ORAL CONTRACEPTIVES, COMBINED

1. Exposure

Combined oral contraceptives consist of the steroid hormone oestrogen in combination with a progestogen, taken primarily to prevent pregnancy. The same hormones can also be used in other forms for contraception. Combined oral contraceptive pills generally refer to pills in which an oestrogen and a progestogen are given concurrently in a monthly cycle. In contrast, a cycle of sequential oral contraceptive pills includes oestrogen-only pills followed by five to seven days of oestrogen plus progestogen pills. Sequential oral contraceptive pills were removed from the consumer market in the late 1970s; they are covered in an IARC monograph (IARC, 1979, 1987). Combined oral contraceptives are thus usually administered as a pill containing oestrogen and progestogen, which is taken daily for 20–22 days, followed by a seven-day pill-free interval (or seven days of placebo), during which time a withdrawal bleed is expected to occur. The most commonly used oestrogen is ethinyloestradiol, although mestranol is used in some formulations. The progestogens most commonly used in combined oral contraceptives are derived from 19-nortestosterone and include norethisterone, norgestrel and levonorgestrel, although many others are available (Kleinman, 1990) (see Annex 2, Table 1).

Chemical and physical data and information on the synthesis, production, use and regulations and guidelines for hormones used in combined oral contraceptives are given in Annex 1. Annex 2 (Table 1) lists the trade names of many contemporary combined oral contraceptives with their formulations.

Combined oral contraceptives are currently available in monophasic, biphasic and triphasic preparations, the terms referring to the number of different doses of progestogen they contain. Monophasic pills maintain a constant dose of oestrogen and progestogen, while multiphasic pills allow a lower total dose of progestogen to be given by reducing the amount of progestogen early in the 20–22-day period of exposure. Biphasic pills contain a lower dose of progestogen early in the cycle followed by a higher dose in the last 11 days. Triphasic pills consist of three doses of progestogen, increasing through the cycle, which may or may not be accompanied by variations in the dose of oestrogen (Kleinman, 1990).

Sequential pills contain only oestrogen during the first part of the cycle and an oestrogen and progestogen thereafter. In older regimens, oestrogen was given alone for the first 16 days of the cycle, followed by five days of combined oestrogen and progestogen. These preparations were withdrawn from use in many countries in the 1970s after concern about their association with endometrial cancer (IARC, 1974, 1979). The sequential combined oral contraceptive regimens available currently include oestrogen alone for a

shorter interval, usually one week, followed by combined oestrogen and progestogen (Wharton & Blackburn, 1988; Kleinman, 1990).

Combined oral contraceptives act primarily by preventing ovulation, by inhibiting pituitary follicle-stimulating hormone and luteinizing hormone and by abolishing the pre-ovulatory surge in luteinizing hormone. The progestogen component renders the cervical mucus relatively impenetrable to sperm and may also reduce the receptivity of the endometrium to implantation (Williams & Stancel, 1996). Together, these actions make combined oral contraceptives very effective in preventing pregnancy, with fewer than one pregnancy per 100 users in the first year of use, when used correctly.

1.1 Historical overview

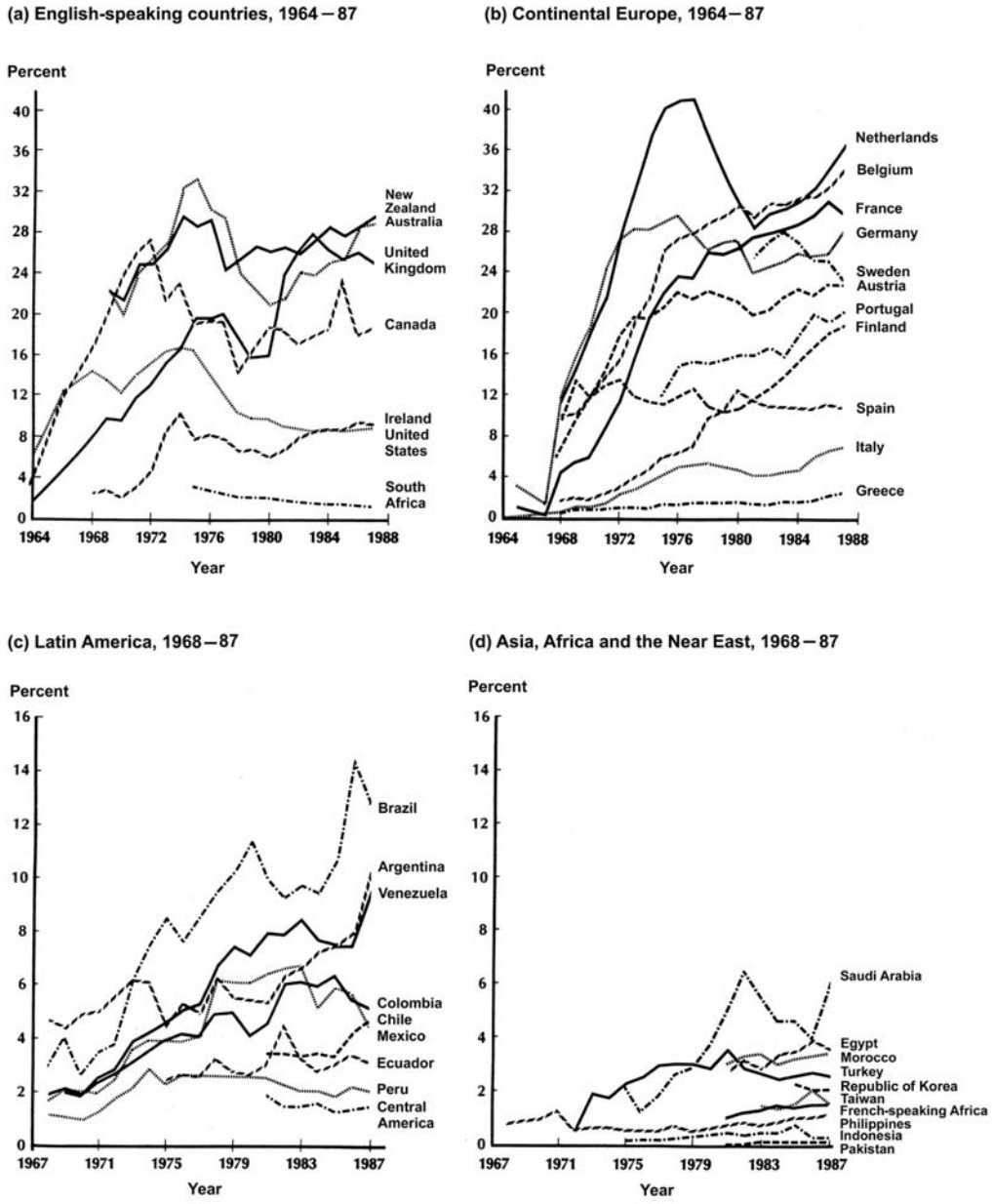
In the late nineteenth century, researchers noted that follicular development and ovulation were suppressed during pregnancy and that extracts of the corpus luteum inhibited ovulation in laboratory animals. In 1921, Ludwig Haberlandt proposed that extracts of the ovary itself could act as a contraceptive (Kleinman, 1990).

Three oestrogens were identified in 1929 and 1930, and progesterone was identified in 1934; however, there were no readily available oral equivalents until 1941, when Russell Marker synthesized diosgenin from extracts of the Mexican yam. Further experimentation yielded the synthesis of norethisterone (norethindrone in the United States) by Carl Djerassi in 1950 and norethynodrel by Frank B. Colton in 1952. These compounds were named progestogens (or progestins) due to their progesterone-like actions (Kleinman, 1990).

In the early 1950s, John Rock investigated the combination of oestrogen and progestogen for the treatment of infertility and found that women who were taking this compound did not ovulate. During 1956, Gregory Pincus, Celso-Ramon Garcia, John Rock and Edris Rice-Wray initiated clinical trials in Puerto Rico of the use of oral norethynodrel as a contraceptive. It was noted that the preparations containing the oestrogen mestranol as a contaminant were more effective in suppressing ovulation than those containing pure norethynodrel. In 1957, the combination of mestranol and norethynodrel was made available in the United States for regulation of menstruation, and in May 1960 it was approved as an oral contraceptive (McLaughlin, 1982; Kleinman, 1990). It was marketed as Enovid® and contained 150 μg mestranol and 9.35 mg norethynodrel (Thorogood & Villard-Mackintosh, 1993). Oral norethisterone (Norlutin®) was approved for menstrual regulation, but was not approved as an oral contraceptive until 1962, when it was combined with mestranol, as Ortho-Novum® (Drill, 1966). Interestingly, in 1959, about 500 000 women in the United States were taking Enovid® or Norlutin® for the treatment of ‘menstrual disorders’ (McLaughlin, 1982). Enovid® became available in the United Kingdom in 1960 (Thorogood & Villard-Mackintosh, 1993). Combined oral contraceptives were introduced throughout Europe and Latin America in the mid- to late 1960s, while use in many countries of Asia, Africa and the Middle East began in the 1970s and early 1980s (Wharton & Blackburn, 1988).

Figure 1 shows sales data for 1964–87 which have been converted into estimates of the percentages of women aged 15–44 buying the combined oral contraceptive pill from

Figure 1. Estimated percentages of women aged 15–44 buying oral contraceptives from pharmacies



Adapted from Wharton and Blackburn (1988)

pharmacies. It shows the rapid increase in the use of the combined oral contraceptive pill in North America, Australia, New Zealand and many European countries in the late 1960s and early 1970s, as well as the decline in use in some countries in the late 1970s, corresponding to the period when the adverse cardiovascular effects of the combined oral contraceptive pill were becoming apparent. They also show the lower but generally increasing rates of combined oral contraceptive use over that time in Latin America, Asia and Africa, although it is important to bear in mind that these figures do not include combined oral contraceptives donated by aid agencies, which constitute up to a third of use in these places (Wharton & Blackburn, 1988).

From the first combined oral contraceptive pill to those available at the time of writing, the doses of oestrogen and progestogen have decreased by at least threefold, and the compositions of treatments have changed, as has the timing of administration of the various component hormones (Piper & Kennedy, 1987). As noted above, the first combined oral contraceptive contained 150 μg mestranol (oestrogen) and 9.35 mg norethynodrel (progestogen); in 1963, just under 50% of combined oral contraceptive pills used by a sample of British women contained 100 μg oestrogen and the remainder contained at least 50 μg oestrogen (Thorogood & Villard-Mackintosh, 1993). Nausea, headaches, vomiting and other side-effects were already thought to be related to high oestrogen levels when research in Britain in the late 1960s linked high oestrogen doses to thromboembolic disease. This finding resulted in the development and prescription of lower-dose pills in the 1970s and 1980s, with the eventual phasing out of those containing more than 50 μg of oestrogen. These lower-dose combined oral contraceptives were found to be just as effective in preventing pregnancy as the high-dose pills, but with fewer side-effects (Wharton & Blackburn, 1988). Most of the combined oral contraceptives prescribed now contain less than 50 μg oestrogen (Wharton & Blackburn, 1988), a dose of 30–35 μg being standard and doses of 20 μg being available (Kleinman, 1990).

The dose of progestogen has also decreased over time, and many different types have been developed (see Annex 2, Table 1). Use of combined oral contraceptives containing a high dose of progestogen peaked in 1972 in the United States, with gradual decreases since, facilitated by the introduction of biphasic and triphasic pills in the 1980s, which allowed the use of even lower doses of progestogen (Piper & Kennedy, 1987; Wharton & Blackburn, 1988). The so-called ‘new-generation’ progestogens (desogestrel, gestodene and norgestimate) were introduced in the mid-1980s, promising lower doses with equivalent efficacy. Studies published around 1995 showed these compounds to be associated with higher rates of venous thromboembolism than those seen with other progestogens (Jick *et al*., 1995; Farley *et al*., 1996), resulting in a decrease in the number of prescriptions of combined oral contraceptives containing new-generation progestogens.

1.2 Patterns of use of combined oral contraceptives

Over 200 million women worldwide have used combined oral contraceptives since 1960 (Kleinman, 1990), and over 60 million are using them currently (Wharton & Blackburn, 1988). The prevalence of combined oral contraceptive use varies enormously

by country and region. Table 1 shows the percentage of married women or women in union aged 15–49 using any form of contraception (including traditional methods) and the percentage taking oral contraceptives. Although progestogen-only oral contraceptives are generally included in this figure, they constitute a relatively small proportion of use, even in the countries where they are most commonly used (see the monograph on 'Hormonal contraceptives, progestogens only'). The percentages are derived mainly from the Demographic and Health Surveys conducted by the United States Aid to International Development.

In 1988, the highest rates of combined oral contraceptive use were found in Europe, with over 40% of women in union of reproductive age using combined oral contraceptives in Belgium, Germany, Hungary and the Netherlands; in most other western European countries and in Australia and New Zealand, current use was 20–40%. Lower rates of use were found in Mediterranean Europe, including Spain, Italy and Greece. Use in the Americas and South-East Asia was generally intermediate, representing around 10–20% of eligible women, while countries in North Africa and the Middle East showed considerable variation in rates of use. The low rates of use of combined oral contraceptives in many countries of sub-Saharan Africa probably reflect low rates of contraceptive use overall and are in keeping with the large 'ideal family size' reported in those countries (Wharton & Blackburn, 1988). The low use in many eastern European and former Soviet Union countries probably reflects reliance on other methods of birth control, including abortion, and use of intrauterine devices (Popov *et al*., 1993). Use of combined oral contraceptives is also uncommon in the Indian sub-continent. They are not licenced for contraceptive use in Japan, although high-dose preparations are available for the treatment of menstrual problems (Kleinman, 1996).

Patterns of use also vary from country to country. Table 2 shows the percentages of women who have ever used combined oral contraceptives by year of birth. The figures are those for the controls of population-based studies of use of combined oral contraceptives and breast cancer. Clearly, in the birth cohorts examined, any use of the pill depends on the age of the woman at the time combined oral contraceptives were introduced into a country as well as the overall prevalence and pattern of use. It is also clear that, in many countries in Europe and in Australia, New Zealand and North America, the vast majority of women born more recently will have taken combined oral contraceptives at some stage. In 1981, 81% of Swedish women aged 25–30 had ever used combined oral contraceptive pills, whereas in 1990–91, 88% of women born in 1960–65 had ever used them; 77% had begun use before the age of 20 (Ranstam & Olsson, 1993). In a United States survey conducted between 1976 and 1980, 15% of 15–19-year-olds and 34% of 20–24-year-olds were currently using combined oral contraceptives (Russell-Briefel *et al*., 1985). In this context, it is important to note that women in high-prevalence countries who have never taken combined oral contraceptives may have particular characteristics, such as psychiatric illness. Indeed, in Sweden, women who have taken combined oral contraceptives are more likely to smoke, drink alcohol, be cohabiting, be older at their first full-term pregnancy and younger at menarche than women who have never taken them (Ranstam & Olsson, 1993).

Table 1. Contraceptive use among married women or women in union, aged 15–49, by country

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Africa					
Algeria	1986–87	36	27		
	1992	51	39	3 300	1 287
Benin	1982	27	0		
	1996	16	1	800	8
Botswana	1984	28	10		
	1988	33	15	100	15
Burkina Faso	1993	8	2	1 600	32
Burundi	1987	9	0.25	800	2
Cameroon	1978	3	0		
	1991	16	1	1 600	19
Central African Republic	1994	15	1	500	5
Comoros	1996	21	3	75	2.2
Côte d'Ivoire	1980–81	4	1		
	1994	11	2	1 900	42
Egypt	1980	24	16		
	1984	30	17		
	1988	38	15		
	1991	48	16		
	1992	47	13		
	1995	48	10	8 300	863
Eritrea	1995	8	2		
Ethiopia	1990	4	2	8 300	158
Gambia	1990	12	3	100	3
Ghana	1979–80	12	3		
	1988	13	2		
	1993	20	3		
	1995	28	7	2 300	161
Kenya	1977–78	7	2		
	1984	17	3		
	1989	27	5		
	1993	33	10	3 100	298
Lesotho	1977	7	2		
	1991–92	23	7	200	14
Liberia	1986	6	3	400	13
Madagascar	1992	17	2	1 700	26
Malawi	1984	7	1		
	1992	13	2	1 400	31
Mali	1987	5	1		
	1995–96	7	3	1 900	59

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Africa (contd)					
Mauritania	1981	1	0		
	1990	4	1	300	3
Mauritius	1975	46	21		
	1985	75	21		
	1991	75	21	200	42
Morocco	1970	1	1		
	1971	3	2		
	1972	4	3		
	1973	6	5		
	1974	7	6		
	1979	16	13		
	1979–80	19	13		
	1983–84	26	16		
	1987	36	23		
	1992	42	28		
	1995	50	32	3 300	1 063
Namibia	1989	26	7		
	1992	29	8	100	8.3
Niger	1992	4	2	1 300	20
Nigeria	1981–82	6	0		
	1990	6	1	18 100	217
Réunion	1990	73	40	100	40
Rwanda	1983	10	0		
	1992	21	3	900	27
Senegal	1978	4	0		
	1986	11	1		
	1992	7	2	1 200	26
South Africa	1975–76	50	14		
	1981–82	48	14		
	1988	50	13	4 300	568
Sudan	1979	5	3		
	1989–90	9	4		
	1992–93	10	5	3 700	185
Swaziland	1988	20	5	100	5.5
Togo	1988	34	0	600	2.4
Tunisia	1978	31	7		
	1983	41	5		
	1988	50	9		
	1994–95	60	7	1 100	80

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Africa (contd)					
Uganda	1988–89	5	1		
	1995	15	3	2 600	68
Zambia	1992	15	4	1 200	52
Zimbabwe	1979	14	5		
	1984	38	23		
	1988	43	31		
	1994	48	33	1 400	463
Europe					
Austria	1981–82	71	40	1 200	480
Belgium	1966	72	5		
	1975	87	30		
	1982	81	32		
	1991	80	47	1 700	792
Bulgaria	1976	76	2	1 600	32
Czech Republic	1993	69	8	1 700	138
Denmark	1970	67	25		
	1975	63	22		
	1988	78	26	700	182
Finland	1971	77	20		
	1977	80	11		
	1989	70	15		
	1994	79	31	700	214
France	1972	64	11		
	1978	79	27		
	1988	80	27		
	1994	75	37	8 500	3 137
Germany	1985	78	34		
	1992	75	59	12 000	7 080
Hungary	1966	67	0		
	1974	74	27		
	1977	73	36		
	1986	73	39		
	1993	84	41	1 800	742
Italy	1979	78	14	9 600	1 344
Lithuania	1994–95	66	5	600	28

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Europe (contd)					
Netherlands	1969	59	27		
	1975	75	50		
	1977	73	40		
	1982	69	39		
	1985	72	40		
	1988	70	43		
	1993	74	47	2 200	1 034
Norway	1977	71	13		
	1988	76	18	500	89
Poland	1972	60	2		
	1977	75	7	6 400	448
Portugal	1979–80	66	19	1 800	344
Romania	1978	58	1		
	1993	57	3	3 800	122
Slovakia	1991	74	5	1 000	50
Slovenia	1989	92	25		
Spain	1977	50	12		
	1985	59	16	6 400	992
Sweden	1981	78	23	1 200	276
Switzerland	1980	71	28		
	1994	82	34	1 000	341
United Kingdom	1970	75	19		
	1975	76	30		
	1976	77	32		
	1983	83	24		
	1986	81	19		
	1989	72	25	9 300	2 325
North America					
Canada	1984	73.1	11	4 200	462
United States	1965	63	15		
	1973	70	25		
	1976	68	23		
	1982	70	13		
	1988	74	15		
	1990	71	15	35 800	5 191
Latin America and the Caribbean					
Bolivia	1983	24	3		
	1989	30	2		
	1994	45	3	1 000	28

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Latin America and the Caribbean (contd)					
Brazil	1986	66	25		
	1996	77	21	23 700	4 906
Colombia	1969	28	5		
	1976	43	14		
	1978	46	17		
	1980	49	17		
	1984	55	21		
	1986	65	16		
	1990	66	14		
	1995	72	13	4 700	606
Costa Rica	1976	68	23		
	1978	64	25		
	1981	65	21		
	1984	65	23		
	1986	68	19		
	1992–93	75	18	400	72
Cuba	1987	70	10	1 900	190
Dominican Republic	1975	32	8		
	1977	31	8		
	1980	42	9		
	1983	28	5		
	1986	50	9		
	1991	56	10		
	1996	64	13	1 000	129
Ecuador	1979	35	10		
	1982	40	10		
	1987	44	9		
	1989	53	9		
	1994	57	10	1 700	173
El Salvador	1975	22	7		
	1976	20	6		
	1978	34	9		
	1985	47	7		
	1988	47	8		
	1993	53	9	700	61
Guadeloupe	1976	44	10	100	10
Guatemala	1978	19	6		
	1983	25	5		
	1987	23	4		
	1995	31	4	1 300	49

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Latin America and the Caribbean (contd)					
Guyana	1975	32	10	200	20
Haiti	1977	19	3		
	1983	7	2		
	1987	8	3		
	1989	10	4		
	1994	18	3	1000	31
Honduras	1981	27	12		
	1984	35	13		
	1987	41	13	700	94
Jamaica	1975–76	41	13		
	1979	55	24		
	1983	51	27		
	1989	55	20		
	1993	62	22	400	86
Martinique	1976	51	17	100	17
Mexico	1973	13	11		
	1976	29	12		
	1978	26	9		
	1979	38	15		
	1982	50	14		
	1987	53	10	13 000	1 261
Nicaragua	1981	27	11		
	1992–93	49	13	500	65
Panama	1976	57	19		
	1979	61	19		
	1984	58	12	300	35
Paraguay	1977	29	12		
	1979	32	10		
	1987	45	13		
	1990	48	14		
	1995–96	56	14	600	81
Peru	1969–70	26	3		
	1977–78	41	5		
	1981	41	5		
	1986	46	7		
	1991–92	59	6		
	1996	64	6	300	19

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Latin America and the Caribbean (contd)					
Puerto Rico	1968	60	11		
	1974	62	20		
	1976	65	13		
	1982	70	9		
	1995–96	78	10	500	49
Trinidad and Tobago	1970–71	44	17		
	1977	54	19		
	1987	53	14	200	28
Venezuela	1977	60	19	2 700	506
Asia					
Bahrain	1989	53	13	100	13
Bangladesh	1975	8	3		
	1977	9	2		
	1979	13	4		
	1980	12	4		
	1981	20	4		
	1983	19	3		
	1985	25	5		
	1989	31	9		
	1991	40	14		
	1993	45	17	21 400	3 724
Burma	1991	17	4		
China	1982	70	6		
	1988	71	4		
	1992	77	3	222 700	5 968
Hong Kong	1969	42	16		
	1972	54	20		
	1977	77	28		
	1982	77	21		
	1984	72	22		
	1987	81	16	900	148
India	1980	32	1		
	1988	43	1		
	1992–93	41	1	159 000	1 908
Indonesia	1973	9	3		
	1976	26	15		
	1979	21	11		
	1980	26	14		
	1985	39	15		

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Asia (contd)					
Indonesia (contd)	1987	51	18		
	1991	50	15		
	1994	55	17	31 400	5 369
Iran	1978	23	20		
	1992	65	23	9 200	2 116
Iraq	1974	14	8		
	1989	14	5	2 500	117.5
Japan	1969	52	1		
	1971	53	1		
	1973	59	1		
	1975	61	2		
	1977	60	2		
	1979	62	2		
	1984	57	1		
	1986	64	1		
	1988	56	1	18 600	186
Jordan	1972	21	13		
	1976	25	12		
	1983	26	8		
	1985	27	6		
	1990	35	5	500	23
Kuwait	1987	35	24	300	72
Malaysia	1966–67	9	4		
	1970	16	12		
	1974	36	18		
	1979	36	25		
	1981	42	17		
	1984	51	12		
	1988	48	15	2 600	390
Nepal	1976	3	1		
	1981	7	1		
	1986	15	1		
	1991	25	1		
	1996	29	1	3 500	49
Oman	1988	9	2	200	4.8
Pakistan	1975	4	1		
	1980	6	1		
	1984–85	9	1		
	1990–91	12	1	18 100	127

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Asia (contd)					
Philippines	1968	15	1		
	1972	8	5		
	1973	18	7		
	1976	22	11		
	1977	22	11		
	1978	37	5		
	1979	37	6		
	1980	45	5		
	1981	48	16		
	1983	33	6		
	1988	36	7		
	1993	40	9		
	1995	53	11		
	1996	48	12	9 700	1 125
Quatar	1987	32	13	100	13
Republic of Korea	1991	79	3	7 600	228
Singapore	1970	45	38		
	1973	60	22		
	1977	71	17		
	1978	71	17		
	1982	74	12	500	57.9
Sri Lanka	1975	32	2		
	1982	55	3		
	1987	62	4	2 700	110.7
Thailand	1970	14	4		
	1973	26	11		
	1975	33	14		
	1978	53	22		
	1981	59	20		
	1984	65	20		
	1985	59	21		
	1987	66	19	9 000	1 674
Turkey	1963	22	1		
	1968	32	2		
	1973	38	4		
	1978	50	8		
	1983	51	8		
	1988	63	6		
	1993	63	5	9 400	461

Table 1 (contd)

Country or region	Year of survey	Any method (%)	Oral contra-ceptives (%)	No. of women (in thousands, 1990)	Calculated no. of oral contra-ceptive users (thousands)
Asia (contd)					
Viet Nam	1988	53	0		
	1994	65	2	10 000	210
Yemen	1979	1	1		
	1991–92	7	3	1 700	54
Oceania					
Australia	1986	76	24	2 600	624
New Zealand	1976	70	29	400	114

From Population Council (1994, 1995); Phai *et al*. (1996); Population Council (1996a,b); United Nations (1996); Population Council (1997a,b,c,d,e,f; 1998a,b); United States Census Bureau (1998)

Sales figures for 1987 show that more than 40% of oral contraceptives purchased by pharmacies in most 'developed' countries were monophasic preparations, containing less than 50 μg oestrogen; approximately 35% were triphasic preparations, 10% were monophasic preparations containing 50 μg oestrogen, about 8% were biphasic preparations containing less than 50 μg oestrogen, about 3% were sequential combined preparations, and around 2% contained progestogen alone. In 'developing' countries, just under 50% of preparations bought by pharmacies were monophasic preparations containing less than 50 μg oestrogen, approximately 10% were triphasic preparations and around 42% were monophasic preparations containing 50 μg oestrogen (Wharton & Blackburn, 1988). Most of the oral contraceptives provided by major aid organizations (United States Aid to International Development, United Nations Family Planning Agency, International Planned Parenthood Federation) contain 30 μg ethinyloestradiol and 150 μg levonorgestrel.

1.3 Exposure to other combinations of oestrogen and progestogen

Injectable combined hormonal contraceptives were first developed in the late 1960s and consist of a depot progestogen and oestrogen administered monthly. Formulations and brands of such preparations are listed in Table 3, with a list of some of the countries in which they are available. They are used in parts of Latin America, China, Spain, Portugal, Thailand, Indonesia and Singapore, although, as can be seen from Table 1 in the monograph on 'Hormonal contraceptives, progestogens only', they are unlikely to constitute a large proportion of the contraceptive use in these countries.

In Latin America, at least 1 million women use dihydroxyprogesterone acetophenide and oestradiol oenanthate, and the combination of dihydroxyprogesterone acetophenide

Table 2. Percentages of women who have ever used oral contraceptives, by year of birth

Country	Year of birth							
	< 1915	1915–19	1920–24	1925–29	1930–34	1935–39	1940–44	1945–49
Australia	0	3	18	36	55	69	80	85
Canada	1	6	26	42	53	67	79	84
China	–	–	1	2	19	36	39	39
Denmark	–	0	4	21	35	46	66	75
France	–	–	–	7	16	38	61	69
Germany	–	–	–	–	40	58	75	86
Italy	0	0.4	0.2	2	3	8	15	25
Netherlands	–	5	16	35	49	69	84	90
New Zealand	–	–	–	50	61	75	84	91
Norway	–	–	–	–	–	–	–	45
Sweden	–	–	–	–	–	–	65	82
United Kingdom	–	3	15	27	41	51	68	83
United States	1	4	14	28	43	60	75	85

From Collaborative Group on Hormonal Factors in Breast Cancer (1996a) Appendix 5

Table 3. Injectable contraceptives containing oestrogen and progesterone given monthly

Brand name	Composition	Dose (mg)	Availability
Anafertin, Yectames	Oestradiol oenanthate Dihydroxyprogesterone acetophenide	5 75	Many Latin American countries and Spain
Chinese injectable No. 1	Oestradiol valerate 17α-Hydroxyprogesterone caproate	5 250	China
Chinese injectable No. 2	Oestradiol Megestrol acetate	3.5 25	China
Cicnor, Damix, Progesterol, Segutalmes	Oestradiol oenanthate Medroxyprogesterone acetate	10 150	Portugal
Ciclofem, Ciclofemina, Cyclofem, Cyclo Geston	Oestradiol cypionate Medroxyprogesterone acetate	5 25	Registered in Guatamala, Indonesia, Mexico, Peru and Thailand
Chinese injectable No. 3, Mesigyna, Norigynon	Oestradiol valerate Norethisterone oenanthate	5 50	Argentina, Brazil and Mexico
Agurin, Ciclovar, Deproxone, Exuna, Horprotal, Neolutin, Normagest, Novular, Perlutal, Perlutale, Perlutan, Proter, Topasel, Uno Ciclo	Oestradiol oenanthate Dihydroxyprogesterone acetophenide	10 150	Many Latin American countries and Spain
Redimen, Soluna, Unijab	Oestradiol benzoate Dihydroxyprogesterone acetophenide	10 150	Peru and Singapore
Unalmes	Oestradiol oenanthate Alfasona acetophenide	10 120	Chile and Paraguay

From Kleinman (1990); Lande (1995)

and hydroxyprogesterone caproate (Chinese injectable No. 1) has been used by about 1 million women in China (Lande, 1995).

A relatively high dose of oestrogen and progestogen can be administered up to 72 h after unprotected intercourse as 'emergency contraception'. It is often given as 100 µg ethinyloestradiol and 0.5 mg levonorgestrel (or 1 mg norgestrel), as two tablets, immediately and a further equal dose 12 h later (Kleinman, 1990). A progestogen-only regimen is also available (see the monograph on 'Hormonal contraceptives, progestogens only').

2. Studies of Cancer in Humans

2.1 Breast cancer

The relationship between the use of combined oral contraceptives and the risk for breast cancer was reviewed by a working group convened by IARC in 1979 (IARC, 1979). At the time, the results from several follow-up (Royal College of General Practitioners, 1974; Ory *et al*., 1976; Vessey *et al*., 1976) and case–control studies (Vessey *et al*., 1972, 1975; Paffenbarger *et al*., 1977; Sartwell *et al*., 1977; Kelsey *et al*., 1978; Lees *et al*., 1978) had been published. The data were sparse even for the analysis of use. The Group concluded that there was no clear evidence that use of combined oral contraceptives influences the risk for breast cancer.

In the two decades since the 1979 report, oral contraceptive formulations have been changed: The doses of oestrogen and progestogen have been lowered, the components used have changed, cyclic preparations with different doses at different times during the menstrual cycle have been introduced, and progestogen-only formulations have become available.

Various aspects of the use of combined oral contraceptives in relation to the incidence of breast cancer have been assessed in numerous epidemiological studies conducted since 1979. Several detailed reviews of the epidemiological evidence have been published (Prentice & Thomas, 1987; Olsson, 1989; Romieu *et al*., 1990; Malone, 1991; Thomas, 1991a; WHO, 1992; Malone *et al*., 1993; Schlesselman, 1995). In addition, a pooled analysis of the individual data from 54 studies was reported (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a,b); the analyses covered an estimated 90% of the data available at that time.

Studies in which cases of breast cancer occurring before 1980 were analysed provide limited information on many aspects of the use of combined oral contraceptives that are of interest, notably use at a young age, long duration of use, recent use and use followed by a long latent period (Ravnihar *et al*., 1979; Jick *et al*., 1980; Brinton *et al*., 1982; Harris *et al*., 1982; Vessey *et al*., 1982; Janerich *et al*., 1983; Hennekens *et al*., 1984; Schildkraut *et al*., 1990; Morabia *et al*., 1993). The early studies have been reviewed in detail (Thomas, 1991a). The studies considered here are based on data collected since 1979 and are limited to those reported in English.

The follow-up studies are summarized in Table 4, the case–control studies in which hospitalized controls were used are summarized in Table 5 and the case–control studies in which controls from other sources were used are summarized in Table 6. When several reports are available on the same study, all are listed; however, the data shown are taken from the report (marked with an asterisk) that was based on the largest numbers. The studies are listed in order of the year of the first publication of results. Thus, follow-up data have been published from the Nurses' Health Study (Colditz *et al*., 1994), in which data on the use of combined oral contraceptives and risk factors were collected by postal questionnaire and the diagnoses were verified from hospital records.

A variety of methods was used in the case–control studies. The data on use of combined oral contraceptives and other risk factors for breast cancer were obtained almost exclusively by personal interview; the diagnoses of breast cancer were generally verified from hospital or cancer registry records. In virtually all of the studies, relative risks were estimated after control for important potential confounding factors, such as reproductive variables and socioeconomic status. The Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b) analysed all of the published and unpublished studies available to them, for a combined total of some 53 000 cases and 100 000 controls. Individual data from each of the studies were analysed centrally; combined relative risk estimates were obtained by a modification of the Mantel-Haenszel procedure, with stratification on study, age at diagnosis, parity and age at the birth of the first child.

Comparisons of any use of combined oral contraceptives ('ever use') with no use ('never use') yielded overall relative risk estimates close to 1.0 in most studies. In the analysis of the Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b), the relative risk estimate was 1.17 [95% confidence interval [CI], 1.1–1.24] on the basis of data from hospital-based case–control studies, 1.0 [95% CI, 0.97–1.1] from case–control studies with population controls and 1.07 [95% CI, 1.00–1.14] from follow-up studies. These estimates were not significantly different. The characteristics of women who had ever used oral contraceptives varied, however, from study to study and changed over time: there was a tendency to use combined oral contraceptives at younger ages and for longer.

In the early and mid-1980s, a number of associations between the use of combined oral contraceptives and an increased risk for breast cancer were observed in subgroups of some epidemiological studies, and hypotheses were raised (and later refuted) to explain those observations. In 1981, Pike *et al*. observed that the risk for breast cancer more strongly tended to increase with increasing duration of use of combined oral contraceptives before the first full-term pregnancy than after, raising the hypothesis that use of these contraceptives before the first full-term pregnancy is more harmful. A few subsequent studies provided some support for this hypothesis (McPherson *et al*., 1987; Rohan & McMichael, 1988; Olsson *et al*., 1989, 1991a), but most studies did not (Meirik *et al*., 1986, 1989; Romieu *et al*., 1989; Stanford *et al*., 1989; UK National Case–Control Study Group, 1989; Paul *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Weinstein *et al*., 1991; Wingo *et al*., 1991; Ewertz, 1992;

Table 4. Follow-up studies of breast cancer associated with use of combined oral contraceptives

Reference	Country	Age at recruitment (years)	Size of cohort	Period of follow-up	No. of cases	Loss to follow-up (%)	Any use (%)	RR (95% CI), any versus none	RR (95% CI) for longest duration
Lipnick *et al*. (1986); Romieu *et al*. (1989); Colditz *et al*. (1994)[*] (Nurses' Health Study)	United States	30–55	118 273	1976–86	1 799	5	48	1.1 (0.97–1.2)	Not reported
Kay & Hannaford (1988)[a] (incidence)	United Kingdom	Not reported	47 000	1968–85	239	[61]	Not reported	Former use (99 cases in 134 079 person–years), 1.2 (0.9–1.6) Current use (44 cases in 104 505 person–years), 1.2 (0.84–1.9)	≥ 10 years, 1.4 (0.91–2.3)
Mills *et al*. (1989)	United States	≥ 25	20 341	1976–82	215	1	27	1.5 (0.94–2.5) based on 29 cases in 31 188 person–years among women ≤ 45 years of age in 1960	≥ 10 years, 1.4 (0.34–6.0) based on 2 cases in 1660 person–years among women ≤ 45 years of age in 1960
Vessey *et al*. (1989a)	United Kingdom	25–39	17 032	1968–87	189	0.3 per year	Not reported	Not reported	Ages 25–44, ≥ 10 years, 0.65/1000 person–years (14 cases) versus 0.62/1000 person–years for no use (49 cases) [RR, 1.0] Ages ≥ 45, ≥ 10 years, 1/1000 person–years versus (8 cases) 2.2/1000 person–years for no use (50 cases) [RR, 0.48]

Table 4 (contd)

Reference	Country	Age at recruitment (years)	Size of cohort	Period of follow-up	No. of cases	Loss to follow-up (%)	Any use (%)	RR (95% CI), any versus none	RR (95% CI) for longest duration
Beral *et al*. (1999)[a]	United Kingdom	Not reported	46 000	1968–93	259 (deaths[b])	25	63	1.1 (0.82–1.4)	≥ 10 years, 1.4 (0.86–2.1) (26 deaths)
Collaborative Group (1996b)	–	–	–	–	6 806	–	[38]	1.07 [1.00–1.14]	≥ 15 years, 1.1 [0.96–1.2]

RR, relative risk; CI, confidence interval
* Report from which data are taken
[a] Data from Royal College of General Pracitioners (1974)
[b] 154 deaths for any use, 105 deaths for no use

Table 5. Case–control studies of use of combined oral contraceptives and breast cancer with hospital controls

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Vessey *et al*. (1983)	United Kingdom	1968–80	16–50	1 176	1 176	Not reported	46/47	0.98 (0.81–1.2)	≥ 97 months versus never 0.99 (0.67–1.4)
Rosenberg *et al*. (1984); Miller *et al*. (1986, 1989); Rosenberg *et al*. (1996)* (surveillance study)	United States	1977–92	25–59	3 540 (white women)	4 488	95	≥ 1 year of use [29/30]	≥ 1 year versus < 1 year 1.1 (1.0–1.3)	≥ 10 years versus < 1 year 0.9 (0.7–1.1)
Talamini *et al*. (1985)	Italy	1980–83	26–79	368	373	99	4/6	0.7 (0.4–1.4)	Not reported
Ellery et *al*. (1986)	Australia	1980–82	25–64	141	279	Not reported	[48/42]	0.9 (0.6–1.5)	≥ 6 years 1.3 (0.7–2.7)
La Vecchia *et al*. (1986, 1989); Tavani *et al*. (1993a)*	Italy	1983–91	< 60	2 309	1 928	98/97	16/14	1.2 (1.0–1.4)	≥ 60 months versus never 0.8 (0.5–1.0)
McPherson *et al*. (1987)	United Kingdom	1980–84	16–44	351	351	Not reported	68/65	Not reported	≥ 12 years versus never 1.8 (0.82–3.9)
			≥ 45	774	774	Not reported	24/27	Not reported	≥ 12 years versus never 0.84 (0.39–1.8)
Ravnihar *et al*. (1988)	Slovenia	1980–83	25–54	534	1 989	Not reported	30/24	1.6 (1.3–2.1)	> 7 years versus never 2.4 (1.5–3.8)
Harris *et al*. (1990)	United States	1979–81	All	401	519	Not reported	19/23	0.8 (0.6–1.2)	≥ 5 years (age < 50) 0.4 (0.2–0.8)

Table 5 (contd)

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
WHO Collaborative Study (1990)*; Ebeling *et al*. (1991); Thomas (1991b); Thomas *et al*. (1991, 1992, 1994)	10 countries: 3 developed, 7 developing	1979–86	< 62	2 116	13 072	Not reported	34/34	1.2 (1.0–1.3)	> 8 years 1.6 (1.2–2.0)
Clavel *et al*. (1991)	France	1983–87	25–56	464	542	99/99	[51/44]	1.5 (1.1–2.1)	≥ 21 years versus never 1.2 (0.4–3.9)
Bustan *et al*. (1993)	Indonesia	1990–91	25–55	119	258	90	32/21	1.8 (1.1–3.0)	> 5 years 1.1 (0.6–2.1)
Gomes *et al*. (1995)	Brazil	1978–87	25–75	300	600	Not reported	21/15	1.8 (1.2–2.9)	Not reported
La Vecchia *et al.* (1995)	Italy	1991–94	< 65	1 991	1 899	96/96	18/14	1.1 (0.9–1.4)	> 8 years versus never 1.2 (0.7–1.9)
Lipworth *et al*. (1995)	Greece	1989–91	All	820	795	95/93	4/4	≤ 45 years of age 1.1 (0.60–2.0) > 45 years of age 1.6 (0.82–3.3)	≤ 45 years of age, ≥ 3 years 0.47 (0.13–1.70) 45 years of age, ≥ 3 years 1.2 (0.32–4.2)
Palmer *et al*. (1995) (surveillance)	United States	1977–92	25–59	524 (black women)	1 021	95	[31/27]	≥ 1 year versus < 1 year 1.6 (1.2–2.1)	≥ 10 years versus < 1 year 1.1 (0.6–2.0)
Levi *et al*. (1996)	Switzerland	1990–95	< 70	206	424	85	37/32	1.5 (1.1–2.3)	≥ 10 years versus never 2.4 (1.4–4.2)

Table 5 (contd)

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Tomasson & Tomasson (1996)	Iceland	1965–89	25–69	1 062	5 622 (cancer detection clinic)	Not reported	Not reported	Not reported	> 8 years 0.96 (0.69–1.3)
Tryggvadóttir *et al*. (1997)	Iceland	1975–95	18–43	204	1 183 (cancer detection clinic)	Not reported	79/81	Not reported	> 8 years 1.3 (p = 0.55)
Collaborative Group (1996a)[a]	–	–	–	15 030	34 565	–	26/31	1.17 [1.1–1.24]	≥ 15 years versus never 1.1 [0.96–1.2]

RR, relative risk; CI, confidence interval
* Report from which data are taken
[a] Includes all studies mentioned above

Table 6. Case–control studies of use of combined oral contraceptives and breast cancer with controls other than hospitalized patients

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Pike *et al.* (1981, 1983); Bernstein *et al.* (1990)*	United States	1972–83	< 37	439 (population-based)	439 (neighbours)	68/not reported	85/85	Not reported	> 8 years versus never 1.7 (p_{trend} < 0.01)
Centers for Disease Control Cancer and Steroid Hormone Study (1983a); Stadel *et al.* (1985); Cancer and Steroid Study (1986)*; Schlesselmann *et al.* (1987, 1988); Stadel *et al.* (1988); Wingo *et al.* (1991); Mayberry & Stoddard-Wright (1992) (CASH Study)	United States	1980–82	20–54	4 711 (population-based)	4 676 (random-digit dialling)	80/83	[63/64]	1.0 (0.9–1.1)	≥ 15 years versus never 0.9 (0.8–1.1)
Meirik *et al.* (1986)*; Lund *et al.* (1989); Meirik *et al.* (1989); Holmberg *et al.* (1994) (Sweden–Norway Joint National Study)	Sweden, Norway	1984–85	< 45	422	722 (population-based)	89/81	77/78	Not reported	≥ 12 years versus never 2.2 (1.2–4.0)
Paul *et al.* (1986, 1990*, 1995) (New Zealand National Study)	New Zealand	1983–87	25–54	891 (population-based)	1 864 (electoral rolls)	95/90	77/83	1.0 (0.82–1.3)	≥ 14 years versus never 1.1 (0.78–1.7)

Table 6 (contd)

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Rohan & McMichael (1988)	Australia	1982–84	20–69	395 (population-based)	386 (electoral rolls)	[81/72]	49/49	1.1 (0.70–1.6)	> 7 years versus never 0.67 (0.38–1.2)
Yuan *et al.* (1988)	China	1984–85	20–69	534	534 (population-based)	94/99	[19/18]	1.1 (0.74–1.5)	≥ 10 years versus never 1.4 (0.62–3.2)
Jick *et al*. (1989)	United States	1975–83	< 43	127 (health plan)	174 (health plan)	Not reported	61/71	0.9 (0.4–1.9)	≥ 10 years 1.4 (0.4–4.6)
Olsson *et al*. (1989*, 1991a)	Sweden	1979–80 1982–85	≤ 46	174 (hospital)	459 (population-based)	100/92	82/72	[1.8]	Not reported
Stanford *et al*. (1989)	United States	1973–80	All	2 022	2 183 (screening programme)	78/83	24/24	1.0 (0.9–1.2)	≥ 15 years versus never 0.65 (0.3–1.6)
UK National Case–Control Study Group (1989*, 1990); Chilvers *et al*. (1994) (United Kingdom National Study)	United Kingdom	1980–85	< 36	755 (population-based)	755 (general practice)	72/89	[91/89]	Not reported	> 8 years versus never 1.7 ($p_{trend} < 0.001$)
Weinstein *et al.* (1991)	United States	1984–86	20–70	1 067 (population-based)	1 066 (drivers' license files)	66/41	26/23	1.2 (0.98–1.5)	≥ 4 years versus never 1.2 (0.82–1.6)

Table 6 (contd)

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Ewertz (1992)	Denmark	1983–84	< 40	203	212	90/88	[81/79]	1.2 (0.73–1.9)	≥ 12 years versus < 4 years 1.3 (0.82–2.0)
			40–59	856	778 (population-based)	89/80	36/37	Not reported	
Rosenberg *et al*. (1992)	Canada	1982–86	< 70	607 (cancer hospital)	1 214 (neighbourhood)	79/65	43/45	Not reported	≥ 15 years versus never 0.9 (0.4–1.7)
Ursin *et al*. (1992)	United States and Canada	1935–89	< 50	149 (2 registries)	243 (sisters)	Not reported	[42/30]	1.7 (1.0–2.9)	≥ 7 years 2.0 (0.93–4.2)
Rookus *et al*. (1994)	Netherlands	1986–89	20–54	918	918 (population-based)	60/72	85/85	1.1 (0.8–1.4)	≥ 12 years versus never 1.3 (0.9–1.9)
White *et al*. (1994)	United States	1983–90	21–45	747 (population-based)	961 (random-digit dialling)	83/78	78 (≥ 1 year)/ 76 (≥ 1 year)	1.0 (0.71–1.5)	≥ 10 years versus never 1.3 (0.92–1.9)
Brinton *et al*. (1995)	United States	1990–92	20–45	1 648 (population-based)	1 505 (random-digit dialling)	86/78	76/71 (≥ 6 months)	Not reported ≥ 6 months to < 5 years versus < 6 months 1.3 (1.1–1.5)	≥ 10 years versus < 6 months 1.3 (1.0–1.6)

Table 6 (contd)

Reference	Country	Years of case diagnosis	Age (years)	No. of cases	No. of controls	Participation rate (%)	Any use (%)	RR (95% CI), ever versus never	RR (95% CI), longest duration
						Cases/Controls	Cases/Controls		
Primic- akelj *et al*. (1995)	Slovenia	1988–90	25–54	624 (hospital)	624 (population-based)	94/83	48/48	1.1 (0.85–1.4)	> 8 years versus never 1.2 (0.76–1.7)
Newcomb *et al*. (1996)	United States	1988–91	< 75	6 751 (population-based)	9 311 (drivers' licenses or Medicare)	81/84	38/39	1.1 (1.0–1.2)	≥ 15 years versus never 1.0 (0.8–1.4)
Rossing *et al*. (1996)	United States	1988–90	50–64	537 (population-based)	545 (random-digit dialling)	81/73	[47/41]	1.1 (0.8–1.4)	> 10 years versus never 0.8 (0.5–1.3)
Collaborative Group (1996b)[a]	–	–	–	31 089	37 676	–	[48/49]	1.0 [0.97–1.1]	≥ 15 years 1.1 (0.96–1.2)

RR, relative risk; CI, confidence interval
* Reports from which data are taken
[a] Includes all studies

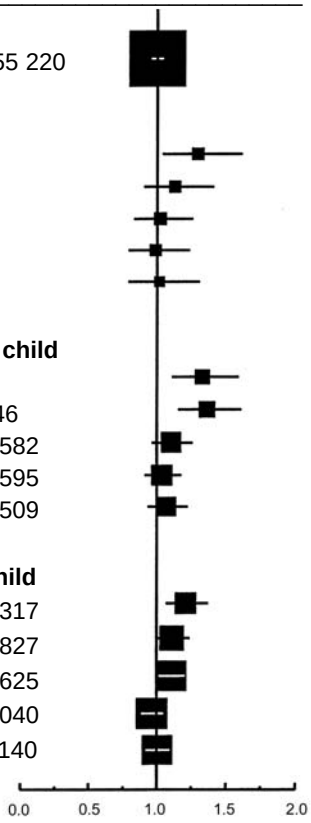
Tavani *et al*., 1993a; White *et al*., 1994; Brinton *et al*., 1995; Palmer *et al*., 1995; Primic-akelj *et al*., 1995; Collaborative Group on Hormonal Factors in Breast Cancer, 1996b; Levi *et al*., 1996; Newcomb *et al*., 1996; Rosenberg *et al*., 1996). When the study from which the hypothesis arose was completed, with larger numbers, the effect was no longer seen (Bernstein *et al*., 1990). Rather, the data now suggested that the increase in risk was related to use before the age of 25. Some subsequent evidence has suggested that the risk is greater the younger the woman is when she first uses combined oral contraceptives (White *et al*., 1994), but most studies have not supported this idea (Meirik *et al*., 1986; Ravnihar *et al*., 1988; Rohan & McMichael, 1988; Stanford *et al*., 1989; UK National Case–Control Study Group, 1989; Paul *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Clavel *et al*., 1991; Weinstein *et al*., 1991; Wingo *et al*., 1991; Ewertz, 1992; Rosenberg *et al*., 1992; Tavani *et al*., 1993a; Brinton *et al*., 1995; Palmer *et al*., 1995; Primic- akelj *et al*., 1995; Levi *et al*., 1996; Newcomb *et al*., 1996; Rosenberg *et al*., 1996; Rossing *et al*., 1996). The study of Rookus *et al*. (1994) suggested an increased risk for breast cancer before the age of 35 for women who started to use combined oral contraceptives at an early age but no increased risk between the ages of 36 and 45. The Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b) provided little support for the idea that the effect of combined oral contraceptives is modified by the timing in relation to the first pregnancy (Figure 2) or the age at first use, except perhaps that the relative risks were somewhat higher in current or recent users who began use before the age of 20 (Figure 3). There is, however, no evidence of any persistent excess risk many years after use has ceased for women who began use before the age of 20.

Data on the use of combined oral contraceptives in relation to age at the time of diagnosis of breast cancer are shown in Table 7. As evidence has accumulated, a relatively consistent finding has been an increased risk for breast cancer occurring before the age of 45, and particularly before 35, among users of combined oral contraceptives (Meirik *et al*., 1986; McPherson *et al*., 1987; Stanford *et al*., 1989; UK National Case–Control Study Group, 1989; Bernstein *et al*., 1990; Paul *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Weinstein *et al*., 1991; Wingo *et al*., 1991; Rookus *et al*., 1994; Brinton *et al*., 1995; Palmer *et al*., 1995; La Vecchia *et al*., 1995; Newcomb *et al*., 1996; Rosenberg *et al*., 1996). Some studies have not shown such an increase, however (Vessey *et al*., 1983; Ravnihar *et al*., 1988; Ewertz, 1992; Rosenberg *et al*., 1992; Tavani *et al*., 1993a; White *et al*., 1994; Primic- akelj *et al*., 1995). Most of the studies show no overall increase in risk for older women, although the relative risk estimates were increased for older women in some studies (Vessey *et al*., 1983; Ravnihar *et al*., 1988; Rookus *et al*., 1994). The Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b) found little difference in risk according to the age at diagnosis of breast cancer once recency of use had been taken into account (Figure 4).

Data on the recency of use of combined oral contraceptives are shown in Table 8. A relatively consistent finding is that the risk for breast cancer is increased among women who have used these oral contraceptives recently, within the previous five to 10 years

Figure 2. Relative risk for breast cancer by time since last use of combined oral contraceptives and in relation to childbearing

Use of combined oral contraceptives in relation to childbearing	RR[a] ± SD	Cases/controls
Never used	1.00 ± 0.018	28 200/55 220
Nulliparous women		
Current user	1.30 ± 0.098	516/883
Last use 1–4 years previously	1.13 ± 0.092	418/639
5–9 years previously	1.02 ± 0.082	472/610
10–14 years previously	0.99 ± 0.086	411/477
≥ 15 years previously	1.02 ± 0.099	338/432
Parous women who began use before the birth of their first child		
Current user	1.33 ± 0.081	605/862
Last use 1–4 years previously	1.36 ± 0.076	744/1 046
5–9 years previously	1.10 ± 0.054	1 072/1 582
10–14 years previously	1.04 ± 0.051	1 159/1 595
≥ 15 years previously	1.07 ± 0.055	1 106/1 509
Parous women who began use after the birth of their first child		
Current user	1.21 ± 0.054	1 142/2 317
Last use 1–4 years previously	1.11 ± 0.045	1 448/2 827
5–9 years previously	1.11 ± 0.036	2 473/4 625
10–14 years previously	0.97 ± 0.032	2 514/5 040
≥ 15 years previously	1.00 ± 0.034	2 611/5 140



Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)

[a] Relative risk (RR) given with 99% confidence interval (CI) relative to no use, stratified by study, age at diagnosis, parity and, where appropriate, age when first child was born and age when the risk for conceiving ceased

Size of square indicates the number of cases

Figure 3. Relative risk for breast cancer for various indices of the timing of combined oral contraceptive use within categories of time since last use

(a) Relative risk for breast cancer by duration of use and time since last use of combined oral contraceptives

	RR[a] ± SD	Cases/controls	RR[a] & 99% CI
Never used	1.00 ± 0.014	28 200/55 220	
Current user			
Duration ≤ 12 months	1.18 ± 0.122	176/621	
1–4 years	1.27 ± 0.079	489/1 158	
5–9 years	1.21 ± 0.061	794/1 338	
≥ 10 years	1.29 ± 0.060	882/1 156	
Last use 1–4 years previously			
Duration ≤ 12 months	1.05 ± 0.080	359/1 021	
1–4 years	1.12 ± 0.064	649/1 240	
5–9 years	1.26 ± 0.059	908/1 369	
≥ 10 years	1.14 ± 0.060	746/1 045	
Last use 5–9 years previously			
Duration ≤ 12 months	1.05 ± 0.056	757/1 712	
1–4 years	1.05 ± 0.043	1 280/2 186	
5–9 years	1.13 ± 0.044	1 340/2 067	
≥ 10 years	1.14 ± 0.062	714/1 060	
Last use 10–14 years previously			
Duration ≤ 12 months	1.00 ± 0.044	1 160/2 337	
1–4 years	0.97 ± 0.037	1 581/2 639	
5–9 years	0.99 ± 0.046	1 075/1 681	
≥ 10 years	1.01 ± 0.083	332/598	
Last use ≥ 15 years previously			
Duration ≤ 12 months	1.05 ± 0.036	1 999/3 470	
1–4 years	1.04 ± 0.041	1 533/2 574	
5–9 years	0.87 ± 0.064	483/946	
≥ 10 years	0.90 ± 0.146	83/196	

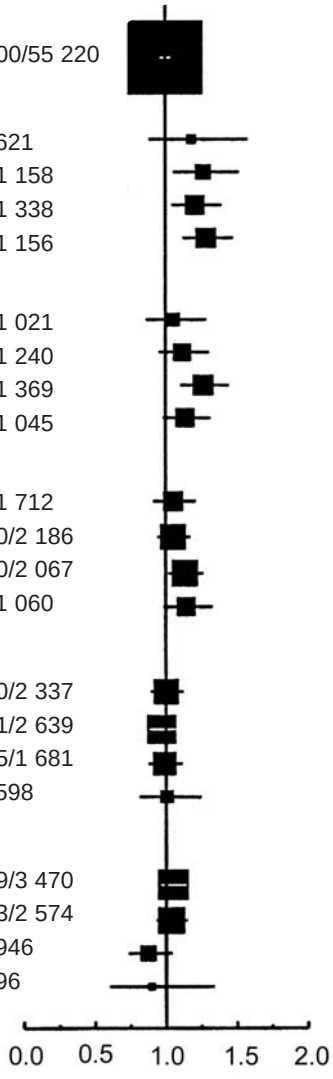


Figure 3 (contd)

(b) Relative risk for breast cancer by age at first use and time since last use of combined oral contraceptives

	RR[a] ± SD	Cases/controls
Never used	1.00 ± 0.018	28 200/55 220
Current user		
Age at first use < 20 years	1.59 ± 0.093	565/945
20–24 years	1.17 ± 0.065	679/1 336
25–29 years	1.16 ± 0.077	421/895
≥ 30 years	1.25 ± 0.069	676/1 097
Last use 1–4 years previously		
Age at first use < 20 years	1.49 ± 0.093	503/794
20–24 years	1.15 ± 0.060	768/1 379
25–29 years	1.09 ± 0.072	483/906
≥ 30 years	1.11 ± 0.055	908/1 596
Last use 5–9 years previously		
Age at first use < 20 years	1.07 ± 0.070	560/938
20–24 years	1.09 ± 0.046	1 224/2 039
25–29 years	1.01 ± 0.052	803/1 551
≥ 30 years	1.18 ± 0.046	1 504/2 497
Last use 10–14 years previously		
Age at first use < 20 years	1.13 ± 0.072	555/771
20–24 years	0.93 ± 0.041	1 249/2 088
25–29 years	1.06 ± 0.051	1 001/1 705
≥ 30 years	0.95 ± 0.042	1 343/2 691
Last use ≥ 15 years previously		
Age at first use < 20 years	1.14 ± 0.077	524/714
20–24 years	1.01 ± 0.045	1 305/1 988
25–29 years	1.01 ± 0.051	1 035/1 854
≥ 30 years	0.99 ± 0.046	1 234/2 630

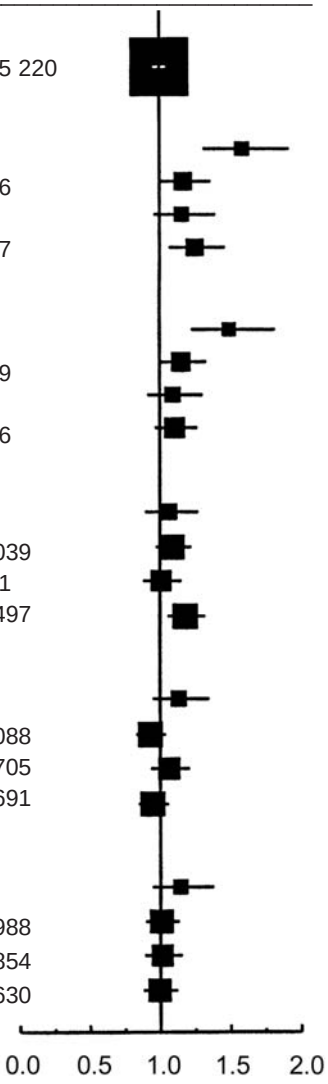
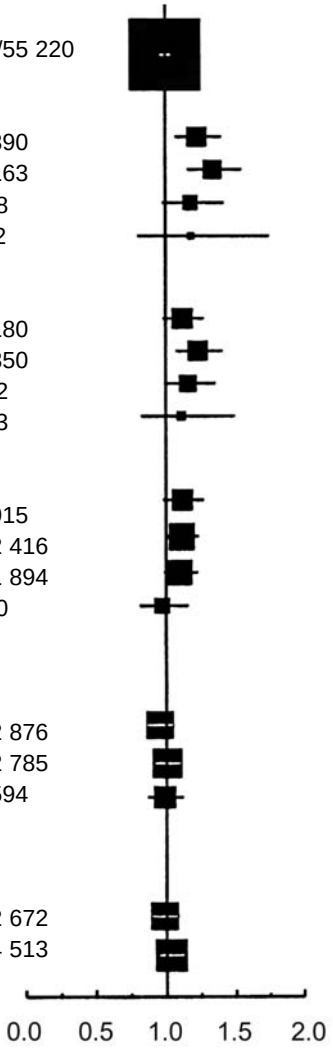


Figure 3 (contd)

(c) Relative risk for breast cancer by time since first use and time since last use of combined oral contraceptives

	RR[a] ± SD	Cases/controls
Never used	1.00 ± 0.015	28 200/55 220
Current user		
First use < 10 years previously	1.22 ± 0.058	947/2 390
10–14 years previously	1.34 ± 0.065	823/1 163
15–19 years previously	1.18 ± 0.079	461/588
≥ 20 years previously	1.18 ± 0.165	110/132
Last use 1–4 years previously		
First use < 10 years previously	1.12 ± 0.054	967/2 180
10–14 years previously	1.23 ± 0.059	869/1 350
15–19 years previously	1.16 ± 0.066	650/902
≥ 20 years previously	1.11 ± 0.121	176/243
Last use 5–9 years previously		
First use < 10 years previously	1.12 ± 0.053	941/1 915
10–14 years previously	1.11 ± 0.043	1 424/2 416
15–19 years previously	1.10 ± 0.045	1 246/1 894
≥ 20 years previously	0.97 ± 0.068	480/800
Last use 10–14 years previously		
First use < 10 years previously	Not applicable	
10–14 years previously	0.95 ± 0.038	1 433/2 876
15–19 years previously	1.01 ± 0.036	1 739/2 785
≥ 20 years previously	0.99 ± 0.049	976/1 594
Last use ≥ 15 years previously		
First use < 15 years previously	Not applicable	
15–19 years previously	0.98 ± 0.038	1 523/2 672
≥ 20 years previously	1.03 ± 0.034	2 575/4 513



Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)

Of 15 tests for heterogeneity, one within each time since last use category, two are statistically significant: age at first use in current users ($\chi^2 = 12.7$, degrees of freedom (d.f.) = 3, $p = 0.005$) and age at first use by women whose last use was 1–4 years previously ($\chi^2 = 12.6$, d.f. = 3, $p = 0.006$).

[a] Relative risk (given with 99% confidence interval) relative to no use, stratified by study, age at diagnosis, parity, and, where appropriate, the age when first child was born and age when risk for conceiving ceased.

Table 7. Use of combined oral contraceptives and risk for breast cancer risk according to age at diagnosis

Reference	Years of diagnosis	Comparison	Age at diagnosis (years)	Users		RR	95% CI
				No. of cases	No. of controls or person–years		
Vessey *et al*. (1983)	1968–80	Ever versus never	< 36	210	210	0.94	0.57–1.5
			36–40	257	257	0.86	0.56–1.3
			41–45	388	388	0.72	0.51–1.0
			46–50	321	321	1.5	1.0–2.2
Meirik *et al*. (1986)	1984–85	≥ 12 years versus never	< 45	39	23	2.2	1.2–4.0
McPherson *et al.* (1987)	1980–84	≥ 12 years versus never	< 45	21	20	1.8	0.82–3.9
			≥ 45	13	23	0.84	0.39–1.8
Ravnihar *et al*. (1988)	1980–83	Ever versus never	< 35	31	96	1.5	0.68–3.4
			35–44	84	249	1.7	1.2–2.4
			45–54	57	122	1.5	1.0–2.3
Romieu *et al*. (1989)	1976–86	Current versus never	30–34	3	8 090[a]	0.71	0.19–2.6
		Past versus never		18	51 417[a]	0.67	0.3–1.4
		Current versus never	35–39	6	6 674[a]	1.0	0.43–2.4
		Past versus never		100	114 278[a]	1.0	0.74–1.5
		Current versus never	40–44	13	4 369[a]	2.7	1.5–4.6
		Past versus never		153	119 882[a]	1.1	0.89–1.5
		Current versus never	45–49	8	2 635[a]	1.6	0.81–3.3
		Past versus never		196	91 394[a]	1.2	0.95–1.4
		Current versus never	50–54	2	777[a]	1.1	0.28–4.4
		Past versus never		133	61 657[a]	1.1	0.90–1.4
		Current versus never	55–59	0	72[a]	–	
		Past versus never		69	29 144[a]	1.0	0.80–1.4
		Current versus never	60–64	0	5[a]	–	
		Past versus never		16	5 056[a]	1.2	0.72–2.1

Table 7 (contd)

Reference	Years of diagnosis	Comparison	Age at diagnosis (years)	Users		RR	95% CI
				No. of cases	No. of controls or person–years		
Stanford *et al*. (1989)	1973–80	Ever versus never	< 40	76	92	1.0	0.5–1.9
			40–44	208	235	1.4	0.9–1.9
			45–49	385	377	1.1	0.8–1.4
			50–54	425	448	0.8	0.6–1.1
			55–59	331	366	0.99	0.6–1.5
			≥ 60	597	665	1.0	0.5–2.2
UK National Case–Control Study (1989)	1982–85	> 8 years versus never	< 35	198	143	1.74	$p_{trend} < 0.001$
Bernstein *et al*. (1990)	1972–83	Ever versus never	< 37			RR, 1.0 per year of use	
Paul *et al*. (1990)	1983–87	Ever versus never	25–34	59	370	1.2	0.44–3.4
			35–44	286	711	1.2	0.78–1.8
			45–54	340	455	1.0	0.77–1.3
WHO Collaborative Study (1990)	1979–86	Ever versus never	< 35	160	1 613	1.3	0.95–1.7
			≥ 35	560	2 814	1.1	0.98–1.3
Weinstein *et al*. (1991)	1984–86	Ever versus never	20–49	175	145	1.4	1.0–2.0
			50–70	101	95	1.1	0.79–1.5
Wingo *et al*. (1991)	1980–82	Ever versus never	20–34	425	547	1.4	1.0–2.1
			35–44	1 190	1 031	1.1	0.9–1.3
			45–54	888	991	0.9	0.8–1.0

Table 7 (contd)

Reference	Years of diagnosis	Comparison	Age at diagnosis (years)	Users		RR	95% CI
				No. of cases	No. of controls or person–years		
Ewertz (1992)	1983–84	≥ 12 years versus never	< 40	20	22	1.1	0.5–2.2
			40–59	83	67	1.3	0.82–2.0
Rosenberg *et al*. (1992)	1982–86	≥ 10 years versus never	< 40	13	27	0.8	0.3–2.5
			40–69	46	95	0.9	0.6–1.3
Tavani *et al*. (1993a)	1983–91	Ever versus never	< 60	371	265	1.2	1.0–1.4
			< 40	130	151	0.9	0.6–1.2
Rookus *et al*. (1994)	1986–89	≥ 12 years versus never	< 35	20	8	2.9	
			36–45	75	79	1.1	
			46–54	41	21	2.3	
White *et al*. (1994)	1983–90	≥ 1 year versus < 1 year	< 46	583	733	1.0	0.81–1.3
Brinton *et al*. (1995)	1990–92	≥ 6 months versus < 6 months or never	< 35	206	193	1.7	1.2–2.6
			35–39	379	336	1.4	1.0–1.8
			40–44	674	545	1.1	0.9–1.4
			45–49	203	184	1.2	0.8–1.8
			50–54	138	142	0.94	0.6–1.4
Palmer *et al*. (1995)	1977–92	≥ 3 years versus < 1 year	< 45	87	142	2.2	1.5–3.2
			45–59	27	31	1.3	0.7–2.4
Primic- akelj *et al*. (1995)	1988–90	Ever versus never	Pre-menopausal	250	249	1.0	0.80–1.4
			Post-menopausal	48	50	1.4	0.82–2.4

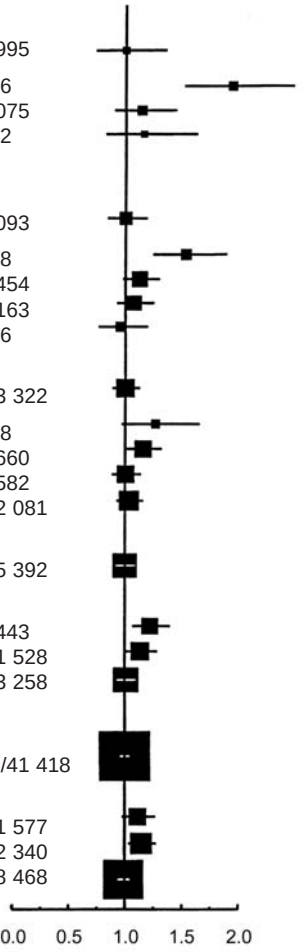
Table 7 (contd)

Reference	Years of diagnosis	Comparison	Age at diagnosis (years)	Users		RR	95% CI
				No. of cases	No. of controls or person–years		
Newcomb *et al.* (1996)	1988–91	Ever versus never	< 35	139	400	1.4	0.8–2.3
			35–44	723	1 155	1.0	0.8–1.3
			45–54	809	1 112	1.1	0.9–1.3
			≥ 55	591	780	1.0	0.9–1.2
Rosenberg *et al.* (1996)	1977–92	≥ 1 year versus < 1 year	25–34	184	422	1.7	1.3–2.3
			35–44	455	606	0.9	0.7–1.0
			45–59	389	333	1.2	1.0–1.4
Rossing *et al.* (1996)	1988–90	Ever versus never	50–64	253	226	1.1	0.8–1.4

RR, relative risk; CI, confidence interval
[a] Person-years

Figure 4. Age-specific relative risk for breast cancer by time since last use of combined oral contraceptives

Age at diagnosis of breast cancer and duration of use of combined oral contraceptives	RR[a] ± SD	Cases/ controls
Age < 30 at diagnosis		
never user	1.00 ± 0.118	290/2 995
< 5 years since last use; < 20 at first use	1.95 ± 0.134	348/916
< 5 years since last use; ≥ 20 at first use	1.14 ± 0.098	254/1 075
5–9 years since last use	1.16 ± 0.143	134/412
≥ 10 years since last use	insufficient data	18/48
Age 30–34 at diagnosis		
never user	1.00 ± 0.067	690/2 093
< 5 years since last use; < 20 at first use	1.54 ± 0.101	437/498
< 5 years since last use; ≥ 20 at first use	1.13 ± 0.058	745/1 454
5–9 years since last use	1.08 ± 0.060	629/1 163
≥ 10 years since last use	0.96 ± 0.085	293/586
Age 35–39 at diagnosis		
never user	1.00 ± 0.047	1 459/3 322
< 5 years since last use; < 20 at first use	1.27 ± 0.116	237/278
< 5 years since last use; ≥ 20 at first use	1.16 ± 0.055	965/1 660
5–9 years since last use	1.00 ± 0.049	899/1 582
≥ 10 years since last use	1.03 ± 0.044	1 286/2 081
Age 40–44 at diagnosis		
never user	1.00 ± 0.035	2 958/5 392
< 5 years since last use; < 20 at first use	insufficient data	45/43
< 5 years since last use; ≥ 20 at first use	1.22 ± 0.057	942/1 443
5–9 years since last use	1.13 ± 0.051	1 024/1 528
≥ 10 years since last use	1.01 ± 0.034	2 222/3 258
Age > 45 at diagnosis		
never user	1.00 ± 0.017	22 803/41 418
< 5 years since last use; < 20 at first use	insufficient data	1/4
< 5 years since last use; ≥ 20 at first use	1.11 ± 0.053	1 029/1 577
5–9 years since last use	1.15 ± 0.043	1 405/2 340
≥ 10 years since last use	0.99 ± 0.021	4 427/8 468



Test for trend with age at diagnosis in women with:
last use < 5 years ago, age at first use < 20: χ^2 (1 d.f.) = 5.2; p = 0.02
last use < 5 years ago, age at first use ≥ 20: χ^2 (1 d.f.) = 0.0; NS
last use 5–9 years ago: χ^2 (1 d.f.) = 1.2; NS
last use ≥ 10 years ago: χ^2 (1 d.f.) = 0.1; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
d.f., degree of freedom
[a] Relative risk (given with 99% confidence interval) relative to no use, stratified by study, age at diagnosis, parity and, where appropriate, the age when her first child was born and the age when her risk for conceiving ceased.

