvist *et al*., 1989; La Vecchia *et al*., 1995), the infrequent use of conjugated oestrogens provided insufficient power for comparative analyses. Oestradiol compounds and conjugated oestrogens seem to have similar oestrogenic effects on the target organs, e.g. with regard to endometrial cancer (Persson *et al*., 1989; Beresford *et al*., 1997) and breast cancer in studies in Europe and the United States (Bergkvist *et al*., 1989; Colditz *et al*., 1995). Neither the pooled analysis of individual data nor studies in the United States (Hulka *et al*., 1982; Hiatt *et al*., 1984; Kaufman *et al*., 1984; Brinton *et al*., 1986; Wingo *et al*., 1987; Stanford *et al*., 1995) showed a difference in risk with dose of conjugated oestrogens (i.e. 0.625 versus 1.25 mg).

Data on risk by type of administration are scarce. No pattern of risk has been related to cyclic versus continuous intake of oestrogens (Hulka *et al*., 1982; Brinton *et al*., 1986). Vaginal application of oestrogen was not related to the risk for breast cancer (Colditz *et al*., 1992), whereas parenteral administration was linked to an increased risk in one study (Hulka *et al*., 1982).

(*f*) *Susceptibility factors*

In epidemiological research, interest has focused on whether certain sub-groups of women are more likely to develop breast cancer after post-menopausal hormonal therapy. Such analyses are often hampered by lack of power. In the pooled analysis of individual data, the only significant effect modifier was body mass index: the adverse effect of hormone treatment was greater for women with a body mass index < 25 kg/m^2. Other types of factor addressed in individual studies are described below.

(i) *Type of menopause*

Since oophorectomy and time of natural menopause are powerful determinants of breast cancer, menopausal status has been examined as a modifier of the risk associated with hormonal therapy. The association in oophorectomized women has been found to be strong in some studies (Hoover *et al*., 1981; Wingo *et al*., 1987; Yang *et al*., 1992; Stanford *et al*., 1995), whereas in other studies, higher risks have been noted for women who had a natural menopause and have intact ovaries (Ross *et al*., 1980; Hulka *et al*., 1982; Ewertz, 1988); some studies found no difference in effect with ovarian status (Kaufman *et al*., 1991; Palmer *et al*., 1991; Newcomb *et al*., 1995).

(ii) *Age at diagnosis*

In the Nurses' Health Study, the increased risk with current intake became progressively more pronounced with increasing age at diagnosis (Colditz *et al*., 1995). An effect of age at diagnosis has also been suggested in some case–control studies (Brinton *et al*., 1981; Wingo *et al*., 1987; Kaufman *et al*., 1991; Palmer *et al*., 1991; La Vecchia *et al*., 1992a). One difficulty in interpreting the data is that the effect of age could be mixed with duration of intake.

(iii) *Body build*

The relative contribution of treatment to post-menopausal oestrogen concentrations is likely to be greater in lean than obese women, since endogenous oestrogen production is enhanced by the amount of fat tissue (Siiteri, 1987). The findings of some epidemiological studies corroborate this hypothesis by showing stronger or unique associations in lean women (Kaufman *et al*., 1991; Palmer *et al*., 1991; Colditz *et al*., 1992; Harris *et al*., 1992a; Newcomb *et al*., 1995; Collaborative Group on Hormonal Factors in Breast Cancer, 1997); conversely, in other studies, the effect was more marked in obese women (Mills *et al*., 1989; La Vecchia *et al*., 1992a).

(iv) *Previous use of combined oral contraceptives*

Current and recent use of combined oral contraceptives has been linked to an increased risk for breast cancer (see the monograph on 'Oral contraceptives, combined'). Few studies have yet been able to address whether the risk associated with hormonal therapy is modified by previous use of combined oral contraceptives, especially for women who have taken high-dose pills. There is no evidence of such an interaction;

however, few data are available (Mills *et al*., 1989; Colditz *et al*., 1992; Schuurman *et al*., 1995; Stanford *et al*., 1995; Collaborative Group on Hormonal Factors in Breast Cancer, 1997). Since cohorts of women who were commonly exposed to combined oral contraceptives are increasingly being treated with hormonal therapy, the possibility of a combined effect becomes an important issue.

(v) *Hereditary breast cancer*

Inherited breast cancer has been studied through the proxy variable of family history, i.e. according to closeness of relationship and age at onset. Further, the share of cancers caused by dominant inheritance is lower for post-menopausal women than for women with breast cancer before the menopause. These circumstances may explain why the findings on the joint effect of family history and hormone use are inconsistent: about as many studies show an increased risk (Hoover *et al*., 1981; Hulka *et al*., 1982; Nomura *et al*., 1986; Wingo *et al*., 1987; Kaufman *et al*., 1991; Newcomb *et al*., 1995) as show an absence of an effect modification (Kaufman *et al*., 1984; Brinton *et al*., 1986; Rohan & McMichael, 1988; Mills *et al*., 1989; Palmer *et al*., 1991; Yang *et al*., 1992; Stanford *et al*., 1995).

(vi) *Benign breast disease*

Women with a history of so-called benign breast disease may have a higher risk of developing breast cancer after post-menopausal hormonal therapy (Ross *et al*., 1980; Brinton *et al*., 1986; Nomura *et al*., 1986; Mills *et al*., 1989), but numerous other studies do not support the association (Hoover *et al*., 1981; Hulka *et al*., 1982; Kaufman *et al*., 1984; Wingo *et al*., 1987; Rohan & McMichael, 1988; Kaufman *et al*., 1991; Palmer *et al*., 1991; Yang *et al*., 1992; La Vecchia *et al*., 1995; Newcomb *et al*., 1995; Stanford *et al*., 1995). Many uncertainties hamper the interpretation of the data, e.g. whether a risk-increasing effect applies to specific types of benign lesions (hyperplasia or atypia) or whether it is use of post-menopausal hormonal therapy before or after diagnosis that is important.

(vii) *Alcohol, reproductive factors*

A few studies have shown an increased risk in association with heavy alcohol consumption (Colditz *et al*., 1992; Gapstur *et al*., 1992). Studies of the combined effects of hormonal therapy and age at menarche, age at first birth, parity and age at menopause have generally yielded null results (Kaufman *et al*., 1984; Nomura *et al*., 1986; Palmer *et al*., 1991; Yang *et al*., 1992; Stanford *et al*., 1995), whereas a few others found stronger associations among users who were older at the time of the birth of their first child (Colditz *et al*., 1992), with multiparity (La Vecchia *et al*., 1992a) and with late menopause (Wingo *et al*., 1987; Ewertz, 1988; Mills *et al*., 1989).

(*g*) *Tumour characteristics*

More intense surveillance of users of hormonal therapy may lead to earlier detection and bias with regard to latency. There is evidence of an increased risk for breast cancer after hormone treatment in two studies performed in a population of women participating in a

breast cancer screening programme in the United States, in which the impact of detection bias should be low. Thus, in a case–control study (Brinton *et al*., 1986), the positive duration-dependent relationship was significant and strongest for in-situ or small (≤ 1 cm) tumours. In a subsequent follow-up study (Schairer *et al*., 1994), a doubling of the relative risk with oestrogen use for more than 10 years was limited to in-situ tumours. Further, in a Swedish record-linkage study, the odds ratio of having a small tumour (≤ 2 cm) and spread to axillary lymph nodes was lower for women prescribed oestrogens (in combination with progestogens), even when adjustment was made for mode of detection (mammography screening or examinations because of symptoms) (Magnusson *et al*., 1996).

In the pooled analysis of individual data, the adverse effect of hormone use was stronger for women with cancers localized to the breast than for those with cancers that had spread beyond the breast (Collaborative Group on Hormonal Factors in Breast Cancer, 1997).

(*h*) *Survival and mortality*

Mortality rates due to breast cancer were analysed during a 12-year follow-up, after correction for the comparative external mortality rates for prevalent breast disease (Yuen *et al*., 1993). The standardized mortality ratio estimates were still slightly below baseline (0.8). A reduction in the rate of mortality from breast cancer among women using hormone treatment as compared with those who were not has been found in other cohort studies (Petitti *et al*., 1987; Hunt *et al*., 1990; Henderson *et al*., 1991; Willis *et al*., 1996). In the Nurses' Health Study, the relative risks for death from breast cancer were 0.76 (95% CI, 0.56–1.02) for current use and 0.83 (95% CI, 0.63–1.1) for past use, but rose to 1.4 (95% CI, 0.82–2.5) for current use for 10 or more years (Grodstein *et al*., 1997). These data should be interpreted cautiously.

In summary, the preponderence of evidence suggests an increase in risk for breast cancer with increasing duration of use of post-menopausal oestrogen therapy for current and recent users.

2.2 Endometrial cancer

2.2.1 *Descriptive studies*

In the United States, use of oestrogens at menopause increased during the 1960s. With increasing evidence of an association between post-menopausal oestrogen therapy and endometrial cancer, the United States Food and Drug Administration issued a warning to physicians in 1976. A decline in post-menopausal oestrogen therapy use ensued and was later followed by an increase in the use of post-menopausal oestrogen–progestogen therapy (Austin & Roe, 1982; Standeven *et al*., 1986; Gruber & Luciani, 1986; Ross *et al*., 1988). The incidence of uterine corpus cancer (as a proxy for endometrial cancer) began to rise in the 1960s, reached a peak in the mid-1970s and then declined until the 1990s (Persky *et al*., 1990). The increased incidence was found primarily among post-menopausal women and followed and then paralleled the increase in the use of post-menopausal oestrogen therapy.

2.2.2 *Cohort studies*

The impact of post-menopausal oestrogen therapy on the occurrence of endometrial cancer has been investigated in eight cohort studies (Table 6). Six of the studies (Hammond *et al*., 1979; Gambrell *et al*., 1980; Vakil *et al*., 1983; Lafferty & Helmuth, 1985; Hunt *et al*., 1987; Ettinger *et al*., 1988) showed an elevated risk associated with any use of post-menopausal oestrogen therapy, without specifying the risk by duration or dose, while two others (Paganini-Hill *et al*., 1989; Persson *et al*., 1989) also provided risk estimates related to the duration of use. Women who had ever used post-menopausal oestrogen therapy were more likely to develop endometrial cancer than non-users in all these studies (relative risk, 1.3–10). In three cohort studies (Hammond *et al*., 1979; Hunt *et al*., 1987; Persson *et al*,. 1989), the increased risk was significant; in two reports (Paganini-Hill *et al*., 1989; Persson *et al*., 1989) described in detail below, the risk estimates for duration of use were given.

In the study of Paganini-Hill *et al*. (1989), the risk for endometrial cancer increased from 5.2 [95% CI not provided] for ≤ 2 years of use to 20 for ≥ 15 years of use (95% CI, 7.2–54; p for trend, < 0.0001). A sustained increase in risk was noted after the cessation of therapy. Women who had stopped post-menopausal oestrogen therapy 15 or more years previously still had a nearly sixfold increase in risk (5.8; 95% CI, 2.0–17) relative to women who had never used them. In an analysis of oestrogen dose in this study, the risk for women using higher doses (≥ 1.25 mg; relative risk, 11.0 [95% CI not provided]) did not differ from that of women using lower doses (≤ 0.625 mg; relative risk, 15.0 [95% CI not provided]).

In the case–cohort study of Persson *et al*. (1989), the risk increased with increasing duration of use, from 1.1 (95% CI, 0.5–2.5) among users for six months or fewer to 1.8 (95% CI, 1.1–3.2) among women who had used post-menopausal oestrogen therapy for 73 months or more. Use of either conjugated oestrogen or oestradiol was associated with an increase in risk, with a relative risk of 1.7 (95% CI, 1.1–2.7) for conjugated oestrogen and 2.1 (95% CI, 1.4–3.0) for oestradiol.

2.2.3 *Case–control studies*

Over 30 studies have been conducted to investigate the association between post-menopausal oestrogen therapy and endometrial cancer (Table 7); all except one (Salmi, 1980) reported an elevated risk for women with any use of post-menopausal oestrogen therapy relative to those who had never used it (relative risks, 1.3–12.0), and in 22 studies the excess was statistically significant. Both qualitative reviews (Herrinton & Weiss, 1993) and a meta-analysis of the published results (Grady *et al*., 1995) have found an overall excess, with increasing risk with increasing duration of use.

The risk for endometrial cancer has been evaluated in relation to the duration of use, the time since last use (recency), oestrogenic potency (dose), type (conjugated, synthetic) and regimen (continuous versus cyclic with breaks) of therapy.

Over 20 studies that provide information on the duration of post-menopausal oestrogen therapy showed that duration is one of the strongest determinants of risk; the risk continues to increase with continuing duration of use (Ziel & Finkle, 1975; Mack *et al*.,

Table 6. Cohort studies on use of unopposed post-menopausal oestrogen therapy and risk for endometrial cancer

Reference, country	Age at beginning of follow-up (years)	Study group of source population	Comparison group	Approximate duration of follow-up (years)	No. of observed cases	No. of expected cases or person–years	Relative risk or SIR	95% CI
Hammond *et al*. (1979), USA	NR	301 women attending hospital clinic, use of OT ≥ 5 years	Rates from Third National Cancer Survey	NR	11	1.18	9.3	4.7–17
Gambrell *et al*. (1980), USA	NR	Women attending medical centre, any use of OT	Women attending medical centre, no use of OT	≤ 11	NR	NR	[1.6]	NR
Vakil *et al*. (1983), Canada	32–62	1 483 women attending gynaecology clinics	Rates from Ontario	≤ 17	8	6.2	1.3	NR, NS
Lafferty & Helmuth (1985), USA	45–60	61 women attending a private clinic, use of OT ≥ 3 years	63 women attending a private clinic, no OT use	3–16	NR	NR	[2.7]	NR
Hunt *et al*. (1987), UK	NR	4 544 women attending 21 menopause clinics	Rates from Birmingham Cancer Registry	5	12	4.2	2.8	1.5–5.0
Ettinger *et al*. (1988)	≥ 53	181 members of health maintenance organization with ≥ 5 years of OT	220 members with ≤ 1 year use of OT	≤ 24	5 years of use: 18; ≤ 1 year of use: 3	2 705 person–years 4 197 person–years	[9.3]	NR
Paganini-Hill *et al*. (1989), USA	44–100	5 160 women in a retirement community	Internal comparison	5	Never use: 45 Any use: 5	11 281 person–years 12 472 person–years	10	NR

Table 6 (contd)

Reference, country	Age at beginning of follow-up (years)	Study group of source population	Comparison group	Approximate duration of follow-up (years)	No. of observed cases	No. of expected cases or person–years	Relative risk or SIR	95% CI
Persson *et al.* (1989), Sweden	≥ 35	23 244 women with ≥ 1 prescription for any OT use	Rates from the Uppsala health care region	6	48	34.3	1.5	1.1–1.9

SIR, standardized incidence ratio; CI, confidence interval; NR, not reported; OT, oestrogen therapy; NS, not significant

Table 7. Case–control studies on any use of oestrogen alone and the risk for endometrial cancer

Reference, country	Age (years)	No. of cases/ no. of controls	Proportion (%) of cases/controls exposed	Odds ratio (95% CI)	Longest duration of OT use (years)	Odds ratio (95% CI) for longest duration of OT use
Smith *et al*. (1975), USA	≥ 48	317/317	48/17	4.5 [3.1–6.6]	NR	NR
Ziel & Finkle (1975), USA	57	94/188	57/15	7.6 [4.7–11]	≥ 7	14 (NR)
Mack *et al*. (1976), USA	≥ 52	63/252	NR/43	5.6 (2.8–11)	≥ 8	8.8 (NR)
Gray *et al*. (1977), USA	57	205/205	16/6	3.1 (1.5–6.8)	≥ 10	12 (1.5–240)
McDonald *et al*. (1977), USA	≥ 25	145/580	27/28	0.9 (0.6–1.4)	≥ 3	7.9 (2.9–21)
Horwitz & Feinstein (1978), USA	62	119/119	29/3	12 (4.0–48)	NR	NR
Hoogerland *et al*. (1978), USA	NR	587/587	18/9	2.2 (1.6–3.2)	≥ 10	6.7 (NR)
Antunes *et al*. (1979), USA	NR	451/888	17/4	5.5 (2.3–13)	≥ 5	15 (4.9–45)
Weiss *et al*. (1979), USA	50–74	322/289	69/25	[6.3] (NR)	≥ 20	8.3 (2.8–25)
Hulka *et al*. (1980), USA	61	256/861	33/35[a]	1.4 (0.9–2.1)	≥ 9.5	5.5 (1.9–16)[a]
Jelovsek *et al*. (1980), USA	58	431/431	12/6	2.4 (1.4–3.9)	≥ 10	2.6 (1.1–5.9)
Salmi (1980), Finland	35–60	282/282	6/15	0.4[b] (0.2–0.7)	NR	NR
Spengler *et al*. (1981), Canada	40–74	88/177	45/22	2.9 (1.7–5.1)	≥ 5	8.6 (3.2–23)
Stavraky *et al*. (1981), Canada	40–80	206/199[c]	47/29	4.8 (2.7–8.4)	≥ 10	14 (5.0–42)
Kelsey *et al*. (1982), USA	45–74	167/903	36/19	1.6 (1.3–2.0)[c]	≥ 10	2.7 (NR)
Henderson *et al*. (1983), USA	≤ 45	127/127	12/7	[1.8] (NR)	≥ 2	3.1 (NR)
La Vecchia *et al*. (1984), Italy	33–74	283/566	25/17	2.3 (1.6–3.2)	NR	'Trend'
Ewertz *et al*. (1984), Denmark	NR	115/115	18/13	4.9 (2.0–12)	≥ 1	1.7 (0.4–6.9)
Shapiro *et al*. (1985), USA/Canada	50–69	425/792	31/15	3.5 (2.6–4.7)	NR	NR
Petterson *et al*. (1986), Sweden	34–90	254/254	16/12	1.3 (0.8–2.1)	≥ 4	4.3 (1.3–14)
Buring *et al*. (1986), USA	40–80	188/428	39/20	2.4 (1.7–3.6)	≥ 10	7.6 (NR)
Ewertz (1988), Denmark	44–89	149/154	56/21	4.7 (2.9–7.7)	NR	NR
Koumantaki *et al*. (1989), Greece	40–79	83/164	10/6	2.0 (0.8–5.1)	NR	NR
Rubin *et al*. (1990), USA	20–54	196/986	24/14	1.9 (1.3–2.8)	≥ 6	3.5 (1.7–7.4)
Voigt *et al*. (1991), USA	40–64	158/182	19/7	3.1 (1.6–5.8)	> 3	5.7 (2.5–13)
Jick (1993), USA	50–64	172/172	75/49	6.5 (3.1–13)	≥ 5	22 (6.5–74)
Levi *et al*. (1993a), Switzerland	32–74	158/468	38/20	2.7 (1.7–4.1)	≥ 5	5.1 (2.7–9.8)

Table 7 (contd)

Reference, country	Age (years)	No. of cases/ no. of controls	Proportion (%) of cases/controls exposed	Odds ratio (95% CI)	Longest duration of OT use (years)	Odds ratio (95% CI) for longest duration of OT use
Brinton *et al*. (1993), USA	20–74	300/207	24/14	3.0 (1.7–5.1)	≥ 5	6.0 (2.7–13)
Finkle *et al*. (1995), USA	29–85	NR	54/44	5.0 (2.9–9.8)	NR	NR
Green *et al*. (1996), USA	45–74	661/865	49/21	[2.0] [1.7–2.5]	> 12	16 (10–26)
Beresford *et al*. (1997), USA	45–74	832/1 114	15/13	2.7 (1.9–4.0)	NR	NR
Goodman *et al*. (1997b), USA	18–84	332/511	50/32	2.6 (1.8–3.8)	≥ 3	3.6 (2.2–6.0)
Pike *et al*. (1997), USA	50–74	833/791	51/33	2.2[d] (1.9–2.5)	NR	NR
Cushing *et al*. (1998), USA	45–64	484/780	30/12	5.4 (2.3–13)	> 8	8.4[e] (4.0–18)

CI, confidence interval; OT, oestrogen therapy; NR, not reported

[a] Only for 321 community controls

[b] Risk for oestriol use; risk for conjugated oestrogen use, 5.0 [CI not reported]

[c] Controls without gynaecological disorders

[d] Risk per five years of use

[e] > 1.25 mg/day

1976; Gray *et al*., 1977; McDonald *et al*., 1977; Hoogerland *et al*., 1978; Antunes *et al*., 1979; Hulka *et al*., 1980; Jelovsek *et al*., 1980; Shapiro *et al*., 1980; Spengler *et al*., 1981; Stavraky *et al*., 1981; Kelsey *et al*, 1982; La Vecchia *et al*., 1984; Shapiro *et al*., 1985; Buring *et al*., 1986; Rubin *et al*., 1990; Brinton *et al*., 1993; Pike *et al*., 1997). Use for less than six months was found not to increase the risk in four studies (McDonald *et al*., 1977; Hoogerland *et al*., 1978; Hulka *et al*., 1980; Spengler *et al*., 1981), while two studies that included the risk of use for six months to one year (McDonald *et al*., 1977; Hoogerland *et al*., 1978) found increased risks in this category of duration also. In the meta-analysis of the published results (Grady *et al*., 1995), the overall relative risk was 2.3 (95% CI, 2.1–2.5) for oestrogen users when compared with non-users. The summary relative risk for less than one year of use was 1.4 (95% CI, 1.0–1.8), whereas that for use for more than 10 years was 9.5 (95% CI, 7.4–12).

In some studies, but not all (Brinton *et al*., 1993; Finkle *et al*., 1995), that addressed the risk associated with recency of post-menopausal oestrogen therapy, the risk for endometrial cancer remained higher than in non-users even 10 years after cessation (Shapiro *et al*., 1985; Levi *et al*., 1993a; Finkle *et al*., 1995; Green *et al*., 1996). Women with the longest durations of post-menopausal oestrogen therapy had especially high excess risks after discontinuation of use (Rubin *et al*., 1990; Green *et al*., 1996). In the meta-analysis of the published results (Grady *et al*., 1995), the summary relative risk was largest for the group of women who had ceased use within one year or less (relative risk, 4.1; 95% CI, 2.9–5.7) but remained elevated (2.3; 95% CI, 1.8–3.1) five years or more after cessation.

An elevated risk for endometrial cancer is associated with all commonly prescribed doses of conjugated oestrogens (Gray *et al*., 1977; Antunes *et al*., 1979; Weiss *et al*., 1979; Hulka *et al*., 1980; Stavraky *et al*., 1981; Jick *et al*., 1993; Cushing *et al*., 1998). Four studies that addressed the effect of a low dose (0.3 mg/day) on the risk for endometrial cancer (Gray *et al*., 1977; Weiss *et al*., 1979; Jick *et al*., 1993; Cushing *et al*., 1998) yielded consistent results: the risk of women using low doses did not differ from that of women using high doses (0.625 mg). In the meta-analysis of the published studies (Grady *et al*., 1995), the summary relative risks were 3.9 (95% CI, 1.6–9.5) for any use of low doses (0.3 mg), 3.4 (95% CI, 2.0–5.6) for intermediate doses (0.625 mg) and 5.8 (95% CI, 4.5–7.5) for high doses (≥ 1.25 mg), but these values did not differ significantly.

Use of oestrogens other than conjugated ones (e.g. oestradiol) was commoner in Europe than in the United States (Persson *et al*., 1989). Other oestrogens have been shown to be related to an increased risk for endometrial cancer in most (Mack *et al*., 1976; Weiss *et al*., 1979; Antunes *et al*., 1979; Buring *et al*., 1986) but not all (Shapiro *et al*., 1980) studies of the type of post-menopausal oestrogen therapy. In the meta-analysis of the published results (Grady *et al*., 1995), users of conjugated oestrogens had greater risk for endometrial cancer (relative risk, 2.5; 95% CI, 2.1–2.9) than users of other oestrogens (1.3; 95% CI, 1.1–1.6).

