COMBINED ESTROGEN–PROGESTOGEN CONTRACEPTIVES

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These substances were considered by a previous Working Group, in June 1998 (IARC, 1999), under the title 'Oral contraceptives, combined'. Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Introduction

Combined hormonal contraceptives consist of an estrogen and a progestogen, and act primarily by preventing ovulation through the inhibition of follicle-stimulating hormone and luteinizing hormone. The progestogen component also renders the cervical mucus relatively impenetrable to sperm and reduces the receptivity of the endometrium to implantation. These mechanisms render combined hormonal contraceptives very effective in the prevention of pregnancy. Annual failure rates vary between 0.02% (two per 10 000 women/year) when full adherence to instructions for use is assumed (Ketting, 1988) and 5% for typical use (Fu *et al.*, 1999).

A variety of innovations have been developed since combined hormonal contraceptives were first made available in the late 1950s, but not all of these have proved valuable in practice. Changes in drug components, doses used and the temporal sequencing of exposure to drugs have incorporated new technologies and responded to suggested risks. While regional variations in use are abundant, the dominant trends have been towards less androgenic progestogens, lower doses of estrogen and progestogen, the near abandonment of hormonal contraceptives with an estrogen-only phase, a proliferation of different product formulations and the continuing development of novel delivery systems.

In combined hormonal contraception, ethinylestradiol is the most common estrogen, although other are used occasionally. A variety of progestogens is available and these differ in their properties with regard to progestogenic and androgenic characteristics. The estrogen and progestogen contained in combined hormonal contraceptives are usually given in a monthly cycle, and a variety of regimens ensure that the doses of the two constituents

produce menstrual cycling. In general, estrogen and progestogen are taken in combination for 21 days followed by 7 drug-free days (often placebo tablets) during which time withdrawal bleeding usually occurs. Other cyclic schedules may be used to reduce or eliminate menses. A constant combination of estrogen and progestogen doses may be used (monophasic) or the doses of progestogen and (less often) estrogen may vary in two (biphasic) or three (triphasic) phases. While oral administration predominates, combined hormonal contraceptives can also be administered by injection, a transdermal patch or a transvaginal device.

Although the primary indication of these medications is to prevent pregnancy through regular use, they are also used to regulate menstrual disorders, to treat acne vulgaris or for emergency contraception. Worldwide, more than 100 million women use combined hormonal contraceptives. While their use is more common in developed countries, substantial consumption also occurs in the developing world. Recent trends suggest that overall use has continued to increase slowly in some regions, while it has remained constant in others. The demographic and social characteristics of combined hormonal contraception users are known to differ from those of non-users of these drugs.

1.2 Historical overview

Researchers in the late nineteenth century 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 similar extracts might act as a contraceptive (IARC, 1999; Fraser, 2000).

Three estrogens were identified in the late 1920s and 1930s — estrone, estriol and estradiol. Progesterone was identified in 1929 and was crystallized in 1934. An oral equivalent of progesterone was not available until 1941, when diosgenin was synthesized from extracts of the Mexican yam. Further experimentation yielded the synthesis of norethisterone (known as norethindrone in the USA) in 1951 and norethynodrel in 1952. These compounds were named progestogens (or progestins) due to their progesterone-like properties (IARC, 1999; Fraser, 2000; Junod & Marks, 2002).

In the early 1950s, the combination of estrogen and progestogen was tested as a treatment for infertility, and it was noted that women who took this combined formulation did not ovulate. In 1956, during clinical trials of oral norethynodrel (a progestogen) as a contraceptive, it was found that preparations that contained mestranol (an estrogen) as a contaminant were more effective in suppressing ovulation than those that contained pure norethynodrel. In 1957, the combination of mestranol and norethynodrel was approved for use in the USA for the regulation of menstruation. Even before this combination was approved as a contraceptive in the USA in 1960, it was already being used for such purposes by 0.5 million women. In the same year, it became available in the United Kingdom. Diffusion of this and a second combined hormonal contraceptive formulation (mestranol and norethisterone) to continental Europe and Latin America occurred somewhat later in 1964–68. By the early 1970s, over 25% of women of child-bearing age in many developed

countries were using combined hormonal contraceptives (IARC, 1999; Fraser, 2000; Junod & Marks, 2002; Shampo & Kyle, 2004).

The doses of combined hormonal contraceptives during this early period were 150 μg mestranol and 9.35 mg norethynodrel (Enovid in 1957), but quickly declined to 100 μg mestranol and 2 mg norethisterone (Ortho-Novum in 1964). Doses were further reduced to 50 μg estrogen as confirmation was received that low-dose formulations remained effective with a consequent reduction in adverse effects that had tended to limit continued use. The ease of use, efficacy and reversibility of hormonal contraceptives, as well as changing sexual behaviours and new expectations regarding the regulation of fertility, contributed to the rapid increase in combined hormonal contraceptive use in the 1960s (IARC, 1999; Junod & Marks, 2002).

The upward trend in the use of combined hormonal contraceptives came to a temporary halt in the early 1970s when adverse events associated with their use were highlighted, particularly in women who smoked cigarettes (WHO, 1995). While a variety of side-effects and a risk for thromboembolic events had been recognized earlier, new reports also focused on the risk for cardiovascular disease (Fraser, 2000). As a result, use of combined hormonal contraceptives declined substantially in most developed nations throughout the 1970s. Partly in response to these concerns, a new generation of combined hormonal contraceptives was developed that featured lower doses of estrogen (30 and 35 μg) and newer, more potent progestogens.

Increased use of combined hormonal contraceptives resumed in 1979–81 in many countries, particularly in the light of studies that suggested their relative safety and potential benefits on some outcomes, including reductions in rates of ovarian and endometrial cancer rates (Burkman *et al.*, 2004). At this time, use of combined hormonal contraceptives also increased in many countries in Asia, Africa and the Middle East, facilitated by international aid programmes that were aimed at alleviating the economic consequences of high rates of fertility (IARC, 1999).

At the same time, dose schedules were also modified and refined. With the introduction of biphasic (1982) and triphasic (1984) combined hormonal contraceptives, doses of progestogen were modulated in a manner thought to mimic physiological patterns, although the objective benefits are subject to debate (Van Vliet *et al.*, 2006a,b,c). The previous practice of sequential exposure to estrogen only, followed by combined exposure to estrogen and progestogen, was abandoned after it was found to be associated with an increased risk for endometrial cancer (IARC, 1999).

Further modifications have been made more recently through the continued development of other progestogens, the use of even lower doses of estrogen and the use of alternative dose schedules. Newer progestogens, such as spironolactone-derived drospirenone and more potent and less androgenic gonanes (desogestrel, gestodene), became more common. These formulations were partly aimed at reducing androgenic side-effects such as hirsutism and weight gain. Estrogen doses were reduced to 20 μg and then 15 μg. These low doses may be unsatisfactory for many women because of breakthrough bleeding and they require stricter adherence to instructions for use in order to be effective (Gallo *et al.*,

2004). Other recent innovations in combined hormonal contraception include devices for transvaginal and transdermal administration, variations in length of schedule (both shorter and longer cycles) and combined injectable formulations.

The increase in the use of combined hormonal contraceptives appeared to diminish in the mid-1990s (IMS Health, 2005), possibly due to renewed concerns regarding adverse effects and the growth of alternative contraceptive technologies (e.g. progestogen-only contraception) in developed countries. Similar declining increases in developing countries may reflect a shift towards greater use of other longer-term contraception, including sterilization, injections of progestogen and intrauterine devices (United Nations, 2004a).

In the 1990s, concerns about potential risks of combined hormonal contraceptives for cardiovascular disease (Hannaford *et al.*, 1994) and thromboembolic events persisted. In addition, the risk for breast cancer, which had been a concern since the introduction of hormonal contraceptives, was also re-emphasized (Collaborative Group on Hormonal Factors in Breast Cancer, 1996a,b). Specific concerns were also raised about the increased incidence of thromboembolism associated with progestogens such as gestodene and desogestrel (Jick *et al.*, 1995; WHO, 1995). In spite of these qualms, the effectiveness, ease of use and the risk profile of combined hormonal contraceptives suggest that they will continue to be used to a significant extent in the future. As in the past, the nature of the exposure associated with the components of combined hormonal contraception will probably continue to evolve.

1.3 Preparations of combined hormonal contraceptives

A plethora of products is available for use in combined hormonal contraceptives. Products that are currently available differ in a number of important aspects, including the estrogen compound used and its dose, the progestogen used, the schedule of exposure to the drugs and the route of administration. In addition, identical formulations may carry different brand names in different countries or even within the same country. These products and their ingredients are presented in Annexes 1–3.

The most common estrogen in combined hormonal contraceptives is ethinylestradiol. Over time, other estrogens have been used, including initially mestranol (a pro-drug of ethinylestradiol) and, more recently, estradiol. In the early development of combined hormonal contraceptives, doses of estrogen in the range of 100–150 μg were commonly used. Contemporary combined hormonal contraceptives may be classified by estrogen dose into 'high-dose' (≥ 50 μg), 'moderate-dose' (30–35 μg) and 'low-dose' (15–20 μg).

A variety of progestogens is used in combined hormonal contraceptives. Currently, they are often distinguished as 'first-generation' estranes (such as norethynodrel or norethisterone), 'second-generation' gonanes (such as levonorgestrel or norgestimate), 'third-generation' gonanes (gestodene and desogestrel) and 'fourth-generation' drospirenone. An additional class of progestogens, the pregnanes (e.g. cyproterone and chlormadinone), may also be used. Estranes are highly androgenic, while pregnanes and drospirenone have antiandrogenic activity. The later gonanes are less androgenic than the earlier compounds in

that series. Lower androgenic activity minimizes androgenic side-effects such as acne, hirsutism, nausea and lipid changes. The affinity of individual progestogens for progesterone receptors varies considerably and determines the daily doses required to produce endometrial differentiation. Drospirenone has the lowest affinity (typical daily dose, 3 mg), while the later gonanes have the greatest affinity (0.05–0.15 mg daily dose) (Hammond *et al.*, 2001).

The schedule by which exposure to the drugs occurs may also vary. Most commonly, a constant combination of estrogen and progestogen is used for 3 weeks of a 4-week cycle (monophasic). The doses of progestogen and (less often) estrogen may vary in two (biphasic) or three (triphasic) phases followed by a drug-free phase. While multiphasic schedules seek to mimic physiological variations in exposure to hormones, they may not produce objective benefits over monophasic schedules (Van Vliet *et al.*, 2006a,b). Sequential exposure regimens that used prolonged exposure to estrogen alone are no longer used (IARC, 1999), but a short, 5-day, estrogen-only sequence has been re-introduced. Cycle lengths shorter and longer than 4 weeks may be used with the aim of limiting the duration of menses or eliminating menses altogether (Sulak, 2004). One-day-only use of hormones may be used for emergency contraception.

While oral administration predominates in combined hormonal contraception, the drugs also can be provided by injection, transdermal patch or transvaginal device. Injection of an estrogen and progestogen was used early in the development of hormonal contraception and is still available. Innovations in drug delivery have generated transdermal patches and a vaginal device.

The vast array of products available allows combined hormonal contraception to be tailored to the specific needs and preferences of individual women. While some of the newer products may offer advantages over the older ones, differences in adverse effects and effectiveness are not clear. [In addition, the proliferation of products also represents market differentiation in a large and profitable, but competitive market.]

It is important to recognize that many products are relatively new to the market, particularly those that provide newer progestogens. These, together with products that are currently under development, create a challenge for the evaluation of long-term risk from this class of pharmaceuticals.

1.4 Patterns of use

This section includes the indications of combined hormonal contraceptives, their current prevalence of use globally and trends in the use of these preparations. The characteristics of women who use combined hormonal contraception are also described. Most information on patterns of use of combined hormonal contraceptives is limited to oral forms, and does not include other routes of exposure except for progestogen-only formulations. However, these non-oral forms are generally much less common and information on oral use provides a reasonable proxy for all combined hormonal contraceptive use.

1.4.1 *Prevalence of use*

Based on a compilation of data sources, Blackburn *et al.* (2000) concluded that approximately 100 million women were current users of combined hormonal contraceptives worldwide and, outside of India and China, which have a very low prevalence of use, that 32% of married women in the developing world had ever used them. While variations in their use were enormous, they were the most widely used method of contraception among married women in two-thirds (44/68) of developing countries.