Table 8. Risk for breast cancer in relation to time since last use (recency of use) of combined oral contraceptives

Reference	Years of case diagnosis	Time since last use (years)	Age (years)	Users (cases/ controls)	RR (95% CI)
Vessey *et al*. (1983)	1968–80	≤ 1	16–50	58/69	0.99 (0.76–1.3)
		> 1–≤ 4		122/119	0.95 (0.7–1.3)
		> 4–≤ 8		125/101	1.3 (0.98–1.8)
		> 8		90/136	0.67 (0.48–0.94)
Meirik *et al*. (1986)	1984–85	Current use	< 45	80/80	1.5 (0.8–2.8)
		1–2		30/25	1.8 (0.9–3.7)
		3–5		45/35	1.9 (1.0–3.3)
		6–8		45/49	1.4 (0.8–2.4)
		9–11		36/55	1.0 (0.6–1.7)
		≥ 12		90/127	0.9 (0.6–1.4)
Rohan & McMichael (1988)	1982–84	≤ 8	20–69	81/71	1.2 (0.7–2.2)
		9–14		51/62	0.87 (0.50–1.5)
		≥ 15		55/52	1.1 (0.62–1.9)
Stanford *et al*. (1989)	1973–80	Current use	All	47/57	0.81 (0.5–1.2)
		1–3		93/96	1.0 (0.7–1.4)
		4–6		102/109	1.0 (0.8–1.4)
		≥ 7		221/251	0.96 (0.8–1.2)
Romieu *et al*. (1989)	1976–86	Current use	30–64	32/22 622[a]	1.6 (1.1–2.2)
		< 1		205/129 638[a]	1.1 (0.97–1.3)
		1–2		156/123 636[a]	1.1 (0.89–1.3)
		3–4		86/72 837[a]	0.97 (0.78–1.2)
		5–9		159/104 277[a]	1.1 (0.96–1.4)
		10–14		57/33 206[a]	1.1 (0.83–1.4)
		≥ 15		6/3 195[a]	1.1 (0.47–2.4)
WHO Collaborative Study (1990)	1979–86	Current use	< 62	127/747	1.7 (1.3–2.1)
		4–35 months		120/751	1.4 (1.1–1.8)
		3–9		234/1 374	1.2 (0.98–1.4)
		> 9		213/1 388	0.91 (0.77–1.1)
Clavel *et al*. (1991)	1983–87	Current use	20–55	41/45	1.4 (0.9–2.4)
		< 5		75/80	1.6 (1.0–2.5)
		5–9		66/56	1.6 (1.0–2.4)
		≥ 10		55/55	1.5 (0.9–2.3)

Table 8 (contd)

Reference	Years of case diagnosis	Time since last use (years)	Age (years)	Users (cases/ controls)	RR (95% CI)
Wingo *et al*. (1991)	1980–82	< 1 1–< 2 2–3 4–5 6–7 8–9 10–11 12–13 14–15 16–17 18–19 ≥ 20	20–34	Not given	1.7 (1.1–2.6) 1.1 (0.6–2.1) 1.2 (0.7–1.9) 1.8 (1.1–3.0) 1.5 (0.9–2.5) 1.5 (0.9–2.6) 1.3 (0.7–2.4) 1.0 (0.5–1.8) p_{trend} = 0.5
			35–44	Not given	1.2 (0.8–1.8) 1.2 (0.7–2.3) 1.5 (1.0–2.2) 1.2 (0.9–1.6) 1.1 (0.8–1.5) 1.0 (0.7–1.3) 1.0 (0.7–1.3) 0.9 (0.7–1.2) 1.0 (0.7–1.3) 1.2 (0.8–1.7) 0.9 (0.5–1.5) 0.6 (0.3–1.5) p_{trend} < 0.01
			45–54	Not given	0.8 (0.4–1.5) 0.8 (0.3–2.2) 1.0 (0.7–1.5) 1.1 (0.8–1.4) 1.0 (0.8–1.3) 1.3 (0.9–1.7) 0.9 (0.7–1.2) 0.8 (0.6–1.1) 0.8 (0.6–1.1) 0.6 (0.5–0.8) 1.0 (0.7–1.5) 0.6 (0.4–0.8) p_{trend} < 0.01

Table 8 (contd)

Reference	Years of case diagnosis	Time since last use (years)	Age (years)	Users (cases/ controls)	RR (95% CI)
Ewertz (1992)	1983–84	< 5	< 40	56/65	1.0 (0.59–1.8)
		5–9		46/37	1.5 (0.81–2.8)
		≥ 10		59/58	1.2 (0.68–2.1)
			40–59	118/92	Reference
				87/70	0.97 (0.64–1.5)
				90/121	0.58 (0.39–0.85)
Tavani *et al*. (1993a)	1983–91	<5	< 60	97/82	1.3 (1.0–1.9)
		5–9		105/75	1.1 (0.8–1.6)
		≥ 10		166/103	1.2 (0.9–1.5)
Rookus *et al*. (1994)	1986–89	< 3	46–54	Not given	1.9 (0.9–4.1)
White *et al*. (1994)	1983–90	Current use	21–45	59/88	1.3 (0.83–1.9)
		< 5		102/131	1.3 (0.91–1.8)
		5–9		135/171	1.0 (0.75–1.4)
		10–14		171/226	0.88 (0.65–1.2)
		≥ 15		116/111	0.96 (0.67–1.4)
Brinton *et al*. (1995)	1990–92	< 5	20–34	135/Not given	2.0 (1.3–3.1)
		5–9		40/Not given	1.5 (0.8–2.6)
		≥ 10		31/Not given	1.2 (0.6–2.2)
			35–39	106/Not given	1.5 (0.9–2.2)
				72/Not given	1.3 (0.9–2.0)
				201/Not given	1.3 (0.9–1.9)
			40–44	57/Not given	1.2 (0.8–2.0)
				91/Not given	1.2 (0.8–1.7)
				526/Not given	1.1 (0.9–1.4)
La Vecchia *et al.* (1995)	1991–94	< 10	< 35	Not given	1.4 (0.7–2.7)
		≥ 10		Not given	1.6 (0.4–6.0)
			35–44	Not given	1.9 (1.2–2.9)
				Not given	1.3 (0.9–2.0)
			45–64	Not given	1.3 (0.8–2.3)
				Not given	1.1 (0.8–1.4)

Table 8 (contd)

Reference	Years of case diagnosis	Time since last use (years)	Age (years)	Users (cases/ controls)	RR (95% CI)
Palmer *et al*. (1995)	1977–92	< 2	25–59 (women with ≥ 3 years of use)	19/29	3.1 (1.5–6.3)
		2–4		6/28	0.9 (0.3–2.4)
		5–9		26/37	2.5 (1.4–4.5)
		10–14		14/35	1.0 (0.5–2.1)
		≥ 15		14/7	4.7 (1.7–13)
Paul *et al*. (1995)	1983–87	< 1	25–34	18/147	1.3 (0.42–4.1)
		1–4		17/85	1.9 (0.61–6.1)
		5–9		20/96	1.4 (0.46–4.3)
		≥ 10		4/42	0.36 (0.08–1.6)
			35–44	31/76	1.2 (0.65–2.1)
				40/93	1.4 (0.82–2.5)
				65/188	1.1 (0.65–1.7)
				150/354	1.2 (0.78–1.9)
			45–54	21/20	1.5 (0.78–2.9)
				30/43	0.90 (0.53–1.5)
				63/78	1.0 (0.69–1.6)
				226/314	0.99 (0.74–1.3)
Primic- akelj *et al*. (1995)	1988–90	< 6 months	25–54	32/16	2.3 (1.2–4.5)
		7 months–5 years		43/38	1.3 (0.78–2.1)
		6–10		54/68	0.89 (0.57–1.4)
		11–15		94/102	1.0 (0.71–1.4)
		> 15		75/75	1.1 (0.76–1.7)
Levi *et al*. (1996)	1990–95	< 5	< 70	22/40	1.9 (0.9–3.6)
		5–14		33/40	2.4 (1.4–4.4)
		≥ 15		22/54	1.0 (0.6–1.8)
Newcomb *et al*. (1996)	1988–91	< 2	< 35	30/109	1.3 (0.6–2.6)
		2–4		25/64	1.9 (0.9–3.8)
		5–9		47/127	1.5 (0.8–2.7)
		≥ 10		37/100	1.1 (0.6–2.2)
			35–44	26/21	2.0 (1.1–3.9)
				19/40	0.7 (0.4–1.3)
				108/164	1.1 (0.8–1.5)
				570/930	1.0 (0.8–2.1)

Table 8 (contd)

Reference	Years of case diagnosis	Time since last use (years)	Age (years)	Users (cases/ controls)	RR (95% CI)
Newcomb *et al.* (1996) (contd)			45–54	8/8	1.4 (0.5–4.0)
				10/12	1.3 (0.5–3.1)
				45/66	0.9 (0.6–1.4)
				746/1 026	1.1 (0.9–1.3)
		< 5	55–74	11/7	2.2 (0.8–5.7)
		5–9		24/41	0.8 (0.5–1.4)
		≥ 10		556/732	1.0 (0.9–1.2)
Rosenberg *et al*. (1996)	1977–92	< 3	25–34	80/184	1.9 (1.3–2.8)
		3–4		18/57	1.8 (0.9–3.3)
		5–9		53/112	1.6 (1.0–2.4)
		10–14		22/48	1.3 (0.7–2.3)
		≥ 15		0/3	–
			35–44	36/94	0.7 (0.5–1.2)
				25/50	0.8 (0.4–1.4)
				92/176	0.8 (0.5–1.0)
				157/159	1.1 (0.8–1.5)
				124/99	0.8 (0.6–1.2)
			45–54	16/29	0.9 (0.5–1.9)
				15/17	1.7 (0.8–3.8)
				70/80	1.0 (0.7–1.5)
				110/79	1.4 (1.0–1.9)
				159/106	1.2 (0.9–1.6)
Rossing *et al.* (1996)	1988–90	≤ 10	50–64	29/24	1.1 (0.6–2.0)
		11–15		57/43	1.4 (0.9–2.1)
		16–20		65/57	1.1 (0.7–1.7)
		21–25		57/59	0.9 (0.6–1.4)
		≥ 26		43/42	0.9 (0.6–1.5)

RR, relative risk; CI, confidence interval

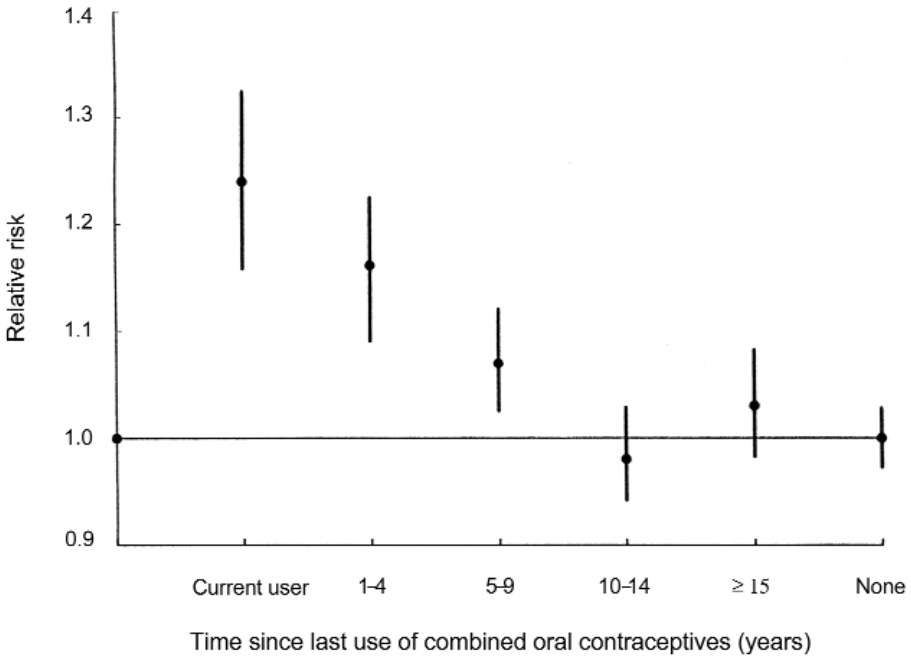
[a] Person–years

(Meirik *et al*., 1986; Rohan & McMichael, 1988; Romieu *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Wingo *et al*., 1991; White *et al*., 1994; Brinton *et al*., 1995; Paul *et al*., 1995; Primic- akelj *et al*., 1995; La Vecchia *et al*., 1995; Levi *et al*., 1996; Newcomb *et al*., 1996; Rosenberg *et al*., 1996). In the study of La Vecchia *et al.* (1995), the increase was greater for women with longer use. Another consistent finding is that there is little or no increase in risk, or possibly even a decrease, among women who last used combined oral contraceptives at least 10 years previously (Meirik *et al*., 1986; Rohan & McMichael, 1988; Romieu *et al*., 1989; Stanford *et al*., 1989; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Wingo *et al*., 1991; White *et al*., 1994; Brinton *et al*., 1995; Paul *et al*., 1995; Primic- akelj *et al*., 1995; Levi *et al*., 1996; Newcomb *et al*., 1996; Rosenberg *et al*., 1996; Rossing *et al*., 1996); however, there was little or no variation in risk with recency of use in the studies of Vessey *et al*. (1983), Stanford *et al*. (1989), Ewertz (1992), Tavani *et al*. (1993a) or Rossing *et al*. (1996). The studies of Clavel *et al*. (1991) and Palmer *et al*. (1995) showed increased risks for users of these oral contraceptives that appeared to be unrelated to the recency of use. The estimated relative risk for breast cancer overall in the collaborative reanalysis was 1.24 (95% CI, 1.17–1.3) for current users, 1.16 (95% CI, 1.1–1.22) for users 1–4 years after stopping, 1.07 (95% CI, 1.0–1.12) for users 5–9 years after stopping and 1.0 (95% CI, 0.96–1.1) for users 10 or more years after stopping (Collaborative Group on Hormonal Factors in Breast Cancer, 1996b; Figure 5).

The relationship between recency of use and the risk for breast cancer at different ages was not assessed in many studies. Among those in which it was, that of Rookus *et al*. (1994) showed an increased risk associated with recent use for older but not younger women. In the studies of Wingo *et al*. (1991), Brinton *et al*. (1995), Paul *et al*. (1995) and Rosenberg *et al*. (1996), the increase in risk for recent users was most apparent in women under 35 years of age; in the study of La Vecchia *et al.* (1995), the increase for recent users was greatest among women aged 35–44; in the Nurses' Health Study (Romieu *et al*., 1989), the point estimates of relative risk were increased for current users aged 40–45 and 45–49 and not younger users, but there were very few current users in any age group. In the study of Newcomb *et al*. (1996), the point estimates of relative risk were elevated for recent users in every age group, from < 35 through 55 and older (see Figure 4).

Data on the duration of use have been inconsistent, some studies suggesting increasing risk with increasing duration of use overall, before a first pregnancy or after starting at a young age. Long use is highly correlated with recent use, and it has been difficult to disentangle their effects. In studies in which recency of use was taken into account, there has been no clear trend for an increased risk with increasing duration (Romieu *et al*., 1989; Paul *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1990; Wingo *et al*., 1991; Palmer *et al*., 1995; Primic- akelj *et al*., 1995; Newcomb *et al*., 1996; Rosenberg *et al*., 1996). It is too early, however, to rule out a greater increase in risk for recent users who have used combined oral contraceptives for a very long time beginning at young ages, because the data on this issue are sparse (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a,b; Figure 3).

Figure 5. Relative risk for breast cancer by time since last use of combined oral contraceptives



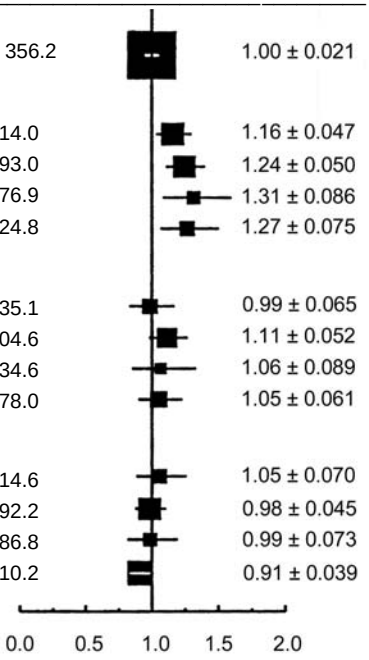
From Collaborative Group on Hormonal Factors in Breast Cancer (1996b)
Relative risk (given with 95% confidence interval (CI)) relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

It has also been suggested that the risk for breast cancer associated with use of combined oral contraceptives varies according to the constituents of the formulation, e.g. that preparations with 'high potency' progestogens (as defined by their effect on the uterus) are the most harmful (Pike *et al.*, 1983). It has been difficult to study individual formulations because there are many of them, there are relatively few users of any particular one, and women tend to use several over the course of their reproductive lives. Little support for a differential effect according to the type of oestrogen or progestogen or the dose is provided in most studies (Vessey *et al.*, 1983; Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development, 1986; Ravnihar *et al.*, 1988; UK National Case–Control Study Group, 1989; Clavel *et al.*, 1991; Ebeling *et al.*, 1991; Thomas *et al.*, 1992; Rookus *et al.*, 1994; Collaborative Group on Hormonal Factors in Breast Cancer, 1996a; Rosenberg *et al.*, 1996), although others indicate an effect (McPherson *et al.*, 1987; Ewertz, 1992; White *et al.*, 1994). Data from the Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b) (Figures 6–10) show that there is little variation according to type or dose of oral contraceptive.

Figure 6. Relative risk (RR) for breast cancer by time since last use and oestrogen and progestogen type and dose of combined oral contraceptives last used

(a) Oestrogen type and dose

Time since last use	Cases/controls	InRR	1	Relative risk[a]	
		var(InRR)	var(InRR)	RR (99% CI)	RR ± SD
Never	15 715/29 503	0.0	2 356.2		1.00 ± 0.021
Last use < 5 years previously					
ethinyloestradiol < 50 µg	1 494/2 217	74.7	514.0		1.16 ± 0.047
ethinyloestradiol = 50 µg	1 203/2 543	107.4	493.0		1.24 ± 0.050
mestranol = 50 µg	427/1 152	48.4	176.9		1.31 ± 0.086
mestranol > 50 µg	568/780	52.9	224.8		1.27 ± 0.075
Last use 5–9 years previously					
ethinyloestradiol < 50 µg	554/796	–3.5	235.1		0.99 ± 0.065
ethinyloestradiol = 50 µg	954/1 699	43.5	404.6		1.11 ± 0.052
mestranol = 50 µg	302/673	8.4	134.6		1.06 ± 0.089
mestranol > 50 µg	676/893	13.7	278.0		1.05 ± 0.061
Last use ≥ 10 years previously					
ethinyloestradiol < 50 µg	555/637	11.4	214.6		1.05 ± 0.070
ethinyloestradiol = 50 µg	1 247/2 124	–8.1	492.2		0.98 ± 0.045
mestranol = 50 µg	423/822	–2.4	186.8		0.99 ± 0.073
mestranol > 50 µg	1 548/2 073	–56.8	610.2		0.91 ± 0.039

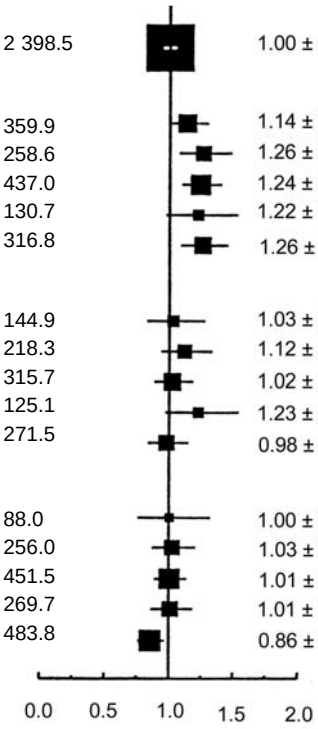


Trend for heterogeneity by type and dose of oestrogen in women with:
Last use < 5 years previously: χ^2 (3 d.f.) = 2.9; NS
Last use 5–9 years previously: χ^2 (3 d.f.) = 2.3; NS
Last use ≥ 10 years previously: χ^2 (3 d.f.) = 4.0; NS

Figure 6 (contd)

(b) Progestogen type and dose

Time since last use	Cases/controls	lnRR var(lnRR)	1 var(lnRR)	Relative risk[a] RR (99% CI)	RR ± SD
Never	15 715/29 503	0.0	2 398.5		1.00 ± 0.020
Last use < 5 years previously					
levonorgestrel < 250 mg	931/1 613	45.6	359.9		1.14 ± 0.056
levonorgestrel > 250 mg	622/1 358	60.2	258.6		1.26 ± 0.070
norethisterone < 1000 mg	1 070/2 075	94.5	437.0		1.24 ± 0.053
norethisterone > 1000 mg	310/457	26.3	130.7		1.22 ± 0.097
other	775/1 213	73.1	316.8		1.26 ± 0.063
Last use 5–9 years previously					
levonorgestrel < 250 mg	331/527	4.3	144.9		1.03 ± 0.084
levonorgestrel > 250 mg	509/956	24.4	218.3		1.12 ± 0.072
norethisterone < 1000 mg	726/1 242	7.6	315.7		1.02 ± 0.057
norethisterone > 1000 mg	324/401	25.4	125.1		1.23 ± 0.099
other	611/975	–5.4	271.5		0.98 ± 0.060
Last use ≥ 10 years previously					
levonorgestrel < 250 mg	212/274	0.4	88.0		1.00 ± 0.107
levonorgestrel > 250 mg	633/1 095	6.6	256.0		1.03 ± 0.063
norethisterone < 1000 mg	1 157/1 683	2.3	451.5		1.01 ± 0.047
norethisterone > 1000 mg	716/960	3.0	269.7		1.01 ± 0.061
other	1 080/1 692	–74.0	483.8		0.86 ± 0.042



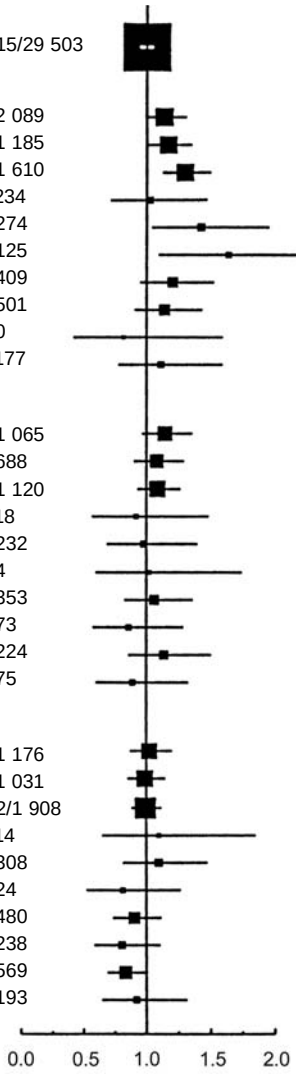
Trend for heterogeneity by type and dose of progestogen in women with:
Last use < 5 years previously: χ^2 (4 d.f.) = 2.6; NS
Last use 5–9 years previously: χ^2 (4 d.f.) = 5.4; NS
Last use ≥ 10 years previously: χ^2 (4 d.f.) = 9.1; p = 0.003

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
d.f., degrees of freedom; NS, not significant; CI, confidence interval; SD, standard deviation
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

Figure 7. Relative risk (RR) for breast cancer by time since last use and type of oestrogen and progestogen in the oral contraceptive

(a) First used

Oestrogen and progestogen	Cases/controls	Relative risk[a] RR & 99% CI	RR ± SD
Never	15 715/29 503		1.00 ± 0.020
Last use < 5 years previously			
ethinyloestradiol and norgestrel	882/2 089		1.14 ± 0.058
ethinyloestradiol and norethisterone	782/1 185		1.17 ± 0.061
mestranol and norethisterone	839/1 610		1.30 ± 0.064
ethinyloestradiol and lynoestrenol	112/234		1.03 ± 0.141
mestranol and lynoestrenol	171/274		1.42 ± 0.146
ethinyloestradiol and ethynodiol	109/125		1.64 ± 0.202
mestranol and ethynodiol	280/409		1.20 ± 0.101
mestranol and chlormadinone or norethynodrel	308/501		1.14 ± 0.095
ethinyloestradiol and desogestrel or gestodene	32/50		0.82 ± 0.234
other	117/177		1.11 ± 0.147
Last use 5–9 years previously			
ethinyloestradiol and norgestrel	540/1 065		1.14 ± 0.070
ethinyloestradiol and norethisterone	499/688		1.08 ± 0.072
mestranol and norethisterone	697/1 120		1.08 ± 0.062
ethinyloestradiol and lynoestrenol	64/118		0.92 ± 0.178
mestranol and lynoestrenol	125/232		0.98 ± 0.136
ethinyloestradiol and ethynodiol	49/84		1.02 ± 0.209
mestranol and ethynodiol	245/353		1.06 ± 0.099
mestranol and chlormadinone acetate	76/173		0.86 ± 0.145
mestranol and norethynodrel	212/224		1.14 ± 0.116
other	90/175		0.89 ± 0.145
Last use ≥ 10 years previously			
ethinyloestradiol and norgestrel	658/1 176		1.02 ± 0.062
ethinyloestradiol and norethisterone	745/1 031		0.99 ± 0.057
mestranol and norethisterone	1 372/1 908		0.99 ± 0.045
ethinyloestradiol and lynoestrenol	55/114		1.10 ± 0.213
mestranol and lynoestrenol	210/308		1.10 ± 0.121
ethinyloestradiol and ethynodiol	69/124		0.81 ± 0.154
mestranol and ethynodiol	338/480		0.91 ± 0.077
mestranol and chlormadinone acetate	128/238		0.81 ± 0.111
mestranol and norethynodrel	430/569		0.84 ± 0.067
other	123/193		0.92 ± 0.132

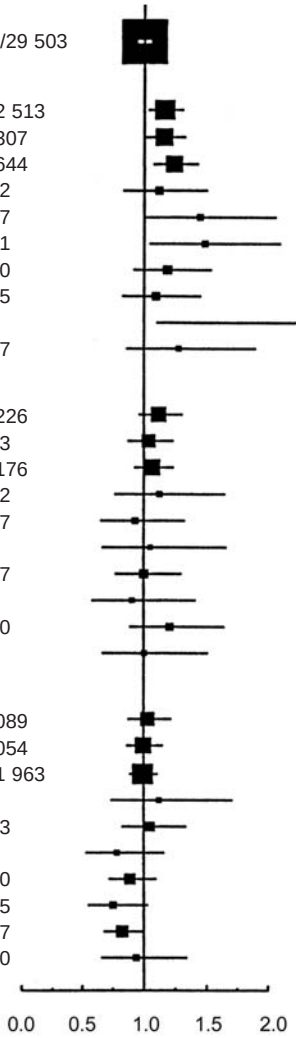


Test for heterogeneity by type of oral contraceptive in women with:
Last use < 5 years previously: χ^2 (9 d.f.) = 13.3; NS
Last use 5–9 years previously: χ^2 (9 d.f.) = 6.0; NS
Last use ≥ 10 years previously: χ^2 (9 d.f.) = 10.8; NS

Figure 7 (contd)

(b) Most used

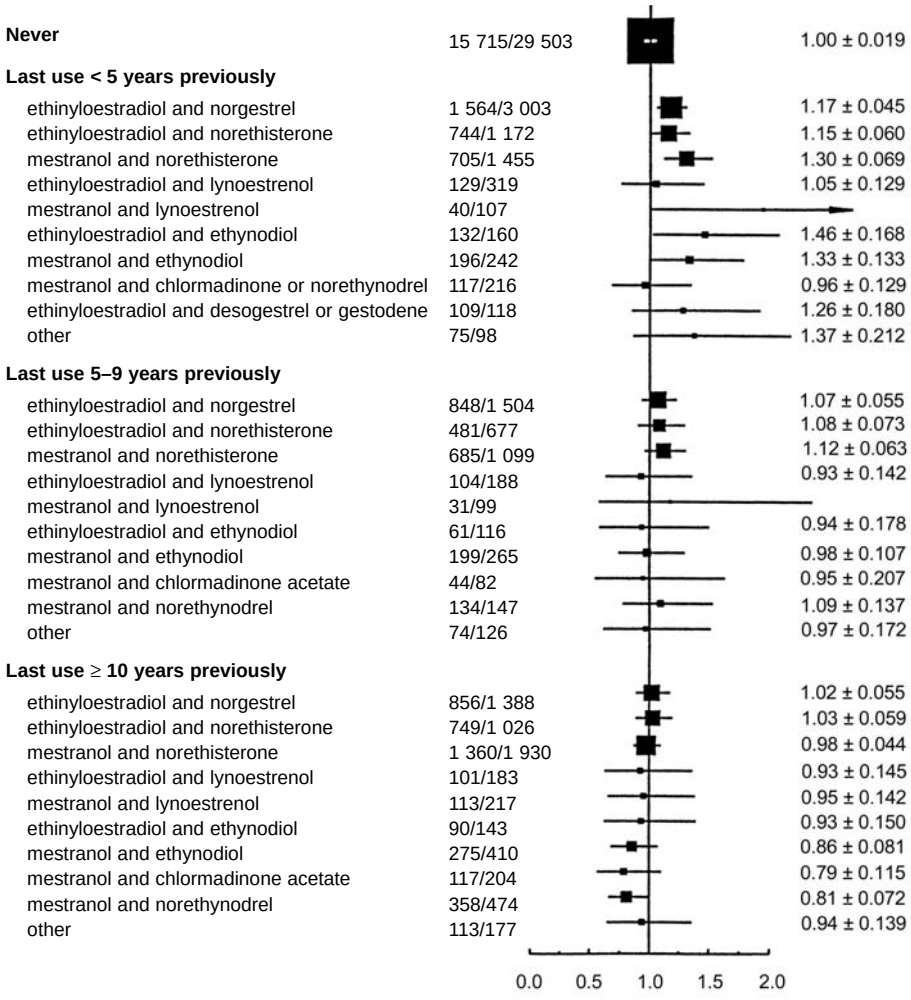
Oestrogen and progestogen	Cases/controls	Relative risk[a] RR & 99% CI	RR ± SD
Never	15 715/29 503		1.00 ± 0.021
Last use < 5 years previously			
ethinyloestradiol and norgestrel	1 168/2 513		1.16 ± 0.051
ethinyloestradiol and norethisterone	835/1 307		1.16 ± 0.058
mestranol and norethisterone	807/1 644		1.24 ± 0.063
ethinyloestradiol and lynoestrenol	163/332		1.12 ± 0.122
mestranol and lynoestrenol	149/217		1.45 ± 0.168
ethinyloestradiol and ethynodiol	137/161		1.49 ± 0.169
mestranol and ethynodiol	241/320		1.19 ± 0.110
mestranol and chlormadinone or norethynodrel	181/305		1.10 ± 0.115
ethinyloestradiol and desogestrel or gestodene	30/22		
other	101/147		1.28 ± 0.175
Last use 5–9 years previously			
ethinyloestradiol and norgestrel	631/1 226		1.12 ± 0.064
ethinyloestradiol and norethisterone	506/723		1.04 ± 0.070
mestranol and norethisterone	729/1 176		1.07 ± 0.060
ethinyloestradiol and lynoestrenol	108/172		1.13 ± 0.159
mestranol and lynoestrenol	123/217		0.93 ± 0.134
ethinyloestradiol and ethynodiol	67/118		1.05 ± 0.183
mestranol and ethynodiol	224/307		1.00 ± 0.102
mestranol and chlormadinone acetate	66/137		0.91 ± 0.165
mestranol and norethynodrel	168/170		1.21 ± 0.133
other	85/162		1.00 ± 0.160
Last use ≥ 10 years previously			
ethinyloestradiol and norgestrel	553/1 089		1.03 ± 0.066
ethinyloestradiol and norethisterone	764/1 054		1.00 ± 0.057
mestranol and norethisterone	1 425/1 963		0.99 ± 0.044
ethinyloestradiol and lynoestrenol	83/148		1.12 ± 0.174
mestranol and lynoestrenol	335/443		1.05 ± 0.097
ethinyloestradiol and ethynodiol	82/153		0.78 ± 0.136
mestranol and ethynodiol	316/460		0.89 ± 0.078
mestranol and chlormadinone acetate	126/235		0.75 ± 0.107
mestranol and norethynodrel	398/527		0.83 ± 0.069
other	120/190		0.94 ± 0.134



Test for heterogeneity by type of oral contraceptive in women with:
Last use < 5 years previously: χ^2 (9 d.f.) = 12.0; NS
Last use 5–9 years previously: χ^2 (9 d.f.) = 4.4; NS
Last use ≥ 10 years previously: χ^2 (9 d.f.) = 14.4; NS

Figure 7 (contd)

(c) Last used

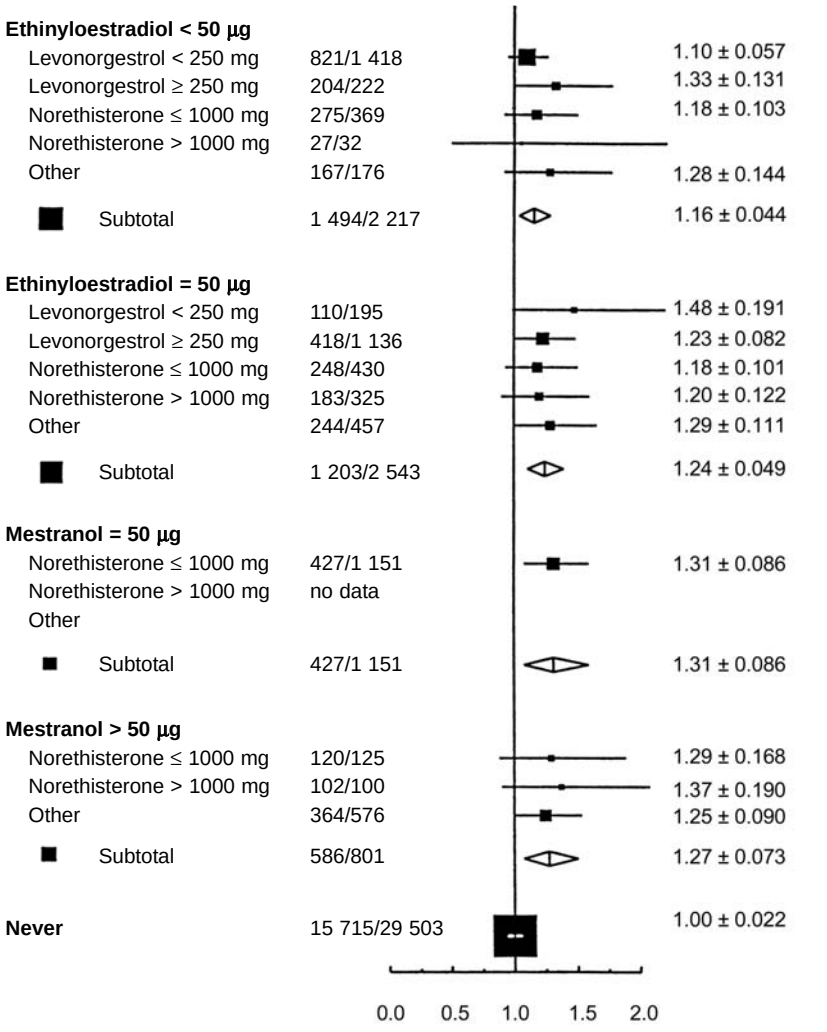


Test for heterogeneity by type of oral contraceptive in women with:
Last use < 5 years previously: χ^2 (9 d.f.) = 13.6; NS
Last use 5–9 years previously: χ^2 (9 d.f.) = 3.4; NS
Last use ≥ 10 years previously: χ^2 (9 d.f.) = 11.5; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
CI, confidence interval; SD, standard deviation; d.f., degrees of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

Figure 8. Relative risk (RR) for breast cancer by type and dose of progestogen in the oral contraceptive last used, grouped according to type and dose of oestrogen

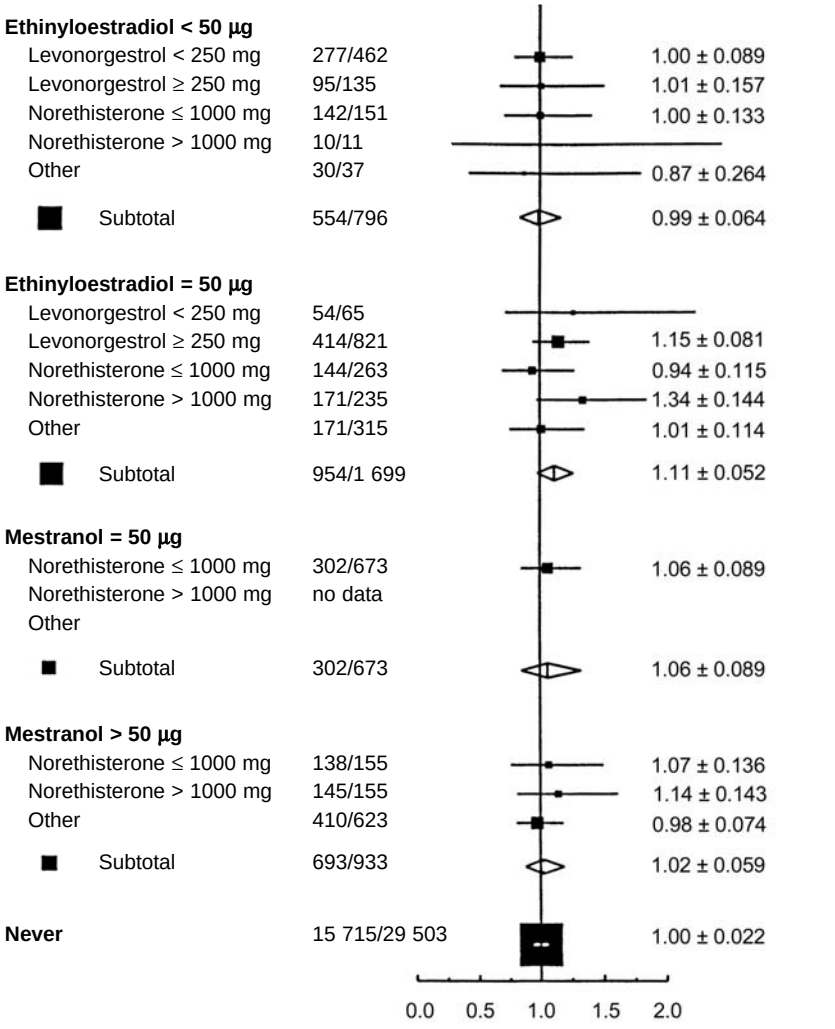
(a) Last use < 5 years previously



Test for heterogeneity by type and dose of progestogen within oral contraceptives containing:
Ethinyloestradiol < 50 µg : χ^2 (4 d.f.) = 3.2; NS
Ethinyloestradiol = 50 µg : χ^2 (4 d.f.) = 1.7; NS
Mestranol > 50 µg : χ^2 (2 d.f.) = 0.3; NS

Figure 8 (contd)

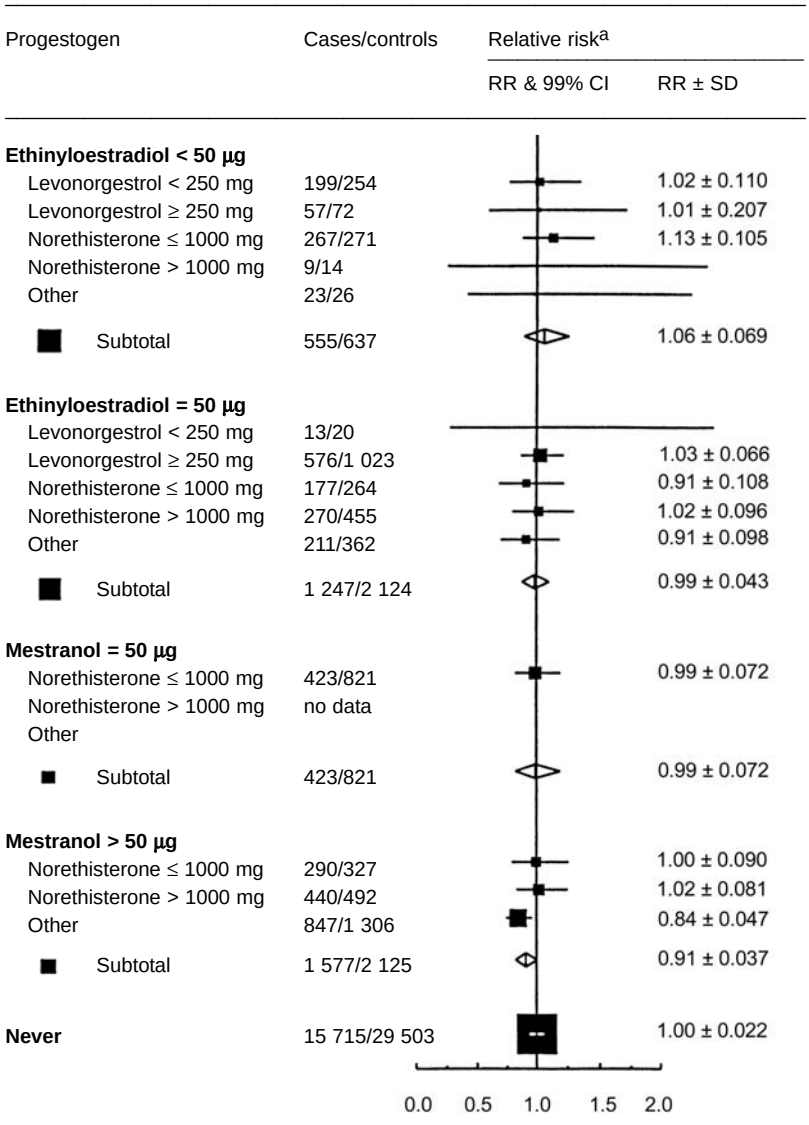
(b) Last use 5–9 years previously



Test for heterogeneity by type and dose of progestogen within oral contraceptives containing:
Ethinyloestradiol < 50 µg : χ^2 (4 d.f.) = 0.9; NS
Ethinyloestradiol = 50 µg : χ^2 (4 d.f.) = 5.6; NS
Mestranol > 50 µg : χ^2 (2 d.f.) = 1.2; NS

Figure 8 (contd)

(c) Last use ≥ 10 years previously

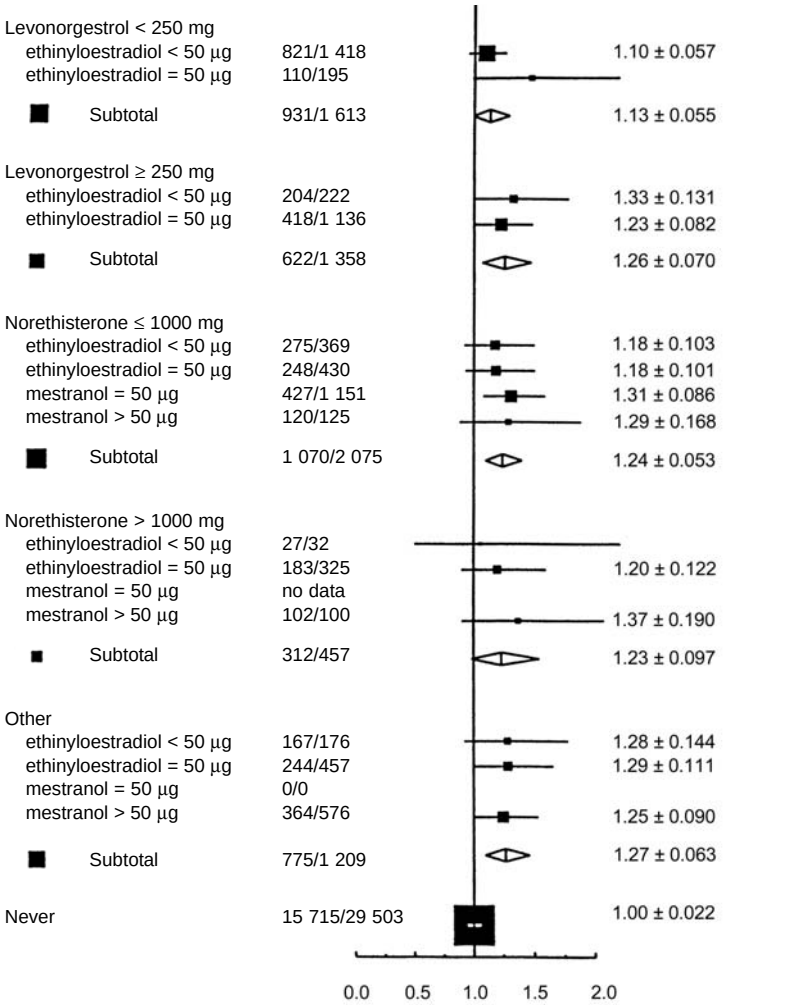


Test for heterogeneity by type and dose of progestogen within oral contraceptives containing:	
Ethinyloestradiol < 50 µg	: χ^2 (4 d.f.) = 1.2; NS
Ethinyloestradiol = 50 µg	: χ^2 (4 d.f.) = 1.8; NS
Mestranol > 50 µg	: χ^2 (2 d.f.) = 5.2; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
CI, confidence interval; SD, standard deviation; d.f., degrees of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

Figure 9. Relative risk (RR) for breast cancer by type and dose of oestrogen in the oral contraceptive last used, grouped according to type and dose of progestogen

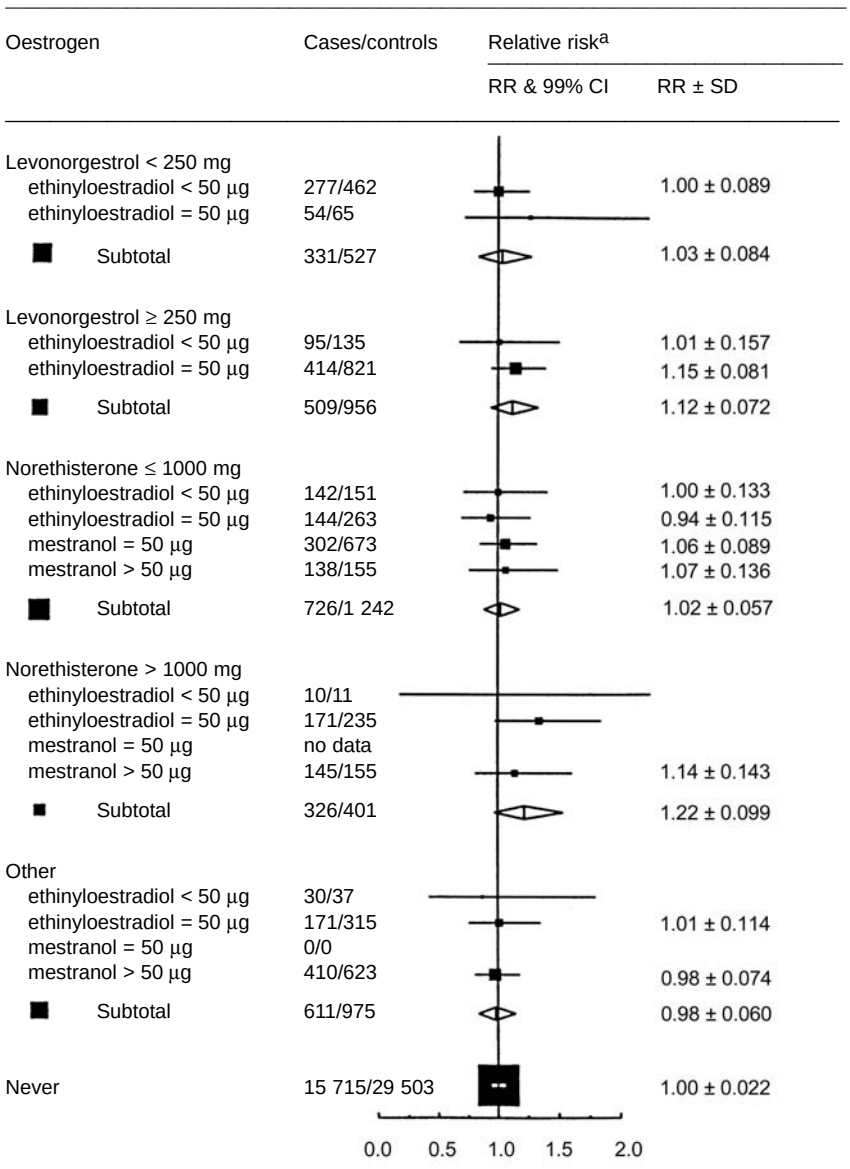
(a) Last use < 5 years previously



Test for heterogeneity by type and dose of oestrogen within oral contraceptives containing:	
Levonorgestrol < 250 mg	: χ^2 (1 d.f.) = 3.2; NS
Levonorgestrol > 250 mg	: χ^2 (1 d.f.) = 0.3; NS
Norethisterone < 1000 mg	: χ^2 (3 d.f.) = 1.2; NS
Norethisterone > 1000 mg	: χ^2 (2 d.f.) = 0.8; NS
Other	: χ^2 (2 d.f.) = 0.1; NS

Figure 9 (contd)

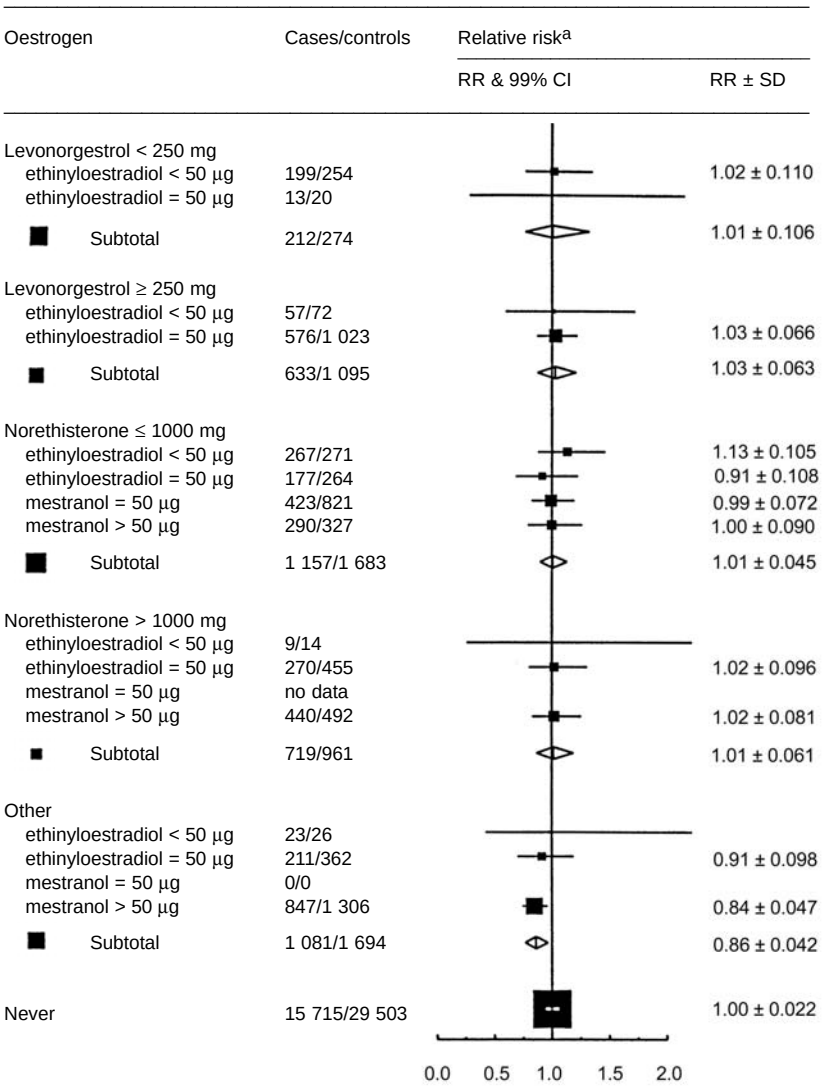
(b) Last use 5–9 years previously



Test for heterogeneity by type and dose of oestrogen within oral contraceptives containing:	
Levonorgestrol < 250 mg	: χ^2 (1 d.f.) = 1.0; NS
Levonorgestrol > 250 mg	: χ^2 (1 d.f.) = 0.5; NS
Norethisterone < 1000 mg	: χ^2 (3 d.f.) = 0.8; NS
Norethisterone > 1000 mg	: χ^2 (2 d.f.) = 2.3; NS
Other	: χ^2 (2 d.f.) = 0.2; NS

Figure 9 (contd)

(c) Last use ≥ 10 years previously

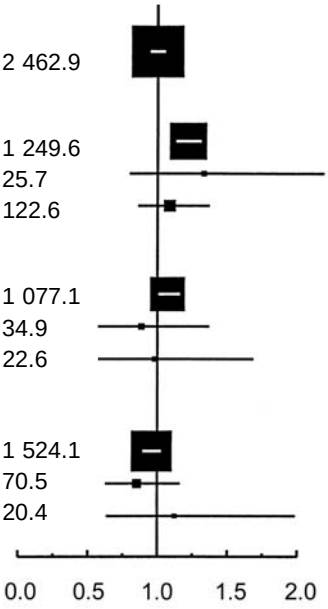


Test for heterogeneity by type and dose of oestrogen within oral contraceptives containing:	
Levonorgestrol < 250 mg	: χ^2 (1 d.f.) = 0.2; NS
Levonorgestrol > 250 mg	: χ^2 (1 d.f.) = 0.0; NS
Norethisterone < 1000 mg	: χ^2 (3 d.f.) = 2.2; NS
Norethisterone > 1000 mg	: χ^2 (2 d.f.) = 0.4; NS
Other	: χ^2 (2 d.f.) = 0.6; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
CI, confidence interval; SD, standard deviation; d.f., degrees of freedom; NS, not significant
[a] Relative to no use, stratifed by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

Figure 10. Relative risk (RR) for breast cancer by time since last use and type of combined oral contraceptive last used

Type of oral contraceptive	Cases/controls	InRR var(InRR)	1 var(InRR)	Relative risk[a] RR & 99% CI	RR ± SD
Never	15 715/29 503	0.0	2 462.9		1.00 ± 0.020
Last use < 5 years previously					
Standard	3 467/6 423	247.7	1 249.6		1.22 ± 0.030
Sequential	56/97	7.4	25.7		1.33 ± 0.229
Phasic	303/392	10.3	122.6		1.09 ± 0.094
Last use 5–9 years previously					
Standard	2 564/4 169	75.9	1 077.1		1.07 ± 0.032
Sequential	71/112	–4.2	34.9		0.89 ± 0.159
Phasic	56/64	–0.5	22.6		0.98 ± 0.208
Last use ≥ 10 years previously					
Standard	4 018/6 048	–62.9	1 524.1		0.96 ± 0.025
Sequential	152/201	–11.1	70.5		0.85 ± 0.110
Phasic	51/50	2.4	20.4		1.12 ± 0.235



Test for heterogeneity by type of oral contraceptives in women with:
Last use < 5 years previously : χ^2 (2 d.f.) = 1.7; NS
Last use 5–9 years previously: χ^2 (2 d.f.) = 1.4; NS
Last use ≥ 10 years previously: χ^2 (3 d.f.) = 1.5; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
CI, confidence interval; SD, standard deviation; d.f., degrees of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conceiving ceased

A few studies indicate that the effect of use of combined oral contraceptives on the risk for breast cancer might be greater among women who have another risk factor than among those without the factor; however, there is no consistent evidence to suggest that the effect of combined oral contraceptives is modified by important risk factors such as benign breast disease, parity and menopausal status. There has been particular concern that a family history of breast cancer might modify an effect of the use of these contraceptives on the risk for breast cancer, but the results to date suggest that the risk is similar among users of these contraceptives with and without a family history of breast cancer (see Figure 11).

Information on the relation of use of oral contraceptives to breast cancer risk among women with mutations in the *BrCA1* or *BrCA2* gene is available from one small study in which 14 such women were compared with 36 women with breast cancer who did not have the mutations (Ursin *et al.*, 1997). A statistically significantly increased relative risk was observed among women who had used oral contraceptives for more than two years before their first full-term pregnancy.

Information on the relationship between use of combined oral contraceptives and the spread of the breast cancer at the time of diagnosis is much sparser than information on overall incidence. The collaborative reanalysis found that the relative risk was greater for localized tumours than for those that had spread beyond the breast (Collaborative Group on Hormonal Factors in Breast Cancer, 1996b). The estimated relative risk for disease localized to the breast was significantly increased for women who had used combined oral contraceptives in the previous five years (1.2), but declined to 1.1 five to nine years after they had stopped use and to 1.0, 10 or more years after stopping. For cancer that had spread beyond the breast, the relative risks were 1.1 for women who had used combined oral contraceptives in the previous five years, 0.96 five to nine years after stopping and 0.93 (significant) 10 or more years after stopping; all of these estimates were compatible with 1.0.

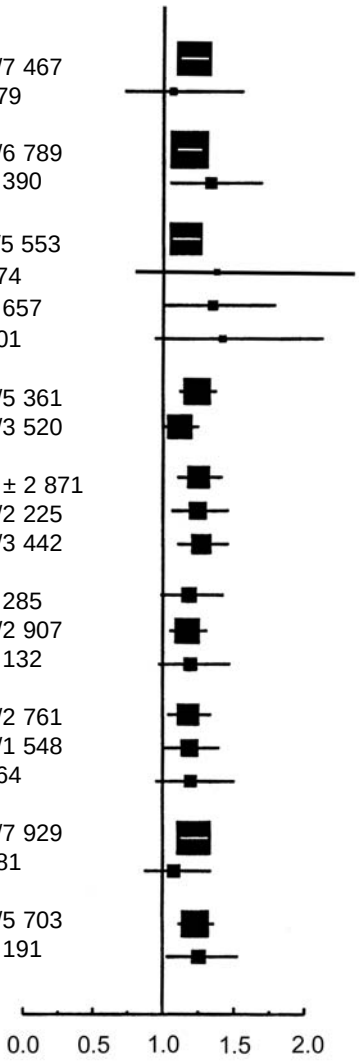
The most consistent findings to date are: a small increase in the risk for breast cancer among current and recent users of combined oral contraceptives; a decline in the risk relative to that of women who have never used them some 10 years after stopping; and little or no increase in risk with increasing duration of use after recency has been taken into account.

The possibility that biased recall might explain the observed increases was assessed in detail by the UK National Case–Control Study Group (1989) and Rookus *et al*. (1994). On the basis of reported use and records of use of combined oral contraceptives, they concluded that only a small part of the observed increase in risk could be explained by reporting bias. Data from follow-up studies are sparser than those from case–control studies. Greater assurance that reporting bias can be ruled out entirely, or that it plays only a small role, will be supplied if positive associations based on larger numbers are produced by the studies now in progress. The important known risk factors for breast cancer, such as age at first birth, parity and age at menopause, were controlled for and they seem unlikely to account for the observed increases. The increases have been observed across

Figure 11. Relative risk (RR) for breast cancer by time since last use of combined oral contraceptives and various characteristics of women

(a) Last use < 5 years previously

Characteristic	RR[a] ± SD	Cases/controls	RR[a] & 99% CI
Mother and/or sister with history of breast cancer			
No	1.21 ± 0.035	3 858/7 467	
Yes	1.06 ± 0.153	454/279	
Country of residence			
Developed	1.18 ± 0.031	4 777/6 789	
Developing	1.33 ± 0.108	296/2 390	
Ethnic origin			
White	1.16 ± 0.036	3 511/5 553	
Black	1.37 ± 0.244	162/274	
Asian	1.35 ± 0.128	241/1 657	
Other	1.42 ± 0.188	155/701	
Years of education			
< 13 years	1.24 ± 0.045	2 638/5 361	
≥ 13 years	1.12 ± 0.046	2 201/3 520	
Age at menarche			
≤ 12 years	1.25 ± 0.054	2 076 ± 2 871	
13 years	1.24 ± 0.069	1 329/2 225	
≥ 14 years	1.27 ± 0.062	1 484/3 442	
Height			
< 160 cm	1.18 ± 0.079	975/1 285	
160–169 cm	1.17 ± 0.047	2 392/2 907	
≥ 170 cm	1.19 ± 0.089	932/1 132	
Weight			
< 60 kg	1.18 ± 0.054	2 098/2 761	
60–69 kg	1.19 ± 0.069	1 305/1 548	
≥ 70 kg	1.20 ± 0.098	642/864	
Menopausal status			
Pre-menopausal	1.22 ± 0.035	4 417/7 929	
Post-menopausal	1.08 ± 0.087	433/881	
Alcohol consumption			
< 50 g per week	1.23 ± 0.044	2 588/5 703	
≥ 50 g per week	1.25 ± 0.087	938/1 191	

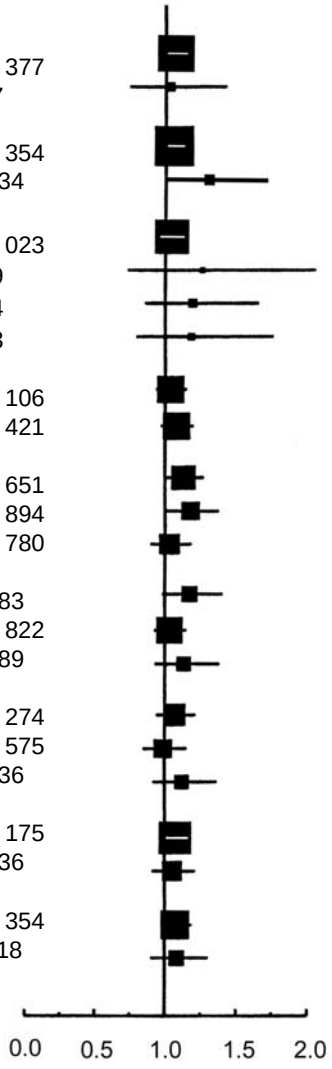


Global test for heterogeneity: χ^2 (14 d.f.) = 11.0; NS

Figure 11 (contd)

(b) Last use 5–9 years previously

Characteristic	RR[a] ± SD	Cases/controls	RR[a] & 99% CI
Mother and/or sister with history of breast cancer			
No	1.07 ± 0.031	3 270/6 377	
Yes	1.03 ± 0.128	468/367	
Country of residence			
Developed	1.06 ± 0.028	4 036/6 354	
Developing	1.31 ± 0.121	203/1 334	
Ethnic origin			
White	1.04 ± 0.033	2 974/5 023	
Black	1.26 ± 0.237	159/229	
Asian	1.19 ± 0.140	165/954	
Other	1.18 ± 0.168	135/503	
Years of education			
< 13 years	1.04 ± 0.041	2 014/4 106	
≥ 13 years	1.08 ± 0.042	2 090/3 421	
Age at menarche			
≤ 12 years	1.13 ± 0.048	1 825/2 651	
13 years	1.18 ± 0.065	1 136/1 894	
≥ 14 years	1.03 ± 0.054	1 179/2 780	
Height			
< 160 cm	1.17 ± 0.076	862/1 183	
160–169 cm	1.03 ± 0.042	1 991/2 822	
≥ 170 cm	1.13 ± 0.082	845/1 089	
Weight			
< 60 kg	1.07 ± 0.051	1 643/2 274	
60–69 kg	0.99 ± 0.059	1 088/1 575	
≥ 70 kg	1.12 ± 0.081	713/1 036	
Menopausal status			
Pre-menopausal	1.07 ± 0.035	2 987/5 175	
Post-menopausal	1.05 ± 0.057	903/1 836	
Alcohol consumption			
< 50 g per week	1.07 ± 0.039	2 121/4 354	
≥ 50 g per week	1.08 ± 0.074	870/1 118	

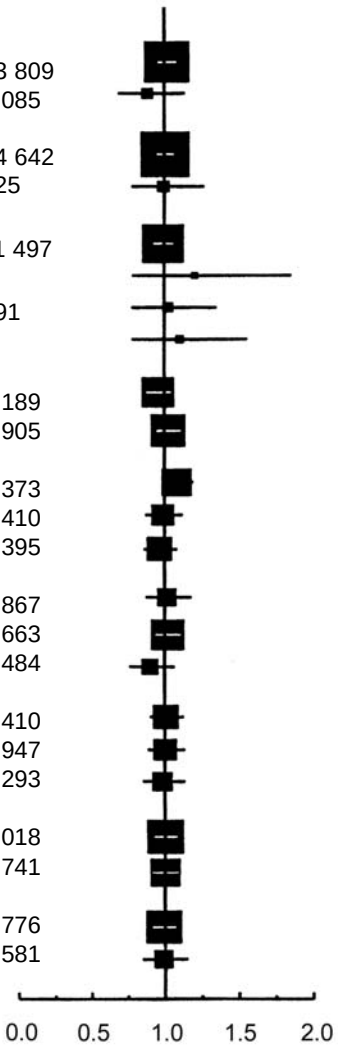


Global test for heterogeneity: χ^2 (14 d.f.) = 14.5; NS

Figure 11 (contd)

(c) Last use ≥ 10 years previously

Characteristic	RR[a] ± SD	Cases/controls	RR[a] & 99% CI
Mother and/or sister with history of breast cancer			
No	1.02 ± 0.025	6 988/13 809	
Yes	0.88 ± 0.092	1 122/1 085	
Country of residence			
Developed	1.01 ± 0.023	8 554/14 642	
Developing	0.99 ± 0.094	264/1 825	
Ethnic origin			
White	0.99 ± 0.027	6 578/11 497	
Black	1.21 ± 0.185	339/463	
Asian	1.02 ± 0.109	255/1 491	
Other	1.10 ± 0.142	225/786	
Years of education			
< 13 years	0.96 ± 0.034	3 613/7 189	
≥ 13 years	1.03 ± 0.033	5 030/8 905	
Age at menarche			
≤ 12 years	1.08 ± 0.039	3 963/6 373	
13 years	0.99 ± 0.049	2 344/4 410	
≥ 14 years	0.96 ± 0.044	2 427/5 395	
Height			
< 160 cm	1.02 ± 0.059	1 724/2 867	
160–169 cm	1.02 ± 0.034	4 494/6 663	
≥ 170 cm	0.90 ± 0.063	1 641/2 484	
Weight			
< 60 kg	1.01 ± 0.044	2 958/4 410	
60–69 kg	1.00 ± 0.048	2 553/3 947	
≥ 70 kg	0.98 ± 0.055	1 986/3 293	
Menopausal status			
Pre-menopausal	1.00 ± 0.031	4 967/8 018	
Post-menopausal	1.00 ± 0.038	2 814/5 741	
Alcohol consumption			
< 50 g per week	0.99 ± 0.031	4 449/8 776	
≥ 50 g per week	0.99 ± 0.059	1 746/2 581	



Global test for heterogeneity: χ^2 (14 d.f.) = 13.5; NS

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
SD, standard deviation; CI, confidence interval; d.f., degrees of freedom; NS, not significant
[a] Relative risk relative to no use, stratified by study, age at diagnosis, parity and, where appropriate, age when first child was born and age when risk for conceiving ceased

case–control studies of various designs, both population- and hospital-based, suggesting that selection bias in the enrolment of cases or controls is not the explanation for the observed increases. The associations have also been observed across different populations. If biased recall, selection bias and confounding are unlikely explanations of the findings, the remaining explanations are that the associations are real (i.e. combined oral contraceptives act as a tumour promoter), that they are due to detection bias (i.e. breast cancer is diagnosed earlier in women who have used combined oral contraceptives) or both. There are few data on the mortality rates of users of these contraceptives, although two studies reported estimates close to 1.0 (Colditz *et al*., 1994; Beral *et al*., 1999).

2.2 Endometrial cancer

Combined and sequential oral contraceptives are discussed separately in relation to the risk for endometrial cancer, as use of these two preparations may have different impacts. Most of the information on the risk for endometrial cancer in relation to use of combined oral contraceptives concerns monophasic pills, i.e. with fixed doses of an oestrogen and a progestogen during a cycle. There is no information about the specific, long-term risk for endometrial cancer associated with use of the multiphasic oral contraceptives available since the early 1980s, in which varying doses of oestrogen and progestogen are given concurrently over one cycle.

2.2.1 *Combined oral contraceptives*

The cohort studies in which use of combined oral contraceptives and the risk for endometrial cancer have been investigated are summarized in Table 9 and the case–control studies in Table 10, with the risk associated with the duration and recency of use when available. Risk estimates by weight, parity (or gravidity) or use of post-menopausal oestrogen therapy are given in the text.

(*a*) *Descriptive studies*

Several analyses have suggested that increased use of combined oral contraceptives can partially explain the decreasing rates of mortality from uterine corpus cancer (i.e. excluding those from cervical cancer) seen between 1960 and the 1980s (Beral *et al*., 1988; Persson *et al*., 1990; dos Santos Silva & Swerdlow, 1995). The decrease is particularly notable among women aged 55 or younger, who are most likely to have used combination oral contraceptives. Interpretation of these trends is complicated by improvements in cancer treatment over time and by lack of correction for the proportion of women who have had their uterus removed and are no longer at risk for developing (or dying from) endometrial cancer. Furthermore, the rate of death from uterine corpus cancer has generally been decreasing since the early 1950s, a decade before oral contraceptives were available. Thus, while it is plausible that increased use of combined oral contraceptives could have preceded and then paralleled the decrease in mortality from endometrial cancer, the magnitude of any decrease in the rate of death from uterine corpus cancer related to increased use of oral contraceptives remains unclear.