Most cases of endometrial cancer related to post-menopausal oestrogen therapy have been of the well-differentiated histological type and at an early clinical stage (McDonald

et al., 1977; Antunes *et al*,. 1979; Buring *et al*., 1986; Rubin *et al*., 1990). Myometrial invasion has been reported in only a few cases (Mack *et al*., 1976; McDonald *et al*., 1977; Antunes *et al*., 1979; Weiss *et al*., 1979; Jelovsek *et al*., 1980; Buring *et al*., 1986). In the meta-analysis of the published results (Grady *et al*., 1995), the summary relative risk for early-stage (0–1) cancer was higher (4.2; 95% CI, 3.1–5.7) than that for later stages (2–4) (1.4; 95% CI, 0.8–2.4). Similarly, the summary risk estimate for non-invasive cancer was higher (6.2; 95% CI, 4.5–8.4) than that for invasive cancer (3.8; 95% CI, 2.9–5.1) (Grady *et al*., 1995). Post-menopausal oestrogen therapy was related to the risk for death from endometrial cancer in four studies (Lafferty & Helmuth, 1985; Petitti *et al*., 1987; Ettinger *et al*., 1988; Paganini-Hill *et al*., 1989) and in the meta-analysis (Grady *et al*., 1995). Each of these studies reported at least a doubling of the risk for death from endometrial cancer among women who had ever used post-menopausal oestrogen therapy as compared with those who had never done so.

An increased risk for endometrial cancer has been associated with both continuous and cyclic oestrogen use (Mack *et al.*, 1976; McDonald *et al.*, 1977; Antunes *et al.*, 1979; Weiss *et al.*, 1979; Hulka *et al.*, 1980; Buring *et al.*, 1986) as well as with intermittent regimens (McDonald *et al.*, 1977; Antunes *et al.*, 1979). There were no differences in the summary relative risk estimates for the continuous regimen (2.9; 95% CI, 2.2–3.8) and intermittent and cyclic regimens (3.0; 95% CI, 2.4–3.8) in the meta-analysis (Grady *et al.*, 1995).

Weight and smoking have been reported to modify the relationship between post-menopausal oestrogen therapy and the risk for endometrial cancer. Some studies (Kelsey *et al.*, 1982; Ewertz *et al.*, 1984; La Vecchia *et al.*, 1984; Ewertz *et al.*, 1988; Levi *et al.*, 1993a) indicate that the effects of obesity and oestrogen use are not multiplicative; leaner women have a higher risk for endometrial cancer than women with higher body mass indices (La Vecchia *et al.*, 1982a). Smoking modified the relationship between post-menopausal oestrogen therapy and endometrial cancer in three case–control studies (Franks *et al.*, 1987; Koumantaki *et al.*, 1989; Levi *et al.*, 1993a). Franks *et al.* (1987) presented risks for endometrial cancer stratified by smoking: post-menopausal non-smoking women using oestrogen therapy had a higher relative risk (3.8; 95% CI, 1.7–8.2) than smokers using such therapy (1.0; 95% CI, 0.4–2.6). Levi *et al.* (1993a) and Koumantaki *et al.* (1989) reported similar risk estimates. [Although the effect of smoking is biologically plausible, it cannot be regarded as protective against endometrial cancer.]

2.3 Cervical cancer

2.3.1 *Methodological considerations*

The methodological issues that arise in studies of oral contraceptives and cervical cancer also apply to studies of post-menopausal oestrogen therapy and this disease (see section 2.3 of the monograph on ‘Oral contraceptives, combined’). Briefly, exogenous oestrogens may affect various stages in the development of cervical cancer, and epidemiological studies of intraepithelial lesions and invasive lesions should therefore be considered separately. There are also two main histological types of invasive disease, squamous-cell

carcinoma and adenocarcinoma, and ideally these also should be considered separately. In assessing associations between exogenous oestrogens and cervical cancer, the potentially confounding effects of sexual factors and infection by oncogenic strains of the human papillomavirus should be considered. Finally, the influence of Papanicolaou (Pap) smear screening should be considered, as women on post-menopausal hormonal therapy may be more likely to have Pap smears than women not on this therapy.

In the study of Persson *et al*. (1997a) in Sweden, women taking post-menopausal hormonal therapy tended to be of lower parity, older at the birth of their first child and have a higher prevalence of hysterectomy or oophorectomy than women who did not receive such therapy. In addition, a higher level of education was associated with long-term exposure to post-menopausal hormonal therapy, as were heavy physical exercise and diets rich in fibre. Women who had used oral contraceptives were more likely to use oestrogens both with and without progestogen than women who had not used oral contraceptives. These observations serve to demonstrate the importance of considering potentially confounding variables when assessing observed relationships between post-menopausal hormonal therapy and the risks for various neoplasms.

2.3.2 *Cohort studies*

The risk for cervical carcinoma in relation to post-menopausal hormonal therapy has been considered in two cohort studies. Adami *et al.* (1989) reported results for a cohort of 23 244 Swedish women who had been given prescriptions for such therapy. The cohort was assembled between 1977 and 1980 and followed through to the end of 1984. The observed numbers of women with various cancers were compared with expected numbers based on the incidence rates in the population from which the cohort members were accrued. Women who had ever used post-menopausal oestrogen therapy had a relative risk for cervical cancer of 0.8 (95% CI, 0.5–1.2) in comparison with women who had never used oestrogens (27 observed and 34.05 expected cases). The risk in relation to duration of use was not calculated. The risk was lower for women who had used conjugated oestrogens or oestradiol (0.6; 95% CI, 0.3–1.0) than for women who had used other compounds, mainly oestriol (1.3; 95% CI, 0.7–2.3), although oestriol is a less potent oestrogen than conjugated oestrogens or oestradiol. Women who were under 60 years of age at entry into the cohort had a relative risk of 0.6 (95% CI, 0.4–1.0) when compared with older women, whose relative risk was 1.2 (95% CI, 0.6–2.3). This difference was observed for use of either conjugated oestrogens or oestradiol and for use of oestriol. The risk was also somewhat lower for women who were followed for more than five years than for women who were followed for a shorter period: the relative risk of women followed from 0–4 years was 0.9 (95% CI, 0.6–1.3) and that for women followed for five or more years was 0.6 (95% CI, 0.2–1.3). The investigators were unable to control for sexual variables, prior Pap smear screening or human papillomavirus infection. An updated report of the same study (Schairer *et al*., 1997) gave a relative risk for dying of cervical cancer in relation to use of post-menopausal hormonal therapy of 1.2 (95% CI, 0.8–1.7), based on 23 deaths after follow-up through the end of 1986.

In a study in Britain (Hunt *et al*., 1990), 4544 women who had received continuous post-menopausal hormone treatment for at least one year were recruited from 21 pre-menopause clinics around the country between 1974 and 1982, and were followed through 1988. During this period, two women died of cervical cancer, whereas the expected number was 6.8 on the basis of mortality rates for England and Wales. This gave a rate ratio of 0.3 (95% CI, 0.0–1.1).

2.3.3 *Case–control studies*

The results of only one case–control study of post-menopausal oestrogen therapy and cervical cancer have been published (Parazzini *et al*., 1997). In this hospital-based study conducted in northern Italy, 645 women with invasive cervical cancer were compared with 749 women admitted to the same hospitals with acute conditions. After adjustment for age, calendar year of interview, number of sexual partners, parity, oral contraceptive use, lifetime number of cervical smears, social class, smoking and menopausal status, the relative risk for women who had ever used post-menopausal oestrogen therapy was estimated to be 0.5 (95% CI, 0.3–0.8). The risk of women who had used post-menopausal oestrogen therapy for fewer than 12 months was 0.6 (95% CI, 0.4–1.1) and that for women who had used it for 12 or more months was 0.5 (95% CI, 0.2–1.0) (*p* value for trend, < 0.01). Consistent with the results of Adami *et al*. (1989) described above, the risk of women who had last used post-menopausal oestrogen therapy more than 10 years previously was 0.4 (95% CI, 0.2–0.7), whereas that for women who had last used these products within the past 10 years was 0.9 (95% CI, 0.5–1.7); the risk was lower for women who had first used these products before the age of 50 (0.4; 95% CI, 0.2–0.7) than for women who had first used them at a greater age (0.8; 95% CI, 0.4–1.5).

2.4 Ovarian cancer

2.4.1 *Descriptive studies*

Over the last few decades, no major or systematic trend in incidence or mortality rates has been observed for ovarian cancer in elderly women (Adami *et al.*, 1990; La Vecchia *et al.*, 1992b; Koper *et al.,* 1996; La Vecchia *et al.*, 1998). Consequently, descriptive data on the incidence of and mortality from ovarian cancer do not indicate an effect of post-menopausal oestrogen therapy.

2.4.2 *Cohort studies*

The main findings of cohort studies on post-menopausal oestrogen therapy and ovarian cancer risk are given in Table 8.

In a 13-year follow-up for mortality, between recruitment in 1968–72 and 1983, in a study in the United States on contraception use in 16 638 women aged 18–59, six deaths from ovarian cancer were observed among women who had ever used post-menopausal oestrogen therapy (relative risk, 0.9; 95% CI, 0.3–2.8) (Petitti *et al.,* 1987).

The relationship between post-menopausal oestrogen therapy and ovarian cancer was also analysed in the data from the American Cancer Society's cancer prevention study (II)

Table 8. Selected cohort studies on post-menopausal oestrogen therapy and ovarian cancer, 1980–97

Reference, country	Outcome	No. of cases	Relative risk for any use (95% CI)	Comments
Petitti *et al.* (1987), USA	Mortality	6	0.9 (0.3–2.8)	13-year mortality follow-up of the study on contraception
Rodriguez *et al.* (1995), USA	Mortality	436	1.2 (0.9–1.4)	Significant: direct relationship with duration ($p = 0.03$). RR, 1.4 (95% CI, 0.9–2.1) for 6–10 years and RR, 1.7 (95% CI, 1.1–2.8) for ≥ 11 years of use
Adami *et al.* (1989), Sweden	Incidence	64	1.0 (0.7–1.2)	Cohort of 23 246 women prescribed post-menopausal oestrogen therapy, followed for an average of 6.7 years
Schairer *et al*. (1997), Sweden	Mortality	52	1.0 (0.8–1.3)	Same cohort as Adami *et al*. (1989); follow-up for mortality, 8.6 years

CI, confidence interval; RR, relative risk

for 240 073 peri- and post-menopausal women enrolled in 1982; 436 deaths from ovarian cancer were registered over seven years of follow-up (Rodriguez *et al.*, 1995). The relative risk was 1.2 (95% CI, 0.9–1.4) for any use of oestrogen and rose to 1.4 (95% CI, 0.9–2.1) for 6–10 years of use and to 1.7 (95% CI, 1.1–2.8) for ≥ 11 years of use. This elevated risk was not explained by allowance for other known or likely risk factors for ovarian cancer.

In a Swedish record-linkage prospective study of 23 246 women who were prescribed menopausal oestrogens, recruited between 1977 and 1980 and followed-up for an average of 6.7 years (Adami *et al.,* 1989), 64 cases of ovarian cancer were observed versus 66.64 expected (relative risk, 1.0; 95% CI, 0.7–1.2). After 8.6 years of follow-up (Schairer *et al*., 1997), 52 deaths from ovarian cancer were observed versus 52.7 expected (relative risk, 1.0; 95% CI, 0.8–1.3).

2.4.3 *Case–control studies*

At least 12 case–control studies published after 1979 and a pooled analysis of individual data from 12 studies of ovarian cancer have provided data on post-menopausal oestrogen therapy (Table 9). Of these, seven studies, including an investigation of 205 cases in the United States (Weiss *et al.*, 1982), a multicentre case–control study of 377 cases in various areas of Canada, Israel and the United States (Kaufman e*t al.*, 1989), a population-based case–control investigation of 367 cases and 564 controls in Ontario,

Table 9. Selected case–control studies on post-menopausal oestrogen therapy and ovarian cancer, 1980–97

Reference, country	Type of study	No. of cases	Age (years)	Relative risk for any use (95% CI)	Comments
Hildreth *et al.* (1981), USA	Hospital-based	62	45–74	0.9 (0.5–1.6)	
Weiss *et al.* (1982), USA	Population-based	205	36–75	1.3 (0.9–1.5)	Stronger association for endometrioid ovarian cancer (3.1; 95% CI, 1.0–9.8)
Franceschi *et al.* (1982), Italy	Hospital-based	161	19–69	[1.0]	No effect
Tzonou *et al.* (1984), Greece	Hospital-based	150	All	1.6 (post-menopausal)	Not significant
Harlow *et al.* (1988), USA	Population-based	116	20–79	0.9	Ovarian neoplasms of borderline malignancy
Kaufman *et al.* (1989), Canada, Israel, USA	Hospital-based	377	18–69	1.1 (0.8–1.6)	Unopposed oestrogens only. No association with combined treatment (RR, 0.7; 95% CI, 0.2–1.8) or with specific histotypes
Booth *et al.* (1989), UK	Hospital-based	225	< 65	1.5 (0.9–2.6)	No association with specific histotypes
Polychronopoulou *et al.* (1993), Greece	Hospital-based	189	< 75	1.4 (0.4–4.9)	Based on 6 exposed cases and 4 controls only
Parazzini *et al.* (1994), Italy	Hospital-based	953	23–74	1.6 (1.2–2.4)	Adjusted for major covariates, including combined oral contraceptive use
Purdie *et al.* (1995), Australia	Population-based	824	18–79	1.0 (0.8–1.3)	Adjusted for major covariates, including oral contraceptive use
Risch *et al.* (1996), Ontario, Canada	Population-based	367	35–79	1.3 (0.9–2.0)	Multivariate RR, 2.0 (95% CI, 1.0–4.0) for serous and 2.8 (95% CI, 1.2–6.9) for endometrioid for ≥ 5 years of use. No association with mucinous tumours

Table 9 (contd)

Reference, country	Type of study	No. of cases	Age (years)	Relative risk for any use (95% CI)	Comments
Hempling *et al.* (1997), USA	Hospital-based	491	NR	0.9 (0.6–1.2)	Other cancers as controls
Re-analysis of original data					
Whittemore *et al.* (1992), USA	Pooled analysis of 12 US hospital- and population-based case-control studies	2 197	All	0.9 (0.7–1.3) (hospital-based) 1.1 (0.9–1.4) (population-based)	Invasive cancers. No trend in risk with duration. RR per year of use, 1.0 for both hospital- and population-based studies
Harris *et al.* (1992b), USA	Pooled analysis of same 12 studies as Whittemore *et al*. (1992) but for tumours of low malignant potential	327	All	1.1 (0.7–1.9)	Ovarian neoplasms of borderline malignancy. No difference between hospital-based and population-based studies. No trend in risk with duration

CI, confidence interval; RR, relative risk; NR, not reported

Canada (Risch, 1996), and four European studies, from the United Kingdom (Booth *et al*., 1989), Greece (Tzonou *et al*., 1984; Polychronopoulou *et al.*, 1993) and Italy (Parazzini *et al.*, 1994), reported relative risks between 1.2 and 1.6. Other case–control studies, including the pooled analysis of individual data from the United States studies (Whittemore *et al.*, 1992), however, showed no consistent association.

Hildreth *et al.* (1981) provided data on 62 cases of ovarian cancer and 1068 controls in seven hospitals in Connecticut, United States, between 1977 and 1979. The response rate was 71% for both cases and controls. The relative risk for any use of post-menopausal oestrogen therapy was 0.9 (95% CI, 0.5–1.6).

Weiss *et al.* (1982) considered 205 cases of epithelial ovarian cancer diagnosed between 1975 and 1979 and 611 population controls in Washington State and Utah, United States. The overall relative risk for any use of post-menopausal oestrogen therapy was 1.3 (95% CI, 0.9–1.8), and there was no consistent time–risk relationship; however, the relative risk was 3.1 (95% CI, 1.0–9.8) for the 17 endometrioid tumours. Allowance was made for age, state of residence and hysterectomy.

Franceschi *et al.* (1982) reported data on 161 cases and 561 population controls interviewed in 1979–80 in greater Milan, northern Italy. Any use of non-contraceptive oestrogens was reported by 17% of cases and 17% of controls, corresponding to an age-adjusted relative risk of [1.0]. The duration of use was also similar in cases and controls.

In a hospital-based case–control investigation of 150 case and 250 control women interviewed in 1980–81 in Athens, Greece (Tzonou *et al.,* 1984), the relative risk for any use of post-menopausal oestrogen therapy was 1.6 (not significant). No information was available on duration of use or other time factors.

Harlow *et al.* (1988) considered 116 cases of ovarian cancer of borderline malignancy and 158 hospital controls in western Washington, United States, diagnosed between 1980 and 1985. The response rate was 68% for cases and 74% for controls. The relative risk for any use of post-menopausal oestrogen therapy was 0.9, in the absence of a consistent duration–risk relationship.

Kaufman *et al.* (1989) conducted a multicentre case–control study in Canada, Israel and the United States on 377 cases of epithelial ovarian cancer and 2030 hospital controls interviewed between 1976 and 1985. The multivariate relative risk for any use of post-menopausal oestrogen therapy was 1.1 (95% CI, 0.8–1.6), after allowance for socio-demographic factors, age at menarche, parity, menopausal status, age at menopause and oral contraceptive use, but it rose to 1.6 (95% CI, 0.8–3.2) for ≥ 10 years of use. The trend in risk with duration was not significant. No appreciable heterogeneity was observed across different histological types.

A study of 235 cases and 451 hospital controls conducted between 1978 and 1983 in London and Oxford, England (Booth *et al.,* 1989), gave a multivariate relative risk for any use of post-menopausal oestrogen therapy of 1.5 (95% CI, 0.9–2.6) after adjustment for age and social class. No data were available on duration of use.

A study of 189 cases and 200 controls conducted in 1989–91 in greater Athens, Greece (Polychronopoulou *et al.,* 1993) gave a relative risk for any use of post-menopausal

oestrogen therapy of 1.4 (95% CI, 0.4–4.9). No information was given on duration or other time–risk relationships. The response rate of cases was almost 90%. Allowance was made for age, education, weight, age at menarche, parity and age at the birth of the first child.

A study of 953 cases diagnosed between 1983 and 1992 in northern Italy and 2503 hospital controls (Parazzini *et al.,* 1994) found a multivariate relative risk (after allowance for socio-demographic factors, parity, age at menarche, type of menopause, age at menopause and oral contraceptive use) of 1.6 (95% CI, 1.2–2.4) for any use of post-menopausal oestrogen therapy. The relative risk for ≥ 2 years of use was 1.7 (95% CI, 0.9–3.4).

Purdie *et al.* (1995) provided data on 824 cases diagnosed between 1990 and 1993 and 860 population controls in three Australian states. The response rate was 90% for cases and 73% for controls. The multivariate relative risk (adjusted for socio-demographic factors, family history of cancers, talc use, smoking and reproductive and hormonal factors) for any use of post-menopausal oestrogen therapy was 1.0 (95% CI, 0.8–1.3). No information was given on duration of use or any other time–risk relationship.

Risch (1996) reported data on post-menopausal oestrogen therapy for 367 patients with invasive epithelial ovarian cancer and 564 population controls in Ontario, Canada, interviewed during 1989–92. The response rate was 71% for cases and 65% for controls. The relative risk for any use of post-menopausal oestrogen therapy was 1.3 (95% CI, 0.9–2.0) for non-mucinous neoplasms and 0.7 (95% CI, 0.2–2.1) for mucinous ones. The association was apparently strongest (1.9; 95% CI, 1.0–3.5) for endometrioid neoplasms, with a significant duration–risk relationship. Allowance was made in the analysis for age, parity, lactation, combined oral contraceptive use, tubal ligation, hysterectomy and family history of breast cancer.

In a study based on data collected between 1982 and 1995 at the Roswell Park Cancer Institute, United States (Hempling *et al.*, 1997), 491 patients with epithelial ovarian cancer were compared with 741 women admitted for non-hormone-related malignancies. The overall relative risk for any use of post-menopausal oestrogen therapy was 0.9 (95% CI, 0.6–1.2); there was no significant trend with duration of use. The relative risk was 0.6 (95% CI , 0.3–1.4) for ≥ 10 years of use. Further, there was no appreciable heterogeneity across histological types. Allowance was made for age, parity, combined oral contraceptive use, smoking, family history of ovarian cancer, age at menarche, menopausal status and socio-demographic factors.

A pooled analysis of individual data from 12 studies of 2197 white cases of invasive epithelial ovarian cancer and 8893 white controls in the United States (Whittemore *et al.*, 1992) gave a pooled multivariate relative risk for invasive ovarian cancer associated with any use of post-menopausal oestrogen therapy for more than three months of 0.9 (95% CI, 0.7–1.3) for hospital-based studies and 1.1 (95% CI, 0.9–1.4) for population-based studies; there was no consistent duration–risk relationship. The relative risk for use for > 15 years was 0.5 (95% CI, 0.2–1.3) for hospital-based and 1.5 (95% CI, 0.8–3.1) for population-based studies. The overall trend per year of use was 1.0 for both types of

study; neither risk estimate was significant. Allowance was made in the analysis for age, study, parity and combined oral contraceptive use.

In a similar pooled analysis of individual data on 327 cases of epithelial ovarian tumours of borderline malignancy, the relative risk for any use of post-menopausal oestrogen therapy was 1.1 (95% CI, 0.7–1.9) (Harris *et al.*, 1992b).

Earlier studies (La Vecchia *et al.*, 1982b; Weiss *et al.*, 1982) had suggested that endometrioid neoplasms are related to post-menopausal oestrogen therapy, but this suggestion was not confirmed in several subsequent studies (Kaufman *et al.*, 1989; Whittemore *et al.*, 1992). It was thus unclear whether post-menopausal oestrogen therapy is consistently related to any specific histotype of ovarian cancer; however, a recent Canadian study (Risch, 1996) gave relative risks of 1.4 for serous, 1.9 for endometrioid and 0.7 for mucinous tumours, and significant trends in risk with duration of use for serous and endometrioid tumours. The issue of a potential histotype-specific relationship is therefore still open to discussion, although it remains possible that ovarian cancer cases in women who had used post-menopausal oestrogen therapy are more often classified as endometrioid. The available data therefore suggest that there is little or no association between use of post-menopausal oestrogen therapy and invasive epithelial ovarian neoplasms or those of borderline malignancy. No adequate data were available on post-menopausal oestrogen therapy and non-epithelial (germ-cell or sex-cord-stromal) ovarian neoplasms.

2.5 Liver cancer

2.5.1 *Cohort studies*

Goodman *et al.* (1995) reported the results of a study of risk factors for liver cancer in Hiroshima and Nagasaki, Japan. Information was collected by questionnaire from 36 133 men and women between 1978 and 1981, who were followed through population-based cancer registries until 1989. There were 242 cases of hepatocellular carcinoma in the two cities, of which 86 were in women; information on use of female hormone preparations was available for 76 of these cases. Details of the type of female hormones used were not collected, but oral contraceptive use is very rare in Japan and it is likely that the hormones were largely given as post-menopausal hormonal therapy. Sixty-nine of the case women had never used hormones, and seven had used these preparations. The risk for any use relative to no use of hormones, adjusted for city, age at the time of the atomic bombing, attained age and radiation dose to the liver, was 1.3 (95% CI, 0.6–2.8). There was no information on infection with hepatitis viruses, but infection with hepatitis B virus is common in western Japan.

Persson *et al.* (1996) studied the cancer risk after post-menopausal hormonal therapy in a population-based cohort of 22 579 women aged 35 or more and living in the Uppsala health care region, Sweden. Women who had ever received a prescription for post-menopausal hormonal therapy between 1977 and 1980 were identified and followed-up until 1991. Information on hormone use was obtained from pharmacy records. The expected numbers of cases were calculated from national incidence rates. There was no information on smoking or alcohol consumption. The standardized incidence ratio for all cancers was

1.0 (95% CI, 0.9–1.0). There were 43 cancers of the hepatobiliary tract, comprising 14 hepatocellular carcinomas, five cholangiocarcinomas, 23 gall-bladder cancers and one unclassified; the expected number was 73.2, giving a standardized incidence ratio of 0.6 (95% CI, 0.4–0.8) for any type of post-menopausal hormonal therapy. The ratios for hepatocellular carcinoma were 0.8 (95% CI, 0.4–1.6) for treatment with oestradiol or conjugated oestrogens and 0.5 (95% CI, 0.2–1.4) for treatment with oestriol and other oestrogens. The relative risks for cholangiocarcinoma were 0.7 (95% CI, 0.1–2.0) for treatment with oestradiol or conjugated oestrogens and 0.3 (95% CI, 0.0–1.7) for treatment with oestriol and other oestrogens. There was no information on infection with hepatitis viruses.