The United Nations (2004b) has compiled data from multiple sources on worldwide patterns of combined hormonal contraceptive use (Table 1). It was estimated that, among women in marital or consensual unions, 7.3% currently use combined hormonal contraception orally and 2.9% currently use hormonal injections or implants. Together, these methods account for 17% of all women who use contraception. Current oral use of combined hormonal contraception is greater in developed nations (15.7%) than in less developed nations (5.8%) (see Table 1), while the converse is true of injectable preparations and implants (0.7% versus 3.3%).

Reported use in the late 1990s varied considerably by region, with a relatively high prevalence of use among women in northern Africa, South-East Asia, South America, North America, New Zealand/Australia and Europe (except eastern Europe) (United Nations, 2004b). On a national level, particularly high prevalences of use were noted in Algeria (44%), Bangladesh (23%), Brazil (21%), Hungary (38%), Iran (21%), Kuwait (29%), Morocco (32%), Thailand (23%) and Zimbabwe (36%). In addition, all countries in western Europe had a prevalence above 30%. In many cases, countries adjacent to those with high prevalence of use had low prevalence: China (2%), India (2%), Peru (7%), Poland (2%), Rwanda (1%), Sudan (5%) and Yemen (4%). A range of factors contribute to these striking differences, including level of economic development, patterns of foreign aid and national family planning programmes (United Nations, 2004c).

Lundberg *et al.* (2004) presented additional information on worldwide variations in use. Current use among women aged 25–44 years varied from < 1 to 58%. In general, the variations within countries were relatively small compared with those between countries. In accordance with other studies, particularly high oral use of combined hormonal contraceptives was noted in western Europe and Australia/New Zealand.

Ross *et al.* (2002) suggested that a hierarchy of preferences for contraceptive methods exists in developing countries and depends on availability of contraception. At the highest level of access, sterilization is generally the method of choice, followed by oral contraceptives, intrauterine devices and condoms in decreasing order of preference. On the contrary, oral contraceptives are the most prevalent method in those countries that have the lowest mean availability of contraception.

Ali and Cleland (2005) also noted substantial variations in oral use of combined hormonal contraceptives within South and Central America where it was fairly prevalent in Brazil and Nicaragua, but low in Peru and Bolivia.

Table 1. Prevalence of oral use of combined hormonal contraceptives worldwide

	No. of women included[a]	Prevalence of oral contraceptive use[b]		Proportion of oral contraceptive use among all forms of contraception (%)[c]	Year of survey
		Mean (%)	Range (%)		
World	1 043 265	7.3		12.1	1998
More developed[d]	170 043	15.7		22.9	1996
Less developed[e]	873 223	5.8		9.8	1998
Africa	117 120	7.3		27.2	1999
Eastern		5.9	1.4–35.5		
Middle		1.6	1.0–16.7		
Northern		17.7	5.1–44.3		
Southern		10.4	5.4–14.7		
Western		2.7	1.8–18.2		
Asia	293 294	4.5		7.1	1997
China		1.7			
India		2.1			
South Central Asia		4.8	0.6–23.0		
South-East Asia		12.8	6.2–23.1		
Western Asia		6.4	1.0–28.8		
Europe	109 277	17.4		26.0	1995
Eastern		6.9	2.3–37.7		
Northern		19.2	3.9–26.0		
Southern		11.8	4.5–21.7		
Western		48.2	30.8–58.6		
Latin America and Caribbean	81 810	13.8		19.5	1997
Caribbean		7.6	2.3–31.5		
Central America		7.5	5.0–18.0		
South America		17.1	3.8–24.5		
USA/Canada	42 029	15.5		20.3	1995
Australia/New Zealand	2 989	23.3		30.7	1998
Oceania (except Australia/ New Zealand)	1 303	4.7	4.4–22.6	17.6	1996

From United Nations (2004b)

[a] Women aged 15–49 in a marital or consensual union (in thousands)

[b] Includes all formulations of oral contraceptives.

[c] Includes oral contraceptives, male and female sterilization, injectable implants, intrauterine devices, condoms, vaginal barriers, other modern methods, rhythm, withdrawal and other traditional methods.

[d] More developed: Europe, USA/Canada, Australia/New Zealand, Japan

[e] Less developed: Latin America and the Caribbean, Africa, Asia (except Japan), Oceania (except Australia and New Zealand)

Yuzpe (2002) reported that 17% of women of reproductive age in the USA were current users of oral contraceptives in 1995. In 2000, it was estimated that there were more than 10 million users in the USA, and that use was more common among younger women. In the USA, 80% of women born after 1945 have used oral contraceptives at some time (Blackburn *et al.*, 2000).

1.4.2 *Trends in prevalence*

Information on trends over time also indicates substantial heterogeneity between countries. Different investigators have reached contradictory conclusions on whether worldwide use is increasing or remains constant. Bongaarts and Johansson (2000) tracked changes in combined oral contraceptive use in the developing world and projected that it would double between 1993 (11% of women) and 2015 (22%). This trend is attributed to improved access, changes in the characteristics of users with better education, a desire for smaller families and new and improved technology. In contrast, Zlidar *et al.* (2003) suggested that use among married women had been more or less constant in 38 developing countries since 1990. However, Blackburn *et al.* (2000) noted very large increases in oral contraceptive use in Bangladesh (from 3 to 21%), Kenya (from 3 to 9%) and Morocco (from 13 to 32%) between 1978 and 1998, while rates declined or remained similar in Colombia, India and Egypt during the same period. A United Nations analysis of data on trend suggested little net change over time. Substantial variations were noted, however, with sizeable increases or decreases in selected countries (United Nations, 2004a).

Data on sales of combined hormonal contraceptives (IMS Health, 2005) confirmed many of the data on prevalence observed in the United Nations data compilation, but indicated increasing use worldwide (Table 2). A worldwide increase of 19% between 1994 and 1999 and a subsequent 21% increase from 1999 to 2004 were noted. The largest relative increases occurred in eastern Europe, the eastern Mediterranean, South-East Asia and the western Pacific. Only modest increases were observed in Africa and South America. It should be noted that these data may not include large quantities of hormonal contraceptives that are provided by national and international family planning programmes. Several other trends are indicated from the sales data: (i) the use of higher estrogen doses (≥ 50 μg) has continued to decline; (ii) growth in the use of products that contain later progestogens (gestodene, desogestrel) has slowed down and in some countries there has been a shift back to earlier progestogens (norethynodrel, norethisterone); and (iii) monophasic hormonal formulations have continued to predominate with some shift away from multiphasic forms (IMS Health, 2005).

On the basis of case–control data from several large cities in the USA, the most frequent duration of use among controls was 1–5 years, although some women reported use for more than 15 years (Marchbanks *et al.*, 2002).

While most use of combined hormonal contraception is for on-going contraception, additional common indications include emergency contraception, regulation of menstrual disorders and treatment of acne. In a study of use in Dutch adolescents (14–17 years old),

Table 2. Trends in sales of combined hormonal contraceptives for selected years (millions of standard units[a])

Regions[b]	1994	1999	2004
Africa	9.5	10.4	10.2
South Africa	8.9	9.7	9.5
West Africa	0.6	0.6	0.7
Eastern Mediterranean	14.8	16.7	20.7
Europe	259.3	293.4	338.1
Eastern Europe	14.3	31.9	46.0
Western Europe	245.0	261.5	292.2
North America	103.3	122.4	161.0
South America	91.1	103.6	110.9
South-East Asia	17.0	45.0	70.1
Bangladesh	6.4	4.2	6.9
India	0	22.1	15.4
Republic of Korea	2.7	2.3	2.9
Rest of South-East Asia	7.9	16.4	44.9
Western Pacific	21.7	24.7	34.6
Australia/New Zealand	16.5	16.6	15.9
China/Hong Kong	0.2	0.7	0.9
Japan	0	0.6	3.0
Taiwan, China	1.4	1.3	1.4
Rest of Western Pacific	3.6	5.4	13.5
Total	516.9	616.4	745.8

From IMS Health (2005)

[a] Standard units, sales in terms of standard dose units; the standard dose unit for oral products is one tablet or capsule.

[b] The countries were grouped according to the WHO classification:
West Africa includes: Benin, Burkina, Cameroon, Congo, Gabon, Guinea, Ivory Coast, Mali, Senegal, Togo;
Eastern Mediterranean includes: Egypt, Jordan, Kuwait, Lebanon, Morocco, Saudi Arabia, Tunisia, United Arab Emirates;
Eastern Europe includes: Belarus, Bulgaria, Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Russian Federation, Slovakia, Slovenia, Ukraine;
Western Europe includes: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Israel, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, Turkey, United Kingdom;
North America includes: Canada, Central America (Costa Rica, El Salvador, Guatemala, Honduras, Nicaragua, Panama), Mexico, Puerto Rico, USA;
Rest of South-East Asia includes: Indonesia, Pakistan, Thailand;
South America includes: Argentina, Brazil, Chile, Colombia, Dominican Republic, Ecuador, Peru, Uruguay, Venezuela;
Rest of Western Pacific includes: Malaysia, Philippines, Singapore.

current use for indications other than contraception (34%) included use for irregular cycles (18%), dysmenorrhoea (25%) and acne (11%) (van Hooff *et al.*, 1998).

The characteristics of women who use combined contraceptives differ from those who do not. Use appears to be more frequent among women who are younger and more highly educated, and increases with access to modern contraceptives (Piccinino & Mosher, 1998; Ross *et al.*, 2002).

Characteristics of users depend on regional differences and have evolved over time. Women have gradually begun to use oral contraceptives at younger ages, and initiation of use at 15–19 years of age is now frequent, while in the past it tended to start at 20–24 years of age. One study in the Netherlands reported a large increase in use among 15–17-year-old girls (Van Hooff *et al.*, 1998). In contrast, Piccinino and Mosher (1998) observed a decline in use among teenagers between 1988 and 1995 in the USA.

2. Studies of Cancer in Humans

2.1 Breast cancer

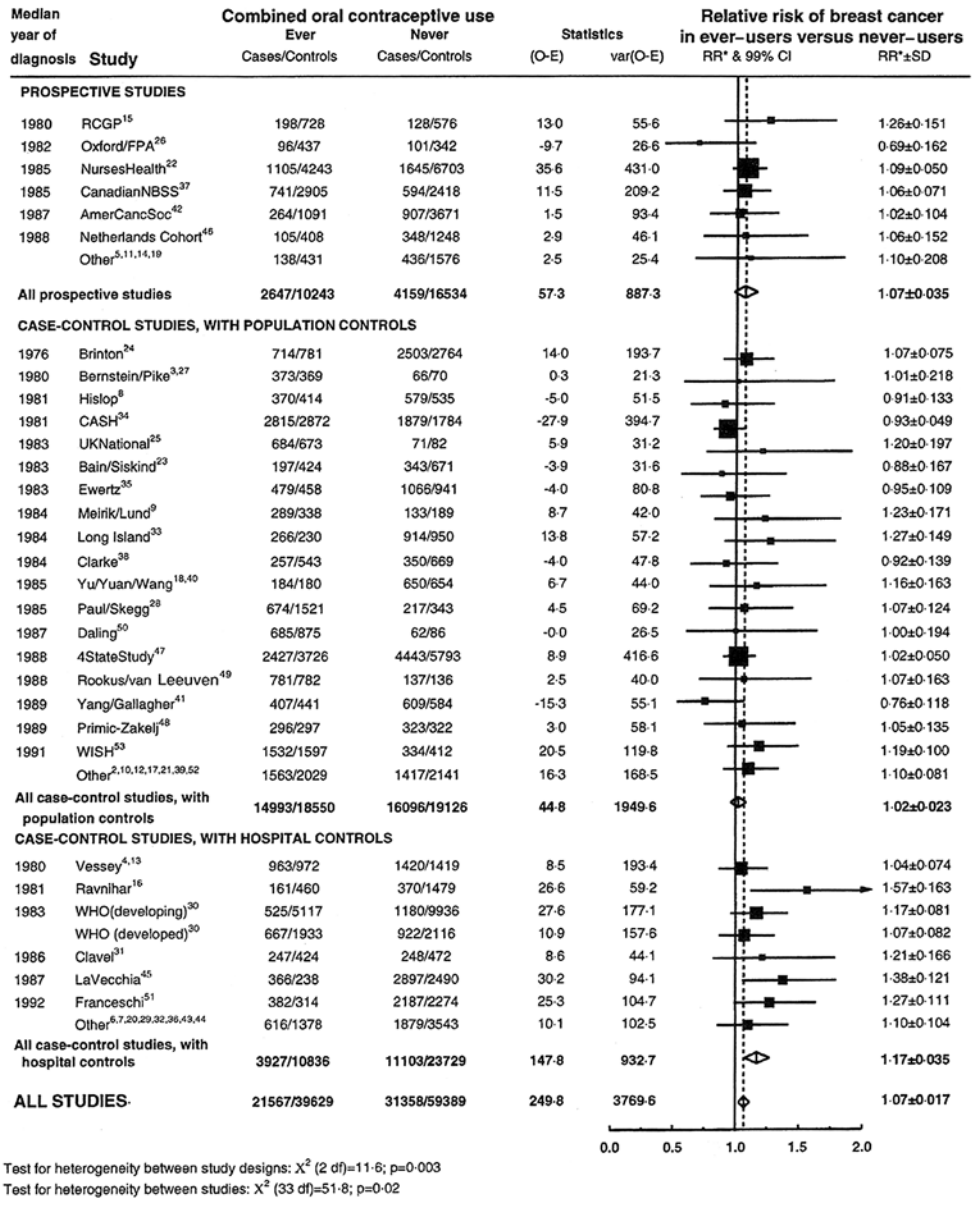
2.1.1 *Background*

In the previous evaluation of exogenous hormones and risk for cancer in women (IARC, 1999), the overall assessment of the use of combined oral contraceptives and the risk for breast cancer relied heavily on the work of the Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b) (Figure 1). More than 50 case–control and cohort studies that included over 53 000 women with breast cancer had assessed the relation between use of combined oral contraceptives and risk for breast cancer. The weight of the evidence suggested a small increase in the relative risk among current and recent users of combined oral contraceptives. The small increase in risk was not related to duration of use, type of use or dose of the preparation used. By 10 years after cessation of use, the risk for breast cancer in women who had used combined oral contraceptives was similar to that of women who had never used this type of contraception (Figure 2). It was concluded that, if the reported association was causal, the excess risk for breast cancer associated with typical patterns of current use of combined oral contraceptives was very small.