Table 9. Cohort studies of use of oral contraceptive pills[a] (not otherwise specified) and risk for endometrial cancer (by duration and recency of use when available)

Reference	Cohort enrolment		End of follow-up	Type/measure of therapy	No. of cases	No. of person–years	RR (95% CI)
	Year/age	Source population/ response/follow-up					
Trapido (1983)	1970/25–57 years	97 300 residents of Boston, USA, and 14 contiguous towns/70%	Dec. 1976	No use	75	296 501	Referent
				Any use	18	124 851	1.4 (NR)
				Duration (months)			
				1–11	6	33 997	1.7 (NR)
				12–23	4	21 978	1.9 (NR)
				24–35	3	21 437	1.6 (NR)
				36–59	2	28 705	0.6 (NR)
				≥ 60	3	18 734	1.5 (NR)
Beral *et al*. (1988, 1999)	May 1968–June 1969	46 000 British women identified by general practitioners/NA	April 1987 (incidence)	No use	16	182 866	Referent
				Any use	2	257 028	0.2 (0.0–0.7)
			Dec. 1993 (mortality)	No use	6	335 998	Referent
				Any use	2	517 519	0.3 (0.1–1.4)
Vessey & Painter (1995)	1968–74/ 25–39 years	17 032 patients at 17 family planning clinics, UK/NA	Oct. 1993	No use	14	NR	Referent
				Any use	1	NR	0.1 (0.0–0.7)

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

[a] May be use of either combined or sequential oral contraceptive pills, but the majority of women used combined

Table 10. Case–control studies of use of oral contraceptive pills and risk for endometrial cancer (by duration and recency of use when available)

Reference	Location/period/age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		OR (95% CI)
				Cases	Controls		Cases	Controls	
Weiss & Sayvetz (1980)	Washington State, USA/ Jan. 1975–Dec. 1977/36–55 years	General population	Personal interviews	83	96	*Combined*			
						No use, < 1 year's use	93	173	Referent
						≥ 1 year's use	17	76	0.5 (0.1–1.0)
Kaufman *et al*. (1980)	USA and Canada/ July 1976–Dec. 1979/ < 60 years	Hospital patients	Personal interviews	96[a]	96[a]	*Combined*			
						No use	136	411	Referent
						Any use	16	99	[0.4 (0.2–0.8)[b]]
						Duration (years)			
						< 1	5	14	0.8 (NR)
						1–2	6	32	0.5 (NR)
						≥ 3	5	53	0.3 (NR)
						Unknown	0	6	
						Recency (years)			
						≥ 5	12	60	0.6 (0.3–1.2)
						With duration ≥ 1 year	8	52	0.5 (0.2–1.0)
Kelsey *et al*. (1982)	Connecticut, USA/ July 1977–Mar. 1979/ 45–74 years	Hospital patients	Personal interviews	67	72	*Sequential/combined*			
						No use	NR	NR	Referent
						For each + 5 years of use	NA	NA	0.6 (0.3–1.5)
						Age 45–55 years			
						No use	31	256	Referent
						Duration (years)			
						≤ 2.5	4	42	0.9 (NR)
						> 2.5	2	44	0.5 (NR)
Hulka *et al*. (1982)	North Carolina, USA/ Jan. 1970–Dec. 1976/ < 60 years	General population	Personal interviews and medical record reviews	90[a]	90[a]	*Combined*			
						No use, < 6 months' use	74	172	Referent
						≥ 6 months' use	5	31	0.4 (NR)
						Recency (years)			
						< 1	0	13	0
						≥ 1	5	14	0.9 (NR)
						Duration (years)			
						< 5	3	14	0.6 (NR)
						≥ 5	2	17	0.3 (NR)

Table 10 (contd)

Reference	Location/period/age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		OR (95% CI)
				Cases	Controls		Cases	Controls	
Henderson *et al*. (1983a)	Los Angeles county, USA/Jan. 1972–Dec. 1979/≤ 45 years	Residents in neighbourhood of cases	Telephone interviews	81	NR	*Combined*			
						No use	67	50	Referent
						Duration (years)			
						< 2	23	22	0.8 (NR)
						2–3	12	11	0.8 (NR)
						4–5	4	9	0.3 (NR)
						≥ 6	4	18	0.1 (NR)
Cancer and Steroid Hormone Study (1987)	Eight US areas/Dec 1980–Dec 1982/ 20–54 years	General population	Personal interviews	73	84	*Combined*			
						No use	250	1 147	Referent
						Combined only	NR	NR	0.5 (0.4–0.6)
						Duration (months)			
						3–6	24	186	0.9 (0.5–1.5)
						–11	13	80	1.3 (0.6–2.6)
						12–23	20	266	0.7 (0.4–1.2)
						24–71	26	576	0.4 (0.3–0.7)
						72–119	12	317	0.4 (0.2–0.8)
						≥ 120	15	241	0.4 (0.2–0.8)
						Recency (years)			
						< 5	12	471	0.3 (0.1–0.5)
						5–9	22	417	0.4 (0.2–0.6)
						10–14	30	368	0.5 (0.3–0.8)
						≥ 15	9	144	0.3 (0.2–0.6)
La Vecchia *et al*. (1986)	Greater Milan, Italy/ Jan. 1979–Nov. 1985/ < 60 years	Hospital patients	Personal interviews	98[c]	98[c]	*Combined*			
						Non-user	163	1 104	Referent
						Any use	7	178	0.5 (0.2–1.1)
Pettersson *et al*. (1986)	Uppsala, Sweden/Jan. 1980–Dec. 1981/ < 60 years	General population	Personal interviews	93	80	*Not specified*			
						No use	96	91	Referent
						Any use	12	22	0.5 (0.2–1.1)
						Duration (years)			
						< 1	5	6	0.8 (0.2–2.7)
						≥ 1	7	16	0.4 (0.2–1.0)
						Any contraceptive			
						Any use	9	22	0.4 (0.2–0.9)
						Duration (years)			
						< 1	5	6	0.8 (0.2–2.7)
						≥ 1	4	16	0.2 (0.1–0.7)

Table 10 (contd)

Reference	Location/period/age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		OR (95% CI)
				Cases	Controls		Cases	Controls	
WHO Collaborative Study (1988); Rosenblatt *et al.* (1991)	Seven countries/Jan. 1979–Feb. 1988/ < 60 years	Hospital patients	Personal interviews	87	93	*Combined*			
						No use	118	687	Referent
						Combined only	14	149	0.5 (0.3–1.0)
						Any contraceptive			
						No use	118	655	Referent
						Any use	12	180	0.5 (0.2–1.1)
						Combined			
						Any contraceptive			
						No use	182	1 072	Referent
						Progestogen content			
						High			
						Duration (months)			
						1–24	1	85	0.1 (0.0–0.7)
						≥ 25	2	69	0.2 (0.0–0.8)
						Recency (months)			
						1–120	1	61	0.1 (0.0–0.8)
						≥ 121	2	93	0.2 (0.0–0.7)
						Low			
						Duration (months)			
						1–24	8	69	1.0 (0.5–2.4)
						≥ 25	1	56	0.1 (0.0–1.1)
						Recency (months)			
						1–120	2	72	0.3 (0.0–1.1)
						≥ 121	7	54	1.1 (0.5–2.8)
Koumantaki *et al.* (1989)	Athens, Greece/1984/ 40–79 years	Hospital patients	Personal interviews	80	95	*Not specified*			
						No use, ≤ 6 months' use	80	151	Referent
						> 6 months' use	3	13	0.6 (0.2–2.0)[d]
Levi *et al.* (1991)	Canton of Vaud, Switzerland/ Jan 1988–July 1990/ 32–75 years	Hospital patients	Personal interviews	85[a]	85[a]	*Combined*			
						No use	105	227	Referent
						Any use	17	82	0.5 (0.3–0.8)
						Duration (years)			
						< 2	9	19	1.0 (0.5–2.3)
						2–5	3	18	0.5 (0.1–1.2)
						5	5	45	0.3 (0.1–0.7)
						Recency (years)			
						< 10	4	30	0.3 (0.1–0.9)
						10–19	7	37	0.4 (0.2–1.0)
						> 19	5	15	0.8 (0.3–2.2)

Table 10 (contd)

Reference	Location/period/age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		OR (95% CI)
				Cases	Controls		Cases	Controls	
Shu *et al.* (1991)	Shanghai, China/April 1988–Jan. 1990/ 18–74 years	General population	Personal interviews	91	96	*Not specified*			
						No use (any birth control)	84	72	Referent
						Any use	32	46	0.8 (0.4–1.8)
						Duration (years)			
						≤ 2	NR	NR	1.4 (0.6–3.0)
						> 2	NR	NR	0.4 (0.1–1.2)
Stanford *et al.* (1993)	Five US areas/June 1987–May 1990/ 20–74 years	General population	Personal interviews	87	66	*Combined*			
						No use	321	187	Referent
						Any use	81	107	0.4 (0.3–0.7)
						Duration (years)			
						< 1	27	21	0.7 (0.3–1.4)
						1–2	16	33	0.3 (0.1–0.6)
						3–4	12	16	0.3 (0.1–0.8)
						5–9	14	15	0.7 (0.3–1.6)
						≥ 10	7	19	0.2 (0.1–0.5)
						Recency (years)			
						< 10	6	18	0.1 (0.0–0.3)
						10–14	15	27	0.3 (0.1–0.7)
						15–19	24	32	0.4 (0.2–0.8)
						≥ 20	33	27	0.7 (0.4–1.3)
						By duration (years)			
						< 3			
						Recency (years)			
						< 15	7	15	0.2 (0.1–0.6)
						15–19	10	16	0.3 (0.1–0.8)
						≥ 20	26	23	0.6 (0.3–1.3)
						≥ 3			
						Recency (years)			
						< 15	14	30	0.2 (0.1–0.5)
						15–19	12	16	0.4 (0.2–1.0)
						≥ 20	7	4	0.8 (0.2–3.3)
Jick *et al.* (1993)	Washington State, USA, Group Health Cooperative/1979–1989/50–64 years	Members of health maintenance organization	Mailed form and pharmacy database	83	79	*Not specified*			
						No use	110	737	Referent
						Any use	26	270	0.5 (0.3–0.9)
						Duration (years)			
						1	7	65	0.4 (0.1–1.4)
						2–5	11	90	0.8 (0.3–1.7)
						≥ 6	8	115	0.3 (0.1–0.9)

Table 10 (contd)

Reference	Location/period/age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		OR (95% CI)
				Cases	Controls		Cases	Controls	
Jick *et al.* (1993) (contd)						*Recency (years)*			
						1–10	5	67	0.4 (0.1–1.1)
						11–15	6	82	0.4 (0.1–1.2)
						16–20	4	57	0.5 (0.1–1.8)
						≥ 21	9	54	0.6 (0.2–2.1)
Voigt *et al.* (1994)[e]	Washington State, USA/1975–77 and 1985–87/40–59 years	General population	Personal interviews	83	95 and 73[f]	*Combined*			
						No use, < 1 year's use	117	284	Referent
						Recency (years)			
						> 10			
						Duration (years)			
						1–5	14	30	0.9 (0.4–1.9)
						> 5	4	16	0.4 (0.1–1.2)
						≤ 10			
						Duration (years)			
						1–5	7	28	1.0 (0.4–2.4)
						> 5	7	74	0.3 (0.1–0.6)
						Progestogen content[g]			
						Low			
						Duration (years)			
						1–5	10	22	1.1 (0.5–2.6)
						> 5	3	32	0.2 (0.1–0.8)
						High			
						Duration (years)			
						1–5	3	14	0.8 (0.2–3.1)
						> 5	3	28	0.3 (0.1–0.9)
Kalandidi *et al.* (1996)	Athens, Greece/1992–94/< 59–≥ 70 years	Hospital patients	Personal interviews	83	88	*Not specified*			
						No use	143	293	Referent
						Any use	2	5	1.3 (0.2–7.7)

OR, odds ratio; CI, confidence interval; NR, not reported; NA, not applicable

[a] Responses reported for case and control women combined

[b] Crude odds ratio and 95% confidence interval calculated from data provided in the published paper by exact methods

[c] Methods state that less than 2% of eligible case and control women refused an interview.

[d] 90% confidence interval

[e] Includes women from the study of Weiss & Sayvetz (1980)

[f] Response for controls identified 1985–1987

[g] Classified according to subnuclear endometrial vacuolization

(*b*) *Cohort studies*

A questionnaire to derive information on oral contraceptive use was sent to approximately 97 300 married women aged 25–57 in eastern Massachusetts, United States, in 1970, who were identified from the 1969 Massachusetts residence lists (Trapido, 1983). The age-adjusted rate ratio for women who had ever used oral contraceptives relative to non-users was 1.4; there was no consistent pattern of a decreasing or increasing rate ratio with longer or more recent use (Table 9). Among nulliparous women, the age-adjusted rate ratio for oral contraceptive users relative to non-users was 2.4 (95% CI, 0.6–9.2), whereas the analogous rate ratio for parous women was 1.4 (95% CI, 0.8–2.4). Among women who also reported any use of post-menopausal oestrogen therapy, the age-adjusted rate ratio for oral contraceptive users relative to non-users was 2.0 (95% CI, 0.9–4.3). No distinction was made between sequential and combined oral contraceptive use, and both preparations were available to the cohort before and during the study follow-up.

Beral *et al.* (1999) followed-up approximately 23 000 oral contraceptive users and a similar number of non-users identified in 1968 and 1969 by the Royal College of General Practitioners. Use of oral contraceptives (not otherwise specified) and the occurrence of uterine cancer were both determined from physicians' reports. Uterine corpus cancer (i.e. excluding the cervix) was diagnosed in two of the oral contraceptive users and 16 of the non-users, resulting in a rate ratio of 0.2 (95% CI, 0.0–0.7) after adjustment for age, parity, smoking, social class, number of previously normal Papanicolaou ('Pap') smears and history of sexually transmitted disease. In a 25-year follow-up of deaths in the cohort, there were eight deaths from endometrial cancer, two of women who had ever used oral contraceptives and six of women who had never used them (rate ratio, 0.3; 95% CI, 0.1–1.9).

The study of the Oxford Family Planning Association included 17 032 married white women identified at 17 family planning clinics in England and Scotland (Vessey & Painter, 1995) who had used oral contraceptives (not otherwise specified), a diaphragm or an intrauterine device for at least five months. Information on contraceptive history and any hospital referrals was obtained from physicians or from the women themselves (for those who stopped attending the clinics) during the study follow-up. A total of 15 292 women remained under observation until the age of 45; only those who had never used oral contraceptives (5881) or had used them for eight years or more (3520) were followed from then on. Endometrial cancer was diagnosed in 15 women, only one of whom had used oral contraceptives (age-adjusted rate ratio, 0.1; 95% CI, 0.0–0.7). In a previous analysis of mortality in this cohort (Vessey *et al.*, 1989b), none of the oral contraceptive users but two of those using a diaphragm or an intrauterine device (the comparison group) had died from uterine corpus cancer.

(*c*) *Case–control studies*

Weiss and Sayvetz (1980) compared 117 women identified from a population-based cancer registry with 395 control women in the general population of western Washington State, United States. Women who had used combined oral contraceptives for one year or

more had half the risk for endometrial cancer of women who were either non-users or had used oral contraceptives for less than one year, after adjustment for age and use of post-menopausal oestrogen therapy (odds ratio, 0.5; 95% CI, 0.1–1.0). No further difference in the duration of use was seen between case and control women. In stratified analyses, the reduced risk was present only for women who had never used post-menopausal oestrogen therapy (odds ratio, 0.4; 95% CI, 0.1–1.1) or who had used it for two years or less (odds ratio, 0.1; 95% CI, 0.01–1.1); no reduction was noted among women who had used it for three years or more (odds ratio, 1.3; 95% CI, 0.3–6.6).

Among 154 women with endometrial cancer and 525 control women in a hospital-based study in the United States and Canada (Kaufman *et al.*, 1980), a 60% reduction in risk was seen among women who used combined oral contraceptives relative to non-users, after adjustment for use of non-contraceptive hormones, parity, body mass, menopausal status, age at menopause, ethnic group, diabetes, education, age and area of residence. The risk for endometrial cancer declined with increasing duration of use, and a sustained reduction in risk was suggested for women who had stopped using oral contraceptives in the previous five or more years. A reduction in risk was noted for women who had used combined oral contraceptives but had never used non-contraceptive oestrogens (odds ratio, 0.4; 95% CI, 0.2–0.8), but not for the women who had ever used both oral contraceptives and non-contraceptive oestrogens (odds ratio, 0.6; 95% CI, 0.3–1.6), although the lack of information on the duration of non-contraceptive oestrogen use makes it difficult to interpret this estimate.

Kelsey *et al.* (1982) studied women admitted to seven hospitals in Connecticut, United States. The 167 newly diagnosed cases of endometrial cancer were compared with 903 control women admitted for non-gynaecological surgical services. Among the study participants aged 45–55 years—the women who had had the opportunity to use oral contraceptives—those who had used oral contraceptives for 2.5 years or more had a 50% decrease in risk.

Among 79 women treated at a hospital in North Carolina, United States, for endometrial cancer, 6.3% had used combined oral contraceptives for six months or more, whereas 15.3% of the 203 control women from 52 counties in the State (the main referral area for the hospital) had done so (Hulka *et al.*, 1982). Since only 15% of the control women reported use of combined oral contraceptives, the risk estimates for more detailed aspects of oral contraceptive use are fairly imprecise (Table 10). There is a suggestion that the risk was lower with longer use (≥ 5 years), with previous use and with use of 'progestogen-predominant' (based on the relative proportions of oestrogens and progestogens in their chemical composition) oral contraceptives. When oral contraceptive use was stratified by use of post-menopausal oestrogens, both users of at least six months' duration (0 cases, 6 controls) and non-users (odds ratio, 0.6 [95% CI not provided]) of post-menopausal oestrogens appeared to have a reduced risk associated with use of oral contraceptives.

Henderson *et al.* (1983a) identified 127 women with endometrial cancer from the population-based cancer registry for Los Angeles County and matched them to control

women of similar age who lived in the same neighbourhood as the matched case. The risk for endometrial cancer decreased with increasing duration of use of combined oral contraceptives, and this pattern remained after further adjustment for parity, current weight, infertility and amenorrhoea. Neither the recency of use of oral contraceptives nor the relative oestrogen and progestogen content of the oral contraceptives had a clear impact on the risk, beyond that explained by the duration of use (data not shown). When the analysis was stratified by body weight, a reduction in risk with longer duration of use was seen among women whose current weight was less than 170 lbs [77 kg] but not among women whose current weight was greater.

In a population-based study conducted by the Centers for Disease Control and the National Institute of Child Health and Human Development in the United States, women with newly diagnosed endometrial cancer, who were 20–54 years of age, were identified from eight cancer registries (Atlanta, Detroit, San Francisco, Seattle, Connecticut, Iowa, New Mexico, and four urban counties in Utah) in the United States Surveillance, Epidemiology and End Results (SEER) Program; 3191 controls were selected from the general population (Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987). Women who had used only combination oral contraceptives had half the risk for endometrial cancer of non-users (age-adjusted odds ratio, 0.5; 95% CI, 0.4–0.6). The risk generally decreased with increasing duration of oral contraceptive use, the greatest reduction in risk being seen among women who had used combined oral contraceptives for two years or more. The strength of the association was similar after adjustment for age alone and after multivariate adjustment for age, parity, education, body mass, menopausal status, geographic region, exogenous oestrogen use and infertility. The risk for endometrial cancer did not vary with recency of use of oral contraceptives or time since first use; both women who had ceased use of oral contraceptives 15 years or more before the study interview and women who had first used oral contraceptives more than 20 years before interview had a lower risk than non-users (age-adjusted odds ratios, 0.3 (95% CI, 0.2–0.6) and 0.4 (95% CI, 0.2–0.7), respectively). When the analysis was stratified by the formulation of the oral contraceptive, all formulations that had been used for at least six months or more were associated with a decreased risk for endometrial cancer. Nulliparous women who had used combined oral contraceptives for one year or more had a larger reduction in risk than non-users (age-adjusted odds ratio, 0.2; 95% CI, 0.1–0.5), but women of high parity had little difference in risk, the age-adjusted odds ratio for women who had had five or more births being 0.8 (95% CI, 0.4–1.9). No difference in risk was reported with body mass, smoking, alcohol consumption, use of exogenous oestrogens or menopausal status (data not shown) or for the different histological subtypes of endometrial cancer (adenocarcinoma, adenoacanthoma and adenosquamous carcinoma).

In a hospital-based study in the area of greater Milan, Italy, La Vecchia *et al.* (1986) compared the use of combined oral contraceptives by women admitted for endometrial cancer and women admitted for traumatic, orthopaedic, surgical and other conditions.

Seven (4%) of the 170 case women and 178 (14%) of the 1282 control women reported use of combined oral contraceptives, resulting in an odds ratio of 0.6 (95% CI, 0.2–1.3) after adjustment for age, marital status, education, parity, age at menarche, age at first birth, age at menopause, body mass index, cigarette smoking and use of non-contraceptive female hormones.

Pettersson *et al.* (1986) studied 254 women residing in the health care region of Uppsala (Sweden) who were referred to the Department of Gynaecologic Oncology with a newly diagnosed endometrial malignancy; each case was matched by age and county of residence to one control woman identified from a population registry. Use of combined oral contraceptives was analysed for women aged 60 or less, resulting in 108 cases and 113 controls. Women who had ever used these contraceptives for one year or more had a lower risk than non-users (odds ratios, 0.5 (95% CI, 0.2–1.1) and 0.4 (95% CI, 0.2–1.0), respectively). Among the women who had used combined oral contraceptives only for contraception, the reductions were slightly greater: odds ratios for any use versus none, 0.4 (95% CI, 0.2–0.9), and for one year versus none, 0.2 (95% CI, 0.1–0.7). It is unclear from the published paper if the estimates were adjusted for potentially confounding factors.

A hospital-based study was conducted in Australia, Chile, China, Colombia, Israel, Kenya, Mexico, the Philippines and Thailand to compare the use of combined oral contraceptives by 140 women with endometrial cancer and 910 women admitted to units other than obstetrics and gynaecology in each centre between 1979 and 1986 (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988). Women who had used only combined oral contraceptives had a lower risk for endometrial cancer than non-users (odds ratio, 0.5; 95% CI, 0.3–1.0), after adjustment for hospital, age, calendar year of interview and race. A reduction in risk was suggested at each level of the factors examined, including gravidity (odds ratios, 0.7 (95% CI, 0.3–1.5) for < 5 pregnancies and 0.3 (95% CI, 0.1–1.5) for ≥ 5 pregnancies), history of infertility (odds ratios, 0.6 (95% CI, 0.3–1.2) for none and 0.4 (95% CI, 0.0–7.3) for a positive history) and use of oestrogens for any other reason except menopausal symptoms (data not shown). The numbers of cases (total, 220) and control women (total, 1537) in this study continued to accrue through 1988 and were then further evaluated by Rosenblatt *et al.* (1991). Among the women who used combined oral contraceptives for contraception only, those who used formulations with a relatively 'high' dose of progestogen (on the basis of the ability of the preparation to induce subnuclear vacuolization in human endometrium) had a lower risk than non-users, regardless of the relative oestrogen dose (odds ratios, 0.2 (95% CI, 0.0–0.5) for high dose and 0 (95% CI, 0.0–1.1) for low dose). In contrast, women who used formulations with a relatively low dose of progestogen had little, if any, reduction in risk, regardless of the relative oestrogen dose (odds ratios, 1.1 (95% CI, 0.1–9.1) for high dose and 0.6 (95% CI, 0.3–1.3) for low dose). Additionally, the reduction in risk did not vary appreciably by the duration or recency of use for the women who used formulations with a relatively high dose of progestogen, whereas the women who used formulations with a relatively low dose of progestogen had a reduction in risk with longer duration of use (odds ratio, 0.1 (95% CI, 0.0–1.1) for ≥ 2 years' use

versus none) or with more recent use (odds ratio, 0.3 (95% CI, 0.1–1.1) for use within the last 10 years versus none). Similar results were seen for first use of oral contraceptives within the previous 14 years. All of these estimates were adjusted for age, gravidity, age at menarche, centre and year of diagnosis.

Koumantaki *et al.* (1989) studied women with endometrial cancer admitted to two hospitals in Athens, Greece, and control women admitted to the Athens Hospital for Orthopaedic Disorders. Only three (4%) of the 83 case women and 13 (8%) of the 164 controls had used oral contraceptives for six or more months (odds ratio, 0.6; 90% CI, 0.2–2.0, adjusted for age, parity, age at menarche, age at menopause, menopausal oestrogen use, years of smoking, height and weight).

Among 122 women treated at a major referral hospital in the Canton of Vaud (Switzerland) for endometrial cancer, 14% had used combined oral contraceptives, as had 27% of the 309 control women admitted to the same hospital for non-neoplastic, non-gynaecological conditions (Levi *et al.*, 1991). The risk decreased from 1.0 (95% CI, 0.5–2.3) for use for less than two years to 0.5 (95% CI, 0.1–1.2) for use for two to five years to 0.3 (95% CI, 0.1–0.7) for use for more than five years. Oral contraceptive use within the previous 10 years (odds ratio, 0.3; 95% CI, 0.1–0.9) or within the previous 10–20 years (odds ratio, 0.4; 95% CI, 0.2–1.0) and first use before the age of 30 (odds ratio, 0.3; 95% CI, 0.1–0.7) were all associated with a reduction in the risk for endometrial cancer. Women who had used oral contraceptives for five years or more had a reduction in risk even if use had occurred 20 or more years previously. The risk estimates were adjusted for age, area of residence, marital status, education, parity, body mass, cigarette smoking and use of post-menopausal oestrogen therapy. Little variation in risk was seen by categories of body mass (odds ratios, 0.6 (95% CI, 0.3–1.0) for < 25 kg/m^2 and 0.2 [95% CI not provided] for ≥ 25 kg/m^2) or cigarette smoking (odds ratios, 0.5 (95% CI, 0.2–1.2) for ever smoked and 0.6 (95% CI, 0.3–1.3) for never smoked). Stratification by use of post-menopausal oestrogen therapy was also presented (odds ratios, 0.4 (95% CI, 0.1–1.2) for ever use and 0.5 (95% CI, 0.3–1.0) for never use), but duration of post-menopausal oestrogen therapy was not analysed. While no reduction in risk was noted for nulliparous women (6 cases and 14 controls) who used oral contraceptives (age-adjusted odds ratio, 0.8; 95% CI, 0.2–2.9), the parous oral contraceptive users (11 cases and 68 controls) did have a reduced cancer risk (age-adjusted odds ratio, 0.3; 95% CI, 0.1–0.7).

Shu *et al.* (1991) studied 268 women with endometrial cancer identified from the population-based Shanghai (China) Cancer Registry and 268 age-matched control women identified from the Shanghai Residents Registry. The risk for endometrial cancer varied little between users of oral contraceptives (not otherwise specified) and women who had never used any type of contraception, after adjustment for age, gravidity and weight (odds ratio, 0.8; 95% CI, 0.4–1.8). When the duration of use was evaluated, there was a suggestion that oral contraceptive use for more than two years was associated with a reduction in risk (odds ratio, 0.4; 95% CI, 0.1–1.2).

In the United States, 405 women with endometrial cancer diagnosed at seven hospitals (in Chicago, Illinois; Hershey, Pennsylvania; Irvine and Long Beach, California;

Minneapolis, Minnesota; and Winston-Salem, North Carolina) and 297 age-, race- and residence-matched control women from the general population agreed to be interviewed (Stanford *et al*., 1993). Use of combined oral contraceptives was reported by 20% of the case women and 36% of the control women (odds ratio, 0.4; 95% CI, 0.3–0.7, after adjustment for age, education, parity, weight and use of post-menopausal oestrogen therapy). There was no clear pattern of a decreasing risk with increasing duration of use (Table 10). Relative to non-users, a strong reduction in risk was noted for women who had used these preparations within the last 10 years (odds ratio, 0.1; 95% CI, 0.0–0.3) and for those who had used them first less than 15 years previously (odds ratio, 0.1; 95% CI, 0.0–0.4); both of these effects waned with more distant oral contraceptive use. The risk estimates varied little by age at first use (< 25, 25–29, 30–34, ≥ 35). When duration and recency were evaluated jointly, use within the previous 20 years was more strongly predictive of a risk reduction than longer duration of use (≥ 3 years). In a joint evaluation with other possible modifying factors, three or more years of combination oral contraceptive use were associated with a reduced risk for endometrial cancer among women of high parity (odds ratio for women with five or more births, 0.2; 95% CI, 0.0–0.6), women who weighed less than 150 lbs [68 kg] (odds ratio, 0.4; 95% CI, 0.2–0.9) and women who had never (odds ratio, 0.2; 95% CI, 0.1–0.6) or briefly (< 3 years) (odds ratio, 0.8; 95% CI, 0.2–3.2) used post-menopausal oestrogen therapy. No reduction and perhaps even an increase in risk was noted for use of combined oral contraceptives of three years or more by women who were nulliparous (odds ratio, 1.9; 95% CI, 0.3–11), weighed more than 200 lbs [91 kg] (odds ratio, 2.7; 95% CI, 0.8–8.5) or had used post-menopausal oestrogen therapy for three years or more (odds ratio, 4.1; 95% CI, 0.4–38). The estimates did not vary appreciably by history of smoking, infertility or menopausal status.

Jick *et al.* (1993) studied women who were members of a large health maintenance organization in western Washington State, United States. Women in whom endometrial cancer had been diagnosed (n = 142) were identified from the organization's tumour registry; the 1042 control women were also members of the organization. Both groups included only women who used the pharmacies of the organization and who had previously completed a questionnaire sent to all female members for a mammography study. Use of oral contraceptives (not otherwise specified), determined from the questionnaire, was reported by 18% of case women and 26% of controls, for an odds ratio of 0.5 (95% CI, 0.3–0.9), adjusted for age, enrolment date in the organization, body mass, age at menopause, parity and current use of post-menopausal oestrogen therapy. In comparison with non-users, the reduced risk for endometrial cancer was most pronounced for women who had used oral contraceptives for six or more years (odds ratio, 0.3; 95% CI, 0.1–0.9) or within the last 10 years (odds ratio, 0.4; 95% CI, 0.1–1.1).

Voigt *et al.* (1994) combined the study population described in the study of Weiss and Sayvetz (1980) with a similar study population identified between 1985 and 1987 in western Washington State, United States. The study included 316 cases and 501 controls. When oral contraceptive use was stratified by use of unopposed oestrogen, women who had used combined oral contraceptives for one year or more and who had also used

unopposed oestrogens for three years or more had no reduction in risk relative to women who had not used oral contraceptives or women who had used them for less than one year (odds ratio, 1.1; 95% CI, 0.4–2.6), whereas a reduction was noted for women who had never used unopposed oestrogens or had used them for less than three years and had used combined oral contraceptives for more than one year (odds ratio, 0.5; 95% CI, 0.3–0.9). Thus, further analyses were restricted to women who had used unopposed oestrogens never or for less than three years. When duration and recency of use of combined oral contraceptives were evaluated jointly, longer use (> 5 years) was associated with a reduced risk for endometrial cancer irrespective of recency (last use, ≤ 10 years ago versus > 10 years ago). When duration and the relative potency of the progestogens in the formulation were evaluated jointly, a longer duration of use (> 5 years), and not progestogen dosage, was most predictive of a reduced risk.

Kalandidi *et al.* (1996) studied 145 women with endometrial cancer admitted to two hospitals in Athens, Greece, and 298 control women admitted to the major accident hospital in Athens with bone fractures or other orthopaedic disorders. Only two (1%) of the case women and five (1.7%) of the controls had ever used oral contraceptives (not otherwise specified). Although a multivariate-adjusted risk estimate was presented (odds ratio, 1.3; 95% CI, 0.2–7.9), no useful inferences can be drawn from this small study.

(*d*) *Summary*

In general, women who have taken combined oral contraceptives have about one-half the risk for endometrial cancer of non-users (Kaufman *et al*., 1980; Weiss & Sayvetz, 1980; Hulka *et al*., 1982; Kelsey *et al*., 1982; La Vecchia *et al.*, 1986; Pettersson *et al*., 1986; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988; Koumantaki *et al*., 1989; Levi *et al*., 1991; Jick *et al*., 1993; Stanford et al., 1993; Vessey & Painter, 1995; Beral *et al*., 1999). The reduction first appears after two to five years of use (Kaufman *et al*., 1980; Hulka *et al*., 1982; Henderson *et al*., 1983a; Pettersson *et al*., 1986; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi *et al*., 1991; Shu *et al*., 1991; Jick *et al*., 1993; Stanford *et al*., 1993; Voigt *et al*., 1994) and continues to decrease as the duration of oral contraceptive use increases (Kaufman *et al*., 1980; Henderson *et al*., 1983a; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi *et al*., 1991; Stanford *et al*., 1993). Some studies have shown a greater reduction in risk with more recent use (Levi *et al*., 1991; Jick *et al.*, 1993; Stanford *et al.*, 1993), but others have found no difference (Kaufman *et al*., 1980; Henderson *et al*., 1983a; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988). When duration and recency of use were evaluated jointly, longer use (≥ 5 years) was associated with a reduced risk, irrespective of recency (Voigt *et al*., 1994), whereas another study showed

that recency (use within the last 15 years) and not duration of use was most predictive of a reduced risk (Stanford *et al*., 1993). Some studies found that the reduction in risk may be greatest with use of oral contraceptives in which progestogen effects predominate (Hulka *et al*., 1982) or that contain higher doses of progestogen (Rosenblatt *et al*., 1991), but another study found that a longer duration of use (≥ 5 years), and not progestogen dose, was most predictive of a reduced risk (Voigt *et al*., 1994).

While no reduction in risk was found for women in the highest categories of body weight in two studies (Henderson *et al*., 1983a; Stanford *et al*., 1993), two others found a reduced risk regardless of weight or body mass (Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi *et al*., 1991). Although one study noted a reduced risk only among oral contraceptive users who were nulliparous (Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987), three others found that the reductions were strongest among parous women (Levi *et al*., 1991) or women of higher parity (≥ 5 births) (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988; Stanford *et al*., 1993). In comparison with women who did not use oral contraceptives, oral contraceptive users who had also used post-menopausal oestrogen therapy for three or more years showed no reduction in risk in two studies (Stanford *et al*., 1993; Voigt *et al*., 1994). While four other studies did find a reduced risk among oral contraceptive users who had ever used post-menopausal oestrogen therapy (Kaufman *et al*., 1980; Hulka *et al*., 1982; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987; Levi *et al*., 1991), the inclusion of women who had used this therapy for fewer than two or three years could have obscured any altered relationship with longer duration of use.

2.2.2 *Sequential oral contraceptives*

(*a*) *Case reports*

In the mid-1970s, case reports appeared in the United States of endometrial abnormalities—ranging from proliferative lesions to severe atypical hyperplasia (Lyon & Frisch, 1976; Kaufman *et al*., 1976; Cohen & Deppe, 1977) to endometrial cancer (Lyon, 1975; Silverberg & Makowski, 1975; Silverberg *et al*., 1977)—among women who had used a sequential oral contraceptive preparation, Oracon®, containing 0.1 mg ethinyloestradiol and 25 mg dimethisterone (Weiss & Sayvetz, 1980). In response to these reports, sequential preparations were removed from the consumer market in the United States and Canada in 1976, but the impact of exposure to these preparations continued to be evaluated in epidemiological studies.

(*b*) *Case–control studies*

The epidemiological studies of sequential oral contraceptive use and endometrial cancer are summarized in Table 11. Weiss and Sayvetz (1980) reported a seven-fold elevation in risk with use of Oracon®, but not with other types of sequential preparations, after adjustment for age, use of combined oral contraceptives and post-menopausal

Table 11. Case–control studies of use of sequential oral contraceptive pills and risk for endometrial cancer

Reference	Location/period/ages	Source of controls	Ascertainment of use	Participation (%)		Type/measure of therapy	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Weiss & Sayvetz (1980)	Washington State, USA/Jan. 1975–Dec. 1977/36–55 years	General population	Personal interviews	83	96	No use Oracon® Other	110 6 1	376 8 11	Referent 7.3 (1.4–39) 0.3 (0.0–2.9)
Kaufman *et al.* (1980)	USA and Canada/July 1976–Dec. 1979/ < 60 years	Hospital patients	Personal interviews	96[a]	96[a]	No use Any use Oracon®	152 2 1	516 9 3	Referent [0.8 (0.2–2.8)][b] [1.1 (0.2–6.0)][b]
Henderson *et al.* (1983a)	Los Angeles county, USA/Jan. 1972–Dec. 1979/< 45 years	General population	Telephone interviews	81	NR	No use Duration (years) < 2 ≥ 2	116 2 9	121 5 1	Referent 0.4 (NR) 4.6 (NR)
Cancer and Steroid Hormone Study (1987)	Eight US areas/ Dec. 1980–Dec. 1982/ 20–54 years	General population	Personal interviews	73	84	No use Only sequential	250 7	1 147 64	Referent 0.6 (0.3–1.3)
WHO Collaborative Study (1988)	Seven countries/Jan. 1979–Feb. 1986/ < 60 years	Hospital patients	Personal interviews	87	93	No use Only sequential	118 1	687 5	Referent 0.9 (0.1–8.3)

CI, confidence interval; NR, not reported

[a] Responses reported for case and control women combined

[b] Crude odds ratio and 95% CI calculated from data provided in published paper using exact methods

oestrogen therapy, among 117 women with endometrial cancer identified from a population-based cancer registry and 395 women from the general population in western Washington State, United States.

Henderson *et al.* (1983a) evaluated oral contraceptive use among 127 white case–control pairs matched for age (in five-year age groups) and area of residence; the case women were identified from the population-based University of Southern California Cancer Surveillance Program and controls from the case's neighbourhood of residence. An almost fivefold increase in risk was found with the use of any type of sequential oral contraceptive for two years or more on the basis of use by nine case women and one control. [The particular brand of sequential oral contraceptive, or the combination of brands, used is not clear from the published paper.]

A study in the United States (Atlanta, Georgia; Detroit, Michigan; San Francisco, California; Seattle, Washington; Connecticut, Iowa, New Mexico and four urban areas of Utah; Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987) found that only seven of 433 case women and 64 of 3191 controls had exclusively used sequential oral contraceptives, resulting in an age-adjusted odds ratio of 0.6 (95% CI, 0.3–1.3). Among the larger group of women with any use of sequential oral contraceptives (26 cases and 152 controls), the risk for endometrial cancer for women who had used them in the previous three to 12 years, for three years or more or who had used Oracon® was 1.5 times that of other sequential oral contraceptive users. No estimates of the risk for these women relative to that of non-users was provided in the published paper.

Two other studies found neither an excess nor a decreased risk among small numbers of women who had used sequential oral contraceptives. In a hospital-based study in several metropolitan areas in the United States and Canada, Kaufman *et al.* (1980) reported that only two (1.3%) of the 154 case women and nine (1.7%) of the 525 control women had reported use of any type of sequential oral contraceptive during personal interviews; one of the case women and three control women reported using Oracon®.

In the international hospital-based study described on p. 120, only one of the 140 case women and five of the 910 control women had exclusively used sequential oral contraceptives (crude odds ratio, 0.9; 95% CI, 0.1–8.3); the specific preparations were not reported (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1988).

In summary, the case reports that preceded the epidemiological studies were important in indicating that the risk for endometrial cancer was potentially elevated among users of sequential oral contraceptives and specifically among users of a particular brand, which contained a relatively potent oestrogen, ethinyloestradiol, and a weak progestogen, dimethisterone. In contrast, it was not clear from the case–control studies whether the increase in risk was restricted to users of this brand or included users of other sequential preparations. This was largely due to the low prevalence of sequential oral contraceptive use in these study populations: only 6% or less of the control women in all of the studies. When the analyses were further stratified by specific preparations, the numbers of women in each category were too small for useful inferences to be drawn from most of these studies.

2.3 Cervical cancer

2.3.1 *Methodological considerations*

(*a*) *Stage of disease and classification*

Cervical cancer is a particularly difficult disease to study with respect to use of oral contraceptives. It is generally accepted that invasive cervical cancer results from a series of changes in the cervical epithelium, from normal epithelial structure to various grades of pre-invasive changes and then on to invasive cervical carcinoma. As oral contraceptives could act at any stage in this process to enhance progression to the next stage, studies should include separate assessment of the effects of steroid contraceptives on risk at different stages of the neoplastic process. Early studies of oral contraceptives and cervical neoplasia included a mixture of lesion types, and these are not considered in this review. In the studies of specific types of preneoplastic lesions, there is considerable variation in the definition of the cases included. In addition, the systems used to classify precancerous cervical lesions histologically and cytologically have changed over time. Early studies included cervical dysplasia (sometimes sub-classified into mild, moderate and severe) and carcinoma *in situ*. In more recent studies, cases have been classified as cervical intraepithelial neoplasia (CIN), with a grading system of I–III to designate the severity of the lesion. Lesions have also been referred to histologically as squamous intraepithelial neoplasia and similarly graded on a scale of I–III to indicate severity. In general, the higher grades correspond roughly to carcinoma *in situ* and severe dysplasia, and the lower grades correspond roughly to mild and moderate dysplasia. In reviewing the literature on non-invasive cervical neoplasia, the terms used by the authors have been retained.

The two generally recognized histological types of invasive cervical carcinoma are squamous-cell carcinoma and adenocarcinoma. In many studies of invasive cervical cancer, these histological types have not been distinguished. In this review, such studies are usually classified with those of squamous-cell carcinoma, because squamous-cell carcinoma was the more common type at the time and in the places where the studies that did not distinguish them were conducted.

(*b*) *Confounding and effect-modifying variables*

Another difficulty in assessing the effect of oral contraceptives on the risk for cervical cancer is that the disease is caused by several types of human papillomavirus (HPV) (IARC, 1995). These viruses are sexually transmitted, and women with cervical neoplasia tend to be those whose sexual behaviour is conducive to the acquisition of sexually transmitted diseases, or who are married to men who have engaged in extramarital sexual relationships conducive to the acquisition of sexually transmitted agents. In some cultures, women who use oral contraceptives tend also to be women whose sexual behaviour is conducive to the acquisition of sexually transmitted agents. Under such circumstances, a spurious association between use of oral contraceptives and cervical neoplasia could be observed, if sexual practices are not controlled for either in the study design or in the statistical analysis. Unless otherwise stated, studies in which the sexual

behaviour of the subjects has not been taken into consideration have been excluded from this review.

In recent studies, attempts have been made to control for HPV infection when assessing possible associations between use of oral contraceptives and cervical neoplasia. To date, however, all attempts to do so have been limited by technical deficiencies. It is generally accepted that cervical neoplasia results from persistent infection with an oncogenic type of HPV. If a woman clears her infection, then she is unlikely to develop a cervical neoplasm. If oral contraceptives were to enhance the risk for cervical cancer by increasing the likelihood that an HPV infection will become persistent, women should be classified according to whether they have persistent infection with an oncogenic HPV. In a case–control study, this would require an adequate serological test for markers of HPV persistence; to date, no such test has been developed. Another approach would be to conduct a prospective follow-up study of a large group of women who have recently acquired an oncogenic HPV type for the development of cervical neoplasia. This approach has several limitations: one is that women could be monitored only until they developed mild or moderate intraepithelial lesions, since it would be unethical not to treat such lesions and allow them to progress to more severe disease; the second problem is that such studies require large numbers of women and a long duration of follow-up. Studies of mild intraepithelial lesions are under way, but the results in relation to use of hormonal contraceptives to date are limited; furthermore, the results of studies of mild lesions may not indicate a relationship between use of oral contraceptives and more severe disease.

Another possibility is that oral contraceptives enhance the risk for cervical cancer in women with persistent HPV infection. In order to address this issue in case–control studies, analyses have been restricted to cases and controls with evidence of HPV DNA in cervical scrapings. In such studies that have been conducted to date, few controls have been found to have HPV, and the relative risk estimates are therefore imprecise.

(*c*) *Studies of oral contraceptives and human papillomavirus infection*

Because oncogenic forms of HPV are involved in the etiology of cervical carcinoma, a number of investigations have been conducted to determine whether infection with HPV is associated with the use of oral contraceptives. It has been clearly shown that the sensitivity and specificity of methods for detecting HPV differ significantly. Methods involving the polymerase chain reaction (PCR) of DNA have been found to be the most sensitive and specific when compared with other methods such as filter *in situ*, dot–blot and Southern blot hybridization (IARC, 1995); and epidemiological studies of cervical carcinoma in which methods other than PCR have been used to detect HPV should be interpreted with the understanding of potential misclassification of HPV status. Studies on younger women have given inconsistent results for an association between the prevalence of HPV infection and oral contraceptive use. The following section is limited to studies in which PCR-based techniques were used.

Hildesheim *et al.* (1993) investigated the risk factors for HPV infection in 404 cytologically normal low-income women in Washington DC, United States, of a median age

of 26 years. The prevalence of HPV infection was found to be higher among current users of oral contraceptives (42.9%) than among women who had never used them (33.3%). Former users (prevalence, 40%) were also at increased risk of having a current HPV infection (difference in prevalence, 2.6%; 95% CI, -10.2–15.5), although these findings were not significant.

Ley *et al.* (1991) found an increased risk for HPV infection with oral contraceptive use in their study of 467 university women of a mean age of 23 years. A higher prevalence of HPV infection was associated with both past (crude odds ratio, 3.0; 95% CI, 1.8–5.0) and current use (crude odds ratio, 3.3; 95% CI, 2.1–5.3).

Bauer *et al.* (1993) examined factors associated with HPV prevalence among 483 cytologically normal women of a median age of 34 years. The prevalence in non-users, former users and current users of oral contraceptives was 5.3, 12.8 and 34.0%, respectively, but this difference, after adjusting for confounding factors, could have occurred by chance.

Burk *et al.* (1996) studied 439 sexually active women in Brooklyn, New York, United States, of an average age of 31 years. Women who had ever used oral contraceptives but were not current users had a higher prevalence of HPV infection (21.9%) than those who had never used them (17.1%); current users had a 14.8% rate of HPV PCR-DNA positivity.

Wheeler *et al.* (1993) found that oral contraceptive use was not associated with HPV infection among 357 cytologically normal university women in New Mexico, United States, of a median age of 23 years. The prevalences of HPV infection in former users (43.9%) and current users (41.8%) were not significantly different from that in women who had never used them (50%) after control for other confounding factors.

Muñoz *et al.* (1996) investigated the association between HPV DNA positivity and risk factors among 810 middle-aged women who were controls in case–control studies of cervical cancer conducted in Spain, Colombia and Brazil. The mean age of these women differed by site: 41.7 years in Spain, 42.8 years in Colombia and 52.7 years in Brazil. Use of oral contraceptives was not significantly associated with HPV DNA positivity. When compared with non-users, women who had used contraceptives for three years or less (odds ratio, 0.7; 95% CI, 0.4–1.4) and more than three years (odds ratio, 0.6; 95% CI, 0.3–1.2) were not at increased risk for HPV infection.

Ho *et al.* (1998), investigating the risk factors for the acquisition of HPV infection in university women, found that oral contraceptive use was not significantly associated.

In a follow-up study of 393 women with normal cervical cytology, Hildesheim *et al.* (1994) found no evidence that persistence of HPV infection was associated with use of oral contraceptives.

The inconsistent results of these studies could be due to differences in the sexual behaviour of oral contraceptive users and non-users in the studies. In the aggregate, they do not provide direct evidence that oral contraceptives interact with HPV to cause cervical cancer. Some are, nevertheless, consistent with a role for oral contraceptives in the genesis of cervical cancer, either by enhancing the likelihood of infection or persistence of infection by oncogenic types or by some direct, synergistic mechanism of HPV and oral contraceptives.

(*d*) *Influence of screening*

A third problem in assessing the effect of hormonal contraceptives on the risk for cervical cancer is the influence of the results of Pap smears. If the cases detected at screening are those more likely to be studied, and if women are more likely to have Pap smears if they have used oral contraceptives, then the women who are studied may be more likely to have used oral contraceptives than other cases in the population. This could lead to spuriously elevated relative risks in relation to oral contraceptive use in case–control studies, particularly for studies of intraepithelial lesions, which are largely asymptomatic and frequently detected at screening. Because of this potential bias, studies of intraepithelial lesions in which both the cases and the controls came from the same screening programme (the preferred design) are distinguished in this review from those in which they were not.

Screening with Pap smears may also influence the results of studies of invasive disease. If having a Pap smear protects against invasive disease, fewer cases will have used oral contraceptives than in the general population, which could result in a spuriously low relative risk. The influence of prior Pap smears must therefore be considered in assessing the risk for both intraepithelial and invasive cervical neoplasms in relation to oral contraceptive use.

2.3.2 *Descriptive studies*

Doll (1985) noted that mortality rates from cervical cancer in Britain increased in women born after 1935, corresponding to some change that took place in about 1960. This is approximately when oral contraceptives came into use, but it is also when women began to change their sexual behaviour, so that the trend could be the result of increased rates of HPV infection.

Peters *et al*. (1986a) reported an increase in the proportion of all newly diagnosed cervical adenocarcinomas in non-Hispanic white women under the age of 35 in Los Angeles County, United States, between 1972 and 1982. There was no increase in the risk for adenocarcinoma in older women, and there was a decreased prevalence incidence ratio for invasive squamous-cell cervical carcinoma in women of all ages during the same time period. The authors hypothesized that the trends were due to the introduction of oral contraceptives, which might preferentially increase the risk for adenocarcinomas over that for squamous-cell carcinomas. Schwartz and Weiss (1986) analysed data from the United States SEER Program and also noted an increase in the risk for adenocarcinomas between 1973 and 1982 in women under the age of 35. No comparable increase in the risk for adenocarcinomas was observed in older women, and no increase in the risk for adenosquamous carcinomas or squamous-cell carcinomas was observed for the same period. In fact, the rates of squamous-cell carcinomas had decreased in all age groups during those same years. The results of this study are thus consistent with those of Peters *et al.* (1986a) and are not inconsistent with the hypothesis that use of oral contraceptives is associated with an increase in the risk for adenocarcinomas. Chilvers *et al*. (1987) reported, however, an increased risk for both adenocarcinoma and squamous-cell carcinoma in women under

the age of 35 in three regions of England between 1968 and 1982, which would argue against a particularly strong increase in risk for adenocarcinomas associated with use of oral contraceptives.

Trends in the incidence rates of adenocarcinoma and adenosquamous carcinoma during the period 1973–91 were examined by Vizcaino *et al*. (1998) in 60 population-based registries in 25 countries. Consistent with the results of Doll (1985), they found a significant increase in the incidence of this condition in many countries between 1973 and 1991. The authors suggested that the increase was due in part to increased transmission of HPV; they also suggested that it was due in part to improvements in screening. With the introduction of the cyto-brush, more cervical adenocarcinomas *in situ* are being detected in some populations, which could result in a decline in the rates of invasive cervical adenocarcinoma. The patterns of the temporal changes across countries do not appear to be explained by variations in the patterns of use of oral contraceptives among these populations; and the observation that the rates of squamous and adenocarcinoma of the cervix are highly correlated among the populations studied suggests that oral contraceptives do not preferentially enhance the risk for adenocarcinomas over that for squamous-cell carcinomas.

2.3.3 *Cohort studies*

(*a*) *Studies of cervical dysplasia and carcinoma* in situ *in the absence of assays for human papillomavirus DNA*

Peritz *et al*. (1977) reported the results of a cohort study of 17 942 women, 18–58 years of age, who received health examinations at the Kaiser Permanente Medical Center in Walnut Creek, California, United States, between 1968 and 1972. They did not provide serial Pap smears but, between 1973 and 1975, all women in the health plan who developed dysplasia or carcinoma *in situ* of the cervix were identified from medical records. After controlling for age, education, marital status, number of Pap smears before entry into the cohort, smoking and selected infections, the relative risk for either cervical dysplasia or carcinoma *in situ* was found to increase with the duration of oral contraceptive use. Carcinoma *in situ* and cervical dysplasia were combined in the estimates of relative risk, but the inclusion of squamous dysplasia in the analyses reduced the strength of the association, suggesting that the association was stronger for carcinoma *in situ* than for dysplasia.

Between 1970 and 1972, approximately 32 000 15–39-year-old women were recruited for a study in Ljubljana, Yugoslavia, through family planning and gynaecological clinics (Andolsek *et al*., 1983). Attempts were made to collect Pap smears from women in the cohort annually, but large numbers of women were lost during the seven-year follow-up period. After adjustment for years of follow-up, age at first pregnancy and number of Pap smears, there was no significant increase in the risk for either carcinoma *in situ* or severe dysplasia in women who had used oral contraceptives. When the two conditions were combined, there was no trend of increase in risk with duration of use.

The results of three cohort studies that specifically assessed the risk for cervical dysplasia in relation to oral contraceptive use are summarized in Table 12. The study of

Table 12. Cohort studies of use of oral contraceptives and cervical dysplasia

Reference (date cohort started)	Comparison groups	No. of cases	Relative risk (95% CI)	Comments
Zondervan *et al*. (1996) (1968–74)	No use	35	1.0	– adjusted for social class, smoking, age at first birth, diaphragm use, condom use; – *p* value of test for trend = 0.2; – 22 years of follow-up; – no increase in risk after 12 months since last use
	Any use	124	1.1 (0.7–1.7)	
	Current use	59	1.7 (1.0–2.8)	
	Months of use			
	1–12	5	0.8 (0.3–2.1)	
	13–24	5	0.7 (0.2–1.9)	
	25–48	11	0.5 (0.3–1.1)	
	49–72	34	1.8 (1.0–3.0)	
	73–96	26	1.2 (0.7–2.2)	
	≥ 97	43	1.1 (0.6–1.8)	
New Zealand Contraception and Health Study Group (1994) (1980–86)	IUD	92	1.0	– adjusted for smoking, age at first intercourse, number of partners, use of depot medroxyprogesterone acetate; – 5.5 years of follow-up
	Use	125	1.2 (0.9–1.6)	
Gram *et al*. (1992) (1979–80)	No use	NR	1.0	– adjusted for marital status, age group, smoking, alcohol abuse, oral contraceptive use; – 7 years' mean follow-up of users; – *p* value of test for trend = 0.05 – 354 women with CIN grade I or II, 44 with CIN grade III, and 3 with carcinoma; results not altered when analysis restricted to grade I or II
	Past use	NR	1.4 (1.0–1.8)	
	Current use	NR	1.5 (1.1–2.1)	
	Age started			
	> 24		1.1 (0.7–1.8)	
	20–24		1.5 (1.1–2.0)	
	< 20		1.3 (0.9–1.9)	

IUD, intrauterine device; NR, not reported; CIN, cervical intraepithelial neoplasia

the Oxford Family Planning Association (Zondervan *et al*., 1996) covered 17 032 women who were recruited at 17 large family planning clinics in England and Scotland between 1968 and 1974. The most recent results represent 22 years of follow-up. No increase in the risk for cervical dysplasia was observed with duration of oral contraceptive use. A small increase in risk, of borderline statistical significance, was observed for current users; however, this possible increase did not persist 12 months after last use.

The New Zealand Contraception and Health Study Group (1994) followed a cohort of 7199 women who had initially had two Pap smears showing no dysplasia for an average of 5.5 years of follow-up. The women were screened annually for cervical abnormalities. When the cohort was established, 2469 women were using oral contraceptives, 2072 women were using an intrauterine device and 1721 women were using depot medroxy-

progesterone acetate. In comparison with women who had used an intrauterine device, women who had used oral contraceptives were not at increased risk for cervical dysplasia. The women in the cohort had used oral contraceptives for an average of 2.5 years.

Between 1979 and 1980, 6622 women between the ages of 20 and 49 in Tromsø, Norway, were interviewed and subsequently followed-up for 10 years (Gram *et al*., 1992) by linking the cohort to computerized information in the pathology registry at the University of Tromsø. Serial Pap smears were not taken from all women, although at least one cytological smear was recorded for 96% of the women in the registry between 1980 and 1989. As most of the cases were CIN-I or -II, this study is summarized in Table 12 with the two studies that provide information on dysplasia. The risk for disease was significantly increased among women who were using oral contraceptives when the cohort was established; it was somewhat lower and of borderline statistical significance for past users. Women who first used oral contraceptives before the age of 24 were at slightly greater risk than were women who began using them later. The difference is not, however, statistically significant and could be due to differences in duration of use among women who began using oral contraceptives at different ages. No information on duration of use was reported.

Table 13 shows the results of two cohort studies of oral contraceptives and cervical carcinoma *in situ*. In the study of the Oxford Family Planning Association (Zondervan *et al*., 1996), the risk of women who had used oral contraceptives for more than 96 months was significantly increased, but no significant trend of increasing risk with duration of use was observed. The risk was also increased in current users of oral contraceptives but not in women who had stopped use for more than one year.

The study of the Royal College of General Practitioners (Beral *et al*., 1988) was begun in 1968. Over 23 000 women who were taking oral contraceptives at the time and an approximately equal number of women who had never taken oral contraceptives were recruited by 1400 general practitioners throughout the United Kingdom, who reported details of oral contraceptive use and the health status of each woman in the study twice each year. After 17–19 years of follow-up, a significantly increased risk for cervical carcinoma *in situ* was found for women who had ever used oral contraceptives. The risk was also observed to increase with duration of use.

It should be noted that information on the number of sexual partners was collected only by the New Zealand Contraception and Health Study Group. All of the associations summarized in Tables 12 and 13 could, therefore, be due to residual confounding by sexual variables. It should also be noted that all of the risk estimates for current users were increased and that the risk decreased after cessation of use. These observations are consistent with a screening bias: women taking oral contraceptives may be more likely to have Pap smears than women who are not. On balance, the results of the cohort studies do not provide strong evidence that cervical dysplasia or carcinoma *in situ* is causally related to use of oral contraceptives.

Table 13. Cohort studies of use of oral contraceptives and cervical carcinoma *in situ*

Reference (date cohort started)	Comparison groups	No. of cases	Relative risk (95% CI)	Comments
Zondervan *et al*. (1996) (1968–74)	No use	22	1.0	– adjusted for social class, smoking, age at first birth, diaphragm use, condom use; – *p* value of test for trend = 0.2; – 22 years of follow-up; – no significant increase in risk after 12 months since last use
	Any use	99	1.7 (1.0–3.0)	
	Current use	45	2.2 (1.2–4.1)	
	Months of use			
	1–12	4	1.4 (0.5–4.4)	
	13–24	7	1.8 (0.7–4.6)	
	25–48	20	1.7 (0.8–3.5)	
	49–72	18	1.5 (0.7–3.0)	
	73–96	11	1.2 (0.5–2.6)	
	≥ 97	39	2.5 (1.3–4.7)	
Beral *et al*. (1988) (1968–70)	No use	34	1.0	– adjusted for age, parity, smoking, social class, number of prior normal Pap smears; – *p* value of test for trend, < 0.001; – follow-up through 1987 (17–19 years)
	Any use	173	2.9 (2.0–4.1)	
	Years of use			
	< 5	84	2.4	
	5–9	66	3.6	
	≥ 10	23	4.8	

CI, confidence interval

(*b*) *Studies of cervical dysplasia in which assays for human papillomavirus DNA were performed*

Three cohort studies of a different design from those summarized in Tables 12 and 13 have been conducted. Koutsky *et al.* (1992) followed-up a cohort of 241 women with normal cervical cytology by cytological and colposcopic examinations every four months for approximately two years. HPV DNA was detected by dot–filter hybridization and Southern blot hybridization for confirmation. The risk for CIN-II or -III was not associated with use of oral contraceptives.

Liu *et al*. (1995) assembled a cohort of 206 women with cervical dysplasia who had been recruited into a randomized trial of the effect of folic acid supplementation on the course of cervical dysplasia; they had provided two to four cervical smears, which were tested for HPV-16 by Southern blotting. Follow-up examinations were conducted every two months for a total of six months. The risk for progression from low- to high-grade dysplasia was not associated with past or current use of oral contraceptives: the relative risk for progression in HPV-16-negative women was 1.6 (95% CI, 0.8–3.1) for past users versus never users and 1.4 (95% CI, 0.7–2.7) for current versus never users, whereas the comparable relative risks in HPV-16-positive women were 0.8 (95% CI, 0.6–1.1) and 0.8 (95% CI, 0.6–1.0), respectively. Although the differences in relative risk estimates for HPV-16-negative and -positive women could have occurred by chance, they are consistent

with the hypothesis that oral contraceptives enhance progression of dysplasia in the absence of HPV-16.

In a study of similar design (Ho *et al*., 1995), 70 women with cervical dysplasia were followed at three-month intervals for 15 months. HPV DNA was assayed by PCR techniques. The risk for persistent dysplasia was not associated with oral contraceptive use after HPV status was taken into account; results stratified by HPV status were not presented.

(*c*) *Studies of invasive cervical carcinoma*

The results of two cohort studies of the risk for invasive cervical carcinoma in relation to oral contraceptive use are summarized in Table 14. HPV status was not considered in either study. The study of the Oxford Family Planning Association (Zondervan *et al*., 1996) found an increased risk for invasive cervical carcinoma in women who had ever used oral contraceptives that was of borderline statistical significance. The risk was particularly enhanced for women who had used oral contraceptives within the past two years. There was no trend of increase in risk with duration of use.

The study of the Royal College of General Practitioners (Beral *et al*., 1988) also showed an increase in risk for invasive cervical carcinoma of borderline statistical significance among women who had ever used oral contraceptives and an increase in risk with duration of use. Beral *et al.* (1999) also found an increase in risk for deaths due to cervical carcinoma. On the basis of 25 years of follow-up and 172 deaths, the relative risk for

Table 14. Cohort studies of use of oral contraceptives and invasive cervical carcinoma

Reference (date cohort started)	Comparison groups	No. of cases	Relative risk (95% CI)	Comments
Zondervan *et al*. (1996) (1968–74)	No use	2	1.0	– adjusted for social class, smoking, age at first birth, diaphragm use, condom use; – *p* value of test for trend, 0.8; – 22 years of follow-up; – no significant increase in risk after 24 months since last use
	Any use	31	4.4 (1.0–32)	
	Use in past 2 years	21	6.8 (1.6–49)	
	Months of use			
	1–24	4	5.5 (0.8–51)	
	25–72	6	2.8 (0.5–23)	
	≥ 73	21	4.7 (1.1–33)	
Beral *et al*. (1988) (1968–70)	No use	16	1.0	– adjusted for age, parity, smoking, social class, number of prior normal Pap smears; – *p* value of test for trend, < 0.001; – follow-up through 1987 (17–19 years)
	Any use	49	1.8 (1.0–3.3)	
	Years of use			
	< 5	21	1.3	
	5–9	17	2.0	
	≥ 10	11	4.4	

CI, confidence interval

dying from cervical cancer among women who had ever used oral contraceptives was 1.7 (95% CI, 0.9–3.2). The relative risk increased with duration of use (*p* value for trend, 0.03) and was 4.1 (95% CI, 1.6–11) for users of 10 or more years' duration. The risk decreased with time since cessation of use and was not significantly increased 10 years after exposure.

Because these results are for invasive cervical cancer, they are unlikely to be due to preferential screening of women taking oral contraceptives. They could, however, be due to incomplete control of the confounding influence of sexual behaviour, since in neither of these studies was a detailed sexual history obtained.

2.3.4 *Case–control studies*

(*a*) *Studies of cervical intraepithelial neoplasia not based on screening programmes*

Ten case–control studies of CIN in relation to use of oral contraceptives are summarized in Table 15. In all of these studies, the cases were selected from clinics, hospitals or tumour registries, and controls were selected from clinics, hospitals or the general population. HPV status was not assessed in any of these investigations. Because the cases and controls were not selected from the same screening programme, these studies are more likely than studies based on screened populations to be influenced by screening bias. Nevertheless, an attempt was made in all of the studies to control for both sexual variables and prior screening, and they therefore provide useful information on the possible association between CIN and oral contraceptive use. A study by Hellberg *et al*. (1985) is omitted from Table 15 because, the controls were pregnant women and, as such, were not representative of the population from which the cases came with respect to contraceptive factors. Furthermore, no relative risk estimates were provided in the report of that study.

The study by Harris *et al*. (1980) was conducted at two hospitals in Oxford, England, between 1974 and 1979. After adjustment for pregnancy outside marriage, cigarette smoking and numerous sexual partners, the risk for carcinoma *in situ* or dysplasia was found to increase significantly with duration of oral contraceptive use.

Clarke *et al*. (1985) studied women attending the dysplasia clinic of the Toronto General Hospital, Canada, between 1979 and 1981 who had histologically confirmed cervical dysplasia. The controls were selected from the same neighbourhood as the corresponding cases. After controlling for number of sexual partners, the relative risk for women who had ever used oral contraceptives was estimated to be 1.7 ($p = 0.14$). Age at first sexual intercourse, smoking status and years of education were also considered as potential confounders. No information was presented on risk in relation to duration of use.

Irwin *et al*. (1988) identified women with carcinoma *in situ* from the population-based cancer registry of Costa Rica between 1982 and 1984. The controls were selected from a national survey. After adjustment for age, history of sexually transmitted disease or pelvic inflammatory disease, gravidity, age at first intercourse, number of sexual partners and history of Pap smears before 1982, a significant trend of increased risk with duration of use was observed. The risk was highest for women who had used oral

Table 15. Case-control studies of use of oral contraceptives and cervical intraepithelial neoplasia (CIN) in which cases and controls were not selected from the same screening programme

Reference	Definition of cases	No. of subjects		Relative risk (95% CI)[a]		Long-term use		Comments
		Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)	
Harris *et al*. (1980)	Carcinoma *in situ* or dysplasia	237	422	Not reported		≥ 10	2.1 (significant trend, $p = 0.003$)	Cases from 2 hospitals, controls largely from gynaecological clinics of the same hospitals
Clarke *et al*. (1985)	Dysplasia	250	500	1.7		Not reported		Cases from dysplasia clinics, neighbourhood controls
Irwin *et al*. (1988)	Carcinoma *in situ*	583	938	1.6 (1.2–2.2)	2.3 (1.5–3.5)	≥ 10	2.0 (1.0–3.6) (p for trend = 0.04)	Cases from tumour registry, general population controls
Brock *et al*. (1989)	Carcinoma *in situ*	117	196	1.5 (0.4–6.6)	1.8 (0.4–8.6)	≥ 6	2.3 (0.5–11) (p for trend = 0.05)	Cases from 2 hospitals, controls from case's family physician's files or files of university-affiliated general practitioners
Jones *et al*. (1990)	Carcinoma *in situ*	293	801	Not reported	1.8 (1.0–3.4)	≥ 10	1.4 (0.8–2.7) (p for trend = 0.04)	Cases from clinics, general population controls
Cuzick *et al*. (1990)	CIN-I CIN-II CIN-III	110 103 284	833 833 833	Not reported		> 9 > 9 > 9	1.8 (NS) 2.5 (NS) 1.3 (NS)	Cases from many clinics, controls from general practitioners and family planning clinics
Coker *et al*. (1992)	CIN-II/-III	103	258	0.7 (0.3–1.6)	1.2 (0.5–2.8)	≥ 5	0.6 (0.2–1.4)	Cases from dysplasia referral clinic, controls from single family practice centre
De Vet *et al*. (1993)	Dysplasia	257	705	Not reported		> 10	2.3 (1.2–4.6)[b]	Cases from 40 municipalities, controls from populations of 6 of these municipalities

Table 15 (contd)

Reference	Definition of cases	No. of subjects		Relative risk (95% CI)[a]		Long-term use		Comments
		Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)	
Kjaer *et al*. (1993)	Carcinoma *in situ*	586	614	1.4 (0.9–2.1)	1.5 (1.0–2.4)	≥ 10	1.7 (1.0–2.7) (p for trend = 0.01)	Cases from tumour registry, controls from general population
Ye *et al*. (1995)	Carcinoma *in situ*	231	8 364	1.0 (0.8–1.4)	1.2 (0.8–1.9)	> 5	1.5 (1.0–2.3) (p for trend = 0.13)	Hospitalized cases and controls; analyses restricted to cases with vaginal bleeding to minimize screening bias

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

[a] Controlled for various potentially confounding variables except human papillomavirus

[b] Among current users

contraceptives within the past year (current users); the relative risk was not increased after five years since cessation of use.

Brock *et al*. (1989) recruited women with histologically confirmed carcinoma *in situ* which had been diagnosed in two hospitals in Sydney, Australia, between 1980 and 1983. The controls were selected from the same clinics from which the cases came. After adjustment for number of sexual partners, age at first sexual intercourse and smoking, the risk for carcinoma *in situ* of women who had ever used oral contraceptives was estimated to be 1.5. The risk was somewhat higher for current users, and a trend of increasing risk with duration of use was observed which was of borderline statistical significance.

Jones *et al*. (1990) recruited cases of cervical carcinoma *in situ* from 24 participating hospitals in five United States cities. Controls from the same communities were ascertained through random-digit dialling. After control for age, race, interval since last Pap smear, number of abnormal smears, number of sexual partners, history of non-specific genital infection or sores and years of cigarette smoking, the relative risk was found to increase slightly with duration of oral contraceptive use. The risk was particularly high for current users of oral contraceptives (borderline statistical significance) and was not significantly elevated in former users.

Cuzick *et al*. (1990) recruited women referred to the Royal Northern Hospital in London, England, by their local general practitioners for evaluation of an abnormal cervical smear which was histologically classified as CIN-I, -II or -III. The controls came largely from one general practice and one family planning clinic. The relative risks for CIN were not significantly increased after more than nine years of oral contraceptive use, and no significant trends of increasing risk with duration of use were observed. The relative risk estimates were adjusted for age, social class, age at first intercourse, number of partners, parity and age at first birth. No information was provided on the risk of current users or risk in relation to time since last use.

Coker *et al*. (1992) recruited cases of CIN-II or CIN-III from a dysplasia clinic; controls were selected from a family practice centre [which might have biased the results with respect to hormonal contraceptive use]. No increase in risk was observed in relation to the features of oral contraceptive use considered, although the highest relative risk was observed for current users.

De Vet *et al*. (1993) studied women with dysplasia who were referred from 40 municipalities in the Netherlands to participate in a randomized clinical trial of the effects of β-carotene on cervical dysplasia. The controls were selected from the general population of six of these municipalities. After adjustment for the number of sexual partners, number of cigarettes smoked per day, marital status, number of children, age at first intercourse, current frequency of intercourse and age, the risk for dysplasia was found to be increased in current users of oral contraceptives who had used these products for over 10 years. The risk was not increased for current users who had used them for a shorter period or for former users.

Kjaer *et al*. (1993) recruited women with cervical carcinoma *in situ* who were living in the greater Copenhagen area between 1985 and 1986 through the Danish Cancer

Registry. The controls were recruited from the general population of Copenhagen. After control for age, years of smoking, number of sexual partners, proportion of sexually active life without use of barrier contraceptives, years of use of an intrauterine device, number of births, age at first episode of genital warts and ever having a Pap smear, the relative risk for cervical carcinoma *in situ* was found to increase significantly with duration of oral contraceptive use. The risk was also increased in current users of oral contraceptives and declined with years since last use, so that the relative risk was 1.0 after nine years since last exposure.

Ye *et al*. (1995) analysed data from the WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Women hospitalized for treatment of carcinoma *in situ* were recruited from one centre each in Mexico and Chile and three centres in Thailand. The controls were women from the same hospitals as the cases but with diseases not considered to be associated with hormonal contraceptive use. Overall, women who had ever used oral contraceptives had a relative risk for cervical carcinoma *in situ* of 1.3 (95% CI, 1.2–1.5) and a strong trend of increasing risk with months of use: the relative risk of women who had used oral contraceptives for more than five years was 2.0 (95% CI, 1.7–2.5; p for trend < 0.001). The risk was also increased for women who had last used oral contraceptives within the previous 12 months (relative risk, 1.7) but not for women who had used them in the more distant past (relative risk, 1.2). To minimize any potential influence of screening bias, additional analyses were restricted to cases that presented with vaginal bleeding and were presumably not diagnosed by screening. In this subset (shown in Table 15), the risk was not significantly increased for women who had ever used oral contraceptives, and no significant trend of risk with duration of use was observed; there was also no increase in the risk of current users. These relative risk estimates were adjusted for age, hospital, marital status, number of pregnancies, history of induced abortion, number of Pap smears six months before the reference date, use of injectable contraceptives and use of condoms. Other potentially confounding variables that were considered but not found to be confounders included use of an intrauterine device or diaphragm, douching after intercourse, age at first sexual relationship, age at menarche, menopausal status, number of visits to a doctor for vaginal discharge, number of sexual relationships, history of any venereal disease or of gonorrhoea or syphilis, tubal ligation, ectopic pregnancy, stillbirth, miscarriage, prior dilatation and curettage, chest X-ray and family history of cancer.

Some consistencies among the results of the studies summarized in Table 15 are generally higher relative risk estimates for current users than for ever users and a tendency for the relative risks to decline with time since use. These findings suggest a bias due to screening in many of these studies. Nonetheless, most of the studies also found that the relative risk estimates were higher among long-term than short-term users of oral contraceptives, and, in many instances, a significant trend of increasing use with duration of use was observed. This too, however, could be due to selective factors. The longer a woman uses oral contraceptives, the more likely she is to have a Pap smear and to be diagnosed with CIN. The study of Ye *et al*. (1995) provides evidence that this kind of bias can occur.

(*b*) *Studies of cervical intraepithelial neoplasia based on screening programmes*

The case–control studies of CIN summarized in Table 16 are those in which the cases and controls were selected from the same screening programme. Thomas (1972) compared women with carcinoma *in situ*, dysplasia and any abnormal Pap smear (class III, IV or V) with women whose Pap smears were normal. All of the subjects were residents of Washington County, Maryland (United States). No increase in the risk for these conditions in relation to ever having used oral contraceptives was observed. These estimates were not appreciably altered by controlling for age, circumcision status of the husband, use of barrier contraceptives, smoking status, frequency of church attendance, evidence of trichomonas on the index smear, history of vaginal discharge, education, having been divorced or separated, having a husband who had previously been married, number of live births, conception of first child before marriage and age at first pregnancy. The risk in relation to duration of use was not reported, but the cases and controls did not differ with respect to mean cumulative dose of oestrogen or of progestogen received. They also did not differ with respect to time since first use of oral contraceptives or current use of oral contraceptives. The mean duration of use of oral contraceptives was slightly, but not significantly, higher for controls (21 months) than for the cases (20 months).

Worth and Boyes (1972) selected cases of carcinoma *in situ* from the British Columbia Screening Programme in Canada. The controls were women in the same medical practices as the cases who had negative Pap smears. The proportions of cases and controls who had ever used oral contraceptives were similar [the age-adjusted relative risk was 1.1], and the mean length in months of oral contraceptive use did not differ between the two groups (25.7 and 21.5 for cases and controls aged 20–24 and 33.9 and 32.0 months for women aged 25–29, respectively). Although the relative risk estimate was not controlled for other potential confounders, it is unlikely that doing so would have increased the relative risk estimate to a significant level. The results of these two early studies, although reassuring, are limited by the short duration of use and a short duration of follow-up.