2.5.2 *Case–control studies*

Yu *et al.* (1991) used a population-based cancer registry to identify histologically confirmed hepatocellular carcinomas diagnosed in women aged 18–74 between 1984 and 1990 who were black or white residents of Los Angeles County, United States. Two neighbourhood controls were sought for each case and matched on sex, year of birth and race. Eighty-four of 412 (20.4%) eligible patients were interviewed (70.6% died before attempted contact), of which 10 were excluded from the analysis because the diagnosis of hepatocellular carcinoma was not confirmed. The response rate among the initially selected controls was 71%. Adjustment for smoking and alcohol did not alter the results. Ten of the 25 case women (40.0%) had used Premarin® or other oestrogens, in comparison with 19 of the 58 female controls (32.8%). The relative risks, adjusted for duration of use of oral contraceptives, were 1.1 (95% CI, 0.3–3.6) for any use and 0.8 (95% CI, 0.2–4.5) for use for up to 12 months, 1.0 (95% CI, 0.2–5.1) for 13–60 months and 1.0 (95% CI, 0.2–6.0) for 61 months or more. Seven case women had one or more markers of hepatitis B and C viral infections; when these cases were excluded, use of Premarin® was still not related to hepatocellular carcinoma after adjustment for duration of use of oral contraceptives.

Tavani *et al.* (1996) studied the relationship between the risk for biliary cancer and factors related to female hormones in Milan, northern Italy, between 1984 and 1993. The cases were in 31 women aged 27–76 with histologically confirmed cancers of the biliary tract (of whom 17 had gall-bladder cancers); the controls were 377 women, age frequency-matched with cases, who were in hospital for acute, non-neoplastic, non-digestive conditions. Post-menopausal oestrogen therapy was used by 4 of 31 cases and 21 of 377 controls, yielding a relative risk, adjusted for age and history of cholelithiasis, of 2.2 (95% CI, 0.7–7.2).

2.6 Colorectal cancer

2.6.1 *Descriptive studies*

The incidence of colon cancer is similar for men and women, while a male predominance is found for rectal cancer. The female:male ratio of colon cancer incidence is relatively higher at pre-menopausal ages, suggesting an influence of some biological correlate

of sex. Over the last two decades, mortality rates from these cancers in many developed countries have declined in women but not in men (La Vecchia *et al.*, 1998).

2.6.2 *Cohort studies*

(*a*) *Colorectal adenomas*

Grodstein *et al*. (1998) reported that 838 of 59 002 post-menopausal women had developed colorectal adenomas. There was no association between hormonal therapy and the incidence of adenomas overall, but current users had a lower risk for large (≥ 1 cm) adenomas than women who had never used hormones (relative risk, 0.74; 95% CI, 0.55–0.99).

(*b*) *Colorectal cancer*

Cohort studies on post-menopausal oestrogen therapy and cancers of the colon and rectum are summarized in Table 10.

Wu *et al*. (1987) followed a cohort of 7345 women in a large retirement community in California, United States, representing 62% of those to whom a questionnaire had been mailed; 4060 women reported ever having used post-menopausal oestrogen therapy of any type. After a four-year follow-up, 68 incident cases of colorectal cancer were identified. No association with risk for colorectal cancer was found (age-adjusted relative risk, 0.98; 95% CI, 0.5–1.8, for < 8 years of use and 1.0, 95% CI, 0.6–1.8 for ≥ 8 years' use).

A cohort of 22 597 Swedish women (mean age, 55 years) who received a prescription for post-menopausal oestrogen therapy were followed-up for cancer incidence and deaths through national cancer registries for an average of 6.7 years (Adami *et al*., 1989) and, subsequently, for 13 years from 1977 through 1991 (Persson *et al*., 1996). Overall, 153 incident cases and 62 deaths due to cancer of the colon and 80 incident cases of rectal cancer were observed. Information on exposure to post-menopausal oestrogen therapy was available only from accumulated pharmacy records; women were categorized into three exclusive compound groups according to the formulation prescribed: any oestradiol compounds or conjugated oestrogens, 11% of whom also received a progestogen; other oestrogens, chiefly a weak oestriol compound; and a fixed oestrogen–progestogen combination. For those for whom oestradiol compounds or conjugated oestrogens had ever been prescribed, the relative risk was 0.9 (95% CI, 0.7–1.1) for incident colorectal cancer and 0.9 (95% CI, 0.7–1.2) for incident rectal cancer (Persson *et al.*, 1996); a significant decrease in risk for mortality from colon cancer was observed (0.6; 95% CI, 0.4–0.9). The corresponding relative risks for women who had received only other oestrogens were 1.0 (95% CI, 0.8–1.3) for new cases of colon cancer, 0.8 (95% CI, 0.5–1.2) for new cases of rectal cancer and 0.8 (95% CI, 0.5–1.2) for death from colon cancer. The relative risk for exposure to any type of oestrogen was 0.9 for the incidence of either colon or rectal cancer and 0.7 (95% CI, 0.5–0.9) for mortality from colon cancer.

In an initial report from the Nurses' Health Study (Chute *et al*., 1991), there was no significant association between hormonal therapy and colon or rectal cancer after an

Table 10. Cohort studies of use of post-menopausal oestrogen therapy and colorectal cancer

Reference, country	Size of cohort	Follow-up (years)	No. of cases of colorectal cancer	Type of use	Relative risk (RR; 95% confidence interval) (any versus no use)					Duration of use	Recency of use	Adjustment, comments
					Colon–rectum	Colon	Right colon	Left colon	Rectum			
Wu *et al.* (1987), California, USA	7 345	4	68	–	1.00 (NS)	–	–	–	–	No effect (RR ≥ 8 years' use, 1.0; 0.6–1.8)	NR	Age
Adami *et al.* (1989); Persson *et al.* (1996), Sweden	23 244	13	233, 62 deaths	OT	0.9 (0.7–1.1)	–	–	–	0.9 (0.7–1.2)	No effect	NR	Age; RR for colon mortality, 0.6 (0.4–0.9)
				Oestriol	1.0 (0.8–1.3)	–	–	–	0.8 (0.5–1.2)			
				Any type	0.9 (0.7–1.2)	–	–	–	0.9 (0.7–1.1)			
Chute *et al.* (1991); Grodstein *et al.* (1998), USA	59 002	14	262			0.64 (0.48–0.85)	0.56 (0.35–0.91)	0.79 (0.50–1.2)	0.67 (0.40–1.1)	No effect (RR ≥ 5 years' use, 0.72; 0.53–0.96)	No risk reduction after 5 years' duration (RR, 0.92; 0.70–1.2)	Age, body mass index, COC use, family history of cancer, diet, alcohol, smoking and age at menopause
				Current users		0.65 (0.50–0.83)						
				Past users		0.86 (0.67–1.1)						
Bostick *et al.* (1994); Folsom *et al.* (1995), Iowa, USA	41 837	6	293	Current users		0.73 (0.47–1.1)	–	–	–	Inverse trend (RR, 0.31 for ≤ 5 years' use)	No effect	Age, body mass index, weight:height ratio, alcohol, exercise and medical history
				Past users		0.80 (0.61–1.1)	–	–	–			

Table 10 (contd)

Reference, country	Size of cohort	Follow-up (years)	No. of cases of colorectal cancer	Type of use	Relative risk (RR; 95% confidence interval) (any versus no use)					Duration of use	Recency of use	Adjustment, comments
					Colon–rectum	Colon	Right colon	Left colon	Rectum			
Calle *et al*. (1995), USA	422 373	7	897 deaths	–	–	0.71 (0.61–0.83)	–	–	–	Significant trend (RR for > 11 years' use, 0.54; 0.39–0.76)	Stronger effect among current users (RR, 0.55; 0.40–0.76)	Age, body mass index, parity, menopause, COC, diet, exercise, race and smoking
Risch & Howe (1995), Canada	33 003	14	230	–	1.0 (0.74–1.5)	1.3 (0.86–1.9)	–	–	0.64 (0.33–1.2)	RR, 0.65 (0.21–2.6) for ≥ 5 years)	Not shown	Age Linkage study
Troisi *et al*. (1997), USA	33 779	7.7	313	Un-opposed OT	–	1.1 (0.7–1.5)	1.6 (1.0–2.7)	0.8 (0.5–1.5)	1.2 (0.7–2.3)	No effect	RR for recent use, 0.78 (0.55–1.1)	Age (but unaltered by education, body mass index, parity and COC use)
				Any OT	0.99 (0.79–1.2)	1.1 (0.81–1.6)	1.7 (1.0–2.7)	0.98 (0.58–1.7)	1.1 (0.59–1.9)			

NS, not significant; OT, oestrogen therapy; NR, not reported; COC, combined oral contraceptives

eight-year follow-up. After 14 years, however, Grodstein *et al*. (1998) reported that 262 of 59 002 post-menopausal women had developed colorectal cancer. In this analysis, current use was associated with a decreased risk, the relative risk adjusted for several potential confounding variables being 0.65 (95% CI, 0.50–0.83). The results were not changed (relative risk, 0.64; 95% CI, 0.49–0.82) after exclusion of women who had undergone a screening sigmoidoscopy, suggesting that the lower risk was not due to more intensive screening of women who had used hormones. This association disappeared five years after hormone use was discontinued (relative risk, 0.92; 95% CI, 0.70–1.2).

In a prospective cohort study of 35 215 women aged 55–69 years with a driver's licence and without a history of cancer in Iowa, United States, from 1986 through 1990 (Bostick *et al*., 1994), 212 new cases of colon cancer were documented. The relative risk for colon cancer associated with post-menopausal oestrogen therapy use, adjusted for age, parity, height, energy and vitamin intake, was 0.93 (95% CI, 0.68–1.3) for former users and 0.82 (95% CI, 0.50–1.3) for current users. The study cohort was updated by Folsom *et al*. (1995), who followed-up 41 837 women aged 55–69 years for two additional years. The relative risk for colon cancer (293 observed cases), adjusted for age, body mass index, waist-to-hip ratio, exercise, alcohol and medical history, was 0.80 (95% CI, 0.61–1.1) in former users and 0.73 (95% CI, 0.47–1.1) in current users. The lowest relative risk was seen for short-term (≤ 5 years) current post-menopausal oestrogen therapy use (relative risk, 0.3; 95% CI, 0.10–0.98).

A cohort of 676 526 female participants (median age, 56) in the Cancer Prevention Study II was recruited in 1982 from all over the United States (Calle *et al*., 1995). By the end of 1989, 43 862 (6.5%) of the women had died. A total of 897 deaths from colon cancer occurred among 422 373 post-menopausal women who had not had cancer at entry to the study. The relative risk associated with any use of post-menopausal oestrogen therapy, adjusted for age, race, body mass index, parity, menopause, combined oral contraceptive use, dietary habits, exercise, smoking and aspirin use, was 0.71 (95% CI, 0.61–0.83). The risk reduction was strongest for women who were current users at the time of entry to the cohort (0.55; 95% CI, 0.40–0.76), and there was a significant trend of decreasing risk with increasing years of use (at entry) among all users (relative risk for users of > 11 years, 0.54; 95% CI, 0.39–0.76). No data on incidence were available.

A record linkage cohort study was carried out in Saskatchewan, Canada, between the Prescription Drug Plan Database (1976–87) and the Provincial Cancer Registry Database (1960–90) on all 33 003 resident women aged 43–49 (Risch & Howe, 1995). Of 32 973 women who did not have colorectal cancer at the beginning of the study, 230 developed this cancer. For users of post-menopausal oestrogen therapy, the age-adjusted relative risk was 1.3 (95% CI, 0.86–1.9) for colon cancer, 0.64 (95% CI, 0.33–1.2) for rectal cancer and 1.0 (95% CI, 0.74–1.5) for both together.

A cohort of 64 182 women was selected for follow-up within the Breast Cancer Detection Demonstration project between 1973 and 1980 in 27 cities of the United States (Troisi *et al*., 1997). Telephone interviews were conducted between 1979 and 1986 with 61 434 women. The analyses were restricted to 33 779 post-menopausal women (41–80 years of

age; mean age, 59) who completed the follow-up questionnaire between 1987 and 1989. After an average follow-up of 7.7 years, 313 cases of colorectal cancer were identified (84 from death certificates). Any use of post-menopausal oestrogen therapy was not related to the risk for colorectal cancer (age-adjusted relative risk, 0.99; 95% CI, 0.79–1.2). The relative risk for recent use of five or more years' duration was 0.75 (95% CI, 0.50–1.1). The risks were similar for colon cancer and rectal cancer. Eighty-four per cent of the person–years of post-menopausal oestrogen therapy use were accounted for by oestrogen use alone: the relative risks for any use of oestrogen alone were similar to those for any use of post-menopausal oestrogen therapy (relative risk, 1.1; 95% CI, 0.7–1.5 for colon; and 1.2; 95% CI, 0.7–2.3 for rectum).

2.6.3 *Case–control studies*

(*a*) *Colorectal polyps*

Potter *et al*. (1996) undertook a case–control study in Minnesota, United States, between 1991 and 1994 of cases in 219 women, aged 30–74, with colonoscopy-proven, pathology-confirmed, adenomatous polyps of the colon and rectum. Two control groups were selected: 438 women without polyps at colonoscopy and 247 community controls matched on age and postal code; the response rates of all three groups were around 65%. The multivariate relative risks for use of post-menopausal oestrogen therapy for fewer than five years, compared with no use, among post-menopausal women were 0.52 (0.32–0.85) in comparison with colonoscopy-negative controls and 0.74 (0.44–1.3) in comparison with community controls. For five or more years of use, the corresponding figures were 0.39 (0.23–0.67) and 0.61 (0.34–1.1).

Jacobson *et al*. (1995) studied patients with colorectal adenomatous polyps between 1986 and 1988 in New York, United States. The cases (128) were in cancer-free women aged 35–84 years in whom an adenoma was detected at the index colonoscopy. The 283 controls were cancer-free women with a normal index colonoscopy at the same institution as the cases. The adjusted relative risk associated with post-menopausal oestrogen therapy was 0.7 (95% CI, 0.3–1.2).

Chen *et al.* (1998) studied 187 women with colorectal polyps and 188 controls, aged 50–75 years, who were members of a prepaid health plan and underwent sigmoidoscopy in 1991–93. For women who used post-menopausal oestrogen therapy in the year before sigmoidoscopy relative to women who did not (37 cases and 38 controls), the relative risk adjusted for age, sigmoidoscopy date, physical activity, bone mass index, smoking and ethnicity was 0.57 (95% CI, 0.35–0.94). The risk for > 5 years of use (16 cases and 30 controls) was 0.49 (95% CI, 0.25–0.97).

(*b*) *Colorectal cancer*

Case–control studies of use of post-menopausal oestrogen therapy and the risks for cancers of the colon and rectum are summarized in Table 11.

A case–control study was conducted in 1976–77 in Washington State, United States, on 143 white women with colorectal cancer, aged 45–74 years, and 707 white women of

Table 11. Case–control studies of use of post-menopausal oestrogen therapy and colorectal cancer

Reference, country	No. of cases/ no. of controls	Type of controls	Type of use	Relative risk (RR; 95% confidence interval) (any versus no use)					Duration of use	Recency of use	Adjustment, coments
				Colon-rectum	Colon	Right colon	Left colon	Rectum			
Weiss *et al*. (1981), Washington, USA	143/707	Popu-lation	≤ 5 years ≥ 6 years	1.1 (0.7–1.9) 1.0 (0.6–1.6)	–	–	–	–	No trend	NR	Age
Potter & McMichael (1983), Adelaide, Australia	155/311	Popu-lation		–	0.8 (0.4–1.5)	–	–	1.5 (0.8–3.0)			Reproductive variables (diet had no effect); use of hormones other than COC
Davis *et al*. (1989), Canada	720/349	Cancer patients	Current users Past users	1.5 (0.8–2.7) 1.1 (0.7–1.9)	–	–	–	–	NR	NR	Age and parity No distinction possible between OT and COC use
Furner *et al*. (1989), Chicago, USA	90/208	Spouses		0.5 (0.27–0.90)	–	0.8 (0.27–2.6)	0.6 (0.27–1.3)	0.2 (0.03–0.77)	No trend	NR	Age, parity, hysterectomy
Fernandez *et al*. (1998), including data from Negri *et al*. (1989); Fernandez *et al*. (1996), Italy	1 536/3 110	Hospital		0.58 (0.44–0.76)	0.59 (0.43–0.82)	0.35 (0.15–0.80)	0.67 (0.44–1.0)	0.48 (0.31–0.75)	Significant (RR for ≥ 2 years' use, 0.46; 0.26–0.81)	RR for ≥ 10 years since last use, 0.52 (0.27–0.99)	Age, education, family history of cancer, body mass index, parity, menopause, COC and energy intake

Table 11 (contd)

Reference, country	No. of cases/ no. of controls	Type of controls	Type of use	Relative risk (RR; 95% confidence interval) (any versus no use)					Duration of use	Recency of use	Adjustment, coments
				Colon-rectum	Colon	Right colon	Left colon	Rectum			
Peters *et al*. (1990), Los Angeles, USA	327/327	Neigh-bours	< 5 years 5–14 years ≥ 15 years		1.3 (0.88–2.0) 1.1 (0.64–1.8) 1.1 (0.58–1.9)	1.4 (0.80–2.6) 1.1 (0.47–2.6) 1.2 (0.51–2.8)	1.2 (0.69–2.3) 1.1 (0.55–2.2) 0.75 (0.30–1.8)	–	No effect	NR	Family history of cancer, parity, menopause, exercise, fat, alcohol and calcium intake
Wu-Williams *et al*. (1991), North America and China	189/494 (North America) 206/618 (China)	Neigh-bours			2.1 $p = 0.14$ 2.9 $p = 0.01$	– –	– –	0.5 $p = 0.23$ 1.3 $p = 0.56$	NR; mostly short duration of use	NR	Use of 'other hormones' Unadjusted but unaltered by exercise, saturated fat intake and years in the USA Artificial menopause was a risk factor in China
Gerhardsson de Verdier & London (1992), Sweden	299/276	Popu-lation		–	0.6 (0.4–1.0)	0.4 (0.2–0.8)	1.0 (0.5–1.9)	0.7 (0.4–1.3)	No trend	NR	Age Hormone use included both OT and COC, but mostly OT
Jacobs *et al*. (1994), Seattle, USA	148/138	Popu-lation		–	0.60 (0.35–1.0)	0.46 (0.23–0.91)	0.74 (0.39–1.4)	–	Significant trend (RR ≥ 5 years' use, 0.47; 0.24–0.91)	RR of current users, 0.53 (0.29–0.96)	Age, vitamin intake and hysterectomy Greater protection for multiparous women

Table 11 (contd)

Reference, country	No. of cases/ no. of controls	Type of controls	Type of use	Relative risk (RR; 95% confidence interval) (any versus no use)					Duration of use	Recency of use	Adjustment, coments
				Colon-rectum	Colon	Right colon	Left colon	Rectum			
Newcomb & Storer (1995), Wisconsin, USA	694/1 622	Popu-lation	Unopposed oestrogen (recent use)		0.54 (0.34–0.88)	–	–	0.90 (0.46–1.76)	Significant trend (p = 0.002)	Lower RR for < 10 years since last use, 0.54 (0.36–0.80) for colon	Age, alcohol, body mass index, family history of cancer and sigmoidoscopy
			Any OT		0.73 (0.56–0.94)	0.43 (0.22–0.84) (recent use)	0.64 (0.39–1.0) (recent use)	1.2 (0.83–1.6)			
Kampman *et al*. (1997), USA	815/1 019	Members of medical care organi-zation		–	0.82 (0.67–0.99)	NR	NR	–	No trend (RR ≥ 10 years' use, 0.86)	RR for recent use, 0.71 (0.56–0.89)	Age, family history of cancer, aspirin, energy intake, COC and exercise
Yood *et al*. (1998), Detroit, USA	60/143	Members of health mainte-nance organi-zation	Current use Past use	0.34 (0.11–0.99) 0.40 (0.12–1.4)	–	0.55 (0.14–2.2)	0.32 (0.30–1.7)	–	NR	NR	Age, race, reproductive variables, dietary habits and colonoscopy

NR, not reported; COC, combined oral contraceptives; OT, oestrogen therapy

the same ages drawn from a population survey in the area (Weiss *et al*., 1981). Use of post-menopausal oestrogen therapy of any type was not related to cancer risk (age-adjusted relative risk, 1.1; 95% CI, 0.7–1.9 for ≤ 5 years' use; and 1.0; 95% CI, 0.6–1.6 for ≥ 6 years' use).

Potter and McMichael (1983) conducted a case–control study in Adelaide, Australia, between 1979 and 1980 on 155 cases of colorectal cancer (out of 212 eligible cases) and 311 control women selected from the local electoral roll. The relative risk, adjusted for reproductive variables, for use of oestrogen therapy of any type, apart from oral contraceptives, was 0.8 (95% CI, 0.4–1.5) for colon cancer and 1.5 (95% CI, 0.8–3.0) for rectal cancer.

A case–control study conducted in Alberta, Canada, between 1969 and 1973 included data on 528 cases of colon cancer, 192 of rectal cancer (i.e. 69% of identified colorectal cancers in the study area) and 349 control women aged 35 and more (Davis *et al*., 1989). The controls were women with cancers at sites not associated with endocrine factors (chiefly cancers of the mouth and stomach). The estimated relative risk for use of exogenous hormones (including post-menopausal oestrogen therapy and combined oral contraceptives) among women over 50, as a surrogate for post-menopausal oestrogen therapy, adjusted for age and parity, was 1.5 (95% CI, 0.8–2.7) for current use and 1.1 (95% CI, 0.7–1.9) for past use.

Ninety women with colorectal cancer and 208 controls who were the wives of colorectal cancer patients were interviewed between 1980 and 1983 in Chicago, United States, representing 63% of the subjects initially contacted (Furner *et al*., 1989). The relative risk associated with post-menopausal oestrogen therapy [not otherwise specified] adjusted for age, parity and hysterectomy was 0.5 (95% CI, 0.27–0.90). The inverse association was stronger for cancer of the rectum (relative risk, 0.2; 95% CI, 0.03–0.77; two cases) than for cancers of the right colon (0.8; 0.27–2.63; six cases) or left colon (0.6; 0.03–0.77; 12 cases). No trend in risk emerged with duration of post-menopausal oestrogen therapy.

A hospital-based case–control study conducted in Milan, Italy, between 1985 and 1992 (Negri *et al*., 1989; Fernandez *et al*., 1996) included 709 women with colon cancer (median age, 61 years) and 992 women in hospital for acute, non-digestive, non-hormone-related disorders. The relative risk for women who had ever used post-menopausal oestrogen therapy, adjusted for age, social class, family history of cancer, menarche and parity was 0.40 (95% CI, 0.25–0.66). The risk decreased with increasing duration of use (0.46 for ≤ 2 years; 0.25 for > 2 years of use). No consistent trend was observed with time since first or last use. Another case–control study conducted with a similar protocol in six Italian areas between 1992 and 1996 (Talamini *et al*., 1998) included 537 women with colon cancer, 291 women with rectal cancer and 2081 control women in hospital for acute conditions unrelated to hormonal or gynaecological diseases. The relative risk for any use of post-menopausal oestrogen therapy, adjusted for age, centre, education, exercise and energy intake, was 0.6 (95% CI, 0.3–1.0) for rectal cancer and 1.0 (95% CI, 0.69–1.5) for colon cancer; however, only about 10% of post-menopausal women had had post-menopausal oestrogen

therapy. A pooled analysis of the two Italian studies described by Fernandez *et al*. (1996) and Talamini *et al*. (1998) included 994 women with cancer of the colon and 542 with cancer of the rectum, in addition to 3110 hospital controls (Fernandez *et al*., 1998). The relative risks for any use, adjusted for age, education, family history of cancer, body mass index, parity, menopause, combined oral contraceptive use and energy intake, were 0.59 (95% CI, 0.43–0.82) for colon cancer and 0.48 (95% CI, 0.31–0.75) for rectal cancer. The inverse association was stronger for cancer of the right colon (0.35; 0.15–0.80) than for that of the left colon (0.67; 0.44–1.03). Significant trends in risk by duration of use emerged for all subsites. The decrease in the relative risk associated with post-menopausal oestrogen therapy was greater 10 or more years after cessation of use (relative risk, 0.50 for colon and 0.54 for rectum) than earlier.