2.1.2 *Use of combined oral contraceptives and detection of breast cancer*

The increase in risk for breast cancer associated with the use of combined oral contraceptives in younger women could be due to more frequent contacts with doctors, which leads to earlier detection of breast cancer through mammography, breast examination or echography. An effect of early detection would normally lead to an increase in the number of women diagnosed with in-situ or early stage breast cancer (i.e. tumour node metastasis stage I or cancer < 2 cm in size).

Figure 1. Relative risk for breast cancer in ever-users compared with never-users of combined oral contraceptives



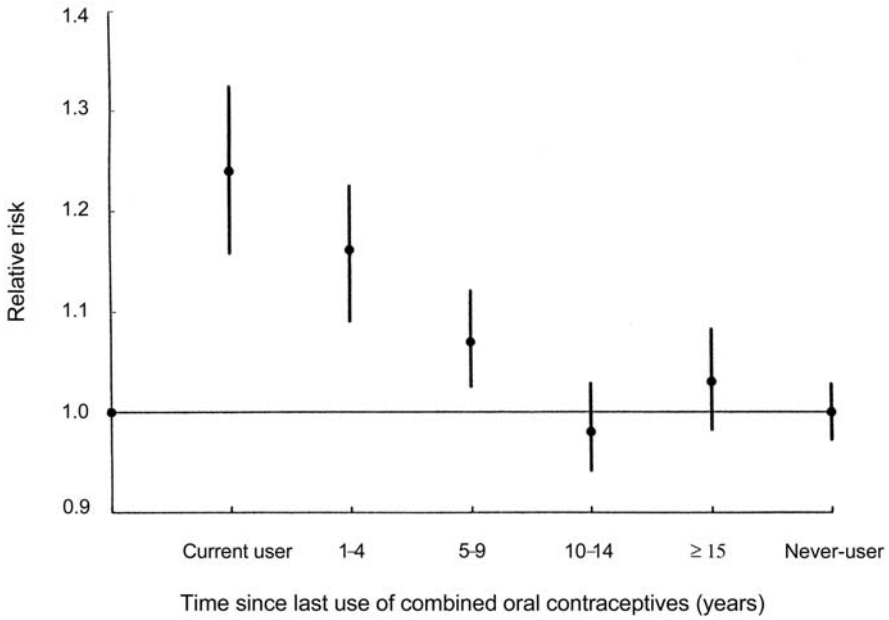
From Collaborative Group on Hormonal Factors in Breast Cancer (1996a)

Separate results are given for individual studies. Each relative risk and its 99% confidence interval (CI) is plotted as a black square and a line. The area of the square is proportional to the amount of statistical information (i.e. to the inverse of the variance of the logarithm of the relative risk). Diamonds indicate 99% CIs for totals. The solid vertical line represents a relative risk of 1.0 and the broken vertical line indicates the overall relative risk estimate for all studies combined.

*Relative risk (given with 99% CI) relative to never-users, stratified by study, age at diagnosis, parity and, where appropriate, the age of a woman when her first child was born and her age when her risk for conception ceased.

The numbers next to the references refer to the citations in the original article.

Figure 2. Relative risk for breast cancer by time since last use of combined oral contraceptives



From Collaborative Group on Hormonal Factors in Breast Cancer (1996a,b)
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 conception ceased.

An analysis of the methods of detection of breast cancer in the Cancer and Steroid Hormone Study (CASH, 1986) found that, among women 20–44 years of age, 86% of breast cancers in oral contraceptive users and 84% of breast cancers in non-users were detected by the women themselves (Schlesselman *et al.*, 1992). In both groups, 2% or fewer of cancers were detected by mammography. Proportions of in-situ carcinomas were 4% and 5% in non-users and users, respectively. On average, the tumour diameter was 0.3 cm smaller in women who had used oral contraceptives ($p < 0.001$). Similar results were found in women aged 45–54 years. In clinical terms, however, that difference in size is small, and the authors concluded that the net effect of any diagnostic bias on advancing the date of diagnosis of cancer was less than 8 weeks. This corresponds to a spurious increase in the risk of early occurring breast cancer in oral contraceptive users of at most 2.4% (relative risk, 1.024).

Two large-scale studies of breast cancer and oral contraceptive use in the USA found significantly increased risks in women under 35 years of age who had used oral contraceptives for 5 years or more (Brinton *et al.*, 1995) or for 10 years or more (White *et al.*, 1994). Both studies examined breast screening and methods of diagnosis in case and control women, and concluded that the increased risks could not be explained by differences in screening or in biopsy rates between oral contraceptive users and non-users.

In the Women's CARE (Contraceptive and Reproductive Experience) study (Marchbanks *et al.*, 2002), the risk for invasive breast cancer with current low-estrogen oral contraceptive use was 1.5 (95% confidence interval [CI], 0.9–2.6) in women aged 45–64 years. In order to exclude a screening effect, the authors analysed the data after exclusion of women with stage I tumours. They did not report the data but stated that the relative risk did not decrease.

In the study conducted in Los Angeles, USA, cases of breast cancer included in-situ and invasive tumours (Ursin *et al.*, 1998). To examine the probability of early detection bias, the authors limited the analysis to invasive cancers and, although results were not reported, they stated that the findings remained unchanged.

2.1.3 *Cohort studies* (Table 3)

Grabrick *et al.* (2000) studied 426 families of women who were diagnosed with breast cancer at a tumour clinic in Minnesota, USA. Among a total of 6150 women who were studied, 239 cases of breast cancer were diagnosed. The aim of the study was to assess whether family history of breast cancer might modify the association between use of combined oral contraceptives and the risk for breast cancer. Among the entire cohort, ever use of oral contraceptives was associated with a relative risk of 1.4 (95% CI, 1.0–2.0) for breast cancer. The risk for 4 or more years of use was 1.3 (95% CI, 0.9–1.9). The relative risk for breast cancer associated with ever use of combined oral contraceptives was 3.3 (95% CI, 1.6–6.7) among sisters and daughters of the probands, 1.2 (95% CI, 0.8–2.0) among granddaughters and nieces of the probands and 1.2 (95% CI, 0.8–1.9) among women who had married into the families. The positive association with breast cancer among relatives of the probands was mainly confined to the use of oral contraceptives before 1975.

The long-term effects of oral contraceptives have been examined in a nested case–control study from the Netherlands. Van Hoften *et al*. (2000) studied the effect of past use of combined oral contraceptives and the long-term risk for developing breast cancer. Within a cohort of more than 12 000 women, 309 cases of breast cancer had developed during 7 years of follow-up, and these were compared with 610 controls. The risk for ever use of combined oral contraceptives was 1.31 (95% CI, 0.96–1.79). Duration of use was not associated with risk for breast cancer (relative risk, 1.43; 95% CI, 0.92–2.22) but, in a sub-analysis of women over 55 years of age who had used oral contraceptives for more than 10 years, the relative risk was 2.1 (95% CI, 1.1–4.0; based on 22 exposed cases) compared with never users.

The Women's Lifestyle and Health cohort combined data from Norway and Sweden, and included more than 103 000 women who were aged 30–49 years at entry into the study in the early 1990s (Kumle *et al*., 2002). The population was followed up for breast cancer incidence by linkage to the Norwegian and the Swedish Cancer Registries; during 10 years of follow-up, 1008 women were diagnosed with invasive breast cancer. The relative risk was 1.3 (95% CI, 1.1–1.5) for ever use of combined oral contraceptives, 1.6 (95% CI, 1.2–2.1) for current use of any type of oral contraceptives at the beginning of follow-up and

Table 3. Cohort studies on the use of oral contraceptives and the risk for breast cancer

Reference	Country	Age at recruitment (years)	Size of cohort	Period of cohort	Histological diagnosis	No. of cases	Any use (%)	Relative risk (95% CI), any versus none	Relative risk (95% CI), longest duration	Relative risk (95% CI), current, recent use
Grabrick *et al.* (2000)[a]	USA	21–88	6 150	1991–96	NS	239	51	1.4 (1.0–2.0)	1.3 (0.9–1.9)	No difference between strata (data not shown)
Van Hoften *et al.* (2000)[b]	Netherlands	42–63	12 184	1982–96	NS	309	62.1	1.31 (0.96–1.79)	1.43 (0.92–2.22)	NS
Kumle *et al.* (2002)	Norway and Sweden	30–49	103 027	1991–99	Invasive	1008	74.11	1.3 (1.1–1.5)	1.3 (1.0–1.8)	1.6 (1.2–2.3)
Dumeaux *et al.* (2003)[c]	Norway	30–70	96 362	1991–97	Invasive	851	61.29	1.25 (1.07–1.46)	1.40 (1.09–1.79)	1.06 (0.39–2.87)
Dumeaux *et al.* (2004)[c,d]	Norway	30–70	86 948	1991–97	Invasive	1130	NS	NS	1.29 (1.05–1.60)	NS

CI, confidence interval; NS, not specified

[a] Cases included high-risk population

[b] Nested case–control study

[c] 63 patients were excluded from the multivariate analysis. Norwegian component of the study by Kumle *et al.* (2002)

[d] Update of Dumeaux *et al.* (2003), with adjustment for alcoholic beverage consumption. Included only women with complete information on alcoholic beverage consumption and duration of oral contraceptive use.

1.2 (95% CI, 1.1–1.4) for past use (before recruitment to the study). The results showed no increase in risk with longest duration of use. In relation to time since last use, the risk appeared to be higher in women who had used oral contraceptives within the last 2 years (relative risk, 1.6; 95% CI, 1.2–2.3) compared with women who had stopped using oral contraceptives 10–14 years previously (relative risk, 1.2; 95% CI, 1.0–1.6). Slightly stronger associations were related to early use (before the age of 20 years) and to relatively long-term use before first birth, but these were of borderline statistical significance.

In the Norwegian component of the previous study (Dumeaux *et al*., 2003), the investigators studied whether specific types of estrogens and progestogens contained in oral contraceptives exert different effects on the risk for breast cancer. Among more than 96 000 women, 851 cases of invasive breast cancer were diagnosed during follow-up. The relative risk for ever use of combined oral contraceptives was 1.25 (95% CI, 1.07–1.46). An increased risk was related to use for 10 years or more (relative risk, 1.40; 95% CI, 1.09–1.79), but no trend in risk related to recency of use ($p = 0.42$) or to time since last use. In this study, the investigators examined the dose of estrogen contained in the respective brands of oral contraceptives, and reported a relative risk of 1.5 (95% CI, 1.1–2.0) associated with a cumulative dose of 100 mg or more. Within the same cohort, Dumeaux *et al*. (2004) studied whether the association with use of combined oral contraceptives may be modified by the use of alcoholic beverages. More than 86 000 women were followed up and included in the analysis, and 1130 cases of invasive breast cancer were diagnosed. The results suggested that combined oral contraceptives had an increasing effect on risk only among low consumers of alcoholic beverages (i.e. < 5 g per day) and not among women who reported regular use of alcoholic beverages ($p \leq 0.0001$).