Molina *et al*. (1988) recruited women with cervical carcinoma *in situ* who were referred from a screening programme to any one of three hospitals in Santiago, Chile. The controls were women with normal Pap smears who were selected from the same screening programme. After adjustment for total number of pregnancies, history of induced abortion, pay status (an indicator of socioeconomic status), age at first intercourse, number of sexual partners, history of vaginal discharge and frequency of prior Pap smears, no increase in the risk for cervical carcinoma *in situ* was observed in women who had ever used oral contraceptives, and no trend in risk with duration of use was observed. An increase in the risk of current users was found, but no increase in risk was observed for previous users.

Parazzini *et al*. (1992) recruited women with CIN from screening clinics in Milan, Italy. The controls were women with normal cervical smears selected from the same screening clinics. No increase in the risk for either CIN-I and -II or CIN-III was observed in women who had ever used oral contraceptives. No information was presented on

Table 16. Case–control studies of use of oral contraceptives and cervical intraepithelial neoplasia (CIN) in which the cases and controls were selected from the same screening programme

Reference	Definition of cases	No. of subjects		Relative risk (95% CI)[a]		Long-term use		Comments
		Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)[a]	
Thomas (1972)	Carcinoma *in situ*	104	302	0.58 (NS)		Not reported		Mean duration of use and cumulative doses of oestrogen and progestogen not higher in cases than controls
	Dysplasia	105	302	1.24 (NS)				
	Pap III, IV, V (all cases)	324	302	0.91 (NS)				
Worth & Boyes (1972)	Carcinoma *in situ*	310	682	[1.1] (NS)[b]				Low response rates; no adjustment for confounders; RR calculated for age group 25–29 years
Molina *et al.* (1988)	Carcinoma *in situ*	133	254	1.0 (0.6–1.7)	3.2 (1.1–9.8)	> 6	0.7 (0.2–2.0)	
Negrini *et al.* (1990)	Low-grade SIL	208	1 423	0.9	0.8	≥ 5	0.5	Results similar in subset tested for and adjusted for HPV infection
	High-grade SIL	19	1 423	2.7	4.7	≥ 5	4.6	
Parazzini *et al.* (1992)	CIN I and II	124	323	0.9 (0.6–1.4)[b]		Not reported		No adjustment for confounders
	CIN III	138	323	1.0 (0.7–1.4)[b]				
Schiffman *et al.* (1993)	CIN	443	439	Not reported	1.3 (0.6–2.8)	Not reported		Adjusted for HPV infection
Muñoz *et al.* (1993)	CIN III							Adjusted for HPV infection
	Spain	249	242	1.3 (0.7–2.3)		≥ 5	1.8 (0.8–3.7)	
	Colombia	276	270	1.0 (0.6–1.6)		≥ 5	0.9 (0.5–1.5)	
Becker *et al.* (1994)	High-grade dysplasia	374	651	0.4 (0.2–0.9)	0.4 (0.2–1.0)	≥ 10	0.6 (0.2–1.4)	Adjusted for HPV infection

CI, confidence interval; RR, relative risk; Pap, Papanicolaou smear; NS not significant; SIL, squamous intraepithelial neoplasia; HPV, human papillomavirus

[a] Controlled for various potentially confounding variables except HPV, unless otherwise stated

[b] Adjusted only for age

duration of use or time since last use; however, because the cases were recruited between 1981 and 1990, it can be assumed that some of the women who had used oral contraceptives had done so for a considerable time.

The remaining four studies summarized in Table 16 differ from the others and from the studies in Table 15 in that the investigators attempted to make some adjustment for HPV status. In the study of Negrini *et al*. (1990), women with cervical intraepithelial lesions were selected from among women who received their diagnosis in 13 clinics associated with three hospitals in the Washington DC area (United States). Women with normal Pap smears were selected from the same clinics to serve as controls. Cervical scrapings were assayed for specific types of HPV by Southern blot analysis. No increase in the risk for low-grade cervical intraepithelial lesions was observed with respect to any use of oral contraceptives, current use or long-term use. While the study was based on small numbers, after adjustment for age, interval since last Pap smear and lifetime number of sexual partners, the risk for high-grade squamous intraepithelial neoplasia was found to be increased for women who had ever used oral contraceptives, for long-term users and for current users. The only estimate that had a 95% CI that included unity was that for women who had used oral contraceptives for more than five years. The results for both low-grade and high-grade squamous intraepithelial neoplasia were not appreciably different from those shown in the Table after stratification on HPV status.

Schiffman *et al*. (1993) selected cases of CIN from a cytological screening programme at Kaiser Permanente in Portland, Oregon (United States). The controls were women with a normal Pap smear. Specific types of HPV DNA were assayed in cervical vaginal lavage specimens by PCR techniques. After adjustment for age and HPV infection, the risk for CIN was not significantly increased in women who had used oral contraceptives in the past or were using them currently. No information was provided on risk in relation to duration of use.

Muñoz *et al*. (1993) selected women with CIN-III from hospitals, pathology laboratories and screening clinics in Spain and Colombia and selected controls from the same place of recruitment as the corresponding case but among women who had normal cytological results on the same date as the case was detected. HPV DNA in cervical scrapings was assayed by PCR. The risk for CIN-III was not increased among women who had ever used oral contraceptives in either Spain or Colombia after adjustment for age, centre, number of sexual partners, age at first intercourse, HPV infection, *Chlamydia trachomatis* infection, husband's sexual partners (in Spain) and smoking status (in Colombia). The risk was also not significantly increased for women who had used oral contraceptives for more than five years, and in neither country was there a significant trend of increasing risk with duration of use. In Spain, however, the risk was somewhat increased in long-term users and the p of the test for trend was 0.08.

Becker *et al*. (1994) recruited women with high-grade dysplasia through the University of New Mexico Women's Health Care and Maternal and Infant Care clinics in the United States. Women who were referred to the University of New Mexico colposcopy clinic and found to have high-grade dysplasia were compared with controls with normal

Pap smears selected from the same clinics from which the cases came. In this study, the term 'high-grade dysplasia' was used to cover moderate dysplasia, severe dysplasia and carcinoma *in situ* combined. Cervical smears were assayed for specific types of HPV DNA by dot–blot hybridization and PCR techniques. The relative risk estimates were adjusted for age, age at first intercourse, lifetime number of sexual partners, ethnicity and HPV infection as identified by PCR. The relative risks for high-grade dysplasia were not increased among women who had ever used oral contraceptives, were current users or were long-term users.

In the aggregate, the results of the eight studies summarized in Table 16 do not provide convincing evidence that use of oral contraceptives enhances the risk for cervical intraepithelial lesions. The large relative risks in the study of Negrini *et al*. (1990) are based on small numbers, and the increase in the risk of current users suggests that the results were influenced by screening bias. With this exception, the results of the studies summarized in the Table are consistent with no influence of oral contraceptives on the risk for these lesions.

(*c*) *Hospital-based studies of invasive squamous-cell cervical carcinoma*

Table 17 summarizes the results of seven hospital-based case–control studies of invasive squamous-cell cervical carcinoma. The case group in the study of Ebeling *et al*. (1987) consisted of 129 women with invasive cervical carcinoma treated at a university hospital or city hospital in Leipzig, Germany. The controls were selected from among women admitted to the same hospitals for skin diseases or orthopaedic conditions. After adjustment for number of pregnancies, age at first pregnancy, number of sexual partners, age at first intercourse, history of vaginal discharge, smoking and months since last Pap smear, the relative risk for invasive squamous-cell carcinoma decreased from 2.1 to 1.5 and was no longer statistically significant. In addition, the trend in risk with duration of use was reduced after adjustment to a non-significant level. The risk was higher for current users than for previous users (1.2; 95% CI, 0.6–2.5). The risk was particularly high for women who had begun use before the age of 25, but, after additional adjustment for age at first use, the relative risk of women who had used oral contraceptives for more than seven years was further reduced to 1.3. The risk of women who had first used oral contraceptives before the age of 25 remained statistically significant at 2.6 after adjustment for duration of use.

Parazzini *et al*. (1990) recruited 367 women under the age of 60 with invasive cervical cancer (assumed to be largely squamous-cell) from among women admitted to four large teaching and general hospitals in Milan, Italy. The controls were patients admitted for acute conditions to one of the hospitals in Milan and to several specialized Milan University clinics. The relative risk of women who had ever used oral contraceptives was 1.9 (95% CI, 1.0–3.1) after control for age, marital status, education, parity, number of sexual partners, age at first intercourse, cigarette smoking, history of Pap smears and use of barrier methods of contraception. The risk was further increased for women who had used oral contraceptives for more than two years, and there was a significant trend of

Table 17. Case–control studies of use of oral contraceptives and invasive squamous-cell cervical carcinoma: hospital controls

Reference	No. of subjects		Relative risk (95% CI)[a]		Long-term use			Comments
	Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)[a]	*p* for trend	
Ebeling *et al.* (1987)	129	275	1.5 (0.8–2.9)	2.0 (1.0–4.1)	≥ 7	1.8 (1.0–3.8)	≥ 0.10	– includes 4 adenocarcinomas; – conducted in eastern Germany; – RR for women who first used oral contraceptives at ≤ 24 years, 3.0 (1.1–8.1)
Parazzini *et al.* (1990)	367	323	1.9 (1.0–3.1)	Not reported	> 2	2.5 (1.2–5.1)	0.007	Histological type not reported
Brinton *et al.* (1990)	667	1 429	1.1 (0.8–1.5)	1.3 (0.9–1.9)	≥ 10	1.1 (0.6–2.0)	Not reported	– conducted in 4 Latin American countries; – hospital and population controls; – RR estimates controlled for HPV 16/18 status
WHO Collaborative Study (1993)	2 361	13 644	1.3 (1.2–1.5)	1.0 (0.8–1.3)	> 8	2.2 (1.8–2.7)	< 0.001	Conducted in 11 centres in 9 countries
Eluf-Neto *et al.* (1994)	197	218	Not reported		≥ 5	2.5 (0.9–7.3)	0.11	– 9 adenocarcinomas, 9 adeno-squamous carcinomas and 3 undifferentiated carcinomas; – conducted in Brazil; – RR estimate controlled for HPV status

Table 17 (contd)

Reference	No. of subjects		Relative risk (95% CI)[a]		Long-term use			Comments
	Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)[a]	*p* for trend	
Chaouki *et al*. (1998)	107	147	1.1 (0.4–3.4)	Not reported	> 5	6.4 (1.3–31)	0.004	– 107 cases and 56 controls with unknown use of oral contraceptives not included; – includes 16 adeno- and adenosquamous carcinomas; – conducted in Morocco; – RR estimate adjusted for HPV infection
Ngelangel *et al*. (1998)	323	380	Not reported		≥ 4	2.0 (0.5–7.6)	(not significant)	– conducted in the Philippines; – RR estimate adjusted for HPV infection

CI, confidence interval; RR, relative risk; HPV, human papillomavirus

[a] Controlled for various potentially confounding variables except HPV, unless otherwise stated

increasing risk with duration of use. The risk decreased slightly with time since last use, from 1.7 (95% CI, 0.8–3.7) for women who had last used oral contraceptives within the past five years to 1.5 (95% CI, 0.9–2.7) for women who had most recently used oral contraceptives more than five years previously.

Brinton *et al*. (1990) conducted a case–control study in selected hospitals in Panama, Costa Rica, Bogota, Colombia, and Mexico City, Mexico, with two age-matched controls selected for each case. In Panama and Costa Rica, one community and one hospital control were selected for each case, while in Bogota and Mexico City, both controls were selected from the same hospital from which the case was recruited. Cervical scrapings from all study subjects were tested for HPV DNA by filter in-situ hybridization. This method is now known to be of low sensitivity and specificity, so that if HPV was found to be associated with use of combined oral contraceptives, there could be residual confounding by HPV infection. After adjustment for age, number of sexual partners, age at first intercourse, interval since last Pap smear, number of births, HPV-16/-18 infection status and education, no increase in risk was seen for women who had ever used oral contraceptives. There was also no trend of increasing risk with increasing duration of use. There was, however, an increased relative risk of 1.7 (95% CI, 1.1–2.6) for women who had used oral contraceptives for more than five years and who had used them most recently within the past three years. The risk for users of this duration who had last used these compounds more than three years previously was not increased. These results are based on 667 cases of squamous-cell carcinoma and 61 cases of adeno-carcinoma. When the analyses were restricted to women with squamous-cell carcinoma, the results were not appreciably different.

The cases in the WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1993) were of invasive cervical squamous-cell carcinoma and were recruited from one or more hospitals in Australia, Chile, Colombia, Israel, Kenya, Mexico, Nigeria and the Philippines. The controls were selected from among women admitted to the same hospitals as the cases for conditions not believed to be associated with the use of hormonal contraceptives. All of the relative risk estimates were controlled for age, centre, number of pregnancies and number of prior Pap smears. Control for additional variables obtained at interview did not appreciably alter the estimated relative risks. The risk of women who had ever used oral contraceptives was estimated to be 1.3 (95% CI, 1.2–1.5). A significant trend of increasing risk with duration of use was observed. Women who had used oral contraceptives in the past year (but not current users) were at increased risk, but a trend of decreasing risk with time since last use was observed. The increase in risk with duration of use was evident four to five years after first exposure, and the risk declined to that of non-users eight years after discontinuation of use.

Eluf-Neto *et al.* (1994) recruited 199 cases of invasive cervical cancer from seven hospitals in São Paulo, Brazil, and 225 controls from the same hospitals. HPV DNA was assayed in cervical scrapings from the study participants by PCR-based methods. After control for HPV status, a nonsignificantly increased risk was observed with duration of oral contraceptive use.

In a study in Rabat, Morocco, Chaouki *et al.* (1998) recruited 214 cases of invasive cervical cancer from a single cancer hospital and 203 controls from the same hospital or a nearby general hospital. HPV DNA was assayed in cervical specimens by a PCR-based assay. On the basis of 107 cases and 147 controls with a known history of use of oral contraceptives, no increase in the risk for cervical cancer was observed among women who had ever used oral contraceptives, after control for HPV status. A significant trend of increasing risk with duration of use was observed, however.

Ngelangel *et al.* (1998) recruited cases of invasive cervical cancer and controls from a single hospital in Manila, the Philippines. PCR-based assays for HPV DNA were performed on cervical scrapings from the study subjects. After control for HPV DNA status, a significant trend of increasing risk with duration of hormonal contraceptive use was observed.

(*d*) *Population-based studies of invasive squamous-cell cervical carcinoma*

The results of seven case–control studies of invasive squamous-cell cervical carcinoma in which population controls were used are summarized in Table 18. Peters *et al*. (1986b) identified 200 cases of invasive squamous-cell cervical carcinoma from the Los Angeles Cancer Registry, United States, and compared them with 200 neighbourhood controls. No trend of increasing risk with increasing duration of oral contraceptive use was observed in univariate analyses. No information on risk in relation to features of use other than duration was reported.

Celentano *et al*. (1987) identified 153 cases of invasive squamous-cell cervical cancer in women who had been admitted to Johns Hopkins Hospital in Baltimore, Maryland (United States) between 1982 and 1984. The controls were selected from among women residing in the same neighbourhood as the cases. No increase in risk was seen for women who had ever used oral contraceptives after control for use of other methods of contraception (condom, intrauterine device, diaphragm and vaginal spermicides), age at first intercourse, years of smoking cigarettes, frequency of Pap smears, use since last Pap smear and having visited an obstetrician–gynaecologist. No additional information was provided on risk in relation to various features of oral contraceptive use.

In a case–control study in the United States (Brinton *et al*., 1986, 1987), cases were recruited from 24 participating hospitals in Birmingham, Chicago, Denver, Miami and Philadelphia between 1982 and 1984. The controls were selected by random-digit dialling from the same populations from which the cases came. After control for age, ethnic origin, number of sexual partners, age at first intercourse, education, interval since last Pap smear and history of a non-specific genital infection or sore, the relative risk for invasive squamous-cell cervical carcinoma among women who had used oral contraceptives for more than 10 years was estimated to be 1.6 (95% CI, 0.9–2.9). This result is based on 417 women with squamous-cell carcinomas; when they were combined with 62 women with adenocarcinomas or adenosquamous carcinomas, the risk increased with duration of use. Analyses of both histological types indicated a higher risk for women

Table 18. Case–control studies of use of oral contraceptives and invasive squamous-cell cervical carcinoma: population controls

Reference	No. of subjects		Relative risk (95% CI)[a]		Long-term use			Comments
	Cases	Controls	Ever	Current	Duration (years)	RR (95% CI)[a]	*p* for trend	
Peters *et al*. (1986b)	200	200	Not reported		≥ 10	1.1 (0.5–2.7)	NS	– conducted in Los Angeles, USA; – risk relative to no use and use of < 2 years – univariate analysis only
Celentano *et al*. (1987)	153	153	0.7 (0.3–1.9)		Not reported			Conducted in Maryland, USA
Brinton *et al*. (1986)	417	789			≥ 10	1.6 (0.9–2.9)		Conducted in 5 US cities
Irwin *et al*. (1988)	129	631	0.8 (0.5–1.3)	0.3 (0.1–0.8)	≥ 5	0.9 (0.5–1.6)	NS	Conducted in Costa Rica
Bosch *et al*. (1992)	432	376	1.3 (0.9–2.0)					– conducted in Colombia and Spain; – RR estimates controlled for HPV status assessed by PCR; – risk increased with duration of use in HPV DNA-positive women only; in comparison with HPV-positive controls, RR = 8.9 (1.1–72)
Kjaer *et al*. (1993)	58	607	1.3 (0.5–3.3)	1.3 (0.5–3.7)	≥ 6	1.3 (0.5–3.5)	0.38	Conducted in Copenhagen, Denmark
Daling *et al*. (1996)	221	466	1.0 (0.6–1.6)		≥ 5	1.3 (0.7–2.2)	NS	– conducted in Washington State, USA; – RR, 2.3 (95% CI, 1.4–3.9) in women who used oral contraceptives before the age of 17, controlling for HPV-16 antibody status

RR, relative risk; CI, confidence interval; NS, not significant; HPV, human papillomavirus; PCR, polymerase chain reaction

[a] Controlled for various potentially confounding variables except HPV, unless otherwise stated

who had used oral contraceptives within the past year than for those who had used them in the more distant past.

In the study conducted in Costa Rica by Irwin *et al*. (1988), described above, 129 women with invasive cervical cancer (assumed to be squamous-cell) were compared with 631 controls selected from the general population of Costa Rica. No increase in risk was seen for women who had ever used oral contraceptives, and no trend of increasing risk with duration of use was found. Women who had used oral contraceptives within the past year were actually at reduced risk for disease, the estimate being 0.3 (95% CI, 0.1–0.8), but this estimate was based on only seven cases and 102 controls. No trend in risk with time since last use was observed. All of the estimates were adjusted for age, history of sexually transmitted disease or pelvic inflammatory disease, gravidity, age at first intercourse, number of sexual partners and history of prior Pap smears.

In a study of risk factors for cervical cancer in Colombia and Spain, Bosch *et al*. (1992) identified 436 women with histologically confirmed squamous-cell carcinoma and selected 387 controls from the general population in which the cases arose. No increase in risk was observed for women who had ever used oral contraceptives. Cervical scrapings from the study subjects were assayed for type-specific HPV DNA by PCR. No trend of increasing risk with duration of use was observed for women who had no HPV DNA, but a trend was observed for women who had HPV DNA, and this observation was statistically significant (p for trend = 0.027). This observation is, however, based on very small numbers of HPV DNA-positive controls: 17 among women who had never used oral contraceptives and one woman each who had used oral contraceptives for 1–9 and 10 or more years. The numbers of cases in these three categories were 110, 12 and 35, respectively. The relative risks in relation to non-users were estimated to be 3.0 (95% CI, 0.3–28) for users of oral contraceptives for 1–9 years and 8.9 (95% CI, 1.1–72) for users of more than 10 years' duration.

Kjaer *et al*. (1993) recruited 59 women with invasive cervical cancer and living in the greater Copenhagen area from the Danish Cancer Registry; the controls were selected from the general female population of greater Copenhagen. The risk for invasive squamous-cell cervical cancer was not significantly increased among women who had ever used oral contraceptives, and no significant trend of increasing risk with duration of use was observed. The relative risk of women who had used oral contraceptives within the past two years was 1.7 (95% CI, 0.6–4.7). The risk decreased to 1.0 for women who had last used oral contraceptives more than two years previously (p for trend = 0.002). The relative risks in this study were adjusted for age, years of school attendance, number of sexual partners, proportion of sexually active life without use of barrier contraceptives, ever having had gonorrhoea and ever having had a Pap smear.

In a population-based case–control study conducted in Washington State, United States, Daling *et al*. (1996) interviewed 221 women with invasive squamous-cell cervical carcinoma and 466 control women selected by random-digit dialling. Serum from most of the study subjects was tested for HPV-16 capsid antibodies. After adjustment for age, number of Pap smears in the last decade and lifetime number of sexual partners, the risk

was not increased for women who had ever used oral contraceptives, and no trend of increasing risk with duration of use was observed. After control for HPV antibody status, the risks relative to that of women who first began using oral contraceptives after the age of 20 were 1.6 (95% CI, 1.0–2.4) for women who had first used them between the ages of 18 and 19 and 2.3 (95% CI, 1.4–3.8) for women who had first begun using them at age 17 or younger. No information was given on risk in relation to time since last use of oral contraceptives.

The results of the studies summarized in Tables 17 and 18 are not totally consistent, but some generalizations can be made cautiously. If the risk is increased in women who have ever used oral contraceptives, then the increase in risk is likely to be modest. Most of the studies do show a small increase in risk for 'ever users', but the risks are small, and the 95% CIs of the estimates in most instances include unity. The relative risk estimates for long-term users are generally higher in the hospital-based studies (Table 17) than in the population-based studies (Table 18), but the estimates are not consistently higher or lower in hospital-based studies in which HPV DNA status was considered than in such studies in which it was not. The higher relative risks in hospital-based than in population-based studies are therefore probably not due to differences in confounding.

None of the studies indicates that risk is increased long after initial exposure to oral contraceptives. The only possible exception is the study of Daling *et al*. (1996), in which it was found that the risk for women who were first exposed to oral contraceptives before the age of 17 was increased. This observation requires independent confirmation.

In the three studies in which risk was considered in relation to use of oral contraceptives among women with and without other risk factors for cervical cancer (Brinton *et al*., 1986; Parazzini *et al*., 1990; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1993), there was some suggestion that the risk in relation to oral contraceptives might be greater in women with than without such sexual risk factors as a history of non-specific genital infection or sore (Brinton *et al*., 1986), absence of use of barrier contraceptives (Brinton *et al*., 1986; Parazzini *et al*., 1990), having had multiple sexual partners (Parazzini *et al*., 1990), a history of sexually transmitted diseases and presence of herpes simplex virus-II antibodies (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1993). These observations are consistent with the idea that oral contraceptives enhance risk in the presence of a sexually transmitted oncogenic agent such as certain strains of HPV.

Table 19 summarizes the results of the four studies (described above) in which the risk for invasive squamous-cell cervical cancer in relation to oral contraceptive use was estimated on the basis of a comparison of cases and controls with evidence of HPV DNA in cervical cells. In each study, the relative risk estimates for women with HPV DNA were increased, and evidence for a trend of increasing risk with duration of use of combined oral contraceptives is provided from three of the studies. These results should be interpreted with caution, however, because few controls were found to be HPV-positive and all of the estimates therefore have wide confidence limits. In addition, three of the four studies shown in Table 19 are hospital-based.

Table 19. Case–control studies of use of oral contraceptives and invasive squamous-cell cervical cancer in which analyses were restricted to women with human papillomavirus (HPV) DNA in cervical scrapings

Reference	Use of oral contraceptives	All subjects			HPV-positive subjects		
		No. of subjects		RR (95% CI)[a]	No. of subjects		RR (95% CI)[a]
		Cases	Controls		Cases	Controls	
Bosch *et al*. (1992)	Never	291	270	1.0	110	17	1.0
	Ever	141	106	1.3 (0.9–2.0)	50	2	6.5 (1.3–31)
Eluf-Neto *et al*. (1994); Bosch *et al*. (1995)	Years of use						
	None	125	152	1.0	97	21	1.0
	1–4	39	44	1.3 (0.7–2.3)	30	9	1.2 (0.4–4.2)
	≥ 5	33	22	2.7 (1.4–5.2)	27	2	9.0 (1.4–57)
Chaouki *et al*. (1998)	Years of use						
	< 1	8	25	1.0	20	7	1.0
	1	14	14	1.4 (0.2–8.1)			
	2–5/2–4[b]	32	35	2.8 (0.6–13)	21	6	1.0 (0.2–6.6)
	> 5/≥ 5[b]	39	42	6.4 (1.3–31)	37	3	16 (2.2–115)
Ngelangel *et al*. (1998)	Years of use						
	None	258	277	1.0	NR	NR	1.0
	1–3	40	80	0.3 (0.1–0.7)	NR	NR	0.3 (0.1–0.8)
	≥ 4	25	23	2.0 (0.5–7.6)	NR	NR	2.8 (0.2–30)
	Total	323	380	–	303	35	–

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

[a] Controlled for various potentially confounding variables

[b] Years of use in HPV-positive subjects

In summary, if there is an increased risk for squamous-cell cervical carcinoma in relation to use of oral contraceptives, it is more likely to be found in relation to invasive rather than in-situ disease. The available evidence indicates that the effect of oral contraceptives on risk probably requires the presence of HPV DNA in the cervical epithelium.

(*e*) *Studies of invasive cervical adeno- and adenosquamous carcinomas*

An early case–control study in Milan, Italy (Parazzini *et al*., 1988), showed a relative risk of 0.8 (95% CI, 0.2–2.4) for cervical adenocarcinoma among women who had ever used combined oral contraceptives. Five case–control studies have been conducted to assess the risk of adenocarcinomas and adenosquamous carcinomas in relation to duration of use of oral contraceptives (Table 20). The study of Brinton *et al*. (1990), described previously, included 41 women with adenocarcinoma and 20 women with adenosquamous carcinoma. The risk for either neoplasm among women who had ever used oral contraceptives was estimated to be 2.4 (95% CI, 1.3–4.6). No trend of increasing risk with duration of use was observed. The relative risk estimates were adjusted for age, number of sexual partners, age at first sexual intercourse, interval since last Pap smear, number of births, HPV-16/-18 infection status and education.

Thomas *et al*. (1996) analysed data from the WHO Collaborative Study of Neoplasia and Steroid Contraceptives, described previously. A total of 271 women with adenocarcinoma and 106 women with adenosquamous carcinoma were included in the study. The risk of women who had ever used oral contraceptives was increased for adenocarcinoma but not for adenosquamous carcinoma. A significant trend of increasing risk for adenocarcinoma was observed with duration of oral contraceptive use; no similar trend was observed for women with adenosquamous carcinoma, but, when both histological types were combined, a significant trend of increasing risk was observed. The risk for adenocarcinoma was highest among women who had used these products within the past year and generally declined with time since last use. These trends were strongest for neoplasms that developed in women under the age of 35. The association with risk was also somewhat stronger for formulations with high-potency progestogens than for low-potency products.

Brinton *et al*. (1986, 1987) analysed data from the population-based case–control study conducted in five US cities described previously to assess the risks for adenocarcinoma, adenosquamous carcinoma and both. As in the study of Thomas *et al*. (1996), the risk of long-term users of oral contraceptives was more strongly related to adenocarcinoma than to adenosquamous carcinoma.

Between 1977 and 1991, Ursin *et al*. (1994) identified 195 cases of adenocarcinoma and adenosquamous carcinoma from the Los Angeles Cancer Registry, United States, which were compared with 386 neighbourhood controls. After adjustment for education, household income, number of sexual partners before the age of 20, number of episodes of genital warts, months of diaphragm use and weight gain between the age of 18 and the time of diagnosis, the relative risk of women who had ever used oral contraceptives was

Table 20. Case–control studies of use of oral contraceptives and cervical adeno- and adenosquamous carcinomas

Reference	Type of case	No. of subjects		Ever use	Long-term use			Comments
		Cases	Controls	RR (95% CI)[a]	Duration (years)	RR (95% CI)[a]	*p* for trend	
Brinton *et al*. (1990)	Adenocarcinoma and adenosquamous carcinoma	61	1 429	2.4 (1.3–4.6)	≥ 10	1.8 (0.5–6.5)	NS	– hospital and population controls; – conducted in 4 Latin American countries
Thomas *et al*. (1996)	Adenocarcinoma	271	2 084	1.6 (1.2–2.1)	≥ 8	2.4 (1.4–4.0)	0.003	– hospital controls; – conducted in 10 centres in 8 countries
	Adenosquamous carcinoma	106	803	1.1 (0.7–1.8)	≥ 8	1.6 (0.6–4.1)	NS	
	Both	377	2 887	1.5 (1.1–1.9)	≥ 8	2.2 (1.4–3.5)	0.003	
Brinton *et al*. (1986, 1987)	Adenocarcinoma	40	801		≥ 10	2.4[b]	0.15	– population controls; – conducted in 5 US cities; – separate analyses of adenocarcinoma and adenosquamous cancer based on very small numbers of women who used oral contraceptives for 10 years or longer (5 and 2, respectively)
	Adenosquamous carcinoma	23	801		≥ 10	1.3[b]	0.77	
	Both	62	789		≥ 10	3.0 (1.1–8.2)		
Ursin *et al*. (1994)	Adenocarcinoma and adenosquamous carcinoma	195	386	2.1 (1.1–3.8)	≥ 12	4.4 (1.8–11)	0.04	– population controls; – conducted in Los Angeles, USA – 150 cases were adenocarcinomas; 15 were adenosquamous; no pathological confirmation of the other cases
Ngelangel *et al*. (1998)	Adenocarcinoma or adenosquamous carcinoma	33	380	Not given	≥ 4	4.3 (0.3–57)	NS	– hospital controls; – conducted in the Philippines; – RR adjusted for HPV infection; – RR based on 4 exposed cases and 23 exposed controls only; – use of all hormonal contraceptives reported, but largely represents use of combined oral contraceptives

RR, relative risk; CI, confidence interval; NS, not significant; HPV, human papillomavirus

[a] Controlled for various potentially confounding variables except HPV, unless otherwise stated

[b] RR adjusted only for age and race

estimated to be 2.1 (95% CI, 1.1–3.8). The risk increased significantly with duration of use. The relative risk of current users of contraceptives was 1.8 (95% CI, 0.6–5.7) and was close to unity for women who had used oral contraceptives more than one year in the past. These results did not change when the analysis was limited to the 150 cases of adenocarcinoma.

In the study in the Philippines (Ngelangel *et al.*, 1998) summarized above, data for 33 cases of adenocarcinoma or adenosquamous carcinoma indicated an increased risk for women who had used oral contraceptives for four years or more. The estimate is based on only three exposed cases, however, and the confidence limits of the estimates are wide and include unity.

In the aggregate, the results of the five studies summarized in Table 20 suggest that long-term use of oral contraceptives increases the risk for cervical carcinomas with adenomatous elements, although confounding by HPV infection cannot be ruled out. The association with use of oral contraceptives appears to be somewhat stronger for adenocarcinoma than for adenosquamous carcinoma.

It has also been suggested that use of oral contraceptives is more strongly related to adenocarcinoma than to squamous-cell carcinoma of the cervix. The results summarized in Tables 17, 18 and 20 are inconsistent in this regard. The study conducted in four Latin American countries (Brinton *et al.*, 1990) provided estimates for users of more than 10 years of 1.1 for squamous-cell carcinoma and 1.8 for adenocarcinoma or adenosquamous carcinoma. The studies in Los Angeles provide estimates of 1.1 for squamous-cell carcinoma in users of more than 10 years' duration (Peters *et al.*, 1986b) and 4.4 for adenocarcinoma or adenosquamous carcinoma combined in users of more than 12 years' duration (Ursin *et al.*, 1994); these results, however, are based on different study populations. The study in the Philippines (Ngelangel *et al.*, 1998) found relative risks of 2.0 and 4.3 in users of four years' or more duration for squamous and adenomatous carcinomas, respectively. The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1993) provided an estimate of 2.2 for both squamous-cell carcinoma and tumours with adenomatous elements (adenocarcinoma and adenosquamous carcinoma combined; Thomas *et al.*, 1996) in women who had used oral contraceptives for more than eight years. In the study conducted in five United States cities (Brinton *et al.*, 1986, 1987), the risks for adenocarcinoma and adenosquamous carcinoma combined of users of more than 10 years' duration was estimated to be 3.0, while the estimate for squamous-cell carcinoma was 1.6. The estimate for squamous-cell carcinoma adjusted only for age and race was 1.2. For adenocarcinoma and adenosquamous carcinoma separately, the relative risks adjusted for age and race were 2.4 and 1.3, respectively. The results of these studies thus do not resolve the question of whether use of oral contraceptives is more strongly related to adenocarcinoma and adenosquamous carcinoma than to squamous-cell carcinoma.

Another method that has been used to address the issue of the relative strength of the association between oral contraceptives and various histological types of cervical carcinoma is comparison of use by women with squamous and adenomatous cervical lesions. Persson *et al.* (1987) compared the oral contraceptive use of 23 women with adeno-

carcinoma with that of 46 women with squamous-cell carcinoma. The proportions of women who had used oral contraceptives in each group were similar, and the duration of use did not differ. Jones and Silverberg (1989) similarly compared 18 cases of endocervical adenocarcinoma with an equal number of cases of squamous-cell carcinoma; both groups included both *in situ* and invasive disease. The proportions of women in the two groups who had used oral contraceptives did not differ significantly. Honoré *et al*. (1991) compared each of 99 women with cervical adenocarcinoma with three comparable women with squamous-cell carcinoma, with matching on age, year of diagnosis and clinical stage. The women in the two groups did not differ with respect to any use of oral contraceptives and, among users, the two groups did not differ with respect to age at start of use, age at discontinuation of use or months of use of oral contraceptives. Hopkins and Morley (1991) compared 61 women with adenocarcinoma and 206 women with squamous-cell carcinoma who were under the age of 40. Thirty-three per cent of the women with adenocarcinomas and 31% of those with squamous-cell carcinomas had ever used oral contraceptives. The results of these clinical studies do not support the hypothesis that use of oral contraceptives is more strongly related to the development of adenocarcinoma than squamous carcinoma of the uterine cervix.

On balance, there appears to be insufficient evidence to conclude firmly that use of oral contraceptives is related to adenocarcinoma of the uterine cervix. The associations observed could be due to residual confounding by HPV infection, and a firm conclusion about the risk for adenocarcinoma of users of oral contraceptives must await the results of investigations that adequately control for HPV infection.

2.4 Ovarian cancer

2.4.1 *Descriptive studies*

Younger women in several developed countries have experienced substantial declines in the incidence and mortality rates of ovarian cancer. Cohort analyses based on data from Switzerland (Levi *et al.*, 1987), England and Wales (Beral *et al.*, 1988; dos Santos Silva & Swerdlow, 1995), Great Britain (Villard-Mackintosh *et al.*, 1989), Sweden (Adami *et al.*, 1990) and the Netherlands (Koper *et al.*, 1996) and a systematic analysis of mortality trends in 16 European countries (La Vecchia *et al.*, 1992, 1998) showed that women born after 1920—i.e. the generations that have used combined oral contraceptives—have consistently reduced ovarian cancer rates. The downward trends were greater in countries where combined oral contraceptives have been most widely used (La Vecchia *et al.*, 1998).

Thus, descriptive data on the incidence and mortality rates of ovarian cancer are consistent with the hypothesis of a favourable effect of combined oral contraceptive use on subsequent ovarian cancer rates.

2.4.2 *Cohort studies*

The results of cohort studies on use of combined oral contraceptives and ovarian cancer are summarized in Table 21. Most of the evidence refers to epithelial neoplasms, unless otherwise specified. Three cohort studies conducted in the United States and the

Table 21. Selected cohort studies on use of combined oral contraceptives and ovarian cancer, 1980–97

Reference	No. of cases (age, years)	Relative risk (95% CI)		Comments
		Any use	Longest use	
Ramcharan *et al*. (1981a), USA	16 (18–64)	0.4 (0.1–1.0)	–	Adjusted for age only; Walnut Creek Study on Contraception
Beral *et al*. (1988), UK	30 (≥ 25)	0.6 (0.3–1.4)	≥ 10 years, 0.3	Royal College of General Practitioners' cohort
Vessey & Painter (1995), UK	42 (all)	0.4 (0.2–0.8)	> 8 years, 0.3 (0.1–0.7)	Oxford Family Planning cohort
Hankinson *et al*. (1995), USA	260 (30–65)	1.1 (0.8–1.4)	≥ 5 years, 0.7 (0.4–1.1)	Nurses' Health Study

CI, confidence interval

United Kingdom provided data on a total of about 100 cases of epithelial ovarian cancer. In the Walnut Creek Study in the United States (Ramcharan *et al.*, 1981a), 16 cases of ovarian cancer were registered between 1968 and 1977, corresponding to an age-adjusted relative risk of 0.4 for any use of combined oral contraceptives.

The Royal College of General Practitioners' study was based on 47 000 women recruited in 1968 in 1400 British general practices (Beral *et al.*, 1988): 30 cases of ovarian cancer were observed up to 1987, corresponding to multivariate relative risks of 0.6 (95% CI, 0.3–1.4) for any use of combined oral contraceptives and of 0.3 for 10 years of use or more. Allowance was made in the analysis for age, parity, smoking and social class. In a subsequent follow-up study of mortality in that cohort up to the end of 1993 (Beral *et al*., 1999), 55 deaths from ovarian cancer were reported; there was a statistically significantly reduced mortality rate from ovarian cancer among women who had ever used oral contraceptives (relative risk, 0.6; 95% CI, 0.3–1.0).

The study of the Oxford Family Planning Association was based on 17 032 women enrolled between 1968 and 1974 from various family planning clinics in the United Kingdom (Vessey & Painter, 1995). Up to October 1993, 42 cases of ovarian cancer were registered, corresponding to relative risks of 0.4 (95% CI, 0.2–0.8) for any use of combined oral contraceptives and 0.3 (95% CI, 0.1–0.7) for more than eight years of use. Adjustment was made for age and parity.

In the Nurses' Health study, based on 121 700 registered nurses aged 30–55 in 1976, 260 cases of ovarian cancer were observed prospectively between 1976 and 1988 (Hankinson *et al.*, 1995). The multivariate relative risk for any use, which essentially reflected former use, was 1.1 (95% CI, 0.8–1.4) but declined to 0.7 (95% CI, 0.4–1.1) for use for five years or more. Adjustment was made for age, tubal ligation, age at menarche, age at menopause, smoking and body mass index.

2.4.3 *Case–control studies*

The epidemiological evidence from case–control studies on use of combined oral contraceptives and ovarian cancer is well defined and consistent: at least 20 out of 21 studies published between 1980 and 1997 found relative risks below unity, the sole apparent outlier being a study conducted in China (Shu *et al.*, 1989).

Table 22 gives the main results of case–control studies of ovarian cancer published between 1980 and 1997 which included information on use of combined oral contraceptives. Table 23 gives age-specific relative risks and 95% CIs, while Table 24 gives the relative risks related to time since last use for studies that provided the relevant information. The findings of two pooled analyses of case–control studies on the issue are also included. These were conducted on 971 cases and 2258 controls in three European countries (Franceschi *et al.*, 1991a) and on 2197 cases and 8893 controls in white women from 12 studies in the United States (Whittemore *et al.*, 1992), for a total of over 3100 cases and 11 000 controls.

In a pooled analysis of individual data from three hospital-based European studies (Franceschi *et al.*, 1991a), the multivariate relative risk was 0.6 (95% CI, 0.4–0.8) for any

Table 22. Selected case–control studies of use of oral contraceptives and ovarian cancer, 1980–97

Reference, location	Type of study	No. of cases (age, years)	Relative risk (95% CI)			Comments
			Any use	Longest use	Duration (years)	
Willett *et al.* (1981), USA	Nested in a cohort	47 (< 60)	0.8 (0.4–1.5)	0.8 (0.3–2.1)	> 3	Prevalent cases from the Nurses' Health cohort study; adjusted for age
Hildreth *et al.* (1981), USA	Hospital-based	62 (45–74)	0.5 (0.1–1.7)	Not reported		Adjusted for age and parity; odds ratio based on 3 cases with use of oral contraceptives
Weiss *et al.* (1981a), USA	Population-based	112 (36–55)	0.6 (not reported)	0.4 (0.2–1.3)	≥ 9	Adjusted for age, demographic factors and parity
Franceschi *et al.* (1982), Italy	Hospital-based	161 (19–69)	0.7 (0.4–1.1)	Not reported		Adjusted for age
Cramer *et al.* (1982), USA	Population-based	144 (< 60)	0.4 (0.2–1.0)	0.6	> 5	Adjusted for age and parity
Rosenberg *et al.* (1982), USA	Hospital-based	136 (< 60)	0.6 (0.4–0.9)	0.3 (0.1–0.8)	≥ 5	Protection by use of combined and sequential oral contraceptives; independent of parity; adjusted for several variables
Risch *et al.* (1983), USA	Population-based	284 (20–74)	[0.5] (not reported)	Not reported		Multivariate odds ratio approximately 0.9 per year of use
Tzonou *et al.* (1984), Greece	Hospital-based	150 (all ages)	0.4 (0.1–1.1)	Not reported		Adjusted for age, parity, age at menopause and use of oestrogen replacement therapy
Cancer and Steroid Hormone Study (1987), USA	Population-based	492 (20–54)	0.6 (0.5–0.7)	0.2 (0.1–0.4)	≥ 10	Consistent results by type of combined oral contraceptive
Harlow *et al.* (1988), USA	Population-based	116 (20–79)	0.4 (0.2–0.9)	0.4 (0.2–1.0)	> 4	Borderline malignancy; adjusted for age and parity

Table 22 (contd)

Reference, location	Type of study	No. of cases (age, years)	Relative risk (95% CI)			Comments
			Any use	Longest use	Duration (years)	
Wu *et al.* (1988), USA	Hospital- and population-based	299 (18–74)	0.7 (0.5–1.1)	0.4 (0.2–0.7)	> 3	Combination of two studies conducted in the 1970s and 1980s
Shu *et al.* (1989), China	Population-based	229 (18–70)	1.8 (0.8–4.1)	1.9 (0.4–9.3)	> 5	Only 23 cases and 12 controls had ever used combined oral contraceptives
WHO Collaborative Study (1989a), 7 countries	Hospital-based	368 (< 62)	0.8 (0.6–1.0)	0.5 (0.3–1.0)	> 5	Similar results in developed and developing countries
Hartge *et al.* (1989a), USA	Hospital-based	296 (20–79)	1.0 (0.7–1.7)	0.8 (0.4–1.5)	> 5	Data collected between 1978 and 1981
Booth *et al.* (1989), UK	Hospital-based	235 (< 65)	0.5 (0.3–0.9)	0.1 (0.01–1.0)	> 10	Consistent results in strata of parity
Parazzini *et al.* (1991a), Italy	Hospital-based	505 (22–59)	0.7 (0.5–1.0)	0.5 (0.3–0.9)	≥ 2	Protective effect present in strata of major risk factors for ovarian cancer
Parazzini *et al.* (1991b), Italy	Hospital-based	91 (23–64)	0.3 (0.2–0.6)	0.2 (0.1–0.6)	≥ 2	Borderline malignancy; adjusted for age, parity, education, age at menopause and oral contraceptive use
Polychronopoulos *et al.* (1993), Greece	Hospital-based	189 (< 75)	0.8 (0.2–3.7)	Not reported		Multivariate RR; only three cases and seven controls had ever used combined oral contraceptives
Rosenberg *et al.* (1994), USA	Hospital-based	441 (< 65)	0.8 (0.6–1.0)	0.5 (0.2–0.9)	≥ 10	Association persisted as long as two decades after stopping and was not confined to any type of oral contraceptive formulation

Table 22 (contd)

Reference, location	Type of study	No. of cases (age, years)	Relative risk (95% CI)			Comments
			Any use	Longest use	Duration (years)	
Risch *et al.* (1994, 1996), Canada	Population-based	450 (35–79)	0.5 (0.4–0.7)	0.3 (0.2–0.6)	≥ 10	The inverse relationship was stronger for non-mucinous (RR, 0.9) for each year of use than for mucinous tumours (RR, 1.0); trend per year of use, 0.9 among all subjects
Purdie *et al.* (1995), Australia	Population-based	824 (18–79)	0.5 (0.4–0.7)	0.3 (0.2–0.4)	≥ 1	Adjusted for parity
Pooled analyses						
Franceschi *et al.* (1991a), Greece, Italy, UK	Three hospital-based studies	971 (< 65)	0.6 (0.4–0.8)	0.4 (0.2–0.7)	≥ 5	Protection was still present ≥ 15 years after stopping use (odds ratio, 0.5).
Whittemore *et al.* (1992), USA	Pooled analysis of 12 US population- and hospital-based case–control studies	2197 (all)	0.7 (0.6–0.8)	0.3 (0.2–0.4)	≥ 6	Invasive epithelial neoplasms in white women; protection present in population- and hospital-based studies
Harris *et al.* (1992), USA	Same pooled analysis as Whittemore *et al.* (1992)	327	0.8 (0.6–1.1)	0.6 (0.4–0.9)	> 5	Epithelial tumours of low malignant potential in white women
John *et al.* (1993), USA	Pooled analysis of 7 of the 12 studies in the pooled analysis of Whittemore *et al.* (1992)	110	0.7 (0.4–1.2)	0.6 (0.2–1.6)	≥ 6	Epithelial ovarian cancers in black women

CI, confidence interval; RR, relative risk

Table 23. Selected case–control studies on use of combined oral contraceptives and ovarian cancer, 1980–97; age-specific relative risks

Reference	Age group (years)	Relative risk (95% CI)
Willett *et al.* (1981)	< 35	0.3 (0.1–1.3)
	35–44	1.1 (0.4–3.2)
	≥ 45	1.3 (0.4–3.9)
Rosenberg *et al.* (1982)	18–29	0.4
	30–39	0.6
	40–49	0.5
	50–59	0.7
Centers for Disease Control (1983b)	20–29	0.3 (0.1–1.4)
	30–39	0.8 (0.3–2.0)
	40–49	0.6 (0.4–1.1)
	50–54	0.6 (0.3–1.1)
Parazzini *et al.* (1991a)	< 35	0.4 (0.2–0.9)
	35–44	0.8 (0.4–1.4)
	45–54	0.7 (0.4–1.3)
	55–59	0.8 (0.1–7.6)
Rosenberg *et al.* (1994)	< 45	0.5 (0.3–0.8)
	45–65	0.7 (0.4–1.2)
Pooled analysis		
Franceschi *et al.* (1991a)	< 45	0.6 (0.3–1.0)
	45–54	0.5 (0.3–1.0)
	55–64	0.6 (0.4–0.9)

CI, confidence interval

use and 0.4 (95% CI, 0.2–0.7) for longest (≥ 5 years) use. Allowance was made in the analysis for age, other socio-demographic factors, menopausal status and parity. The protection persisted for at least 15 years after use had ceased.

In a pooled analysis of individual data from 12 studies in the United States (Whittemore *et al.*, 1992), the corresponding values were 0.7 (95% CI, 0.6–0.8) for any use and 0.3 (0.2–0.4) for use for more than six years in the population-based studies. Adjustment was made for age, study and parity. The results were similar when the hospital-based and population-based studies were considered separately: the relative risks were 0.7 in both types of study for any use of combined oral contraceptives, 0.6 in hospital-based studies and 0.3 in population-based studies for longest use (> 6 years) and 0.95 (not significant) and 0.90 ($p < 0.001$), respectively, per added year of use.

An inverse association was also observed in a further analysis of seven studies of 110 cases and 251 controls in black women in the United States. The relative risk was 0.7 for any use and 0.6 for use for six years or more (John *et al.*, 1993). The United States pooled

Table 24. Selected case–control studies on use of combined oral contraceptives and ovarian cancer, 1980–97; results according to time since last use

Reference	Time since last use (years)	Relative risk (95% CI)
Cramer *et al.* (1982)	< 2	2.1 (NR)
	2–< 6	0.7 (NR)
	6–< 10	0.7 (NR)
	≥ 10	0.3 (NR)
Rosenberg *et al.* (1982)	< 1	0.3 (NR)
	1–4	0.4 (NR)
	5–9	0.8 (NR)
	≥ 10	0.5 (NR)
Centers for Disease Control (1983b)	< 1	1.0 (0.4–2.2)
	1–4	0.6 (0.3–1.1)
	5–9	0.5 (0.3–0.9)
	≥ 10	0.5 (0.3–0.9)
Harlow *et al.* (1988)	≤ 5	0.3 (0.1–0.9)
	> 5	0.6 (0.3–1.4)
WHO Collaborative Study (1989a)	< 0.5	0.9 (0.5–1.8)
	0.5–< 5	0.9 (0.5–1.5)
	5–< 10	0.8 (0.5–1.4)
	≥ 10	0.5 (0.3–0.9)
Hartge *et al.* (1989a)	< 1	0.5 (0.1–1.6)
	1–9	0.9 (0.5–1.6)
	≥ 10	1.4 (0.7–2.6)
Parazzini *et al.* (1991a)	< 10	0.5 (0.3–0.8)
	≥ 10	0.9 (0.5–1.5)
Rosenberg *et al.* (1994)	< 15	0.4 (0.2–0.8)
	15–19	0.5 (0.3–1.0)
	≥ 20	0.8 (0.4–1.5)

CI, confidence interval; NR, not reported

analysis also included data on 327 cases of epithelial ovarian neoplasms of borderline malignancy in white women. The relative risks were 0.8 (95% CI, 0.6–1.1) for any use of combined oral contraceptives and 0.6 (0.4–0.9) for five years of use or more (Harris *et al.*, 1992).

The most convincing aspect of the inverse relationship between use of combined oral contraceptives and risk for ovarian cancer is the consistency of the results, independently of the type of study (hospital- or population-based), geographical area (Australia, Europe, North America and developing countries) and type of analysis, including allowance for covariates which differed from study to study, although more variables tended to be included in most recent ones. Likewise, the inverse relationship

between use of combined oral contraceptives and ovarian cancer was observed for most types of formulations considered, including those with low doses (Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development, 1987; Rosenblatt *et al.*, 1992; Rosenberg *et al.*, 1994).

The overall estimate of protection for any use is approximately 40%, and a steady inverse relationship exists with duration of use. The decrease in risk was over 50%, and probably around 60% for use for more than five years; however, in contrast to the findings of the Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development (1987), no protection was evident after very short-term use, i.e. three to six months, in an analysis of factors associated with the short-term use of oral contraceptives by Gross *et al.* (1992) on the data of the above study.

Willett *et al.* (1981) conducted a case–control study of 47 cases of ovarian cancer and 470 controls nested in the Nurses' Health Study cohort (based on 121 964 registered nurses aged 30–55 in 1986 and residing in 11 large American states). They found an age-adjusted relative risk of 0.8 (95% CI, 0.4–1.5) for any use of combined oral contraceptives and 0.2 (95% CI, 0.1–1.0) for women aged 35 or younger, who were more likely to be current users.

Hildreth *et al.* (1981) considered 62 cases of epithelial ovarian cancer and 1068 hospital controls aged 45–74 in Connecticut, United States, that had been diagnosed between 1977 and 1979. The response rate was 71% for both cases and controls. The multivariate relative risk for any use of combined oral contraceptives, after allowance for age and parity, was 0.5 (95% CI, 0.2–1.7).

Weiss *et al.* (1981a), in a population-based case–control study of 112 cases diagnosed between 1975 and 1979 in Washington and Utah, United States, found a relative risk (adjusted for age, demographic factors and parity) of 0.6 for any use and 0.4 (95% CI, 0.2–1.3) for longest use, which was of borderline statistical significance ($p = 0.04$). The response rate was 66% for cases and 92% for controls.

Franceschi *et al*. (1982) considered data on 161 cases of epithelial ovarian cancer and 561 hospital controls in women interviewed in Milan, Italy, in 1979–80. The age-adjusted relative risk for ever use was 0.7 (95% CI, 0.4–1.1).

Cramer *et al.* (1982) conducted a case–control study of 144 cases and 139 population controls in 1978–81 in the Greater Boston area (United States) and found a relative risk, adjusted for age and parity, of 0.4 (95% CI, 0.2–1.0) for any use of combined oral contraceptives, in the absence of a consistent duration–risk relationship (relative risk, 0.6 for > 5 years). The latter may be due to the small number of cases. The response rates were around 50% for both cases and controls.

Rosenberg *et al*. (1982), in a hospital-based case–control study of 136 cases and 539 controls collected between 1976 and 1980 from various areas of the United States and Canada, found an age-adjusted relative risk of 0.6 (95% CI, 0.4–0.9) for any use and 0.3 for use for five years or more. The response rates were 94% for both cases and controls, and the results were not materially modified by multivariate analysis.

Risch *et al.* (1983) provided data from a case–control study of 284 cases and 705 controls from Washington and Utah (United States) diagnosed between 1975 and 1979, giving a significant multivariate relative risk estimate of 0.9 per year for use of combined oral contraceptives. The response rates were 68% for cases and 95% for controls.

In a case–control study conducted in 1980–81 on 150 cases and 250 hospital controls in Athens, Greece, Tzonou *et al.* (1984) found a multivariate relative risk (adjusted for age, parity, age at menopause and use of post-menopausal oestrogen therapy) of 0.4 (95% CI, 0.1–1.1). The lack of significance may be due to the low frequency of use of combined oral contraceptives in this study, which was only 2.7% in cases and 7.2% in controls.

The Centers for Disease Control Cancer and Steroid Hormone Study (1983b) and the Cancer and Steroid Hormone Study of the Centers for Disease Control and National Institute of Child Health and Human Development (1987) was a population-based investigation conducted between December 1980 and December 1982 in eight areas of the United States on 546 women 20–54 years of age with ovarian cancer and 4228 controls. The response rates were 71% for cases and 83% for controls. The multivariate relative risk, adjusted for age and parity, for any use of combined oral contraceptives was 0.6 (95% CI, 0.5–0.7), which decreased to 0.2 (0.1–0.4) for use for10 years or more. The results were consistent when specific formulations of combined oral contraceptives were considered separately.

Harlow *et al.* (1988) provided information on use of combined oral contraceptives in 116 cases of epithelial ovarian cancers of borderline malignancy diagnosed between 1980 and 1985 and 158 controls. The relative risk for any use, adjusted for age and parity, was 0.4, in the absence, however, of a consistent duration–risk relationship.

Wu *et al.* (1988), in a hospital-based case–control study of 299 cases diagnosed in 1983–85 and 752 hospital controls and 259 population-based controls from the San Francisco Bay area, United States, found a relative risk, adjusted for parity, of 0.7 (95% CI, 0.5–1.1) for any use and 0.4 (95% CI, 0.2–0.7) for more than three years of use. The overall relative risk per year of use was 0.9 (95% CI, 0.8–0.9). The response rate was about 70% for both cases and controls.

Shu *et al.* (1989), in a case–control study conducted during 1984–86 in Shanghai, China, on 229 ovarian cancer cases (172 epithelial) and an equal number of controls, found a relative risk (adjusted for education, parity, ovarian cysts and age at menarche) of 1.8 (95% CI, 0.8–4.1) for any use of combined oral contraceptives. Only 23 cases and 12 controls had ever used such preparations. The response rates were 89% for cases and 100% for controls. In China, use of combined oral contraceptives might have been an indication of a westernized life style.

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1989a) included data on 368 cases of histologically confirmed cases of epithelial ovarian cancer and 2397 hospital controls. The patients were interviewed between 1979 and 1986 in seven countries, with response rates of 73% for cases and 94% for controls. The multivariate relative risk (adjusted for age, centre, year of interview and parity) for any use of combined oral contraceptives was 0.8 (95% CI, 0.6–1.0) and decreased to 0.5 (95% CI,

0.3–1.0) for five years of use or more. The reduction in risk was of a similar magnitude in developed and developing countries (Thomas, 1991).

In a case–control study conducted in 1978–81 in the Washington DC area of the United States with 296 patients with epithelial ovarian cancer and 343 hospital controls, Hartge *et al.* (1989a) found relative risks (adjusted for age and race) of 1.0 (95% CI, 0.7–1.7) for any use of combined oral contraceptives and 0.8 (95% CI, 0.4–1.5) for use for more than five years. The response rates were 74% for cases and 78% for controls.

Booth *et al.* (1989), in a hospital-based case–control study of 235 patients and 451 controls interviewed between 1978 and 1983 in London and Oxford, England, found multivariate relative risks of approximately 0.5 (95% CI, 0.3–0.9) for any use and 0.1 (0.01–1.0) for use for more than 10 years. They reported a significant inverse trend in risk with duration of use. Allowance was made for age, social class, gravidity and duration of unprotected intercourse.

Parazzini *et al.* (1991a) provided data on 505 cases of epithelial ovarian cancer in women under 60 years of age and 1375 hospital controls interviewed between 1983 and 1989 in northern Italy. The multivariate relative risk (adjusted for age, sociodemographic factors, parity, age at menarche, lifelong menstrual pattern, menopausal status and age at menopause) for any use of combined oral contraceptives was 0.7 (95% CI, 0.5–1.0), which decreased to 0.5 (0.3–0.9) for two years of use or more, with a significant inverse trend in risk with duration. The response rate was 98% for both cases and controls.

Parazzini *et al.* (1991b) also considered 91 patients with epithelial ovarian cancer of borderline malignancy and 237 hospital controls who were interviewed between 1986 and 1990 in northern Italy. The multivariate relative risk (adjusted for age, education, parity and age at menopause) for any use of combined oral contraceptives was 0.3 (95% CI, 0.2–0.6), and that for two years of use or more was 0.2 (0.1–0.6). The response rate was 98% for both cases and controls.

In a case–control study of 189 cases and 200 controls conducted in 1989–91 in greater Athens, Greece (Polychronopoulou *et al*., 1993), only three cases and seven controls had any use of combined oral contraceptives, corresponding to a multivariate relative risk of 0.8 (95% CI, 0.1–3.7). The response rate for cases was about 90%.

Rosenberg *et al.* (1994) updated their 1982 report, providing data collected between 1977 and 1991 on 441 cases of epithelial ovarian cancer and 2065 hospital controls from various areas of the United States. The response rate was 94% for both cases and controls. The multivariate relative risk for any use (adjusted for parity, hysterectomy, monolateral oophorectomy, tubal ligation, family history of ovarian cancer and sociodemographic factors) was 0.8 (95% CI, 0.6–1.0). No significant protection was observed with up to three years of use, but the relative risk declined to 0.5 (95% CI, 0.2–0.9) for 10 years of use or more. The risk estimates were similar for various types of combined oral contraceptive formulations.

Risch *et al.* (1994, 1996) provided data on 450 cases of epithelial ovarian cancer in women aged 35–79 diagnosed between 1989 and 1992 and 564 controls in Ontario, Canada. The response rates were 71% for cases and 65% for controls. The odds ratio,

adjusted for age and parity, for any use of oral contraceptives was 0.5 (95% CI, 0.4–0.7); after 10 or more years of use, it was 0.3 (0.2–0.6). The overall multivariate odds ratio per each year of use of combined oral contraceptives, adjusted for age, parity, lactation, use of postmenopausal oestrogen therapy, tubal ligation, hysterectomy and family history of breast cancer, was 0.90 (95% CI, 0.86–1.0), and the protection was stronger for serous and endometrioid cancers than for mucinous neoplasms.

Purdie *et al.* (1995) in a population-based study of 824 cases diagnosed between 1990 and 1993 and 860 controls in three Australian states found a relative risk of 0.6 (95% CI, 0.5–0.7) for any use, which declined to 0.3 (0.2–0.4) for 10 years of use or more. The response rates were 90% for cases and 73% for controls. Allowance was made in the analysis for sociodemographic factors, family history of cancer, talc use, smoking and reproductive and hormonal factors.

Parity is a well-recognized protective factor for ovarian cancer (Parazzini *et al.*, 1991c) and is a correlate of the use of combined oral contraceptives, i.e. a potentially relevant confounder. The inverse relationship between use of combined oral contraceptives and ovarian cancer was also observed, however, after adequate allowance had been made for parity and was reproduced consistently in several studies across separate strata of parity, age and other potential covariates, including marital status, education, menopausal status, other types of contraceptive use and other selected menstrual and reproductive factors.

The association between oral contraceptive use and the risk for ovarian cancer has been assessed in women with germ-line mutations in the *BRCA-1* or *BRCA-2* gene (Narod *et al.*, 1998). Thus, 207 women with such mutations and ovarian cancer were compared with 53 of their sisters who had one of these mutations. The relative risk for ovarian cancer was estimated to be 0.4 (95% CI, 0.2–0.7) for women who had ever used oral contraceptives and 0.3 (0.1–0.7) for women who had used oral contraceptives for six or more years.

At least two studies (Harlow *et al.*, 1988; Parazzini *et al.*, 1991b) and the pooled analysis of 12 United States studies (Harris *et al.*, 1992) also considered epithelial ovarian tumours of borderline malignancy. An inverse relationship was seen for these neoplasms, suggesting that combined oral contraceptives exert protection against the whole process of epithelial ovarian carcinogenesis.

Little information is available on the different histological types of epithelial ovarian cancer. In a Canadian study (Risch *et al.*, 1996), the inverse association was apparently stronger for non-mucinous (odds ratio per year of use, 0.9; 95% CI, 0.85–0.93) than for mucinous (odds ratio per year of use, 0.97; 0.93–1.04) tumours. This observation, however, requires confirmation.

In the case of non-epithelial ovarian cancers, 38 germ-cell neoplasms and 45 sex-cord-stromal neoplasms were identified from the collaborative analysis of four United States case–control studies (Horn-Ross *et al.*, 1992). The multivariate relative risks for any use of combined oral contraceptives were 2.0 (95% CI, 0.8–5.1) for germ-cell cancers and 0.4 (0.2–0.8) for sex-cord-stromal neoplasms. The data were inadequate to evaluate

duration of use or any other time–risk relationship. Similarly, the few available data indicate a consistent inverse association between use of combined oral contraceptives and benign epithelial tumours (ovarian cysts) (Parazzini *et al.*, 1989; Booth *et al.*, 1992) but not benign ovarian teratomas (Westhoff *et al.*, 1988; Parazzini *et al.*, 1995).

The favourable effect of use of combined oral contraceptives on the risk for epithelial ovarian cancer seems to persist for at least 10–15 years after use of the contraceptives has ceased (Cancer and Steroid Hormone Study of the Centers for Disease Control and National Institute of Child Health and Human Development, 1987; Franceschi *et al.*, 1991a; Whittemore *et al.*, 1992; Rosenberg *et al.*, 1994) and is not confined to a particular formulation (Rosenblatt *et al.*, 1992; Rosenberg *et al.*, 1994). There is some suggestion that formulations with lower doses of oestrogen are slightly less protective: in the WHO Collaborative Study on Neoplasia and Steroid Contraceptives (Rosenblatt *et al.*, 1992), the relative risk for ovarian cancer associated with any use of combined oral contraceptives was 0.7 (95% CI, 0.4–1.1) for high-dose preparations and 0.8 (95% CI, 0.5–1.3) for low-dose ones. The available data do not provide definite evidence of an inverse association between use of combined oral contraceptives with low-dose oestrogen and ovarian cancer for longer periods or in relation to recency of use.

The suppression of ovulation induced by oral contraceptives has been suggested to explain the inversion association, since it protects the ovarian epithelium from recurrent trauma and contact with follicular fluid (Fathalla, 1971; Casagrande *et al.*, 1979; Parazzini *et al.*, 1991c). Combined oral contraceptives may also protect against ovarian cancer by reducing exposure to pituitary gonadotropins, which stimulate the growth of cell lines derived from human ovarian carcinoma (Simon *et al.*, 1983). The lack of apparent protection by post-menopausal oestrogen therapy, however, does not support the existence of a favourable role of gonadotropin stimulation on ovarian carcinogenesis.

Since the incidence of ovarian cancer is already appreciable in middle age, and survival from the disease is unsatisfactory, the protection attributable to use of oral contraceptives is important and is therefore one of the major issues in any risk–benefit, public health evaluation of the use of combined oral contraceptives (Gross & Schlesselman,1994; La Vecchia *et al.*, 1996).

2.5 Cancers of the liver and gall-bladder

The vast majority of primary liver cancers are hepatocellular carcinomas. Chronic infection with hepatitis B (HBV) or C virus causes hepatocellular carcinoma, the relative risk exceeding 50 in many studies (IARC, 1994). Drinking of alcoholic beverages also causes liver cancer (IARC, 1988). Cholangiocarcinoma is much less common, although it is frequent in parts of South-East Asia and can be caused by infection with liver flukes (Parkin *et al.*, 1991).

2.5.1 *Descriptive studies*

Forman *et al.* (1983) analysed the rates of mortality from primary liver cancer among men and women in England and Wales between 1958 and 1981. The age-standardized

death rate in women aged 20–39 increased from 0.9 per million in 1970–75 to 1.8 per million in 1976–81 ($p < 0.005$), whereas changes in death rates between these periods among women aged 40–54 and among men were small and not statistically significant. The authors suggested that the change was consistent with the idea that oral contraceptives caused some cases of liver cancer, but noted that no such trend was apparent in Australia, western Germany, the Netherlands or the United States, other countries where oral contraceptive use had been similar to that in England and Wales. In an analysis of subsequent secular trends in mortality in England and Wales, Mant and Vessey (1995) concluded that the rate of mortality from liver cancer had remained constant in women in age groups that had had major exposure to oral contraceptives, and Waetjen and Grimes (1996) found no evidence for an effect of oral contraceptive use on secular trends in liver cancer death rates in Sweden or the United States.

2.5.2 *Cohort studies*

Colditz *et al*. (1994) studied a cohort of 121 700 female registered nurses aged 30–55 in the United States in 1976 who were followed-up for deaths until 1988. Women who reported angina, myocardial infarct, stroke and cancer (other than non-melanoma skin cancer) at baseline were excluded, leaving 116 755 women for follow-up. Of these, 55% reported having used oral contraceptives, and 5% reported current use. It was estimated that 98% of the deaths were ascertained. Incidence rates with person–months of follow-up were used as the denominator and oral contraceptive use at recruitment as the exposure. The relative risks were adjusted for age and for potential confounders including smoking but not alcohol consumption. There were 2879 deaths after 1.4 million women–years of follow-up. The risks associated with any use of oral contraceptives relative to no use, adjusted for age, smoking, body mass index and follow-up interval, were 0.93 (95% CI, 0.85–1.0) for death from any cause and 0.9 (0.8–1.0) for death from any cancer. There were 10 deaths from primary liver or biliary-tract cancer during the 12 years of follow-up, two of which were among women who had used oral contraceptives, with a relative risk of 0.4 (95% CI, 0.1–2.4). No information was provided on infection with hepatitis viruses.

Hannaford *et al.* (1997) described the relationships between use of oral contraceptives and liver disease in two British prospective studies by the Royal College of General Practitioners and the Oxford Family Planning Association. In the first study, 46 000 women, half of whom were using combined oral contraceptives, were recruited in 1968–69 and followed-up until they changed their general practitioner or until 1995. Cancer diagnoses were categorized according to the woman's contraceptive status at the time. There were five cases of liver cancer, comprising one hepatocellular carcinoma in a woman who had never used oral contraceptives, three cholangiocarcinomas in women who had formerly used oral contraceptives and one cholangiocarcinoma in a woman who had never used oral contraceptives. The risk for cholangiocarcinoma associated with former use of oral contraceptives in relation to no use was 3.2 (95% CI, 0.3–31). In a study of mortality in the same cohort after 25 years of follow-up, there were five deaths

from liver cancer among women who had used combined oral contraceptives and one in a woman who had never used them, for a relative risk of 5.0 (95% CI, 0.6–43) (Beral *et al*., 1999). In the study of the Oxford Family Planning Associaiton, 17 032 women were recruited between 1968 and 1974, and most were followed-up until 1994. Three liver cancers were reported, comprising two hepatocellular carcinomas and one cholangiocarcinoma, all in women who had formerly used oral contraceptives. No information on infection with hepatitis viruses was provided.

2.5.3 *Case–control studies*

(*a*) *Benign neoplasms of the liver*

Edmondson *et al.* (1976) interviewed by telephone 34 of 42 eligible women who had undergone surgery for hepatocellular adenoma in Los Angeles, United States, between 1955 and 1976. One age-matched friend control was interviewed for each case. Twenty-eight of the 34 cases (82%) and 19 of 34 controls (56%) had used oral contraceptives for more than 12 months. The risks relative to use of oral contraceptives for less than 12 months were 1.3 for 13–36 months of use, 5.0 for 61–84 months, 7.5 for 85–108 months and 25 for 109 months and longer.

Rooks *et al.* (1979) interviewed 79 of 89 eligible in women aged 16–50 in whom hepatocellular adenoma had been diagnosed between 1960 and 1976 at the Armed Forces Institute of Pathology, Washington DC, United States. Three age-matched neighbourhood controls were sought for each case, and 220 were interviewed. Seventy-two of the 79 cases (91%) and 99 of 220 controls (45.0%) had used oral contraceptives for more than 12 months. The risks relative to use of oral contraceptives for less than 12 months were 9 for 13–36 months of use, 116 for 37–60 months, 129 for 61–84 months and 503 for 85 months and longer.

(*b*) *Malignant tumours of the liver*

The studies on malignant tumours of the liver described below are summarized in Table 25.