Peters *et al*. (1990) conducted a population-based case–control study in Los Angeles, United States, between 1983 and 1986. A total of 327 white women with colon cancer (out of 472 eligible cases) and 327 individually matched neighbourhood controls were interviewed. The relative risks, adjusted for age, family history of cancer, parity, menopause, exercise, fat, calcium and alcohol intake, for < 5, 5–14 and ≥ 15 years' duration of use, were 1.3 (95% CI, 0.88–2.0), 1.1 (95% CI, 0.64–1.8) and 1.0 (95% CI, 0.58–1.9), respectively. The risk estimates were similar for cancers of the right and left colon.

A population-based case–control study was conducted among Chinese women in western North America and China between 1981 and 1986 with a common protocol (Wu-Williams *et al*., 1991). It included 395 women with colorectal cancer, 189 from North America and 206 from China, and 1112 age-matched controls, 494 and 618, respectively. The unadjusted relative risk for rectal cancer associated with the use of hormones other than combined oral contraceptives was 0.5 in North America and 1.3 in China (neither significant). The relative risk for colon cancer was 2.1 ($p = 0.14$) in North America and 2.9 ($p = 0.01$) in China. About 90% of the post-menopausal oestrogen therapy users had used hormones for one year or less.

A population-based case–control study was performed in Stockholm, Sweden, in 1986–88, which included 299 cases and 276 controls (i.e. about 80% of eligible subjects) (Gerhardsson de Verdier & London, 1992). The questionnaire used did not allow distinction between post-menopausal oestrogen therapy and combined oral contraceptives. The age-adjusted relative risk for hormone use was 0.6 (95% CI, 0.4–1.0) for colon cancer, 0.7 (95% CI, 0.4–1.3) for rectal cancer, 0.4 (95% CI, 0.4–1.0) for cancer of the right colon and 1.0 (95% CI, 0.5–1.9) for cancer of the left colon.

Jacobs *et al*. (1994) conducted a case–control study among women aged 30–62 years in Seattle, United States, between 1985 and 1989. It included 193 new cases of colon cancer (out of 295 eligible cases) and 194 controls (out of 227 eligible controls) selected by random-digit dialling. Among post-menopausal women aged ≥ 45 years (i.e. 148 cases and 138 controls), the relative risk associated with use of post-menopausal oestrogen therapy, adjusted for age, vitamin intake and hysterectomy, was 0.60 (95% CI, 0.35–1.0) for colon cancer, with estimates of 0.47 (95% CI, 0.24–0.91) for ≥ 5 years' use and 0.53 (95% CI, 0.29–0.96) for current use.

A case–control study was conducted between 1990 and 1991 in Wisconsin, United States (Newcomb & Storer, 1995). After exclusion of pre-menopausal women, 694 women with colorectal cancer (480 colon and 214 rectum) and 1622 control women (randomly selected from lists of licensed drivers and Medicare beneficiaries) were included. The relative risk for any use of post-menopausal oestrogen therapy, adjusted for age, alcohol, body mass index, family history of cancer and history of sigmoidoscopy, was 0.73 (95% CI, 0.56–0.94) for colon cancer and 1.2 (95% CI, 0.83–1.6) for rectal cancer. Among recent users, the relative risk for colon cancer was 0.54 for both use of post-menopausal oestrogen therapy of any type and use of oestrogens only. The inverse association was stronger for recent use ($p < 0.001$).

Kampman *et al*. (1997) conducted a case–control study between 1992 and 1995 in the United States among women aged 30–79 who were members of a medical care programme, covering 894 cases of colon cancer (out of 1521 eligible cases) and 1120 control women (63% of those who were contacted). The relative risk for colon cancer (adjusted for age, family history of cancer, aspirin and energy intake) of post-menopausal women (i.e. 815 cases and 1019 controls) for use of oestrogen therapy for longer than three months was 0.82 (95% CI, 0.67–0.99). The inverse assocation was confined to recent users (i.e. < 1 year before diagnosis) (relative risk, 0.71; 95% CI, 0.56–0.89). No trend with duration of post-menopausal oestrogen therapy was observed (relative risk for ≥ 10 years of use, 0.86). The reduced relative risk associated with post-menopausal oestrogen therapy use did not appear to be explained by confounding factors such as dietary habits, body mass index or physical activity. Although the number of routine sigmoidoscopies did not differ significantly between women who had ever and never had post-menopausal oestrogen therapy, those who had used it had undergone more sigmoidoscopies because of symptoms.

A case–control study was conducted among members of a large health maintenance organization in Detroit, United States. The preliminary results (Yood *et al*., 1998) on 60 women with colorectal cancer and 143 population controls showed an adjusted relative risk associated with post-menopausal oestrogen therapy of 0.34 (95% CI, 0.11–0.99) for current users and 0.40 (95% CI, 0.12–1.40) for past users.

2.7 Cutaneous malignant melanoma

2.7.1 *Descriptive studies*

The incidence of melanoma has increased at a rate of 3–7% per year in most Caucasian populations in the last decades (Armstrong & Kricker, 1994). Changes in recreational patterns of exposure to the sun and, to some extent, increasing detection account for the observed rises. The incidence rates are similar in men and women, although a female excess is found in some countries, e.g. the United Kingdom and the Nordic countries.

Cohort and case–control studies of cutaneous and ocular malignant melanoma and post-menopausal oestrogen therapy are summarized in Table 12.

Table 12. Studies on use of post-menopausal oestrogen therapy (OT) or combined hormonal therapy (HT) and cutaneous malignant melanoma

Reference, country	No. of cases/ no. of controls	Type of controls	Type of use	RR (95% CI) for any versus no use	Duration of use	Recency of use	Adjustment, comments
Cohort							
Persson *et al.* (1996), Sweden	22 597 (60 cases)		Any OT HT	0.9 (0.7–1.1) 0.6 (0.3–1.1)	NR	NR	Age 13 years' follow-up. Standardized mortality ratio, 0.5 (95% CI, 0.2–1.0) No association with non-melanomatous skin cancer
Case–control							
Holly *et al*. (1983) Seattle, USA	87/863	Population	1–3 years 4–7 years ≥ 8 years	1.1 0.85 1.0	No effect	NR	Age RR very similar for 61 cases of SSM
Lew *et al*. (1983), Massachusetts, USA	111/107	Friends of cases	–	–	–	–	No difference in OT use
Beral *et al*. (1984), Sydney, Australia	287/574	Hospital and population		1.4 (0.78–2.6)	NR	NR	Age
Holman *et al*. (1984), Western Australia	276/276	Population		1.5 (0.87–2.7)	No trend	NR	Age and residence RR very similar for SSM (1.9; 0.88–4.2)
Gallagher *et al*. (1985), Canada	361/361	Members of health plans	< 1 year 1–4 years ≥ 5 years	1.0 1.0 0.9	No trend	No effect	Age, education, phenotype and freckling RR similar for SSM
Green & Bain (1985), Queensland, Australia	91/91	Population		–	–	–	Age 11 cases and 11 controls reported use of hormonal therapy other than COC

Table 12 (contd)

Reference, country	No. of cases/ no. of controls	Type of controls	Type of use	RR (95% CI) for any versus no use	Duration of use	Recency of use	Adjustment, comments
Østerlind *et al*. (1988), Denmark	151/297	Population	Oestrogen, unopposed	1.3 (0.8–2.1)	No trend (RR for > 7 years' use, 1.2; 0.7–2.2)	NR	Age, naevi and sunbathing No difference in risk between histological subtypes
			Oestrogen, opposed	1.5 (0.8–2.8)			
Holly *et al*. (1994), San Francisco, USA	452/935	Population	Conjugated oestrogens, after oophorectomy	2.4 (1.1–5.2)	No trend	NR	Age and education but unaltered by phenotype and sun Similar risks for SSM
			Any OT, after natural menopause	0.88 (0.50–1.6)			
			Hysterectomy with no or one ovary removed	2.0 (0.8–5.0)			
			Any OT after bilateral oophorectomy	2.2 (1.0–4.7)			
Westerdahl *et al*. (1996), Sweden	403/707	Population	HT, any	1.0 (0.5–1.8)	No trend	No effect of age at first or last use	Phenotype, naevi and sunburns Risks were similar at different anatomical sites
Ocular melanoma							
Hartge *et al*. (1989), Philadelphia, USA	214/209	Detached retina		2.0 (1.2–3.0)	No effect (RR for ≥ 6 years' use, 2.2; 0.9–5.8)	NR	Age and oophorectomy Similar risks for users of conjugated oestrogens and users of other formulations

RR, relative risk; CI, confidence interval; NR, not reported; SSM, superficial spreading melanoma; COC, combined oral contraceptives

2.7.2 *Cohort studies*

One cohort investigation (see section 2.6.2) provided information on post-menopausal oestrogen therapy and the risk for cutaneous malignant melanoma (Persson *et al*., 1996), expressed as incidence and mortality rates, among 22 597 Swedish women. After 13 years of follow-up, 60 new cases and eight deaths from cutaneous malignant melanoma were recorded. The age-adjusted standardized incidence ratios for any use of post-menopausal oestrogen therapy were 0.9 (95% CI, 0.7–1.1) for a diagnosis of cutaneous malignant melanoma and 0.5 (95% CI, 0.2–1.0) for death from this condition.

2.7.3 *Case–control studies*

Holly *et al*. (1983) conducted a case–control study in Seattle, United States, between 1976 and 1979 of 87 women aged 37–74 years (out of 124 eligible) with histologically confirmed cutaneous malignant melanoma, 61 of whom had superficial spreading melanomas. The controls were 863 age-matched women (response rate, 93%), who represented a random sample of the population from which the cases were derived. The age-adjusted relative risks for any use of post-menopausal oestrogen therapy among women ≥ 45 were close to unity: 1.1, 0.85 and 1.0 for 1–3, 4–7 and ≥ 8 years of use, respectively, for all histological types combined. Very similar risks were found when the analysis was restricted to women with superficial spreading melanomas (1.1, 1.1 and 0.98, respectively).

Lew *et al*. (1983) studied 111 women with cutaneous malignant melanoma in Massachusetts, United States, during 1978–79 and 107 controls chosen among friends of the cases. No difference in the frequency of post-menopausal oestrogen therapy use was reported between cases and controls, but detailed data were not shown.

Beral *et al*. (1984) investigated 287 women aged 15–54 years in Sydney, Australia, between 1978 and 1980, who had received a diagnosis of cutaneous malignant melanoma (new and prevalent cases) between 1974 and 1980, and 574 age-matched controls, who were hospital patients for the new cases and from the population for prevalent cases. Post-menopausal oestrogen therapy use was slightly more frequent among cases (6.6%) than controls (4.7%; unadjusted relative risk, 1.4; 95% CI, 0.78–2.6).

In another case–control study carried out in Western Australia between 1980 and 1981 (Holman *et al*., 1984), the cases were those of 276 women under the age of 80 (mean age, 45) with histologically proven pre-invasive or invasive cutaneous malignant melanoma (out of 373 eligible women). The controls were 276 age-matched women extracted from the electoral roll (out of 458 sampled women). Fourteen percent of subjects had ever taken hormone tablets or injections containing an oestrogen but no progestogen; of these, 59% had taken them for menopausal symptoms. The relative risk associated with any post-menopausal oestrogen therapy, adjusted for age and area of residence, were 1.5 (95% CI, 0.87–2.7) for all cutaneous malignant melanoma and 1.9 (95% CI, 0.88–4.2) for superficial spreading melanoma. No trend in risk with duration of use was seen.

Gallagher *et al*. (1985) conducted a case–control study in western Canada between 1979 and 1981 that included 361 women aged 20–79 years (out of 412 eligible cases) with cutaneous malignant melanoma, of whom 269 had superficial spreading melanoma, and

361 age-matched control women selected from medical plan listings (59% response rate). No association was found with any post-menopausal oestrogen therapy; the relative risks, adjusted for age, education, skin colour, hair colour and freckling, were 1.0 for < 1 or 1–4 years of use and 0.9 for ≥ 5 years of use. The risks for superficial spreading melanoma were identical.

Green and Bain (1985) studied the effect of female hormones on the incidence of cutaneous malignant melanoma in Queensland, Australia, between 1979 and 1980 in 91 women aged 15–81 years (92% of eligible women) with a first cutaneous malignant melanoma; lentigo maligna melanomas were not included. The control women consisted of a random sample of 91 women drawn from the electoral rolls, who were matched with cases by age and residence. The frequency of use of hormones other than oral contraceptives was low, and it was the same in cases and controls.

A case–control study on cutaneous malignant melanoma was carried out in Denmark between 1982 and 1985 (Østerlind *et al*., 1988). The case series consisted of 280 women aged 20–79 with newly diagnosed cutaneous malignant melanoma, of whom 207 had superficial spreading melanoma (out of 304 eligible women); lentigo maligna melanomas were not included. The controls consisted of 536 women selected from the National Population Register (out of 677 originally identified). Among post-menopausal women (i.e. 151 cases and 297 controls), the relative risk for cutaneous malignant melanoma among users of unopposed post-menopausal oestrogen therapy (adjusted for age, naevi and sunbathing) was 1.3 (95% CI, 0.8–2.1). For users of post-menopausal oestrogen therapy of any type, the relative risk was 1.1 (95% CI, 0.7–1.7). No trend in risk with increasing duration of use was found (relative risk for ≥ 7 years of use, 1.2; 95% CI, 0.7–2.2). There was no difference in risk for subtypes of melanoma, including superficial spreading melanoma.

Holly *et al*. (1994) carried out a case–control study of cutaneous malignant melanoma between 1981 and 1987 in San Francisco, United States, among 452 white women aged 25–59, 355 of whom had superficial spreading melanoma; 79% of those eligible were interviewed. Random-digit dialling was used to identify 935 control women of the same age (77% of those contacted). The relative risks associated with post-menopausal oestrogen therapy in pre-menopausal or naturally menopaused women were close to 1.0: for women with a natural menopause, the relative risk was 0.88 (95% CI, 0.50–1.6). The relative risk of women who had undergone a hysterectomy without removal of both ovaries was 2.0 (95% CI, 0.85–4.5), and that for women who had had bilateral oophorectomy was 2.2 (95% CI, 1.0–4.7). No difference was found according to the dose of conjugated oestrogens.

Westerdahl *et al*. (1996) carried out a case–control study on exposure to hormones in southern Sweden between 1988 and 1990. The cases were those of 403 women with a first histopathological diagnosis of cutaneous malignant melanoma, and the 707 age-matched control women were randomly selected from the National Population Registry. Post-menopausal oestrogen therapy had been used by 13% of the cases and 14% of the controls, giving a relative risk, adjusted for phenotype, naevi and sunburns, of 1.0 (95% CI, 0.5–1.8). No associations were found between cutaneous malignant melanoma and duration of post-

menopausal oestrogen therapy, age at first use or age at latest use. Consistent results were found for cutaneous malignant melanoma at different anatomical sites.

2.8 Intraocular malignant melanoma

A case–control study carried out between 1979 and 1980 in Philadelphia, United States, of ocular melanoma included 239 women (mean age, 58) with intraocular malignant melanoma (out of 444 eligible cases) and 223 control matched by age and race (Hartge *et al*., 1989). The controls were patients with detached retinas. The relative risk for post-menopausal women who reported using oestrogen therapy, adjusted for age and history of oophorectomy, was 2.0 (95% CI, 1.2–3.0) and that for ≥ 6 years of use was 2.2 (95% CI, 0.9–5.8) (Table 12).

2.9 Thyroid cancer

2.9.1 *Descriptive studies*

Cancer of the thyroid is a rare, very heterogeneous disease. The rate of mortality from this cancer has been falling slowly, whereas the incidence has been increasing in most developed countries over the last three decades (Franceschi & La Vecchia, 1994). The incidence rates are two- to threefold higher for women than for men, and the difference is greatest for well-differentiated papillary carcinomas for women aged 25–44 (Franceschi & Dal Maso, 1998). A positive correlation between parity and the incidence of thyroid cancer was reported from individual data on all (1.1 million) Norwegian women born 1935–69 (Kravdal *et al*., 1991).

2.9.2 *Case–control studies*

These studies are summarized in Table 13.

The case–control study of McTiernan *et al*. (1984) was carried out in Seattle, United States, between 1980 and 1981. The cases were those of 183 women aged 18–80 with papillary, follicular and mixed thyroid carcinomas, diagnosed between 1974 and 1979, who represented 65% of those identified through the cancer surveillance system of western Washington, United States. The controls were women aged 18–80 identified through random-digit dialling; of 478 eligible controls, 394 were interviewed. The majority of the patients and controls (87%) were white. Among women over 30 years of age (153 cases and 281 controls), the age-adjusted relative risk for any use of post-menopausal oestrogen therapy (34% of cases and 27% controls) was 1.4 (95% CI, 0.89–2.3) for thyroid cancer overall and 1.9 (95% CI, 1.1–3.4) for tumours of the papillary type. The relative risk for ≥ 3 years' duration of use was 1.2 for all thyroid cancers and 1.6 for papillary thyroid cancer. The association was slightly stronger when only women whose tumours were found by a physician (rather than by the woman herself) were included.

A case–control study carried out in Connecticut, United States, between 1978 and 1980 by Ron *et al.* (1987) included 159 women aged 20–76 with thyroid cancer, i.e. 80% of those identified through the Connecticut Tumor Registry. Tumour slides were reviewed centrally. The controls were 285 women frequency-matched to the cases on age. Random-digit

Table 13. Case–control studies on use of oestrogen therapy and thyroid cancer

Reference, country	No. of cases/ no. of controls	Type of controls	RR (95% CI) for any versus no use	Duration of use	Adjustment, comments
McTiernan *et al*. (1984), Seattle, USA	153/281	Population	All 1.4 (0.89–2.3) Papillary 1.9 (1.1–3.4)	No trend (RR for ≥ 3 years' use, 1.2)	Age Risk slightly higher for cases found by physicians
Ron *et al*. (1987), Connecticut, USA	71/123	Population, ≥ 35 years	0.5 (NS)	NR	Age, parity, radiotherapy and benign thyroid disease
Franceschi *et al*. (1990), Italy	71/94	Hospital	0.3 (0.1–1.1)	NR	Age and residence
Kolonel *et al*. (1990), Hawaii, USA	140/328	Population	0.9 (0.5–1.7)	NR	Age and ethnic group
Levi *et al*. (1993b), Switzerland	91/306	Hospital	1.8 (0.6–5.2)	NR	Age and history of benign thyroid disease
Hallquist *et al*. (1994), northern Sweden	123/140		1.1 (0.3–3.7)	NR	Age
Galanti *et al*. (1996), Norway and Sweden	74/134	Population, post-menopausal	0.98 (0.39–2.5)	No effect (RR for > 2 years' duration, 1.2; 0.25–5.3)	Age and parity

RR, relative risk; CI, confidence interval; NS, not significant; NR, not reported

dialling was used to select controls under 65 years of age, while controls over 65 years were chosen from the Medicare roster. Post-menopausal oestrogen therapy had ever been used by 8/71 cases and 23/123 controls over the age of 35. The relative risk, adjusted for age, parity and history of radiotherapy and benign thyroid diseases was 0.5 (not significant).

Franceschi *et al*. (1990) conducted a case–control study in the provinces of Pordenone, Padua and Milan, northern Italy, between 1986 and 1992, which included 165 women under 75 years old with newly diagnosed, histologically confirmed thyroid cancer. The control women, frequency-matched by age to the cases, were 214 in-patients identified in the same network of hospitals with diagnoses of acute, non-neoplastic, non-hormonal diseases. The response rates exceeded 95% among both cases and controls. All of the study subjects were interviewed during their hospital stay. Among post-menopausal women, any use of oestrogen therapy was reported by five of 71 cases and 17 of 94 controls (relative risk adjusted for age and area of residence, 0.3; 95% CI, 0.1–1.1).

A case–control study of thyroid cancer was conducted between 1980 and 1987 in Hawaii, United States (Kolonel *et al*., 1990), and consisted of 140 women from five ethnic groups, 18 years or older, with thyroid cancer identified through the Hawaii Tumour Registry. The histological type of the tumours was reviewed centrally. The controls were 328 women, age-matched to cases, selected from among people participating in a concurrent health surveillance programme. Questionnaires were administered at home, with response rates of 79% for cases and 74% for controls. The relative risk for women who had ever used post-menopausal oestrogen therapy (15% of control women), adjusted for age and ethnic group, was 0.9 (95% CI, 0.5–1.7).

Levi *et al*. (1993b) studied risk factors for thyroid cancer in the Canton of Vaud, Switzerland, between 1988 and 1990. The cases were those of 91 women, aged 12–72, with histologically confirmed thyroid cancer. The controls were 306 women admitted to the same hospital as the cases for acute conditions. Post-menopausal oestrogen therapy had ever been used by seven of 91 cases and 31 of 306 controls. The relative risk, adjusted for age and history of benign thyroid diseases, was 1.8 (95% CI, 0.6–5.2).

Hallquist *et al*. (1994) conducted a case–control study on thyroid cancer in northern Sweden between 1980 and 1989. The cases were those of 123 women, aged 20–70, with histopathologically confirmed thyroid cancer, identified through the Swedish Cancer Registry. The controls were 240 women randomly drawn from the National Population Registry of the same counties as the cases. All of the study subjects returned a mailed questionnaire. Use of post-menopausal oestrogen therapy was uncommon (five cases and nine controls) and unrelated to the risk for thyroid cancer; the age-adjusted relative risk was 1.1 (95% CI, 0.3–3.7).

Galanti *et al*. (1996) carried out a case–control study on thyroid cancer in northern Norway and central Sweden between 1985 and 1993. The cases were those of 191 women aged 17–72 years with histologically confirmed papillary, follicular or mixed carcinoma of the thyroid gland. The controls were 341 age-matched women selected from the national population registries. Information was based on a mailed questionnaire, with a response rate of over 90%. Among post-menopausal women, use of any type of oestrogen

therapy was reported by 13 of 74 cases and 24 of 134 controls; the relative risk, adjusted for age and parity, was 0.98 (95% CI, 0.39–2.5). The relative risk for use for more than two years was 1.2 (95% CI, 0.25–5.3).

2.10 Other cancers

La Vecchia *et al*. (1994) assessed the role of female hormones in gastric cancer in Milan, Italy, between 1985 and 1993. The cases were those of 229 post-menopausal women with newly diagnosed, histologically confirmed gastric cancer. The controls were 614 post-menopausal women in hospital for acute, non-neoplastic, non-digestive tract conditions. The relative risk for users of post-menopausal oestrogen therapy, adjusted for age, education, family history of cancer and dietary habits, was 0.54 (95% CI, 0.3–1.1).

Chow *et al*. (1995) studied 165 cases of renal-cell cancer in women aged 20–79 and 227 age- and frequency-matched population controls in Minnesota, United States, between 1988 and 1990. Among post-menopausal women (134 cases and 173 controls), the relative risk for any use of post-menopausal oestrogen therapy, adjusted for age, smoking and body mass index, was 1.8 (95% CI, 1.1–3.0). There was no trend with duration of use.

Lindblad *et al*. (1995) evaluated the effect of exogenous hormones on the incidence of renal-cell cancer in Australia, Denmark, Germany, Sweden and the United States during 1989–91. The cases were those of 608 women aged 20–79 with histologically confirmed renal-cell cancer identified mainly through local cancer registries. The controls were 766 women sampled from local residential lists and frequency-matched by age to the cancer cases. The relative risk of post-menopausal oestrogen therapy users, adjusted for age, smoking and body mass index, was 1.0 (95% CI, 0.8–1.4). For > 7 years of use, the relative risk was 1.2 (95% CI, 0.7–2.0). The risk did not vary with the age at starting post-menopausal oestrogen therapy.

In the record-linkage study of a cohort of 22 597 Swedish women to whom post-menopausal oestrogen therapy had ever been prescribed (Persson *et al*., 1996), a 13-year follow-up showed the following standardized incidence ratios for cancer: vulva/vagina, 1.2 (95% CI, 0.7–1.8); pancreas, 1.1 (95% CI, 0.9–1.4); brain, 0.8 (95% CI, 0.6–1.0); lung, 1.0 (95% CI, 0.8–1.2); urinary bladder, 0.9 (95% CI, 0.7–1.1); other skin cancers, 0.9 (95% CI, 0.7–1.3); endocrine glands other than thyroid, 1.0 (95% CI, 0.8–1.3) and connective tissue, 1.6 (95% CI, 1.0–2.4).

3. Studies of Cancer in Experimental Animals

3.1 Studies reviewed previously

The conclusions with regard to carcinogenicity in experimental animals for oestrogens used in post-menopausal oestrogen therapy in the previous monograph (IARC, 1979) are summarized below.