2.1.4 *Case–control studies* (Table 4)

A case–control study in the USA assessed whether the combined use of oral contraceptives at a young age may increase the risk for breast cancer (Brinton *et al*., 1998). The participants were under 55 years of age and included 1031 cases of breast cancer and 919 population controls. The study reported that the relative risk associated with ever use was 1.14 (95% CI, 0.9–1.4).

In Taiwan, China, where the incidence of breast cancer is generally low, Chie *et al*. (1998) studied the association between the use of combined oral contraceptives and subsequent risk for breast cancer in a case–control study of 174 cases and 453 hospital-based controls. The odds ratio for ever versus never use of oral contraceptives was 1.7 (95% CI, 0.9–3.2), and appeared to be somewhat higher among women who had started using oral contraceptives before the age of 25 years (odds ratio, 3.5; 95% CI, 1.2–9.7) and women who had used them for 5 years or more (odds ratio, 2.1; 95% CI, 0.8–5.6).

In a case–control study from California, Ursin *et al*. (1998) examined the use of combined oral contraceptives and the risk for breast cancer in young women. The aim of the study was to assess aspects of oral contraceptive use that may be important for the increased risk related to current or recent use in young women. The study included more

Table 4. Case–control studies of the use of oral contraceptives and the risk for breast cancer

Reference, location	Years of case diagnosis	Age (years)	Histology	Use	Ever versus never				Longest duration of use					Current/recent use				Time since last use
					Cases	Controls	Odds ratio	95% CI	Cases	Controls	Odds ratio	95% CI	Use (years)	Cases	Controls	Odds ratio	95% CI	
Brinton *et al.* (1998), USA	1990–92	< 55	In-situ or invasive	Never	283	278	Ref.		283	278	Ref.							
				Ever	748	641	1.14	0.9–1.4	173	127	1.27	0.9–1.7	≥ 10					
Chie *et al.* (1998), Taiwan, China	1993–94	NS	NS	Never	149	406	Ref.		149	406	Ref.							
				Ever	25	47	1.7	0.9–3.2	9	15	2.1	0.8–5.6	≥ 5					
Ursin *et al.* (1998), USA	1983–88	≤ 40	In-situ and invasive	Never	124	116	Ref.		124	116	Ref.			124	116	Ref.		
				Ever	618	626	0.83	0.62–1.12	52	30	1.4	0.81–2.40	> 12	111	84	1.14	0.75–1.72	< 1 year
Magnusson *et al.* (1999), Sweden	1993–95	50–74	Invasive	Never	1733	1938	Ref.		1733	1938	Ref.			1733	1938	Ref.		
				Ever	898	889	0.98	0.86–1.12	357	353	0.98	0.82–1.18	≥ 5	73	59	1.0	0.69–1.44	< 10 years
Ursin *et al.* (1999), USA	1983–87	20–55	NS		383	594	Ref.		383	594	Ref.			383	594	Ref.		
					207	351	0.91	0.72–1.15	45	87	0.71	0.47–1.07	> 5	29	63	0.68	0.41–1.14	< 5 years
Shapiro *et al.* (2000), South Africa	1994–97	20–54	Invasive	Never	264	992	Ref.		264	992	Ref.			264	992	Ref.		
				Ever	220	633	1.2	1.0–1.5	16	39	1.2	0.7–2.3	> 10	16	53	1.2	0.7–2.0	< 5 years
Tessaro *et al.* (2001), Brazil	1995–98	20–60	NS	Never	45[a]	141[a]	Ref.		45[a]	141[a]	Ref.							
					42[b]	112[b]	Ref.		41[b]	111[b]	Ref.							
				Ever	127[a]	375[a]	1.1	0.7–1.6	38[a]	92[a]	1.2	0.7–2.0	> 12					
					126[b]	392[b]	0.9	0.6–1.6	35[b]	123[b]	1.0	0.5–1.8						
Heimdal *et al.* (2002), Norway	1999	40–60	NS	Never	NR	NR	Ref.							NR	NR			
				Ever	NR	NR	0.9	0.68–1.18						NR	NR	1.99	0.80–4.98	0–4 years
Marchbanks *et al.* (2002), USA	1994–98	35–64	Invasive	Never	1032	980	Ref.		1032	980	Ref.			1032	980	Ref.		
				Ever	3497	3658	0.9	0.8–1.0	234	202	1.0	0.8–1.3	> 15	200	172	1.0	0.8–1.3	< 7 months

Table 4 (contd)

Reference, location	Years of case diagnosis	Age (years)	Histology	Use	Ever versus never				Longest duration of use					Current/recent use				Time since last use
					Cases	Controls	Odds ratio	95% CI	Cases	Controls	Odds ratio	95% CI	Use (years)	Cases	Controls	Odds ratio	95% CI	
Narod *et al.* (2002)[c], 11 countries	1977–2001	47.3 ± 10	Invasive	Never	NR	NR	Ref.											
				Ever	NR	NR	1.2	1.02–1.52						NR	NR	0.83	0.66–1.04	< 1 year
Althuis *et al.* (2003), USA	1990–92	20–44	In-situ and invasive	Never	371	406	Ref.											
				Ever	1269	1086	1.24	1.0–1.5						309	258	1.47	1.2–1.9	≤ 5 years
Claus *et al.* (2003), USA	1994–98	20–79	Ductal carcinoma *in situ*	Never	425	465	Ref.		425	465	Ref.			425	465	Ref.		
				Ever	404	522	1.0	0.8–1.2	47	61	0.9	0.6–1.5	≥ 10	17	38	0.6	0.3–1.3	< 1 year
Newcomer *et al.* (2003), USA	NS	< 75	Lobular and ductal		Lobular													
				Never	334	5864	Ref.							Lobular				
				Ever	159	3447	1.2	0.9–1.6						6	141	2.6	1.0–7.1	
					Ductal									Ductal				
				Never	3391	5864	Ref.							47	141	1.2	0.8–1.9	
				Ever	1676	3447	1.0	0.9–1.1										

CI, confidence interval; NR, not reported; NS not specified; Ref., reference

[a] Hospital cases/controls

[b] Neighbourhood cases/controls

[c] Results only for *BRCA1* mutation carriers

than 700 women under 40 years of age who had invasive breast cancer or ductal carcinoma *in situ* and 744 population controls matched to the cases. The relative risk for ever versus never use was 0.83 (95% CI, 0.62–1.12) and that for use of oral contraceptives for 12 years or more was 1.4 (95% CI, 0.8–2.4). These results were adjusted for age, age at menarche, age at first birth, parity, duration of breast feeding and physical activity.

In another report restricted to Asian immigrants to California, Ursin *et al*. (1999) studied the relation between combined oral contraceptive use and risk for breast cancer. The study included nearly 600 cases of breast cancer and 1000 population controls. The results showed that the use of oral contraceptives increased with increasing time since migration, but there was no indication that use of oral contraceptives increased the risk for breast cancer. The relative risk for ever versus never use was 0.91 (95% CI, 0.72–1.15). The investigators conducted several subgroup analyses, according to age when the women started using oral contraceptives, duration of use and time since last use, but found no consistent association related to the use of oral contraceptives and the risk for breast cancer in any of these analyses.

In a large case–control study from Sweden, Magnusson *et al*. (1999) studied reproductive factors and the risk for breast cancer among women 50–74 years of age. As part of the study, the investigators also collected information on past use of combined oral contraceptives and were thus able to evaluate whether use in the past influenced the risk for breast cancer after the menopause. The study included 3016 women with invasive breast cancer and 3263 population controls without breast cancer, but information on oral contraceptives was available only for a subset of the population. The results showed no clear association with ever use of oral contraceptives (relative risk, 0.98; 95% CI, 0.86–1.12) and no association related to duration of use (p for trend = 0.88). The odds ratio for last use < 10 years previously was 1.00 (95% CI, 0.69–1.44) compared with never users. These results were adjusted for age, age at menarche, parity, age at first birth, menopausal status, age at menopause, height, body mass index and use of combined hormonal menopausal therapy.

The use of oral contraceptives in relation to the risk for breast cancer has been assessed in a case–control study in South Africa (Shapiro *et al.*, 2000). The primary aim of the study was to examine the effects of injectable contraceptives, but information was also collected on the use of oral contraceptives. Shapiro *et al*. (2000) included women aged 20–54 years who came from a defined area close to Cape Town, and were of either black or mixed racial descent. The odds ratio associated with ever use of oral contraceptives was 1.2 (95% CI, 1.0–1.5), that for use of 10 or more years was 1.2 (95% CI, 0.7–2.3) and that associated with use within the last 5 years was 1.2 (95% CI, 0.7–2.0). In analyses within age groups, the relative risk for ever use was 1.7 (95% CI, 1.0–3.0; based on 36 exposed cases) in women under 35 years of age, 1.2 (95% CI, 0.8–1.6; based on 91 exposed cases) in women aged 35–44 years and 1.1 (95% CI, 0.8–1.6; based on 93 exposed cases) in women aged 45–54 years.

A case–control study from Brazil (Tessaro *et al*., 2001) reported the use of combined oral contraceptives and the risk for breast cancer using different groups of controls, but the results showed no association with ever use or with duration of use.

In a case–control study of high-risk families, Heimdal *et al*. (2002) studied the use of combined oral contraceptives in carriers of the *BRCA1* mutation in relation to risk for breast cancer. The hazard ratio for ever use of oral contraceptives was 0.90 (95% CI, 0.68–1.18) in the total data set and 3.00 (95% CI, 0.36–10.2) in *BRCA1* carriers.

A large case–control study in the USA also aimed at studying whether the use of combined oral contraceptives at a relatively young age is associated with risk for breast cancer later in life (Marchbanks *et al*., 2002). The study included more than 4500 women with breast cancer and a similar number of controls from the same area as the cases, identified by random-digit dialling, aged 35–64 years. There was no association with ever use of oral contraceptives (odds ratio, 0.9; 95% CI, 0.8–1.0); the odds ratio was 1.0 (95% CI, 0.8–1.3) for current use and 0.9 (95% CI, 0.8–1.0) for use in the past. There was no increase in risk related to duration of use, age at first use or time since last use, and no increase in risk for breast cancer associated with any use of oral contraceptives that contained a relatively high dose of estrogen (relative risk, 0.8; 95% CI, 0.7–0.9; based on 1082 exposed cases). The relative risk associated with first use between 15 and 19 years was 1.0 (95% CI, 0.8–1.1; based on 1239 exposed cases). The results did not differ for black and white women, and there was no increase in risk associated with oral contraceptive use of women who had a family history of breast cancer. These results were adjusted for age, age at menarche, age at first birth, parity, body mass index, menopausal status and use of combined hormonal therapy.

Women who are carriers of *BRCA1* and *BRCA2* mutations have an inherited increase in risk for breast cancer. Narod *et al*. (2002) investigated whether women with these characteristics who have used combined oral contraceptives are at particularly high risk in a matched case–control study of 1311 pairs of women with known *BRCA1* or *BRCA2* mutations. Use of oral contraceptives was not associated with an increase in risk for those who were carriers of *BRCA2* mutations. However, the results suggested that *BRCA1* carriers may be at slightly elevated risk if they had used oral contraceptives before 1975, if they had used them before the age of 30 years and if the use had lasted for at least 5 years.

Althuis *et al.* (2003) studied risk for breast cancer related to current or recent use of combined oral contraceptives in a case–control study of women aged 20–44 years. The study involved more than 1600 women with invasive breast cancer and a comparable number of community controls. The odds ratio for ever use of oral contraceptives was 1.24 (95% CI, 1.0–1.5). Women who had used oral contraceptives within the last 5 years had a relative risk of 1.47 (95% CI, 1.2–1.9) and the odds ratio in women who had stopped taking oral contraceptives at least 10 years previously was 1.13 (95% CI, 0.9–1.4). Compared with never users, women who had used oral contraceptives that contained more than 35 μg ethinylestradiol were at higher risk (relative risk, 1.99; 95% CI, 1.2–3.2; based on 54 exposed cases) than women who used lower-dose preparations (relative risk, 1.27; 95% CI,

0.9–1.7; based on 161 exposed cases). In the subgroup of women under 35 years of age, the relative risk associated with ever use was 2.05 (95% CI, 1.3–3.2; based on 209 exposed cases), that for use within the last 5 years was 2.22 (95% CI, 1.4–3.5; based on 135 exposed cases) and that for cessation of use 10 or more years previously was 1.52 (95% CI, 0.8–3.0; based on 31 exposed cases). In this subgroup, dose of estrogen was also associated with an increased risk for breast cancer: the odds ratio associated with using oral contraceptives that contained high-dose estrogen was 3.62 (95% CI, 1.7–7.9; based on 27 exposed cases) and that for use of low-dose estrogen contraceptives was 1.91 (95% CI, 1.1–3.2; based on 81 exposed cases), compared with the risk in never users.