Henderson *et al.* (1983b) studied women in Los Angeles County, United States, in whom liver cancer had been diagnosed and confirmed histologically during 1975–80 when they were 18–39 years of age. Two neighbourhood controls were sought for each case and matched on age and ethnic group. Twelve cases of liver cancer were identified, and interviews were obtained with 11 of the patients: eight with hepatocellular carcinoma, one with a giant-cell carcinoma, one with a sclerosing duct-forming carcinoma and one with a papillary carcinoma. Four out of 22 identified controls refused to be interviewed and were replaced, giving a response rate among those first selected of 82%; the true response rate was probably lower because the census information used to identify controls could not be obtained for 4.3% of the houses surveyed. Three patients, two with hepatocellular carcinoma, were interviewed in person by telephone; next-of-kin respondents were used for the others. None of the patients or controls reported a prior history of hepatitis or jaundice; none of the four cases had antigens to HBV surface antigen (HBsAg);

Table 25. Case–control studies of use of combined oral contraceptives and cancers of the liver

Reference and study area (period of diagnosis)	Cancer type	Combined oral contraceptives			Relative risk (95% CI)	Comments
		Use	No. of cases	No. of controls		
Henderson *et al.* (1983b) USA (1975–80)	Hepatocellular	Never	1[a]	8	[1.0]	No association with alcohol use; none of the 4 cases tested had antibodies to HBV surface antigen
		Ever	7	8	[7.0 (0.7–71)] [unmatched analysis]	
	Other	Never	0	1		
		Ever	3	5		
Neuberger *et al.* (1986) UK (1976–85)	Hepatocellular	*Total group*		*Expected no.*		No information on alcohol use
		Never	8	7.3	1.0	
		Ever	18	18.7	1.0 (0.4–2.4)	
		< 4 years	4	11.4	0.3 (0.1–1.1)	
		4–7 years	5	5.0	0.9 (0.3–3.4)	
		≥ 8 years	9	2.3	4.4 (1.5–13)	
		Excluding HBV-positive		*Expected no.*		
		Never	5	5.9	1.0	
		Ever	17	16.1	1.5 (0.5–4.4)	
		< 4 years	4	9.8	0.5 (0.1–1.9)	
		4–7 years	5	4.5	1.5 (0.4–6.3)	
		≥ 8 years	8	1.8	7.2 (2.0–26)	
Forman *et al.* (1986) UK (1979–82)	Hepatocellular	Never	4	68	1.0	No information on alcohol use; cases with hepatitis or cirrhosis excluded; no information on HBV status
		Ever	15	79	3.8	
		< 4 years	8	56	3.0	
		4–7 years	4	19	4.0	
		≥ 8 years	3	4	20	
	Cholangio-carcinoma	Never	8	68	1.0	
		Ever	3	79	0.3	
		< 4 years	1	56	0.1	
		≥ 4 years	2	23	0.9	

Table 25 (contd)

Reference and study area (period of diagnosis)	Cancer type	Combined oral contraceptives			Relative risk (95% CI)	Comments
		Use	No. of cases	No. of controls		
Palmer *et al*. (1989) USA (1977–85)	Hepatocellular	Never	1	29	[1.0]	No information on alcohol use or HBV status; one case of hepatocellular carcinoma had cirrhosis
		Ever	8	16	[14 (1.7–126)]	
		< 2 years	1	7	–	
		2–4 years	4	4	20 (2.0–190)	
		≥ 5 years	3	5	20 (1.6–250)	
	Cholangio-carcinoma	Never	0	8		
		Ever	2	2		
WHO Collaborative Study Group (1989b) Chile, China, Colombia, Israel, Kenya, Nigeria, Philippines, Thailand (1979–86)	Hepatocellular	Never	29	197	1.0	No significant difference in alcohol drinking habits between cases and controls; no information on HBV status, but all centres except one were in endemic areas
		Ever	7	69	0.6 (0.2–1.6)	
		≤ 2 years	6	45	0.8 (0.3–2.2)	
		> 2 years	1	24	0.2 (0.0–1.9)	
	Cholangio-carcinoma	Never	19	162	1.0	
		Ever	11	72	1.2 (0.5–3.1)	
		≤ 2 years	6	41	1.2 (0.4–3.7)	
		> 2 years	5	30	1.3 (0.4–4.1)	
Kew *et al.* (1990) South Africa (< 1989)	Hepatocellular	Never	39	84	1.0	Association unaltered by adjustment for alcohol use; 19 cases had antibodies to HBV surface antigen
		Ever	7	8	1.9 (0.6–5.6)	
		< 4 years	3	3	2.1 (0.4–11)	
		4–8 years	1	1	2.0 (0.1–33)	
		> 8 years	3	4	1.5 (0.3–7.2)	
Vall Mayans *et al*. (1990) Spain (1986–88)	Hepatocellular	Never	23	54	1.0	Association unaltered by adjustment for alcohol use; none of the oral contraceptive users had antibodies to HBV surface antigen
		Ever	6	3	[4.7 (1.1–20)]	

Table 25 (contd)

Reference and study area (period of diagnosis)	Cancer type	Combined oral contraceptives			Relative risk (95% CI)	Comments
		Use	No. of cases	No. of controls		
Yu *et al.* (1991) USA (1984–90)	Hepatocellular	Never	12	40	1.0	Association unaltered by adjustment for alcohol; 7 cases had antibodies to HBV or HCV: exclusion of these increased the association with oral contraceptives
		Ever	13	18	3.0 (1.0–9.0)	
		< 1 year	4	7	2.3 (0.5–11)	
		1–5 years	3	7	1.7 (0.3–9.1)	
		> 5 years	6	4	5.5 (1.2–25)	
Hsing *et al.* (1992) USA (1985–86)	Hepatocellular	*All subjects*				Adjusted for alcohol use; subjects with cirrhosis were excluded; no information on HBV status
		Never	33	306	1.0	
		Ever	39	243	1.6 (0.9–2.6)	
		< 5 years	16	121	1.2 (0.6–2.4)	
		5–9 years	13	61	2.0 (1.0–4.4)	
		≥ 10 years	8	41	2.0 (0.8–4.8)	
		Spouse or parent respondent				
		Never	17	211	1.0	
		Ever	35	180	2.7 (1.4–5.3)	
		< 5 years	15	93	2.1 (0.9–4.6)	
		5–9 years	13	48	3.9 (1.6–9.6)	
		≥ 10 years	7	26	4.8 (1.7–14)	
	Cholangio-carcinoma	*Spouse or parent respondent*				
		Never	7	211	1.0	
		Ever	6	180	0.8 (0.3–2.7)	
		< 5 years	2	93	0.5 (0.1–2.7)	
		5–9 years	1	48	0.6 (0.1–5.4)	
		≥ 10 years	3	26	3.3 (0.7–16)	

Table 25 (contd)

Reference and study area (period of diagnosis)	Cancer type	Combined oral contraceptives			Relative risk (95% CI)	Comments
		Use	No. of cases	No. of controls		
Tavani *et al.* (1993b) Italy (1984–92)	Hepatocellular	Never	34	173	1.0	Association unaltered by adjustment for alcohol use; no information on HBV status; relative risk, 4.3 (1.0–18) > 10 years after last use
		Ever	9	21	2.6 (1.0–7.0)	
		≤ 5 years	5	17	1.5 (0.5–5.0)	
		> 5 years	2	4	3.9 (0.6–25)	
Collaborative MILTS Project Team (1997) France, Germany, Greece, Italy, Spain, UK (1990–96)	Hepatocellular	*All subjects*				Association unaltered by adjustment for alcohol
		Never	145	693	1.0	
		Ever	148	1 086	0.8 (0.5–1.0)	
		1–2 years	26	238	0.8 (0.5–1.3)	
		3–5 years	26	201	0.6 (0.3–1.1)	
		≥ 6 years	90	638	0.8 (0.5–1.1)	
		No cirrhosis or HBV or HCV				
		Never	16	250	1.0	
		Ever	35	324	[1.7 (0.9–3.1)] [unmatched analysis]	
		1–2 years	5	74	1.3 (0.4–4.0)	
		3–5 years	5	57	1.8 (0.5–6.0)	
		≥ 6 years	25	193	2.8 (1.3–6.3)	

CI, confidence interval; HBV, hepatitis B virus; HCV, hepatitis C virus

[a] This case had received injections of hormones of undetermined type for nine months.

none of the patients reported exposure to any known hepatotoxin such as vinyl chloride, and there was no difference in the frequency of alcohol consumption between cases and controls. Smoking histories were not reported. Ten of the 11 patients (seven of the eight cases of hepatocellular carcinoma) had used oral contraceptives, and the eleventh had received hormone injections of an undetermined type; 13 of the 22 controls had used oral contraceptives. The average duration of use of oral contraceptives was 64.7 months for the patients and 27.1 months for the controls (one-sided matched $p < 0.005$). [The relative risk for any use of oral contraceptives was 7.0 (95% CI, 0.7–71) for hepatocellular carcinoma and 6.9 (0.7–64) for all liver cancers (unmatched analyses).]

Neuberger *et al.* (1986) studied 26 women in whom hepatocellular carcinoma had been diagnosed and confirmed histologically in a non-cirrhotic liver when they were under the age of 50. The cases were referred from all over Britain to the Liver Unit at King's College School of Medicine and Dentistry, London, between 1976 and 1985. The controls were 1333 women who were hospital controls in a case–control study of breast cancer and had been interviewed during 1976–80; the response rate was not given. The source of information on the exposures of the cases is not specified, but may have been interviews. The results were not adjusted for smoking or alcohol use. Eighteen of the 26 case women had taken oral contraceptives. The controls were used to calculate the expected numbers of cases for each duration of pill use, within age and calendar groups. The expected number of women who had ever used oral contraceptives was 18.7, giving a relative risk of 1.0 (95% CI, 0.4–2.4). The relative risks for durations of use were 0.3 (95% CI, 0.1–1.1) for < 4 years, 0.9 (0.3–3.4) for 4–7 years and 4.4 (1.5–13) for ≥ 8 years. None of the case women had HBsAg, but one had antisurface antibodies and three had anticore antibodies. Exclusion of these four cases changed the relative risks associated with oral contraceptive use to 1.5 (95% CI, 0.5–4.4) for any use, 0.5 (0.1–1.9) for < 4 years, 1.5 (0.4–6.3) for 4–7 years and 7.2 (95% CI, 2.0–26) for ≥ 8 years. Three cases in this study were also included in the study of Forman *et al.* (1986), described below.

Forman *et al.* (1986) identified all women certified to have died from liver cancer at the age of 20–44 in England and Wales between 1979 and 1982. Deaths from secondary liver cancer or from benign liver tumours were excluded. Two controls were selected for each case from among women who had died from cancer of the kidney, cancer of the brain or acute myeloid leukaemia, and, for 1982 only, two further controls were selected for each case from among women who had died as a result of a road traffic accident. Information on exposure was obtained from a questionnaire sent to the general practitioners of cases, and information was obtained for 46 of 85 (54.1%) potential cases and for 147 of 233 (63.1%) eligible controls. Further information, including pathological data, was sought for potential cases and resulted in 35 confirmed cases of primary liver cancer, of which 24 were hepatocellular carcinoma and 11 were cholangiocarcinoma. Five of the deaths from hepatocellular carcinoma were excluded from the analysis, two because they had had chronic active hepatitis, two because they had had severe alcoholic disease and associated liver cirrhosis and one because she had had Down's syndrome, which might have prejudiced the prescription of oral contraceptives. Eighteen of the 30 case women

(15 of the 19 with hepatocellular carcinoma) had used oral contraceptives, compared with 79 of the 147 controls. Information on smoking and alcohol habits was not available. The relative risks, adjusted for age and year of birth, were: for hepatocellular carcinoma, 3.8 for any use, 3.0 for use for < 4 years, 4.0 for 4–7 years and 20.1 for ≥ 8 years; for cholangiocarcinoma, 0.3 for any use, 0.1 for < 4 years and 0.9 for ≥ 4 years. [The published relative risks were adjusted for age and year of birth, but confidence intervals were not given. The unadjusted relative risks and 95% confidence intervals, calculated from the published data, were: hepatocellular carcinoma, any use, 3.2 (95% CI, 1.0–10); < 4 years, 2.4 (0.7–8.5); 4–7 years, 3.6 (0.8–16); and ≥ 8 years, 13 (2.1–78); cholangiocarcinoma, any use, 0.3 (95% CI, 0.1–1.3); < 4 years, 0.2 (0.0–1.3); ≥ 4 years, 0.7 (95% CI, 0.2–3.7).] There was no information on infection with hepatitis viruses. Three cases in this study were also included in the study of Neuberger *et al.* (1986), described above.

Palmer *et al.* (1989) conducted a hospital-based case–control study of women in whom liver cancer had been diagnosed when they were 19–54 years of age in five United States cities in 1977–85. They identified 12 cases of liver cancer, of which nine were hepatocellular carcinoma, two were cholangiocarcinoma and one was undetermined. None of the case women reported a history of hepatitis, nor was there mention in their hospital discharge summaries of HBV infection; liver cirrhosis was discovered at the time of surgery in one case of hepatocellular carcinoma. Five controls were selected for each case and matched on hospital, age and date of interview; the diagnoses of controls were trauma for 16, eight herniated discs, five acute respiratory infections and 31 eye, ear and gastrointestinal conditions. Information on exposure was obtained from case and control women at interview. Overall, 95% of the subjects approached were interviewed. Smoking status was not reported, but alcohol intake was similar in cases and controls. Eleven of the 12 case women (eight of the nine cases of hepatocellular carcinoma) and 20 of the 60 controls had used oral contraceptives. The risk for hepatocellular carcinoma relative to women who had used oral contraceptives for < 2 years was 20 (95% CI, 2.0–190) for 2–4 years of use and 20 (1.6–250) for ≥ 5 years of use. [The unmatched relative risk for any use was 15 (95% CI, 1.7–126).]

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1989b) was a hospital-based case–control study conducted in eight countries. The eligible cases were those of women in whom liver cancer was diagnosed between 1979 and 1986 and who were born after 1924 or 1929. A total of 168 eligible cases were identified; 122 (72.6%) of the diagnoses were confirmed, and these women were interviewed. Histological typing was available for 69 cases: 36 were hepatocellular carcinoma, 29 were cholangiocarcinoma, one was an adenocarcinoma and three were other types. Controls were selected from among individuals admitted to the same hospitals as the cases with conditions not thought to be related to use of oral contraceptives. The aim was to select two controls for each case, but controls were not individually matched to cases; there was thus a pool of over 14 000 controls, from whom up to eight were selected for each case of liver cancer, matched on age, study centre and year of interview. The overall response rate of controls was 94.3%. All case and control women were interviewed. Information

on smoking was not collected; there was no statistically significant difference between case and control women in alcohol consumption, 17.2% of the cases and 26% of the controls having ever drunk alcohol. The finding that 25 of the 122 cases (20.5%) and 216 of the 802 controls (26.9%) had used oral contraceptives gave relative risks, adjusted for number of live births and occupation, of 0.7 (95% CI, 0.4–1.2) for any use, 0.8 (0.4–1.5) for use for 1–12 months, 0.7 (0.3–1.7) for 13–36 months and 0.7 (0.3–1.7) for ≥ 37 months. The relative risks for any use by histological subtype were 0.6 (95% CI, 0.2–1.6) for hepatocellular carcinoma, 1.2 (0.5–3.1) for cholangiocarcinoma and 0.5 (0.2–1.3) for a clinical diagnosis with no histological confirmation. Information on prior infection with hepatitis viruses was not collected, but all except one of the study centres were in countries with high rates of liver cancer and where HBV infection is endemic.

Kew *et al.* (1990) conducted a hospital-based case–control study in Johannesburg, South Africa, among patients in whom histologically confirmed hepatocellular carcinoma was diagnosed when they were aged 19–54. Two controls per case were selected and matched on age, race, tribe, rural or urban birth, hospital and ward. Patients with diseases in which contraceptive steroids might be causally implicated were not considered eligible as controls. All of the subjects were interviewed, but the response rates were not given. Smoking and alcohol intake were associated with the risk for liver cancer, but inclusion of these variables in the analysis did not alter the results. Seven of 46 cases (15.2%) and eight of 92 controls (8.7%) had used oral contraceptives, giving an overall relative risk of 1.9 (95% CI, 0.6–5.6). The relative risks were 2.1 (95% CI, 0.4–11) for use for < 4 years, 2.0 (0.1–33) for 4–8 years and 1.5 (95% CI, 0.3–7.2) for > 8 years. Nineteen of the 46 cases were HBsAg-positive, 25 had evidence of past infection with HBV, and two had never been infected. The relative risk for hepatocellular carcinoma in HBsAg-negative patients who used contraceptive steroids of any type was 0.4 (95% CI, 0.2–1.0).

Vall Mayans *et al.* (1990) conducted a hospital-based case–control study in Catalonia, north-eastern Spain, where 96 patients admitted to the Liver Unit of the University Hospital in Barcelona between 1986 and 1988 were identified, 74 of whom had histologically or cytologically confirmed hepatocellular carcinoma. Liver cirrhosis was present in 83 (86.5%) cases. For the 29 cases in women, two controls were selected per case and matched on sex, age, hospital and time of admission. Patients with diagnoses related to use of oral contraceptives were considered ineligible as controls. One control was excluded from the analysis because of later confirmation of liver cirrhosis. Serum from all patients was tested for HBsAg, antibody to hepatitis B core antigen and antibody to hepatitis surface antigen. All patients were interviewed, but the response rates were not given. Smoking was not associated with risk, and adjustment for alcohol intake did not alter the results. Six of the 29 female cases (20.7%) and three of the 57 female controls (5.3%) had used oral contraceptives [unmatched relative risk, 4.7 (95% CI, 1.1–20)]. Overall, 9.4% of cases and 2.1% of controls were HBsAg-positive, and all of the users of oral contraceptives were HBsAg-negative.

Yu *et al.* (1991) used a population-based cancer registry to identify cases of histologically confirmed hepatocellular carcinoma diagnosed in black or white non-Asian

women residents aged 18–74 in Los Angeles County, United States, between 1984 and 1990. Two neighbourhood controls were sought for each case and matched on sex, year of birth and race. Eighty-four of 412 eligible patients (20.4%) were interviewed (70.6% died before contact could be made), of which 10 were excluded from the analysis because the diagnosis of hepatocellular carcinoma was not confirmed. The response rate among the controls first selected was 71%. Adjustment for smoking and alcohol did not alter the results. Thirteen of the 25 case women (52%) and 18 of the 58 controls (31%) had used oral contraceptives. The relative risks were 3.0 (95% CI, 1.0–9.0) for any use, 2.3 (0.5–11) for use for ≤ 12 months, 1.7 (95% CI, 0.3–9.1) for 13–60 months and 5.5 (95% CI, 1.2–25) for ≥ 61 months. For the 11 case women who had formerly used oral contraceptives, the mean time since last use was 14.5 years. Seven case women had antibodies to one or more markers of hepatitis viral infection; when these cases were excluded, the association between use of oral contraceptives and the risk for hepatocellular carcinoma became stronger.

Hsing *et al.* (1992) studied deaths from primary liver cancer among women aged 25–49 in the United States (except Oregon) in 1985 and in the National Mortality Followback Survey in 1986. Of the 203 deaths from liver cancer identified, 52 cases not specified as primary, four cases of chronic liver disease and 29 cases with a history of liver cirrhosis were excluded. This left 98 cases for analysis, of which 76 were primary liver cancer and 22 were cholangiocarcinoma. Controls were selected from among women in the National Mortality Followback Study who had died in 1986 from causes other than liver cancer and whose next-of-kin returned the questionnaire. Potential controls with evidence of chronic liver disease or whose causes of death were thought to be associated with oral contraceptive use were excluded, leaving 629 controls for analysis. Information on exposure was obtained from next-of-kin by postal questionnaire. The results were presented both for all subjects and for subjects for whom the respondent was the spouse or parent (thought to be more reliable). The relative risks were adjusted for smoking and alcohol use. For all subjects with complete data, 39 of 72 cases (54.2%) and 243 of 549 controls (44.3%) had ever used oral contraceptives; the relative risks were 1.6 (95% CI, 0.9–2.6) for any use, 1.2 (0.6–2.4) for use for < 5 years, 2.0 (1.0–4.4) for 5–9 years and 2.0 (0.8–4.8) for ≥ 10 years. For subjects whose spouse or parent responded, the relative risks were 2.7 (95% CI, 1.4–5.3) for any use, 2.1 (0.9–4.6) for use for < 5 years, 3.9 (1.6–9.6) for 5–9 years and 4.8 (1.7–14) for ≥ 10 years. When the four Asian cases and 10 controls, from populations presumed to have a higher prevalence of HBV infection, were excluded from the analysis, higher risk estimates were seen for any use (2.8; 95% CI, 1.4–5.5) and for long-term (≥ 10 years) use (5.2; 1.7–15). The relative risks for the 13 cases of cholangiocarcinoma were 0.8 (95% CI, 0.3–2.7) for any use, 0.5 (0.1–2.7) for < 5 years of use, 0.6 (0.1–5.4) for 5–9 years and 3.3 (0.7–16) for ≥ 10 years.

Tavani *et al.* (1993b) conducted a hospital-based case–control study of women with histologically or serologically confirmed hepatocellular carcinoma diagnosed at the age of 28–73 in the greater Milan area, Italy, between 1984 and 1992. The controls were women admitted to hospital for acute non-neoplastic diseases (37% traumas, 13% other ortho-

paedic disorders, 40% acute surgical conditions, 10% other). Since none of the women aged 60 or over had ever used oral contraceptives, the analysis was restricted to women under that age. All of the participating subjects were interviewed; the response rates were not given but were close to 100% in other reports of this study. The results were not adjusted for smoking or alcohol use. Nine of the 43 cases (20.9%) and 21 of the 194 controls (10.8%) had ever used oral contraceptives. The relative risks, adjusted for age, education and parity, were 2.6 (95% CI, 1.0–7.0) for any use, 1.5 (0.5–5.0) for use for ≤ 5 years and 3.9 (0.6–25) for use for > 5 years. In relation to time since oral contraceptives were last used, the relative risks were 1.1 (95% CI, 0.3–4.6) for ≤ 10 years and 4.3 (1.0–18) for > 10 years. There was no information on infection with hepatitis viruses.

The Multicentre International Liver Tumour Study (Collaborative MILTS Project Team, 1997) included women with hepatocellular carcinoma diagnosed before the age of 65 between 1990 and 1996 in seven hospitals in Germany and one each in France, Greece, Italy, Spain and the United Kingdom. The diagnoses were based on histological examination or on imaging and increased α-fetoprotein concentration. An average of four controls was sought for each case: two general hospital controls without cancer, one hospital control with an eligible tumour diagnosis and one population control. The controls were frequency matched for age, and living controls were obtained for cases who had died. Of the 368 eligible cases, 317 (86.1%) were included in the study, although 24 of these were excluded from the analysis because of missing information on confounding factors. Information was obtained at interview, except for 136 case women (42.9%) who had died or who could not be interviewed for other reasons, for whom a next-of-kin was interviewed. The overall response rate for controls was not given, but that for hospitalized patients (cases and hospital controls) varied from 68 to 100% between centres, whereas the response rate for population controls varied from 60 to 80% between countries. Smoking and alcohol use were considered as confounders but were not included in the models presented. Oral contraceptive use was reported for 148 of the 293 cases (50.5%) and 1086 of the 1779 controls (61.0%). The relative risk for any use of oral contraceptives was 0.8 (95% CI, 0.5–1.0), and those for durations of use were 0.8 (0.5–1.3) for 1–2 years, 0.6 (0.3–1.1) for 3–5 years and 0.8 (95% CI, 0.5–1.1) for ≥ 6 years. For use of oral contraceptives containing cyproterone acetate, the relative risks were 0.9 (95% CI, 0.5–1.6) for any use, 0.9 (0.4–2.4) for use for 1–2 years, 0.9 (0.3–2.4) for 3–5 years and 0.9 (95% CI, 0.4–2.0) for ≥ 6 years. When the analysis was restricted to the 51 cases without liver cirrhosis or evidence of infection with hepatitis viruses, the relative risks were 1.3 (95% CI, 0.4–4.0) for use of any oral contraceptives for 1–2 years, 1.8 (0.5–6.0) for 3–5 years and 2.8 (1.3–6.3) for ≥ 6 years.

(*c*) *Gall-bladder*

Yen *et al.* (1987) studied extrahepatic bile-duct cancers in Massachusetts and Rhode Island, United States, between 1975 and 1979 in 27 women with histologically confirmed bile-duct cancer and 152 controls, who were patients with a variety of other cancers. All of the subjects were interviewed. Of the women under 60 years of age, four of 10 cases

and six of 76 controls reported use of combined oral contraceptives (age-adjusted relative risk, 7.8; 95% CI, 2.0–30).

The relationship between use of combined oral contraceptives and risk for primary gall-bladder cancer was examined in 58 case and 355 control women who were participating in an international hospital-based case–control study during 1979–86 (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1989c). Any use of combined oral contraceptives was not associated with risk, adjusted for age and history of gall-bladder disease (relative risk, 0.6; 95% CI, 0.3–1.3), and no increase in risk was seen among women who had taken combined oral contraceptives for more than three years (0.6; 0.2–2.2) or more than 12 years before cancer diagnosis (0.6; 0.2–1.8).

2.6 Colorectal cancer

2.6.1 *Cohort studies*

The Walnut Creek Contraceptive Drug Study (1981) showed no association between use of combined oral contraceptives and all cancers of the digestive tract (age-adjusted relative risk, 0.9; 95% CI, 0.4–2.0). [The majority of these cancers were probably colorectal cancers.] The results of cohort studies that specifically addressed colorectal cancers are shown in Table 26.

Reports on the association between use of combined oral contraceptives and the risk for colorectal cancer were made from the the Nurses' Health Study (Chute *et al*., 1991; Martinez *et al.*, 1997). Follow-up until 1992 of 89 448 nurses for over 1 million person–years, with 501 incident cases of colorectal cancer, showed a relative risk adjusted for age, body mass index, exercise, family history of cancer, aspirin use, alcohol use, smoking, meat intake and reproductive factors of 0.8 (95% CI, 0.7–1.0). Women who had used combined oral contraceptives for 96 months or more were at significantly lower risk (0.6; 95% CI, 0.4–0.9). The results for colon cancer were similar to those for rectal cancer.

In the Iowa Women's Health Study cohort (Bostick *et al*., 1994), described in the monograph on 'Post-menopausal oestrogen therapy', the prevalence of any use of combined oral contraceptives was 17% among women with colon cancer and 19% among women without colon cancer. Any use was associated with a relative risk, adjusted for age, height, parity, energy intake and vitamin intake, of 1.0 (95% CI, 0.7–1.4).

Beral *et al.* (1999) reported on a 25-year follow-up of 46 000 women who were recruited in 1968-69 by general practitioners throughout Britain. At recruitment, 49% of the women were using combined oral contraceptives; by the end of the follow-up, 63% had used them at some time, the median duration of use being four years. The relative risk for death from colorectal cancer among women who had ever used combined oral contraceptives, adjusted for age, parity, social class and smoking, was 0.6 (95% CI, 0.4–0.9). The trend in risk by duration of use was not significant. The relative risk of women who had last used combined oral contraceptives 15 years or more previously was 1.0 (95% CI, 0.5–2.0) and that for death from all cancers combined for any use was 1.0 (95% CI, 0.8–1.1).

Table 26. Cohort studies of use of combined oral contraceptives and colorectal cancer

Reference	Country	Study population (follow-up) no. of cancers	RR (95% CI) (any versus no use)			Duration of use	Adjustment/comments
			Colon–rectum	Colon	Rectum		
Martinez *et al.* (1997) (Nurses Health Study)	USA	89 448 (12 years) 501	0.8 (0.7–1.0)	0.6 (0.4–1.0)	0.8 (0.5–1.2)	Significant trend (RR for ≥ 8 years' use, 0.6; 95% CI, 0.4–0.9)	Age, body mass index, exercise, family history of cancer, aspirin use, alcohol use, smoking, meat intake and reproductive factors Prevalence of combined oral contraceptive use, 32%; mostly past use
Bostick *et al.* (1994)	Iowa, USA	35 215 (4 years) 212	–	1.0 (0.7–1.4)	–	Not reported	Adjusted for age, height, parity, energy and vitamin intake Prevalence of combined oral contraceptive use, 19%
Beral *et al.* (1999)	United Kingdom	46 000 (25 years) 170 deaths	0.6 (0.4–0.9)	–	–	No trend	Mortality rates, adjusted for age, parity, social class and smoking RR for ≥ 10 years of use, 0.3 (0.1–1.2); RR for last use ≥ 15 years previously, 1.0 (0.5–2.0)

RR, relative risk; CI, confidence interval

2.6.2 *Case–control studies*

(*a*) *Colorectal polyps*

In a study by Jacobson *et al.* (1995) in New York City, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', a lower frequency of any use of combined oral contraceptives was found among cases of colorectal polyps (72/280) than among control women (19/126) (relative risk, 0.6; 95% CI, 0.3–1.1).

Potter *et al.* (1996) in a study in Minnesota, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', found similar proportions of women who had ever used combined oral contraceptives among women with and without polyps; the risk associated with ≥ 5 years of use relative to that of women who had not undergone colonoscopy was 0.8 (95% CI, 0.5–1.4) and that relative to community controls was 1.1 (0.6–1.8).

(*b*) *Colorectal cancer*

Several of the case–control investigations on use of combined oral contraceptives and colorectal cancer risk are also described in the monographs on 'Post-menopausal oestrogen therapy' and 'Post-menopausal oestrogen–progestogen therapy', and are summarized only briefly here. Table 27 lists the studies summarized below.

Weiss *et al*. (1981b), in a study in the United States described in detail in the monograph on 'Post-menopausal oestrogen therapy', reported that any use of combined oral contraceptives was commoner among cases (33%) than among controls (23%), but the difference was not significant. The age-adjusted relative risks were 1.3 (95% CI, 0.5–3.1) for < 5 years of use and 2.0 (0.7–5.2) for ≥ 5 years of use. The relative risks for any use were 1.0 for colon cancer and 2.6 ($p = 0.09$) for rectal cancer.

Potter and McMichael (1983), in Adelaide, Australia, found that use of combined oral contraceptives was slightly less common among cases of colon cancer than among controls, with a relative risk adjusted for reproductive variables of 0.5 (95% CI, 0.3–1.2) for any versus no use. The relative risk for rectal cancer was 0.7 (95% CI, 0.3–1.8), with a trend of decreasing risk with increasing duration of use (relative risk for ≥ 25 months of use, 0.2; 95% CI, 0.0–1.0).

In the case–control study of Furner *et al*. (1989) described in detail in the monograph on 'Post-menopausal oestrogen therapy', the crude relationship between colorectal cancer and the use of combined oral contraceptives was 0.6 (95% CI, 0.3–1.3); only nine case and 32 control women had ever used combined oral contraceptives.

A case–control study conducted by Kune *et al*. (1990) in Melbourne, Australia, between 1980 and 1981 included all local incident cases of colorectal cancer (108 colon and 82 rectum) and 200 age-matched female controls representing a random sample of local population. The relative risks, adjusted for reproductive factors, of women who had ever used combined oral contraceptives were 1.2 (95% CI, 0.6–2.3) for colon and 2.0 (95% CI, 1.0–4.1) for rectal cancer. The relative risks associated with more than nine years' duration of use, however, were 0.7 for colon and 0.9 for rectal cancer [confidence intervals not given].

Table 27. Case–control studies of use of combined oral contraceptives and colorectal cancer

Reference	Country	Cases : controls (type of controls)	RR (95% CI) (any versus no use)			Duration of use	Recency of use	Adjustments/Comments
			Colon–rectum	Colon	Rectum			
Weiss *et al*. (1981b)	Washington, USA	143 : 707 (population)	≤ 5 years, 1.3 (0.5–3.1) ≥ 5 years, 2.0 (0.7–5.2)	1.0	2.6 (p = 0.09)	No significant trend	Not reported	Age Prevalence of combined oral contraceptive use was about 30% (22% for ≥ 5 years' use)
Potter & McMichael (1983)	Adelaide, Australia	155 : 311 (population)	–	0.5 (0.3–1.2)	0.7 (0.3–1.8)	Inverse trend (RR for > 2 years' use, 0.20; 95% CI, 0.0–1.0)	Not reported	Reproductive variables (diet was influential) Prevalence of combined oral contraceptive use among controls, 18%
Furner *et al*. (1989)	Chicago, USA	90 : 208 (spouses)	0.6 (0.3–1.3)	–	–	Not reported	Not reported	Unadjusted Prevalence of combined oral contraceptive use among controls, 6%
Kune *et al*. (1990)	Melbourne, Australia	190 : 200 (population)	–	1.2 (0.6–2.3)	2.0 (1.0–4.1)	No effect (RR for > 9 years use, 0.7 for colon and 0.9 for rectum; not significant)	Not reported	Age, parity and age at birth of first child Prevalence of combined oral contraceptive use among controls, 20%
Fernandez *et al*. (1998a)	Italy	1232 : 2793 (hospital)	0.6 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–1.1)	No effect	Stronger protection from recent use (RR for < 10 years, 0.4; 95% CI, 0.3–0.7)	Age, education, cancer family history, body mass index, oestrogen replacement therapy, parity, menopause and energy intake Prevalence of use of combined oral contraceptives among controls, 12%

Table 27 (contd)

Reference	Country	Cases : controls (type of controls)	RR (95% CI) (any versus no use)			Duration of use	Recency of use	Adjustments/Comments
			Colon–rectum	Colon	Rectum			
Peters *et al.* (1990)	Los Angeles, USA	327 : 327 (neighbours)	–	**< 5 years**: 1.0 (0.6–1.8); right colon, 1.4 (0.6–3.3); left colon, 0.7 (0.3–1.5) **≥ 5 years**: 1.1 (0.4–2.9); right colon, 1.3 (0.3–5.5); left colon, 1.0 (0.2–4.8)	–	No effect	Not reported	Family history of cancer, parity, exercise, fat, alcohol and calcium intake Prevalence of combined oral contraceptive use among controls, 19%
Franceschi *et al.* (1991b)	North-eastern Italy	89 : 148 (hospital)	0.2 (0.0–2.0)	–	–	Not reported	Not reported	Unadjusted Only 1 case and 9 controls had ever used combined oral contraceptives
Wu-Williams (*et al.* (1991)	North America and China	395 : 1112 (neighbours)		North America: 1.2 (p = 0.67); China: 0.6 (p = 0.27)	North America: 0.4 (p = 0.04); China: 0.7 (p = 0.34)	No trend	Not reported	Unadjusted (but unaltered by exercise, saturated fat and years in the USA) Prevalence of combined oral contraceptive use among controls, 16% in North America and 12% in China
Jacobs *et al.* (1994)	Seattle, USA	193 : 194 (population)	–	1.2 (0.7–1.9); right colon, 1.2 (0.7–2.3); left colon, 1.1 (0.6–2.1)	–	No trend	Not reported	Age, age at birth of first child and vitamin intake Prevalence of combined oral contraceptive use among controls, 27%

Table 27 (contd)

Reference	Country	Cases : controls (type of controls)	RR (95% CI) (any versus no use)			Duration of use	Recency of use	Adjustments/Comments
			Colon–rectum	Colon	Rectum			
Kampman *et al.* (1997)	USA	894 : 1120 (members of medical care programme)	–	0.9 (0.7–1.1)	–	Not reported	Not reported	Age, family history of colorectal cancer, aspirin use, energy intake, post-menopausal oestrogen therapy and exercise Prevalence of combined oral contraceptive use among controls, 25%

RR, relative risk; CI, confidence interval

Franceschi *et al*. (1991b) carried out a case–control study in north-eastern Italy which included a very few users of combined oral contraceptives (one case and nine controls). The crude relative risk was 0.2, but the 95% CI (0.0–2.0) was very broad.

A pooled analysis of a case–control study from Milan (Negri *et al.,* 1989; Fernandez *et al*., 1996) and a multicentre study from Italy (Talamini *et al*., 1998) which involved 803 cases of colon cancer, 429 of rectal cancer and 2793 hospital controls (Fernandez *et al.*, 1998) provided a relative risk estimate (adjusted for age, education, family history of cancer, body mass index, parity, menopause, use of post-menopausal oestrogen therapy and energy intake) of 0.6 (95% CI, 0.5–0.9) for colon cancer and 0.7 (0.4–1.0) for rectal cancer for women who had ever used combined oral contraceptives. Increasing duration of use was related to a decreasing risk for colon cancer. The relative risk for recent users (< 10 years since last use) was 0.4 (95% CI, 0.3–0.7). Similar patterns of risk were found for various strata of age, educational level, parity, family history of colorectal cancer and body mass index.

In the case–control study of Peters *et al*. (1990) in Los Angeles, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', use of combined oral contraceptives was not associated with an increased risk for colon cancer. The relative risk, adjusted for family history of cancer, parity, exercise and fat, alcohol and calcium intake, was 1.1 (95% CI, 0.4–2.9) for ≥ 5 years of use. This estimate was based on very few long-term users (13 cases and 15 controls).

The study by Wu-Williams *et al*. (1991), among Chinese women in North America and China, also described in the monograph on 'Post-menopausal oestrogen therapy', included small proportions of women who had ever used combined oral contraceptives: 16% in North America and 12% in China. The crude relative risks for rectal cancer were 0.4 ($p = 0.04$) in North America and 0.7 ($p = 0.34$) in China, and those for colon cancer were 1.2 ($p = 0.67$) and 0.55 ($p = 0.27$), respectively.

In the study of Jacobs *et al*. (1994) in Seattle, United States, described in the monograph on 'Post-menopausal oestrogen therapy', any use of combined oral contraceptives was reported by about 25% of both women with colon cancer and controls. The relative risk, adjusted for age and vitamin intake was 1.2 (95% CI, 0.7–1.9).

Kampman *et al*. (1997), in a study described in the monograph on 'Post-menopausal oestrogen therapy', did not find a significant association between the risk for colon cancer and use of combined oral contraceptives, which was reported by about 25% of cases and controls. The relative risk, adjusted for age, family history of colorectal cancer, aspirin use and energy intake, was 0.9 (95% CI, 0.7–1.1).

2.7 Cutaneous malignant melanoma

2.7.1 *Cohort studies*

In the late 1960s, three large cohort studies of users of combined oral contraceptives were begun (Table 28). All three provided information on the risk for cutaneous malignant melanoma according to use of combined oral contraceptives, but were based on small numbers of observed cases. Furthermore, it was not possible in any of the studies

Table 28. Cohort studies of use of combined oral contraceptives and risk for cutaneous malignant melanoma

Reference	Country, study	Population (follow-up), no. of cancers	RR (95% CI) any versus no use	Duration of use	Recency of use	Adjustments/comments
Hannaford *et al*. (1991)	United Kingdom, Oxford Family Planning Association	17 032 (15 years) 32	0.8 (0.4–1.8)	No trend (RR for ≥ 10 years' use, 1.0; 95% CI, 0.2–1.6)	No effect	Age, parity, social class and smoking No increase in risk for any combined oral contraceptive formulation
	United Kingdom, Royal College of General Practitioners	23 000 (20 years) 58	0.9 (0.6–1.5)	No trend (RR for ≥ 10 years' use, 1.8; 95% CI, 0.8–3.9)	No effect	Age, parity, social class and smoking No risk increase for any combined oral contraceptive formulation
Ramcharan *et al*. (1981b)	California, USA, Walnut Creek Contraceptive Drug Study	17 942 (8 years) 20	3.5 (1.4–9.0)	No trend	Not reported	Age
Bain *et al*. (1982)	USA, Nurses' Health Study	121 964 (at start) 141	0.8 (0.5–1.3) women < 40 years, 1.4 (0.8–2.5)	No trend	No effect	Age, parity, height and hair dye use Nested case–control investigation (141 non-fatal cutaneous malignant melanomas and 2820 age-matched controls)

RR, relative risk; CI, confidence interval

to make allowance for major determinants of cutaneous malignant melanoma such as solar exposure and phenotypic characteristics.

Between 1968 and 1974, 17 032 white married women aged 25–39 were recruited at 17 family planning clinics in the United Kingdom, in the framework of a study by the Oxford Family Planning Association (Adam *et al*., 1981; Hannaford *et al*., 1991). On entry, 56% of women were taking oral contraceptives, 25% were using a diaphragm and 19% were using an intrauterine device. Since, during the course of the study, each woman's oral contraceptive status could change, users of these preparations might have contributed periods of observation for either current or former users. After 266 866 woman–years of follow-up, 32 new cases of cutaneous malignant melanoma were recorded, 17 of which were among women who had ever used oral contraceptives (relative risk, 0.8; 95% CI, 0.4–1.8). None of the rates observed in any category of duration of use was materially different from that seen in women who had never used these preparations. The relative risks, adjusted for age, parity, social class and smoking, were 0.6 (95% CI, 0.2–1.6) for < 5 years of use, 1.0 (0.4–2.6) for 5–9 years and 1.0 (0.2–3.1) for ≥ 10 years. There was no relationship between time since stopping use of oral contraceptives and the risk for cutaneous malignant melanoma. None of the formulations resulted in a specific risk pattern. The distribution of cutaneous malignant melanomas by site was similar in users and non-users of oral contraceptives.

Between 1968 and 1969, 1400 general practitioners throughout the United Kingdom recruited 23 000 women who were using oral contraceptives and a similar number of age-matched women who had never used them, in the framework of the study of the Royal College of General Practitioners (Kay, 1981; Hannaford *et al*., 1991). After 482 083 woman–years of follow-up, 58 new cases of cutaneous malignant melanoma had been recorded, 31 of which were among women who had ever used combined oral contraceptives; the relative risk, adjusted for age, parity, social class and smoking, was 0.9 (95% CI, 0.6–1.5). No significant trend of increasing risk with duration of use was seen, the relative risk for 10 years or more of use being 1.8 (95% CI, 0.8–3.9), and the relative risk did not vary according to recency of use, the oestrogen or progestogen content of the contraceptives or the site of cutaneous malignant melanoma.

A cohort study of 17 942 women who were members of the Kaiser-Permanente Health Plan, in California, United States, aged 18 and older, was established in 1970 within the Walnut Creek Contraceptive Drug Study. Ramcharan *et al*. (1981b) updated the preliminary findings of Beral *et al*. (1977) to approximately eight years of follow-up and observed 20 cases of cutaneous malignant melanoma (age-adjusted relative risk, 3.5; 95% CI, 1.4–9.0). All five cases in women 18–39 years of age occurred among users of combined oral contraceptives. The influence of duration and recency of use was not assessed. The percentage distribution of hours of exposure to the sun by current, past or no use of combined oral contraceptives was similar.

In a postal survey of 121 964 registered nurses in the United States in 1976 (Bain *et al*., 1982), no overall relationship was found between risk for cutaneous malignant melanoma and use of combined oral contraceptives among 141 women with non-fatal

cutaneous malignant melanoma and 2820 age-matched control women. The relative risk, adjusted for age, parity, height and hair dye use, was 0.8 (95% CI, 0.5–1.3). No significant trends emerged with duration of use or time since first use. For women who were under the age of 40 at the time cutaneous malignant melanoma was diagnosed, the relative risk was 1.4 (95% CI, 0.8–2.5). For women under 40 who had used combined oral contraceptives for more than two years at least 10 years before diagnosis of cutaneous malignant melanoma, the relative risk was 2.3 (95% CI, 0.8–6.9). An analysis restricted to the 84 histologically documented cases of cutaneous malignant melanoma showed similar results [not shown].

2.7.2 *Case-control studies*

These studies are summarized in Table 29.

Adam *et al*. (1981) investigated 169 cases of cutaneous malignant melanoma in women aged 15–49 years that had been notified to the cancer registries of south-western England during 1971–76, and 507 age-matched control women drawn from the lists of the same general practitioners as the cases. Data were obtained from the general practitioners' records and for about 70% of the study women from postal questionnaires. The risk for cutaneous malignant melanoma was not significantly increased among women who had ever used combined oral contraceptives, the unadjusted relative risk being 1.3 (95% CI, 0.9–2.0) from the practitioners' records and 1.1 (95% CI, 0.7–1.8) from the postal questionnaires.

In an Australian investigation by Green and Bain (1985), described in detail in the monograph on 'Post-menopausal oestrogen therapy', there was no increased risk for cutaneous malignant melanoma in relation to use of combined oral contraceptives, with an age-adjusted relative risk of 0.7 (95% CI, 0.4–1.5), and no trend of increasing risk with increasing duration of use, the relative risk for > 4 years' use being 0.4 (95% CI, 0.2–1.1). The risk was also not elevated among women who had first used combined oral contraceptives 10 or more years before diagnosis of cutaneous malignant melanoma, with a relative risk of 0.9 (95% CI, 0.4–2.2).

In the case–control study of Holly *et al*. (1983) in Seattle, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', use of combined oral contraceptives for five years or more was commoner among cases than controls, with age-adjusted relative risks of 1.5 for 5–9 years of use and and 2.1 for ≥ 10 years' duration (not significant). This relationship was seen only with long-term use of combined oral contraceptives among women with superficial spreading melanoma, with relative risks of 2.4 for 5–9 years and 3.6 for ≥ 10 years of use, and a highly significant trend ($p = 0.004$) with increasing duration of use. Adjustment was not made for the pattern of exposure to the sun.

In the study of Lew *et al*. (1983), in Massachusetts, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', no data were given on hormonal treatment, but it was reported that cases and controls did not differ with respect to use of combined oral contraceptives.

Table 29. Case–control studies of use of combined oral contraceptives and malignant melanoma

Reference	Country	Cases : controls (type of controls)	Subgroup	RR (95% CI) any versus no use	Duration of use	Recency of use	Adjustment/Comments
Adam *et al.* (1981)	England	169 : 507 (same general practitioner)	General practitioners' records, postal questionnaires	1.3 (0.9–2.0) 1.1 (0.7–1.8)	No significant trend (RR for ≥ 5 years, 1.6; 95% CI, 0.8–3.0)	No effect	Unadjusted Responses to postal questionnaire (response rate about 70%) did not show an association between use of combined oral contraceptives and exposure to the sun
Green & Bain (1985)	Queensland, Australia	91 : 91 (population)		0.7 (0.4–1.5)	No trend (RR for > 4 years' use, 0.4; 95% CI, 0.2–1.1)	No effect (RR for use ≥ 10 years before diagnosis, 0.9; 95% CI, 0.4–2.2)	Age After allowance for phenotypic characteristics and solar exposure, RR for > 4 years' use, 0.4 (95% CI, 0.1–2.0)
Holly *et al.* (1983)	Seattle, USA	87 : 863 (population)	1–4 years 5–9 years ≥ 10 years	1.0 1.5 (NS) 2.1 (NS)	Significant trend only for SSM (RR ≥ 10 years use, 3.6)	Increased risk for ≥ 12 years since first use: 4.4 (95% CI, 2.0–9.7)	Age No data on solar exposure
Lew *et al.* (1983)	Massachusetts USA	111 : 107 (friends of cases)	–	–	–	–	No difference in combined oral contraceptive use
Beral *et al.* (1984)	Sydney, Australia	287 : 574 (hospital and population)		1.0 (NS)	No significant trend	No significant effect	Unadjusted (but altered by education, phenotype, history of sunburn and solar exposure) Increased risk for women who had begun taking combined oral contraceptives at least 10 years before and with ≥ 5 years' duration of use: 1.5 (95% CI, 1.0–2.1). No difference by location, thickness or type of CMM

Table 29 (contd)

Reference	Country	Cases : controls (type of controls)	Subgroup	RR (95% CI) any versus no use	Duration of use	Recency of use	Adjustment/Comments
Helmrich *et al.* (1984)	United States and Canada	160 : 640 (hospital)		0.8 (0.5–1.3)	No trend (RR for ≥ 10 years' use, 1.0; 95% CI, 0.4–2.9 [only age-adjusted])	No effect of time since first use (RR for first use ≥ 10 years previously, 1.1; 95% CI, 0.7–1.8)	Age, area, religion, education and hormone-related variables
Holman *et al.* (1984)	Western Australia	276 : 276	CMM SSM	1.0 (0.6–1.6) 1.1 (0.6–2.2)	No significant trend (RR for ≥ 5 years use, 1.1; 95% CI, 0.6–2.0)	No effect (RR for ≥ 10 years' use before diagnosis, 1.1; 95% CI, 0.7–1.7)	Age and residence
Gallagher *et al.* (1985)	Canada	361 : 361 (members of health plans)	CMM < 1 year 1–4 years ≥ 5 years SSM < 1 year 1–4 years ≥ 5 years	 1.0 0.9 0.8 1.1 1.1 0.9	No trend	No effect (RR for use ≥ 10 years prior to diagnosis, 1.0)	Age, education, phenotype and freckling Allowance for phenotypic characteristics
Østerlind *et al.* (1988)	Denmark	280 : 536	CMM SSM	0.8 (0.5–1.2) 0.9 (0.6–1.3)	No trend (RR for ≥ 10 years' use, 1.0; 95% CI, 0.6–1.7)	No effect (RR for use ≥ 10 years before diagnosis, 1.3; 95% CI, 0.7–2.2)	Age, phenotype and sunbathing No difference according to type and potency of combined oral contraceptives
Zanetti *et al.* (1990)	Northern Italy	186 : 205 (population)	CMM SSM	1.0 (0.5–1.9) 1.3 (0.4–4.5)	No trend (RR for ≥ 3 years' use, 1.0; 95% CI, 0.5–2.7)	No effect	Age, education, phenotype and sunbathing Risk did not change according to CMM type or location, age or combined oral contraceptive potency

Table 29 (contd)

Reference	Country	Cases : controls (type of controls)	Subgroup	RR (95% CI) any versus no use	Duration of use	Recency of use	Adjustment/Comments
Augustsson *et al.* (1991)	Sweden	69 : 196 (population)			Not reported		No difference in combined oral contraceptive use
Lê *et al.* (1992)	France	91 : 149 (hospital)	< 10 years ≥ 10 years	1.1 (0.6–2.0) 2.1 (0.7–5.9)	No significant trend	No effect of use 15–20 years before diagnosis (RR, 1.9; 95% CI, 0.8–4.5)	
Palmer *et al.* (1992)	Philadelphia and New York, USA	615 : 2107	Severe Not severe	1.1 (0.8–1.5) 1.5 (1.1–2.4)	No trend (RR for not severe for ≥ 10 years' use, 2.0; 95% CI, 0.9–4.3)	No effect (RR for first use ≥ 20 years before severe CMM, 1.1; 95% CI, 0.7–1.8)	Age, education, body mass index, menopause and phenotype Elevated risk among not severe cases of CMM was attributed to surveillance bias; similar RR for different types
Zaridze *et al.* (1992)	Moscow, Russian Federation	96 : 96		0.04 (0.0–0.5)	Not reported	Not reported	Phenotype, naevi and sunbathing Only one case and seven controls
Holly *et al.* (1995)	San Francisco, USA	452 : 930 (population)	CMM SSM	0.7 (0.5–0.9) 0.7 (0.5–1.0)	No trend (RR for ≥ 10 years' use: CMM, 0.8; 95% CI, 0.5–1.3; SSM, 1.0; 95% CI, 0.6–1.6)	No effect (RR for use ≥ 17 years before diagnosis, 0.6; 95% CI, 0.4–0.7)	Age (unaltered by education, phenotype and solar exposure)

Table 29 (contd)

Reference	Country	Cases : controls (type of controls)	Subgroup	RR (95% CI) any versus no use	Duration of use	Recency of use	Adjustment/Comments
Westerdahl *et al.* (1996)	Sweden	180 : 292 (population)		1.6 (0.9–2.8)	No effect (RR for > 8 years' use, 1.0; 95% CI, 0.5–2.0)	No effect	Phenotype, naevi and sunburns Age at use and timing of use in relation to first child did not influence risk
Ocular melanoma							
Hartge *et al.* (1989b)	Wilmington and Philadelphia, USA	238 : 223 (detached retina)		0.9 (0.4–1.7)	No trend (RR for ≥ 10 years' use, 0.2; NS)	No effect	Age

RR, relative risk; CI, confidence interval; NS, not significant; CMM, cutaneous malignant melanoma; SSM, superficial spreading melanoma

In the study of Beral *et al*. (1984) in Sydney, Australia, described in detail in the monograph on 'Post-menopausal oestrogen therapy', women who had ever used combined oral contraceptives were not at increased risk for cutaneous malignant melanoma (relative risk, 1.0). There was, however, an increased risk for women who had used these formulations for five years or more and who had begun use at least 10 years before diagnosis of cutaneous malignant melanoma, with a relative risk of 1.5 (95% CI, 1.0–2.1). The increase in risk persisted after control for phenotypic characteristics, number of moles and measures of exposure to ultraviolet light. The risk did not vary according to the location, thickness or type of melanoma.

In a case–control study carried out in several parts of the United States and Canada between 1976 and 1982 (Helmrich *et al*., 1984), the case series consisted of 160 women aged 20–59 years with a recent histological diagnosis of cutaneous malignant melanoma, and the controls were 640 women aged 20–59 years admitted to hospital for trauma or orthopaedic and surgical conditions. The age-adjusted relative risk for those who had ever used combined oral contraceptives was 0.8 (95% CI, 0.5–1.3), and there was no trend in risk with increasing duration of use, the relative risk for ≥ 10 years of use being 1.0 (95% CI, 0.4–2.9). For the 40 case and 140 control women who had first used combined oral contraceptives at least 10 years previously, the relative risk was 1.1 (95% CI, 0.7–1.8) and for women with more advanced cutaneous malignant melanoma (i.e. Clark's level IV and V), the relative risk was 0.6 (95% CI, 0.2–2.3).

In the study of Gallagher *et al*. (1985), in Canada, described in detail in the monograph on 'Post-menopausal oestrogen therapy', no association was seen between the risk for cutaneous malignant melanoma and use of combined oral contraceptives in 361 cases and an equal number of controls aged 20–69. The relative risks for < 1, 1–4 and ≥ 5 years' use, adjusted for age, phenotypic characteristics and freckling, were 1.0, 0.9 and 0.8, respectively. No association was seen between type of superficial spreading melanoma and duration of use or years since last use, the relative risk for women who had used combined oral contraceptives 10 or more years before diagnosis of cutaneous malignant melanoma being 1.0.

In the Danish study of Østerlind *et al*. (1988), described in detail in the monograph on 'Post-menopausal oestrogen therapy', use of oral contraceptives was not related to the risk for cutaneous malignant melanoma (relative risk adjusted for age, phenotypic characteristics and sunbathing, 0.8; 95% CI, 0.5–1.2) or superficial spreading melanoma (relative risk, 0.9; 95% CI, 0.6–1.3), and there was no evidence of a dose–response relationship, the relative risk for ≥ 10 years' use being 1.0 (95% CI, 0.6–1.7). No specific risk pattern was seen with the type of oral contraceptive, such as sequential, progestogen only and high-potency combined oral contraceptives, assessed separately, but there were few women in each group.

Zanetti *et al*. (1990) carried out a case–control study in Turin, northern Italy, between 1984 and 1987 of 186 women aged 19–92 with histologically confirmed cutaneous malignant melanoma out of 211 identified from the Turin Cancer Registry and 205 control women aged 17–92 drawn from the National Health Service Registry (out of the 300 initially contacted). Use of combined oral contraceptives, analysed only in women

aged 60 or younger, was not associated with cutaneous malignant melanoma, the relative risk adjusted for age, education, phenotypic characteristics and sunbathing being 1.0 (95% CI, 0.5–1.9); no association was seen with superficial spreading melanoma (relative risk, 1.3; 95% CI, 0.4–4.5). Similarly, the longest duration of use (≥ 3 years: 1.0; 95% CI, 0.5–2.7) or use that had started 10 or more years before the diagnosis of cutaneous malignant melanoma was not associated with an increased risk. The relative risks were identical for use of combined oral contraceptives containing high oestrogen doses (≥ 50 μg) and low oestrogen doses.

Augustsson *et al*. (1991) studied 69 cases of cutaneous malignant melanoma in Swedish women aged 30–50 and compared them with 196 controls drawn from the same population. Skin type, phenotypic characteristics, number of naevi and dysplastic naevi were taken into account. Although the relative risk was not reported, no difference in the use of combined oral contraceptives was reported between cases and controls.

Lê *et al*. (1992) assessed the effect of use of combined oral contraceptives on the risk for cutaneous malignant melanoma risk in France between 1982 and 1987. The cases were those of 91 white women under 45 years of age who had new, histologically confirmed melanomas, and the controls were 149 women consulting for diagnosis or treatment of diseases unrelated to use of combined oral contraceptives, including skin diseases. No significant association was found between the total duration of use of combined oral contraceptives (relative risk, adjusted for age at menarche for ≥ 10 years' use, 2.1; 95% CI, 0.7–5.9) or the time since first use (relative risk 15–20 years since first use, 1.9; 95% CI, 0.8–4.5) and the risk for cutaneous malignant melanoma, and no difference was found between superficial spreading melanoma and other types of cutaneous malignant melanoma. The relative risk for 49 case women and 78 matched controls who were aged 30–40 and had used oral contraceptives for 10 or more years, however, was significantly increased: 4.4 (95% CI, 1.1–17). In a subgroup of 57 case women and 65 controls for whom allowance could be made for phenotypic characteristics and solar exposure, the relative risks were similar.

A case–control study on cutaneous malignant melanoma was carried out between 1979 and 1991 in Philadelphia and New York, United States (Palmer *et al*., 1992), in which the cases were in 615 women under the age of 70 (median age, 40) who had recently received a first diagnosis of cutaneous malignant melanoma. Patients with melanoma *in situ* were not included. Two control groups of white women with a median age of 41 years with other malignancies (610 patients) or non-malignant illnesses (1497 patients) judged to be unrelated to use of combined oral contraceptives were selected. In order to address the possibility of selection bias due to differential surveillance of combined oral contraceptive users and non-users, the cases were subdivided by severity. For severe cases (thickness ≥ 0.75 mm, or Clark's level IV or V), the relative risks adjusted for age, education, menopause and phenotypic characteristics were 1.1 (95% CI, 0.8–1.5) for any use, 1.1 (0.6–2.1) for ≥ 10 years' use and 1.1 (0.7–1.8) for ≥ 20 years' use. For non-severe cases, increased risks were found for any use (1.5; 95% CI, 1.1–2.2) and for ≥ 10 years' use (2.0; 0.9–4.3). The relative risks did not vary by type of cutaneous

malignant melanoma. According to the authors, the increased risks seen for non-severe cases of cutaneous malignant melanoma were probably due to greater surveillance of combined oral contraceptive users.

Zaridze *et al*. (1992) evaluated risk factors in 96 cases of cutaneous malignant melanoma in Moscow, Russian Federation. Controls matched by age were recruited from among persons visiting cancer patients. Use of combined oral contraceptives could be analysed for 54 women with cutaneous malignant melanoma and 54 controls and showed a strong inverse association: the relative risk, adjusted for phenotypic characteristics, naevi and sunbathing, was 0.04 (95% CI, 0.0–0.5). Only one case and seven controls, however, had ever used combined oral contraceptives.

In the study of Holly *et al*. (1995), described in detail in the monograph on 'Post-menopausal oestrogen therapy' (Holly *et al*., 1994), 72% of the cases of cutaneous malignant melanoma and 79% of the control subjects in San Francisco, United States, reported ever having used combined oral contraceptives. The age-adjusted relative risk was 0.7 (95% CI, 0.5–0.9) for all cutaneous malignant melanoma and 0.7 (95% CI, 0.5–1.0) for superficial spreading melanoma. Examination by latency and duration of use showed no significant trend. The relative risk for ≥ 10 years' use was 0.8 (95% CI, 0.5–1.3) for all cutaneous malignant melanoma and 1.0 (95% CI, 0.6–1.6) for superficial spreading melanoma. Use beginning ≥ 17 years before diagnosis was associated with relative risks of 0.6 (95% CI, 0.4–0.7) for cutaneous malignant melanoma and 0.6 (95% CI, 0.4–0.8) for superficial spreading melanoma.

In the Swedish study of Westerdahl *et al*. (1996), described in the monograph on 'Post-menopausal oestrogen therapy', any use of combined oral contraceptives (40% of cases and 37% of controls) was associated with a non-significantly elevated risk of 1.6 (95% CI, 0.9–2.8) after adjustment for phenotypic characteristics, naevi and sunburns. No trend in risk was seen with duration of use (relative risk for > 8 years' use, 1.0; 95% CI, 0.5–2.0), age at first use or age at last use.

A meta-analysis of 18 published case–control studies on cutaneous malignant melanoma and use of combined oral contraceptives, including 17 of the papers reviewed here and that of Beral *et al*. (1977), showed a pooled relative risk of 1.0 (95% CI, 0.9–1.0) (Gefeller *et al*., 1997). The data for 3796 cases and 9442 controls showed no significant heterogeneity of the effect of combined oral contraceptives in the different studies, and analysis of various subgroups, defined by the design characteristics of the studies, did not materially alter this result.

2.8 Retinal melanoma

In a case–control study of ocular melanoma in the United States (Hartge *et al*., 1989b), described in the monograph on 'Post-menopausal oestrogen therapy', use of combined oral contraceptives was reported by about 13% in both cases and controls, to give an age-adjusted relative risk of 0.9 (95% CI, 0.4–1.7). The estimated risk was not related to duration of use (relative risk for ≥ 10 years' use, 0.2; 95% CI, 0.3–1.2) or to the interval since first or last use.

2.9 Thyroid cancer

None of the cohort studies provided information on use of combined oral contraceptives and the risk for thyroid cancer. The case–control studies are summarized in Table 30.

In the case–control study of McTiernan *et al*. (1984), in Seattle, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', the use of combined oral contraceptives (prevalence: 93/141 cases and 130/219 controls) was associated with a slightly increased risk for thyroid cancer (1.6; 95% CI, 1.0–2.5). The magnitude of the excess risk did not increase with increasing duration of use (relative risk for > 3 years' duration, 1.2). The risk was higher among women with follicular thyroid cancer (3.6; 95% CI, 1.1–12.8) and among those women who discovered their own tumours as compared with those whose tumour was found by a physician.

Preston-Martin *et al*. (1987) evaluated the risk factors for thyroid cancer in women aged 40 or less in Los Angeles, United States, between 1980 and 1981. The cases were in 108 white women with papillary, follicular or mixed thyroid cancer (out of 135 identified through Southern California Cancer Surveillance Program) and controls were 108 age-matched women who lived near the case women (neighbourhood controls). More cases (67/78) than controls (76/106) had ever used combined oral contraceptives (unadjusted relative risk, 2.4; 95% CI, 1.1–5.7). Cases and controls did not differ with respect to age at first use. There was no trend of increasing risk with increasing duration of use, the relative risk for > 5 years' duration of use being 2.4 (95% CI, 0.9–6.9).

In a study conducted in Connecticut, United States (Ron *et al*., 1987), described in the monograph on 'Post-menopausal oestrogen therapy', similar proportions of cases (55/109) and controls (110/208) had ever used combined oral contraceptives, the relative risk adjusted for age, parity, radiotherapy to the head and neck and benign thyroid disease being 0.8 (not significant). For women under the age of 35 at the time of diagnosis, the relative risk was 1.8 (not significant). Duration and latency of use were not assessed.

Franceschi *et al*. (1990) found relatively few users of combined oral contraceptives among cases of thyroid cancer in Italy (23/165 cases and 28/214 controls). The age-adjusted relative risks were 1.1 (95% CI, 0.5–2.4) for use for < 24 months and 1.1 (95% CI, 0.4–3.0) for use for ≥ 24 months.

In a case–control study in Hawaii (Kolonel *et al*., 1990), described in the monograph on 'Post-menopausal oestrogen therapy', women who had ever used combined oral contraceptives (43% among controls) showed no increased risk for thyroid cancer. The relative risk, adjusted for age and ethnic group was 0.9 (95% CI, 0.5–1.5). The effects of duration and latency of use were not reported.

Levi *et al*. (1993), in study in Switzerland, described in detail in the monograph on 'Post-menopausal oestrogen therapy', found a prevalence of any use of combined oral contraceptives of 56% among thyroid cancer cases and 44% among control women; the relative risk, adjusted for age and a history of benign thyroid disease, was 1.2 (95% CI, 0.7–2.3). There was no trend of increasing risk with increasing duration of use, the relative risk for ≥ 5 years' use being 1.4 (95% CI, 0.7–2.7). Analyses restricted to women under 45 years of age or to cases of papillary thyroid cancer yielded similar risk estimates.

Table 30. Case–control studies on use of combined oral contraceptives and thyroid cancer

Reference	Country	Cases : controls (type of controls)	RR (95% CI), any versus no use	Duration of use	Adjustment/comments
McTiernan *et al*. (1984)	Seattle, USA	141 : 319 (population)	1.6 (1.0–2.5)	No trend (RR for > 3 years' use, 1.2)	Age Greatest risk increase seen for follicular thyroid cancer (RR, 3.6; 95% CI, 1.1–13)
Preston-Martin *et al*. (1987)	Los Angeles, USA	108 : 108 (population)	2.4 (1.1–5.7)	No trend (RR for > 5 years' use, 2.4; 95% CI, 0.9–6.9)	Unadjusted. Only women aged 40 or less
Ron *et al*. (1987)	Connecticut, USA	109 : 208 (population)	0.8	Not reported	Age, parity, radiotherapy to the head and neck and benign thyroid diseases RR for women < 35 was 1.8 (not significant)
Franceschi *et al*. (1990)	Italy	165 : 214 (hospital)	< 2 years, 1.1 (0.5–2.4) ≥ 2 years, 1.1 (0.4–3.0)	No effect	Age and area of residence
Kolonel *et al*. (1990)	Hawaii, USA	140 : 328 (population)	0.9 (0.5–1.5)	Not reported	Age and ethnic group Increased risk for women with difficulty in conceiving (RR, 1.8; 95% CI, 1.0–3.1) and those who used fertility drugs (RR, 4.2; 95% CI, 1.5–11)
Levi *et al*. (1993)	Vaud, Switzerland	91 : 306 (hospital)	1.2 (0.7–2.3)	No trend (RR for ≥ 5 years' use, 1.4; 95% CI, 0.7–2.7)	Age and history of benign thyroid disease Similar risk estimates for women under 45 and for papillary thyroid cancer
Preston-Martin *et al*. (1993)	Shanghai, China	207 : 207 (population)	1.7 (1.0–3.1)	No trend (RR for > 5 years' use, 0.9; 95% CI, 0.4–2.4)	Age
Wingren *et al*. (1993)	South-eastern Sweden	93 : 187 (population)	No risk (RR not reported)	Not reported	Only papillary carcinomas

Table 30 (contd)

Reference	Country	Cases : controls (type of controls)	RR (95% CI), any versus no use	Duration of use	Adjustment/comments
Hallquist *et al*. (1994)	Northern Sweden	123 : 240 (population)	All, 0.8 (0.5–1.4) Papillary, 0.6 (0.3–1.2)	No trend	Age Risk did not vary by timing in relation to age at first pregnancy
Galanti *et al*. (1996)	Sweden and Norway	191 : 341 (population)	0.9 (0.6–1.5)	No trend (> 2 years' use RR, 0.8; 95% CI, 0.5–1.3)	Age and parity

RR, relative risk; CI, confidence interval

Preston-Martin *et al*. (1993) carried out a study in Shanghai, China, between 1981 and 1984, which included 207 women aged 18–54 listed in the Shanghai Cancer Registry as having a histologically confirmed thyroid cancer; 20% of the cases were reviewed by a pathologist. The 207 control women, matched to the cases by year of birth, were chosen randomly from the Shanghai Residents' Registry; over 90% of the eligible subjects were interviewed. Few women had used combined oral contraceptives (43/207 cases and 29/207 controls), but any use of such formulations was associated with a marginally increased risk (unadjusted relative risk, 1.7; 95% CI, 1.0–3.1). Among users, however, there was no trend in risk with the duration of use, the relative risk for > 5 years' use being 0.9 (95% CI, 0.4–2.4).

Wingren *et al*. (1993) studied 93 cases of thyroid cancer and 187 controls aged 20–60 in south-east Sweden and reported that use of combined oral contraceptives was not associated with an increased risk. No data were shown.

Hallquist *et al*. (1994), in Sweden, reported that 42/123 cases and 92/240 controls had reported any use of combined oral contraceptives, giving an age-adjusted relative risk of 0.8 (95% CI, 0.5–1.4). The corresponding relative risk for papillary thyroid cancer was 0.6 (95% CI, 0.3–1.2). The risk did not vary by duration of use, being 0.6 (95% CI, 0.2–1.5) for ≥ 7 years' use, or by timing of use in relation to age at first pregnancy.

Galanti *et al*. (1996), in a study in Sweden and Norway described in the monograph on 'Post-menopausal oestrogen therapy', reported that 98/179 cases and 180/334 controls had used combined oral contraceptives. No relation was found between use and the risk for thyroid cancer; the age- and parity-adjusted relative risk for any use was 0.9 (95% CI, 0.6–1.5). Use for > 2 years was associated with a relative risk of 0.8 (95% CI, 0.5–1.3).

2.10 Other cancers

A 25-year follow-up of 46 000 women in Great Britain in the framework of a study on oral contraceptives by the Royal College of General Practitioners did not show significant excess mortality from lung cancer (relative risk, 1.2; 95% CI, 0.8–1.8) or any other cancer (Beral *et al*., 1999).

In a study by La Vecchia *et al*. (1994), from Milan, Italy, described in detail in the monograph on 'Post-menopausal oestrogen therapy', use of combined oral contraceptives was not related to the risk for gastric cancer. Six of 229 cases and 19 of 614 controls had ever used such formulations, giving a relative risk adjusted for age, education, a family history of cancer and dietary habits of 1.3 (95% CI, 0.5–3.5).

Chow *et al*. (1995) in a study in Minnesota, United States, described in detail in the monograph on 'Post-menopausal oestrogen therapy', found no relation between use of combined oral contraceptives and the risk for renal-cell cancer; the relative risk, adjusted for age, smoking and body mass index was 0.8 (95% CI, 0.4–1.3). For use longer than 10 years, the relative risk was 0.3 (95% CI, 0.1–1.0).

The risk for renal-cell cancer and use of combined oral contraceptives was also evaluated in an international study by Lindblad *et al*. (1995), described in detail in the monograph on 'Post-menopausal oestrogen therapy'. Any use of combined oral contraceptives

was associated with a relative risk, adjusted for age, smoking and body mass index, of 0.7 (95% CI, 0.5–0.9). There was an inverse trend in risk with increasing duration of use, the relative risk for > 10 years' use being 0.5 (95% CI, 0.3–0.9).

3. Studies of Cancer in Experimental Animals

In this section, only relevant studies on oestrogens and progestogens alone and in combination that were published subsequent to or not included in Volume 21 of the IARC Monographs (IARC, 1979) are reviewed in detail. Studies reviewed previously are summarized briefly.

3.1 Oestrogen–progestogen combinations

3.1.1 *Studies reviewed previously*

Mouse

The results of studies reviewed previously (Committee on Safety of Medicines, 1972; IARC, 1979) on the carcinogenicity of combinations of oestrogens and progestogens in mice are as follows:

Chlormadinone acetate in combination with mestranol tested by oral administration to mice caused an increased incidence of pituitary adenomas in animals of each sex. Oral administration of chlormadinone acetate in combination with ethinyloestradiol to mice resulted in an increased incidence of mammary tumours in intact and castrated males.

After oral administration of ethynodiol diacetate and mestranol to mice, increased incidences of pituitary adenomas were observed in animals of each sex. The combination of ethynodiol diacetate plus ethinyloestradiol, tested by oral administration to mice, increased the incidences of pituitary adenomas in animals of each sex and of malignant tumours of connective tissues of the uterus.

Lynoestrenol in combination with mestranol was tested in mice by oral administration. A slight, nonsignificant increase in the incidence of malignant mammary tumours was observed in females which was greater than that caused by lynoestrenol or mestranol alone.

The combination of megestrol acetate plus ethinyloestradiol, tested by oral administration to mice, caused an increased incidence of malignant mammary tumours in animals of each sex.

Noresthisterone acetate plus ethinyloestradiol, tested by oral administration to mice, increased the incidences of pituitary adenomas in animals of each sex, but the incidences were comparable to those induced by ethinyloestradiol alone. The combination of norethisterone plus ethinyloestradiol was also tested in mice by oral administration; an increased incidence of pituitary adenomas was observed in females. Norethisterone plus mestranol increased the incidences of pituitary adenomas in animals of each sex.

Norethynodrel in combination with mestranol was tested by oral administration in mice. Increased incidences of vaginal or cervical tumours were found in female mice and of pituitary adenomas in males and females. In female mice, an increased incidence of

malignant mammary tumours was observed, but the incidence was not greater than that seen with norethynodrel alone. In castrated male mice, the combined treatment resulted in an increased incidence of mammary tumours.

The combination of norgestrel plus ethinyloestradiol was tested in mice by oral administration; no increase in the incidence of tumours was observed.

Rat

The results of studies reviewed previously (Committee on Safety of Medicines, 1972; IARC, 1979) on the carcinogenicity in rats of several combinations are as follows:

Ethynodiol diacetate plus ethinyloestradiol was tested for carcinogenicity by oral administration to rats. The incidence of malignant mammary tumours was increased in animals of each sex. In combination with mestranol, the incidence of mammary tumours was increased in one study but not in another.

Lynoestrenol in combination with mestranol was tested in female rats by oral administration. No increase in tumour incidence was observed.

Megestrol acetate plus ethinyloestradiol was tested by oral administration to rats. The incidence of benign liver tumours was increased in animals of each sex, but not to a level greater than that observed with ethinyloestradiol alone. In male rats, there was a small increase in the incidence of benign and malignant mammary tumours; females showed a small increase in the incidence of malignant mammary tumours.

The combination of noresthisterone acetate plus ethinyloestradiol, tested by oral administration to rats, increased the incidences of benign mammary tumours and liver adenomas in males. Norethisterone plus mestranol increased the incidence of malignant mammary tumours in female rats and increased the incidence of liver adenomas in males.

Norethynodrel in combination with mestranol was tested by oral administration to rats. In males, increased incidences of liver adenomas, pituitary adenomas and benign and malignant mammary tumours were observed, but the incidences were no greater than those with norethynodrel alone. In females, the incidences of pituitary adenomas and malignant mammary tumours were increased.