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.

Oestradiol and its esters were tested in mice, rats, hamsters, guinea-pigs and monkeys by subcutaneous injection or implantation and in mice by oral administration. Subcutaneous administration of oestradiol 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 to mice led to an increased incidence of mammary tumours. Subcutaneous injections to neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life and an increased incidence of mammary tumours.

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.

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.

3.2 New studies of oestrogens used in post-menopausal oestrogen therapy

3.2.1 *Conjugated oestrogens*

(*a*) *Subcutaneous implantation*

Hamster: Groups of eight or nine adult, castrated, male Syrian golden hamsters, 50–55 days of age, were administered equilin or d-equilenin by subcutaneous implantation of a pure pellet (20 ± 1.4 mg) in the shoulder region; to maintain constant levels, the pellets were reimplantated at three-month intervals. The mean daily absorption of equilin and d-equilenin was 147 ± 22 μg and 145 ± 15 μg, respectively. After nine months of treatment, renal adenocarcinomas were detected microscopically in frozen serial sections (at least 25–30 sections from each kidney) stained histochemically for esterase. Equilin produced renal carcinoma in 6/8 hamsters (number of tumours per animal, 5.5 ± 0.9), whereas no detectable tumours were found in nine hamsters after d-equilenin treatment (Li *et al*., 1983). [The Working Group noted the small number of animals, that only a single dose was used and that only the kidney was examined microscopically.]

Groups of six to eight adult, castrated, male Syrian golden hamsters (weighing 85–95 g) were implanted with pellets containing deconjugated hormones designed to provide absorption of 111 ± 11 μg oestrogen per day. Additional pellets were implanted every 2.5 months. The duration of treatment was nine months. Renal tumours were detected in

frozen sections stained for nonspecific esterase activity (Li *et al*., 1995). The incidence in untreated controls was not reported; historically, it was 0 under these experimental conditions (Liehr *et al*., 1986a). All animals developed microscopic renal carcinomas. The numbers of tumours in the two kidneys combined were 15 ± 3 in animals given oestrone, 18 ± 1 in those given equilin plus d-equilenin and 16 ± 2 in those given Premarin®.

(*b*) *Administration with known carcinogens*

Rat: In a study reported in more detail in the monograph on 'Post-menopausal oestrogen–progestogen therapy', one group of seven ovariectomized rats treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) received Premarin® at a concentration of 18.75 mg/kg diet (ppm) for 285 days. Mammary tumours occurred in 0/7 ovariectomized controls given DMBA, 6/7 intact controls given DMBA and 5/7 ovariectomized rats given both DMBA and Premarin® (Sakamoto *et al*., 1997).

3.2.2 *Oestradiol*

(*a*) *Oral administration*

Mouse: Groups of 200–227 female C3H/HeJ mice, six weeks of age, with a high titre of antibodies to the mouse mammary tumour virus (MTV^+) factor were fed diets containing 0, 100, 1000 or 5000 μg/kg diet (ppb) oestradiol for 104 weeks. Interim kills were carried out at 26, 52 and 78 weeks, and all surviving animals were killed at 104 weeks. At that time, the incidence of cervical adenosis was increased in 8/20 mice at 1000 ppb and 3/6 at 5000 ppb, and the incidence of uterine adenocarcinomas was increased in the latter group (5/207 compared with 0/227 controls). Mammary hyperplastic alveolar nodules were increased by this dose, from 0/57 in controls to 5/78 at weeks 40–65, 3/29 in controls to 5/19 at weeks 66–91 and 6/50 in controls to 6/17 at weeks 92–105; the time to development of mammary adenocarcinomas was also shortened, the tumour incidences being 4/91 in controls and 5/93 at the high dose at weeks 0–39, 15/57 in controls and 34/78 at the high dose at weeks 40–65, 13/29 in controls and 11/19 at the high dose at weeks 66–91 and 19/50 in controls and 8/17 at the high dose at weeks 92–105 (Highman *et al*., 1980).

(*b*) *Subcutaneous and/or intramuscular administration*

Rat: Groups of 2–16 female Fischer 344 rats, seven weeks of age, were each injected subcutaneously with 5 mg oestradiol dipropionate once every two weeks for 13 weeks. Treated animals were killed at two-week intervals during the study. Ten untreated female rats were used as controls, five rats being killed at week 7 and at week 13. No pituitary tumour was observed in control animals, but pituitary adenomas were observed in 1/2 treated animals killed at week 5 and 11/12 killed at week 7, and carcinomas were observed in 1/12 rats killed at week 7, 6/6 at week 9, 4/4 at week 11 and 16/16 at week 13 (Satoh *et al*., 1997).

(*c*) *Subcutaneous implantation*

Rat: A group of 21 intact ACI rats, 61–63 days of age, received subcutaneous implants of Silastic tubing containing 27.5 mg crystalline oestradiol. A group of three untreated females served as controls. Treatment with oestradiol resulted in rapid development of palpable mammary tumours, which were first observed 99 days after treatment; 100% of the treated group developed tumours within 197 days. The mean time to appearance of the first palpable tumour was 145 ± 26 days. All of the mammary tumours were classified as carcinomas, and invasive features were observed. The average concentration of circulating oestradiol in the serum of treated animals at the time of killing was 185 pg/mL. Mammary tumours were not observed in intact controls or in 11 ovariectomized female rats treated at 45 days of age with oestradiol for 140 days. Intact and ovariectomized rats had similar incidences of oestradiol-induced pituitary tumours (Shull *et al*., 1997).

Hamster: Male Syrian golden hamsters were orchiectomized at seven weeks of age; then, four weeks later, they received implants every three months of pellets containing 20 mg oestradiol. After 5.3 months, renal-cell dysplasia and infiltrating and non-infiltrating renal carcinoma were observed in 5/5 oestradiol-treated animals. No tumour was observed in untreated control hamsters (Goldfarb & Pugh, 1990).

Li *et al*. (1983) reported renal carcinomas in 6/6 castrated male hamsters treated similarly with pellets of 20 mg oestradiol for 8.3 months.

(*d*) *Administration with known carcinogens*

Mouse: Groups of virgin female Swiss mice, 12–13 weeks of age, receive no treatment (10 mice), beeswax-impregnated cotton threads inserted into the cervix (10 mice), 0.1 mL olive oil weekly by injection (4 mice), an intracervical insertion of beeswax-impregnated threads containing approximately 600 µg 3-methylcholanthrene (MCA) and weekly injections of 0, 0.01, 0.1, 5 or 50 µg oestradiol for 16 weeks (18–25 mice), insertion of beeswax-impregnated threads and weekly injections of 0.01, 0.1, 5 and 50 µg oestradiol throughout the period of observation (6–9 mice) or weekly injections of 0.01, 0.1, 5 and 50 µg oestradiol alone (5–8 mice). Placement of thread containing MCA resulted in the emergence of precancerous and cancerous lesions in the cervical epithelium. Weekly administration of oestradiol resulted in incidences of cervical squamous-cell carcinomas of 16/24, 16/26, 10/18 and 8/19 at the four doses, respectively, as compared with 16/21 mice given only MCA by the same regimen. The decrease in the incidence of carcinomas was significant ($p < 0.05$) with the high dose of oestradiol. The occurrence of hyperplastic and dysplastic changes was not correlated with treatment (Das *et al*., 1988). [The Working Group noted that the effect could have been due to interference with the metabolism of MCA.]

Groups of 30 or 31 female ICR mice, 10 weeks of age, were given 10 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) by intravaginal instillation once a week for three weeks and then fed a diet containing 0 or 5 ppm oestradiol for 20 weeks, starting one week after the last exposure to MNU; a third group of 31 mice was given oestradiol in the diet, and a fourth group of 15 mice received basal diet. At the termination of the experiment at

week 23, the incidence of endometrial adenocarcinomas in the groups receiving both MNU and oestradiol (15/31) was significantly higher ($p = 0.001$) than that in the group given MNU alone (2/29) and significantly higher ($p = 0.001$) than that given oestradiol alone (7/31). The incidence of endometrial preneoplastic lesions in the group receiving oestradiol alone was 48%. No endometrial lesions or carcinomas were observed in the 15 controls on basal diet. Small numbers of squamous-cell carcinomas and preneoplastic lesions (dysplasia and hyperplasia) were also seen in the uterine cervix of mice given MNU alone or MNU plus oestradiol (Niwa *et al*., 1991). [The Working Group noted that no statistical comparison was presented between the untreated controls and the group receiving oestradiol alone.]

Groups of 30 female ICR mice, 10 weeks of age, were given 10 mg/kg bw MNU into the left uterine corpus and normal saline into the right. One week later, the animals received a diet containing 0 or 5 ppm oestradiol for 30 weeks. At that time, the incidence of endometrial adenocarcinomas in the group given MNU plus oestradiol (8/24) was higher than that in mice given MNU alone (3/26), but the difference was not statistically significant. The incidence of preneoplastic endometrial lesions (atypical and adenomatous hyperplasia) was somewhat increased in the group given MNU plus oestradiol in comparison with those given MNU alone (Niwa *et al*., 1993).

Groups of 24–73 female ICR mice, 10 weeks of age, were fed a diet containing 0 or 5 ppm oestradiol from the beginning of the experiment up to 16 weeks and an intravaginal instillation of 10 mg/kg bw MNU once a week for three weeks from week 4 (73 mice), oestradiol only (41 mice), MNU only (41 mice) or were untreated (24 mice). Mice from each group were killed and necropsied at weeks 8, 12, 16, 23 and 30. Oestradiol induced cystic glandular hyperplasia and adenomatous and atypical hyperplasia of the endometrium, and MNU induced adenomatous and atypical hyperplasia. Oestradiol did not induce endometrial carcinoma. Data presented in bar graphs indicate that the incidence of endometrial adenocarcinoma was approximately 10% in the mice given MNU alone and approximately 30% in those given MNU plus oestradiol [no statistics specified] (Niwa *et al*., 1996).

Three groups of 25–29 CD-1 mice, 10 weeks of age and in persistent oestrous, were given either a single intrauterine administration of polyethylene glycol, 12.5 mg/kg bw *N*-ethyl-*N*-nitrosourea (ENU) dissolved in polyethylene glycol or ENU in the same manner plus subcutaneously implanted oestradiol pellets one week before ENU administration, the pellets being renewed after eight weeks of the experiment. At termination of the experiment at week 15 after ENU treatment, all surviving mice were killed for assessment of proliferative uterine lesions. All groups had endometrial hyperplasia, the severity being greatest in mice given oestradiol plus ENU. The incidence of adenocarcinomas in this group (20/29) was significantly greater ($p < 0.01$) than that in mice given the vehicle (0/25) or in mice given ENU alone (0/29) (Takahashi *et al*., 1996).

Rat: Groups of 19 female Sprague-Dawley rats were ovariectomized at 60 days of age and given a single dose of 0 or 0.25 mg MNU by vaginal instillation, followed one week later by subcutaneous implantation of long-term release Silastic pellets containing

5 mg/mL oestradiol in sesame oil. After 16 months, an increase in the incidence of benign vaginal stromal polyps (4/19) was found in the MNU plus oestradiol group. No vaginal polyps were seen in groups given either MNU alone (0/19) or oestradiol alone (0/17). A number of non-neoplastic changes also seen in the vagina and uterus were due to oestradiol treatment either with or without MNU (Sheehan *et al*., 1982).

Groups of 29–30 female Sprague-Dawley rats, 50 days of age, received an intravenous injection of 50 mg/kg bw MNU and, 10 days later, subcutaneous injections of 20 µg oestradiol, 4 mg progesterone, 20 µg oestradiol plus 4 mg progesterone or sesame oil on five days a week for 40 days. A further group were ovariectomized at 60 days of age and received no further treatment. Administration of oestradiol delayed the appearance of mammary carcinomas, reduced the incidence (13/30 compared with 27/30 with MNU alone) and decreased the number of tumours per rat (0.6 versus 3.5). Concomitant administration of oestradiol and progesterone after initiation with MNU was as effective as ovariectomy in inhibiting mammary carcinogenesis after initiation with MNU: 4/29 and 4/29, respectively, compared with 27/30 with MNU alone (Grubbs *et al*., 1983).

Groups of 10 male Fischer 344 rats, weighing 130–150 g, were given 0 or 0.05 mg/kg bw oestradiol after partial hepatectomy. After a 13-day recovery phase, all animals received 0.02% 2-acetylaminofluorene in the diet for two weeks, with a further growth stimulus in the form of 2 mL/kg bw carbon tetrachloride given by intragastric instillation on day 7 of the feeding period. There was no significant difference in the incidence of γ-glutamyltranspeptidase-positive foci [putative preneoplasic lesions] in oestradiol and control groups (Schuppler *et al*., 1983).

Groups of 12 or 14 male Wistar Furth rats were castrated at 40 days of age and received subcutaneous implants of pellets containing 5 mg oestradiol, which were replaced every two months throughout the 12-month experiment. Twelve rats at 50–55 days of age were further given 5 mg/kg bw *N*-butyl-*N*-nitrosourea. None of the 14 castrated rats given oestradiol alone developed hepatic tumours. Treatment with the nitrosourea plus oestradiol did not elicit any hepatic tumours; however, oestradiol alone or in combination with the nitrosourea resulted in high incidences of pituitary adenomas (9/14 and 8/11, respectively). No control data were reported (Sumi *et al*., 1984).

Groups of 12 or 13 female Sprague-Dawley rats, seven weeks of age, were subjected to partial hepatectomy; 24 h later, they received 5 mg/kg bw *N*-nitrosodiethylamine (NDEA) and then 0 or 0.6 ppm oestradiol in the diet for nine months. In NDEA-initiated rats, oestradiol did not increase the number of γ-glutamyltranspeptidase-positive lesions per liver or the incidence of hepatic nodules or hepatocellular carcinoma (Yager *et al*., 1984).

A group of 30 female Sprague-Dawley rats, 50–55 days of age, received implants of 3 mg oestradiol-containing silicone wafers; 48 h later, all animals were given 20 mg DMBA by oral gavage. The animals were palpated for mammary tumours after one month and twice weekly thereafter. Oestradiol treatment was continued for 160 days in 15 animals, and the implants from 15 rats were removed after 14 days. After 160 days, 90% of the surviving animals treated continuously with oestradiol had developed palpable

mammary tumours; this incidence was similar to that in rats from which the implant had been removed at 14 days (Wotiz *et al*., 1984).

Groups of 24–31 female Sprague-Dawley rats, 40 days of age, were given 0 or 20 μg oestradiol and/or 4 mg progesterone by subcutaneous injection on five days a week for five weeks. A dose of 50 mg/kg bw MNU was administered at 96 and 103 days of age, three and four weeks, respectively, after the last hormone injection. The incidence of mammary adenocarcinomas was 48% in the MNU plus oestradiol group, 42% in the MNU plus progesterone group, 13% in the MNU plus oestradiol plus progesterone group ($p < 0.05$) and 61% in the group given MNU alone (Grubbs *et al*., 1985).

Groups of six female Sprague-Dawley rats, weighing 200–250 g, received 5 mg/kg bw oestradiol 1 or 24 h before an intraperitoneal injection of 0 or 50 mg/kg bw NDEA and were killed after eight weeks. The numbers of γ-glutamyltranspeptidase-positive foci per cm^3 of liver increased from 364 ± 57 in animals given only NDEA to 1149 ± 186 in those receiving oestradiol 24 h before NDEA ($p < 0.01$); the number was increased to 3779 ± 280 ($p < 0.001$) when the hormone was injected 1 h before the carcinogen, i.e. about 25% of the number of foci scored in control rats receiving NDEA 24 h after partial hepatectomy (Taton *et al*., 1990).

Female Wistar MS rats were ovariectomized at 23 days of age and, at 60 days of age, were divided into groups of 20–25 rats. The animals were given subcutaneous injections of 50 μg oestradiol benzoate in 0.2 mL olive oil, 5 mg progesterone in 0.2 mL olive oil or 50 μg oestradiol benzoate plus 5 mg progesterone daily for 14 days. At this time, rats were irradiated with 260 cGy γ-rays, followed 30 days later by subcutaneous implantation of pellets containing diethylstilboestrol (estimated release rate, 0.38 μg per day). The rats were observed for the appearance of palpable mammary tumours for up to one year. The tumour incidences were 6/23 in the controls, 12/21 in rats given oestradiol benzoate alone ($p < 0.05$), 8/25 in rats given progesterone alone and 9/23 in those given oestradiol plus progesterone. These increases were accompanied by significant increases in DNA synthesis in the mammary gland, as determined on the final day of oestrogen or progestogen at the time of radiation treatment (Inano *et al*., 1995).

(*e*) *Carcinogenicity of metabolites*

Hamster: In two studies, castrated male Syrian golden hamsters were given the 2-hydroxy- and 4-hydroxy metabolites of oestradiol. In the first study, the oestrogen-containing pellets (25 mg) were implanted at 0 and three months and left for six months. In the second study, the oestrogen-containing pellets were implanted every three months and left for 9–10 months. Oestradiol produced renal-cell carcinomas in 4/5 and 6/6 hamsters, respectively; 2-hydroxyoestradiol in 0/5 and 0/6 hamsters, respectively; and 4-hydroxyoestradiol in 4/5 and 5/5 hamsters, respectively (Liehr *et al*., 1986a; Li & Li, 1987).

3.2.3 *Oestriol*

Mouse: Groups of 30 female ICR mice, 10 weeks of age, received 10 mg/kg bw MNU solution into the left uterine corpus and saline into the right. One week later, animals

received a diet containing 0 or 25 mg/kg diet (ppm) oestriol for 30 weeks. At that time, all surviving mice were necropsied and underwent histological examination. Endometrial adenocarcinomas developed in both groups: the incidence was 7/25 in mice given MNU plus oestriol and 3/26 in controls, but the difference was not statistically significant (Niwa *et al*., 1993).

Rat: Two groups of 30 female Sprague-Dawley rats, 55 days old, received subcutaneous implants of Silastic wafers containing 0 or 5 mg oestriol; 48 h later, all rats were given 20 mg DMBA by oral gavage. The animals were examined one month after DMBA treatment and thereafter once weekly. Seven weeks after the onset of the first mammary tumour (day 42 after DMBA treatment), palpable mammary tumours were found in all of the 28 surviving animals given DMBA alone and in 6/26 given DMBA plus oestriol; 180 days after the onset of the first mammary tumour, 13/26 given DMBA plus oestriol had palpable mammary tumours (Wotiz *et al*., 1984).

Thirty female Sprague-Dawley rats, 50–55 days old, received subcutaneous implants of Silastic wafers containing 5 mg oestriol; 48 h later, all animals received 20 mg DMBA by oral gavage. The implants were removed from 15 animals after 14 days. At the termination of the experiment at 180 days, the incidence of mammary tumours was 60% after two weeks of oestriol treatment and 20% with continuous oestriol treatment (Wotiz *et al*., 1984).

Groups of 19 female Sprague-Dawley rats, 35–50 days of age, received 20 mg DMBA in 1.5 mL sesame oil by oral gavage; two weeks later, one group received subcutaneous implants of crystalline pellets containing 638 μg oestriol each month for 10 months. The incidences of mammary carcinomas at one year were 12/19 in the group receiving DMBA plus oestriol and 18/19 in those given DMBA alone ($p < 0.05$, χ^2 test) (Lemon, 1987).

Groups of 8–26 virgin female Sprague-Dawley rats, 40–50 days of age, were irradiated from a cobalt-60 gamma source delivering 3.5 Gy to the dorsal area of the rats. Crystalline sodium chloride pellets containing oestriol (638 ± 175 μg per month) were implanted subcutaneously into the anterior dorsal area each month for life. Control rats were irradiated without oestriol treatment. Oestriol treatment was begun one to three days before irradiation or 5, 13 or 15 days after irradiation. The rats were weighed and examined every 10–14 days during their natural life span after irradiation. Biopsies were performed on persistent and growing tumours within two to four weeks of discovery, and biopsy tissues were examined histopathologically. Tumour-free rats were observed until death, at which time they underwent necropsy. Of 142 irradiated controls, 93 developed mammary carcinomas; two-thirds of the tumours appeared more than 300 days after irradiation. When oestriol administration was begun one to three days before or five days after irradiation, no significant reduction in mammary carcinoma incidence (29/54 controls versus 50/113 oestriol-treated) was observed. When oestriol administration was further delayed, a significant reduction in mammary carcinogenesis was observed: 7/12 controls versus 6/14 given oestriol 15 days after irradiation ($p < 0.07$) and 14/20 controls versus 6/18 rats given oestriol 13 days after irradiation ($p < 0.02$). This inhibition was stated to be associated with the rapid differentiation of the mammary gland (Lemon *et al*., 1989).

3.2.4 *Oestrone*

(*a*) *Subcutaneous implantation*

Hamster: Implantation of 20-mg pellets of oestrone resulted in microscopic renal carcinomas in 8/10 male castrated Syrian hamsters after 8.5 months of treatment (Li *et al*., 1983).

(*b*) *Administration with known carcinogens*

Mouse: Groups of 30 female ICR mice, 10 weeks of age, were given 10 mg/kg bw MNU into the left uterine corpus and normal saline into the right. One week later, the mice received a diet containing 0 or 25 mg/kg diet (ppm) oestrone for 29 weeks. At that time, the incidence of adenocarcinoma in the group given MNU plus oestrone (9/23) was significantly higher ($p < 0.05$) than that in mice given MNU alone (3/26). In addition, the incidences of preneoplastic endometrial lesions (atypical and adenomatous glandular hyperplasia) in mice receiving oestrone with or without MNU were higher than that in controls (Niwa *et al*., 1993).

Toad: Groups of 100 female toads (*Bufo regularis*), weighing approximately 50 g, received either subcutaneous injections of 1 mL amphibian saline containing 3 mg *N*-nitrosodimethylamine (NDMA) into the dorsal lymph sac once a week, subcutaneous injections of 0.1 mg oestrone dissolved in 1 mL corn oil once a week or 3 mg NDMA followed by direct injection of 0.1 mg oestrone in 1 mL corn oil once a week. The duration of the experiment was 14 weeks. The incidence of hepatocellular carcinomas was 17/99 in toads given NDMA alone, the first tumour appearing at week 8. In toads treated with oestrone alone, the incidence was 4/97, the first tumour appearing at week 12 after the first injection. The incidence of liver tumours was 23/94 in toads treated with NDMA plus oestrone, the first tumour appearing at week 6 after initiation (Sakr *et al*., 1989).

(*c*) *Carcinogenicity of metabolites*

Rat: Groups of 20 female Crl:CD(SD)BR rats, 30 days of age, were given 100 µL dimethyl sulfoxide (DMSO) containing 30 µmol/rat oestrone-3,4-quinone [purity not specified], DMSO alone or 1.2 µmol/rat *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (purity, > 99%), which were used as vehicle and positive controls, respectively. One-sixth of the total dose was injected under each of six nipples on the left side of each rat, whereas DMSO only was injected under the nipples on the right side. The thoracic mammary glands of the rats were treated at 30 days of age, and those located in the inguinal area were treated on the following day. Rats were fed a high-fat AIN76A diet (23.5% corn oil) throughout the course of the experiment. The experiment was terminated 44 weeks after treatment. The positive control induced mammary tumours in 20/20 rats, but there was no difference in tumour incidence or multiplicity among rats receiving DMSO (3/20) and those treated with oestrone-3,4-quinone (4/20) (El-Bayoumy *et al*., 1996).

Hamster: Li and Li (1987) investigated the carcinogenicity of the 2-hydroxy and 4-hydroxy metabolites of oestrone in castrated male Syrian golden hamsters given implants

of oestrogen-containing pellets every three months for 9–10 months. Renal tumours were found in 8/10 hamsters given oestrone, 0/6 given 2-hydroxyoestrone and 2/6 given 4-hydroxyoestrone.

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

4.1 Absorption, distribution, metabolism and excretion

The disposition of oestradiol, oestrone and oestriol is considered together, because there is interconversion between oestradiol and oestrone *in vivo* in both humans and other mammals, and the latter is converted to oestriol (Figure 4).