In a case–control study in the USA, Claus *et al*. (2003) assessed whether the use of combined oral contraceptives was associated with the development of ductal breast carcinoma *in situ* by comparing more than 800 cases with this condition and approximately 1000 control women aged 20–79 years. The results showed no association between any use of oral contraceptives and the risk for breast cancer *in situ* (odds ratio, 1.0; 95% CI, 0.8–1.2).

The association between oral contraceptive use and the risk for breast cancer was investigated in a population-based case–control study in the USA (Norman *et al*., 2003) that included 1847 postmenopausal women with invasive breast cancer and 1932 controls (not shown in Table 4). The aim of the study was to assess the combined effect of use of oral contraceptives at a young age and use of hormonal therapy after the menopause. The report did not present detailed results related to the use of oral contraceptives alone, but stated that the use of oral contraceptives, independent of the use of hormonal therapy, was not associated with a risk for breast cancer.

Whether the use of combined oral contraceptives has different effects on various histological subtypes of breast cancer was investigated by Newcomer *et al*. (2003) in a case–control study of women under 75 years of age (mean age, 57.5 years). The study involved 493 women with lobular breast cancer, 5510 women with ductal carcinoma and 9311 randomly selected controls. The odds ratio for ever versus never use of oral contraceptives was 1.2 (95% CI, 0.9–1.6) for lobular breast carcinoma and 1.0 (95% CI, 0.9–1.1) for ductal carcinoma. The odds ratio associated with current use was 2.6 (95% CI, 1.0–7.1; based on six exposed cases) for lobular carcinoma, and there was a significant trend ($p = 0.02$) of increased risk with more recent use. Current use of oral contraceptives was not clearly associated with risk for ductal carcinoma (odds ratio, 1.2; 95% CI, 0.8–1.9). Among women who had started using oral contraceptives before the age of 20 years, the odds ratio was 1.0 (95% CI, 0.5–1.9; based on 17 exposed cases) for lobular carcinoma and 1.0 (95% CI, 0.8–1.2; based on 217 exposed cases) for ductal carcinoma.

2.2 Endometrial cancer

When the association between endometrial cancer and use of combined oral contraceptives was last reviewed, relevant data were available from three cohort studies and 16 case–control studies (IARC, 1999). The results from these studies consistently showed that the risk for endometrial cancer was reduced in women who had used oral contraceptives,

and the reduction in risk was greater with longer duration of use. The evidence for combined oral contraceptives (including studies that were reviewed in 1999) is summarized here, together with new studies.

The cohort and case–control studies in which use of combined oral contraceptives and the risk for endometrial cancer has been investigated are summarized below and, when available, the risk associated with duration and recency of use is given. Risk estimates for women of different weight and parity (or gravidity) or who are users of hormonal menopausal therapy are given when available.

2.2.1 *Descriptive studies*

Several analyses have suggested that increased use of combined oral contraceptives can partially explain the decreasing rates of mortality from cancer of the uterine corpus (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 evident among women aged 55 years or younger, who most probably used combined oral contraceptives. Interpretation of these trends is complicated by improvements in cancer treatment over time and by a lack of correction for the proportion of women who had their uterus removed and were no longer at risk for developing (or dying from) endometrial cancer. Furthermore, the rates of death from cancer of the uterine corpus have generally decreased 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 cancer of the uterine corpus that is related to increased use of oral contraceptives remains unclear.

2.2.2 *Cohort studies*

A questionnaire to obtain information on oral contraceptive use was sent to approximately 97 300 married women aged 25–57 years in eastern Massachusetts, USA, in 1970, who were identified from the 1969 Massachusetts residence lists (Trapido, 1983). The age-standardized rate ratio for women who had ever used oral contraceptives relative to non-users was 1.4 (95% CI, 0.9–2.4); there was no consistent pattern of a risk with longer or more recent use (Table 5). 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 menopausal estrogen 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.* (1988) 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

Table 5. Cohort studies of the use of oral contraceptive pills[a] and the risk for endometrial cancer

Reference, location	Age (range/ median)	Source population	Follow-up	Type/measure of therapy	No. of cases	No. of person–years	Relative risk (95% CI)
Trapido (1983), USA	25–57 years	97 300 residents of Boston and 14 contiguous towns	1970–76	No use	75	296 501	1.0
				Any use	18	124 851	1.4 (0.9–2.4)
				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), United Kingdom	49 years	46 000 British women identified by general practitioners	1968–87 (incidence) Dec. 1993 (mortality)	No use	16	182 866	1.0
				Any use	2	257 028	0.2 (0.0–0.7)
				No use	6	335 998	1.0
				Any use	2	517 519	0.3 (0.1–1.4)
Vessey & Painter (1995), United Kingdom	25–39 years	17 032 patients at 17 family planning clinics	1968–93	No use	14	NR	1.0
				Any use	1	NR	0.1 (0.0–0.7)
Kumle *et al*. (2003), Norway	30–70 years	102 443 Norwegian women	1991–99	No use			1.0
				Ever use			0.59 (0.38–0.92)
				Duration (years)			
				< 5	23	28 115	0.66 (0.39–1.10)
				5–9	8	12 159	0.65 (0.31–1.39)
				> 10	5	8 840	0.41 (0.15–1.13)
				No information	38	53 328	

CI, confidence interval; NR, not reported

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

Practitioners. Use of oral contraceptives (not otherwise specified) and the occurrence of uterine cancer were both determined from physicians' reports. Endometrial cancer was diagnosed in two of the oral contraceptive users and 16 of the non-users, which resulted in a rate ratio of 0.2 (95% CI, 0.0–0.7) after adjustment for age, parity, tobacco 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 (Beral *et al*., 1999), eight deaths from endometrial cancer occurred, two among women who had ever used oral contraceptives and six among women who had never used them (rate ratio, 0.3; 95% CI, 0.1–1.4).

The Oxford Family Planning Association Study included 17 032 white married 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 5 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 years; only those who had never used oral contraceptives (5881) or had used them for 8 years or more (3520) were followed from that time onwards. 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*., 1989), none of the oral contraceptive users but two of those who used a diaphragm or an intrauterine device (the comparison group) had died from endometrial cancer.

Kumle *et al*. (2003) followed 102 443 Norwegian women aged 30–70 years who were recruited into a cohort study in 1991–97. Endometrial cancers were identified by linkage to the Cancer Registry of Norway. Follow-up was through to December 1999, during which time 110 endometrial cancers were diagnosed. The relative risk associated with use of combined oral contraceptives was 0.59 (95% CI, 0.38–0.92). For use of less than 5 years, 5–9 years and more than 10 years, the relative risks were 0.66 (95% CI, 0.39–1.10), 0.65 (95% CI, 0.31–1.39) and 0.41 (95% CI, 0.15–1.13), respectively. Among the users of oral contraceptives, there was a significant trend of decreasing risk for endometrial cancer with increasing duration of use of oral contraceptives ($p = 0.03$).

2.2.3 *Case–control studies* (Table 6)

Among 152 women who had endometrial cancer and 516 controls in a hospital-based study in the USA and Canada (Kaufman *et al.*, 1980), a 60% reduction in risk was seen among women who used combined oral contraceptives relative to non-users. The risk for endometrial cancer declined with increasing duration of use, and a sustained reduction in risk was suggested among women who had stopped using oral contraceptives during the previous 5 years or more.

Weiss and Sayvetz (1980), in a population-based case–control study from western Washington State (USA), found that women who had used combined oral contraceptives for

Table 6. Case–control studies of use of combined oral contraceptives and the risk for endometrial cancer (by duration and recency of use when available)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Kaufman *et al.* (1980), Canada and USA	< 60 years	Hospital patients	Personal interviews	96[a]	96[a]	No use	136	411	1.0
						Any use	16	105	[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 of use			
						≥ 5 years	12	60	0.6 (0.3–1.2)
						Use ≥ 1 year	8	52	0.5 (0.2–1.0)
Weiss & Sayvetz (1980), Washington State, USA	36–55 years	General population	Personal interviews	83	96	No use or < 1 year of use	93	173	1.0
						≥ 1 year of use	17	76	0.5 (0.1–1.0)
Hulka *et al.* (1982), North Carolina, USA	< 60 years	General population	Personal interviews and medical record reviews	90[a]	90[a]	No use or < 6 months' use	74	172	1.0
						≥ 6 months' use	5	31	0.4 (NR)
						Duration (years)			
						< 5	3	14	0.6 (NR)
						≥ 5	2	17	0.3 (NR)
						Recency (years)			
						< 1	0	13	–
						≥ 1	5	14	0.9 (NR)
Kelsey *et al.* (1982), Connecticut, USA	45–74 years	Hospital patients	Personal interviews	67	72	No use			
						Each 5 years of use	NR	NR	1.0
						Age 45–55 years	NR	NR	0.6 (0.3–1.5)
						No use			
						Duration (years)	31	256	1.0
						≤ 2.5	4	42	0.9 (NR)
						> 2.5	2	44	0.5 (NR)

Table 6 (contd)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Henderson *et al*. (1983a), Los Angeles county, USA	≤ 45 years	Residents in neighbourhood of cases	Telephone interviews	81	NR	No use	67	50	1.0
						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)
La Vecchia *et al*. (1986), greater Milan, Italy	< 60 years	Hospital patients	Personal interviews	98[c]	98[c]	Non-user	163	1104	1.0
						Any use	7	178	0.50 (0.23–1.12)
Pettersson *et al*. (1986), Uppsala, Sweden	≤ 60 years	General population	Personal interviews	93	80	No use	96	91	1.0
						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)
CASH (1987a), eight US areas	20–54 years	General population	Personal interviews	73	84	No use	250	1147	1.0
						Ever use	NR	NR	0.5 (0.4–0.6)
						Duration (months)			
						3–6	24	186	0.9 (0.5–1.5)
						7–11	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)

Table 6 (contd)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
WHO Collaborative Study (1988); Rosenblatt *et al.* (1991), nine centers in seven countries: Australia, Chile, China, Israel, Mexico, the Philippines, Thailand	< 60 years	Hospital patients	Personal interviews	87	93	No use	182	1072	1.0
						Progestogen content			
						High			
						Ever use	3	156	0.1 (0.1–0.4)
						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			
						Ever use	9	132	0.6 (0.3–1.2)
						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	40–79 years	Hospital patients	Personal interviews	80	95	No use or ≤ 6 months' use	80	151	1.0
						> 6 months' use	3	13	0.6 (0.2–2.0)[d]
Levi *et al.* (1991), Canton of Vaud, Switzerland	32–75 years	Hospital patients	Personal interviews	85[a]	85[a]	No use	105	227	1.0
						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 6 (contd)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Shu *et al*. (1991), Shanghai, China	18–74 years	General population	Personal interviews	91	96	No use of any birth control	84	72	1.0
						Any use of oral contraceptives	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)
Jick *et al*. (1993), Washington State, USA	50–64 years	Members of health maintenance organization	Mailed form and pharmacy database	83	79	No use	110	737	1.0
						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)
						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)
Stanford *et al*. (1993), five US areas	20–74 years	General population	Personal interviews	87	66	No use	321	187	1.0
						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)

Table 6 (contd)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Voigt *et al.* (1994)[e], Washington State, USA	40–59 years	General population	Personal interviews	83	95 and 73[f]	No use or < 1 year of use	117	284	1.0
						Recency of use			
						> 10 years			
						Duration (years)			
						1–5	14	30	0.9 (0.4–1.9)
						> 5	4	16	0.4 (0.1–1.2)
						≤ 10 years			
						Duration (years)			
						1–5	7	28	1.0 (0.4–2.4)
						> 5	7	74	0.3 (0.1–0.6)
						Progestogen content			
						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	< 59–≥ 70 years	Hospital patients	Personal interviews	83	88	No use	143	293	1.0
						Any use	2	5	1.3 (0.2–7.7)
Salazar-Martinez *et al.* (1999), Mexico City, Mexico	37.1 (mean) years	Hospital patients	Personal interviews	100	93	No use	71	473	1.0
						Duration (years)			
						< 1	6	78	0.56 (0.22–1.30)
						> 1	7	117	0.36 (0.15–0.83)
Weiderpass *et al.* (1999), Sweden	50–74 years	General population	Self-completed questionnaire	75	80	No use	551	2252	1.0
						Duration (years)			
						< 3	91	421	1.0 (0.7–1.3)
						> 3	45	518	0.5 (0.3–0.7)