Norgestrel plus ethinyloestradiol, tested for carcinogenicity in rats by oral administration, caused a small increase in the incidence of benign mammary tumours in males.

Dimethisterone and oestradiol were tested in dogs, with no increase in the incidence of mammary tumours

3.1.2 *New studies*

(*a*) *Oral administration*

Rat

Schuppler and Gunzel (1979) summarized data from a study by the Committee on Safety of Medicines (1972) in the United Kingdom on the incidence of hepatocellular adenomas in groups of 24–124 male and female rats [strain not specified] treated orally with combinations of various oestrogens and progestogens at doses up to 400 times the

human contraceptive dose. The statistically significant increases indicated in their report are indicated by a '+' in Table 31.

Table 31. Effects of progestogen–oestrogen combinations on the incidence of hepatocellular adenomas in rats

Progestogen	Oestrogen	Ratio	Males	Females
Norethynodrel	Mestranol	66:1	+	–
		25:1	–	–
Norethisterone	Mestranol	20:1	+	–
Lynestrenol	Mestranol	33:1	Not tested	–
Megestrol acetate	Ethinyloestradiol	5:1	+	–
		80:1	+	–

From Schuppler & Gunzel (1979)

Groups of 10 female Sprague-Dawley rats, seven weeks of age, were treated with Enovid E (100 μg mestranol + 25 mg norethynodrel) in the diet for nine months, with daily intakes of 0.02–0.03 and 0.5–0.75 mg/kg bw. The numbers of altered γ-glutamyl transpeptidase (γ-GT)-positive hepatic foci, considered to be preneoplastic lesions, were counted at autopsy. A statistically significant ($p < 0.001$) increase in the number of foci (2.8 foci/cm^2) was observed in comparison with untreated controls (0.2 foci/cm^2). No increase in the incidence of hepatic nodules or carcinomas was observed at this time (Yager & Yager, 1980).

Female Wistar rats, 15–17 weeks of age, were treated with quingestanol acetate plus quinestrol, which are 3-cyclopentyl ether derivatives of norethisterone acetate and ethinyloestradiol, respectively, as a 2:1 mixture suspended in sesame oil containing piperidine (0.05% v/w) by stomach tube. A group of 75 rats was treated once weekly with 30 mg/kg bw, 60 rats were treated with 1.2 mg/kg bw per day, and 75 rats were used as vehicle controls; all treatments were given for 50 weeks, followed by 30 weeks of observation for reversibility of any lesions. Groups of 10 animals were killed at 25 and 50 weeks; at 66 weeks, five rats from each treatment group and three vehicle controls were killed, and at 80 weeks all survivors in the treated groups and 10 vehicle controls were killed. Treatment was associated with irreversible hair loss, a reversible decrease in body weight and reversible ataxia. The only treatment-related tumours were mammary masses and adenocarcinomas: at 40–50 weeks, the incidences of adenocarcinomas were 10/33 in rats at 30 mg/kg bw, 15/27 in those at 1.2 mg/kg bw and 1/12 in vehicle controls [statistics not specified]. After treatment was suspended, the incidences of mammary adenocarcinomas were reduced, with 1/8 at weeks 51–66 and 0/12 at weeks 67–80 in animals at 30 mg/kg bw, 3/7 and 2/8 at those times in animals at 1.2 mg/kg bw and 0/10 and 1/13 in vehicle controls (Lumb *et al*., 1985).

Male and female Wistar rats, four weeks old, were given ethinyloestradiol (0.075 mg) + norethisterone acetate (6 mg) dissolved in olive oil by gavage daily, ethinyloestradiol +

norethisterone acetate + 10% ethanol in the drinking-water on five days a week, olive oil + ethanol or olive oil alone. The animals were treated for up to 12 months, with interim kills at two, four, six, eight and 12 months, when the livers were analysed for the presence of hepatocellular carcinomas and hyperplastic nodules. In females, ethinyloestradiol + norethisterone acetate induced a 100% incidence of hyperplastic nodules by four months and hepatocellular carcinomas in 2/25 animals at 12 months. Ethanol increased the incidence of hepatocellular carcinoma at 12 months to 9/22; no hyperplastic nodules or hepatocellular carcinomas were seen in the controls receiving ethanol alone. In males, ethinyloestradiol + norethisterone acetate induced hyperplastic nodules in 6/20 animals at 12 months but no hepatocellular carcinomas. Ethanol increased the incidence of hyperplastic nodules to 100%, beginning at four months, and that of hepatocellular carcinomas to 2/17 at 12 months. Again, ethanol alone had no effect on the incidences of hyperplastic nodules or hepatocellular carcinoma, but it enhanced nuclear and cytosolic oestrogen receptors and DNA adduct formation, as detected by ^{32}P-postlabelling (Yamagiwa *et al*., 1991, 1994).

Monkey

Norlestrin (50:1 norethisterone acetate + ethinyloestradiol) was given to groups of 15–17 young adult female rhesus (*Macaca mulatta)* monkeys weighing 2.8–5.7 kg at the beginning of the study. Norlestrin powder was blended with soft fruit and vegetables and was administered over 10 years as 21 consecutive daily doses followed by seven days without treatment. The daily doses were 0, 0.051, 0.51 and 2.55 mg/kg bw which represented 0, 1, 10 and 50 times the human contraceptive dose. There were no effects on survival and no treatment-related alterations in coagulation or other clinical parameters. Only a few tumours appeared but were found in all groups (Fitzgerald *et al*., 1982).

(*b*) *Administration with known carcinogens*

Mouse

Groups of 20 female B6AF$_1$ mice, 12 weeks of age, were treated with 3-methylcholanthrene by insertion of an impregnated silk thread into the cervical canal and through the uterine wall; a control group was treated with silk thread not impregnated with 3-methylcholanthrene. Pellets containing steroids were then implanted subcutaneously and renewed every three weeks for 15 weeks. The doses given every three weeks were 15 mg norethynodrel and 0.5 mg mestranol per mouse, alone and in combination. No tumours developed in mice that had not received 3-methylcholanthrene, but the steroids caused some histopathological changes in the mucosa of the cervix, uterus and vagina. The incidences of uterine adenoacanthomas were increased ($p < 0.01$) by all three treatments: control, 5/35; mestranol, 10/19; norethynodrel, 11/14; mestranol + norethynodrel, 10/18. Squamous-cell carcinomas of the cervix were observed in all groups, but the incidences were not statistically significantly increased in those receiving steroids (Blanzat-Reboud & Russfield, 1969).

The effects of two formulations, Ovral, consisting of 0.05 mg ethinyloestradiol + 0.5 mg norgestrel, and Noracycline, consisting of 0.05 mg ethinyloestradiol + 1 mg lynoestrenol, on

carcinomas of the uterine cervix induced by 3-methylcholanthrene were studied in groups of 10–30 Swiss albino female mice, eight to nine weeks of age. The mice treated with 3-methylcholanthrene received a sterile cotton thread impregnated with beeswax containing approximately 300 μg of the carcinogen into the uterine cervix. The oral contraceptive combinations were administered orally at doses of 1/2000th of a pill (0.025 μg ethinyloestradiol + 0.25 μg norgestrel), 1/200th of a pill (0.25 μg ethinyloestradiol + 2.5 μg norgestrel) and 1/20th of a pill (2.5 μg ethinyloestradiol + 25 μg norgestrel) of Ovral, and 1/2000th of a pill (0.025 μg ethinyloestradiol + 0.5 μg lynoestrol), 1/200th of a pill (0.25 μg ethinyloestradiol + 5 μg lynoestrol) and 1/20th of a pill (2.5 μg ethinyloestradiol + 50 μg lynoestrol) of Noracycline. Treatment was for 30, 60 or 90 days. Animals that did not receive 3-methylcholanthrene did not develop cervical tumours at any dose of oral contraceptive. In contrast, treatment with either formulation caused dose-dependent, biphasic effects on the incidence of squamous-cell carcinomas induced by 3-methylcholanthrene. At the two lower doses, they were protective in comparison with treatment with the carcinogen alone ($p < 0.05$), while at the high dose they enhanced carcinogenesis: The incidence of squamous-cell carcinomas was 6/23 with 3-methylcholanthrene alone and 8/17 with the high dose of Ovral at 90 days, although the difference was not indicated as being statistically significant. With Noracycline, the enhancement was statistically significant after both 60 days (13/24 versus 2/23, $p < 0.05$) and 90 days (12/19 versus 6/23, $p < 0.05$). At all doses and at all times, both formulations also significantly enhanced the incidence of cervical hyperplasia (Hussain & Rao, 1992).

In a more recent study with the same model, Ovral was administered to Swiss mice at the same two lower doses as the previous study, with 3-methylcholanthrene at a higher dose of 600 μg. The cotton thread was inserted into the right uterine horn. After 90 days, the incidence of tumours in the uterine endometrium was 8/15 in mice receiving the carcinogen alone and 1/16 in the group receiving the carcinogen + the 1/2000th dose of Ovral ($p < 0.05$). No tumours were seen in the group treated with 3-methylcholanthrene + Ovral at the 1/200th dose (Chhabra *et al*., 1995).

Rat

Groups of 9–10 female Sprague-Dawley rats, seven weeks of age, were initiated by treatment with 5 mg/kg bw *N*-nitrosodiethylamine (NDEA) 24 h after partial hepatectomy. Twenty-four hours later they were fed a diet containing mestranol + norethynodrel at concentrations providing 0.02–0.03 and 0.5–0.75 mg/kg bw per day, respectively. After nine months, a statistically significant ($p < 0.001$) increase in the number of γ-GT-positive altered hepatic foci was observed (7.3 versus 0.3 foci/cm^2), with no significant increase in the incidence of nodules or carcinomas (Yager & Yager, 1980).

Groups of female weanling Wistar rats received an intraperitoneal injection of 0 or 200 mg/kg bw NDEA. One month later, half the animals in each group (5–6 rats) received 1/10th of a tablet of Ovulen-50 (5 μg ethinyloestradiol + 100 μg ethynodiol diacetate) daily orally in 0.1 mL propylene glycol for 60 weeks; the other half of the rats

in each group received the vehicle. The livers were examined histochemically for γ-GT-positive foci and histologically. None of the rats developed liver tumours. In rats that had not been initiated with NDEA, Ovulen-50 increased the incidences of γ-GT-positive foci and of microscopic hyperplastic nodules in all five rats; such foci and nodules were not seen in other groups. The authors speculated that the absence of foci and nodules in NDEA-initiated rats with and without treatment with Ovulen-50 may have been due to an interplay of the drug-metabolizing enzymes and that the Ovulen-50 steroids were more rapidly metabolized by the NDEA-initiated rats (Annapurna *et al*., 1988). [The Working Group noted that opposite effects, i.e. enhancement of foci and nodules in initiated livers by oral contraceptive steroids, have been seen in many other studies and that the results of this study must be considered an exception to the general finding and that they lack a mechanistic explanation.]

The results of the previous and new studies on oestrogen–progestogen combinations are summarized in Tables 32 and 33.

3.2 Oestrogens used in combined oral contraceptives

3.2.1 *Studies reviewed previously*

Mouse

Ethinyloestradiol administered to mice increased the incidence of pituitary adenomas and malignant mammary tumours in animals of each sex and the incidences of uterine and cervical tumours in females.

Mestranol increased the incidences of pituitary adenomas and malignant mammary tumours in animals of each sex.

Rat

When ethinyloestradiol was tested for carcinogenicity in rats, the incidences of liver adenomas were increased in animals of each sex and that of liver carcinomas in females.

Administration of mestranol increased the incidence of malignant mammary tumours in females in one of two treated groups.

3.2.2 *New studies*

(*a*) *Oral administration*

Mouse

Schuppler and Gunzel (1979) summarized data on the incidence of hepatic adenomas in groups of 40–120 male and female mice of three strains after oral administration of ethinyloestradiol or mestranol for 20 months at up to 400 times the human contraceptive dose. Only mice of strain BDH-SPF showed a small increase in incidence after receiving ethinyloestradiol.

Rat

Schuppler and Gunzel (1979) reported that the study of the Committee on Safety of Medicines (1972) found no increase in the incidence of liver adenoma in female rats

Table 32. Effects of combinations of various progestogens and oestrogens on tumour incidence in mice

Combination	Pituitary adenomas		Mammary tumours			Uterine tumours	Cervical/ vaginal tumours
	Male	Female	Benign (males)	Malignant			
				Male	Female		
Chlormadinone acetate + mestranol	+	+					
Chlormadinone acetate + ethinyloestradiol			+/c				
Ethynodiol diacetate + mestranol	+	+					
Ethynodiol diacetate + ethinyloestradiol	+	+				+	
Lynoestranol + mestranol					+/–		
Lynoestranol + ethinyloestradiol + 3-methylcholanthrene							+[a]
Megestrol acetate + ethinyloestradiol				+	+		
Norethisterone acetate + ethinyloestradiol	+/?	+/?					
Norethisterone + ethinyloestradiol		+					
Norethisterone + mestranol	+	+					
Norethynodrel + mestranol	+	+	c		+/?		+
Norethynodrel + mestranol + 3-methylcholanthrene						+	–
Norgestrel + ethinyloestradiol + 3-methylcholanthrene							+[a]

+, increased tumour incidence; +/-, slighly increased tumour incidence; +/c, increased tumour incidence in intact and castrated animals; c, increased tumour incidence in castrated animals; +/?, increased tumour incidence, but not greater than that with the oestrogen or progestogen alone

[a] Protection at doses 1/2000th and 1/200th that of a pill for women; enhancement at a dose of 1/20th that of a pill for women

Table 33. Effects of combinations of various progestogens and oestrogens on tumour incidence in rats

Combination	Pituitary adenomas		Mammary tumours			Liver				
	Male	Female	Benign (males)	Malignant		Adenoma		Carcinoma		Foci (females)
				Male	Female	Male	Female	Male	Female	
Ethynodiol diacetate + ethinyloestradiol				+	+					
Ethynodiol diacetate + mestranol				?	?					
Megestrol acetate + ethinyloestradiol			+/–	+/–	+/–	+/?	+/?			
Norethisterone acetate + ethinyloestradiol			+			+		–	+	
Norethisterone + mestranol					+	+	–			
Norethynodrel + mestranol	+/?	+	+/?	+/?	+	+/?	–	–	–	+
Norethynodrel + mestranol + *N*-nitrosodiethylamine							–		–	+
Norgestrel + ethinyloestradiol			+/–							

+, increased tumour incidence; +/–, slighly increased tumour incidence; +/?, increased tumour incidence, but not greater than that with the oestrogen or progestogen alone; ? conflicting; –, no effect

treated orally with mestranol. Of four studies on the incidences of hepatocellular adenoma and carcinoma in groups of 40–120 male and female rats treated orally with ethinyloestradiol at doses up to 400 times the human contraceptive dose, only one showed a statistically significant increase in the incidence of hepatocellular adenoma in males (0% controls, 15.3% treated) and in females (8% controls, 23.5% treated); the incidence of hepatocellular carcinoma was significantly increased only in females (0% controls, 7.4% treated).

Groups of female Sprague-Dawley rats received ethinyloestradiol or mestranol in the diet from about seven weeks of age at concentrations of 0.1 or 0.5 mg/kg diet (ppm) mestranol for nine and 12 months or 0.5 ppm ethinyloestradiol for nine months. The ingested doses were approximately equivalent to 6 or 30 μg/kg bw per day or 3–15 times the human contraceptive dose. Ethinyloestradiol caused a statistically significant ($p < 0.05$) increase in the number of γ-GT-positive, altered hepatic foci, but not in the volume percentage of liver occupied by foci, after nine months. No increase in the incidence of nodules or carcinomas was observed. The high dose of mestranol had similar effects after nine months, but after 12 months, mestranol caused a statistically significant ($p < 0.05$) increase in both the number of altered hepatic foci, and the volume percentage of the liver occupied by foci showed a significant ($p < 0.05$) dose–response relationship. Furthermore, after 12 months, the high dose of mestranol caused a significant ($p < 0.05$) increase in the incidence of hepatic nodules and carcinomas combined (4/16 compared with 0/15 in controls) (Yager *et al*., 1984).

Female Wistar rats, four weeks of age, were treated with 0 (control), 75 or 750 μg ethinyloestradiol in 0.5 mL olive oil by gavage daily for various times up to 12 months. By four months, the incidence of glutathione *S*-transferase-positive, altered hepatic foci, considered to be preneoplastic lesions, was 100% in both groups. At 12 months, hepatocellular carcinomas were found in 2/23 rats at 75 μg ethinyloestradiol and 10/26 at 750 μg, with none in 24 controls. This response correlated with increased oxidative damage to liver nuclear DNA. Antioxidant vitamins (vitamins C and E and β-carotene) slightly reduced the oxidative DNA damage, significantly reduced the number of altered hepatic foci and reduced the hepatocellular carcinoma incidence (Ogawa *et al*., 1995).

Dog

Groups of 15 female beagles, 10–14 months of age at the start of the experiment, received mestranol at a dose of 0.02 or 0.05 mg/kg bw per day for cycles of 21 days followed by seven days with no drug; a group of 18 bitches served as controls. All of the animals were hysterectomized at two years of age. No mammary tumours were detected after five years (Kwapien *et al*., 1980).

(*b*) *Subcutaneous implantation*

Rat

Holtzman (1988) studied the effects of retinyl acetate on ethinyloestradiol-induced mammary carcinogenesis. A group of 24 female ACI rats aged 59–65 days received sub-

cutaneously implanted ethinyloestradiol in cholesterol pellets (1 mg/20 mg pellet). All treated rats developed pituitary tumours, and 21/24 developed mammary gland carcinomas within 25 weeks. Retinyl acetate did not significantly decrease the mammary tumour incidence but reduced the tumour multiplicity by about 50%.

Hamster

A group of 15 male Syrian golden hamsters weighing 90–100 g received 20-mg pellets of ethinyloestradiol in the shoulder region. The pellets were replaced at three-month intervals and oestrogen treatment was continued for seven to eight months. Three animals developed microscopic renal-cell carcinomas. It was also reported that all 10 castrated male hamsters receiving similar treatment but fed 0.2% α-naphthoflavone developed hepatocellular carcinomas compared with 0/10 hamsters receiving only α-naphthoflavone in the diet (Li & Li, 1984).

(*c*) *Administration with known carcinogens*

Liver models

Mouse: Lee *et al*. (1989) compared the effects of several promoters, including ethinyloestradiol, in three strains of NDEA-initiated male mice. Six-week-old C3H/HeN (C3H), C57BL/6N (C57) and BALB/cA (BALB) mice underwent a two-thirds hepatectomy, followed 20 h later by an intraperitoneal injection of 20 mg/kg bw NDEA; 6 h later, the animals were given a diet containing phenobarbital at a concentration of 50 mg/kg diet (ppm), clofibrate at 1000 ppm and ethinyloestradiol at 10 ppm. The animals were killed after 20 weeks for detection of glucose 6-phosphatase-deficient, altered hepatic foci. The mouse strains differed widely with regard to the mean liver volume occupied by foci after receiving NDEA and in their sensitivity to promotion. The most sensitive strain was C3H. The mean volume ($\times 10^6$ μm^3) of the liver foci was 13.2 ± 1.8 in 18 mice that received NDEA only, 460 ± 72 in 20 mice given phenobarbital and 28 ± 6 in 20 mice given clofibrate; 11 mice given ethinyloestradiol showed no effect, the mean liver volume being 12 ± 10. When the data were expressed as total volume of foci/cm^3 liver $\times 10^6$ μm^3, ethinyloestradiol was seen to be protective, reducing the value of 710 ± 128 in 18 controls to 34 ± 22. Similar results were found in C57 and BALB mice.

Female $B6C3F_1$ mice, 12 days of age, were treated with 5 mg/kg bw NDEA by intraperitoneal injection. At five to seven weeks of age, the mice were randomly assigned to groups of 12 which were exposed by inhalation to unleaded gasoline at 0, 292 or 2056 ppm for 6 h per day on five days per week for 16 weeks, to ethinyloestradiol in the diet at a concentration of 1 ppm or to 1 ppm ethinyloestradiol + 2056 ppm unleaded gasoline. Altered hepatic foci were determined in standard histological sections. The percentage of the liver volume occupied by foci was significantly reduced in ethinyloestradiol-treated mice, from 1.1 ± 0.7 in NDEA controls to 0.26 ± 0.31; however, the volume of foci was significantly increased by the high dose of unleaded gasoline, to 4.31 ± 2.51, and further increased to 18 ± 5 by ethinyloestradiol + the high dose of unleaded gasoline (Standeven *et al*., 1994).

Rat: Ethinyloestradiol and mestranol promoted the appearance of altered hepatic foci and the development of hepatic nodules (adenomas) and carcinomas in initiated male and female rats (Wanless & Medline, 1982; Mayol *et al*., 1991; Hallstrom *et al*., 1996; Yager & Liehr, 1996). On the basis of dose and time responses, these synthetic oestrogens are strong promoters of hepatocarcinogenesis (Yager *et al*., 1991). Selected studies that support this conclusion are summarized below.

Groups of 12–18 female Sprague-Dawley rats, approximately seven weeks of age, were subjected to a two-thirds partial hepatectomy to induce cell proliferation and initiated by intraperitoneal injection of 20 mg/kg bw NDEA; 24 h later, they were fed a semi-purified diet containing mestranol at a concentration of 0.1 or 0.5 ppm for 9 or 12 months or ethinyloestradiol at 0.5 ppm for nine months. The daily intakes of mestranol were approximately 6 and 30 μg/kg bw or 3–15 times the human contraceptive dose. All survivors were killed at 9 or 12 months, and the livers were evaluated for γ-GT-positive foci and the presence of nodules (adenomas) and carcinomas. By nine months, ethinyloestradiol and mestranol had caused a significant ($p < 0.05$) increase in the number of γ-GT foci but no increase in the incidence of nodules or hepatocellular carcinoma. Mestranol induced a significant, dose-dependent increase in the incidence of hepatocellular carcinomas by 12 months, with incidences of 6/15 animals given NDEA alone, 7/17 animals given NDEA plus mestranol at 0.1 ppm and 11/14 animals given NDEA + 0.5 ppm mestranol. A similar number of foci developed in rats fed 0.5 ppm mestranol and in rats fed a diet containing 50 ppm phenobarbital (Yager *et al*., 1984).

Ovariectomized Sprague-Dawley rats, 70 days of age, were given a single intraperitoneal injection of 200 mg/kg bw NDEA; beginning on day 80 and every 28 days thereafter for various periods, the rats were treated with subcutaneous implants of Silastic tubing containing a mixture of ethinyloestradiol and cholesterol. The doses of ethinyloestradiol delivered were calculated to be 0, 16, 37, 90 and 230 μg/kg bw per day. After 30 weeks, the proportion of the liver volume occupied by γ-GT-positive, altered hepatic foci showed a linear increase with dose. The increase was statistically significant at 90 and 230 μg/kg bw per day. In initiated rats treated with ethinyloestradiol at 90 μg/kg bw per day, the incidences of hepatic tumours (adenomas + carcinomas) were significantly greater ($p < 0.05$) than in NDEA-initiated controls with cholesterol implants after 30, 40 and 60 weeks of promotion (Campen *et al*., 1990).

Female Sprague-Dawley rat pups, five days of age, were initiated by an intraperitoneal injection of 10 mg/kg bw NDEA or received no treatment. At weaning, groups of 8–12 rats were fed a semi-synthetic basal diet (controls) or basal diet containing mestranol at a concentration of 0.02 or 0.2 mg/kg (0.02 and 0.2 ppm, respectively) for eight months. When administered alone, mestranol did not induce the appearance of placental glutathione *S*-transferase-positive foci; however, in NDEA-initiated rats, mestranol at a concentration of 0.2 ppm significantly increased ($p < 0.05$) the percentage of the liver volume occupied by foci over that in NDEA-initiated rats fed basal diet. No increase was observed at 0.02 ppm (Dragan *et al*., 1996).

Three studies have been conducted to determine whether ethinyloestradiol and mestranol initiate carcinogenesis in the liver.

Female Sprague-Dawley rats fed a semi-purified diet underwent a two-thirds hepatectomy and 24 h later, at the peak of regenerative DNA synthesis, groups of 10 rats were treated by gavage with corn oil or mestranol at a dose of 100 or 500 mg/kg bw. A positive control group was injected intraperitoneally with 10 mg/kg bw NDEA. After another 24 h, the rats were transferred to a diet containing 0.05% phenobarbital to promote any hepatocytes that had been initiated. The rats were killed four months later and their livers analysed for γ-GT-positive foci. NDEA initiation caused a more than 10-fold increase in the number of foci/cm^2, but the number was not significantly increased in rats treated with 100 mg/kg bw mestranol. While there was an approximately fivefold increase in the number of foci in the group fed 500 mg/kg bw, the effect was not statistically significant (Yager & Fifield, 1982).

Male Fischer 344 rats weighing 130–165 g were given various oestrogens and progestogens by intraperitoneal injection approximately 18 h after a two-thirds hepatectomy. Positive controls were treated with *N*-nitrosomorpholine. Two weeks later, the rats were given 0.02% 2-acetylaminofluorene in the diet for two weeks and carbon tetrachloride by gavage at the end of the first week. The animals were then killed, and the numbers of γ-GT-positive foci were determined in 9–15 rats per group. Ethinyloestradiol at a dose of 0.05 mg/kg bw did not increase the number of γ-GT-positive foci over that in controls (Schuppler *et al*., 1983).

Groups of 12 female Sprague-Dawley rats weighing 140–160 g were fed a semi-purified diet containing ethinyloestradiol at a concentration of 10 ppm for six weeks and then returned to basal diet; controls received basal diet alone. On day 7, all rats were given a two-thirds hepatectomy to increase cell proliferation. After one week on basal diet (week 6–7), the rats were given 0.02% 2-acetylaminofluorene in the diet for two weeks with carbon tetrachloride by gavage at the end of the first week to induce regenerative growth and rapid growth of any initiated foci. The rats were then killed and the numbers of γ-GT-positive foci determined. Ethinyloestradiol caused a significant ($p < 0.01$) fourfold increase in the number of foci/cm^2 and a sixfold increase in focal area as a percentage of liver volume (Ghia & Mereto, 1989). [The Working Group noted that ethinyloestradiol was administered for five weeks as opposed to a single treatment, as in the previous two studies.]

Prostate models

Ethinyloestradiol has been used in experimental models of prostate cancer to cause reversible atrophy of the prostate. When treatment is withdrawn, the prostate undergoes regrowth and DNA synthesis, setting the stage for initiation by chemical carcinogens. Shirai *et al*. (1986, 1990), Takai *et al*. (1991) and Mori *et al*. (1996) used this protocol. [The Working Group was aware of these studies but did not consider them relevant for evaluating the carcinogenicity of ethinyloestradiol or combinations containing it.]

Kidney models

Rat: Groups of 19–27 male Fischer 344 rats, six weeks of age, were fed diets containing 0.05% *N*-nitrosobis(2-hydroxypropyl)amine (NDHPA), 0.1% *N*-nitrosoethyl-*N*-hydroxyethylamine (NEHEA), 0.03% *N*-nitrosopiperidine (NPip), 0.02% 2-acetylaminofluorene or 0.5% *N*-nitrosobutyl-*N*-(4-hydroxybutyl)amine (NBHBA) for two weeks, followed by 0.001% (10 ppm) ethinyloestradiol for 49 weeks. At that time, ethinyloestradiol was found to have enhanced the incidences of liver hyperplastic nodules in rats initiated with NDHPA, NEHEA, 2-acetylaminofluorene or NPip and to have enhanced the incidence of hepatocellular carcinoma in rats initiated with NEHEA compared with controls; this nitrosamine also enhanced the incidence of kidney adenomas and renal-cell carcinomas. Tumorigenesis was inhibited in the lungs and urinary bladder of rats initiated with NDHPA or NBHBA. Ethinyloestradiol alone had no tumorigenic effect (Shirai *et al*., 1987).

Hamster: Syrian golden hamsters, five weeks of age, were separated into groups of 30 animals that received four weekly subcutaneous injections of 10 mg/kg bw *N*-nitrosobis(2-oxopropyl)amine (NBOPA) to initiate renal tumorigenesis. These groups then received either control diet or a diet containing 1 ppm ethinyloestradiol for 27 weeks. An additional group of animals was fed the diet containing ethinyloestradiol. Ethinyloestradiol alone did not cause renal tumours or dysplasia. Initiation with NBOPA alone caused the appearance of nephroblastoma in 1/21 animals and 469 dysplastic tubules. Ethinyloestradiol increased the incidence of renal tumours in NBOPA-initiated animals to 4/27 (adenomas) compared with 1/21 (a nephroblastoma) and significantly ($p < 0.001$) increased the number of dysplastic tubules (1602 compared with 469) (Mitsumori *et al*., 1994).

The results of previous and new studies on oestrogens in mice and rats are summarized in Tables 34 and 35.

3.3 Progestogens used in combined oral contraceptives

3.3.1 *Studies reviewed previously*

Mouse

Chlormadinone acetate tested by oral administration to mice slightly increased the incidence of benign liver tumours in treated males.

Oral administration of ethynodiol diacetate to mice increased the incidence of benign liver tumours in males and increased the incidence of mammary tumours in castrated males.

Lynoestrenol increased the incidence of benign liver tumours in males and that of malignant mammary tumours in females.

Megestrol acetate increased the incidence of malignant mammary tumours in females.

Norethisterone acetate increased the incidence of benign liver tumours in males.

Norethisterone increased the incidences of benign liver tumours in males and of pituitary adenomas in females.

Oral administration of norethynodrel increased the incidences of pituitary adenomas in animals of each sex, of mammary tumours in castrated males and of malignant mammary tumours in females.

Table 34. Effects of ethinyloestradiol and mestranol alone and with known carcinogens on tumour incidence in mice

Oestrogen	Pituitary adenoma		Malignant mammary tumours		Uterine tumours	Vaginal/ cervical tumours	Liver		
	Male	Female					Adenoma		Foci (females)
			Male	Female			Male	Female	
Ethinyloestradiol	+	+	+	+	+	+	+	+	
Mestranol	+	+	+	+			–	–	
Ethinyloestradiol + *N*-nitrosodiethylamine									Protective
Ethinyloestradiol + *N*-nitrosodiethylamine + unleaded gasoline									+

+, increased tumour incidence; –, no effect

Table 35. Effects of ethinyloestradiol and mestranol alone and with known carcinogens on tumour incidence in rats

Oestrogen	Pituitary adenoma (females)	Malignant mammary tumours (females)	Liver					Kidney	
			Adenoma		Carcinoma		Foci (females)	Adenoma (males)	Carcinoma (females)
			Male	Female	Male	Female			
Ethinyloestradiol	+	+	+	+		+	+		
Mestranol		+				+/–	+		
Ethinyloestradiol + *N*-nitrosoethyl-*N*-hydroxyethylamine					+			+	+
Ethinyloestradiol + *N*-nitroso-diethylamine			+	+	+	+	+[a]		
Mestranol + *N*-nitrosodiethylamine			+	+	+	+	+		

+, increased tumour incidence; –, no effect; +/–, slightly increased tumour incidence

[a] In one of three studies, ethinyloestradiol initiated hepatocarcinogenesis

After oral administration of norgestrel to mice, no increase in tumour incidence was observed.

Rat

In rats, oral administration of chlormadinone acetate, megestrol acetate or norgestrel did not increase the incidence of any tumour type.

Ethynodiol diacetate, tested by oral administration to rats, increased the incidence of benign mammary tumours in males.

Lynoestrenol slightly increased the incidence of malignant mammary tumours in females.

Norethisterone increased the incidence of benign liver tumours in males and caused small increases in the incidences of benign and malignant mammary tumours in males and of malignant mammary tumours in females.

Norethynodrel increased the incidences of benign and malignant liver-cell tumours, pituitary adenomas and benign and malignant mammary tumours in males and increased the incidence of benign liver tumours in females.

3.3.2 *New studies*

(*a*) *Oral administration*

Mouse

Schuppler and Gunzel (1979) summarized data from the study of the Committee on Safety of Medicines (1972) in the United Kingdom and from additional studies on the hepatocarcinogenicity of the progestogens, norgestrel, norethisterone acetate, norethisterone, chlormadione acetate, ethynodiol diacetate, norethynodrel, megestrol acetate and lynoestrenol, in mice. Increased incidences of liver tumours were detected in groups of 40–80 male CF-LP mice treated with norethisterone acetate, norethisterone, chlormadinone acetate or ethynodiol diacetate and in groups of 40–80 female CF-LP mice treated orally with norethynodrel for 20 months, but the increases were not significant at the 5% level. It was also reported that megestrol acetate given orally at up to 400 times the human contraceptive dose caused a statistically significant increase in the incidence of hepatocellular adenoma in females, from approximately 1% (25 mice) to 5% (73 mice) ($p < 0.05$). Groups of 120 male and female mice [strain not indicated] were treated orally with lynoestrenol at doses up to 400 times the human contraceptive dose for 20 months. The incidence of hepatocellular adenomas was significantly ($p < 0.05$) increased (from approximately 1 to 8%) in males. The incidences induced by megestrol acetate and lynoestrenol were given only as the average for three dose groups, making it impossible to determine a dose–response relationship. There were no statistically significant effects on liver tumour incidence in males or females treated orally with dl-norgestrel alone for 20 months (Schuppler & Gunzel, 1979). [The Working Group noted discrepancies in the numbers of animals and tumour incidences in these two reports but was unable to resolve the differences in the absence of the original data.]

Groups of 40 male and 40 female C57BL/10J mice, seven weeks of age, were fed a diet containing cyproterone acetate obtained by grinding 50-mg tablets of Androcur™ and mixing the powder into the diet at a concentration of 800 mg/kg (ppm) (calculated intake, 125 mg/kg bw per day) for 104 weeks. A control group consisted of eight males and eight females. Cyproterone acetate increased the mortality rate in both males and females after 40 weeks on test: no females survived past 97 weeks, and only four males survived to 104 weeks. The weight of the liver was increased in animals of each sex, and the increase in males was in excess of 100%. In addition, weight gain was reduced such that, at the end of a separate 13-week treatment period, the cyproterone acetate-treated mice weighed 33% less than controls. The causes of death were uterine enlargement in female mice and neoplastic diseases in males. The liver tumour incidences are shown in Table 36. Overall, hepatocellular tumours developed in 44% of the males and 22% of the females. In addition, 85% of the animals developed adenomatous polyps of the pyloric antrum and pancreatic islet hyperplasia (Tucker & Jones, 1996; Tucker *et al*., 1996). [The Working Group noted that this study has been criticized since the dose of cyproterone acetate administered clearly exceeded the maximum tolerated dose (Schauer *et al*., 1996).]

Table 36. Effects of cyproterone acetate (CPA) on liver tumour incidence in C57BL/10J mice

Liver tumour	Males		Females	
	Control	CPA	Control	CPA
Hepatocellular adenoma	0/8	7/39	0/8	2/37
Hepatocellular carcinoma	0/8	12/39	0/8	8/37

From Tucker & Jones (1996); Tucker *et al*. (1996)

Rat

Schuppler and Gunzel (1979) summarized data from the study of the Committee on Safety of Medicines (1972) on the hepatocarcinogenicity in rats of a number of progestogens. Rats [strain unspecified] were treated orally with the progestogens for two years at doses up to 400 times the human contraceptive dose. Table 37 summarizes the results presented in their paper, which indicate statistically significant increases. Cyproterone acetate at doses 200–400 times the human contraceptive dose did not increase the incidence of hepatocellular adenomas in another study in this report. In a further study, groups of 35 male and 35 female rats were treated orally with cyproterone acetate at doses of 250, 1250 or 6250 times the human contraceptive dose and were observed for 20 months. In males, a significant ($p < 0.01$) increase in the incidence of liver adenomas occurred only at 6250 times the human contraceptive dose, while in females a significant ($p < 0.01$) increase was observed at both 1250 and 6250 that dose (Schuppler *et al*., 1977; Schuppler & Gunzel, 1979).

Table 37. Effects of various progestogens on the incidence of hepatocellular adenomas in rats

Progestogen	Males	Females
Norgestrel	–	–
Norethisterone	+	–
Chlormadinone acetate	–	–
Ethynodiol diacetate	–	–
Norethynodrel	+	+
Lynoestrenol	–	–
Megestrol acetate	–	–

From Committee on Safety of Medicines (1972)

Albino Sprague-Dawley-derived rats were fed diets containing 7.5 or 75 ppm norethisterone acetate, which provided intakes approximately 10 and 100 times the human contraceptive dose. The actual progestogen intake was stated to be 0.303 mg/kg bw for males and 0.397 mg/kg bw for females at the low dose and 3.18 mg/kg bw for males and 4.15 mg/kg bw for females at the high dose. Survival over the two-year study was greater in the treated (22%) than in control (10%) rats. Dose-related effects were seen in liver enlargement, numbers of altered hepatic foci and liver neoplastic nodules (adenomas or regenerative nodules) and the incidence of uterine polyps [details not reported]. No statistically significant increase in the incidence of malignant tumours was observed in the liver or other organs (Schardein, 1980).

Male Fischer 344 rats weighing 130–150 g were subjected to a partial hepatectomy 18 h before treatment with a microcrystalline suspension of cyproterone acetate (purity analytically confirmed) in saline as a single intraperitoneal injection of 100 mg/kg bw. Thirteen days later, the rats were fed a diet containing 0.02% 2-acetylaminofluorene to inhibit normal hepatocyte growth, and seven days later, the rats were given 2 mL/kg bw carbon tetrachloride to cause hepatocyte necrosis and stimulate regenerative growth. One week later, the rats were killed and their livers analysed for γ-GT-positive foci. Cyproterone acetate did not significantly increase the number of γ-GT-positive foci over control values (Schuppler *et al*., 1983). [The Working Group noted the use of a single dose and only male rats.]

The tumour initiating activity of cyproterone acetate was tested in groups of six female Sprague-Dawley rats, 22 days of age at the start of treatment, given 0 (vehicle control), 25, 50 or 100 mg/kg bw orally in olive oil on five consecutive days. One week after the last treatment, the rats were given 10 mg/kg bw Clophen A50 (a technical mixture of polychlorinated biphenyls) as a tumour promoter twice weekly for 11 weeks. One group of four animals was untreated. The livers were analysed for the presence of ATPase-deficient and γ-GT-positive foci. The numbers and area of these foci were significantly increased in a dose-dependent manner by cyproterone acetate (Deml *et al*., 1993).

Dog

Groups of 16 young pure-bred beagle bitches received lynoestrenol orally in tablet form at a dose representing 10, 50 and 125 times the human contraceptive dose daily for 364 weeks; controls received a placebo tablet. The results are summarized in Table 38. A biphasic dose–response effect on mammary tumorigenesis was seen: at the low dose, lynoestrenol appeared to protect against the development of mammary tumours, but at the intermediate and high doses, it was associated with increased incidences of mammary nodules and carcinomas [statistics not specified] (Misdorp, 1991).

Table 38. Effects of lynoestrol on mammary tumour incidence in beagle bitches

Treatment	Nodule incidence	Nodule latency (weeks)	Carcinoma incidence
Control	5/16	323	1/16
10 × HCD	0/16*		[0]
50 × HCD	16/16	191**	3/16 [NR]
125 × HCD	16/16	152**	7/16 [NR]

From Misdorp (1991); HCD, human contraceptive dose; [NR], statistical analysis not reported
*Significantly lower than in other groups ($p < 0.05$)
**Significantly earlier than in controls ($p < 0.05$)

In a study to determine the six-month toxicity of the progestogen STS 557, levonorgestrel was administered as control to four female and four male beagles, 7–12 months of age, at a dose of 1 mg/kg bw orally seven times a week for six months. Mammary hyperplasia but no nodules or malignant tumours was observed (Hoffmann *et al*., 1983). [The Working Group noted the short duration of the study.]

(*b*) *Administration with known carcinogens*

Mouse

Groups of 20 female B6AF$_1$ mice, 12 weeks of age, received a silk thread impregnated with 3-methylcholanthrene inserted into the cervical canal and passed through the uterine wall; a control group received unimpregnated silk thread. Pellets containing 15 mg per mouse norethynodrel and 0.5 mg per mouse mestranol, alone and in combination, were then implanted subcutaneously and were renewed every three weeks for a total of 15 weeks. No tumours developed in the mice that did not receive 3-methylcholanthrene, but the steroids caused various histopathological changes in the mucosa of the cervix, uterus and vagina. Norethynodrel alone promoted the incidence of uterine tumours (11/14 compared with 5/35 in controls) but not of cervical or vaginal tumours (Blanzat-Reboud & Russfield, 1969).

Rat

Female Sprague-Dawley rats, seven weeks of age, were initiated with NDEA 24 h after partial hepatectomy; 24 h later, they were fed a diet containing norethynodrel, providing intakes of 0.5–0.75 mg/kg bw per day for nine months. After four months, a statistically significant ($p < 0.05$), sixfold increase in the number of γ-GT-positive, altered hepatic foci was observed in comparison with rats given NDEA alone. At nine months, the number of foci was reduced and significantly greater than with NDEA alone only when one norethynodrel-treated rat with a large number of foci was deleted from the analysis. No significant increase in the incidence of nodules or carcinomas was observed after nine months (Yager & Yager, 1980).

Male Fischer 344 rats, weighing 130–150 g, were subjected to a partial hepatectomy and 18 h later were given norethynodrel or norethisterone acetate (purity confirmed analytically) by intraperitoneal injection of 100 mg/kg bw as a microcrystalline suspension in saline; 13 days later, the rats were fed a diet containing 0.02% acetylaminofluorene to inhibit normal hepatocyte growth, and seven days later the rats were given 2 mL/kg bw carbon tetrachloride to cause hepatocyte necrosis and stimulate regenerative growth. One week later, the rats were killed and their livers were analysed for γ-GT-positive foci. Neither norethynodrel nor norethisterone acetate significantly increased the number of γ-GT-positive foci over control values (Schuppler *et al.*, 1983).

Hamster: Groups of 30 Syrian golden hamsters, five weeks of age, received four weekly subcutaneous injections of *N*-nitrosobis(2-oxypropyl)amine (NBOPA) at a dose of 10 mg/kg bw to initiate renal tumorigenesis and then received either control diet or a diet containing 10 mg/kg diet (ppm) levonorgestrel for 27 weeks. A third group of animals was not treated with the nitrosamine but was fed the diet containing levonorgestrol. Levonorgestrel alone did not cause renal tumours or dysplasia. Initiation with NBOPA caused nephroblastoma in 1/21 animals and 469 dysplastic tubules. Levonorgestrel did not significantly enhance the incidence of renal tumours in initiated animals (2/27 nephroblastomas and 2/27 renal adenomas) or increase the total number of dysplastic tubules (747) (Mitsumori *et al.*, 1994).

The results of previous and new studies on progestogens are summarized in Tables 39–41.

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

4.1 Absorption, distribution, metabolism and excretion

The disposition of various formulations of oral contraceptives used in humans differs. In general, both the oestrogenic and progestogenic compounds in combined oral contraceptives are absorbed by the gut and metabolized largely in the liver. A fraction of the absorbed dose of ethinyloestradiol and several progestogens is excreted in the bile during

Table 39. Effects of various progestogens alone and with a known carcinogen on tumour incidence in mice

Progestogen	Pituitary adenoma		Mammary tumours		Uterine tumours	Vaginal/ cervical tumours	Liver			
	Male	Female	Benign (males)	Malignant (females)			Adenoma		Carcinoma	
							Male	Female	Male	Female
Chlormadinone acetate							+/–			
Cyproterone acetate							+[a]	+/–[a]	+[a]	+[a]
Ethynodiol diacetate			c				+/–			
Lynoestrenol				+			+			
Megestrol acetate				+				+		
Norethisterone acetate							+/–			
Norethisterone		+					+/–			
Norethynodrel	+	+	c	+				+/–		
Norethynodrel + 3-methyl-cholanthrene					+	–				

+, increased tumour incidence; +/–, slightly increased tumour incidence; –, no effect; c, increased incidence in castrated males

[a] Dose exceeded the maximum tolerated daily dose

Table 40. Effects of various progestogens alone and with a known carcinogen on tumour incidence in rats

Progestogen	Pituitary adenoma (males)	Mammary tumours			Liver				
		Benign (males)	Malignant		Adenoma		Carcinoma (males)	Foci	
			Male	Female	Male	Female		Male	Female
Cyproterone acetate					+[a]	+[a]			+[b]
Ethynodiol diacetate		+							
Lynoestrenol				+/–					
Norethisterone acetate					+	+		+	+ or –[c]
Norethisterone		+/–	+/–	+/–	+				
Norethynodrel	+	+	+		+	+	+		–[c]
Norethynodrel + *N*-nitrosodiethylamine									+

+, increased tumour incidence; +/–, slightly increased tumour incidence; –, no effect

[a] Liver adenomas detected only at high doses

[b] Tested for initiating activity; the results were positive in one study in which it was administered for five days and negative when administered as a single dose

[c] Tested as a single dose for initiating activity

Table 41. Effects of various progestogens on mammary tumour incidence in bitches

Progestogen	Benign	Malignant
Chlormadinone acetate	+	+
Lynoestrenol	+[a]	+[a]
Megestrol acetate	+	+

+, increased tumour incidence

[a] In this study, lynoestrenol had a biphasic effect, with protection at the low dose (10 times the human contraceptive dose) and enhancement at 50 and 125 times the human contraceptive dose.

its first transit through the liver. Although some of these compounds are partially re-absorbed via the enterohepatic circulation, a fraction may be lost in this 'first pass', reducing the overall bioavailability. The absorption rates are usually rapid, peak serum values being observed between 0.5 and 4 h after intake. Serum concentrations rise faster with multiple treatments than single doses and achieve higher steady-state levels, which are still punctuated by rises after each daily dose. The rise in steady-state levels with multiple doses may reflect the inhibitory effect of both oestrogens and progestogens on cytochrome P450 metabolic enzyme activities. Alternatively, oestrogens may induce the production of sex hormone-binding globulin, which may increase the capacity of the blood to carry progestogens. Binding of progestogen to the sex hormone-binding globulin may displace oestrogens and androgens, which may then cause adverse androgenic side-effects and alter serum lipid concentrations. The metabolism of progestogens and ethinyloestradiol typically involves oxidative modifications. In some cases, metabolism converts an inactive pro-drug into a hormonally active compound. Oxidized metabolites are typically conjugated as glucuronides or sulfates, and most are eliminated rapidly, with half-lives of 8–24 h.

Kopera (1985) reviewed the drug interactions associated with administration of progestogens to patients receiving other medications. Progestogens adversely affect the metabolism of various drugs and, in turn, the metabolism of progestogens is affected by the other drugs. These effects occur presumably as a consequence of effects on the induction of metabolic enzymes or on competition for metabolic pathways or for binding to serum carrier proteins.

Thomas *et al*. (1993) studied a single menstrual cycle in 25 pre-menopausal women who smoked five or more cigarettes per day and 21 non-smoking women to compare the plasma concentrations of luteinizing hormone, follicle-stimulating hormone, oestradiol, progesterone, testosterone, androstenedione, dehydroepiandrosterone sulfate and sex hormone-binding globulin and urinary excretion of oestradiol, oestriol and oestrone. No significant differences were found between the two groups for these parameters or in the lengths of the follicular and luteal phases.

Kuhnz and Löfberg (1995) evaluated the ratio of 6β-hydroxycortisol to cortisol excreted in urine as a measure of drug metabolizing activity. Groups of 12–15 women received combined oral contraceptives containing levonorgestrel, gestodene or cyproterone acetate in combination with ethinyloestradiol, or levonorgestrel or gestodene alone. Little or no difference in the ratio was observed between groups.

Coenen *et al*. (1996) gave groups of 22 women oral monophasic combined contraceptives containing 35, 30, 30 or 20 μg ethinyloestradiol with 250 μg norgestimate, 75 μg gestodene, 150 μg desogestrel or 150 μg desogestrel, respectively. Each woman received a dose once a day for 21 days of a 28-day cycle for six cycles. All of the steroidal serum parameters tested (total testosterone, free testosterone, dihydrotestosterone, androstenedione) were significantly decreased, and the concentrations of the steroid-binding proteins, sex hormone-binding globulin and cortisol-binding globulin were significantly increased, irrespective of the oral contraceptive preparation used. Differences between the groups were observed only in dehydroepiandrosterone sulfate and cortisol-binding globulin.

4.1.1 *Ethinyloestradiol*

(*a*) *Humans*

Goldzieher and Brody (1990) reviewed information about the pharmacokinetics of ethinyloestradiol and mestranol given in a dose of 35 and 50 μg, respectively, in combination with 1 mg norethisterone. A group of 24 women received ethinyloestradiol and 27 women received mestranol. Serum ethinyloestradiol concentrations were measured after treatment with either oestrogen. Both treatments produced equal average serum concentrations of about 175 pg/mL, but there was wide inter-individual variation. The maximal serum concentrations were achieved in about 1–2 h, and the half-life for elimination ranged from 13 to 27 h. Intra-individual variation in the plasma concentration of ethinyloestradiol derived from mestranol did not differ significantly from that observed after ethinyloestradiol treatment. The oral bioavailability of ethinyloestradiol was only 38–48%. The authors also reviewed their earlier studies of patterns of urinary conjugates, glucuronides and sulfates in women from Nigeria, Sri Lanka and the United States after oral administration of radiolabelled ethinyloestradiol. The proportions of glucuronides and sulfates were about 70 and 18%, respectively, in each population; however, the Nigerian women had the lowest concentrations of oxidative metabolites and the American women the highest. [The basis for this diversity, whether genetic, nutritional or environmental, was unclear.]

Hümpel *et al*. (1990) obtained serum samples from a group of 30 women during one cycle of a combined oral contraceptive containing ethinyloestradiol and desogestrel and from a group of 39 women taking ethinyloestradiol and gestodene. The mean serum concentrations were 186–226 nmol/L sex hormone-binding globulin, 89–93 mg/L cortisol-binding globulin and 280–281 μg/L cortisol. The serum concentrations of ethinyloestradiol reached mean maximum levels of 106–129 pg/mL 1.6–1.8 h after pill intake.

Kuhnz *et al*. (1990a) compared the pharmacokinetics of ethinyloestradiol given as a single dose in combination with either gestodene or desogestrel to 18 women. In contrast to previous reports that the bioavailability of ethinyloestradiol differed according to the associated progestogen, this study showed no significant difference. The maximum concentration of ethinyloestradiol was found 1.9 h after ingestion and reached 101 and 104 pg/mL for the two combinations, respectively. The values for maximum concentrations and for the integral of the serum concentration over time (area under the concentration curve) differed between individuals, but, for each individual, the concentration of ethinyloestradiol reached with the two contraceptives was usually about the same.

(*b*) *Experimental systems*

Standeven *et al*. (1990) studied the metabolism of ethinyloestradiol in primary cultures of rat hepatocytes. At 4, 24 or 48 h after establishment in culture, the cells maintained their ability to metabolize up to 90% of ethinyloestradiol substrate (4 nmol/L or 2 μmol/L) to polar conjugates during a 4-h incubation. The metabolites formed were reported to differ both quantitatively and qualitatively from those formed in rats *in vivo*.

The major pathway of ethinyloestradiol metabolism in humans and animals is 2-hydroxylation, which is presumably catalysed by the 3A4 isoform of cytochrome P450 (Guengerich, 1988; Yager & Liehr, 1996). Like catechols of oestrone and oestradiol, hydroxylated metabolites of ethinyloestradiol can also undergo redox cycling and damage DNA (Yager & Liehr, 1996).

4.1.2 *Mestranol*

(*a*) *Humans*

The pharmacokinetics of mestranol has been investigated (Goldzieher & Brody, 1990) and reviewed (Bolt, 1979; Kuhl, 1990).

Mestranol is a pro-drug that binds poorly to the oestrogen receptor until it is demethylated in the gastrointestinal tract to its active form, ethinyloestradiol; 54% of mestranol is converted to ethinyloestradiol (Bolt & Bolt, 1974). Since the demethylation is not complete, more mestranol than ethinyloestradiol must be administered to achieve the same effect. The pharmacokinetics of mestranol corresponds to that of ethinyloestradiol, except that the peak concentrations are lower. Since mestranol is more lipophilic than ethinyloestradiol, it can be stored in fatty tissues (Bolt, 1979).

In a study by Goldzieher and Brody (1990), 24 women received ethinyloestradiol and 27 were given mestranol, both in combination with norethisterone. The bioavailability and maximum concentration of mestranol were about 30% lower than those of ethinyloestradiol. A 50-μg oral dose of mestranol was bioequivalent to a 35-μg dose of ethinyloestradiol, both administered in combination with 1 mg norethisterone. Administration of 50 μg mestranol resulted in a mean maximum concentration of ethinyloestradiol of 175 pg/mL at 1.9 h. Intra-individual differences in the plasma concentration of ethinyloestradiol over 24 h were large, however, when the effects of single doses were compared in the same individual at different times. The metabolites of mestranol found

in urine are, apart from ethinyloestradiol, 2-hydroxyethinyloestradiol, 2-methoxyethinyloestradiol and 2-hydroxyethinyloestradiol-3-methyl ether (reviewed by Bolt, 1979).

(*b*) *Experimental systems*

Studies in rats have shown that metabolites of mestranol undergo enterohepatic circulation, which may be affected by antibiotics such as neomycin (Brewster *et al*., 1977). Further metabolism of demethylated mestranol is species-specific; for example, 2-hydroxylation occurs in rats and D-homo-annulation in rabbits and guinea-pigs (Abdel-Aziz & Williams, 1969; Ball *et al*., 1973).

4.1.3 *Chlormadinone acetate*

(*a*) *Humans*

The pharmacokinetics of chlormadinone acetate has been reviewed by Kuhl (1990) and in previous *IARC Monographs* (IARC, 1974, 1979); no recent data are available in humans, probably because there has been no or limited use since the early 1970s.

After intravenous injection of radiolabelled chlormadinone acetate, the steroid and its metabolites have an initial rapid half-life of 2.4 h, followed by a slow half-life of 80.1 h. The mean metabolic clearance rate is 126 L/day for chlormadinone acetate and 42.6 L/day for chlormadinone acetate and its metabolites. The long half-life and slow elimination rate are probably due to accumulation of the drug in fat tissue (Dugwekar *et al*., 1973).

(*b*) *Experimental systems*

The major metabolites of chlormadinone acetate are 2α-hydroxychlormadinone acetate and 3β-hydroxychlormadinone acetate. Incubation of chlormadinone acetate with human or rat liver microsomes produces mainly the 3β-hydroxy metabolite. In contrast, incubation with microsomes from phenobarbital-treated rats produces the 2α-hydroxy metabolite, indicating that the metabolite pattern is dependent on the hepatic monooxygenase state (Handy *et al*., 1974).

4.1.4 *Cyproterone acetate*

(*a*) *Humans*

A group of eight young women were treated with a single oral dose of 100 mg cyproterone acetate followed by a single intramuscular dose of 300 mg four weeks later, and the plasma concentration of both parent compound and the 15β-hydroxy metabolite were quantified in seven of the women. The bioavailability of cyproterone acetate after oral administration was about 88%; the mean maximum serum concentration reached 255 ng/mL between 2 and 3 h, and thereafter decreased biphasically, reaching a terminal half-life of about 3.6 days. After intramuscular injection, the serum concentration reached 191 ng/mL after two to three days and then declined, with a half-life of about 4.3 days. The serum concentrations of the 15β-hydroxy metabolite exceeded those of the parent compound 6 h after oral administration and four days after intramuscular injection.

Thereafter, the concentration of the 15β-hydroxy metabolite decreased at a rate parallel to that of cyproterone acetate, indicating that the formation of this metabolite was the rate-limiting metabolic step (Huber *et al*., 1988).

A group of 15 women was treated with a single oral dose of 2.0 mg cyproterone acetate plus 0.035 mg ethinyloestradiol. After one week, three cycles of multiple treatments were started with the same preparation. After the single dose, the maximum concentration of cyproterone acetate was 15.2 ng/mL, which decreased biphasically with half-lives of 0.8 and 54 h, respectively; 3.5% of the dose was free, while 96.5% was bound to serum proteins. During the multiple treatment cycles, a twofold higher accumulation of cyproterone acetate was observed, and its half-life increased to 78 h (Kuhnz *et al*., 1993a).

In a study to determine the bioequivalence of one 100-mg and two 50-mg tablets and to compare two analytical methods for cyproterone acetate, 36 young men received one 100-mg dose followed three weeks later by two 50-mg tablets. The mean maximum concentrations of cyproterone acetate in serum were 200–260 ng/mL 2–3 h after dosing, followed by a second peak between 6 and 12 h. Thereafter, the concentrations decreased biphasically until 120 h after dosing, reaching a mean half-life of about 50 h (Baumann *et al*., 1996).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.5 *Desogestrel*

(*a*) *Humans*

McClamrock and Adashi (1993) reported that desogestrel is metabolized rapidly and completely in the liver and gut wall. It is metabolized to 3-keto-desogestrel, which mediates its progestogenic effects, and it is not metabolized further to another progestogen. The serum concentrations of 3-keto-desogestrel reached maximum levels within 2–3 h after oral administration of desogestrel and were subsequently cleared with a half-life of 12–24 h. In a review (Stone, 1995), it was reported that desogestrel reaches a steady-state serum concentration within 8–10 days. In serum, about 5% of desogestrel circulates freely, while 65% is bound to albumin and 30% to sex hormone-binding globulin.

Madden *et al*. (1990) studied the metabolism of desogestrel in microsomes from six human livers *in vitro*. The main metabolite formed was 3-keto-desogestrel; 3α-hydroxy-desogestrel and 3β-hydroxydesogestrel were also detected. The metabolism of desogestrel was inhibited by 50% by primaquine at a concentration of 30 μmol/L, but not by levonorgestrel at 250 μmol/L.

Nineteen women were given three cycles of a triphasic oral contraceptive with combinations of desogestrel and ethinyloestradiol at doses of 50 and 35 μg for the first seven days, 100 and 30 μg for days 8–14 and 150 and 30 μg for days 15–21, respectively, followed by seven days without hormone. Multiple blood samples were taken from the women throughout this interval, and serum concentrations of 3-keto-desogestrel, ethinyl-

oestradiol and sex hormone-binding globulin were determined, together with the elimination half-life and dose proportionality. The concentration of 3-keto-desogestrel reached steady-state level at each desogestrel dose, and the pharmacokinetics was proportional to dose. The concentration of ethinyloestradiol also reached a steady state, and the pharmacokinetics was constant thereafter. The concentration of sex hormone-binding globulin was significantly increased between days 1 and 7 of the cycle but not between days 7, 14 and 21 (Archer *et al*., 1994).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.6 *Gestodene*

(*a*) *Humans*

Gestodene is an active progestogen that has an oral bioavailability of almost 100% and shows pharmacokinetics linear to dose. The serum concentrations are four times higher after multiple treatment cycles than after one cycle, and the area under the concentration curve increases by five- to eightfold after multiple cycles of gestodene plus ethinyloestradiol. Gestodene is metabolized primarily in the liver by P450 CYP 3A4, and it is a strong inducer of this enzyme. Although ethinyloestradiol is also metabolized by CYP 3A4, gestodene does not appear to inhibit its metabolism. Known metabolites of gestodene include dihydrogestodene, 3,5-tetrahydrogestodene and hydroxygestodene. After a single 75-µg dose of gestodene alone, 64% of the compound was bound to sex hormone-binding globulin in the serum, 34% was bound to albumin and about 1.3% was free. Clearance is dependent on the concentration of free gestodene. The half-life of clearance and elimination is 10–18 h and is higher after multiple doses than after a single dose of gestodene plus ethinyloestradiol. Monophasic preparations typically contain 75 µg gestodene plus 20 or 30 µg ethinyloestradiol, given for 21 days per 28-day cycle. Triphasic preparations contain 50, 70 or 100 µg of gestodene combined with 30, 40 and 30 µg ethinyloestradiol, respectively, in phases administered for weeks 1, 2, and 3 of a four-week cycle. Gestodene does not reduce the oestrogen-induced increases in the concentration of sex hormone-binding globulin and does not affect serum testosterone levels (Shoupe, 1994; Kuhl *et al*., 1995; Wilde & Balfour, 1995). Täuber *et al*. (1990) found that orally administered gestodene is completely absorbed and exhibits dose-linear pharmacokinetics. The maximum serum concentrations reached 1, 3 and 5 ng/mL after single doses of 25, 75 and 125 µg, respectively. Only 0.6% was not bound to protein, while 75% was bound to sex hormone-binding globulin and 24% to albumin.

Kuhnz *et al*. (1990b) studied the binding of gestodene to serum protein in 37 women who had taken a combined oral contraceptive containing gestodene plus ethinyloestradiol for at least three months: 0.6% was free, while 24% was bound to albumin and 75% to sex hormone-binding globulin .

Kuhnz *et al*. (1991) examined the effects of single and multiple administrations of a triphasic combined oral contraceptive containing gestodene and ethinyloestradiol on the

concentrations of ethinyloestradiol and testosterone in 10 women. After a single oral dose of 0.1 mg gestodene plus 0.03 mg ethinyloestradiol, the serum ethinyloestradiol concentration reached 100 pg/mL in about 1.9 h; thereafter, the concentration declined, with a half-life of 11 h. On day 21 of the treatment cycle, the maximum concentrations reached 140 pg/mL 1.6 h after pill intake. In comparison with pretreatment concentrations, those of total and free testosterone were reduced by about 60%.

Kuhnz *et al*. (1993b) treated 14 women with a combined oral contraceptive containing 0.1 mg gestodene plus 0.03 mg ethinyloestradiol as a single dose or for three months as a triphasic regimen. The maximum serum concentrations of gestodene were 4.3 ng/mL after a single dose, 15 ng/mL at the end of the first cycle and 14.4 ng/mL at the end of three cycles, reached 30 min after dosing. A half-life for clearance of 18 h was observed after a single treatment, the volume of distribution being 84 L. Multiple treatments increased the clearance half-life to 20–22 h and reduced the distribution volume to about 18 L. The serum sex hormone-binding globulin concentration increased with multiple treatments, presumably as an effect of ethinyloestradiol; this change in serum protein concentration is thought to account for the observed change in the distribution of gestodene, from 1.3% free, 69% bound to sex hormone-binding globulin and 29% bound to albumin after a single treatment, to 0.6% free, 81% bound to sex hormone-binding globulin and 18% bound to albumin after multiple treatments.

Heuner *et al*. (1995) treated 14 women with a combined oral contraceptive containing 0.1 mg gestodene plus 0.03 mg ethinyloestradiol as a single administration or for three months as a triphasic regimen. The serum concentrations of gestodene, ethinyloestradiol, cortisol-binding globulin, sex hormone-binding globulin and testosterone were followed after the single treatment and through cycles 1 and 3. The serum concentration of ethinyloestradiol reached a peak of about 65 pg/mL by 1.7 h after oral administration; after multiple treatments, the maximum was as high as 90 pg/mL, but the time to reach the maximum concentration was unchanged. The concentration of gestodene reached a maximum of 3.5 ng/mL within 0.7 h after a single dose and 8.7 ng/mL within 0.9 h after multiple doses. The clearance half-time for a single dose of gestodene also increased, from 12.6 h to nearly 20 h. There was a large increase in the concentration with time after multiple treatments. After a single dose, 1.3% of gestodene in serum was unbound, while 30% was bound to albumin and 68% was bound to sex hormone-binding globulin.

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.7 *Levonorgestrel* (see also the monograph on ‘Hormonal contraceptives, progestogens only’, section 4.1.2)

(*a*) *Humans*

The clinical pharmacokinetics and metabolic effects of levonorgestrel have been reviewed (Fotherby, 1995; Lachnit-Fixson, 1996). Lipid metabolism appears to be largely unaffected by three-phasic administration of levonorgestrel, most studies showing no

significant change in the concentrations of high- or low-density lipoprotein or cholesterol. Effects on carbohydrate metabolism have been described, but the results are not consistent. Since levonorgestrel binds strongly to sex hormone-binding globulin, its pharmacokinetics is affected by the large number of factors that affect this globulin.

Stanczyk and Roy (1990) reviewed the metabolism of levonorgestrel in women treated orally with radioactively labelled compound. Levonorgestrel was found mostly untransformed in serum within 1–2 h after administration, but the concentrations of conjugated metabolites increased progressively between 4 and 24 h after ingestion. Most of the conjugates were sulfates and glucuronides. In addition to the remaining unconjugated levonorgestrel, considerable amounts of unconjugated and sulfate-conjugated forms of 3α,5β-tetrahydrolevonorgestrel were found; smaller quantities of conjugated and unconjugated 3α,5α-tetrahydrolevonorgestrel and 16β-hydroxylevonorgestrel were also identified (Sisenwine *et al*., 1975a). About 45% of radioactively labelled levonorgestrel was excreted via the urine and about 32% via the faeces. The major urinary metabolites were glucuronides—most abundantly 3α,5β-tetrahydrolevonorgestrel glucuronide—and smaller quantities of sulfates (Sisenwine *et al*., 1975b).

Carol *et al*. (1992) evaluated the pharmacokinetics of levonorgestrel in groups of 11–20 women given single or multiple treatments with combined oral contraceptive preparations containing 125 μg levonorgestrel plus 30 or 50 μg ethinyloestradiol. The serum concentrations of levonorgestrel reached a maximum of about 4 ng/mL 1–2 h after a single treatment with either preparation. After 21 days of treatment, the peak and sustained concentrations of levonorgestrel were about twice as high as those after a single treatment. The serum concentration of sex hormone-binding globulin increased after treatment with both contraceptives but to a greater extent with the contraceptive containing 50 μg ethinyloestradiol, indicating the important role of the oestrogen in induction of this protein.

Kuhnz *et al*. (1994a) treated 14 women with a combined oral contraceptive containing 0.125 mg levonorgestrel plus 0.03 mg ethinyloestradiol as a single dose or for three months as a triphasic regimen. The serum concentration of free levonorgestrel reached a peak of 0.06–0.08 ng/mL about 1 h after treatment. In contrast, the calculated values of the area under the concentration curve more than doubled, from 0.32 to 0.75–0.77 ng × h/mL, during the first and third multiple treatment cycles. The serum concentrations of cortisol-binding globulin and sex hormone-binding globulin more than doubled after multiple treatments with the contraceptive. After a single dose, 1.4% of the levonorgestrel in serum was free, while 43% was bound to albumin and 55% to sex hormone-binding globulin. After multiple treatments, only 0.9–1.0% levonorgestrel in serum was free and 25–30% was bound to albumin, while the amount bound to sex hormone-binding globulin increased to 69–74%. The concentrations of free and total testosterone decreased from 3 and 460 pg/mL, respectively, before treatment to 1 and 270 pg/mL, respectively, at the end of one treatment cycle, but had increased again to 2 and 420 pg/mL by the first day of the third cycle.

Kuhnz *et al*. (1992) treated groups of eight to nine women with a combined oral contraceptive containing 0.15 mg levonorgestrel plus 0.03 mg ethinyloestradiol as a single

dose or for three months on a monophasic regimen. The peak concentrations of levonorgestrel were found 1 h after single or multiple treatments, but the peak serum concentrations were 3.1 and 5.9 ng/mL, respectively. The area under the concentration curve increased by two- to fourfold for total and free levonorgestrel when a single dose was compared with multiple treatments. The distribution of free, albumin-bound and sex hormone-binding globulin-bound levonorgestrel was similar in women who had received one or multiple treatments, but the serum concentration of the globulin increased significantly after multiple treatments.

(*b*) *Experimental systems*

Kuhnz *et al*. (1995) studied aspects of the pharmacokinetics of levonorgestrel, norgestimate and levonorgestrel-oxime in rats, the last two compounds being pro-drugs of levonorgestrel. The maximum concentration of levonorgestrel was reached about 1 h after treatment and decreased thereafter. In animals treated with norgestimate or levonorgestrel-oxime, the serum concentration of levonorgestrel increased up to about 8 h after treatment and decreased only slightly thereafter up to 24 h after treatment. The total dose ingested, measured as the area under the concentration curve, for levonorgestrel during a 24-h interval was related linearly to the administered dose of each compound.

In a trial of drugs for pregnancy maintenance, Kuhnz and Beier (1994) administered levonorgestrel at a dose of 10–300 μg/day or norgestimate at 30–1000 μg/day subcutaneously to pregnant rats which had been ovariectomized on day 8 of pregnancy. These rats also received a daily dose of 1 μg oestrone. Doses of 300 μg/day of either compound fully maintained pregnancy. In serum samples collected from each animal, the concentration of levonorgestrel increased up to 2–8 h after administration and remained at a plateau thereafter up to 24 h. The area under the concentration curve during the 24-h interval after administration was linearly related to the administered dose of levonorgestrel.

4.1.8 *Megestrol acetate*

(*a*) *Humans*

After administration of megestrol acetate at 160 mg/day to post-menopausal women with advanced breast cancer, the maximum concentration in serum was reached within 2–4 h. Co-administration of megestrol acetate and aminoglutethimide decreased the serum concentration of megestrol acetate by 74% (Lundgren *et al*., 1990).

Megestrol acetate is hydroxylated at various positions of the steroid molecule (Cooper & Kellie, 1968; Lundgren *et al*., 1990). It is metabolized more slowly than progesterone. The 17α-acetoxy group and the 6(7)-double bond are considered to provide resistance to metabolism by liver enzymes (Cooke & Vallance, 1965). The major route of elimination in humans is via the urine. After administration of 4–90 mg radiolabelled megestrol acetate to patients, 56–78% was excreted in the urine and only 7–30% in faeces; 5–8% of that in urine was present as metabolites (Cooper & Kellie, 1968).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.9 *Norethisterone* (see also the monograph on 'Hormonal contraceptives, progestogens only, section 4.1.3)

(*a*) *Humans*

Although norethisterone is absorbed almost completely, it undergoes first-pass metabolism, which decreases its bioavailability to an average of 64%. There is wide inter-individual variation in its absorption, which is estimated to be as high as three- to fivefold. Norethisterone is absorbed rapidly, achieving maximum serum concentrations within 1–4 h. After doses of 0.5, 1 and 3 mg, the serum concentrations peaked at 2–5, 5–10 and up to 30 ng/mL, respectively. When given in combination with ethinyloestradiol, norethisterone reaches higher serum levels, which also increase with multiple doses until they reach a steady state at high concentrations. The higher steady-state level has been attributed to a reduced rate of metabolism when norethisterone and ethinyloestradiol are combined. Furthermore, the oestrogen induces sex hormone-binding globulin which binds norethisterone and changes the relative distribution of free and albumin-bound norethisterone. The half-life for elimination is about 8–10 h. Norethisterone is stored in various target organs, and about 22% of the dose accumulates in fat (Kuhl, 1990).

The major metabolites of norethisterone are isomers of 5α-dihydronorethisterone and tetrahydronorethisterone, which are excreted largely as glucuronides. Because of steric hindrance of the bulky ethinyl group at position 17α, only a small percentage of norethisterone metabolites are conjugated at the 17β-hydroxy group. The ethinyl group remains intact in 90% of metabolites (Kuhl, 1990; Shenfield & Griffin, 1991).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.10 *Lynoestrenol, ethynodiol diacetate and norethynodrel*

(*a*) *Humans*

Lynoestrenol, ethynodiol diacetate and norethynodrel are pro-drugs of norethisterone. Both lynoestrenol and norethynodrel are converted into the active steroids in the gastrointestinal tract and liver, and the conversion is so fast that, 30 min after ingestion, ethynodiol diacetate cannot be detected in serum. The metabolic pathways of lynoestrenol and ethynodiol diacetate involve ethynodiol as the intermediate. The disposition of the three progestogens is largely similar to that of norethisterone, except that the terminal half-life after ingestion of lynoestrenol is longer (Kuhl, 1990).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.11 *Norgestimate*

(*a*) *Humans*

Alton *et al*. (1984) studied the metabolism of ^{14}C-labelled norgestimate in four women over two weeks. An average of 36.8% of the radiolabel was recovered in faeces and 46.8% in urine. Of the urinary metabolites, 57% was released by enzymatic hydrolysis while 12% was unconjugated. The metabolites were separated by chromatography and shown to include norgestrel, 16β-hydroxynorgestrel, 2α-hydroxynorgestrel, 3α,5β-tetrahydronorgestrel, 3,16-dihydroxy-5-tetrahydronorgestrel and an unidentified trihydroxylated metabolite of norgestrel.