Various preparations of oestradiol, such as crystalline oestradiol, micronized oestradiol and esterified oestradiol (e.g. oestradiol valerate, oestradiol 3-benzoate, oestradiol dipropionate), are used for post-menopausal hormonal therapy. The absorption of these oestradiol preparations differs, while the route of exposure remains the same. For example, crystalline oestradiol applied dermally in a cream diffuses more readily through the skin to the systemic circulation than esterified oestradiol, because oestradiol is more lipophilic than its ester derivative. Similarly, micronized oestradiol is absorbed more rapidly than crystalline oestradiol because of its small particle size. The absorption of these oestradiol preparations also depends on the dose administered and the route of administration.

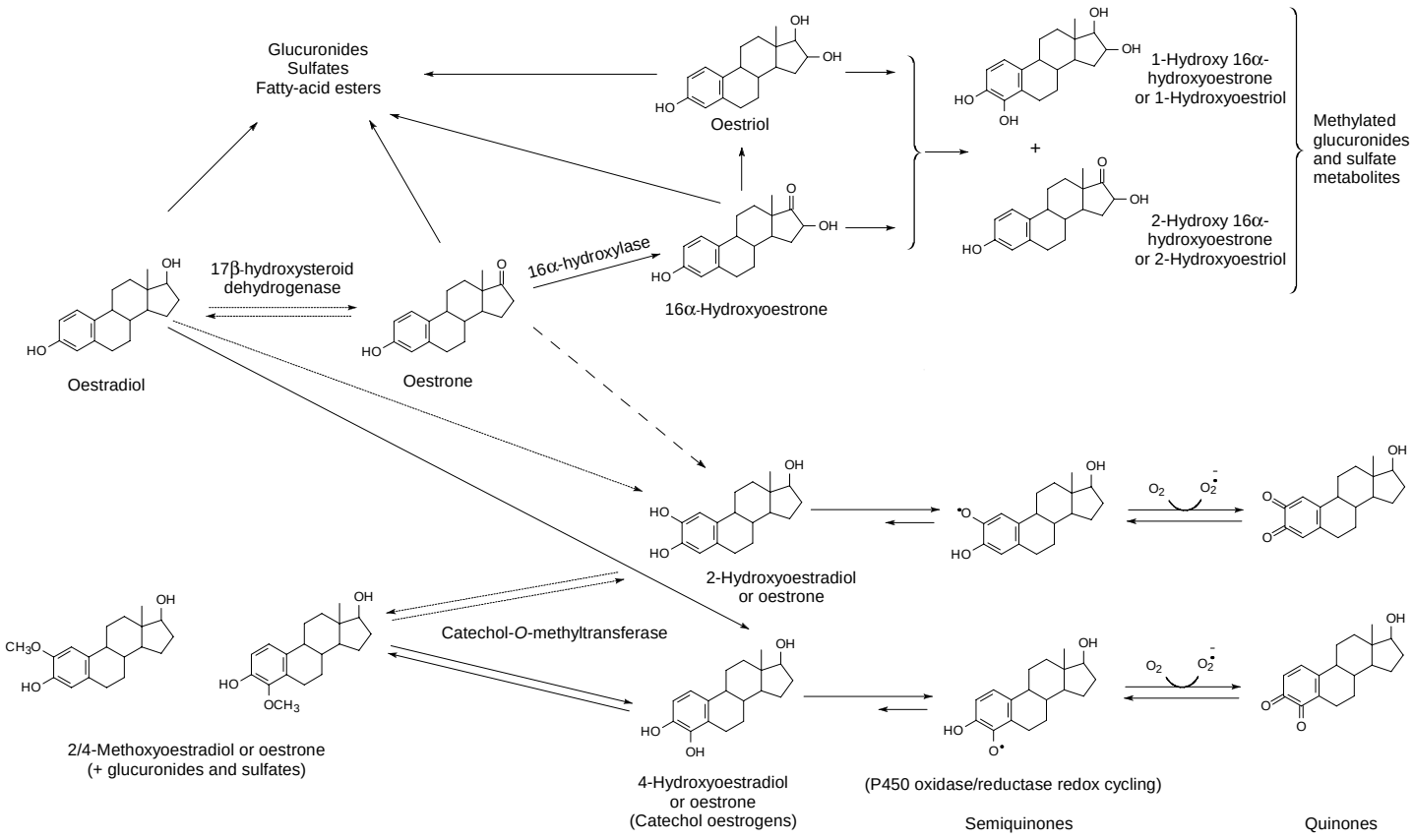
The pharmacokinetics of conjugated equine oestrogens is complicated because so many different kinds of oestrogens are present, including oestrone sulfate (15%), equilin sulfate (25%), dihydroequilin sulfate (15%) and several other oestrogen sulfates. All of these oestrogens undergo metabolic conversions in the gastrointestinal tract and liver. Equilin sulfate and oestrone sulfate are the major components (approximately 40%) of the equine oestrogen preparation Premarin®, which is the most widely prescribed oestrogen used in therapy for post-menopausal women in the United States.

4.1.1 *Humans*

The absorption of oestradiol in humans has been studied extensively; however, the results are difficult to compare as different preparations of oestradiol and different routes of administration have been used.

Daily oral administration of oestradiol tablets results in large pulses of oestradiol and oestrone and exposes women to high concentrations of these compounds. Oral administration of the first oestradiol tablet, 2 mg micronized oestradiol, to 32 healthy post-menopausal women resulted in a maximal plasma oestradiol concentration of 1084 pg/mL 49 min after administration, which decreased rapidly during the subsequent 3 h. Progressive accumulation of oestradiol occurred until a steady state was reached. After the fifth tablet, the average concentration of oestradiol was about 418 pg/mL, which was 12 times greater than that found when a transdermal patch was used. The oestrone concentration reached a peak of 334 pg/mL 4.3 h after the first administration and reached a steady state

Figure 4. Pathways for the metabolism and redox cycling of oestradiol, oestriol and oestrone



Modified from Yager & Liehn (1996)

after the 14th daily administration. This average concentration of oestrone was 9.4 times greater than that found when a transdermal patch was used (Setnikar *et al*., 1996).

After a single oral dose of 2.0 mg/day oestradiol valerate to post-menopausal women, the maximal plasma concentrations were 0.96 nmol/L oestrone, 0.19 nmol/L oestradiol, 44.4 nmol/L oestrone sulfate, 0.6 nmol/L oestradiol sulfate and 0.19 nmol/L oestriol sulfate. The times to reach the maximal concentration were 5.2 h for oestrone, 3.2 h for oestradiol, 4.1 h for oestrone sulfate, 5.0 h for oestradiol sulfate and 8.8 h for oestriol sulfate (Aedo *et al*., 1990).

A comparison of the pharmacokinetic parameters of oral and sublingual administration of micronized oestradiol to post-menopausal women revealed that the time to the maximal concentration of oestradiol was significantly different by the two routes of administration, being 1 h or less for sublingual administration and 6.5–7.6 h for oral administration. The maximal plasma concentration, terminal half-life, area under the curve for the integral of the serum concentration over time (area under the curve) and oral clearance were also different with the two routes of administration. For example, after sublingual administration of 1 mg micronized oestradiol, the maximal plasma oestradiol concentration was 451 pg/mL, the terminal half-life was 18 h, the area under the curve was 2109 pg/mL per h and the oral clearance was 7.6 L/h per kg bw; after oral administration, these values were 34 pg/mL, 20.1 h, 823 pg/mL per h and 27.2 L/h per kg bw, respectively. The concentrations of oestrone were not dependent on route of administration. Sublingual administration resulted in a significantly lower ratio of oestrone to oestradiol than oral administration during the 24-h period (Price *et al*., 1997).

Because oestrogen penetrates normal skin easily (Jewelewicz, 1997), various preparations based on this property have been evaluated, including subcutaneous implants, vaginal creams and rings, percutaneous gels and transdermal therapeutic systems. Transdermal oestradiol provides physiological levels of oestradiol at a constant rate; the transdermal route avoids loss of drug by the hepatic first-pass effect and minimally affects hepatic protein metabolism. As a result, the oestradiol:oestrone ratios more closely resemble those of pre-menopausal women. Maximal serum concentrations of oestradiol are reached within 2–8 h of application of a transdermal system. When 50 µg/day transdermal therapy is used on a long-term basis, the mean steady-state serum concentrations can be 20–50 pg/mL. In a study of 100 µg/day transdermal therapy, the mean oestradiol concentration was 46–152 pg/mL. Within 24 h of removal of the transdermal delivery system, the plasma concentrations of oestradiol and oestrone and the urinary excretion of oestradiol and oestrone conjugates generally returned to pre-treatment levels (Balfour & Heel, 1990).

The pharmacokinetic profiles of oestradiol and oestrone have been reported in 16 healthy post-menopausal women after twice weekly applications for three weeks of an oestradiol transdermal patch, which contains 4 mg oestradiol and delivers 50 µg oestradiol daily. During the first application, oestradiol reached effective concentrations of 30 pg/mL or more 12 h after application; during the following five applications, the concentration remained constant at an average of 35 pg/mL. After removal of the patch, the concentration

returned to basal level within 12 h. The oestrone concentration reached a maximum of 48 pg/mL 41 h after the first application and then remained constant (Setnikar *et al*., 1996).

Seven days' use of patches containing 0.1 or 0.05 mg/day oestradiol resulted in peak average blood concentrations of 100 and 50 pg/mL, respectively. Values approximating 90% of the maximal level were achieved within 12 h after patch application and were maintained for up to 48 h. The mean steady-state blood concentrations over seven days were approximately 70 and 35 pg/mL, respectively. After removal of the patch, the concentrations fell to near baseline within 12 h (Gordon, 1995).

Subcutaneous administration of oestradiol pellets containing 25–200 μg pure crystalline oestradiol resulted in good bioavailability, as seen from the oestradiol:oestrone ratios, because minimal metabolism occurs in subcutaneous tissues (Jewelewicz, 1997). Pellets of 25 mg and 50 mg oestradiol produced serum concentrations of oestradiol of approximately 50–70 pg/mL and 100–120 pg/mL, respectively, for up to several months (Stumpf, 1990). Although the serum oestradiol concentrations were reported to be stable with this route of administration, 12 women of similar age and body weight in one small open study showed striking variations in hormone concentrations over 12 months of follow-up (Jewelewicz, 1997).

In a study of 24 women with vaginal atrophy treated daily with vaginal tablets containing 10 or 25 μg oestradiol, the oestradiol plasma concentrations reached 45 and 60 pmol/L after two weeks, respectively. The plasma oestrone concentration was unchanged by treatment (Johnston, 1996).

Oestradiol-containing polydimethylsiloxane rings inserted into the vagina release oestradiol continuously, and the substance is readily absorbed by the vaginal mucosa. Relatively constant serum concentrations of approximately 150 pg/mL oestradiol and oestrone were achieved for 21 days from a ring containing 400 mg oestradiol (Kuhl, 1990). The bioavailability of oestradiol from vaginal rings has been reported to be 13 ± 7% (range, 7–27%) in post-menopausal women (Gabrielsson *et al*., 1995).

After intramuscular injection of oestradiol esters in oily solution, the compound is released slowly from the primary depot at the injection site and/or from secondary depots in fat tissue (Kuhl, 1990).

Oral intake of 8 mg oestriol resulted in a maximum plasma concentration of 75 pg/mL unconjugated oestriol after 2 h, and the concentrations increased to up to 130 pg/mL after continued daily ingestion of 8 mg oestriol for 30 days, although the serum concentration of conjugated oestriol remained unaltered (Schiff *et al*., 1978). Orally administered oestriol was almost completely conjugated in the intestine to glucuronides (80–90%) and sulfates (10–20%); only 1–2% of the parent steroid reached the circulation (Kuhl, 1990).

Oestriol undergoes much less metabolism after vaginal application than after oral ingestion, and 20% of the dose appears as unconjugated steroid in the blood. At a dose of 0.5 mg, peak levels of 100–150 pg/mL were observed within 2 h. The maximal concentrations of oestriol after vaginal application of 0.5 mg were similar to those obtained after oral intake of 8–12 mg oestriol (Kuhl, 1990). There was no significant difference in the

plasma oestriol concentrations after vaginal administration of 1 mg and oral administration of 10 mg oestriol (Heimer, 1987). Daily administration of 1 g of a cream containing 500 µg oestriol to 11 post-menopausal women for eight weeks caused a mean rise in plasma oestriol from unmeasurable (< 35 pmol/L) to 87 pmol/L (Haspels *et al.*, 1981). Treatment with a low dose of oestriol by the vaginal route may therefore induce systemic effects comparable to those achieved with high oral doses.

After a single oral dose of 2.5 mg/day piperazine oestrone sulfate to post-menopausal women, the maximal plasma concentrations were 1.3 nmol/L oestrone, 0.25 nmol/L oestradiol, 54 nmol/L oestrone sulfate, 0.9 nmol/L oestradiol sulfate and 0.23 nmol/L oestriol sulfate. The time to reach the maximal plasma concentration was 6.4 h for oestrone, 9.8 h for oestradiol, 4.4 h for oestrone sulfate, 6.5 h for oestradiol sulfate and 4.9 h for oestriol sulfate (Aedo *et al.*, 1990).

Most of the available data on distribution are based on studies of intravenous administration. After intravenous administration of oestradiol to post-menopausal women, a high clearance (1.8 ± 0.6 L/min) and a low distribution volume (51 ± 28 L) were found. Oestrogens circulate in the blood bound to albumin (about 60%), sex hormone-binding globulin (about 38%), α_1-glycoproteins and transcortin. Oestradiol binds weakly to albumin (low affinity/high capacity; plasma concentration, 40 g/L), about one-third is tightly bound to sex hormone-binding globulin (high affinity/low capacity) and a small fraction (< 3%) is 'free'. After intravenous administration, the distribution volume of oestradiol at steady state was only about 70 L, representing 1.5–2 times the total body water in fertile women. Its low level of distribution is consistent with its high level of binding to plasma proteins. The production of sex hormone-binding globulin is stimulated by increasing oestrogen concentration. Because of the high concentration of albumin (about five orders of magnitude higher than sex hormone-binding globulin) and its rapid dissociation, albumin may serve a more important regulating role (Gabrielsson *et al.*, 1996).

After daily transdermal treatment of post-menopausal women with 0.1 mg oestradiol for three consecutive cycles, there was no significant difference in the distribution of oestradiol and oestrone between free and protein-bound oestrogen fractions in peripheral plasma (Jasonni *et al.*, 1988).

Oestrone is not tightly bound to plasma proteins and therefore has a higher clearance rate than oestradiol. Oestrone sulfate is quantitatively the most important plasma oestrogen metabolite and is bound with high affinity to albumin, 90% circulating in bound form. Oestrone sulfate is considered to have a large, slowly metabolized reservoir, with 90% of its mass bound to albumin; accordingly, it has a low metabolic clearance rate (about 150 L/24 h) and low renal clearance (Anderson, 1993).

Few studies have addressed the accumulation and storage of oestradiol, oestrone and oestriol after exogenous administration. All three are distributed to various target and non-target organs through the systemic circulation but are also produced locally and accumulate in target tissues particularly rich in fat. Knowledge about the metabolism of oestradiol, oestrone and oestriol in humans has not advanced much since the last evaluation (IARC, 1979), except for hydroxylation of oestradiol.

The metabolic disposition of oestrogens includes oxidative metabolism (largely hydroxylation) and conjugative metabolism by glucuronidation, sulfonation and/or *O*-methylation (reviewed by Zhu & Conney, 1998). Oestradiol is converted to oestrone by a 17β-hydroxysteroid dehydrogenase; the oestrone produced is further metabolized to 16α-hydroxyoestrone and then to oestriol (Johnston, 1996). Hydroxylation of oestradiol at the 2 position is a major metabolic pathway in the liver (Kerlan *et al*., 1992). There are large inter-individual differences in oestradiol 2-hydroxylation in human liver samples, which may be reflected by differences in oestrogenic action. 4-Hydroxylation of oestradiol to a catechol is a minor pathway (usually < 15% of 2-hydroxylation) in the liver. Recent studies have shown that 4-hydroxylation of oestradiol is the dominant pathway of catechol oestrogen formation in human breast and uterus (Figure 4; Liehr *et al*., 1995; Liehr & Ricco, 1996). In humans, 4-hydroxylation of oestradiol is catalysed by the cytochrome P450 enzyme CYP1B1 (Hayes *et al*., 1996). Oestradiol and oestrone hydroxylated can undergo metabolic redox cycling *in vitro* to generate free radicals such as superoxide and the chemically reactive oestrogen semiquinone/quinone intermediates (Liehr & Roy, 1990). In the presence of fatty acid acyl-coenzyme A, oestradiol can be converted at the C-17 position to very lipophilic oestrogen fatty acid esters by enzymes present in liver and in oestrogen target organs such as breast and placenta (Adams *et al*., 1986).

Oestrone sulfate is the oestrogen found at the highest concentration in plasma and seems to constitute a storage form for circulating oestrogens. Oestrone sulfate can be hydrolysed to oestrone by arylsulfatases, which are widely distributed in human tissues (Rozenbaum, 1996).

There is a reversible equilibrium between oestradiol, oestrone and oestrone sulfate, which are interconverted by oestradiol dehydrogenase, sulfotransferase and aryl sulfatase. The two pathways of phase I oestrone inactivation are ring A metabolism, which produces catechol–oestrogens and is favoured in underweight women and hyperthyroid patients, and ring D metabolism, which leads to oestriol production and is increased in obese women and hypothyroid patients. Phase II metabolism involves the formation of several oestrogen conjugates; the sulfates circulate in high concentrations in the blood, and the glucuronides are excreted with the bile and in urine. After oral administration of micronized oestradiol, the serum oestrone:oestradiol ratio, which is 1:2 in fertile women and 2:1 in post-menopausal women, increased to 4:1. Prolonged percutaneous or transdermal treatment with oestradiol in conjunction with subcutaneous implantation of oestradiol pellets has been reported to lead to continuously elevated oestrogen levels and an oestrone:oestradiol ratio of 1:1 to 1:2 (Kuhl, 1990).

Conjugated equine oestrogens are hydrolysed to their active form in the gastrointestinal tract and also undergo considerable hepatic metabolism before entering the bloodstream in an active form (Ansbacher, 1993). Most sulfate esters are hydrolysed to free or unconjugated oestrogen by enzymes in the lower gut; the free oestrogen is absorbed by intestinal tissue, where it can be reconjugated with sulfate. Therefore, the oestrogen sulfate found in the bloodstream is not the same sulfate that was administered. The rate of dissolution is important because it influences where the active ingredients of the product

are released in the gastrointestinal tract, a factor which may affect the amounts of the oestrogen that are activated and the patterns of active and inactive metabolites. Equilin and equilenin are interconverted to 17β-dihydroequilin and 17β-dihydroequilenin and correspond to the interrelation between oestrone and oestradiol. As in the case of natural oestrogen in women, there is an equilibrium between equilin, equilenin and their metabolites and the respective sulfates (Kuhl, 1990).

The sulfate esters of equilin, oestrone and oestradiol do not bind sex hormone-binding globulin; however, equilin sulfate and oestrone sulfate interact with serum albumin with high affinity ($0.9–1.1 \times 10^5$/mol per L). Up to 74% of total equilin sulfate and 85–90% of oestrone sulfate were bound to serum albumin (Bhavnani, 1998). The peak concentration of equilin sulfate is found after 4 h. Equilin is rapidly absorbed and converted to 17β-dihydroequilin. The volume of distribution at steady state was 6 ± 0.5 L for 17β-dihydroequilin sulfate, 23 ± 1.3 L for 17β-dihydroequilin and 12.4 ± 1.6 L for equilin sulfate (O'Connell, 1995).

Six healthy post-menopausal women were seen each month during a six-month trial of cyclic therapy with conjugated equine oestrogens (Premarin®), ingested at 1.25 mg per day for 21 days followed by seven days without therapy. The average serum concentrations in samples taken within 2 h of the last ingestion of drug in a given cycle were 1850 pg/mL unconjugated equilin, 162 pg/mL oestrone and 106 pg/mL oestradiol. Three months after completion of therapy, the oestrone and oestradiol concentrations had returned to pre-treatment levels, but equilin was still detected in serum at a concentration of 144 pg/mL in all three of the women who were investigated (Whittaker *et al*., 1980).

After administration of ^{3}H-equilin sulfate and ^{3}H-17β-dihydroequilin sulfate to post-menopausal women, less than 50% of the administered dose was excreted in the urine. The majority (63–74%) of the radiolabelled metabolites excreted were in the form of glucuronides, whereas 16–17% were found as sulfates and 1–2% in the unconjugated fractions (Bhavnani, 1998). About 40–50% of the radiolabel from injected oestradiol is excreted in the bile (Sandberg & Slaunwhite, 1957).

These studies show that the bioavailability of oestrone, oestradiol and oestriol depends on the formulation, route of exposure and dose of oestradiol administered. Generalizations cannot be made about the disposition of oestrogen because of differences in the regimens, products and route of exposure and other factors such as age and inter- and intra-individual variations. It can be concluded, however, that transdermal administration by patch, percutaneous administration or transvaginal administration allow more circulating oestrogen for a longer time than oral administration. Comparative trials of various oestrogen products are urgently needed, keeping in consideration the route of exposure and the composition of the different products, to allow quantitative evaluation of the disposition of oestrogens.

4.1.2 *Experimental systems*

Few studies have been carried out in experimental systems on the disposition of oestrone, oestradiol and oestriol products since the previous evaluation (IARC, 1979), and

limited experimental studies are available on the absorption, distribution and excretion of oestradiol. Oestrone, oestradiol and oestriol undergo various phase I and phase II metabolic reactions in humans and animals (reviewed by Zhu & Conney, 1998). During phase I metabolism, oestrone, oestradiol and oestriol serve as substrates for aromatic hydroxylation, and these reactions are catalysed by cytochrome P450 enzymes. Oestrone and oestradiol are converted to 2- and 4-hydroxyoestrone and 2- and 4-hydroxyoestradiol, respectively, the ratio of hydroxylated products depending on the target tissues and animal species. Oestradiol and its metabolites serve as substrates for sulfation, methylation and glutathione conjugation. The metabolism depends on the species, strain and sex of the experimental animals and on the experimental conditions.

In the early 1970s and 1980s, it was postulated that oestradiol is converted to reactive intermediates during its metabolism, and direct evidence of reactive metabolites of oestradiol has now been obtained (Liehr *et al*., 1986b; Roy *et al*., 1991). Catechol oestrogens undergo microsomal cytochrome P450-mediated redox cycling reactions (Liehr *et al*., 1986b; Liehr & Roy, 1990; Roy *et al*., 1991), resulting in the formation of reactive metabolites. Both catechols of oestradiol are converted to their respective quinones in the presence of metabolic activation systems. Nuclei can also catalyse redox cycling of oestrogens (Roy & Thomas, 1994). *In vitro*, oestradiol in the presence of a metabolic activation system can be converted to DNA-binding oestrogen quinone metabolite(s) (Liehr *et al*., 1993), but DNA binding has not been detected *in vivo*.

Oestrone, oestradiol and oestriol are excreted in the bile as glucuronides and undergo enterohepatic recirculation. Their glucuronides are hydrolysed in the intestine, and unconjugated oestradiol or oestrone is reabsorbed from the intestine by enterohepatic cycling (Zhu & Conney, 1998).

Topical application of radiolabelled oestradiol to shaved skin of the dorsal neck of rats at a dose of 30.1, 120.4 or 301 pmol/cm^2 and autoradiography revealed the presence of oestradiol in epidermis, sebaceous glands, dermal papillae of hair and fibroblasts 2 h after application. A high concentration and retention of oestradiol in sebaceous glands was observed for more than 24 h, suggesting that sebaceous glands serve as a second storage site for oestradiol (Bidmon *et al*., 1990). An effect of dose and the area of topical application of oestradiol has also been observed in a hairless strain of rats. After a single dose of 50 nmol applied topically, the bioavailability, determined by urinary and faecal excretion of radiolabel after four days, was not affected by the area of the application surface. When the applied doses were increased from 50 to 1000 and 10 000 nmol, the percentage of percutaneous absorption decreased with reduction in the area of application (Chanez *et al*., 1989). Nasal administration of oestradiol to rats resulted in significantly higher blood levels than after intraduodenal administration, the bioavailability being 50% after a dose of 5 μg, 71% after 10 μg and 84% after 20 μg/rat compared with 2–5% via the intraduodenal route for the same doses (Bawarshi-Nassar *et al*., 1989). In rats and rabbits, nasal administration of oestradiol with dimethyl-β-cyclodextrin as a solubilizer and absorption enhancer resulted in significantly more absorption of the oestrogen than when it was given in suspension. Nasal administration of oestradiol–dimethyl-β-cyclodextrin

resulted in an absolute bioavailability of 94.6% in rabbits and 67.2% in rats in relation to an intravenous injection (Hermens *et al*., 1990).