Table 6 (contd)

Reference, location	Age	Source of controls	Ascertainment of use	Participation (%)		Type/measure of use	No. of subjects		Odds ratio (95% CI)
				Cases	Controls		Cases	Controls	
Jain *et al*. (2000), Ontario, Canada	30–79 years	Population	Personal interview	70	59	No use	317	265	1.0
						Any use	195	248	0.66 (0.51–0.84)
Parslov *et al*. (2000), Denmark	> 50 years	Population	Self-completed questionnaire	93	91	No use	90	75	1.0
						Duration (years)			
						< 1	52	95	0.4 (0.3–0.7)
						1–5	50	210	0.2 (0.1–0.3)
						> 5	45	158	0.2 (0.1–0.4)
Newcomb & Trentham-Deitz (2003), Wisconsin, USA	40–79 years	Population	45-min telephone interview	87	85.2	No use	460	1494	1.0
						Duration (years)			
						< 3	74	260	1.04 (0.74–1.40)
						> 3	54	275	0.73 (0.52–1.02)

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 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 in 1985–87

1 year or more had half the risk for endometrial cancer of women who were either non-users or had used combined oral contraceptives for less than 1 year (odds ratio, 0.5; 95% CI, 0.1–1.0). The risk estimates were adjusted for age and use of menopausal estrogen therapy. No further difference in duration of use was seen between cases and controls. In stratified analyses, the reduced risk was present only for women who had never used menopausal estrogen therapy (odds ratio, 0.4; 95% CI, 0.1–1.1) or who had used it for 2 years or less (odds ratio, 0.1; 95% CI, 0.01–1.1); no risk reduction was noted among women who had used it for 3 years or more (odds ratio, 1.3; 95% CI, 0.3–6.6).

Among 79 women treated at a hospital in North Carolina, USA, for endometrial cancer, 6.3% had used combined oral contraceptives for 6 months or longer compared with 15.3% of the 203 controls from 52 counties in the State (the main referral area for the hospital) (Hulka *et al*., 1982).

Kelsey *et al.* (1982) studied women who were admitted to seven hospitals in Connecticut, USA. A total of 167 newly diagnosed cases of endometrial cancer were compared with 903 control women admitted for non-gynaecological surgery. Among the study participants aged 45–55 years — 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 for endometrial cancer.

Henderson *et al.* (1983a) identified 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 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 their relative estrogen and progestogen content had a clear impact on the risk, beyond that explained by 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 who weighed less than 170 lbs [77 kg] but not among women who weighed more.

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 by 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, which resulted in an odds ratio of 0.50 (95% CI, 0.23–1.12) 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 who resided in the health care region of Uppsala (Sweden) and 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 who was identified from a population registry. Use of combined oral contraceptives was analysed for women aged 60 years or under, and 108 cases and 113 controls were analysed. Women who had ever used contraceptives and

users for 1 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). [The Working Group noted that it is unclear whether the estimates were adjusted for potentially confounding factors.]

In a population-based study conducted by the Centers for Disease Control and the National Institute of Child Health and Human Development in the USA, women 20–54 years of age with newly diagnosed endometrial cancer were identified from eight cancer registries (Atlanta, Detroit, San Francisco, Seattle, Connecticut, Iowa, New Mexico and four urban counties in Utah) in the US Surveillance, Epidemiology and End Results (SEER) Program; 3191 controls were selected from the general population (CASH, 1987a). Women who had used only combined 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, and the greatest reduction in risk was seen among women who had used combined oral contraceptives for 2 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, geographical region, exogenous estrogen use and infertility. The risk for endometrial cancer did not vary with recency of use of combined oral contraceptives or with time since first use; 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 the 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 formulation of the oral contraceptive, all formulations that had been used for at least 6 months or more were associated with a decreased risk for endometrial cancer.

In a worldwide multicentre hospital-based study (nine centres in seven countries), the use of combined oral contraceptives was compared in 132 women who had endometrial cancer and 836 control women who were 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 combined oral contraceptives only had a lower risk for endometrial cancer than non-users (odds ratio, 0.5; 95% CI, 0.3–1.0), after adjustment for socioeconomic status, source of referal, use of injectable contraceptives, menopausal status, age of menopause, total number of pregnancies and years until becoming pregnant after infertility. The numbers of cases (total, 220) and control women (total, 1537) in this study continued to accrue through to 1988 and these were then further evaluated by Rosenblatt *et al.* (1991). Oral contraceptives were classified as being low-estrogen dose if they contained less than 50 μg ethinylestradiol or less than 100 μg mestranol and as being high-estrogen dose if they contained larger amounts of these estrogens. Based on their ability to induce subnuclear vacuolization in the endometrium, progestogens in oral contraceptives were classified as being high and low potency. The level of reduction in risk for endometrial cancer was related to both the estrogen dose and progestogen potency of the preparation: odds ratios for ever use were 1.1 (95% CI, 0.1–9.1) for high estrogen–low progestogen, 0.6 (95% CI, 0.3–1.3) for low estrogen–low progestogen,

0.2 (95% CI, 0.05–0.5) for high estrogen–high progestogen and 0.0 (95% CI, 0.0–1.1) for low estrogen–high progestogen preparations.

Koumantaki *et al.* (1989) studied women who had endometrial cancer and were admitted to two hospitals in Athens, Greece, and control women who were 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 combined oral contraceptives for 6 months or longer (odds ratio, 0.6; 90% CI, 0.2–2.0, adjusted for age, parity, age at menarche, age at menopause, menopausal estrogen use, years of smoking, height and weight).

Among 122 women who were 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 of less than 2 years to 0.5 (95% CI, 0.1–1.2) for use of 2–5 years and 0.3 (95% CI, 0.1–0.7) for use of more than 5 years. Oral contraceptive use within the previous 10 years (odds ratio, 0.3; 95% CI, 0.1–0.9) or within the previous 10–19 years (odds ratio, 0.4; 95% CI, 0.2–1.0) and first use before the age of 30 years (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 5 years or longer had a reduction in risk even when 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 menopausal estrogen therapy. Little variation in risk was seen by categories of body mass (odds ratios, 0.6 for < 25 kg/m^2 and 0.2 for ≥ 25 kg/m^2) or cigarette smoking (odds ratios, 0.5 for ever smokers and 0.6 for never smokers). Stratification by use of menopausal estrogen therapy was also presented (odds ratios, 0.4 for ever use and 0.5 for never use). There was no significant difference in the relative risk between nulliparous women (six cases and 14 controls) who used oral contraceptives (age-adjusted odds ratio, 0.8; 95% CI, 0.2–2.9) and the parous oral contraceptive users (11 cases and 68 controls; age-adjusted odds ratio, 0.3; 95% CI, 0.1–0.7).

Shu *et al.* (1991) studied 116 Chinese women who had endometrial cancer identified from the population-based Shanghai Cancer Registry and 118 control women identified from the Shanghai Residents' Registry. The odds ratio for use of oral contraceptives (not otherwise specified) compared with never use of this type of contraception, after adjustment for age, gravidity and weight, was 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 2 years was associated with a greater reduction in the risk for endometrial cancer (odds ratio, 0.4; 95% CI, 0.1–1.2).

Jick *et al.* (1993) studied women who were members of a large health maintenance organization in western Washington State, USA. Women in whom endometrial cancer had been diagnosed were identified from the organization's tumour registry; the controls 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 cases and 26% of controls which gave an odds ratio of 0.5 (95% CI, 0.3–0.9), adjusted for age, date of enrolment in the organization, body mass, age at menopause, parity and current use of menopausal estrogen therapy. In comparison with non-users, the reduced risk for endometrial cancer was most pronounced for women who had used oral contraceptives for 6 years or more (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).

In the USA, 402 women who had endometrial cancer diagnosed at seven hospitals (in Chicago, IL; Hershey, PN; Irvine and Long Beach, CA; Minneapolis, MN; and Winston-Salem, NC) and 294 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 cases and 36% of the controls (odds ratio, 0.4; 95% CI, 0.3–0.7, after adjustment for age, education, parity, weight and use of menopausal estrogen therapy). There was no clear pattern of decreasing risk with increasing duration of use. 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 first used them 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 years). When duration and recency were evaluated jointly, use within the previous 20 years was more strongly predictive of a reduction in risk than longer duration of use (≥ 3 years). In a joint evaluation with other possible modifying factors, 3 or more years of use of combined oral contraceptive was 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 menopausal estrogen therapy.

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, USA. A reduction in risk for endometrial cancer associated with combined oral contraceptive use was present only among users of 5 or more years of duration, and even then only in women who were not long-term users of unopposed postmenopausal estrogens. Among these women, the risk did not substantially vary according to progestogen potency of the combined oral contraceptive used. 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 dose, was most predictive of a reduced risk.

Kalandidi *et al.* (1996) studied 145 women who had endometrial cancer and were admitted to two hospitals in Athens, Greece, and 298 control women who were admitted to the major accident hospital in Athens with bone fractures or other orthopaedic disorders.

Only two (1%) of the cases and five (1.7%) of the controls had ever used oral contraceptives (not otherwise specified). The multivariate-adjusted risk estimate was 1.3 (95% CI, 0.2–7.7).

Salazar-Martinez *et al*. (1999) conducted a hospital-based case–control study in Mexico City that involved 84 women who had endometrial cancer diagnosed in 1995–97 and 668 controls from 63 hospitals. The odds ratio for use of oral contraceptives for less than 1 year was 0.56 (95% CI, 0.22–1.30) and that for use for more than 1 year was 0.36 (95% CI, 0.15–0.83).

Weiderpass *et al*. (1999) conducted a population-based case–control study in Sweden that involved 687 women aged 50–74 years who had had endometrial cancer diagnosed in 1994–95 and 3191 controls. The odds ratio for use of oral contraceptives of less than 3 years was 1.0 (95% CI, 0.7–1.3) and that for use of 3 or more years was 0.5 (95% CI, 0.3–0.7; based on 45 exposed cases and 518 exposed controls). All analyses were adjusted for age, age at menopause, parity and age at last birth, body mass index and duration of previous use of various types of menopausal hormonal therapy.

Jain *et al*. (2000) conducted a case–control study in Ontario, Canada, that involved 512 women who had had endometrial cancer diagnosed in 1994–98 and 513 controls. The odds ratio for ever use of oral contraceptives was 0.66 (95% CI, 0.51–0.84). The estimate of relative risk was adjusted for age, education, parity, weight, age at menarche, tobacco smoking, education, calorie intake and expenditure. No results were given according to duration or recency of use of oral contraceptives.

Parslov *et al*. (2000) conducted a population-based case–control study in Denmark that involved 237 women aged under 50 years who had had endometrial cancer diagnosed in 1987–94 and 538 controls. The odds ratio was 0.4 (95% CI, 0.3–0.7) for use of oral contraceptives of less than 1 year, 0.2 (95% CI, 0.1–0.3) for use of 1–5 years and 0.2 (95% CI, 0.1–0.4) for use of more than 5 years.

Newcomb and Trentham-Deitz (2003) conducted a population-based case–control study in Wisconsin, USA, that involved 591 women aged 40–79 years who had had endometrial cancer diagnosed in 1991–94 and 2045 controls. The relative risk for endometrial cancer was 1.04 (95% CI, 0.74–1.40) for use of oral contraceptives of less than 3 years and 0.73 (95% CI, 0.52–1.02) for use of more than 3 years.

2.3 Cervical cancer

2.3.1 *Introduction*

Five cohort and 16 case–control studies of oral contraceptive use and cervical cancer were evaluated previously (IARC, 1999). On aggregate, the studies showed a small increase in relative risk associated with long-term use. This was observed in four studies in which some analyses were restricted to cases and controls who tested positive for human papillomavirus (HPV) infection. However, the Working Group concluded that biases related to

sexual behaviour, screening and other factors could not be ruled out as possible explanations for the observed associations.

Studies to determine whether the use of hormonal oral contraceptives increases the risk for HPV infection have been reviewed previously (IARC, 1999); it was concluded that oral contraceptives probably do not enhance susceptibility to HPV infection (although in some cultures the sexual behaviours of oral contraceptive users may be more conducive to the acquisition of HPV infection than that of non-users).