McGuire *et al*. (1990) reviewed previous studies on norgestimate and noted that the ^{14}C-labelled compound was rapidly absorbed and reached maximum levels in serum within 0.5–2 h. The estimated half-life for elimination was 45–71 h. The pattern of metabolites separated and identified by gas chromatography and mass spectroscopy indicated the progressive steps of metabolism: norgestimate undergoes hydrolysis at the 17 position, cleavage of the oxime at position 3, followed by reduction of the ketone, hydroxylation in the A and D rings, reduction of the double bond between carbons 4 and 5, and subsequent conjugation to a sulfate or glucuronide. In a study of 10 women who received one or multiple oral doses of 180 µg norgestimate plus 35 µg of ethinyloestradiol, norgestimate was found to be absorbed rapidly, with a maximum serum concentration of 100 pg/mL reached 1 h after treatment. The concentrations declined rapidly thereafter, and none was detectable by 5 h with the techniques used.

The metabolism of norgestimate was investigated in fragments of human colon and in microsomes isolated from human liver. Two hours after addition of labelled norgestimate to the colon tissue, 38% unaltered norgestimate, 49% 17-deacetylnorgestimate and 8.1% conjugated metabolites were found. Five hours after addition of norgestimate to human liver microsomes, there was some deacetylation of norgestimate to 17-deacetylnorgestimate in the absence of NADPH; in the presence of NADPH, only 30% unaltered norgestimate remained, with 39% 17-deacetylnorgestimate, less than 2% 3-ketonorgestimate, 10% norgestrel and 15% unidentified metabolites. The metabolism of 17-deacetylnorgestimate by human liver microsomes was NADPH- and oxygen-dependent and yielded norgestrel and other metabolites (Madden & Back, 1991).

Kuhnz *et al*. (1994b) treated 12 women with single doses of combined oral contraceptives containing either 250 µg levonorgestrel plus 50 µg ethinyloestradiol or 250 µg norgestimate plus 35 µg ethinyloestradiol. About 22% of the dose of norgestimate became available systemically as levonorgestrel.

(*b*) *Experimental systems*

Norgestimate is metabolized mainly to levonorgestrel. In rabbits, norgestimate had no greater androgenic activity than progesterone *in vivo* or *in vitro*. It showed very poor affinity for androgen receptors and did not bind to human sex hormone-binding globulin (Phillips *et al*., 1992).

After subcutaneous administration of norgestimate to immature, castrated male rats and pregnant female rats, levonorgestrel was the principal metabolite. The progestational and androgenic pharmacological responses to treatment with norgestimate were equivalent to those observed at the concentrations of levonorgestrel achieved after that dose (Kuhnz & Beier, 1994).

4.1.12 *Norgestrel*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Hussain *et al*. (1991) examined the effects of an oral contraceptive containing ethinyloestradiol at 50 μg and norgestrel at 0.5 mg, on hepatic cytochrome P450 and cytochrome b5 activity in microsomes and glutathione *S*-transferase activity in cytosol. Doses spanning two orders of magnitude (1/20th–1/2000th of the pill dose) were administered to mice daily for 15 days before the study *in vitro*. The intermediate doses significantly decreased cytochrome P450 and cytochrome b5 activity and increased the sulfhydryl group concentration but had no effect on glutathione *S*-transferase activity; the highest dose (1/20th of the pill), however, decreased the activity of this enzyme.

4.2 Receptor-mediated effects

4.2.1 *Combined oral contraceptives*

Anderson *et al*. (1989) obtained tissue from breast biopsies taken from 347 pre-menopausal women and determined the incorporation of tritiated thymidine into the DNA of epithelial cells. The labelling index (the percentage of cells that had incorporated tritiated thymidine) was higher in women who used combined oral contraceptives than in women who did not during the first 13 days and last seven days of the menstrual cycle. The difference was significant for days 6–13 (approximately 80% increase for 38–44 women per group) but not for days 21–28 (15–20% increase for 43–49 women per group). Multivariate analysis indicated that the effect of current oral contraceptive use increased cell proliferation significantly ($p < 0.01$); the effect appeared to be confined to nulliparous women ($p < 0.005$). The women reported use of at least 20 different brands of oral contraceptive, and the heterogeneity in response in terms of labelling index was statistically significant in the multivariate analysis. There was an apparent relation between ethinyloestradiol dose and labelling index, which increased from 0.66% (95% CI, 0.52–0.85; $n = 83$) and 0.89% (95% CI, 0.65–1.2; $n = 55$) for users of less than 35 μg per day to 1.3% (95% CI, 0.82–1.9; $n = 15$) for women taking 35 μg per day and 3.5% (95% CI, 3.2–3.9) for two women using 50 μg per day. There was no apparent effect of progestogen dose, which was associated with a labelling index of 0.97–0.98% in 21 women using low-progestogen doses, i.e. norgesterel or desogestrel, and 51 women using high-progestogen doses, i.e. norethisterone, lynoestrenol or ethynodiol acetate. This value was similar to the labelling index found in 36 women using triphasic oral contraceptives (0.94%). In a study by

Williams *et al*. (1991), of similar design, 49 oral contraceptive users were compared with 127 women who were not. The observation of an increased breast epithelial cell labelling index in users during the second week of the menstrual cycle was confirmed. Furthermore, throughout the menstrual cycle, fewer cells expressed oestrogen receptor in users than in non-users, the major difference also occurring during week 2. Two further studies did not, however, find an increased labelling index in breast epithelium of women using combined oral contraceptives (Anderson *et al*., 1982; Going *et al*., 1988). In a smaller study (Olsson *et al*., 1996), breast epithelium staining for Ki-S5 antibody (a marker of DNA synthesis) was investigated in reduction mammoplasty samples from 58 women aged 17–47 years; 18 women were current users of oral contraceptives, 34 were past users, and six had never been exposed. There was no difference in labelling index in the three groups or between parous and nulliparous women. There was, however, a significant increase in labelling in 41 women who had used oral contraceptives before a first full-term pregnancy and in 31 who had used them before the age of 20 in comparison with the other women (n = 17 and 27, respectively). Taken together, these studies clearly demonstrate that combined oral contraceptive use increases breast epithelial cell proliferation; the study of Anderson *et al*. (1989) suggests that the dose of ethinyloestradiol influences the magnitude of this effect in the presence of progestogens.

4.2.2 *Ethinyloestradiol*

(*a*) *Humans*

Odlind *et al*. (1980) studied the effects of combined oral contraceptive use on the concentrations of sex hormone-binding globulin in five healthy pre-menopausal women. A dose of 35 μg per day ethinyloestradiol in combination with 0.5 mg per day norethisterone or a dose of 50 μg per day mestranol combined with 1 mg norethisterone given for the duration of one menstrual cycle increased the concentrations by approximately 100%. Administration of 60 μg ethinyloestradiol every other day in combination with 0.5 mg per day norethisterone caused a 50% increase, and a combination of 50 μg per day ethinyloestradiol with 3 mg norethisterone acetate or 2.5 mg lynoestrenol caused a 20% increase. The same dose of 50 μg per day ethinyloestradiol in combination with 1 mg per day lynoestrenol increased the concentration of sex hormone-binding globulin by approximately 80%.

(*b*) *Experimental systems*

The synthetic oestrogen ethinyloestradiol has been shown to bind to the oestrogen receptor of calf and rabbit uterus, rat liver and human oviduct (Kappus *et al*., 1973; Eisenfeld *et al*., 1978; Muechler & Kohler, 1980; Powell-Jones *et al*., 1980; Aten & Eisenfeld, 1982; Lubahn *et al*., 1985). Its relative binding affinity to the human oviductal receptor was about equal to that of oestradiol (Muechler & Kohler, 1980). Binding to the calf uterine receptor was 2–2.5 times higher than that of oestradiol (Lubahn *et al*., 1985).

Ethinyloestradiol transiently enhanced replicative DNA synthesis (tritiated thymidine incorporation) in female rat liver. After subcutaneous implantation of time-release pellets

providing 2.5 μg/rat ethinyloestradiol per day, DNA synthesis peaked between 24 and 72 h and slowly returned to control values within 7–14 days (Yager *et al*., 1986). Similar findings were obtained with doses of 2 μg/kg per day to 3 mg/kg per day delivered by subcutaneous injection. A daily dose of 0.5 mg/kg (approximately 80 μg/rat) caused an increase in liver weight (by about 60% in comparison with pair-fed controls, the latter showing a 35% reduction in liver weight in comparison with control rats fed *ad libitum*) and in liver DNA content (by approximately 30% in comparison with pair-fed controls) (Ochs *et al*., 1986). For these two effects, the relationship between dose and response was approximately log-linear over the range of doses tested (Ochs *et al*., 1986; Schulte-Hermann *et al*., 1988). Oral administration of ethinyloestradiol was less effective than subcutaneous injection (Ochs *et al*., 1986). The effects at the lower doses are probably mediated by the oestrogen receptor, because the increase in DNA synthesis was inhibited by treatment with the anti-oestrogen tamoxifen (15 μg/rat per day), which by itself did not alter hepatic DNA synthesis (Yager *et al*., 1986).

Prolonged exposure of female rats to ethinyloestradiol at a dose of 2.5 or 5 μg/rat per day from time-release pellets stimulated replicative DNA synthesis in the liver during the first week, but strongly inhibited this process after 28 days (72% inhibition) and 42 days (88% inhibition) of ethinyloestradiol treatment in comparison with untreated controls. Treatment with 5 μg/rat per day ethinyloestradiol for 21 days inhibited the regenerative growth response (tritiated thymidine incorporation) usually seen during the first four days after partial hepatectomy. Epidermal growth factor receptor levels were decreased after seven days of ethinyloestradiol treatment, but had returned to control levels after 21 days (Yager *et al*., 1994).

Moser *et al*. (1996) treated 12-day-old B6C3F1 mice with a single intraperitoneal dose of 5 mg/kg bw NDEA followed four weeks later by administration of ethinyloestradiol at 1 mg/kg diet for 16 weeks. Treatment with NDEA and oestrogen did not change the DNA labelling index (0.4–0.8%; bromodeoxyuridine (BrdU) incorporation) observed in normal hepatocytes, but ethinyloestradiol reduced by approximately 70% the markedly increased labelling index (18%) caused by NDEA in hepatic foci of cellular alteration. This ethinyloestradiol-induced decrease in DNA synthesis in foci was accompanied by a decrease in the size of these foci and by a reduction in the number of foci with a decreased (as compared with normal hepatocytes) content of transforming growth factor (TGF)-β1 and of the mannose-6-phosphatase/insulin–like growth factor-II receptor, which is involved in activation of latent TGF-β1.

Vickers *et al*. (1989) and Vickers and Lucier (1991, 1996) gave ovariectomized rats a single intraperitoneal dose of 200 mg/kg bw NDEA, followed by a daily dose of 90 μg/kg bw ethinyloestradiol by slow-release implant for 30 weeks. This treatment restored the decreased liver weights to the values in intact controls, increased the uterine weights above those of intact controls, and restored the decreased total nuclear and cytosolic oestrogen receptor concentrations and the nuclear hepatic oestrogen receptor occupancy in elutriated hepatic parenchymal cells to values greater than those in intact controls. Pretreatment with the chemical carcinogen slightly enhanced these effects of

ethinyloestradiol (Vickers & Lucier, 1991). Very similar effects were found in isolated hepatic sinusoidal endothelial and Kupffer cells enriched by centrifugal elutriation. These cell fractions, derived from female rats treated with a single intraperitoneal dose of 200 mg/kg bw NDEA with or without ethinyloestradiol at 90 μg/kg bw per day for 30 weeks, showed a 5–6.5-fold increase in nuclear oestrogen receptor levels and a two- to three-fold increase in receptor occupancy (Vickers & Lucier, 1996).

In vitro, ethinyloestradiol at 15×10^{-6} mol/L induced mitogenesis in primary cultures of female rat hepatocytes, increasing tritiated thymidine incorporation by two- to three-fold 30 h after exposure (Shi & Yager, 1989; Ni & Yager, 1994a). Although ethinyloestradiol by itself therefore appeared to have only weak mitogenic effects on rat hepatocytes, as a co-mutagen with epidermal growth factor it strongly enhanced the induction of hepatic DNA synthesis when this factor was added during the last 12 h of the 30-h exposure to oestrogen. Thus, Shi and Yager (1989) demonstrated that 25 ng/mL epidermal growth factor increased tritiated thymidine incorporation by almost ninefold at an ethinyloestradiol concentration of 2.5 μmol/L, and almost 14-fold at 15 μmol/L. An 18-h exposure to 2 μmol/L ethinyloestradiol doubled the number of epidermal growth factor receptors per cell, providing a rational explanation for the increased sensitivity of ethinyloestradiol-exposed hepatocytes to epidermal growth factor. A similar effect occurred *in vivo* 24 h after a single 2.5-μg dose of ethinyloestradiol given to female rats: binding of radiolabelled epidermal growth factor started to increase after 8 h and reached twofold maximum enhancement after 18 h. The amount of epidermal growth factor receptor protein increased proportionally, and its half-life increased by 4.3-fold, while receptor mRNA synthesis was not affected by ethinyloestradiol. Thus, stabilization of the epidermal growth factor receptor protein appeared to be the mechanism by which ethinyloestradiol co-stimulated epidermal growth factor-induced mitogenesis in female rat hepatocytes. Epidermal growth factor-induced growth of male rat hepatocytes, however, was inhibited by oestrogen treatment (Francavilla *et al*., 1989). Although this result suggests that marked sex differences exist in the mitogenic effects of epidermal growth factor and oestrogens on rat liver, the use of a different cell culture medium may also have played a role (Yager & Liehr, 1996).

In the presence of 30 nmol/L dexamethasone, ethinyloestradiol treatment at concentrations of 1×10^{-5}–3×10^{-5} mol/L for five days induced γ-GT activity in cultured rat hepatocytes (Edwards & Lucas, 1985).

4.2.3 *Mestranol*

(*a*) *Humans*

Odlind *et al*. (1980) studied the effects of combined oral contraceptive use on the plasma concentrations of sex hormone-binding globulin in five healthy pre-menopausal women. A dose of 50 μg/day mestranol in combination with 1.0 mg/day norethisterone given for the duration of one menstrual cycle to five pre-menopausal women increased the concentration of sex hormone-binding globulin by approximately 100%.

(*b*) *Experimental systems*

Mestranol does not bind to the oestrogen receptor in rabbit uterus (Kappus *et al*., 1973) but bound to those in calf uterus and human oviduct (Muechler & Kohler, 1980; Lubahn *et al*., 1985), although its relative binding affinity was about two orders of magnitude less than that of oestradiol or ethinyloestradiol.

Mestranol caused a threefold increase in replicative DNA synthesis in female rat liver 24 h after the insertion of slow-release pellets delivering 2.5 or 5 μg/rat per day. This effect may be mediated by the oestrogen receptor because the increase in DNA synthesis induced by 2.5 μg per day mestranol was inhibited by concomitant treatment with 15 μg/rat per day tamoxifen, which by itself did not alter hepatic DNA synthesis (Yager *et al*., 1986). The mestranol-induced increase in DNA synthesis was confirmed in experiments in which mestranol was given at a dose of 0.2 mg/kg diet to female rats for eight months. This treatment effectively promoted the induction of enzyme-altered foci in the liver by a single dose of NDEA, as judged from a threefold increase in the BdrU incorporation index in these preneoplastic foci relative to the labelling index in the surrounding normal hepatocytes, which was also increased (Dragan *et al*., 1996).

In vitro, mestranol at doses of 10^{-8}–10^{-5} mol/L enhanced mitogenesis in HepG2 human hepatocarcinoma cells by up to 80% in comparison with control cells (Coezy *et al*., 1987), and it was co-mitogenic at 10^{-6}–10^{-5} mol/L in primary female rat hepatocytes cultured in the presence of TGF-α (Ni & Yager, 1994b). In another study, however, mestranol inhibited the growth of Hep3B human hepatoma cells at 10^{-5} mol/L under conditions in which it did not significantly affect the growth of HepG2 cells (Jiang *et al*., 1995). Tamoxifen at 10^{-6}–10^{-5} mol/L eliminated the mestranol-induced mitogenesis in oestrogen receptor-containing HepG2 carcinoma cells, which points to an oestrogen-mediated mechanism (Coezy *et al*., 1987). In Hep3B hepatoma cells, which do not express oestrogen receptor, tamoxifen inhibits all cell growth. In the presence of mestranol, an additive inhibitory effect was observed, which suggests that the growth inhibition by mestranol observed in these cells is an oestrogen receptor-independent process (Jiang *et al*., 1995).

Mestranol *per se* induced γ-GT activity in cultured rat hepatocytes at concentrations of 3×10^{-6}–10^{-4} mol/L in the presence of 30 nmol/L dexamethasone (Edwards & Lucas, 1985).

The combination of norethynodrel (0.5 or 5 mg/rat per day) and mestranol (7.5 or 75 μg/rat per day) given as a pellet implant to female Sprague-Dawley rats, starting at 45, 55, 65 or 75 days of age, caused changes in the mammary gland that resulted in protection against induction of mammary cancer by a single dose (80 mg/kg bw) of 7,12-dimethylbenz[*a*]anthracene (DMBA) (Russo *et al*., 1989). The hormone treatment was given for 21 days, followed by 21 days' recovery, at which time some rats were killed to study mammary gland morphology, while others received DMBA. The hormone treatment at both doses decreased the number of terminal end-buds per mammary gland and increased the number of alveolar buds, but did not alter the number of terminal ducts; cell proliferation, measured as the DNA-labelling index, was reduced in the terminal ducts and alveolar buds but remained unchanged in the terminal end-buds (Russo *et al*.,

1989; Russo & Russo, 1991). In these experiments, a trend was observed for the hormonal treatment to produce less effect when initiated at a later age. The reduction in cell proliferation in terminal end-buds and terminal ducts, the target tissues for DMBA, may explain the protective effect of the hormone combination on the development of mammary cancer.

In a study with rhesus monkeys (Tavassoli *et al*., 1988), mestranol alone at 0.02 or 0.1 mg/kg per day and combinations of mestranol and ethynerone, chlorethinyl norgestrel and anagestone acetate were given for 10 years in 28-day cycles consisting of 21 days of administration followed by seven days without treatment. Mestranol alone induced minimal to moderate proliferative and atypical alterations in the mammary gland in 8/34 animals, whereas minimal to mild changes occurred in 2/16 controls. With the various mestranol–progestogen combinations, mild to severe atypical hyperplasia was observed in 22–25/52 animals, about 12% in each group showing severe lesions that could not be distinguished from human mammary carcinoma *in situ*. Minimal to severe proliferative atypia were found in 11/15 animals given one of the progestogens, ethynerone; two of these animals had a severe lesion similar to carcinoma *in situ* and one had invasive breast cancer.

4.2.4 *Chlormadinone acetate*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

The progestogen chlormadinone acetate inhibited the induction by ethinyloestradiol of nuclear and cytoplasmic progesterone receptor in human endometrium (Kreitmann *et al*., 1979), and it has been found to bind strongly to the human uterine progesterone receptor, as determined in a competitive binding assay with the 20 000 × *g* supernatant fraction of human endometrium and myometrium (Briggs, 1975). It reduced the binding of oestradiol to rat uterine oestrogen receptor both *in vivo* and *in vitro* (Di Carlo *et al*., 1983). Chlormadinone acetate did not have any detectable oestrogenic activity when tested for induction of alkaline phosphatase activity as an indicator of oestrogen response in oestrogen receptor-containing and oestrogen-sensitive Ishikawa human endometrial cancer cells (Botella *et al*., 1995).

In vitro, chlormadinone acetate at 10^{-6} mol/L stimulated the growth of androgen-sensitive mouse mammary carcinoma Shionogi cells, with a reduction in doubling time of approximately 50%. This effect could be inhibited by a 5×10^{-6} mol/L excess of the androgen receptor-blocking anti-androgen hydroxyflutamide, which by itself did not stimulate the growth of these cells (Luthy *et al*., 1988). Consistent with these observations, chlormadinone acetate weakly bound to the rat ventral prostate androgen receptor (Botella *et al*., 1987).

The growth stimulatory effect of chlormadinone acetate on Shionogi cells was confirmed *in vivo* in DD/S mice: the tumour size was increased by more than threefold

over that in controls after 21 days of treatment with two daily dose of 250 μg/mouse (Plante *et al*., 1988). When tested in castrated male rats at a dose of 10 mg twice daily for 14 days, chlormadinone acetate increased ventral prostate weight by about 50% and stimulated the activity of the cell proliferation-related enzyme ornithine decarboxylase in the ventral prostate by almost 12-fold; effects of similar magnitude were found with 5α-dihydrotestosterone at a dose of 0.15 mg twice daily. Thus, chlormadinone acetate has weak androgenic activity, while no evidence for anti-androgenic activity was detected in these studies (Labrie *et al*., 1987).

Studies with the human breast cancer cell line ZR-75-1, which contains functional oestrogen, progesterone and androgen receptors, suggested that chlormadinone acetate inhibited the growth of these cells by an interaction of androgen and progesterone receptor-mediated mechanisms (Poulin *et al*., 1990).

Chlormadinone acetate inhibited the activity of microsomal oestrone sulfatase in human breast carcinoma tissue *in vitro*, suggesting that it may reduce the formation of biologically active oestrogen in human breast cancer cells *in vivo* (Prost-Avallet *et al*., 1991). It also reduced the activity of 5α-reductase and increased the activity of hepatic 3α- and 3β-hydroxysteroid dehydrogenase in male and female rats (Lax *et al*., 1984).

4.2.5 *Cyproterone acetate*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

Cyproterone acetate is an anti-androgen that has been shown to act at the level of both the (peripheral) androgen receptor and the hypothalamus-pituitary, suppressing gonadotrophin release. Interestingly, it also had intrinsic androgenic activity when tested for its ability to increase the weight of the ventral prostate of castrated male rats (Poyet & Labrie, 1985). In comparison with 5α-dihydrotestosterone, however, it bound only weakly to the rat ventral prostate androgen receptor (Botella *et al*., 1987). In a test system comprising steroid receptor-deficient CV-1 monkey kidney cells stably transfected with androgen receptor and a reporter plasmid containing the mouse mammary tumour virus promotor linked to the chloramphenicol acetyltransferase gene, transcriptional activation of chloramphenicol acetyltransferase has been used to show both androgenic activity of cyproterone acetate (Warriar *et al*., 1993) and lack of androgenic activity (Fuhrmann *et al*., 1992). The human androgen receptor was used in the former study and the rat androgen receptor in the latter, but it is not clear whether this difference was responsible for the discordant findings. In both studies, excess cyproterone acetate inhibited the effect of androgens.

Cyproterone acetate stimulated the growth of androgen-sensitive mouse mammary carcinoma Shionogi cells *in vivo* in DD/S mice; the tumour size was increased 11-fold over that in controls after 21 days of treatment with two daily doses of 250 μg/mouse (Plante *et al*., 1988).

Cyproterone acetate did not stimulate and, indeed, even inhibited the growth of the original MCF-7 human breast cancer cell line at concentrations of 10^{-7}–10^{-5} mol/L, as measured by tritiated thymidine incorporation (Lippman *et al*., 1976). In a later study, stimulation of the growth of the oestrogen-sensitive breast cancer cell lines MCF-7 and EFM-19 was found at concentrations of 10^{-8}–10^{-6} mol/L cyproterone acetate. This effect was influenced by competition with 5α-dihydrotestosterone but not oestradiol, indicating involvement of the androgen receptor but not the oestrogen receptor (Hackenberg *et al*., 1988). In contrast, studies with the human breast cancer cell line ZR-75-1, which contains functional oestrogen, progesterone and androgen receptors, indicated that cyproterone acetate inhibits the growth of these cells, suggesting that this occurs via an interaction of androgen and progesterone receptor-mediated mechanisms (Poulin *et al*., 1990).

Cyproterone acetate is also a progestogen and has been demonstrated to bind to the progesterone receptor of human uterus (Grill *et al*., 1985) and MCF-7 human breast cancer cells (Bergink *et al*., 1983). Cyproterone acetate had oestrogenic activity in ovariectomized mice, as was evident from the observed vaginal keratinization and increases in uterine weight and protein content (Lohiya & Arya, 1981). It did not alter the uterine hyperplastic response to conjugated equine oestrogen in ovariectomized rats (Kumasaka *et al*., 1994).

Cyproterone acetate has considerable effects on the rodent liver: it stimulates the proliferation of hepatocytes, resulting in liver enlargement due to hyperplasia, in the absence of hepatotoxic effects. After three to six daily administrations by gavage of 40–130 mg/kg cyproterone acetate dissolved in oil to female and male rats, the increase in the ratio of liver weight:body weight reached a plateau at 1.5 times to more than twice the values in vehicle-treated controls, while the hepatic DNA content nearly doubled (Bursch *et al*., 1986; Schulte-Hermann *et al*., 1988; Roberts *et al*., 1995). A threshold dose of 5–10 mg/kg per day was found for these effects in female Wistar rats, male rats being less sensitive and showing less pronounced growth of the liver. With a lag of 12–14 h, replicative DNA synthesis was induced by cyproterone acetate in female Wistar rats, reaching a maximum 18–24 h after the first dose, with a predominant response of periportal hepatocytes (Schulte-Hermann *et al*., 1980a). Cyproterone acetate given at a dose of 125 mg/kg bw per day in the diet to C57BL/10J mice increased the BrdU nuclear labelling index in the liver, the effect being statistically significant in females (Tucker & Jones, 1996; Tucker *et al*., 1996). As many as 75% of all hepatocytes responded to cyproterone acetate with proliferation (Schulte-Hermann *et al*., 1980b). Several studies have demonstrated that after cessation of cyproterone acetate treatment, the liver regresses to its normal size, due to massive induction of apoptosis (Bursch *et al*., 1986; Roberts *et al*., 1995). Cyproterone acetate induced the synthesis of TGF-β1 which is possibly involved in the apoptotic response of hepatocytes after withdrawal of cyproterone acetate (Bursch *et al*., 1993; Oberhammer *et al*., 1996).

The mitogenic activity of cyproterone acetate in rat hepatocytes is apparently a direct effect, since the compound stimulated replicative DNA synthesis in female rat hepatocytes cultured in serum-free medium at non-cytotoxic concentrations of 10^{-7}–10^{-4} mol/L

(Parzefall *et al*., 1989); however, proliferation of hepatocytes isolated from human surgical specimens was, on average, not increased by exposure to cyproterone acetate for 24 h at concentrations of 10^{-5} mol/L. This lack of effect was seen with and without subsequent addition of epidermal growth factor during 24 h. In contrast, cyproterone acetate and epidermal growth factor acted in an additive manner in stimulating DNA synthesis in rat hepatocytes, whereas epidermal growth factor *per se* enhanced the growth of both human and rat cultured liver cells (Parzefall *et al*., 1991). The observations with human hepatocytes were limited to cells obtained from seven subjects; while in most cases no effect was observed, a dose-related increase in proliferation was induced by cyproterone acetate in hepatocytes from one of the subjects and a dose-related decrease in cells from another. More observations are therefore needed before a firm conclusion can be reached about the possible proliferative effects of cyproterone acetate on human liver.

Cyproterone acetate caused a shift of the cell cycle of cultured rat hepatocytes from G_0 to the G_1 phase (Duivenvoorden & Maier, 1994), with concomitant induction of c-*myc* and c-*fos* expression (Duivenvoorden *et al*., 1995).

Female rats were subjected at six weeks of age to a carcinogenic regimen of a two-thirds hepatectomy followed 20 h later by gastric intubation with 30 mg/kg NDEA and, one week later, administration of 0.1% phenobarbital in the drinking-water for four to six months. In cultured hepatocytes derived from three rats, cyproterone acetate at 5×10^{-6} mol/L induced a fourfold increase in replicative DNA synthesis in putatively preneoplastic γ-GT-positive cells and a twofold increase in γ-GT-negative hepatocytes. These effects required the presence of both epidermal growth factor and insulin, which by themselves increased proliferation 10-fold over that in controls but did not differentially affect the proliferation of γ-GT-positive and γ-GT-negative cells (Neumann *et al*., 1992). In the same series of experiments, stimulation of DNA repair synthesis by cyproterone acetate was observed in hepatocytes from both carcinogen-treated and untreated rats, and in medium without epidermal growth factor. This raises the possibility that cyproterone acetate has tumour-initiating potential. Cyproterone acetate *per se* at concentrations of 10^{-6}–10^{-5} mol/L induced γ-GT activity in cultured rat hepatocytes (Edwards & Lucas, 1985).

The exact mechanism by which cyproterone acetate induces liver-cell proliferation and hepatic hyperplasia is not understood. Although it stimulated incorporation of tritiated thymidine into cultured hepatocytes from carcinogen-treated female rats at concentrations of 2×10^{-6}–10^{-5} mol/L, another anti-androgen, flutamide, inhibited stimulation of hepatocyte proliferation induced by epidermal growth factor and insulin (Neumann *et al*., 1992). These findings suggest that the hyperplastic effects of cyproterone acetate are not related to its anti-androgenic properties. It is, however, conceivable that the effects are, at least in part, related to the aforementioned androgenic properties of cyproterone acetate, possibly mediated by the androgen receptor. Unlike compensatory liver cell proliferation, which occurs in rats in response to surgical or toxic reduction of the liver mass, direct hepatic hyperplasia induced by cyproterone acetate *in vivo* did not involve up-regulation of the immediate–early response proto-oncogenes c-*fos*, c-*jun* and c-*myc* or induction of

the transcription factors NF-κB and AP-1 (Coni *et al*., 1993; Menegazzi *et al*., 1997). *In vitro*, however, cyproterone acetate-induced hepatocyte proliferation was accompanied by increased expression of not only c-*fos* but also c-*myc* (Duivenvoorden *et al*., 1995). These observations suggest that the stimulation of rat hepatocyte proliferation by cyproterone acetate *in vivo* may differ from that in culture.

Cyproterone acetate also increased the activity of 5α-reductase and decreased the activity of 3α- and 3β-hydroxysteroid dehydrogenases in the livers of male and female rats (Lax *et al*., 1984).

4.2.6 *Desogestrel*

(*a*) *Humans*

Ruokonen and Käär (1985) studied the effects of desogestrel at a dose of 125 μg per day for 60 days in 30 healthy pre-menopausal women with regard to the serum levels of ceruloplasmin and cortisol-binding globulin, as indicators of oestrogenic activity. The concentrations of these proteins were not affected by the treatment, indicating a lack of oestrogenic activity of desogestrel in these women. The serum concentration of sex hormone-binding globulin was markedly decreased by the treatment, to 70 and 60% of pre- and post-treatment values at 30 and 60 days, respectively.

(*b*) *Experimental systems*

The progestogen desogestrel is converted to its unique, directly acting metabolite 3-keto-desogestrel, and this metabolite was used in all of the studies conducted *in vitro*. The relative binding affinity of 3-keto-desogestrel to the rabbit uterine progesterone receptor has been reported to be approximately equal to (Fuhrmann *et al*., 1995) or nine times higher than that of progesterone (Phillips *et al*., 1990).

The progestational activity of desogestrel *in vivo*, measured by inhibition of ovulation and endometrial stimulation in rabbits, was similar to that of progesterone (Phillips *et al*., 1987).

The 3-keto metabolite of desogestrel also bound with high affinity to androgen and glucocorticoid receptors (Kloosterboer *et al*., 1988; Juchem & Pollow, 1990; Phillips *et al*., 1990; Fuhrmann *et al*., 1995), but not to oestrogen or mineralocorticoid receptors (Juchem & Pollow, 1990; Fuhrmann *et al*., 1995; Schoonen *et al*., 1995a,b). The relative binding affinity for the rat ventral prostate androgen receptor was approximately 12% that of dihydrotestosterone (Phillips *et al*., 1990). In *trans*-activation assays, 3-keto-desogestrel had clear androgenic activity and weak glucocorticoid activity, but no agonist or antagonist activity was found in assays that involved the mineralocorticoid receptor (Fuhrmann *et al*., 1995).

In comparison with 5α-dihydrotestosterone, desogestrel had modest androgenic activity *in vivo*, as measured by stimulation of ventral prostate growth in castrated rats (Phillips *et al*., 1987).

3-Keto-desogestrel stimulated the growth of most oestrogen-sensitive human mammary cancer cell lines tested (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994;

Schoonen *et al*., 1995a,b). MCF-7 cell proliferation was stimulated by 3-keto-desogestrel at a concentration of 10^{-6} mol/L, but only in the presence of insulin added to the medium at ≥ 10 ng/mL (van der Burg *et al*., 1992). In experiments in which growth stimulation by 3-keto-desogestrel was compared in various cell lines, it did not appear to require insulin or epidermal growth factor. The growth stimulation was dose-dependent, beginning at concentrations of 10^{-7} mol/L for MCF-7 cells and 10^{-10} mol/L for T47D cells obtained from two different sources (Kalkhoven *et al*., 1994). These dose–response results were confirmed in studies in which the same and two additional sub-lines of MCF-7 and one of the T47D cell lines were used, while the other T47D line did not respond to 3-keto-desogestrel (Schoonen *et al*., 1995a,b). The experiments were performed with breast cancer cell lines grown in phenol red-free medium containing steroid-devoid (dextran-coated charcoal-stripped) serum (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). Under the conditions of these experiments, progesterone receptor expression in both MCF-7 and T47D cells was maintained and was 40-fold higher in the T47D than in the MCF-7 cells. Furthermore, expression of the progesterone-inducible gene encoding fatty acid synthase was more strongly up-regulated by 3-keto-desogestrel in T47D than in MCF-7 cells. By use of a reporter construct containing two progesterone response elements in front of the thymidine kinase promotor coupled to the chloramphenicol acetyltransferase gene transfected into both cell lines, progesterone receptor-mediated *trans*-activation was observed at a concentration of 3-keto-desogestrel as low as 10^{-9} mol/L (Kalkhoven *et al*., 1994). In other experiments, the stimulating effects on cell growth of 3-keto-desogestrel at concentrations of 10^{-7}–10^{-6} mol/L were not blocked by simultaneous treatment of the cells with anti-progestogens such as RU486, whereas the anti-oestrogens 4-hydroxytamoxifen and ICI164,384 (at 10^{-7} mol/L) did inhibit this stimulation (van der Burg *et al*., 1992; Schoonen *et al*., 1995a,b). Growth stimulation of T47D cells by 3-keto-desogestrel at 10^{-10} mol/L was inhibited by RU486 and not by 4-hydroxytamoxifen (both at 10^{-7} mol/L) (Kalkhoven *et al*., 1994). These findings suggest that stimulation of cell proliferation by 3-keto-desogestrel is mediated by the oestrogen receptor at high concentrations and by the progesterone receptor at low concentrations. This is apparently not related to effects at the level of receptor–ligand interaction: 3-keto-desogestrel causes *trans*-activation of reporter constructs containing oestrogen or progesterone response elements transfected into MCF-7 and T47D cells at 10^{-6} and 10^{-9} mol/L, respectively, while 4-hydroxytamoxifen, but not RU486, inhibited *trans*-activation of the oestrogen response element-containing construct, and RU486, but not 4-hydroxytamoxifen, inhibited *trans*-activation of the progesterone response element-containing construct. The expression of the oestrogen-inducible *pS2* gene in MCF-7 cells was slightly inhibited by 3-keto-desogestrel at 10^{-9} mol/L and was not affected at 10^{-6} mol/L (Kalkhoven *et al*., 1994).

Oestradiol at concentrations of 10^{-10} mol/L and higher strongly induced the growth of the MCF-7 and T47D cell lines, regardless of the sub-line used (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). The growth stimulation of MCF-7 cells by oestrogen at 10^{-10} mol/L was inhibited by 3-keto-desogestrel at a concentration of

10^{-8} mol/L but not by the anti-progestogen RU38486 (Schoonen *et al*., 1995a). Oestrogen-induced growth in T47D cells was not blocked by 3-keto-desogestrel at 10^{-6} mol/L in one sub-line (T47D-A) but was totally inhibited in another sub-line (T47D-S) at a concentration of 10^{-10}–10^{-8} mol/L. These two sub-lines differ considerably, in that RU38486, but not 4-hydroxytamoxifen or ICI164,384, blocked oestrogen-stimulated growth in the T47D-A cell line, while both anti-progestogens and anti-oestrogens inhibited T47D-S (Schoonen *et al*., 1995b).

3-Keto-desogestrel at concentrations of 10–40 ng/mL inhibited the growth of endothelial cells derived from human decidual endometrium; the growth of these cells was stimulated by exposure to oestradiol at 5 ng/mL (Peek *et al*., 1995).

3-Keto-desogestrel, but not the parent compound desogestrel, showed moderate affinity for and slow dissociation from sex hormone-binding globulin in human serum (Juchem & Pollow, 1990). Its strong interaction with this globulin could lead to displacement of testosterone and to an increased concentration of free testosterone; however, the decrease in serum sex hormone-binding globulin after progestogen treatment is probably more important in this respect (Nilsson & von Schoultz, 1989).

4.2.7 *Ethynodiol diacetate*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

The progestogen, ethynodiol diacetate, binds with low affinity (K_i 1.3×10^{-7} mol/L) to both the oestrogen and the progesterone receptor in rabbit uterine cytosol (Tamaya *et al*., 1977) but hardly at all to the human endometrial progesterone receptor (Briggs, 1975; Shapiro *et al*., 1978). It also has been reported to have androgenic properties (Darney, 1995), but no information was available on its receptor-mediated effects.

4.2.8 *Gestodene*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

The progestogen gestodene binds to the rabbit uterine progesterone receptor with a relative binding affinity reported to be similar (Fuhrmann *et al*., 1995) or nine times higher than that of progesterone itself (Phillips *et al*., 1990), and 8–10 times higher than that of progesterone in human endometrial, breast and liver tissue (Iqbal & Colletta, 1987).

Gestodene also bound with high to moderate affinity to the androgen, mineralocorticoid and glucocorticoid receptors (Kloosterboer *et al*., 1988; Juchem & Pollow, 1990; Phillips *et al*., 1990; Fuhrmann *et al*., 1995), but did not bind to the oestrogen receptor (Juchem & Pollow, 1990; Pollow *et al*., 1990; Fuhrmann *et al*., 1995). Oestrogen receptor

binding of gestodene has, however, been reported to occur in malignant breast tissue with threefold higher affinity than that of oestradiol (Iqbal *et al*., 1986). Oestradiol and tamoxifen did not interfere with gestodene binding, but gestodene in excess amounts could reduce oestradiol binding (Iqbal & Valyani, 1988). High-affinity binding of gestodene was found in all breast cancer cell lines tested, but not in endometrial carcinoma cells. Cytosolic gestodene binding could not be inhibited by excess oestradiol, although nuclear binding was abolished (Colletta *et al*., 1989). On the basis of these observations, a novel binding site was postulated (Iqbal & Valyani, 1988; Colletta *et al*., 1989). These findings should be re-evaluated in the light of the identification of the oestrogen receptor-β and current knowledge about oestrogen receptor action. The relative binding affinity of gestodene for the rat ventral prostate androgen receptor was approximately 15% that of dihydrotestosterone (Phillips *et al*., 1990). In *trans*-activation assays, gestodene had clear androgenic activity and weak glucocorticoid activity, but antagonist activity was found for the mineralocorticoid receptor (Fuhrmann *et al*., 1995).

Gestodene has been shown to be a potent competitor for binding of 5α-dihydrotestosterone to the androgen receptor in human foreskin fibroblasts, with activity similar to that of testosterone (Breiner *et al*., 1986).

Gestodene stimulated the growth of most oestrogen-sensitive human mammary cancer cells lines tested (van der Burg *et al*., 1992; Catherino *et al*., 1993; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). In one study, stimulation of cell proliferation by gestodene at a concentration of 10^{-6} mol/L was found in MCF-7 cells but only in the presence of insulin at ≥ 10 ng/mL (van der Burg *et al*., 1992). In subsequent experiments, stimulation by gestodene was compared in various cell lines and appeared not to require insulin or epidermal growth factor. Furthermore, a dose-dependent stimulation of cell growth was observed, beginning at a concentration of 10^{-7} mol/L for MCF-7 cells and 10^{-10} mol/L for T47D cells obtained from two sources (Kalkhoven *et al*., 1994). In other experiments with similar but not identical culture conditions, gestodene induced near-maximal growth stimulation of MCF-7 cells, at a concentration of 10^{-7} mol/L (Catherino *et al*., 1993). These dose–response results were confirmed in studies with the same and two additional sub-lines of MCF-7; one of two T47D sub-lines tested did not respond to gestodene (Schoonen *et al*., 1995a,b). All of the experiments were performed with breast cancer cell lines grown in phenol red-free medium which, except in one study (Catherino *et al*., 1993), contained steroid-free (dextran-coated charcoal-stripped) serum (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). Under the conditions of these experiments, progesterone receptor expression in both cell types was maintained and was 20–40-fold higher in the T47D cells than in the MCF-7 cells (Kalkhoven *et al*., 1994; see also Sutherland *et al*., 1988). Furthermore, expression of the progesterone-inducible gene encoding fatty acid synthase was more strongly up-regulated by gestodene in T47D than in MCF-7 cells (Kalkhoven *et al*., 1994). With reporter constructs containing two progesterone response elements in front of the *tk* promotor coupled to the chloramphenicol acetyltransferase gene transfected into both cell lines, *trans*-activation was observed at gestodene concentrations as low as 10^{-9} mol/L, clearly demonstrating expression of functional

progesterone receptor in these cell lines without (Kalkhoven *et al*., 1994) or with addition of oestradiol to the medium to boost receptor expression (Catherino *et al*., 1993). The stimulating effects of gestodene at 10^{-7}–10^{-6} mol/L were not blocked, however, by simultaneous treatment of the cells with anti-progestogens such as RU38486, whereas they were inhibited by the anti-oestrogens 4-hydroxytamoxifen (10^{-7} mol/L) and ICI164,384 (10^{-7}–10^{-6} mol/L) (van der Burg *et al*., 1992; Catherino *et al*., 1993; Schoonen *et al*., 1995a,b). Stimulation of the growth of T47D cells by gestodene at a lower concentration (10^{-10} mol/L) was inhibited by RU486 and not by 4-hydroxytamoxifen (both at 10^{-7} mol/L), suggesting that the cell proliferation-stimulating effects of gestodene are mediated via the oestrogen receptor at high concentrations and by the progesterone receptor at low concentrations (Kalkhoven *et al*., 1994). This effect is apparently not related to effects at the level of receptor–ligand interaction, because gestodene causes *trans*-activation of reporter constructs containing oestrogen or progesterone response elements transfected into MCF-7 and T47D cells at 10^{-10}–10^{-6} mol/L. Furthermore, 4-hydroxytamoxifen and ICI164,384, but not RU486, inhibited *trans*-activation of the oestrogen response element-containing construct, while RU486, but not 4-hydroxytamoxifen or ICI164,384, inhibited *trans*-activation of the progesterone response element-containing construct (Catherino *et al*., 1993; Kalkhoven *et al*., 1994). The expression of the oestrogen-inducible *pS2* gene in MCF-7 cells was slightly inhibited by gestodene at a low concentration (10^{-9} mol/L), but was not affected at 10^{-6} mol/L (Kalkhoven *et al*., 1994).

Oestradiol at concentrations of 10^{-10} mol/L and higher strongly induces the growth of the MCF-7 and T47D cell lines, regardless of the sub-line used (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). The growth stimulation of MCF-7 cells by oestrogen at 10^{-10} mol/L was inhibited by gestodene at a concentration of 10^{-8} mol/L, and this effect was not blocked by RU38486 (Schoonen *et al*., 1995a). Oestrogen-induced growth in T47D cells was not blocked by gestodene at 10^{-6} mol/L in one sub-line (T47D-A) but was totally inhibited in another sub-line (T47D-S) at a concentration of 10^{-10}–10^{-8} mol/L. These two sub-lines differ considerably, in that RU38486, but not 4-hydroxytamoxifen or ICI164,384, blocked oestrogen-stimulated growth in the T47D-A cell line, while both anti-progestogens and anti-oestrogens were inhibitory for T47D-S (Schoonen *et al*., 1995b).

Gestodene induced a large increase in secretion of TGF-β by T47D breast cancer cells, but not HEC-1B human endometrial cancer cells, and the inhibitory effect of gestodene on oestrogen-stimulated T47D cell proliferation was reduced by treatment with a polyclonal antiserum to TGF-β (Colletta *et al*., 1991). Gestodene also inhibited oestrogen-stimulated T47D cell proliferation in sub-lines that had lost their sensitivity to TGF-β to the same extent as in sub-lines that retained their sensitivity to this growth inhibiting factor (Kalkhoven *et al*., 1996); therefore, the involvement of TGF-β in the growth modulating effects of gestodene remains unclear.

Gestodene showed high affinity for and slow dissociation from sex hormone-binding globulin in human serum (Juchem & Pollow, 1990).

When given to female Wistar rats at a dose of 10 mg/kg per day for seven days, gestodene had a slight but significant growth-stimulating effect on the liver, as seen in a 10–15% increase in DNA content without a change in weight (Schulte-Hermann *et al*., 1988).

4.2.9 *Levonorgestrel* (see also the monograph on 'Hormonal contraceptives, progestogens only', section 4.2.2)

(*a*) *Humans*

In the study of Ruokonen and Käär (1985), described in section 4.2.6, the serum concentrations of ceruloplasmin and cortisol-binding protein were not affected, indicating a lack of oestrogenic activity of levonorgestrel in these women. The serum concentration of sex hormone-binding globulin was markedly decreased, to 50–55% of pre- and post-treatment values at both 30 and 60 days.

Ten women were given 30 μg per day levonorgestrel orally on days 7–10 of the menstrual cycle, and endometrial biopsy samples were taken on the 11th day of the previous cycle and on the day after the last dose (also day 11 of the cycle). Levonorgestrel had no effect on the number of glandular and stromal cell mitoses, basal-cell vacuolation or the diameter and epithelial thickness of the endometrial glands (Landgren *et al*., 1990).

(*b*) *Experimental systems*

Levonorgestrel binds with high affinity to progesterone receptors (Lemus *et al*., 1992); its relative binding affinity has been reported to be 1.25 (Kuhnz *et al*., 1995) to five times (Phillips *et al*., 1990) higher than that of progesterone itself for the rabbit uterine progesterone receptor and 1.43 and 1.25 times higher for human uterine and recombinant progesterone receptors, respectively (Kuhnz *et al*., 1995). Metabolites of levonorgestrel showed less or no binding to the progesterone receptor (Lemus *et al*., 1992).

Levonorgestrel had clear progestational activity *in vivo*, both in a pregnancy maintenance test in female rats (Kuhnz & Beier, 1994) and as measured by inhibition of ovulation and endometrial stimulation in rabbits, indicating that it is slightly less active than progesterone (Phillips *et al*., 1987).

Levonorgestrel also bound with high affinity to androgen, mineralocorticoid and glucocorticoid receptors (Kloosterboer *et al*., 1988; Juchem & Pollow, 1990; Phillips *et al*., 1990), but not to oestrogen receptors (Iqbal *et al*., 1986; Juchem & Pollow, 1990; Lemus *et al*., 1992). The relative binding affinity of levonorgestrel for the rat ventral prostate androgen receptor was approximately 20% that of 5α-dihydrotestosterone (Phillips *et al*., 1990).

Levonorgestrel had moderate androgenic activity *in vivo*, in comparison with 5α-dihydrotestosterone, as measured by stimulation of ventral prostate growth in immature, castrated rats (Phillips *et al*., 1987; Kuhnz & Beier, 1994).

Levonorgestrel stimulated the growth of oestrogen-sensitive human mammary cancer cells lines. MCF-7 cell proliferation was stimulated by levonorgestrel at a concen-

tration of 10^{-6} mol/L, but only in the presence of insulin added to the medium at ≥ 10 ng/mL (van der Burg *et al.*, 1992). In experiments in which stimulation by levonorgestrel was compared in three sub-lines of MCF-7 and two of T47D cells, stimulation occurred at concentrations of 10^{-7} mol/L and higher in all cell lines except one of the T47D sub-lines (Schoonen *et al.*, 1995a,b). The experiments were performed with breast cancer cell lines grown in phenol red-free medium which contained steroid-free (dextran-coated charcoal-stripped) serum (van der Burg *et al.*, 1992; Schoonen *et al.*, 1995a,b). Under the conditions of these experiments, progesterone receptor expression in both cell types was maintained and was 20–40-fold higher in T47D cells than in MCF-7 cells (van der Burg *et al.*, 1992; Kalkhoven *et al.*, 1994). The stimulating effects of levonorgestrel at 10^{-7}–10^{-6} mol/L were not blocked by simultaneous treatment of the cells with anti-progestogens such as RU486, whereas the anti-oestrogens 4-hydroxytamoxifen (at 10^{-7} mol/L) and ICI164,384 (at 10^{-7}–10^{-6} mol/L) inhibited this stimulation (van der Burg *et al.*, 1992; Schoonen *et al.*, 1995a,b). These findings suggest that the cell proliferation-stimulating effects of levonorgestrel are not mediated via the progesterone receptor but via the oestrogen receptor (Kalkhoven *et al.*, 1994).

Levonorgestrel increased the reductive activity of 17β-hydroxysteroid dehydrogenase in an oestrogen- and progestogen-stimulated MCF-7 cell line in phenol red-free medium. This effect would increase the formation of oestradiol, indicating a possible mechanism by which this progestogen may increase breast cell proliferation *in vivo* (Coldham & James, 1990).

Levonorgestrel had oestrogenic activity at concentrations of 10^{-8}–10^{-6} mol/L when tested for induction of alkaline phosphatase activity as an indicator of oestrogen response in oestrogen receptor-containing and oestrogen-sensitive Ishikawa human endometrial cancer cells (Botella *et al.*, 1995).

Oestradiol at concentrations of 10^{-10} mol/L and higher strongly induced the growth of MCF-7 and T47D cell lines, regardless of the sub-line used (van der Burg *et al.*, 1992; Kalkhoven *et al.*, 1994; Schoonen *et al.*, 1995a,b). The growth stimulation of MCF-7 cells by 10^{-10} mol/L oestrogen was inhibited by 10^{-9} mol/L levonorgestrel in one sub-line, and the effect was not blocked by RU486; no effect was seen in another sub-line (Schoonen *et al.*, 1995a). Oestrogen-induced growth in T47D cells was not blocked by 10^{-6} mol/L levonorgestrel in one sub-line but was totally inhibited in another sub-line at concentrations of 10^{-10}–10^{-8} mol/L. These two T47D sub-lines differ considerably, in that RU486, but not 4-hydroxytamoxifen or ICI164,384, blocked oestrogen-stimulated growth in the former sub-line, while both anti-progestogens and anti-oestrogens inhibited the other (Schoonen *et al.*, 1995b).

Levonorgestrel at concentrations of 0.1–2 ng/mL inhibited the growth of decidual endothelial cells derived from human endometrium, the growth of which was stimulated by exposure to oestradiol at 5 ng/mL but inhibited by lower concentrations and not affected by higher concentrations (Peek *et al.*, 1995).

Levonorgestrel had high affinity for and slow dissociation from sex hormone-binding globulin in human serum (Juchem & Pollow, 1990). It displaced testosterone,

thus at least theoretically resulting in an increase in free testosterone (Nilsson & von Schoultz, 1989).

Protein and mRNA expression of vascular endothelial growth factor was increased in the endometrium of cynomolgus monkeys treated with levonorgestrel for 20 days as compared with endometrial samples from luteal-phase monkeys. These effects were limited to stromal cells for protein expression detected by immunohistochemistry and to the vascular endothelial growth factor-189 isoform for mRNA expression (Greb *et al*., 1997).

Levonorgestrel had no significant effect on the growth of the liver in female Wistar rats (Schulte-Hermann *et al*., 1988).

4.2.10 *Lynoestrenol*

(*a*) *Humans*

Maudelonde *et al*. (1991) studied the effects of lynoestrenol given at a dose of 10 mg/day on days 5–25 of each menstrual cycle for one to three months to 31 pre-menopausal women with biopsy-confirmed benign breast disease, by comparing them with a group of 16 untreated women with similar clinical characteristics. Fine-needle aspirates were obtained at the start of the study and at the end of the one- to three-month treatment. The mean percentage of cells staining positively for oestrogen receptor decreased from about 60 to 20%, while the number of cells staining positively for cathepsin D (as an indicator of oestrogenic activity) remained the same. The pre-treatment values for these two parameters were not significantly different from those found in the untreated controls. The reduction in the number of oestrogen receptor-positive cells was viewed by the authors as consistent with the anti-oestrogenic activity of lynoestrenol.

Ruokonen and Käär (1985) studied the effects of lynoestrenol at a dose of 5 mg/day for 60 days in 30 healthy pre-menopausal women on serum levels of ceruloplasmin and cortisol-binding globulin, as indicators of oestrogenic activity. The concentrations of these two proteins were slightly (10–20%) elevated after 30 and 60 days of treatment as compared with pre-treatment, but this was significant only 30 days after the start of treatment. Nevertheless, the results indicated weak oestrogenic activity of lynoestrenol in these women. The serum concentration of sex hormone-binding globulin was markedly decreased by the treatment, to 60 and 50% of pre-treatment values at 30 and 60 days, respectively.

In the study of Odlind *et al*. (1980), described in section 4.2.3, a dose of 1 mg/day lynoestrenol in combination with 50 μg/day ethinyloestradiol given for the duration of one menstrual cycle increased the concentration of sex hormone-binding globulin by approximately 100%, but a combination with a higher lynoestrenol dose of 2.5 mg/day caused only a non-significant, 17% increase.

(*b*) *Experimental systems*

The progestogen lynoestrenol was found to bind with low affinity to both the oestrogen and the progesterone receptor in rabbit uterine cytosol (Tamaya *et al*., 1977) and to the human endometrial progesterone receptor (Briggs, 1975).

Lynoestrenol has been reported to have oestrogenic activity *in vivo* (Lax, 1987). It enhanced the activity of microsomal oestrone sulfatase in human breast carcinoma tissue, suggesting that it could stimulate the formation of biologically active oestrogen in human breast cancer cells (Prost-Avallet *et al.*, 1991).

Lynoestrenol has also been reported to have androgenic properties (Darney, 1995).

4.2.11 *Megestrol acetate*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

The progestogen megestrol acetate has been found to bind strongly to the human uterine progesterone receptor, as determined in a competitive binding assay with 20 000 × *g* supernatants of human endometrium and myometrium (Briggs, 1975). Its 19-nor analogue nomegestrol acetate has very high affinity for the progesterone receptor in rat uterus (Botella *et al.*, 1990). Neither megestrol acetate nor nomegestrol acetate affected the growth of the mammary cancer cell lines, MCF-7 and T47D:A18, or *trans*-activated an oestradiol-responsive reporter construct containing oestrogen response elements (Catherino & Jordan, 1995). Nomegestrol acetate also had no oestrogenic activity, as demonstrated by the lack of induction of alkaline phosphatase activity in oestrogen receptor-containing and oestrogen-sensitive Ishikawa human endometrial cancer cells (Botella *et al.*, 1995).

In vitro, megestrol acetate stimulated the growth of androgen-sensitive mouse mammary carcinoma Shionogi cells, with a reduction in the doubling time of approximately 50% at a concentration of 10^{-6} mol/L. This effect was counteracted by a 5×10^{-6} mol/L excess of the androgen receptor blocking anti-androgen, hydroxyflutamide, which itself did not stimulate the growth of these cells (Luthy *et al.*, 1988). Consistent with these observations, megestrol acetate bound weakly to the rat ventral prostate androgen receptor, with an affinity approximately equal to that of testosterone (Botella *et al.*, 1987).

When tested in castrated male rats at a dose of 10 mg given subcutaneously twice daily for 14 days, megestrol acetate increased the ventral prostate weight by about 50% and induced a 13-fold stimulation of the activity of the cell proliferation-related enzyme ornithine decarboxylase in the ventral prostate; similar effects were found with a dose of 0.15 mg 5α-dihydrotestosterone twice daily (Labrie *et al.*, 1987). When castrated rats that had received testosterone re-substitution (via silastic implants) were treated with megestrol acetate at 20 mg/kg per day subcutaneously for 14 or 28 days, however, the prostate weights were reduced by 49 and 65%, respectively (Burton & Trachtenberg, 1986). Thus, megestrol acetate has weak androgenic activity in castrated male rats (Labrie *et al.*, 1987), while it has clear anti-androgenic activity in intact rats (Burton & Trachtenberg, 1986).

Megestrol acetate bound to the glucocorticoid receptor in human mononuclear leukocytes and induced glucocorticoid-like effects in these cells, including inhibition of proliferative responses to mitogenic stimuli (Kontula *et al.*, 1983).

Studies with the human breast cancer cell line ZR-75-1, which contains oestrogen, progesterone and androgen receptors, suggested that megestrol acetate inhibits the growth of these cells through an interaction of androgen and progesterone receptor-mediated mechanisms (Poulin *et al*., 1990).

Megestrol acetate weakly inhibited the induction of angiogenesis by basic fibroblast growth factor and TGF-α in rabbit cornea *in vitro*. This anti-angiogenic activity was not correlated with its binding to glucocorticoid, progesterone or androgen receptors (Yamamoto *et al*., 1994).

4.2.12 *Norethisterone*

(*a*) *Humans*

In the study of Odlind *et al*. (1980), described in section 4.2.3, a dose of 0.5 or 1.0 mg/day norethisterone in combination with 35 μg/day ethinyloestradiol or 50 μg/day mestranol, respectively, given for the duration of one menstrual cycle increased the concentration of sex hormone-binding globulin by approximately 100% in both cases, but the daily dose of 0.5 mg norethisterone in combination with 60 μg ethinyloestradiol given every other day caused only a 45% increase. The dose of 3 mg/day norethisterone acetate in combination with 50 μg/day ethinyloestradiol increased the concentration of sex hormone-binding globulin by approximately 25%.

Ten women were given 300 μg/day norethisterone orally on days 7–10 of the menstrual cycle, and endometrial biopsy samples were taken on the 11th day of the previous cycle and on the day after the last dose (also day 11 of the cycle). The treatment reduced the number of glandular cell mitoses by 65% and markedly increased the number of vacuolated cells in the endometrium, from 0 to 5.5% (Landgren *et al*., 1990).

(*b*) *Experimental systems*

Norethisterone bound with an affinity close to that of the natural ligand to the progesterone receptor in rabbit uterine cytosol (Tamaya *et al*., 1977) and to the nuclear and cytosolic progesterone receptors in human uterine endometrium and myometrium (Briggs, 1975; Shapiro *et al*., 1978; Kasid & Laumas, 1981). It bound with low affinity to the nuclear and the cytosolic progesterone receptors in cultured MCF-7 human breast tumour cells (Kloosterboer *et al*., 1988). In an assay of progestogen-specific stimulation of alkaline phosphatase activity in T47D human breast cancer cells, slightly less than full agonist activity was demonstrated for norethisterone in comparison with progesterone (Markiewicz & Gurpide, 1994). In human endometrial stromal cells in culture, however, norethisterone and progesterone were equally effective in stimulating protein and mRNA expression of insulin-like growth factor binding protein-2 (Giudice *et al*., 1991).

In comparison with progesterone, norethisterone had weak to moderate mixed antagonist/agonist progestational activity; *in vivo* it effectively interfered with pregnancy in the post-nidation period in rats and somewhat less effectively in hamsters, but it also inhibited progesterone-supported pregnancy in ovariectomized rats (Reel *et al*., 1979). Furthermore, it showed weak inhibitory activity on ovulation and endometrial stimu-

lation in rabbits (Phillips *et al*., 1987). In immature female rabbits, norethisterone induced increased expression of uteroglobin in both protein and mRNA (Cerbón *et al*., 1990). This effect is mediated by the progesterone receptor, because it is abolished by RU486 (Pasapera *et al*., 1995).

Norethisterone was found to bind with lower affinity than the natural ligand to the oestrogen receptor in rabbit uterine cytosol (Tamaya *et al*., 1977) and rat uterine homogenate (van Kordelaar *et al*., 1975). Norethisterone acetate inhibited specific binding of oestradiol in the cytosolic fraction of female rat liver at concentrations of 10^{-5}–10^{-4} mol/L, and injection of norethisterone acetate *in vivo* induced nuclear translocation of the oestrogen receptor, i.e. cytosol receptor depletion, in the livers of female rats (Marr *et al*., 1980).

Norethisterone at concentrations of 10^{-7}–10^{-6} mol/L showed weaker oestrogenic activity than oestradiol when tested for its stimulatory effect on alkaline phosphatase activity in Ishikawa human endometrial cancer cells, which is an oestrogen-specific response inhibited by 4-hydroxytamoxifen (Markiewicz *et al*., 1992; Botella *et al*., 1995). Binding of oestradiol to rat uterine oestrogen receptors was reduced by norethisterone both *in vivo* and *in vitro* (Di Carlo *et al*., 1983). In addition, several anti-oestrogenic effects were found *in vivo*: in ovariectomized rats treated subcutaneously with oestradiol valerate at 50 µg/rat once a week, norethisterone acetate at a daily dose of 1 mg was about equally effective as tamoxifen at a daily dose of 0.06 mg/rat in reducing the oestrogen-induced increase in uterine weight and serum prolactin (Spritzer *et al*., 1995). Norethisterone also reduced the hyperplastic response of the uterus in ovariectomized rats after treatment with conjugated equine oestrogen; tamoxifen did not have this effect (Kumasaka *et al*., 1994).

Norethisterone stimulated the growth of most oestrogen-sensitive human mammary cancer cells lines tested (Jeng & Jordan, 1991; Jeng *et al*., 1992; Schoonen *et al*., 1995a,b). It stimulated cell proliferation at concentrations of 10^{-8}–10^{-7} mol/L in studies with the oestrogen receptor-positive MCF-7 and T47D:A18 cell lines (Jeng *et al*., 1992), and these results were confirmed in studies with three sub-lines of MCF-7 and two other T47D cell lines of different origin, except that one of the latter did not respond to norethisterone (Schoonen *et al*., 1995a,b). All of these experiments were performed with cells grown in phenol red-free medium which contained steroid-free (dextran-coated charcoal-stripped) serum (Jeng *et al*., 1992; Schoonen *et al*., 1995a,b). Norethisterone induced *trans*-activation of reporter constructs containing an oestrogen response element coupled to the chloramphenicol acetyltransferase gene transfected into these cells (Jeng *et al*., 1992); however, the cell growth-stimulating and reporter gene *trans*-activating effects of norethisterone at 10^{-6} mol/L were blocked by simultaneous treatment of the cells with the anti-oestrogens 4-hydroxytamoxifen (10^{-7} mol/L) and ICI164,384 (10^{-7}–10^{-6} mol/L), but not by anti-progestogens such as RU486 (Jeng & Jordan, 1991; Jeng *et al*., 1992; Schoonen *et al*., 1995a,b). This suggests that the stimulatory effects of norethisterone on cell proliferation are mediated via the oestrogen receptor, but not the progesterone receptor. Support for this notion was provided by studies indicating that

norethisterone did not stimulate the growth of the oestrogen receptor-negative human breast cancer cell lines MDA-MB-231, BT-20 and T47D:C4 (Jeng *et al*., 1992). Furthermore, the stimulation of MCF-7 cell proliferation by norethisterone was accompanied by a marked decrease in TGF-β2 and TGF-β3 mRNA levels, while the level of TGF-β1 mRNA was not affected. The inhibitory effect on TGF-β2 and TGF-β3 mRNA could be blocked by addition of 4-hydroxytamoxifen (Jeng & Jordan, 1991).

Norethisterone increased the reductive activity of 17β-hydroxysteroid dehydrogenase in an oestrogen- and progestogen-stimulated MCF-7 cell line cultured in the absence of phenol red (Coldham & James, 1990), which indicates that this progestogen stimulates breast cell proliferation *in vivo* by increasing the formation of oestradiol.

Oestradiol at concentrations of 10^{-10} mol/L and higher strongly induced the growth of the MCF-7 and T47D cell lines, regardless of the sub-line used (van der Burg *et al*., 1992; Kalkhoven *et al*., 1994; Schoonen *et al*., 1995a,b). The stimulation of MCF-7 cell growth by oestrogen at 10^{-10} mol/L was not significantly inhibited by norethisterone at the concentrations tested (up to 10^{-6} mol/L) (Schoonen *et al*., 1995a). Oestrogen-induced growth in T47D cells was not blocked by norethisterone at 10^{-6} mol/L in one sub-line (T47D-A), but it was completely inhibited in another sub-line (T47D-S) at a concentration of 10^{-8} mol/L. These two sub-lines differ considerably, in that RU486, but not 4-hydroxytamoxifen or ICI164,384, blocked oestrogen-stimulated growth in the T47D-A cell line, while both anti-progestogens and anti-oestrogens were inhibitory for T47D-S (Schoonen *et al*., 1995b).

In vivo, norethisterone had no androgenic activity, as judged by the lack of stimulation of ventral prostate growth in castrated rats (Phillips *et al*., 1987); however, norethisterone reduced the activity of 5α-reductase in the livers of male and female rats and also decreased the activity of hepatic 3β-hydroxysteroid dehydrogenase in castrated male rats. These effects were not blocked by flutamide or oestradiol, suggesting that androgen receptor-mediation was not involved. The oestrogen-like activity of norethisterone, i.e. the suppression of 3β-hydroxysteroid dehydrogenase, can probably be ascribed to an effect of 'high oestrogen dose' (Lax *et al*., 1984).

Studies with the human breast cancer cell line ZR-75-1, which contains functional oestrogen, progesterone and androgen receptors, suggest that norethisterone inhibits the growth of these cells by a combined action of androgen and progesterone receptor-mediated mechanisms in the presence of oestrogens. In oestrogen-free medium, however, norethisterone stimulated the growth of these cells, an effect that was counteracted by the anti-oestrogen EM-139 (Poulin *et al*., 1990).

Norethisterone did not bind to the glucocorticoid receptor on human mononuclear leukocytes (Kontula *et al*., 1983). It showed moderate affinity for human sex hormone-binding globulin, which could only slightly increase the level of free testosterone (Nilsson & von Schoultz, 1989).

Norethisterone increased secretion of vascular endothelial growth factor by the human breast cancer cell line T47D to a similar extent (two- to threefold over basal levels) as progesterone. This effect, which was progestogen-specific and did not occur in

MCF-7, ZR-75 or MDA-MB-231 cells, suggests an angiogenic response of these cells to norethisterone (Hyder *et al*., 1998).

4.2.13 *Norethynodrel*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

Norethynodrel is metabolized *in vivo* to norethisterone, which binds the progesterone receptor in rabbit uterine cytosol and human uterine endometrium and myometrium, whereas no binding to the progesterone receptor was reported in cultured MCF-7 human breast tumour cells, as pointed out above in the section on norethisterone. Shapiro *et al*. (1978) reported that the binding affinity of norethynodrel itself to the human uterine progesterone receptor is 23% that of progesterone. Progestogen-specific stimulation of alkaline phosphatase activity in T47D human breast cancer cells revealed the full agonist activity of norethynodrel, which was as strong as that of progesterone (Markiewicz & Gurpide, 1994).

The affinity with which norethynodrel bound to the oestrogen receptor in whole rat uterine homogenate was closest to that of the natural ligand of all the progestogens tested (van Kordelaar *et al*., 1975). Norethynodrel at concentrations of 10^{-8}–10^{-6} mol/L showed moderate oestrogenic activity in comparison with oestradiol when tested for its stimulatory effect on alkaline phosphatase activity in Ishikawa human endometrial cancer cells, which is an oestrogen-specific response inhibited by 4-hydroxytamoxifen (Markiewicz *et al*., 1992; Botella *et al*., 1995).

Norethynodrel stimulated the growth of the oestrogen receptor-positive human breast cancer cell lines MCF-7 and T47D-A18 at concentrations of 10^{-8}–10^{-7} mol/L in experiments performed with cells grown in phenol red-free medium which contained steroid-free (dextran-coated charcoal-stripped) serum. Norethynodrel induced *trans*-activation of reporter constructs containing an oestrogen response element coupled to the chloramphenicol acetyltransferase gene transfected into these cells; however, the cell growth-stimulating and reporter gene-*trans*-activating effects of norethynodrel at 10^{-7} mol/L were blocked by simultaneous treatment of the cells with the anti-oestrogens 4-hydroxytamoxifen (10^{-7} mol/L) and ICI164,384 (10^{-7}–10^{-6} mol/L), but not by anti-progestogens such as RU486. These findings suggest that the stimulation of cell proliferation by norethynodrel is mediated via the oestrogen receptor, not the progesterone receptor. Support for this notion is provided by studies indicating that norethynodrel does not stimulate the growth of the oestrogen receptor-negative human breast cancer cell lines MDA-MB-231, BT-20 and T47D:C4 (Jeng *et al*., 1992).

The androgenic activity of norethynodrel has not been studied, but its metabolite norethisterone has androgenic activity *in vivo* (Phillips *et al*., 1987; Duc *et al*., 1995).

Norethynodrel increased the secretion of vascular endothelial growth factor by the human breast cancer cell line T47D to a similar extent (two- to threefold over basal

levels) as progesterone. This effect, which was progestogen-specific and did not occur in MCF-7, ZR-75 or MDA-MB-231 cells, suggests an angiogenic cellular response to norethynodrel (Hyder *et al*., 1998).

The combination of mestranol (7.5 or 75 μg/rat per day) and norethynodrel (0.5 or 5 mg/rat per day) given as a pellet implant to female Sprague-Dawley rats, starting at 45, 55, 65 or 75 days of age, caused changes in the developing mammary gland that resulted in protection against induction of mammary cancer by a single dose (80 mg/kg bw) of DMBA (Russo *et al*., 1989). The hormone treatment was given for 21 days, followed by 21 days' recovery, at which time some rats were killed to study the morphology of their mammary glands, while other rats received DMBA. The hormone treatment at both doses decreased the number of terminal end-buds per mammary gland and increased the number of alveolar buds but did not alter the number of terminal ducts; cell proliferation, measured as the DNA-labelling index, was reduced in the terminal ducts and alveolar buds but remained unchanged in the terminal end-buds (Russo *et al*., 1989; Russo & Russo, 1991). In these experiments, a trend was observed for the hormonal treatment to produce less effect when initiated at a later age. The reduction in cell proliferation in terminal end-buds and terminal ducts, the target tissues for DMBA, may explain the protective effect of the hormonal combination on the development of mammary cancer.

Reboud and Pageaut (1977) administered norethynodrel by subcutaneous implantation of resin pellets to female BALB/C, B6AF1 and C57BL6 mice for two weeks at a dose of 15 mg/mouse once a week and for nine weeks or eight months at 15 mg/mouse every three weeks. Under each of the exposure conditions and in all strains, norethynodrel caused irregular hyperplasia of the vagina and exo-cervix similar to that observed during oestrus, unlike progesterone which caused mucoid and dysplastic cervical changes at the same dose. Progesterone, but not norethynodrel, is a cervical tumour promoter in mice treated with 3-methylcholanthrene.

Norethynodrel had a strong growth-stimulating effect on the livers of female Wistar rats when given at a dose of 10–100 mg/kg bw for seven days, as was evident from a 15–25% increase in liver weight and an approximately 40% increase in hepatic DNA content (Schulte-Hermann *et al*., 1988).

Norethynodrel *per se* at concentrations of 3×10^{-6}–10^{-4} mol/L induced γ-GT activity in cultured rat hepatocytes in the presence of 30 nmol/L dexamethasone (Edwards & Lucas, 1985).