In a study in rats given oestradiol–bisphosphonate conjugates with different esterase-sensitive linkers between the two molecular moieties, the conjugate with the low-cleavage resistance doubled the serum half-life of oestradiol (3.78 h), and the high-cleavage resistance conjugate resulted in a serum half-life approximately four times higher (8.36 h) than that of free oestradiol (Bauss *et al*., 1996).

After administration of oestriol to rats, glucuronides and sulfates of 16-keto-oestradiol and of 2- and 3-methyl esters of 2-hydroxyoestriol and 2-hydroxy-16-ketooestradiol were excreted in the bile (Bolt, 1979).

Oestradiol represented only 6% of the total oestrogen detected in the hepatic portal vein after oestradiol was placed in the stomach of a prepubertal pig; thus, most of the oestradiol was converted or conjugated before entering the hepatic portal vein. The blood concentrations of oestradiol glucuronide, oestrone glucuronide and oestrone sulfate but not of oestradiol or oestrone in the jugular vein rose and remained elevated for several hours, indicating that oestradiol and oestrone are completely converted and/or removed by the liver (Ruoff & Dziuk, 1994a).

In a comparison of the serum and tissue concentrations of oestradiol in fertile female and in castrated male Syrian golden hamsters, oestradiol pellets (20 mg) were implanted into the shoulder region of groups of four to six hamsters every 45 days to maintain the hormone concentration, and the animals were killed after 15 days and at 30-day intervals. The average serum oestradiol concentration in the cycling female hamsters was 79 pg/mL on days 1–2 and 311 pg/mL on days 3–4, attaining a maximum of 358 pg/mL on day 4 of the cycle. The concentrations on days 3–4 of the cycle were threefold higher than those on day 1 in uterine tissue, twofold higher in renal tissue and 2.6-fold higher in hepatic tissue. As was to be expected, the serum oestradiol concentrations of untreated castrated male hamsters did not vary appreciably over the six months of the study, and the average was about 32 pg/mL. Under conditions that produced essentially 100% renal tumour incidence, the serum oestradiol concentration rose rapidly to an average of 71-fold the untreated level. A steady-state serum concentration of 2400–2700 pg/mL was maintained during 45–180 days of continuous oestrogen treatment. The renal concentration of oestradiol in hamsters given this hormone rose by an average of only 5.4-fold between days 15 and 180 of treatment, and the serum concentrations were 5.7- to 8.0-fold higher than those in cycling female hamsters on days 3 and 4, with, however, no apparent effect on weight or mortality rate (Li *et al*. 1994).

A 10-mg dose of crystalline oestradiol placed in the rectum of prepubertal gilts resulted in increased concentrations of oestradiol, oestrone, oestradiol glucuronide, oestrone glucuronide and oestrone sulfate in the hepatic portal vein within 30 min, and the concentrations remained elevated for several hours (Ruoff & Dziuk, 1994b). After oestradiol was placed in the stomach of the prepubertal gilts, the concentrations of oestradiol, oestrone, oestradiol glucuronide, oestrone glucuronide and oestrone sulfate in the hepatic portal vein rose within 5 min and remained elevated for several hours (Ruoff &

Dziuk, 1994a). Most of the conjugated metabolites in liver and kidney of cattle are glucuronides (85–95%) (Kaltenbach *et al*., 1976).

In pregnant rhesus monkeys, oestradiol was eliminated from the maternal circulation principally by conversion to glucuronide conjugates (Hill *et al.*, 1980; Slikker *et al*., 1982). After pulse injection of ^{3}H-oestrone sulfate to adult female rhesus monkeys, the initial volume of distribution was 4.6 ± 0.9 L, and the metabolic clearance rate was 42 ± 2.9 L/day. Infusion of ^{3}H-oestrone sulfate or ^{14}C-oestrone resulted in a metabolic clearance rate of 67.5 ± 8.3 L/day. The conversion ratio of oestradiol to oestrone sulfate was 0.054 ± 0.016; the interconversion value was 43.6 ± 3.4% for oestrone sulfate to oestrone and 33.5 ± 6.6% for oestrone to oestrone sulfate. Thus, oestrone sulfate is cleared slowly and is converted to both oestrone and oestradiol (Longcope *et al.*, 1994).

The disposition of equilin sulfate was determined in female dogs receiving 2.5 mg/kg bw ^{3}H-equilin sulfate orally. The drug was rapidly absorbed (time to reach maximal concentration in plasma, 1 h) and had a moderate half-life (16.3 ± 9.6 h) in plasma. An average of 26.7 ± 4.4% of the administered radiolabel was excreted in urine. When ^{3}H-equilin sulfate was administered as part of a conjugated equine oestrogen preparation, a lower peak concentration, a lower area under the curve, a longer terminal half-life and a lower elimination percentage in urine were observed, indicating that the absorption of equilin sulfate was altered by other components in the preparation. Both plasma and urine contained equilin, equilenin, 17β-dihydroequilenin, 17β-dihydroequilin, 17α-dihydroequilenin and 17α-dihydroequilin. 17β-Dihydroequilin and equilin were the two major chromatographic peaks in plasma, whereas 17β-dihydroequilenin and 17β-dihydroequilin were the major metabolites in urine. The reduction of the 17-keto group and aromatization of ring-B are the major metabolic pathways of equilin in dogs (Chandrasekaran *et al*., 1995).

Uptake of oestrone sulfate has been reported by isolated rat hepatocytes. Accumulation in the cell remained linear with time up to 1 min and then began to decrease. The K_m was 16 ± 6 μmol/L, and the V_m was 0.85 ± 0.56 nmol/min per 10^6 cells. The uptake of oestrone sulfate involves a Na^+- and energy-dependent transport protein and a Na^+-independent anionic transport or multiple organic anion transport (Hassen *et al.*, 1996).

4-Hydroxylation of equilenin was reported in hamster liver microsomes (Sarabia *et al*., 1997). *In vitro*, 4-hydroxyequilenin can participate in redox cycling, and its quinone metabolite (4-hydroxyequilenin-*ortho*-quinone) is more reactive than 4-hydroxyoestrone-*ortho*-quinone (Shen *et al.*, 1997).

4.2 Receptor-mediated effects

4.2.1 *Humans*

In hyperplastic endometrial samples from four women who had been exposed to oestrogen [not further specified], the expression of keratinocyte growth factor mRNA was suppressed to the concentrations found in endometrial samples from unexposed women during the late proliferative phase of the menstrual cycle, whereas the expression of keratinocyte growth factor receptor mRNA was increased by approximately 35% over the enhanced levels found in the same control women (Siegfried *et al*., 1995).

In endometrial biopsy samples from post-menopausal women who had received 1.25 mg Premarin® daily for at least three months, the in-vitro DNA labelling index (incorporation of tritiated thymidine) in glandular epithelial cells and the nuclear oestradiol receptor content were increased and were similar to those found in proliferative-phase endometrium from eight unexposed women. The number of tritiated thymidine-labelled cells per microscopic high-power field was increased threefold by the treatment (four women) in comparison with the pre-menopausal control values (eight women) (Siddle *et al*., 1982).

Groups of post-menopausal women continuously received either oral doses of 2 mg per day oestradiol valerate, 1.5 mg per day oestropipate, 0.625 or 1.25 mg per day conjugated equine oestrogens (Premarin®), 50-mg oestradiol implants or 5 g of a skin cream containing 3 mg oestradiol; most women received a progestogen during the last 7–10 days of each month. Endometrial biopsy samples were obtained, and the receptor content was measured. The content of soluble progesterone receptor was not affected in 13 women taking Premarin® when compared with the level in proliferative-phase endometrium from 12 unexposed women. The nuclear oestradiol receptor content was slightly elevated (by 30%) in the endometrial samples from the 15 women who had received the high dose of Premarin® (1.25 mg/day) as compared with the level in proliferative-phase endometrium from 16 unexposed women. The percentage of endometrial glandular cells that had incorporated tritiated thymidine *in vitro*, examined only in cells from five women given 1.25 mg per day Premarin®, appeared to be minimally elevated (by approximately 10%) over the labelling index observed in proliferative-phase endometrium of 12 women; the endometrial oestrogen receptor content was increased by approximately 25% in these samples. All of the studies indicated a slight increase in cell proliferation in Premarin®-exposed women (Whitehead *et al*., 1981).

The effect of oral treatment for 90 days with 0.2 mg/day of a constituent of conjugated equine oestrogens, 17α-dihydroequilin sulfate, or 1.25 mg per day oestrone sulfate or a combination of these two steroids was examined in groups of seven women in whom menopause had been surgically induced. The serum concentration of sex hormone-binding globulin, measured as an indicator of oestrogenic activity, was increased after the oestrone sulfate treatment, by 20% after 30 days and by 60% after 90 days. Exposure to 17α-dihydroequilin, however, caused a 21 and 12% reduction of this parameter after these time intervals, whereas the combined treatment synergistically increased the concentrations of sex hormone-binding globulin by approximately 100% after 30 or 90 days (Wilcox *et al*., 1996). Ingestion by post-menopausal women of conjugated equine oestrogens at doses of 0.9–1.25 mg/day significantly increased the cortisol-binding capacity of transcortin, whereas lower doses of conjugated equine oestrogens (0.3 and 0.6 mg/day) did not (Schwartz *et al*., 1983).

Post-menopausal women received oral doses of conjugated equine oestrogens (0.625 mg per day Premarin®) for 24 days each month or transdermal doses of oestradiol via adhesive patches delivering 0.05 mg/day every fourth day for the first 24 days of each month. Some women also received a progestogen (dydrogesterone) during the last 12 days

of each month. The serum concentrations of insulin-like growth factor-I were decreased as compared with the pre-treatment levels in the Premarin®-treated group only. The growth hormone and sex hormone-binding globulin concentrations were increased in this group but not in the women receiving transdermal oestrogen (Campagnoli *et al*., 1993).

4.2.2 *Experimental systems*

(*a*) *Conjugated oestrogens*

The equine oestrogens equilin and equilenin bound to the oestrogen receptor with low affinity (less than oestrone) when examined by displacement of radiolabelled 17β-dihydroequilin in rat and human uterine tissue (Bhavnani & Woolever, 1991).

Oestrone sulfate at concentrations of 10^{-7} mol/L and higher stimulated the growth of MCF-7 human breast cancer cells determined after six days; however, when the mitotic index was used as the indicator of cell proliferation, stimulation was already maximal at a concentration of 10^{-10} mol/L, reaching the same level as that achieved with oestradiol at 10^{-12}–10^{-11} mol/L. This effect of oestrone sulfate was inhibited by simultaneous exposure of the cells to the pure anti-oestrogen ICI164,384. Induction of progesterone receptor or production of the pS2 protein, both indicators of cellular oestrogenic effects, occurred only at a concentration of 10^{-6} mol/L and was weaker than that after oestradiol treatment. Three days of treatment of MCF-7 cells with 10^{-7} or 10^{-9} mol/L oestrone sulfate resulted in concentrations in the medium of 4.4×10^{-9} mol/L oestrone and 1.0×10^{-9} mol/L oestradiol (average of two experiments at the high dose) in comparison with 3.3×10^{-11} mol/L oestrone and 4.4×10^{-12} mol/L oestradiol (at the low dose). Importantly, treatment of MCF-7 cells with 10^{-7} mol/L oestrone sulfate resulted in considerable accumulation in isolated nuclei of free oestrone (560 pg/mg DNA) and oestradiol (180 pg/mg DNA) but very litte oestrone sulfate (13 pg/mg DNA) (Santner *et al*., 1993).

Twenty-two adult female cynomolgus monkeys that had undergone surgical menopause were given Premarin® in a diet in which 40% of the calories were from fat; 26 animals received control diet. The daily dose of Premarin® was approximately 7.2 μg per animal for the first eight months of the experiment and 166 μg per animal for the subsequent duration of the 30-month study; the latter dose was stated by the authors to be equivalent to a human dose of 0.625 mg per day. The oestrogen treatment increased the concentration of circulating oestradiol from 5 to 167 pg/mL, increased the thickness of the mammary tissue by 50% and significantly enlarged the estimated surface area of lobular tissue. The mean percentage of epithelial breast cells that stained for Ki-67 MIB-1 antibody (a marker of cell proliferation) was increased from 2.5 to 5.4% in alveoli, from 0.6 to 2.1% in terminal ducts and from 1.2 to 3.0% in major mammary ducts. The mean percentage of epithelial breast cells that stained for progesterone receptors (with antibody techniques) was increased four- to sixfold in these mammary glands, but the percentage of cells that stained for oestrogen receptor was not significantly affected. Oestrogen treatment induced mammary gland hyperplasia in 9/22 of the monkeys as compared with none of the control group (Cline *et al*., 1996).

Hydrolysed Premarin® stimulated cell proliferation in primary cultures of renal proximal tubular cells isolated from castrated male Syrian golden hamsters at concentrations of 10^{-9} and 10^{-8} mol/L, with no effect at either higher or lower concentrations. Treatment of these hamsters with pellets that released 111 ± 11 μg hydrolysed Premarin® per animal per day resulted in a 100% tumour incidence in the kidney within approximately nine months (Li *et al*., 1995).

Equilin bound to sex hormone-binding globulin, displacing 5α-dihydrotestosterone with an affinity that was 50% that of oestradiol and 5.6% that of testosterone (Pan *et al*., 1985).

(*b*) *Oestradiol*

Oestradiol is the natural ligand for the oestrogen receptor. It drives the oestrogen responses of the uterus and mammary gland, which are typical oestrogen-sensitive organs, and in many other tissues. Classical responses to oestradiol mediated by the oestrogen receptor include uterine growth in immature or ovariectomized rodents and transcriptional activation of the progesterone receptor (see, e.g., Musgrove & Sutherland, 1997; Rutanen, 1997). The response of these tissues to oestradiol and the effects observed in cells and with oestrogen-responsive reporter gene constructs, are usually taken as a standard against which the oestrogenicity of other compounds is measured (see, e.g., Jeng *et al*., 1992; Katzenellenbogen *et al*., 1993; Parker, 1995; Kuiper *et al*., 1996). Similarly, displacement of radiolabelled oestradiol is the standard by which binding affinity to the oestrogen receptor is determined. The nature of these oestrogen responses depends mainly on the stage of development of the tissues and the age of the organism. Two high-affinity–low-capacity forms of the receptor have been identified: the oestrogen receptors α and β (Koike *et al*., 1987; Parker, 1995; Kuiper *et al*., 1996). Both types are widely distributed and have been isolated from several mammalian tissues (Greene *et al*., 1986; Krust *et al*., 1986). The role of the oestrogen receptor-β is still largely unclear, and almost all of the reports in the literature about oestrogen binding, oestrogen receptor–ligand interactions and transcriptional regulation by the oestrogen receptor–ligand complex pertain to the oestrogen receptor-α. The oestrogen responses that have been observed in many tissues and cells are probably, however, a result of the compound involvement of the oestrogen receptors α and β, although the relative contribution of each is still unknown. Anti-oestrogens, such as tamoxifen, which also has weak agonist activity, and pure anti-oestrogens, such as ICI164,384 and ICI182,780, compete with oestradiol for oestrogen receptor binding and are typically used to demonstrate that oestrogenic responses are mediated by this receptor (Jeng *et al*., 1992; McDonnell *et al*., 1995). In the context of this monograph, the responses to exogenous oestradiol in various tissues of pre- and post-menopausal women and the effects of oestradiol in appropriate animal or in-vitro models are the most relevant and are summarized here.

Oestradiol strongly induced growth of both MCF-7 and T47D human breast cancer cell lines and other human breast cancer cell lines that contain oestrogen receptors, but it did not stimulate growth of oestrogen receptor-negative breast cancer cell lines (Jeng

et al., 1992; Catherino & Jordan, 1995; Schoonen *et al*., 1995a,b). In MCF-7 cells, the growth-stimulating effect of oestradiol was already observed at a concentration of 10^{-12} mol/L; it increased to a maximum at a concentration of about 10^{-10} mol/L and decreased at concentrations higher than 10^{-9} mol/L (Jeng *et al*., 1992). These growth-stimulating effects were abolished not only by anti-oestrogens such as tamoxifen and ICI182,780 but also by anti-progestogens such as RU486 (Catherino & Jordan, 1995; Schoonen *et al*., 1995a,b). The effects were demonstrated in experiments performed with breast cancer cell lines grown in phenol red-free medium which contained steroid-free (dextran-coated charcoal-stripped) serum (Jeng *et al*., 1992; Schoonen *et al*., 1995a,b).

At concentrations that stimulate growth of human breast cancer cells, oestradiol induced expression of oestrogen-responsive genes, such as the *pS2* gene, at the transcriptional level (Brown *et al*., 1984; Kalkhoven *et al*., 1994) and *trans*-activated oestradiol-responsive reporter constructs containing oestrogen response elements in oestrogen receptor-positive cells (Jeng *et al*., 1992; Kalkhoven *et al*., 1994; Catherino & Jordan, 1995). These effects were inhibited by anti-oestrogens. Most of the effects of oestradiol are believed to be mediated by triggering of the secretion of growth factors by autocrine and paracrine action (Lippman & Dickson, 1989). Transforming growth factor (TGF)-α and epidermal growth factor are the most extensively studied mediators of the cell growth-stimulating effects of oestradiol in breast and uterine tissues (see for reviews Boyd, 1996; McLachlan & Newbold, 1996; Snedeker & Diaugustine, 1996). Both up-regulation of these growth-enhancing factors and down-regulation of growth-inhibiting factors may be involved in the action of oestradiol. For example, oestradiol was shown to affect the production of the growth inhibitor TGF-β by human breast cancer cells (Knabbe *et al*., 1987; Arrick *et al*., 1990). It markedly down-regulated the mRNA expression of growth-inhibiting TGF-β2 and -β3 in MCF-7 cells at a concentration of oestradiol that stimulated the growth of these cells (10^{-8} mol/L), but it did not affect the mRNA expression of TGF-β1 (Arrick *et al*., 1990; Jeng & Jordan, 1991). Other cytokines that are not under oestrogen regulation may also influence the growth of breast cancer cells, but oestradiol may affect the responsiveness of the cells to these factors. For example, a six-day exposure of oestrogen receptor-positive ZR-75-1 human breast cancer cells to 10^{-9} mol/L oestradiol reduced by 20–40% the expression of binding sites for interferon, which has anti-proliferative activity for these cells (Martin *et al*., 1991).

Oestradiol at 10^{-10}–10^{-9} mol/L increased the reductive activity of 17β-hydroxysteroid oxidoreductase [17-hydroxysteroid dehydrogenase] in an oestrogen- and progestogen-sensitive MCF-7 cell line in phenol red-free medium (Coldham & James, 1990). This activity may be involved in the mechanism of breast cancer cell growth stimulation through the induction of oestradiol formation.

Oestradiol at concentrations of 5×10^{-12}–10^{-7} mol/L stimulated alkaline phosphatase activity in Ishikawa human endometrial cancer cells, which is an oestrogen-specific response inhibited by 4-hydroxytamoxifen (Markiewicz *et al*., 1992; Markiewicz & Gurpide, 1994; Botella *et al*., 1995). This assay has been used as a marker for oestrogenic activity in endometrial cells.

Oestradiol at a concentration of 10^{-8} mol/L increased anchorage-independent growth of an SV40-immortalized human endometrial stromal cell line by almost 80% (Xu *et al*., 1995).

Oestradiol given subcutaneously to ovariectomized rats at a dose of 200 μg/kg bw per day for four days slightly decreased the gene expression of mitogen-activated protein kinase (MAPK) and increased MAPK tyrosine phosphorylation and membrane-associated MAPK enzymatic activity in uterine smooth-muscle tissue (Ruzycky, 1996). In the same model, oestradiol increased the membrane-associated protein expression of seven protein kinase C isozymes by 1.5–2.25-fold, whereas no such change occurred in cardiac muscle. These studies indicate that MAPK and protein kinase C play a role in two of the signal transduction pathways that regulate cell proliferation and are affected by oestradiol (Ruzycky & Kulick, 1996).

Groups of 10 female rats received 20 g DMBA by gavage over two weeks to induce mammary tumours. Ovariectomy suppressed MAPK expression in these tumours, and daily subcutaneous injections of oestradiol (10 μg/rat) induced activation of this enzyme in tumours in ovariectomized rats (Koibuchi *et al*., 1997).

In ovariectomized BALB/c mice, a single subcutaneous dose of 20 μg oestradiol 3-benzoate induced an approximately threefold induction of mRNA expression of the RXRα and RARγ retinoic acid receptor subtypes in cervical tissue within 0.5 and 4 h, respectively (Celli *et al*., 1996).

Treatment of cultures of normal human endometrial stromal cells with 10^{-8} mol/L oestradiol with or without 10^{-7} mol/L medroxyprogesterone acetate increased mRNA expression of vascular endothelial growth factor by 4.7- and 3.1-fold, respectively, over control values (Shifren *et al*., 1996); however, in ovariectomized nude mice carrying a human endometrial carcinoma xenograft, implantation of oestradiol pellets that maintained serum levels of this steroid at 200–300 pg/mL did not alter the expression of vascular endothelial growth factor in the tumour tissue (Kim *et al*., 1996). Oestradiol did not alter the secretion of vascular endothelial growth factor by T47D human breast cancer cells (Hyder *et al*., 1998) and had no effect on angiogenesis induction by basic fibroblast growth factor or TGF-α in rabbit cornea *in vitro* (Yamamoto *et al*., 1994).

Oestradiol decreased by approximately 50–60% the growth of decidual endothelial cells derived from human endometrium at concentrations of 0.5 and 2.5 ng/mL, increased the growth of these cells by approximately 30% at 5 ng/mL and had no effect at concentrations of 10 ng/mL and higher (Peek *et al*., 1995).

Oestradiol at concentrations above 10^{-10} mol/L stimulated the migration of human endometrial cancer cells (Ishikawa, HEC-1 or HHUA cells) through an artificial basement membrane and suppressed the mRNA expression of the cell adhesion-related molecules E-cadherin and α- and β-catenin in Ishikawa cells (Fujimoto *et al*., 1996a,b). At concentrations of 10^{-10} mol/L and higher, oestradiol up-regulated the mRNA expression of fibroblast growth factors-1 and -2 but not of fibroblast growth factor-4 in Ishikawa human endometrial cancer cells, with a maximal effect at 10^{-8} mol/L (Fujimoto *et al*., 1997). In contrast, no effect of oestradiol was found on endometrial adenocarcinoma SNG-M cells

in migration and invasion assays, which involve cell growth along a fibronectin gradient, or on their growth or locomotion as determined in a monolayer wounding model *in vitro*. The secretion of matrix metalloproteinases and stromelysin by these cells was not affected (Ueda *et al*., 1996).

Oestradiol has been shown to induce liver cell growth, increasing both liver weight and hepatic DNA content in female Wistar rats when given by subcutaneous injection (1–200 μg/kg bw per day) or in the diet (30–300 μg/kg bw per day) for seven days. The relationship between dose and response for these two parameters was approximately linear over the range of subcutaneous doses (Ochs *et al*., 1986; Schulte-Hermann *et al*., 1988). Oral administration of oestradiol was less effective than subcutaneous injection (Ochs *et al*., 1986).

Oestradiol at 3×10^{-5} mol/L for 48 h induced a two- to threefold increase in tritiated thymidine incorporation into DNA of cultured primary female rat hepatocytes. Although oestradiol by itself thus appeared to have only weak mitogenic effects on primary rat hepatocytes, it strongly enhanced the induction of hepatic DNA synthesis by epidermal growth factor or TGF-α (Ni & Yager, 1994a,b). Epidermal growth factor-induced growth of male rat hepatocytes, however, was inhibited by concomitant oestradiol treatment at concentrations of 2.5×10^{-6}–10^{-5} mol/L (Francavilla *et al*., 1989), suggesting marked sex differences in the mitogenic effects of epidermal growth factor and oestrogens on rat liver. Differences in culture medium composition may also have contributed to these discrepancies (Yager & Liehr, 1996).