It is now generally accepted that persistent infection by one of several carcinogenic strains of sexually transmitted HPV is prerequisite for the development of most cervical carcinomas. However, most infected women do not develop cervical cancer, which indicates that additional co-factors may play an etiological role. The uterine cervical epithelium at the squamocolumnar junction is the tissue from which cervical carcinomas arise and is responsive to estrogens and progestogens. It is therefore reasonable to question whether combined oral contraceptives that contain these two types of hormone act as co-factors to alter the risk for cervical cancer.

Invasive cervical cancer is the final result of a presumed series of events: initial HPV infection, establishment of a persistent infection, resultant development of cervical intraepithelial neoplasia (CIN) of increasing severity (from dysplasia or low-grade CIN to carcinoma *in situ* or CIN3) and finally invasive carcinoma. In-situ and invasive carcinomas are classified histologically as squamous-cell carcinoma, adenocarcinoma or adenosquamous carcinoma if they have both squamous and adenomatous elements. Combined hormonal contraceptives could theoretically increase the risks for cervical cancer by increasing susceptibility to an HPV infection, increasing the probability of persistence in infected women, enhancing the development of mild intraepithelial lesions in women with persistent infection, increasing progression from mild intraepithelial lesions to carcinoma *in situ* or promoting in-situ lesions to invade surrounding tissues (development of invasive carcinomas). Studies of different design are thus needed to address different hypotheses regarding the possible role of hormonal contraceptives in the development of cervical cancer.

It was long acknowledged that cervical cancer was probably caused by one or more sexually transmitted agent (IARC, 2007). A major difficulty in studying the possible role of oral use of combined hormonal contraceptives in the development of cervical cancer was to account adequately for the potential confounding influence of sexual behaviour on the results. After the establishment of the role of HPV in the genesis of cervical carcinoma, studies of oral use of hormonal contraceptives as a possible co-factor were conducted in which HPV infection was assessed using serological tests for HPV antibodies or tests for HPV DNA in cervical scrapings. Cases and controls were classified as HPV-positive or HPV-negative, and estimates of the relative risk for cervical cancer in relation to various features of oral contraceptive use were either calculated separately for HPV-positive women or controlled for HPV status. However, this has been of limited value in assessing the role of oral use of hormonal contraceptives in cervical carcinogenesis because of the lack of sensitivity of the serological tests, and the difficulty in interpreting both negative and positive HPV DNA assays in controls. Another problem in assessing

observed associations of cervical cancer with the oral use of hormonal contraceptives has been the difficulty to rule out the possible effect of selective screening of users (IARC, 2007).

2.3.2 *Meta-analysis*

Since the previous evaluation (IARC, 1999), Smith *et al*. (2003) performed a meta-analysis of data from 28 studies or groups of studies that had been reported up to July 2002; results of the meta-analysis are summarized in Table 7 by duration of use. Risk for cervical cancer was found to increase significantly with duration of oral contraceptive use in most, but not all, case–control and cohort studies, and there was a statistically significant divergence of results in some of the analyses. The combined relative risks from all studies for in-situ and invasive cancers combined in women who used oral contraceptives for < 5, 5–9 and ≥ 10 years were 1.1 (95% CI, 1.1–1.2), 1.6 (95% CI, 1.4–1.7) and 2.2 (95% CI, 1.9–2.4), respectively. The association was stronger in the cohort studies, and risks were higher for in-situ than invasive carcinomas and for adenocarcinomas than squamous-cell carcinomas. An increase in risk with duration of use was apparent in most studies and in all studies combined, but the risk tended to decline with time since cessation of oral contraceptive use regardless of duration of use (Table 8). Invasive and in-situ carcinomas were not separated in these analyses. The association was stronger in women who tested positively for HPV DNA than in those who tested negatively, and the trend in risk with duration of use remained after adjustment for HPV status. The associations were not materially

Table 7. Summary of results of a meta-analysis of data from 28 studies on the risk for cervical cancer in relation to years of oral use of hormonal contraceptives

Type of study or carcinoma	Approximate duration of use (years)		
	< 5	5–9	≥ 10
All cervical cancer types			
Cohort	1.8 (1.4–2.4)	2.2 (1.7–2.9)	3.3 (2.4–4.5)
Case–control	1.1 (1.0–1.2)	1.5 (1.4–1.7)	2.0 (1.8–2.3)
All studies	1.1 (1.1–1.2)	1.6 (1.4–1.7)	2.2 (1.9–2.4)
HPV positive subjects	0.9 (0.7–1.2)	1.3 (1.0–1.9)	2.5 (1.6–3.9)
HPV negative subjects	0.9 (0.6–1.4)	0.9 (0.5–1.4)	1.3 (0.9–1.9)
Adjusted for HPV status[a]	0.9 (0.7–1.1)	1.3 (1.0–1.7)	1.7 (1.3–2.3)
Invasive carcinoma	1.1 (1.0–1.2)	1.4 (1.2–1.6)	2.0 (1.8–2.4)
Carcinoma *in situ*	1.3 (1.2–1.4)	2.1 (1.4–2.4)	2.4 (1.9–2.9)
Squamous-cell carcinoma	1.1 (1.0–1.2)	1.5 (1.3–1.7)	2.0 (1.7–2.3)
Adenocarcinoma	1.5 (1.2–1.8)	1.7 (1.2–2.3)	2.8 (2.0–3.9)

From Smith *et al.* (2003)

[a] Nine studies measured HPV DNA using polymerase chain reaction (PCR)-based assays, one study used HPV antibodies.

Table 8. Summary of results of a meta-analysis of data from four studies[a] on the risk for cervical cancer in relation to years of use and time since last use of oral hormonal contraceptives

Years since last use	Approximate duration of use (years)	
	< 5	≥ 5
< 8	1.4 (1.2–1.5)	2.1 (1.8–2.4)
≥ 8	1.1 (1.0–1.2)	1.4 (1.1–1.9)

From Smith *et al.* (2003)
[a] Including two multicentre collaborative studies

altered after adjustment for numbers of sexual partners, cervical cancer screening, tobacco smoking and use of barrier contraceptives, and were observed in selected studies in both developed and developing countries.

2.3.3 *Methodological considerations*

In some of the analyses of Smith *et al.* (2003), cases of in-situ and invasive carcinoma were not distinguished. Since bias due to selective screening of users of oral contraceptives more probably affected results of studies of in-situ diseases, they were considered separately in this review. In addition, studies of carcinomas *in situ* in screened women were considered separately from studies in general populations.

Although Green *et al.* (2003) reviewed the prevalence of HPV DNA in cancer-free women in 19 case–control studies of cervical cancer and in surveys of HPV prevalence, such studies cannot distinguish between recent, transient HPV infections and persistent infections. Women who have long-term (persistent) infections are more likely to test positive at a single point in time than those who have short-term transient infections; therefore, women who tested positive in these studies may represent persistent infections. No consistent associations were found between prevalence of high- or low-risk types of HPV and any use, current use or long-term use of oral contraceptives. One prospective study of 1995 women in Bogota, Colombia (Molano *et al.*, 2003), reported that clearance of HPV infection was slightly more frequent among women who had ever used oral contraceptives than among non-users (hazard ratio adjusted for age, HPV type, multiplicity of HPV types and parity, 1.38; 95% CI, 1.07–1.77), whereas another prospective study of 621 female university students followed over 24 months (Richardson *et al.*, 2005) found that the use of oral contraceptives was unrelated to clearance of high-risk (age-adjusted hazard ratio, 0.8; 95% CI, 0.3–1.3) or low-risk (hazard ratio, 1.1; 95% CI, 0.6–1.9) HPV infection. It thus seems unlikely that oral contraceptives play a role in the persistence of HPV infections. These results provide evidence that associations of cervical cancer with oral contraceptive use are probably not a result of confounding by detection of HPV.

Infection by high-risk types of HPV has been assessed in two ways: serological tests for HPV antibodies and tests for HPV DNA in cervical tissue (usually using polymerase chain reaction (PCR)-based technology). A small proportion of DNA-based assays and approximately half of the serological tests in cases of cervical cancer give negative results, although nearly all cases are presumably a result of HPV infection, which indicates that these tests are not 100% sensitive (IARC, 2007). Therefore, in some case–control analyses, either all cases (on the assumption that they are all HPV-related) or HPV-positive cases have been compared with HPV-positive controls. In this review, studies based on HPV serology and those based on HPV DNA assays are considered separately, and studies in which cases are compared with HPV-positive controls are distinguished from those in which HPV status is controlled for in the statistical analyses (IARC, 2007).

2.3.4 *Studies of in-situ and invasive cervical cancer in which HPV antibodies were measured*

In two of the most recent studies that presumably used the most sensitive tests available, only 43 (19.5%) of 221 women who had invasive cervical cancer tested positive for antibodies to HPV 16-E7 in an enzyme-linked immunosorbant assay (ELISA) with synthetic peptides (Berrington *et al*., 2002) and 156 (66.4%) of 235 women who had invasive squamous-cell cervical carcinoma tested positive for antibodies to types 16, 18, 31, 45 or 52 using a polymer-based viral-like particle ELISA (Shields *et al*., 2004). Since these tests do not identify correctly all women who have been infected with a high-risk type of HPV, residual confounding can occur in studies in which relative risk estimates are stratified on or controlled for HPV status (IARC, 2007). It has been proposed that all cases (regardless of their HPV antibody status) be compared with HPV-positive controls, but if the proportion of controls that test positive for HPV antibodies varies by use of oral contraceptives, spurious associations with oral contraceptives can occur.

The results of three studies of cervical neoplasia and the use of oral contraceptives, in which HPV antibodies were measured, that have been published since the previous review (IARC, 1999) are summarized in Table 9.

Two studies provided estimates of relative risk for cervical carcinoma in relation to duration of oral contraceptive use after controlling for HPV antibody status. Madeleine *et al*. (2001) compared 150 cases of adenocarcinoma *in situ* in western Washington State, USA, with 651 controls selected from the same population. Berrington *et al*. (2002) compared 221 women aged 20–44 years who had invasive cervical cancer diagnosed in the United Kingdom between 1984 and 1988 with 393 control women selected from the same general practitioners' registers as the cases. In contrast to the study of Shields *et al*. (2004), the percentage of controls with HPV antibodies decreased with duration of oral contraceptive use in this study. [The trend in risk ratios for seropositivity was not statistically significant, but the variables that were controlled for in their calculation was not indicated.] In both of these studies, risk increased with duration of use before controlling for HPV antibody status. Berrington *et al*. (2002) found similar trends in women with and

Table 9. Relative risks for three types of cervical cancer in relation to duration of oral use of hormonal contraceptives controlled for the presence or absence of human papillomavirus (HPV) antibodies

Reference	Lesion	Years of use	No. of subjects		Relative risk adjusted for HPV serology	
			Cases	Controls	No	Yes
Madeleine *et al.* (2001)	Adeno-carcinoma *in situ*	None	8	74	1.0	–
		Ever	124	384	2.7 (1.2–5.8)	4.0 (1.7–9.4)
		1–5	64	250	2.1 (1.0–4.8)	–
		6–11	40	101	3.4 (1.5–8.0)	–
		≥ 12	20	33	5.5 (2.1–14.6)	–
					p for trend < 0.0001	
Berrington *et al.* (2002)	Invasive cervical cancer	None	12	49	[1.0][a]	1.0 (0.5–2.1)
		1–4	73	159	[1.9][a]	1.6 (1.2–2.2)
		5–9	76	117	[2.6][a]	1.9 (1.3–2.6)
		≥ 10	60	68	[3.6][a]	2.8 (1.9–4.2)
Shields *et al.* (2004)	Invasive squamous - cell	None	123	81		1.0[b]
		< 5	59	69	Data not given	0.6 (0.4–0.9)[b]
		5–10	33	30		0.7 (0.4–1.3)[b]
		> 10	20	26		0.5 (0.3–1.0)[b]

[a] Crude estimates calculated by the Working Group from the numbers of cases and controls shown in the table

[b] Results restricted to women with a positive test for HPV antibodies

without HPV antibodies (data not shown) but, after controlling for HPV antibodies, the trend was slightly attenuated; however, Madeleine *et al.* (2001) found that the risk estimate in women who ever had used oral contraceptives was increased after controlling for HPV antibodies.