4.2.14 *Norgestimate*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

The binding affinity of norgestimate for human and rabbit uterine progesterone receptors has been reported to be 1–3% that of progesterone itself (Juchem *et al*., 1993; Kuhnz *et al*., 1995). Other studies showed a 10-fold lower (Killinger *et al*., 1985) or even

a somewhat higher binding affinity (Phillips *et al*., 1990) relative to progesterone. The apparent discrepancies in the observed progesterone receptor binding may be due to the fact that metabolites of norgestimate, levonorgestrel (see above) and levonorgestrel-17-acetate, bind with approximately eight- and fourfold higher affinity, respectively, to this receptor in human myometrial tissue (Juchem *et al*., 1993).

Norgestimate had clear progestational activity *in vivo*, both in a test for pregnancy maintenance in female rats (Kuhnz & Beier, 1994) and as measured by inhibition of ovulation and endometrial stimulation in rabbits (Killinger *et al*., 1985; Phillips *et al*., 1987). In these three endocrine bioassays, norgestimate was 3–10 times more active than progesterone (Phillips *et al*., 1987).

In vivo, norgestimate had little or no androgenic activity in comparison with 5α-dihydrotestosterone, as measured by stimulation of ventral prostate growth in immature castrated rats (Phillips *et al*., 1987, 1990; Kuhnz & Beier, 1994).

At concentrations as low as 10^{-10} mol/L, norgestimate up-regulated the expression of the prostate-specific antigen at the mRNA and protein level in T-47D human breast cancer cells. Expression of the antigen in these cells was also stimulated by other progestogens, androgens and corticosteroids (Zarghami *et al*., 1997).

Norgestimate also bound with very low affinity to the androgen receptor but not at all to the oestrogen receptor (Juchem & Pollow, 1990; Phillips *et al*., 1990). The relative binding affinity for the rat ventral prostate androgen receptor was approximately 0.3% that of 5α-dihydrotestosterone (Phillips *et al*., 1990).

Norgestimate did not bind to sex hormone-binding globulin in human serum (Juchem & Pollow, 1990).

4.2.15 *Norgestrel*

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

Norgestrel binds strongly to the progesterone receptor in human uterus, with an affinity equal to 50 or > 90% that of progesterone (Briggs, 1975; Shapiro *et al*., 1978). A similar result was reported for receptor binding in the chick oviduct (Haukkamaa *et al*., 1980). Norgestrel bound with sixfold higher affinity than progesterone to the progesterone receptor in rabbit lung (Nielsen *et al*., 1987).

In an assay of progestogen-specific stimulation of alkaline phosphatase activity in T47D human breast cancer cells, moderate agonist activity was demonstrated for norgestrel in comparison with progesterone (Markiewicz & Gurpide, 1994).

Norgestrel at concentrations of 10^{-8}–10^{-6} mol/L stimulated the growth of the oestrogen receptor-containing and oestrogen-sensitive mammary cancer cell lines MCF-7 and T47D:A18; this activity was inhibited by the anti-oestrogens ICI182,780 and ICI164,384 (at a concentration of 10^{-6} mol/L), but not by RU486 (at a concentration of 10^{-7} mol/L) (Jeng *et al*., 1992; Catherino *et al*., 1993; Catherino & Jordan, 1995). Norgestrel did not

affect the growth of the oestrogen receptor-negative and oestrogen-independent mammary cancer cell lines MDA-MB-231, BT-20 and T47DC4 (Jeng *et al.*, 1992). Progesterone receptor expression was maintained in both cell types. With reporter constructs containing two progesterone response elements in front of the *tk* promotor coupled to the chloramphenicol acetyltransferase gene transfected into the MCF-7 cell line, transcriptional activation was observed with norgestrel at a concentration of 10^{-6} mol/L, clearly demonstrating expression of functional progesterone. During these experiments, 10^{-10} mol/L oestradiol was present in the medium to boost receptor expression (Catherino *et al.*, 1993). These findings suggest that the stimulating effects of norgestrel on cell proliferation are mediated via the oestrogen receptor. This is apparently not related to effects at the level of receptor–ligand interaction, because norgestrel at concentrations of 10^{-9}–10^{-6} mol/L causes *trans*-activation of reporter constructs containing oestrogen response elements (from the vitellogenin or *pS2* gene) or progesterone response elements transfected into MCF-7 cells; ICI164,384, but not RU486, inhibited *trans*-activation of the oestrogen response element-containing constructs, while RU486, but not ICI164,384, inhibited *trans*-activation of the progesterone response element-containing construct (Catherino *et al.*, 1993; Catherino & Jordan, 1995). Norgestrel also stimulated the protein expression of the progesterone receptor in MCF-7 cells at 10^{-6} but not at 10^{-8} mol/L (Catherino *et al.*, 1993).

Norgestrel showed much weaker binding than the natural ligand to the oestrogen receptor in whole rat-uterine homogenate (van Kordelaar *et al.*, 1975), while displacement of ^{3}H-oestradiol binding to the cytosolic fraction of female rat liver occurred only at norgestrel concentrations of 10^{-5}–10^{-4} mol/L (Marr *et al.*, 1980). The binding of oestradiol to rat uterine oestrogen receptor was reduced, however, by norgestrel, both *in vivo* at 1 h after a single oral dose of 15 mg/kg bw and *in vitro* (Di Carlo *et al.*, 1983). *In vivo*, norgestrel partially reversed the hyperplastic and metaplastic changes found in oestrogen-exposed rat uterus (White *et al.*, 1982).

Norgestrel had much weaker oestrogenic activity than oestradiol at concentrations greater than 1×10^{-6} mol/L when tested for its stimulatory effect on 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).

Norgestrel was shown to be a potent competitor for binding of 5α-dihydrotestosterone to the androgen receptor in human foreskin fibroblasts, with an activity similar to that of testosterone (Breiner *et al.*, 1986).

Studies with the human breast cancer cell line ZR-75-1, which contains functional oestrogen, progesterone and androgen receptors, suggest that norgestrel inhibits the growth of these cells via an interaction of androgen and progesterone receptor-mediated mechanisms in the presence of oestrogens. In oestrogen-free medium, however, norgestrel stimulated the growth of these cells, an effect that was counteracted by the anti-oestrogen EM-139 (Poulin *et al.*, 1990).

Norgestrel increased the secretion of vascular endothelial growth factor by the human breast cancer cell line T47D to an extent (two- to threefold over basal levels)

similar to progesterone. This effect, which was progestogen-specific and did not occur in MCF-7, ZR-75 or MDA-MB-231 cells, suggests an angiogenic response of T47D cells to norgestrel (Hyder *et al*., 1998).

4.3 Genetic and related effects

Most, if not all, of the genetic and related effects associated with use of oral contraceptives can be explained by oestrogen and progestogen receptor mechanisms (King, 1991), but non-receptor processes may also exist (Duval *et al.*, 1983; Yager & Liehr, 1996). The following descriptions indicate how the doses of hormone used relate to receptor and non-receptor mechanisms and to the concentrations achieved *in vivo* in women who use oral contraceptives or post-menopausal hormonal therapy. The concentrations in such formulations are usually several micrograms per kilogram body weight per day, which generate plasma concentrations of nanograms per millilitre for progestogens and picograms per millilitre for oestrogens (Orme *et al.*, 1983; Barnes & Lobo, 1987). Those are the concentrations at which receptor-mediated events can be saturated *in vitro*. Appreciably higher concentrations were used in many of the studies listed in Tables 42–46. The significance of the presence and absence of effects at these concentrations is uncertain, as is the mode of action in the case of effects.

4.3.1 *Combined oral contraceptives*

Genetic changes in cells from women taking steroid hormones have been compared with those in cells from unexposed women in five studies, in all of which few details are given about the hormonal exposure; however, use of oral contraceptives predominated.

Two of three reports described the effects of steroids on lymphocytes. Ghosh and Ghosh (1988) noted an increased frequency of sister chromatid exchange in lymphocytes from 51 healthy, non-smoking Indian women (mean age, 34.5 years) exposed to ethinyloestradiol plus levonorgestrel [doses not given] for 4–28 months as compared with 38 unexposed referents (mean age, 35.6 years). The numbers of sister chromatid exchanges per cell were 5.56 ± 0.21 for the referents and 8.63 ± 0.29 for women taking oral contraceptives ($p < 0.001$).

In contrast, a study in Denmark showed no effect on the sister chromatid exchange frequency in lymphocytes of exposure to oestrogen and progestogen [types and doses not stated] for a minimum of two months (Husum *et al*., 1982). There were 25 non-smoking, healthy women aged 15–42 years in the referent group, who had 8.42 ± 0.21 sister chromatid exchanges per cell and 15 women with otherwise similar characteristics who used oral contraceptives and had 8.54 ± 0.24 sister chromatid exchanges per cell. Smoking of > 20 cigarettes per day produced the expected increase in sister chromatid exchange frequency, but no significant difference was observed between oral contraceptive users who smoked this number of cigarettes and comparable controls: 9.52 ± 0.30 sister chromatid exchanges per cell in 13 referents and 10.36 ± 0.75 sister chromatid exchanges per cell in six oral contraceptive users.

Chromosomal abnormalities were quantified in lymphocytes from 88 women aged 16–35 years in South Africa, equally divided into controls who had never used hormonal contraception and women who had used oral contraceptives [types and doses not stated] for 7–98 months. The groups were pair-matched for race, age, parity and condition of offspring, occupation, medication, X-irradiation and smoking habits (Pinto, 1986). Abnormal chromosomes were found in 31% (410/1286) of lymphocytes from oral contraceptive users and 18% (233/1255) of control cells ($p < 0.0001$). The abnormalities were subclassified into those possibly caused by technical handling (0.29 ± 0.13 and 0.19 ± 0.07 abnormalities per cell in oral contraceptive users and controls, respectively ($p < 0.0001$)) and those not likely to be generated in this way (0.105 ± 0.077 and 0.018 ± 0.029 abnormalities per cell in oral contraceptive users and controls, respectively ($p < 0.0001$)).

Indications of hormone-related genetic damage in lymphocytes in two of the three well-conducted studies raised questions about the potential genotoxicity of steroid hormones in humans. As the relevance of effects in blood lymphocytes to mechanisms of carcinogenesis in tissues such as breast epithelium is unclear, two good analyses of the effects of oral contraceptives on subsequent changes in breast cancer DNA are noteworthy. The two studies were based on the same library of stored breast cancer tissues from women in Sweden whose previous exposure to oral contraceptives was known. At the time of first diagnosis of the cancer, pre-menopausal women were questioned about their earlier life style, including the age at which they had started using oral contraceptives. Tumours removed from these women were stored and subsequently used to analyse ploidy, aneuploidy and cell proliferative activity by flow cytometry (Olsson *et al*., 1991b) and oncogene amplification (Olsson *et al*., 1991c).

In the study of ploidy (Olsson *et al*., 1991b), 175 breast tumours from pre-menopausal women aged 26–52 years were used. Of the tumours from women who had started using oral contraceptives before 20 years of age, 81% ($n = 27$) were aneuploid, whereas only 53% ($n = 59$) of those from women who had never used oral contraceptives were aneuploid ($p < 0.04$). Tumours from women who had started using oral contraceptives at ages 20 to ≥ 24 years had intermediate percentages of aneuploid cells. There was a highly significant ($p = 0.0001$) correlation between early oral contraceptive use and age at diagnosis and other parameters such as proliferative activity, measured as the fraction of cells in S-phase. The statistical significance of the association between early oral contraceptive use and biological effects on the cancer cells was maintained when multivariate analysis was performed.

In the study of oncogenes (Olsson *et al*., 1991c), *erbb*2 (*HER*/neu) and *int*2 gene amplifications were assessed in 72 tumours from 28–50-year-old women. More cancers from women who had started using oral contraceptives before the age of 20 had *erbb*2 amplifications (11/19 or 58% of cancers) than those from women who started after that age (11/53 or 21% of cancers). The odds ratio for this difference was significant in both univariate (odds ratio, 5.3; 95% CI, 1.6–17) and multivariate (odds ratio, 6.8; 95% CI, 1.3–35) analyses. No link was seen between early oral contraceptive use and *int*2 amplification, but this effect was positively associated with any use of progestogens (multivariate

odds ratio, 17; 95% CI, 1.8–170); amplification of *erbb*2 was not related to progestogen use. Other variables considered were age at abortion and first full-term pregnancy, parity, age at diagnosis and tumour stage. The authors recognized the problems associated with interpreting data from such analyses in terms of cause and effect and correctly concluded that they should not be ignored.

4.3.2 *Ethinyloestradiol and some derivatives alone and in combination with progestogens* (Table 42)

No gene mutation was induced in *S. typhimurium* after treatment with ethinyloestradiol. In single studies, small increases in the frequency of unscheduled DNA synthesis were demonstrated in primary cultures of rat hepatocytes treated with ethinyloestradiol, particularly in cells from male rats, and cell transformation was demonstrated *in vitro* in BALB/c 3T3 mouse cells treated with ethinyloestradiol.

In vivo, covalent binding to DNA was demonstrated in the liver, pancreas and kidney of rats and in the kidneys of Syrian hamsters treated with ethinyloestradiol. Chromosomal aberrations were induced *in vivo* in kidney cells of exposed, castrated male Syrian hamsters and in bone-marrow cells of mice treated with high doses of ethinyloestradiol. Paradoxically, ethinyloestradiol at these high doses did not induce micronuclei in bone-marrow cells of mice.

Covalent binding to DNA was observed in the kidneys of Syrian hamsters treated with ethylethinyloestradiol and in those of animals that had received an implant of methylethinyloestradiol. Methoxyethinyloestradiol (Moxestrol) did not induce cell transformation in BALB/c 3T3 mouse cells *in vitro*, but it bound to DNA in the kidneys of Syrian hamsters that had received a Moxestrol implant.

Combinations of ethinyloestradiol and gestodene did not induce gene mutation in various *S. typhimurium* strains. Chromosomal aberrations were induced in the bone marrow of male mice treated *in vivo* with combinations of ethinyloestradiol and norethisterone acetate. In a single study, micronuclei were not induced in female mice exposed *in vivo* to ethinyloestradiol and norethisterone acetate. Primary cultures of baby rat kidney cells were transformed to anchorage-independent growth, and these cells induced tumour formation in syngeneic animals infected with HPV-16 DNA and Ha-*ras*-1 and exposed to an ethanolic extract of oral contraceptive tablets containing ethinyloestradiol and levonorgestrel.

DNA covalent binding was demonstrated in the liver, pancreas and kidney of male rats treated with ethinyloestradiol and tamoxifen.

4.3.3 *Mestranol alone and in combination with progestogens* (Table 43)

Gene mutations were not induced in *S. typhimurium* after treatment with mestranol itself or with ethanolic extracts of Ovulen 21 tablets, containing mestranol, or Enovid tablets, containing mestranol and norethynodrel. Also, gene mutations were not induced in *S. typhimurium* in a host-mediated assay in which the bacteria were recovered from the livers of mice. It has been reported, however, that mestranol and extracts of Ovulen 21 and

Table 42. Genetic and related effects of ethinyloestradiol and its derivatives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Ethinyloestradiol				
Escherichia coli rec strains, differential toxicity	NT	–	1000 µg/plate	Mamber *et al*. (1983)
Bacillus subtilis rec strains, differential toxicity	–	NT	5000 µg/plate	Tanooka (1977)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2500 µg/plate	Lang & Redmann (1979)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Dayan *et al*. (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	500 µg/plate[c]	Lang & Reimann (1993)
Aneuploidy, male Chinese hamster DON cells *in vitro*	+	NT	22.2	Wheeler *et al*. (1986)
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	–	29.6	Drevon *et al*. (1981)
Gene mutation, Chinese hamster lung V79 cells, ouabain resistance *in vitro*	–	–	29.6	Drevon *et al*. (1981)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	–	NT	4	Ishidate *et al*. (1978)
Cell transformation, BALB/c 3T3 mouse cells	–	NT	5.0	Dunkel *et al*. (1981)
Cell transformation, BALB/c 3T3 mouse cells	+	NT	3.0	Liehr *et al*. (1987a)
Cell transformation, Syrian hamster embryo cells, clonal assay	–	NT	50	Dunkel *et al*. (1981)
Cell transformation, RMuLV/Fischer rat embryo cells	+	NT	10.7	Dunkel *et al*. (1981)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	100	Stenchever *et al*. (1969)
Micronucleus induction, mice *in vivo*	–		0.2 po × 15	Shyama & Rahiman (1996)
Chromosomal aberrations, male Syrian hamster kidney cells *in vivo*	+		185 µg/d imp.; 5 mo	Banerjee *et al*. (1994)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		0.12 po × 15	Shyama & Rahiman (1996)
Binding (covalent) to DNA, female rat liver, pancreas, kidney *in vivo*	+		75 µg/d po; 12 mo	Shimomura *et al*. (1992)
Binding (covalent) to DNA, Syrian hamster kidney *in vivo*	+		22 mg imp. × 2	Liehr *et al*. (1987b)
Inhibition of metabolic cooperation, Chinese hamster V79 cells *in vitro*	(+)	NT	0.74	Yager (1983)

Table 42 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Ethinyloestradiol + gestodene (1 part + 2.5 parts)				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate[c,d]	Lang & Reimann (1993)
Ethinyloestradiol + norethisterone acetate				
Chromosomal aberrations, female Swiss mouse bone-marrow cells *in vivo*	+		0.8 po × 15[d]	Shyama *et al.* (1991)
Micronucleus formation, female Swiss mouse bone-marrow cells *in vivo*	–		8.0 po × 15[d]	Shyama *et al.* (1991)
Ethinyloestradiol + (l)-norgestrel				
Cell transformation, primary baby rat kidney+ HPV-16 + H-*ras*-1	+	NT	0.3 (ethanol extract)[d]	Pater *et al.* (1990)
Ethinyloestradiol + tamoxifen				
Binding (covalent) to DNA, female rat liver, pancreas, kidney *in vivo*	+		75 µg + 500 µg/d po, 12 mo	Shimomura *et al.* (1992)
Ethylethinyloestradiol				
Binding (covalent) to DNA, Syrian hamster kidney *in vivo*	+		25 mg imp. × 2	Liehr *et al.* (1987b)
Methoxyethinyloestradiol (Moxestrol)				
Cell transformation, BALB/c 3T3 mouse cells *in vitro*	–	NT	16.3	Liehr *et al.* (1987a)
Binding (covalent) to DNA, Syrian hamster kidney *in vivo*	+		25 mg imp. × 2	Liehr *et al.* (1986)
Methylethinyloestradiol				
Binding (covalent) to DNA, Syrian hamster kidney *in vivo*	+		25 mg imp. × 2	Liehr *et al.* (1986, 1987b)

[a] +, positive; (+), weak positive; –, negative; NT, not tested; ?, inconclusive
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; po, oral; imp., implant; d, day; mo, month
[c] Toxicity was observed at higher dose(s) tested
[d] Total mixture

Table 43. Genetic and related effects of mestranol

Test system	Result[b]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mestranol				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	12.5 µg/plate	Rao *et al*. (1983)
Salmonella typhimurium TA100, TA1535, TA97a, TA98, reverse mutation	–	–	1000 µg/plate[c]	Dhillon *et al*. (1994)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		50000	Aguiar & Tordecilla (1984)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	1	Dhillon *et al*. (1994)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	10	Stenchever *et al*. (1969)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	100	Dhillon *et al*. (1994)
DNA strand breaks, cross-links or related damage, Sprague-Dawley rats *in vivo*	–		250 ip × 1	Yager & Fifield (1982)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	–		100 po × 1	Ansari & Adhami (1977)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		0.01 ip × 1	Dhillon *et al*. (1994)
Micronucleus induction, mouse bone-marrow cells *in vivo*	+		1 ip × 1	Dhillon *et al*. (1994)
Sister chromatid exchange, mouse bone-marrow cells *in vivo*	+		0.1 ip × 1	Dhillon *et al*. (1994)
Host mediated assay (male Swiss albino mouse, intravenous inoculation), *S. typhimurium* TA100, TA1535, TA98, TA97a	–		100 ip × 1	Dhillon *et al.* (1994)
Inhibition of metabolic cooperation, Chinese hamster V79 cells *in vitro*	(+)	NT	0.78	Yager (1983)
Mestranol + 2-acetylaminofluorene (3 µg)				
Salmonella typhimurium TA100, TA98, reverse mutation	NT	+	0.62 µg/plate	Rao *et al*. (1983)
Mestranol + norethisterone				
Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	0.0038 + 0.075	Dutkowski *et al*. (1983)
Micronucleus induction, human lymphocytes *in vitro*	–	NT	0.0038 + 0.075	Dutkowski *et al*. (1983)

Table 43 (contd)

Test system	Result[b]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Enovid extract (mestranol + norethynodrel)				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	625 µg/plate	Rao *et al*. (1983)
Enovid extract (mestranol + norethynodrel) + 2-acetylaminofluorene (3 µg)				
Salmonella typhimurium TA100, TA98, reverse mutation	–	+	31.3 µg/plate	Rao *et al*. (1983)
Enovid extract (mestranol + norethynodrel) + *N*-nitrosopiperidine (250 µg)				
Salmonella typhimurium TA100, reverse mutation	–	+	62.5 µg/plate	Rao *et al*. (1983)
Salmonella typhimurium TA1535, reverse mutation	–	+	31.3 µg/plate	Rao *et al*. (1983)
Ovulen 21 extract (mestranol)				
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	50 µg/plate	Rao *et al*. (1983)
Ovulen 21 extract (mestranol) + 2-acetylaminofluorene (3 µg)				
Salmonella typhimurium TA98, reverse mutation	–	+	31.3 µg/plate	Rao *et al*. (1983)
Ovulen 21 extract (mestranol) + *N*-nitrosopiperidine (250 µg)				
Salmonella typhimurium TA1535, reverse mutation	–	+	31.3 µg/plate	Rao *et al*. (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; ip, intraperitoneal; po, oral
[c] Toxicity was observed at higher dose(s)

Enovid enhanced the mutation yield obtained with an ineffective dose of 2-acetylaminofluorene (3 μg/plate). The extracts also enhanced the mutation yield obtained with an ineffective dose of *N*-nitrosopiperidine (250 μg/plate).

In a single study, mestranol induced sister chromatid exchange and chromosomal aberrations in human lymphocytes *in vitro* and sister chromatid exchange, chromosomal aberrations and micronuclei in bone-marrow cells from mice treated *in vivo*.

Negative results were obtained with a combination of mestranol and norethisterone in a study of sister chromatid exchange and micronucleus formation in human lymphocyte cultures. The concentrations of the test material used in this study were in the nanogram per millilitre range, which are those that might be expected during human use.

4.3.4 *Cyproterone acetate, metabolites and derivatives* (Table 44)

Gene mutations were not induced in *Salmonella typhimurium* by cyproterone acetate or 6,7-epoxycyproterone acetate. Covalent binding to DNA was observed with cyproterone acetate in cultured rat liver cells, the binding being greater in cells from females than from males in all cases, according to one study. Cyproterone acetate also bound to DNA in human and porcine hepatocytes in culture, while a metabolite, 3-hydroxycyproterone acetate, and a derivative, 3-*O*-acetylcyproterone acetate, also bound to isolated calf thymus DNA. DNA strand breakage was induced in female rat hepatocytes *in vitro* in one study. No DNA breakage was observed in male human hepatocytes *in vitro*. DNA repair, including unscheduled DNA synthesis, of damage induced by cyproterone acetate appears to be sex specific, since these processes occur in cultured liver cells from female but not male rats. Gene mutations were not induced at the *hprt* locus of Chinese hamster V79 cells by cyproterone acetate in two studies; in one of the studies, the cells were co-cultured with rat hepatocytes. The frequency of chromosomal aberrations was not increased in a single study with cyproterone acetate in Chinese hamster V79 cells co-cultured with rat hepatocytes, whereas a study of the frequency of micronucleus formation *in vitro* in hepatocytes from female rats gave inconclusive results.

In vivo, covalent binding to DNA has been demonstrated in the livers of male and female rats and female mice (weak binding). No binding to male mouse liver DNA was observed. Unscheduled DNA synthesis was induced in one study in hepatocytes from rats exposed to cyproterone acetate. Also in single studies, this compound increased the frequency of micronucleus formation in hepatocytes from exposed female rats and induced γ-GT-positive foci in the livers of female rats. In one study, the frequency of mutation of the *Lac*I transgene was significantly increased in female BigBlue® transgenic Fischer 344 rats after exposure to 3-*O*-acetylcyproterone acetate. DNA adducts were quantified in the same experiment; the mutation frequency started to increase at doses at which the number of DNA adducts had already reached a plateau.

4.3.5 *Norethisterone alone and in combination with an oestrogen* (Table 45)

Gene mutation was not induced in *S. typhimurium* after treatment with norethisterone. Chromosomal aberrations were induced, but the frequency of micronucleus

Table 44. Genetic and related effects of cyproterone acetate and some derivatives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cyproterone acetate				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	250 µg/plate[c]	Lang & Reimann (1993)
DNA strand breaks, alkaline elution assay, female rat hepatocytes *in vitro*	+	NT	20.85	Martelli *et al.* (1995)
DNA repair exclusive of unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	0.83	Neumann *et al.* (1992)
DNA repair exclusive of unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	0.83	Topinka *et al.* (1995)
DNA repair exclusive of unscheduled DNA synthesis, male rat hepatocytes *in vitro*	–	NT	20.9	Topinka *et al.* (1995)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	1.32	Kasper *et al.* (1995)
Unscheduled DNA synthesis, male rat hepatocytes *in vitro*	–	NT	20.85	Martelli *et al.* (1995)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	0.42	Martelli *et al.* (1995)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	0.84	Martelli *et al.* (1996a)
Gene mutation,Chinese hamster V79 cells, *hprt* locus *in vitro*	–	–	80[d]	Lang & Reimann (1993)
Gene mutation, Chinese hamster V79 cells, *hprt* locus *in vitro* (co-cultured with hepatocytes)	NT	–	41.7	Kasper *et al.* (1995)
Chromosomal aberrations, Chinese hamster V79 cells *in vitro* (co-cultured with hepatocytes)	NT	–	41.7	Kasper *et al.* (1995)
Micronucleus formation, female rat hepatocytes *in vitro*	(+)	NT	0.42	Kasper *et al.* (1995)
DNA strand breaks, alkaline elution assay, male human hepatocytes *in vitro*	–	NT	20.85 (1 sample)	Martelli *et al.* (1995)
DNA strand breaks, alkaline elution assay, female human hepatocytes *in vitro*	(+)	NT	20.8 (3/4 samples)	Martelli *et al.* (1995)
Unscheduled DNA synthesis, male and female human hepatocytes *in vitro*	+	NT	0.42	Martelli *et al.* (1995)
Unscheduled DNA synthesis, male and female human hepatocytes *in vitro*	+	NT	0.42	Martelli *et al.* (1996a)

Table 44 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Cyproterone acetate (contd)				
Unscheduled DNA synthesis, female rat hepatocytes *in vivo*	+		100 po × 1	Kasper & Mueller (1996)
Gene mutation, female *lacI* transgenic rat (BigBlue®) *in vivo*	+		75 po × 1	Krebs *et al*. (1998)
Micronucleus formation, female rat hepatocytes *in vivo*	+		100 po × 1	Martelli *et al*. (1996b)
γ-Glutamyl transpeptidase-positive foci, female Sprague-Dawley rat liver *in vivo*	+		100 po × 6 (weekly)	Martelli *et al*. (1996b)
Binding (covalent) to DNA, female rat hepatocytes *in vitro*	+	NT	0.013	Topinka *et al*. (1993, 1995)
Binding (covalent) to DNA, male rat hepatocytes *in vitro*	+	NT	0.42	Topinka *et al*. (1993, 1995)
Binding (covalent) to DNA, human (male and female), rat (female) hepatocytes *in vitro*	+	NT	4.2	Werner *et al*. (1996)
Binding (covalent) to DNA, pig (male and female), rat (male) hepatocytes *in vitro*	(+)	NT	4.2	Werner *et al*. (1996)
Binding (covalent) to DNA, female rat liver *in vivo*	+		0.1 po × 1	Topinka *et al*. (1993)
Binding (covalent) to DNA, male rat liver *in vivo*	+		3 po × 1	Topinka *et al*. (1993)
Binding (covalent) to DNA, female rat liver *in vivo*	+		10 po × 1	Werner *et al*. (1995)
Binding (covalent) to DNA, male rat liver *in vivo*	+		100 po × 1	Werner *et al*. (1995)
Binding (covalent) to DNA, male rat liver *in vivo*	+		100 po × 1	Werner *et al*. (1996)
Binding (covalent) to DNA, male C57BL/6 mouse liver *in vivo*	–		35 po × 1	Werner *et al*. (1996)
Binding (covalent) to DNA, female rat liver *in vivo*	+		10 po × 1	Werner *et al*. (1996)
Binding (covalent) to DNA, female C57BL/6 mouse liver *in vivo*	(+)		35 po × 1	Werner *et al*. (1996)
Binding (covalent) to DNA, female rat liver *in vivo*	+		25 po × 1	Krebs *et al*. (1998)

Table 44 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
6,7-Epoxycyproterone acetate				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	500 µg/plate[c]	Lang & Reimann (1993)
α-Hydroxycyproterone acetate				
Binding (covalent) to DNA, calf thymus *in vitro*	+	NT	1	Kerdar *et al*. (1995)
3-*O*-Acetyl cyproterone acetate (not a metabolite)				
Gene mutation, female *lacI* transgenic rat (BigBlue®) *in vivo*	+		75 po × 1	Krebs *et al*. (1998)
Binding (covalent) to DNA, calf thymus *in vitro*	+	NT	1	Kerdar *et al*. (1995)
Binding (covalent) to DNA, female rat liver *in vivo*	+		25 po × 1	Krebs *et al*. (1998)

[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; po, oral
[c] Toxicity was observed at this dose in some or all strains
[d] Some toxicity was observed

Table 45. Genetic and related effects of norethisterone and its ester

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Norethisterone				
Salmonella typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	–	–	1000 µg/plate	Peter *et al*. (1981)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	750 µg/plate	Lang & Reimann (1993)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	–	NT	150	Blakey & White (1985)
Unscheduled DNA synthesis, male rat hepatocytes *in vitro*	(+)	NT	15	Blakey & White (1985)
Micronucleus formation, female Swiss albino mouse bone-marrow cells *in vivo*	–		30 po × 15	Shyama & Rahiman (1993)
Chromosomal aberrations, female Swiss albino mouse bone-marrow cells *in vivo*	–		70 po × 1	Ansari & Adhami (1977)
Chromosomal aberrations, female Swiss albino mouse bone-marrow cells *in vivo*	+		3 po × 15	Shyama & Rahiman (1993)
Norethisterone acetate				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2500 µg/plate	Lang & Redmann (1979)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Dayan *et al*. (1980)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	500 µg/plate[c]	Lang & Reimann (1993)
Salmonella typhimurium TA100, TA1535, TA97a, TA98, reverse mutation	–	–	1000 µg/plate[c]	Dhillon & Dhillon (1996)
Sister chromatid exchange, human male lymphocytes *in vitro* (24-h treatment)	+	NT	1	Dhillon & Dhillon (1996)
Sister chromatid exchange, human male lymphocytes *in vitro* (90-min treatment)	+	+	1	Dhillon & Dhillon (1996)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	100	Stenchever *et al*. (1969)
Chromosomal aberrations, human male lymphocytes *in vitro* (72-h treatment)	+	NT	1	Dhillon & Dhillon (1996)
Chromosomal aberrations, human male lymphocytes *in vitro* (6-h treatment)	–	+	10	Dhillon & Dhillon (1996)
Host-mediated assay, male Swiss albino mouse (intravenous inoculation), *S. typhimurium* TA97a, TA98, TA100, TA1535	–		100 ip × 1	Dhillon & Dhillon (1996)
Sister chromatid exchange, Swiss albino mice bone-marrow cells *in vivo*	+		1 ip × 1	Dhillon & Dhillon (1996)
Micronucleus formation, male Swiss albino mouse bone-marrow cells *in vivo*	+		1 ip × 1	Dhillon & Dhillon (1996)
Dominant lethal mutation induction, C3H and NMRI mice *in vivo*	(+)		1 mg/animal po daily × 4 wk	Rohrborn & Hansmann (1974)
Aneuploidy, C3H mice *in vivo*	+		10 mg/animal po daily × 4 wk	Rohrborn & Hansmann (1974)

Table 45 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Norethisterone + mestranol				
Sister chromatid exchange, human lymphocytes *in vitro*	–	NT	0.075 + 0.0038	Dutkowski *et al*. (1983)
Micronucleus formation, human lymphocytes *in vitro*	–	NT	0.075 + 0.0038	Dutkowski *et al*. (1983)
Norethisterone acetate + ethinyloestradiol				
Chromosomal aberrations, female Swiss albino mouse bone-marrow cells *in vivo*	+		0.79 + 0.01 po × 15	Shyama *et al*. (1991)
Micronucleus formation, female Swiss albino mouse bone-marrow cells *in vivo*	–		7.9 + 0.1 po × 15	Shyama *et al*. (1991)

[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; po, oral; ip, intraperitoneal; wk, week
[c] Precipitation and/or toxicity was observed at higher dose(s).

formation was not increased in bone-marrow cells of female Swiss albino mice treated *in vivo* with norethisterone.

In a single study, the frequencies of sister chromatid exchange and micronuclei were not increased in cultured human lymphocytes treated with a combination of norethisterone and mestranol.

4.3.6 *Norethisterone acetate alone and in combination with an oestrogen* (Table 45)

Norethisterone acetate did not induce gene mutation in various strains of *S. typhimurium* with or without an exogenous metabolic activation system. In a single study, the frequencies of sister chromatid exchange and chromosomal aberrations were increased in human lymphocytes treated *in vitro* with norethisterone acetate. In the same study, sister chromatid exchange and micronucleus formation were induced in the bone-marrow cells of Swiss albino mice treated *in vivo* with norethisterone acetate, whereas gene mutations were not induced in *S. typhimurium* in a mouse host-mediated assay in which the bacteria were recovered from the livers of animals after treatment with norethisterone acetate.

The combination of norethisterone acetate plus ethinyloestradiol induced chromosomal aberrations in bone-marrow cells of female Swiss albino mice exposed *in vivo*, whereas micronuclei were not induced in the bone-marrow cells of these mice.

4.3.7 *Chlormadinone acetate* (Table 46)

After rat liver cells were incubated with chlormadinone acetate *in vitro*, covalent DNA binding was observed, particularly in cells from females. Unscheduled DNA synthesis was reported in female but not male rat hepatocytes *in vitro* and in male and female human hepatocytes *in vitro* after treatment with chlormadinone acetate. The BdUr density shift assay to determine DNA repair in female and male rat hepatocytes exposed to chlormadinone acetate *in vitro* gave negative results.

No DNA binding was observed in female rat liver *in vivo*, but micronuclei were induced in female rat hepatocytes *in vivo*.

4.3.8 *Gestodene* (Table 46)

Gene mutations were not induced in *S. typhimurium* by gestodene or combinations of gestodene and ethinyloestradiol.

4.3.9 *Megestrol acetate* (Table 46)

Low levels of covalent DNA binding were observed in rat liver cells treated with megestrol acetate *in vitro*, particularly in cells from females. Megestrol acetate induced unscheduled DNA synthesis in rat primary hepatocytes and gave rise to DNA repair in female and male rat liver cells and in human hepatocytes *in vitro*.

Weak covalent DNA binding was observed in female rat liver *in vivo*; however, micronucleus formation was not induced in female rat hepatocytes, and γ-GT-positive foci were not induced in rat liver *in vivo*.

Table 46. Genetic and related effects of other progestogens used in combined oral contraceptives

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chlormadinone acetate				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	–	–	1000 µg/plate	Dayan *et al*. (1980)
DNA repair exclusive of unscheduled DNA synthesis, female and male rat hepatocytes *in vitro*	–	NT	20.3	Topinka *et al*. (1995)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	0.81	Martelli *et al*. (1996a)
Unscheduled DNA synthesis, male rat hepatocytes *in vitro*	–	NT	8.1	Martelli *et al.* (1996a)
Cell transformation, rat liver cells treated *in vivo* scored *in vitro*	–		100 po × 6	Martelli *et al*. (1996b)
Unscheduled DNA synthesis, male and female human hepatocytes *in vitro*	+	NT	0.81	Martelli *et al*. (1996a)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	100	Stenchever *et al*. (1969)
Micronucleus formation, female rat hepatocytes *in vivo*	+		100 po × 1	Martelli *et al*. (1996b)
Binding (covalent) to DNA, female rat hepatocytes *in vitro*	+	NT	1.2	Topinka *et al*. (1995)
Binding (covalent) to DNA, male rat hepatocytes *in vitro*	(+)	NT	1.2	Topinka *et al*. (1995)
Binding (covalent) to DNA, female rat liver *in vivo*	–		100 po × 1	Topinka *et al*. (1995)
Gestodene				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	5 µg/plate	Lang & Reimann (1993)
Gestodene + ethinyloestradiol (2.5 parts + 1 part)				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	200 µg/plate[c,d]	Lang & Reimann (1993)
Megestrol acetate				
DNA repair exclusive of unscheduled DNA synthesis, female and male rat hepatocytes *in vitro*	–	NT	19.3	Topinka *et al*. (1995)
Unscheduled DNA synthesis, female rat hepatocytes *in vitro*	+	NT	1.93	Martelli *et al.* (1996a)

Table 46 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Megestrol acetate (contd)				
Unscheduled DNA synthesis, male rat hepatocytes *in vitro*	–	NT	19.3	Martelli *et al*. (1996a)
γ-Glutamyl transpeptidase-positive foci induction, female Sprague-Dawley rat liver *in vivo*	–		100 po × 6 (weekly)	Martelli *et al*. (1996b)
Unscheduled DNA synthesis, male and female human hepatocytes *in vitro*	+	NT	0.77	Martelli *et al*. (1996a)
Chromosomal aberrations, human lymphocytes *in vitro*	–	NT	10	Stenchever *et al*. (1969)
Micronucleus formation, female rat liver *in vivo*	–		100 po × 1	Martelli *et al*. (1996b)
Binding (covalent) to DNA, female rat hepatocytes *in vitro*	+	NT	1.2	Topinka *et al*. (1995)
Binding (covalent) to DNA, male rat hepatocytes *in vitro*	(+)	NT	1.2	Topinka *et al*. (1995)
Binding (covalent) to DNA, female rat liver *in vivo*	(+)		10 po × 1	Topinka *et al*. (1995)
Norethynodrel				
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Lang & Redmann (1979)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	250 µg/plate	Rao *et al*. (1983)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate[d]	Lang & Reimann (1993)
Inhibition of metabolic cooperation, Chinese hamster V79 cells *in vitro*	(+)	NT	0.75	Yager (1983)
Norethynodrel + 2-acetylaminofluorene (3 µg)				
Salmonella typhimurium TA100, TA1535, TA98, reverse mutation	NT	–	150 µg/plate	Rao *et al*. (1983)

Table 46 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Levonorgestrel				
Salmonella typhimurium TA100,TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	2500 µg/plate	Lang & Redmann (1979)
Salmonella typhimurium TA100,TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	1000 µg/plate	Dayan *et al*. (1980)
Salmonella typhimurium TA100,TA1535, TA1537, TA1538, TA98, reverse mutation	–	–	500 µg/plate	Lang & Reimann (1993)
Drosophila melanogaster, sex-linked recessive lethal mutations	–		3120	Parádi (1981)
Drosophila melanogaster, sex-linked recessive lethal mutations	(+)		5000	Aguiar & Tordecilla (1984)

[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; po, oral; ip, intraperitoneal

[c] Total mixture

[d] Toxicity was observed at higher dose(s)

4.3.10 *Norethynodrel* (Table 46)

Gene mutations were not induced in *S. typhimurium* by norethynodrel, and it did not enhance the mutagenicity of a sub-threshold mutagenic dose of 2-acetylaminofluorene.

4.3.11 *Levonorgestrel* (Table 46)

Gene mutations were not induced in *S. typhimurium* by levonorgestrel. Ethanolic extracts of combinations of levonorgestrel and ethinyloestradiol induced cell transformation in baby rat kidney cells infected with HPV-16 and carrying the Ha-*ras*-1 oncogene (see Table 42).

4.4 Reproductive and prenatal effects

The literature up to 1979 on the developmental effects of sex hormones was reviewed in Volume 21 of the *IARC Monographs* (IARC, 1979). It has been shown in both humans and experimental animals that sex hormones can interfere with normal genital development. The effects observed with synthetic sex hormones are variable, and oestrogenic, androgenic and progestogenic effects may frequently be observed with one chemical, depending on the target tissues and the background levels of natural hormones acting at specific times. The effects on embryofetal development also depend on the relative importance of numerous conditioning factors and are not always easy to predict; however, masculinization of female fetuses and feminization of male fetuses are observed. Effects are found in many organ systems, and genital development, central nervous system development and sexual differentiation may be affected. The timing of exposure relative to embryofetal and postnatal development is critical in determining the type and site of the defect produced.

The literature on the effects of exposure to sex hormones during pregnancy on induction of other types of congenital malformation is much more controversial. Early case reports and epidemiological studies suggested that a wide variety of defects, affecting most organ systems, could be produced. Syndromes such as the VACTERL syndrome were reported, which involves malformations of one or more of the vertebral, anal, cardiac, tracheal, oesophageal, renal and limb systems. Numerous other studies failed to support the suggestion that these defects were related to hormonal treatment.

Three categories of exposure in pregnancy were considered. Accidental exposure to oral contraceptives comprised the major group, with the least convincing evidence for a connection with birth defects. The evidence related to use of hormonal pregnancy tests was a little stronger but still unsubstantiated; the use of such tests was discontinued many years ago. The third category is use of hormones to treat women with pregnancy problems, such as intermittent bleeding, repeated or threatened abortion and luteal failure. In those cases in which the pregnancy is maintained but the fetus has malformations, it is difficult to decide whether the cause was the hormonal treatment or the underlying disease.

Since the last IARC monograph on this subject (IARC, 1979), many papers have been published on the topic of exposure to hormones during pregnancy, and some have been reviewed. Schardein (1980b) reviewed the literature up to that time on the induction

of genital and non-genital defects. He concluded that the commonest association for genital defects was masculinization of females, generally seen as clitoral hypertrophy, with exposure at around week 8–10 of pregnancy; the prevalence was low and the risk was estimated to be about 1% of exposed infants. The evidence for feminization of males was reported to be less convincing. Hypospadia has been the commonest defect reported, but recent analysis of more than 2000 cases of hypospadia has shown no association with maternal use of oral contraceptives (Källén *et al*., 1991). A meta-analysis of pregnancy outcomes after exposure to sex hormones during the first trimester showed no excess of genital malformations (Raman-Wilms *et al*., 1995).

The evidence accumulated since 1979 on the involvement of exposure to sex hormones in other non-genital malformations has been largely in favour of no association. In a short paper, Brent (1994) reviewed some of the reasons why false associations between congenital heart malformations and hormones may have been concluded in the past. These include the grouping of many different types of congenital heart disorder with different causes, inadequate knowledge about the critical times of exposure for specific defects, failure to differentiate between the actions of oestrogens and progestogens, and inclusion in some studies of syndromes with a known high incidence of heart defects. The paper lists 20 reviews on the subject of exposure to hormones and non-genital congenital malformations, none of which found a causal association. It can be concluded that most epidemiological studies do not indicate that progestogens are teratogenic for the cardiovascular system; secular trend data do not support an association between exposure to sex hormones and cardiovascular disease; a very large number of experimental studies show no relationship between exposure to sex hormones and cardiac malformations; there are no sex hormone receptors in developing cardiac tissue; and no consistent syndrome of non-genital defects has been reported. In 1988, as a result of a meeting to review the evidence, the United States Food and Drug Administration removed the warning label on oral contraceptives which had previously stated that exposure during pregnancy could cause cardiac and limb defects (Brent, 1989).

Useful reviews of the data on congenital malformations have been published (Wilson & Brent, 1981; Polednak, 1985; Simpson, 1985; Bracken, 1990; Simpson & Phillips, 1990). Several case–control studies have been conducted that show little or usually no evidence of an association between birth defects and hormonal exposure (Lammer & Cordero, 1986; Hill *et al*., 1988; Ananijevic-Pandey *et al*., 1992; Pradat, 1992; Martínez-Frías *et al*., 1998). In a large cohort study in the United States (Harlap *et al*., 1985a), no increase in the incidence of malformations was found in relation to the use of oral or other methods of contraception. In a study of women in Thailand (Pardthaisong *et al*., 1988), no increase in the incidence of defects of the heart, central nervous system or limbs was found in the offspring of women using oral or injectable contraceptives, but an increased incidence of polysyndactyly and chromosomal anomalies was observed in women who had previously used medroxyprogesterone acetate (Depo Provera). The small numbers of affected children, the long interval between injection of medroxyprogesterone and the conception of the affected offspring and the unrelated nature of the

effects led the authors to conclude that a causal relationship between treatment and effect was unlikely. Overall, the prevalence of major malformations was significantly lower in the oral contraceptive users than in the non-users.

A study of oral contraceptive use in 730 mothers of children with Down syndrome and 1035 mothers of children with other malformations (Lejeune & Prieur, 1979) showed that more of the mothers aged 30–38 years of children with Down syndrome had ceased contraceptive use within the six months prior to conception than in the other group. Three other studies, two of them prospective studies in which data on contraceptive use was obtained before the outcome of the pregnancy was known (Ericson *et al.*, 1983; Källén, 1989), and a smaller case–control study of contraceptive use in the mothers of Down cases and in mothers of normal children (Janerich *et al.*, 1976), found no association between use of oral contraceptives and the subsequent birth of a child with Down syndrome.

A study of chromosomal abnormalities in 33 551 births and abortions after 20 weeks was reported by Harlap *et al*. (1985b). No increased risk was found for women who used oral contraceptives prior to conception or who were still using contraceptives when they became pregnant.

4.4.1 *Ethinyloestradiol*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

A study was carried out with the inbred mouse strain 129SV-S1 C P, which is heterozygous for the *S1* gene shown to affect the development of primordial germ cells, and which has a 7% spontaneous incidence of testicular teratoma. Pregnant mice were injected subcutaneously with 0.02 or 0.2 mg/kg bw ethinyloestradiol in corn oil on days 11 and 12 of gestation (the day a vaginal plug was observed was considered to be day 0), which had been shown previously to be the critical period for induction of teratoma. The male pups were killed at 15 days of age and the testes examined for teratomas. A dose-related increase in the incidence of cryptorchid testes was found, with 4/107 in controls, 10/109 at the low dose and 23/115 at the high dose ($p < 0.0001$ for trend). A small increase was observed in the incidence of teratoma which was neither dose-related nor significant (odds ratio, 2.4; 95% CI, 0.7–9.1) for the pooled data. The authors suggested that different mechanisms are involved in the etiology of cryptorchidism and teratoma, although both may be induced by oestrogen stimulation (Walker *et al.*, 1990).

Oral administration of 0, 0.02, 0.2 or 2.0 mg/kg bw ethinyloestradiol in olive oil to pregnant Jcl:ICR mice (8–15 litters per group) on days 11–17 of gestation (the presence of a vaginal plug being considered day 0) resulted in a dose-dependent increase in the incidences of ovotestis and cryptorchidism in males, with persistent Müllerian and Wolffian ducts, when fetuses were examined on day 18 of gestation. Leydig-cell proliferation was also seen at the two higher doses with alterations in cellular morphology

suggestive of preneoplastic changes. Female fetuses showed ovarian hypoplasia, with decreased numbers of primordial follicles and increased follicular degeneration (Yasuda *et al.*, 1985, 1986).

In a later study (Yasuda *et al*., 1988), pregnant ICR mice were given a daily dose of 0.02 or 0.2 mg/kg bw ethinyloestradiol in olive oil orally on days 11–17 of gestation. Dams at the low dose were allowed to deliver, and their male offspring were reared to maturity (20–22 months). The animals given the high dose had no live offspring. At day 18, male fetuses were recovered from mice at each dose, and the concentrations of testosterone and oestradiol were measured in testes. Ethinyloestradiol treatment significantly ($p < 0.001$) reduced the concentrations of testosterone in the testes of 18-day-old fetuses, from a mean of 5.21 ± 0.13 pg/testis in the controls ($n = 32$ testes) to 0.89 ± 0.11 pg/testis ($n = 30$) at the low dose and 0.40 ± 0.13 pg/testis ($n = 52$) at the high dose. Treatment at the high dose also reduced the oestradiol levels in the testis from 78.80 ± 0.49 pg/testis in controls to 42.25 ± 1.56 pg/testis ($p < 0.05$). The testosterone:oestradiol ratios were reduced from 1:15 in controls, to 1:77 at the low dose and 1:106 at the high dose. At 20–22 months, the offspring from the low-dose group were killed, and the testes and epididymides were removed, examined histologically and analysed for testosterone and oestradiol. There were significant ($p < 0.05$) increases in the frequency of testicular atrophy, Leydig-cell hyperplasia and absence of epididymal sperm in the treated compared with the control mice. The testosterone concentration was significantly decreased, from 84 to 28 ng/testis ($p < 0.01$), and the oestradiol level was increased, from 356 to 564 ng/testis ($p < 0.05$). The authors suggested that the dramatic fall in the testosterone:oestradiol ratio in the fetal testis results from greatly increased conversion of testosterone to oestradiol by the Leydig cells, so that insufficient amounts of testosterone are available for regulation of pro-spermatogenesis in the developing testis and spermatogenesis, eventually resulting in sterility.

4.4.2 *Mestranol*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Daily oral administration of 0.05 or 0.2 mg/kg bw mestranol to female NMRI mice and $AFAF_1$ hybrid mice, on days 4–8 after mating, inhibited implantation and increased the number of resorptions. The fetuses of NMRI mice had accessory ribs. Treatment on days 7–11 with doses of 0.1–0.2 mg/kg bw induced abortions but had no teratogenic effects (Heinecke & Klaus, 1975).

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

Charles River rats received daily oral doses of 0.05–0.2 mg/kg bw Enovid (2.5 mg norethynodrel, 0.1 mg mestranol) or 0.01–0.1 mg/kg bw mestranol throughout gestation

and for 21 days after parturition. The highest dose of mestranol terminated a significant percentage of pregnancies. No genital defects were observed in surviving male offspring, but female offspring showed enlarged urethral papillae and prematurely opened vaginas, even at lower doses of mestranol. The fertility of female offspring of rats treated with 0.1 mg Enovid was impaired by 55%. Higher doses of Enovid or 0.02 mg mestranol induced complete sterility in female offspring; examination of the ovaries showed no corpora lutea and follicles of reduced size (Saunders, 1967).

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

In rabbits, pregnancy was terminated by daily oral doses of > 0.02 mg/kg bw mestranol on days 0–28 or 0.05 mg/kg bw [lower doses not tested] on days 10–28 of gestation and by daily subcutaneous doses of 0.005 mg/kg bw on days 0–28 or > 0.002 mg/kg bw on days 10–28. Doses that did not terminate pregnancy had no effects on litter size or the weights of the offspring (Saunders & Elton, 1967).

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

When adult beagle bitches received 5 mg/kg bw mestranol orally on day 6 or 21 of gestation, the embryonic losses, based on corpora lutea counts, were 95.5% with early treatment and 67.3% with late mestranol treatment in comparison with 34.5% in controls. The surviving offspring appeared normal (Kennelly, 1969).

4.4.3 *Chlormadinone acetate*

(*a*) *Humans*

No increase in the incidence of malformations was reported in 305 infants whose mothers had been exposed to chlormadinone and oestrogens during pregnancy (Goldzieher *et al.*, 1968; Lepage & Gueguen, 1968; Larsson-Cohn, 1970).

(*b*) *Experimental systems*

Groups of 8–12 male Sprague-Dawley Crl:CD(SD)Br rats were castrated and injected immediately thereafter twice daily for 14 days with one of a number of synthetic progestogens, including chlormadinone acetate, used in the treatment of prostate cancer. Controls received the vehicle, 1% gelatine in 0.9% saline. Dihydrotestosterone was injected at a dose of 150 μg twice daily for 14 days as a positive control. All animals were killed on the morning after the last day of treatment, and the ventral prostate and adrenals were removed and weighed; furthermore, the prostatic content of ornithine decarboxylase was

measured, as it is considered to be a highly specific, sensitive marker of androgenic activity in the prostate. Dihydrotestosterone increased the ventral prostate weight to 43% above that of castrated controls. Chlormadinone acetate was less potent than dihydrotestosterone but caused significant increases in prostate weight, by about 22% at 3 mg and 36% at 10 mg per injection. Whereas dihydrotestosterone caused a 14-fold increase in ornithine decarboxylase activity in the prostate, chlormadinone acetate caused a 5.3-fold increase at 3 mg and an 11.8-fold increase at 10 mg. Chlormadinone acetate thus has weak but significant androgenic activity in the rat ventral prostate (Labrie *et al*., 1987).

Pregnant Wistar rats were given 1, 5 or 10 mg chlormadinone acetate orally once a day for four days on days 17–20 of pregnancy, and the fetuses were removed on day 21. After fixation, histological sections of the pelvic region were examined and the uro-vaginal septum length measured. Masculinization of female fetuses was not observed, in the absence of change in the development of the urogenital septum (Kawashima *et al*., 1977).

Chlormadinone acetate given orally at doses of 1–50 mg/kg bw on days 8–15 of pregnancy to Japanese ddS and CF1 mice caused a significant increase in the incidence of cleft palate. A dose of 10 mg/kg bw, but not of 1 or 3 mg/kg bw, given orally on days 8–20 of gestation to Japanese albino rabbits increased the incidence of cleft palate, abdominal wall defects and wrist contractures (Takano *et al*., 1966).

4.4.4 *Cyproterone acetate*

(*a*) *Humans*

Two men treated for prostatic carcinoma with high oral doses of cyproterone acetate (2×100 mg per day for seven months) had widespread testicular damage, with disappearance of Sertoli cells and spermatogonia and involution of Leydig cells (Re *et al*., 1979). When cyproterone acetate was given at doses of 5–10 mg per day as a contraceptive in several other studies, decreased sperm concentration and motility and increased abnormal morphology, with—except in one study—decreased power to penetrate the mucus, were observed. Variable effects on plasma gonadotrophins and testosterone levels have been reported (Føgh *et al*., 1979; Roy & Chatterjee, 1979; Moltz *et al*., 1980; Wang & Yeung, 1980). Doses of 50–100 mg cyproterone acetate per day combined with testosterone induced azoospermia and decreased testis size in each of 10 subjects. All of the effects were reversible (Meriggiola *et al*., 1996).

In women, ovulation is inhibited by 2 mg per day cyproterone acetate when given in combination with 35 µg ethinyloestradiol (Spona *et al*., 1986). No controlled studies on developmental effects are available.

(*b*) *Experimental systems*

Cyproterone acetate has been reported to have both androgenic and anti-androgenic activity in experimental animals (see also section 4.2.5).

Groups of 8–12 male Sprague-Dawley Crl:CD(SD)Br rats were castrated and then injected twice daily for 14 days with one of a number of synthetic progestogens,

including cyproterone acetate. Treatment was begun one day after castration. Controls were injected with the vehicle, 1% gelatine in 0.9% saline. Dihydrotestosterone was injected twice daily at a dose of 125 µg for 14 days as a positive control. All animals were killed on the morning after the last day of treatment, and the ventral prostate and adrenals were removed and weighed. Dihydrotestosterone increased the ventral prostate weight to approximately five time that of castrated controls. Cyproterone acetate was less potent than dihydrotestosterone but caused a significant increase in prostate weight, by 60% at a dose of 5 mg per injection twice daily. Cyproterone acetate thus has weak but significant androgenic activity in the rat ventral prostate (Poyet & Labrie, 1985).

Anti-androgenic effects have also been reported. Groups of 10 albino Wistar mice were treated subcutaneously with vehicle alone or with 1 mg per animal per day of cyproterone acetate for seven days. The animals were killed on the eighth day and the testes removed for histological and morphometric examination. Treatment caused marked decreases in the volume, surface area and length of the seminiferous tubules, and it inhibited spermatogenesis (Umapathy & Rai, 1982).

The anti-androgenic activity of cyproterone and cyproterone acetate has been shown in mice (Umapathy & Rai, 1982; Homady *et al*., 1986), rats (El Etreby *et al*. 1987), guinea-pigs (Tam *et al*., 1985), ferrets (Kästner & Apfelbach, 1987), goats (Panda & Jindal, 1982; Kumar & Panda, 1983) and monkeys (Lohiya *et al*., 1987; Kaur *et al*., 1990, 1992). The effects observed include decreased sexual behaviour and inter-male aggression, reduced weights of testis and inhibition of spermatogenesis. Fertility can be reduced by low doses of cyproterone acetate even in the absence of reduced spermatogenesis (Rastogi *et al*., 1980), which may be due to an effect on epididymal processing of sperm. In addition to reduced secretion of testosterone and luteinizing hormone (Clos *et al*., 1988), there is also evidence that translocation of the testosterone receptor to the nucleus may be affected (Brinkmann *et al*., 1983).

In rodents, cyproterone acetate has oestrogenic properties, increasing uterine weight and causing vaginal cornification in ovariectomized rats (Arya *et al.*, 1979). When the compound was administered to pregnant rats, feminization of male fetuses, including development of a vagina, has been reported (Neumann *et al.*, 1966; Forsberg & Jacobsohn, 1969).

Treatment of NMRI mice with doses of 5–900 mg/kg bw cyproterone acetate subcutaneously on day 2 of gestation (the day a vaginal plug was observed was considered to be day 0) or with 30 mg/kg bw on single days of pregnancy from day 1 to 12, resulted in a clear dose- and time-related increase in the incidences of cleft palate and of urinary tract and respiratory tract malformations, with up to 64% of fetuses affected after the single 900-mg/kg bw dose (Eibs *et al.*, 1982). Administration in late pregnancy or in the neonatal period can produce permanent changes in neuroendocrine and sexual function of rats. Groups of 15 male and 16 female offspring of rats treated subcutaneously with 1 mg cyproterone acetate on days 15–20 of gestation [strain and number of pregnant animals not specified] were studied when two to three months of age. The weight of the brain was reduced in animals of each sex and the weight of the testis in males. Cell

density in the ventromedial nucleus of the hypothalamus was increased in males in comparison with females. The prolactin concentration in the pituitary was increased in animals of each sex (Rossi *et al.*, 1991). Groups of newborn male Swiss CD1 mice were injected with cyproterone acetate on days 1–10 (200 μg/day), 11–20 (400 μg), 21–30 (800 μg) or 31–40 (1 mg) of age. In all groups, there was an immediate reduction in the weights of the testis, epididymis, vas deferens, preputial gland and seminal vesicles relative to body weight. This reduction in the weight of the accessory sex organs was permanent in animals injected up to day 20 of age but was reversible in animals treated after day 20. In mice injected on days 1–10 of age, marked, permanent infertility was observed when they became adults, but spermatogenesis, the androgen concentration in plasma and sexual behaviour were not affected. The infertility appeared to be due to failure of sperm in the epididymis to mature (Jean-Faucher *et al.*, 1985).

4.4.5 *Levonorgestrel* (see also the monograph on 'Hormonal contraceptives, progestogens only', section 4.4.2)

(*a*) *Humans*

No relevant data were available to the Working Group.

(*b*) *Experimental systems*

Groups of six pregnant rats [strain not specified] were ovariectomized on day 8 of gestation and treated subcutaneously with doses of 0.01–0.3 mg levonorgestrel daily on days 8–21 of gestation; at the same time they received an injection of 1.0 μg oestrone. The rats were killed on day 22 to measure maintenance of pregnancy; satisfactory maintenance was achieved with 0.1 and 0.3 mg levonorgestrel. Immature castrated male rats [age not specified] were treated subcutaneously daily for 13 days with doses of 0.1–3 mg levonorgestrel and were killed the day after the last treatment; the weights of the prostate and seminal vesicles were measured. Levonorgestrel showed androgenic activity, as judged from the increased weight of both tissues (Kuhnz & Beier, 1994).

Groups of 10–12 Prob:WNZ New Zealand white rabbits were mated with Prob:KAL Californian rabbits, producing hybrid fetuses; the day of mating was called day 1. The animals were treated with 0.5 mg/kg bw levonorgestrel in sesame oil by gavage on days 5–25 of gestation and were killed on day 21. [The Working Group concluded that the animals must have been killed after day 25, but the fetal body weights were very low for full-term offspring.] The fetuses were examined macroscopically, and half of them were sliced for visceral examination and the other half examined for skeletal and cartilage malformations. No adverse effect of treatment on pregnancy rate, number of implantations, number of resorptions or number of live or dead fetuses was observed. The female fetal body weight at term was slightly but significantly reduced (15.2 ± 0.61 g versus 17.4 ± 0.74 g in the sesame oil control group). No malformations were observed (Heinecke & Kohler, 1983).

4.4.6 *Lynoestrenol*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Groups of 14–20 inseminated belted Dutch rabbits were given lynoestrenol at a dose of 0, 0.1, 0.5 or 2.5 mg/kg bw orally on days 6–18 of gestation (the day of insemination being considered day 0). The does were killed on day 29 for examination of the fetuses. Increased post-implantation loss was observed at all doses: 1.3% in controls, 13.2% at the low dose, 17.1% at the intermediate dose and 90.9% at the high dose. A wide range of malformations of the brain and eye was observed in all groups, affecting 31–50% of fetuses and only 12% of controls. Cardiovascular malformations were observed in 2, 5 and 20% of fetuses, respectively, at the three doses. No masculinization of female fetuses was observed. In a second experiment with groups of five to six belted Dutch rabbits, lynoestrenol was administered at a dose of 0, 0.1 or 0.5 mg/kg bw on days 6–18 of gestation. The does were allowed to deliver naturally and raise their pups to four weeks of age. At the higher dose, the litter size was decreased and there was clear evidence of central nervous system abnormalities in surviving pups, with ataxia, disorientation, posterior paralysis and rotation of one or both hindlimbs. Anophthalmia and microphthalmia were seen in 3/10 pups at the high dose. Histological examination of the central nervous system revealed pathological changes in the ventral horns of the spinal cord, with a marked reduction in the number of neurons (Sannes *et al.*, 1983).

Pregnant Wistar rats were given 1 or 5 mg lynoestrenol orally once a day for four days on days 17–20 of gestation, and the fetuses were removed on day 21. After fixation, histological sections of the pelvic region were examined and the urovaginal septum length measured. Masculinization of female fetuses was seen at 5 mg, as evidenced by decreased development of the urogenital system (Kawashima *et al.*, 1977).

4.4.7 *Megestrol acetate*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Megestrol acetate has some androgenic activity in rats (Poyet & Labrie, 1985; Labrie *et al.*, 1987). In the study of Labrie *et al.* (1987), described in section 4.4.3, the positive control dihydrotestosterone increased the ventral prostate weight to 43% above that of castrated controls. Megestrol acetate was less potent, but caused significant increases in prostate weight, by about 35% at 3 mg and 59% at 10 mg per injection. Whereas dihydrotestosterone caused a 14-fold increase in the activity of orthinine decarboxylase in the prostate, megestrol acetate caused an 11-fold increase at 3 mg and a 13-fold increase at 10 mg. Megestrol acetate thus has weak but significant androgenic activity in the rat ventral prostate.

In the study of Kawashima *et al*. (1977) described in section 4.4.3, megestrol acetate at a dose of 5 mg induced masculinization of female fetuses.

4.4.8 *Norethisterone*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Ten groups of three timed-mated pregnant rhesus monkeys (*Macaca mulatta*) were treated with Norlestrin (norethisterone acetate, 2.5 mg and ethinyloestradiol, 0.05 mg per tablet) orally at a dose of 5, 10, 25 or 50 mg per monkey per day (on the basis of the norethisterone acetate content). [These doses are equivalent to 0.83, 1.67, 4.17 and 8.33 mg/kg bw daily on the basis of the information in the paper that the doses are equivalent to 20, 40, 100 and 200 times the human contraceptive dose.] The animals at the three lower doses were treated daily during early (days 21–35) or late (days 33–46) organogenesis or throughout (days 21–46) organogenesis and were allowed to deliver at term (165 days' gestation). Animals at 50 mg/day were dosed on days 21–35 only and delivered by caesarean section on day 50 of gestation for serial sectioning and histological examination of the fetuses. Of 26 animals that were allowed to deliver at term, 16 delivered morphologically normal infants (nine male, seven female), eight aborted, and two had stillbirths, a rate of 7.4%, which was not different from that of controls. The overall pre-natal mortality rate was higher in the treated animals (10/26, 38.5%) than in the control colony (55/262, 21%). Two of nine animals at each of the 5- and 10-mg doses aborted, in comparison with 4/9 at 25 mg/day. Among those treated on days 21–35 or 21–46 of gestation, six (37.5%) aborted, in comparison with 2/9 (22.2%) of those treated later in organogenesis, on days 33–46. Only three aborted embryos were recovered, and all three were much smaller than expected for their gestational age; however, they were too severely autolysed for further examination. No morphological or histological abnormalities were detected in fetuses recovered on day 50 of gestation from females at 50 mg. The infants were followed up for a maximum of 2.5 years, and all three animals that died and the five that were sacrificed were necropsied and examined histopathologically. No malformations or significant lesions were found. Detailed physical examination of the live infants showed no morphological changes, and the body weights and other measures were no different from those of controls. The serum oestrogen and progesterone concentrations of females treated with 25 mg on days 21–35 of gestation were measured daily on days 26–44 by immunoassay. The oestrogen concentrations were significantly lower ($p < 0.05$) than those of controls, but the progesterone levels were similar. As it has been shown in other studies that monkeys can be ovariectomized on day 23 of gestation without fetal loss, although the plasma oestrogen concentration falls almost to zero, the authors suggested that the reduction in oestrogen concentration was not the cause of the observed pre-natal deaths. They proposed that Norlestrin has a direct embryolethal effect. The normal progesterone concentrations indicate that

placental synthesis of progesterone is unaffected. The authors also pointed out that as the periods of treatment in this study did not extend into the early fetal period after day 46, when external genital development occurs in the rhesus monkey, genital malformations would not be expected to occur (Prahalada & Hendrickx, 1983).

Eight of the offspring in the study described above from females dosed with 5, 10 or 25 mg Norlestrin were subjected to limited behavioural examination up to 11 months of age. No serious deficiencies in the regulation of activity, motor maturity, manual dexterity or discrimination learning were observed at three to five months of age. Age-appropriate sex-differentiated behaviour was seen at five and 11 months of age. The authors noted, however, that Norlestrin was not given during the period of sexual differentiation of the brain in rhesus monkeys (Golub *et al.*, 1983).

In the study of Kawashima *et al.* (1977), described in section 4.4.3, norethisterone caused masculinization of female fetuses at doses of 5 or 10 mg but not at 1 mg.

4.4.9 *Norethynodrel*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

Mice given 10 mg/kg bw norethynodrel (with 2% mestranol) orally, daily on days 8–15 of gestation, had a very high rate of embryonic death (98.9%), but not when the dose was given on days 14–17 of gestation (Takano *et al.*, 1966).

In mice given 0.2–2.4 mg/kg bw norethynodrel or its 3-hydroxy metabolite as an oral or parenteral dose, either singly or on three consecutive days between days 6 and 16 of gestation, congenital anomalies were observed in near-term fetuses. A single dose of 1.2 mg/kg bw norethynodrel or its metabolite given between days 8 and 16 of gestation produced congenital abnormalities (retarded development, hydrocephalus, club-foot and minor skeletal anomalies) in 10–30% of offspring (Andrew *et al.*, 1972).

Mice that received a single subcutaneous injection of 0.1 mg/kg bw norethynodrel in combination with 1.5 μg/kg mestranol (Enovid) on day 7, 10, 12, 15 or 17 of gestation had normal fetuses, with no external or internal genital anomalies; however, treatment on day 10 of gestation led to a significant decrease in aggressive behaviour of male offspring later in life (Abbatiello & Scudder, 1970).

Oral administration of norethynodrel or its metabolites, 17α-ethynyl-oestr-5(10)-ene-3α,17β-diol and 17α-ethynyl-oestr-5(10)-ene-3β,17β-diol, at a daily dose of 0.15, 0.3 or 0.6 mg/kg bw on days 8–10 or 11–13 of gestation resulted in increased numbers of resorptions and intrauterine deaths on days 11–13. The teratogenic effects included exencephaly after treatment during days 8–10 and hydrocephalus and partial cryptorchidism after treatment on days 11–13. The most effective agent was 17α-ethynyl-oestr-5(10)-ene-3β,17β-diol (Gidley *et al.*, 1970).

Subcutaneous administration of 0.5 or 1 mg/kg bw norethynodrel to pregnant rats on days 2–4 of gestation terminated a significant number of pregnancies (Saunders, 1965).

Subcutaneous administration of 0.083–2.5 mg/kg bw per day norethynodrel to rats on days 10–17 of gestation induced 100% fetal resorptions, whereas a dose of 0.0083 mg/kg bw per day induced 42% resorptions; no virilizing effect was observed in females, but in males the weight of the testes was significantly lowered and the descent of testes was delayed in 35.5% of animals (Roy & Kar, 1967).

In the study of Tuchmann-Duplessis and Mercier-Parot (1972), described in section 4.4.2, complete fetal resorption occurred rapidly in 30 animals in which treatment with Enidrel (mestranol/norethynodrel) was continued for the first 15 days of gestation; however, after two weeks without treatment, the fertility rates and litter sizes were normal. In the 30 animals in which treatment was discontinued, the fertility and pre- and post-natal development of the offspring were normal. No teratogenic effects were observed.

Subcutaneous injection of 1 mg norethynodrel (Enovid) to guinea-pigs daily on days 18–60 of gestation prevented pregnancy (Foote *et al.*, 1968).

4.4.10 *Norgestimate*

(*a*) *Humans*

No data were available to the Working Group.

(*b*) *Experimental systems*

In the study of Kuhnz and Beier (1994), described in section 4.4.5, groups of rats were given doses of 0.03–1 mg/day norgestimate. Satisfactory maintenance of pregnancy was achieved with 0.3 and 1 mg. In immature male rats treated with 1–30 mg norgestimate, the weights of the prostate and seminal vesicles were increased, indicating androgenic activity, but norgestimate was less active than levonorgestrel. The blood concentrations of levonorgestrel were measured in animals treated with levonorgestrel or norgestimate, as levonorgestrel is the major metabolite of norgestimate. Both the pregnancy maintenance and the androgenic activity could be accounted for by the concentrations of levonorgestrel produced as a metabolite.

4.4.11 *Norgestrel*

(*a*) *Humans*

As the active isomer of norgestrel is levonorgestrel, the reader is referred to section 4.4.5 of this monograph.

(*b*) *Experimental systems*

Mature female $B3C6F_1$ mice were superovulated with serum gonadotropin from pregnant mares and 48 h later by human chorionic gonadotrophin; they were then mated overnight with males known to be fertile. The females were killed, and the fertilized pre-embryos were collected from the fallopian tubes 24 h (one-cell stage) or 48 h (2–4-cell stage) after the injection of human chorionic gonadotrophin. The pre-embryos were then cultured for up to 72 h in the absence or presence of 4 ng/mL (dl)-norgestrel, which is the peak plasma concentration of levonorgestrel found in women who use norgestrel as

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

5. Summary of Data Reported and Evaluation

5.1 Exposure

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

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

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

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

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

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

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

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

5.2 Human carcinogenicity

Breast cancer

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

Cervical cancer

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

Endometrial cancer

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

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

Ovarian cancer

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

Cancers of the liver and gall-bladder

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

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

Colorectal cancer

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

Cutaneous malignant melanoma

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

Thyroid cancer

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

5.3 Carcinogenicity in experimental animals

Oestrogen–progestogen combinations

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

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

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

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

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

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

Oestrogens

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

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

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

Ethinyloestradiol increased the incidence of cervical tumours in female mice.

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

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

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

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

Progestogens

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

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

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

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

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

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

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

5.4 Other relevant data

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

5.5 Evaluation

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

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

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

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

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

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

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

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

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

Overall evaluation

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

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

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