Within 6–12 h after oestradiol was given at a dose of 5 mg/kg bw to castrated Syrian golden hamsters by intraperitoneal injection, renal ornithine decarboxylase activities were increased threefold above the control level. Similarly, in hamsters that received subcutaneous implants of pellets of 20 mg oestradiol to maintain chronically high oestrogen levels, the renal activity of ornithine decarboxylase was 1.5–1.9 times the corresponding activity in control animals after 60–80 days of treatment. With a series of oestrogen analogues, there was a direct correlation between the increase in renal ornithine decarboxylase activity *in vivo* and binding to renal oestradiol receptor sites *in vitro*. The concentrations of the polyamines putrescine, spermidine and spermine in hamster kidney all declined during the 180-day experimental period (Nawata *et al*., 1981).

(*c*) *Oestriol*

Oestriol bound with low affinity to oestrogen receptors in calf, rat and human uterine tissue and in Ishikawa human endometrial cancer cells (Katzenellenbogen, 1984; Lubahn *et al*., 1985; Botella *et al*., 1995), with a relative binding affinity about 5–12% that of oestradiol (Batra *et al*., 1984; Katzenellenbogen, 1984; Botella *et al*., 1995).

Incorporation of tritiated thymidine by primary cultures of hepatocytes from female Fischer 344 or Lewis rats was minimally (1.5-fold) stimulated by addition of oestriol to the medium for 48 h at a concentration of 3×10^{-5} mol/L. Simultaneous addition of 15 ng/mL TGF-α stimulated DNA synthesis in these cells by approximately 100-fold in

a synergistic fashion, while TGF-α alone at this concentration stimulated DNA synthesis by 54-fold (Ni & Yager, 1994a,b).

(*d*) *Oestrone*

Oestrone bound with low affinity to the oestrogen receptor in calf, rat and human uterine tissue (Lubahn *et al*., 1985; Bhavnani & Woolever, 1991) and in Ishikawa human endometrial cancer cells (Botella *et al*., 1995). Its relative binding affinity to the receptor in human endometrial cytosol is 2–10% that of oestradiol (Lubahn *et al*., 1985; Bhavnani & Woolever, 1991; Botella *et al*., 1995).

Oestrone stimulated the incorporation of tritiated thymidine into the DNA of MCF-7 human breast cancer cells at concentrations of 10^{-11} mol/L and higher, reaching a plateau of stimulation at 10^{-9} mol/L; this was approximately 20% lower than the maximal stimulation achieved by oestradiol at a concentration of 10^{-10} mol/L (Kitawaki *et al*., 1992). Oestrone treatment of MCF-7 human breast cancer cells stimulated their growth, as seen from the increase in cell number, over a six-day period at concentrations of 10^{-10} mol/L and higher, reaching a maximum at between 10^{-9} and 10^{-8} mol/L. When the mitotic index was used as an indicator of cell proliferation, however, stimulation was already maximal at an oestrone concentration of 10^{-13} mol/L and was similar to that achieved by oestradiol at 10^{-11} mol/L. This effect of oestrone was inhibited by simultaneous exposure of the cells to the pure anti-oestrogen ICI164,384. Three days of treatment of MCF-7 cells with 10^{-8} mol/L oestrone resulted in a concentration of 3.3×10^{-9} mol/L oestradiol in the culture medium (Santner *et al*., 1993).

Oestrone given to female Sprague Dawley rats after ovariectomy at a subcutaneous dose of 1 μg/rat twice daily stimulated the growth of DMBA-induced mammary tumours by 225% after 65 days of treatment; this effect was abolished by simultaneous administration of an anti-oestrogen, EM-800, at 2.5 mg/kg bw per day or medroxyprogesterone acetate at 1 mg/rat subcutaneously twice daily (Luo *et al*., 1997).

Incorporation of tritiated thymidine by cultured primary hepatocytes from female Fischer 344 or Lewis rats was stimulated threefold by addition of oestrone to the medium for 48 h at a concentration of 3×10^{-5} mol/L. Simultaneous addition of 15 ng/mL TGF-α stimulated DNA synthesis by approximately 100-fold in a synergistic fashion, while TGF-α alone at this concentration stimulated DNA synthesis by 54-fold (Ni & Yager, 1994b).

Oestrone at concentrations of 10^{-10}–10^{-8} mol/L stimulated cell proliferation in primary cultures of renal proximal tubular cells isolated from castrated male Syrian hamsters, being maximally effective at 10^{-9} mol/L and not effective at higher or lower concentrations. Treatment of these hamsters with pellets that released 111 ± 11 μg oestrone per animal per day resulted in a 100% tumour incidence in the kidney in approximately nine months (Li *et al*. (1995).

Oestrone bound to sex hormone-binding globulin, displacing 5α-dihydrotestosterone with an affinity that was 25% that of oestradiol and 1.8% that of testosterone (Pan *et al*., 1985).

4.3 Genetic and related effects

Receptor mechanisms are fundamental to the responses to oestrogens (King, 1991), although non-receptor mechanisms may also be important (Duval *et al.*, 1983; Yager & Liehr, 1996).

4.3.1 *Humans*

No data were available on the genetic effects of unopposed oestrogens in humans.

4.3.2 *Experimental systems*

The genetic and related effects of oestrogens are summarized in Table 14.

In one study, oestradiol decreased the formation of single- and double-strand breaks in ΦX-174 RFI DNA induced by hydrogen peroxide alone or with Cu^{2+} (Tang & Subbiah, 1996). Gene mutations were not induced in *Salmonella typhimurium* by oestradiol. DNA strand breaks were not induced by oestradiol in rat hepatocytes or hamster ovary cells in the absence or presence of a metabolic activation system. Oestradiol weakly induced DNA breakage in mouse brain cells. It did not induce DNA repair in a mouse mammary cell line and did not give rise to unscheduled DNA synthesis in hamster embryo cells. It did not induce gene mutations at either the *hprt* or Na^+/K^+ ATPase loci, or sister chromatid exchange or chromosomal aberrations in hamster embryo cells, whereas the formation of micronuclei was increased in these cells in a single study. In several studies, aneuploidy was induced in hamster embryo cells, male hamster cells and human foreskin fibroblasts. In rodent cells, oestradiol was shown to cause cell transformation in five studies with different experimental designs, but it gave negative results in two studies. In hamster cells, it gave rise to the formation of DNA adducts, but it did not cause oxidative DNA damage. In human lymphocytes, oestradiol caused micronucleus formation but no chromosomal aberrations or sister chromatid exchange; it weakly induced aneuploidy.

Studies conducted with oestrogens *in vivo* allow the examination of sex- and organ-specific effects. Induction of covalent modifications in DNA was demonstrated by ^{32}P-postlabelling in the kidneys and liver of hamsters and rats after a subcutaneous implant of oestradiol. In a single study, apparent covalent binding to DNA was not induced in the kidneys or liver of rats or hamsters treated by oral administration with oestradiol; the authors of the study reported that the radiolabel detected in some DNA samples could have been due to protein contamination.

DNA strand breakage was induced in the kidneys but not the livers of hamsters after subcutaneous implantation of oestradiol at low doses and in both liver and kidney after a much higher dose; an even higher dose of oestradiol administered to male hamsters intraperitoneally had no effect on either kidneys or liver.

Oestradiol induced sister chromatid exchange in mouse uterine cells but not in kidney cells *in vivo*, but it did not cause aneuploidy in either cell type. It gave rise to chromosomal aberrations and aneuploidy in renal cells of hamsters exposed *in vivo*.

In a single study, K-, H- or N-*ras* and *p53* gene mutations were not found in endometrial lesions of mice fed a diet containing MNU and oestradiol (Murase *et al.*, 1995).

Table 14. Genetic and related effects of oestradiol and oestrone and their derivatives and of oestriol

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Oestradiol				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2500 µg/plate	Lang & Redman (1979)
Salmonella typhimurium TA1535, TA1537, TA1538, reverse mutation	–	–	500 µg/plate	Ingerowski *et al*. (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	500 µg/plate[c]	Lang & Reimann (1993)
DNA strand breaks, cross-links or related damage, mouse brain DNA *in vitro*	(+)	NT	27.2	Yamafuji *et al*. (1971)
DNA strand breaks, cross-links or related damage, Chinese hamster V79 cells *in vitro*	–	–	816	Swenberg (1981)
DNA single-strand breaks, rat hepatocytes *in vitro*	–	NT	82	Sina *et al*. (1983)
DNA repair exclusive of unscheduled DNA synthesis, female C57BL mouse mammary epithelial cells *in vitro*	–	NT	0.2	Telang *et al*. (1992)
Unscheduled DNA synthesis, Syrian hamster embryo cells *in vitro*	–	NT	10	Tsutsui *et al*. (1987)
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	27.2	Drevon *et al*. (1981)
Gene mutation, Chinese hamster lung V79 cells, ouabain *in vitro*	–	–	27.2	Drevon *et al*. (1981)
Gene mutation, Syrian hamster embryo cells, *hprt* and Na^+/K^+ ATPase loci *in vitro*	–	NT	10	Tsutsui *et al*. (1987)
Sister chromatid exchange, mouse cervical fibroblasts and kidney cells *in vitro*	–	NT	2.7	Hillbertz-Nilsson & Forsberg (1985)
Sister chromatid exchange, Syrian hamster embryo cells *in vitro*	–	NT	10	Tsutsui *et al*. (1987)
Micronucleus formation, Syrian hamster embryo cells *in vitro*	+[c]	NT	2.72	Schnitzler *et al*. (1994)
Micronucleus formation, ovine seminal vesicle cells *in vitro*	+[c]	NT	2.72	Schnitzler *et al*. (1994)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	10	Tsutsui *et al*. (1987)
Chromosomal aberrations, Syrian hamster embryo cells *in vitro*	–	NT	8.17	Tsutsui *et al*. (1997)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Aneuploidy, male Chinese hamster DON cells *in vitro*	+	NT	13.6	Wheeler *et al*. (1986)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	10	Tsutsui *et al*. (1987, 1990)
Aneuploidy, Syrian hamster embryo cells *in vitro*	+	NT	0.82	Tsutsui *et al*. (1997)
Cell transformation, BALB/c 3T3 embryo-derived mouse fibroblasts	+	NT	5.5	Liehr *et al*. (1987a)
Cell transformation, C3H 10T1/2 mouse cells	+	NT	0.27	Kennedy & Weichselbaum (1981)
Cell transformation, Syrian hamster embryo cells	+	NT	3	Tsutsui *et al*. (1987)
Cell transformation, Syrian hamster embryo cells	NT	+	3	Hayashi *et al*. (1996)
Cell transformation, Syrian hamster embryo cells	+	NT	2.72	Tsutsui *et al*. (1997)
Cell transformation, female C57BL mouse mammary epithelial cells	–	NT	0.2	Telang *et al*. (1992)
Cell transformation, primary baby rat kidney + HPV16 + *ras*	–	NT	0.27	Pater *et al*. (1990)
Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	13.6	Hill & Wolff (1983)
Sister chromatid exchange, human lymphocytes *in vitro*	–	–	27.2	Banduhn & Obe (1985)
Micronucleus formation, human lymphocytes *in vitro*	+	NT	1.3	Banduhn & Obe (1985)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	100	Stenchever *et al*. (1969)
Chromosomal aberrations, human lymphocytes *in vitro*	–	–	27.2	Banduhn & Obe (1985)
Aneuploidy, human lymphocytes *in vitro*	(+)	NT	13.6	Banduhn & Obe (1985)
Aneuploidy, human foreskin JHU-1 fibroblasts *in vitro*	+	NT	20	Tsutsui *et al*. (1990)
DNA strand breaks, cross-links or related damage, male Syrian hamster kidney and liver *in vivo*	(+)		22.5 mg imp × 1, 2 wk	Han & Liehr (1994)
DNA strand breaks, cross-links or related damage, male Syrian hamster kidney *in vivo*	+		250 µg/d imp × 1, 7 d	Han & Liehr (1994)
DNA strand breaks, cross-links or related damage, male Syrian hamster liver *in vivo*	–		250 µg/d imp × 1, 7 d	Han & Liehr (1994)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA strand breaks, cross-links or related damage, male Syrian hamster kidney and liver *in vivo*	–		150 ip × 1	Han & Liehr (1994)
Sister chromatid exchange, female NMRI mouse uterine cervix and uterine horn epithelial cells *in vivo*	+		5 µg sc × 1	Forsberg (1991)
Sister chromatid exchange, female NMRI kidney cells *in vivo*	–		5 µg sc × 1	Forsberg (1991)
Chromosomal aberrations, male Syrian hamster renal cortical cells *in vivo*	+		125 µg/d imp × 1, 5 mo	Banerjee *et al*. (1994)
Aneuploidy, female NMRI mouse uterine cervix and uterine horn epithelial cells *in vivo*	–		5 µg sc × 1	Forsberg (1991)
Aneuploidy, female NMRI mouse kidney cells *in vivo*	–		5 µg sc × 1	Forsberg (1991)
Aneuploidy, male Syrian hamster renal tubular cells *in vivo*	+		20 mg imp × 1, 3.5 mo	Li *et al*. (1993)
Increase in nuclear DNA content (aneuploidy), female BALB/c mouse cervicovaginal epithelium *in vivo*	+		25 µg sc × 5	Hajek *et al*. (1993)
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	–	54.5	Han & Liehr (1995)
Binding (covalent) to DNA, Syrian hamster embryo cells *in vitro*	NT	(+)	1	Hayashi *et al*. (1996)
Binding (covalent) to DNA, female Sprague-Dawley rat liver *in vivo*	–		0.3 po × 1	Caviezel *et al*. (1984)
Binding (covalent) to DNA, male and female Syrian hamster kidney and liver *in vivo*	–		0.3 po × 1	Caviezel *et al*. (1984)
Binding (covalent) to DNA, male Syrian hamster kidney cortex *in vivo*	+		31 mg imp × 1, 2.5 mo	Liehr *et al*. (1986c)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to DNA, male Syrian hamster kidney *in vivo*	+		22.5 mg imp × 1, 7 mo	Lu *et al*. (1988)
Oestradiol + testosterone				
Binding (covalent) to DNA, male NBL/Cr rat dorsolateral prostate *in vivo*	+		NR	Han *et al*. (1995)
Binding (covalent) to DNA, male NBL/Cr rat ventral and anterior prostate *in vivo*	–		NR	Han *et al*. (1995)
17α-Oestradiol				
Chromosomal aberrations, male Syrian hamster renal cortical cells *in vivo*	–		105 µg/d imp × 1, 5 mo	Banerjee *et al*. (1994)
4-Hydroxyoestradiol				
Cell transformation, Syrian hamster embryo cells	NT	+	0.3	Hayashi *et al*. (1996)
DNA strand breaks, cross-links or related damage, male Syrian hamster kidney cells *in vivo*	+		250 µg/d imp × 1, 7 d	Han & Liehr (1994)
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	+	57.9	Han & Liehr (1995)
Binding (covalent) to DNA, Syrian hamster embryo cells *in vitro*	NT	+	1	Hayashi *et al*. (1996)
Binding (covalent) to calf thymus DNA *in vitro*	NT	+[d]	66.7	Cavalieri *et al*. (1997)
Binding (covalent) to DNA, female Sprague-Dawley rat mammary cells *in vivo*	+		250 µg i-mam × 1	Cavalieri *et al*. (1997)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
2-Hydroxyoestradiol				
Cell transformation, Syrian hamster embryo cells	NT	+	1	Hayashi *et al*. (1996)
DNA strand breaks, cross-links or related damage, male Syrian hamster kidney cells *in vivo*	-		250 µg/d imp × 1, 7 d	Han & Liehr (1994)
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	–	57.9	Han & Liehr (1995)
Binding (covalent) to DNA, Syrian hamster embryo cells *in vitro*	NT	+	1	Hayashi *et al*. (1996)
Oestradiol-3,4-quinone				
Binding (covalent) to calf thymus DNA *in vitro*	+	NT	200	Cavalieri *et al*. (1997)
Binding (covalent) to DNA, female Sprague-Dawley rat mammary cells *in vivo*	+		250 µg i-mam × 1	Cavalieri *et al*. (1997)
Oestrone				
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	27	Drevon *et al*. (1981)
Gene mutation, Chinese hamster lung V79 cells, ouabain *in vitro*	–	–	27	Drevon *et al*. (1981)
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	–	54.1	Han & Liehr (1995)
Binding (covalent) to DNA, female Sprague-Dawley rat liver *in vivo*	–		0.3 po × 1	Caviezel *et al*. (1984)
Binding (covalent) to DNA, male Syrian hamster kidney and liver *in vivo*	–		0.3 po × 1	Caviezel *et al*. (1984)
Oestrone-3,4 quinone				
DNA strand breaks, cross-links or related damage, human MCF-7 cells *in vitro*	+	NT	7.1	Nutter *et al*. (1994)
Binding (covalent) to calf thymus DNA *in vitro*	(+)	NT	200	Cavalieri *et al*. (1997)

Table 14 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
16α-Hydroxyoestrone				
DNA repair exclusive of unscheduled DNA synthesis, female C57BL mouse mammary epithelial cells *in vitro*	+	NT	0.2	Telang *et al*. (1992)
Cell transformation, female C57BL mouse mammary epithelial cells	+	NT	0.2	Telang *et al*. (1992)
2-Hydroxyoestrone				
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	–	57.5	Han & Liehr (1995)
4-Hydroxyoestrone				
Binding (covalent) to calf thymus DNA *in vitro*	NT	+[d]	66.7	Cavalieri *et al*. (1997)
Binding (covalent) to DNA, male Syrian hamster liver, 8-hydroxy-2′-deoxyguanosine formation *in vitro*	NT	+	57.5	Han & Liehr (1995)
Oestriol				
DNA repair exclusive of unscheduled DNA synthesis, female C57BL mouse mammary epithelial cells *in vitro*	–	NT	0.2	Telang *et al*. (1992)
Aneuploidy, male Chinese hamster DON cells *in vitro*	+	NT	21.6	Wheeler *et al*. (1986)
Cell transformation, female C57BL mouse mammary epithelial cells	–	NT	0.2	Telang *et al*. (1992)
Sister chromatid exchange, human lymphocytes *in vitro*	(+)	NT	14	Hill & Wolff (1983)

[a] +, positive; (+), weak positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw per day; wk, week; d, day; mo, months; imp, subcutaneous implant; ip, intraperitoneal injection; sc, subcutaneous injection; po, oral; i-mam, intramammary; NR, not reported

[c] 41–68% of the induced micronuclei contained CREST-reactive kinetochores

[d] Horeradish peroxidase-activated or lactoperoxidase-activated or S9 activated system

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.

Oestradiol was tested in two-stage carcinogenesis models in mice with the known carcinogens *N*-methyl-*N*-nitrosourea, *N*-ethyl-*N*-nitrosourea or 3-methylcholanthrene and in two-stage carcinogenesis models in rats with *N*-methyl-*N*-nitrosourea, 2-acetylaminofluorene, *N*-nitrosodiethylamine, 7,12-dimethylbenz[*a*]anthracene or *N*-butyl-*N*-nitrosourea. In mice, oestradiol enhanced the incidences of endometrial adenomatous hyperplasia, atypical hyperplasia and adenocarcinomas induced by *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea. A continuously high serum concentration of oestradiol and a low concentration of progesterone appeared to be important for the development of endometrial adenocarcinomas in mice. Oestradiol suppressed the development of uterine cervical carcinomas induced by 3-methylcholanthrene. In rats, large doses of oestradiol alone or oestradiol with progesterone suppressed the development of mammary carcinomas induced by *N*-methyl-*N*-nitrosourea. Combined treatment of ovariectomized rats with oestradiol and *N*-methyl-*N*-nitrosourea induced vaginal polyps. In a two-stage model of liver carcinogenesis in rats, oestradiol showed no initiating activity. It did not show promoting effects in the livers of rats initiated with *N*-nitrosodiethylamine. In one study pretreatment with oestradiol increased the number of liver foci positive for γ-glutamyl transferase induced by *N*-nitrosodiethylamine. Oestradiol did not affect mammary tumour development in intact or ovariectomized female rats treated with 7,12-dimethylbenz[*a*]-anthracene. Oestradiol benzoate enhanced the incidence of mammary tumours in rats treated with γ-rays.

Oestriol

Oestriol was tested for carcinogenicity by subcutaneous implantation in one study in castrated mice and in one study in hamsters. In mice, oestriol increased the incidence and accelerated the appearance of mammary tumours in both male and female mice. In hamsters, oestriol produced kidney tumours.

In female mice, oestriol slightly increased the incidence of *N*-methyl-*N*-nitrosourea-induced endometrial adenocarcinomas. In several studies in female rats, oestriol inhibited the induction of mammary tumours by 7,12-dimethylbenz[*a*]anthracene when administered before the carcinogen; continuous treatment with oestriol resulted in a decreased incidence of mammary tumours. In one study in female rats, oestriol inhibited the induction of mammary carcinomas when administered 13–15 days after irradiation with γ-rays.

Oestrone

Oestrone was tested for carcinogenicity by oral administration in two studies in castrated male mice. The incidence of mammary tumours was increased. In one study in which oestrone was administered by skin application to mice, the incidence of mammary tumours was increased in males and that of pituitary tumours in animals of each sex. In studies in which oestrone was tested by subcutaneous and/or intramuscular administration, mammary tumours were induced in male mice, and the average age at the time of appearance of mammary tumours in female mice was reduced. In castrated male and female rats, subcutaneous injection of oestrone resulted in mammary tumours.

In three studies of subcutaneous or intramuscular administration, oestrone benzoate induced mammary tumours in male mice. In one study in rats, subcutaneous injection of oestrone benzoate induced mammary and pituitary tumours in animals of each sex. In several studies involving subcutaneous implantation of oestrone, the incidences of mammary and lymphoid tumours were increased in mice, and those of mammary and pituitary tumours were increased in rats. In one study in rats, implantation of low-dose oestrone pellets induced adrenal cortical tumours, but high-dose pellets reduced the incidence. In intact and castrated male hamsters, implantation of oestrone resulted in malignant kidney tumours. The oestrone metabolite, 4-hydroxyoestrone, induced kidney tumours at a low incidence in castrated male hamsters.

Oestrone-3,4-quinone, a metabolite of oestrone, was tested for carcinogenicity by direct injection into the mammary glands of rats fed a high-fat diet. There were no significant differences in mammary tumour incidence or multiplicity in comparison with controls that did not receive the metabolite.

The incidence of endometrial adenocarcinomas induced by *N*-methyl-*N*-nitrosourea in the uterine corpus of mice was significantly increased in those receiving an oestrone-containing diet; furthermore, the incidences of preneoplastic endometrial lesions in the *N*-methyl-*N*-nitrosourea-treated and untreated uterine corpora were significantly increased in mice receiving the oestrone-containing diet. In one study in female toads, subcutaneous administration of oestrone enhanced the incidence of hepatocellular carcinomas induced by subcutaneous injection of *N*-nitrosodimethylamine.

5.4 Other relevant data

Oestrogens administered orally are absorbed rapidly and achieve maximum serum levels quickly. Although the major route of metabolism for oestrogens inactivates them and facilitates their excretion, a minor metabolic pathway activates a small proportion of oestrogen to catechol intermediates, with significant potential for damaging DNA, and may also yield reactive oxygen species that damage DNA. Some oestrogens, including conjugated oestrogens, have been reported to have genotoxic activity in experimental systems. At higher concentrations, which may or may not involve receptor mediation, oestrogens have been reported to induce changes in DNA and chromosomes. Oestradiol binds to oestrogen receptors with higher affinity than oestriol or oestrone. Oestrogens can increase the number of proliferating cells in the human endometrium *in vivo*. It has been reported that oestrogens increase cell proliferation in normal breast cells in monkeys and in cultured human breast cancer cells. At higher concentrations, oestrogens stimulated cell proliferation in rat liver *in vivo* and in cultured rat hepatocytes *in vitro*. No information was available on whether the effect of oestrogens on the mammary gland is modified by body weight or by the recency or duration of exposure to oestrogens in experimental systems. Similarly, no information was available on the possible relationship between exposure to oestrogens and the degree of malignancy of breast tumours.

5.5 Evaluation

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

There is *sufficient evidence* in experimental animals for the carcinogenicity of oestradiol and oestrone.

There is *limited evidence* in experimental animals for the carcinogenicity of conjugated equine oestrogens, equilin and oestriol.

There is *inadequate evidence* in experimental animals for the carcinogenicity of d-equilenin.

Overall evaluation

Post-menopausal oestrogen therapy is *carcinogenic to humans (Group 1).*

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