A case–control study in the USA (Shields *et al.*, 2004) compared 235 cases of invasive squamous-cell cancer (all reasonably presumed to have been exposed to HPV) with 206 (43.0%) of 486 population-based controls who tested positive for antibodies against HPV types 16, 18, 31, 45 or 52. In relation to duration of oral contraceptive use, there was no trend in risk for cervical cancer when cases were compared with all controls [data not presented in the published report], but the prevalence of HPV antibodies in the controls increased, which resulted in a decrease in risk in analyses that compared cases with seropositive controls. [Although the results of the US study were controlled for time since last Pap smear, screening bias could have influenced the results if most cases were detected at screening and if oral contraceptive users were more likely to have been screened than non-users. The discrepant results in the relationship of HPV antibody prevalence in the controls with duration of oral contraceptive use in the studies of Berrington *et al.* (2002) and Shields *et al.* (2004) render the results of both investigations questionable.]

2.3.5 *Studies in which cervical tissue was assayed for HPV DNA*

(*a*) *Cervical carcinoma* in situ

Among women with HPV infection at enrolment into a cohort in Manchester, United Kingdom (Deacon *et al.*, 2000), relative risks for subsequent development of CIN3 in current and former users of oral contraceptives were 1.3 (95% CI, 0.7–2.5) and 1.2 (95% CI, 0.6–2.1), respectively. Risk did not vary linearly with increasing duration of use or with time since last use, although the relative risk in women with more than 8 years of use was 1.5 (95% CI, 0.8–2.9).

Castle *et al.* (2002) followed 1812 women who had tested positive for high-risk HPV DNA when they enrolled in a 10-year prospective study of cervical neoplasia at Kaiser Permanente in Portland, OR, USA, for a period of 122 months. The risks for developing CIN3 in current users of oral contraceptives versus non-users was 0.84 (95% CI, 0.49–1.5).

It was shown in the studies below that women who tested positive for HPV DNA in their cervical tissue had an increasing risk for in-situ cervical cancer with increasing duration of oral contraceptive use (Table 10).

In a multicentre case–control study of squamous-cell carcinoma and adenocarcinoma of the cervix in the USA (Lacey *et al*., 1999), cases were selected from hospital admissions and population controls were selected by random-digit dialling. Forty-eight cases of squamous-cell carcinoma *in situ* and 33 cases of adenocarcinoma *in situ* (regardless of their HPV DNA status) were compared with 48 controls who tested positive to one or more of 18 high-risk types of HPV. As shown in Table 10, the relative risk in current users was increased for in-situ adenocarcinoma but not significantly for in-situ squamous-cell carcinoma, and a trend in risk with duration of use was observed only for adenocarcinoma (p for trend = 0.03).

In a study nested in a cohort of screened women in Uppsala county, Sweden, Ylitalo *et al*. (1999) compared 178 cases of carcinoma *in situ* (most presumably squamous-cell) who had HPV type 16 or 18 in pre-diagnostic smears with 178 matched controls with the same HPV types. Risk was higher in current than in former users of oral contraceptives and increased with duration of use (p-value of test for trend = 0.12). A similar trend was observed for women who tested negative for HPV 16 or 18.

In a collaborative study in Colombia and Spain (Moreno *et al*., 2002), 211 cases of squamous-cell carcinoma *in situ* (selected from hospitals, pathology laboratories and screening clinics) who tested positive for one of 14 high-risk types of HPV were compared with 28 controls with normal cervical cytology (selected from the same place of recruitment as the cases) but who had similar positive tests for a high-risk type of HPV. Risk increased with duration of use of oral contraceptives.

[The Working Group noted that the results of the studies of Lacey *et al.* (1999) and Moreno *et al.* (2002) were based on a very small number of controls who were HPV-positive. The relative risk estimates therefore had wide confidence intervals.]

In conclusion, the results from two case–control studies of screened women (Ylitalo *et al.*, 1999; Moreno *et al.*, 2002) showed a significant increase in risk for squamous-cell

Table 10. Relative risks for in-situ cervical neoplasia in relation to oral use of hormonal contraceptives in case–control studies in which cases were compared with controls with high-risk types of HPV DNA in their cervical tissue

Reference, country	Type of study	Reported diagnosis in the cases	Oral contraceptive use	No. of subjects		Relative risk (95% CI)
				Cases	Controls	
Lacey *et al.* (1999), USA	Population-based case–control	Squamous-cell carcinoma *in situ*	Never	7	11	1.0 (reference)
			Former	32	27	1.0 (0.4–2.8)
			Current	9	10	1.3 (0.5–3.6)
			Years of use			
			≤ 2	10	9	0.9 (0.3–3.0)
			3–6	15	12	0.9 (0.3–2.8)
			> 6	16	16	1.2 (0.3–3.9)
		Adeno-carcinoma *in situ*	Never	2	11	1.0 (reference)
			Former	13	27	2.0 (0.4–9.9)
			Current	18	10	12.6 (2.5–64.2)
			Years of use			
			≤ 2	7	9	3.2 (0.6–17.2)
			3–6	7	12	1.7 (0.3–9.5)
			> 6	17	16	6.0 (1.2–30.7)
Ylitalo *et al.* (1999), Sweden	Case–control nested in screening cohort	Carcinoma *in situ*	Never	48	84	1.0 (reference)
			Former	241	239	1.5 (0.8–3.1)
			Current	77	48	2.7 (1.1–6.7)
			Years of use			
			< 2			1.6 (0.7–3.7)
			2–9			2.2 (1.0–4.9)
			≥ 10			2.8 (1.1–6.9)
Moreno *et al.* (2002), Spain and Columbia	Screening-based case–control	Squamous-cell carcinoma *in situ*	Never	65	14	1.0 (reference)
			Ever	146	14	2.5 (1.0–6.8)
			> 5 years of use	92	9	2.9 (1.2–7.1)

CI, confidence interval; HPV, human papillomavirus

intraepithelial cervical lesions with duration of oral contraceptive use, but the population-based (but not screening-based) study did not show such an association (Lacey *et al.*, 1999); two prospective studies showed no significant association of use of oral contraceptives with risk for CIN3 (Deacon *et al.*, 2000; Castle *et al.*, 2002). The studies of Lacey *et al.* (1999) and Madeleine *et al*. (2001) showed a strong increasing trend in risk for adenocarcinoma *in situ* with duration of oral contraceptive use, but in neither study were the controls selected from screening programmes. [Since most cases of adenocarcinoma *in situ* are detected at screening, these studies could have yielded spurious results if the probability of being screened is related to duration of oral contraceptive use.]

(*b*) *Invasive cervical neoplasia*

In a study in Thailand (Thomas *et al*., 2001a), 126 women with in-situ or invasive cervical cancer that contained HPV 16 and 42 women with HPV 18-associated adenomatous cervical carcinoma were compared with 250 hospital control women who had no evidence of HPV infection in their cervical scrapings. Relative risks for HPV 16- and HPV 18-associated tumours in women who had ever used oral contraceptives were estimated to be 1.3 (95% CI, 0.8–2.0) and 1.4 (95% CI, 0.7–2.7), respectively. No trends in risk with duration of oral contraceptive use were found. [Thus, if oral contraceptives do enhance the risk for cervical cancer, they appear to do so equally for women who are infected with HPV types 16 and 18.]

Two case–control studies of invasive cervical cancer, in which PCR-based assays for HPV DNA were used and in which there was sufficient use of oral contraceptives for meaningful analysis, are summarized in Table 11 (Moreno *et al.*, 2002; Shapiro *et al.*, 2003).

Moreno *et al*. (2002) conducted a multinational pooled analysis with studies from Brazil, Columbia, Morocco, Paraguay, Peru, the Philippines, Spain and Thailand which included overall more than 1400 cases and 1900 controls. Cases were women who had been newly diagnosed with invasive squamous-cell cervical carcinoma and admitted to participating hospitals and controls were women with no cervical cancer who were selected from

Table 11. Relative risks for invasive cervical carcinoma in relation to oral use of hormonal contraceptives in two case–control studies in which cases were compared with controls with high-risk types of HPV DNA in their cervical tissue

Reference, country	Oral contraceptive use	No. of women		Relative risk (95% CI)
		Cases	Controls	
Moreno *et al.* (2002), eight countries	Never	1006	149	1.0
	Ever	459	78	1.3 (0.88–1.91)
	> 5 years of use	239	19	4.0 (2.0–8.0)
Shapiro *et al.* (2003), South Africa	Never	364	166	1.0
	Ever	160	88	0.9 (0.7–1.3)
	Years of use			
	< 1	63	41	0.8 (0.5–1.2)
	1–4	58	32	0.9 (0.6–1.6)
	≥ 5	32	13	1.3 (0.6–2.7)
	Years since last use			
	current/< 1	16	9	1.3 (0.7–2.4)
	1–4	17	8	1.9 (1.0–3.5)
	5–9	20	11	1.0 (0.6–1.6)
	10–14	25	19	0.8 (0.5–1.3)
	≥ 15	73	38	0.7 (0.5–0.9)

CI, confidence interval; HPV, human papillomavirus

the same hospitals as the cases. Cervical tissue from all cases and controls was tested for high-risk types of HPV DNA using PCR-based technology. In analyses in which HPV DNA-positive cases and controls were compared, the estimate of the relative risk in women who had ever used oral contraceptives was 1.3 (95% CI, 0.88–1.91), and the individual estimates among the participating countries were significantly heterogeneous (not shown). However, there was a clear increase in risk for more than 5 years of duration of use, and the estimates of relative risks in such users were not significantly heterogeneous among the study centres.

In a large study of invasive cervical cancer published after the meta-analysis by Smith *et al.* (2003), Shapiro *et al*. (2003) recruited 484 women who had invasive squamous-cell cervical cancer and 40 women who had invasive cervical adenocarcinoma from two tertiary care hospitals in South Africa. A total of 1541 control women were recruited from the same hospitals, or from local hospitals or community health centres from which the cases were referred. The overall results are consistent with those of the meta-analysis of Smith *et al.* (2003). No significant trend in risk was seen with duration of use, but a statistically non-significant increase in risk was observed for duration of use of ≥ 5 years. The relative risks for users of oral contraceptives for < 1, 1–4, ≥ 5 years were 0.8 (95% CI, 0.5–1.2), 0.9 (95% CI, 0.6–1.6) and 1.3 (95% CI, 0.6–2.7), respectively. As in the meta-analysis, the risk declined with time since last use: the relative risks for current users and for women who had last used oral contraceptives < 1, 1–4, 5–9, 10–14 and ≥ 15 years previously were 1.3 (95% CI, 0.7–2.4), 1.9 (95% CI, 1.0–3.5), 1.0 (95% CI, 0.6–1.6), 0.8 (95% CI, 0.5–1.3) and 0.7 (95% CI, 0.5–0.9), respectively. Cervical scrapings from all controls were tested for 13 high-risk types of HPV and 254 (16.5%) were positive. As shown in Table 11, when the cases (all reasonably presumed to be HPV-positive) were compared with the HPV-positive controls, risk was not significantly increased in women who had ever used oral contraceptives. Among the controls, the proportions that tested positive for HPV DNA in users and non-users of oral contraceptives were 14.6 and 17.7%, respectively. Although this proportion declined slightly with duration of oral contraceptive use (16.3, 14.7 and 10.7% in users of < 1, 1–4 and ≥ 5 years), it did not vary consistently by time since last use, and the relative risks did not differ appreciably from those based on the comparison of cases to all controls (not shown).

In a study in Latvia (Silins *et al*., 2004), 223 women who had invasive cervical carcinoma were identified in the oncology centre, which treats about 90% of all cases in the country, and were compared with 239 healthy controls selected from the Latvian population registry. Serum from all women were tested for immunoglobulin G (IgG) antibodies to HPV types 6, 11, 16, 18 and 33, and cervical scrapings were tested for 18 high-risk and nine low-risk HPV types. The relative risk for cervical cancer in women who had ever used oral contraceptives was 0.4 before controlling for HPV, 0.4 (95% CI, 0.2–1.0) after controlling for HPV 16/18 antibodies and 0.4 (95% CI, 0.2–1.1) after controlling for HPV in cervical tissue.

In a hospital-based study of 198 cases of invasive cervical cancer and 202 controls conducted in Algeria (Hammouda *et al.*, 2005), relative risks for users of oral contra-

ceptives for < 5, 5–9 and ≥ 10 years were 0.6 (95% CI, 0.3–1.2), 0.5 (95% CI, 0.3–1.1) and 0.8 (95% CI, 0.4–1.6), respectively. Although cases and controls were tested for HPV DNA, the results were presented separately or were adjusted for HPV DNA status.

On aggregate, there is inconsistent evidence that the oral use of hormonal contraceptives alters the risk for invasive cervical carcinoma, although studies in which all (Shapiro *et al*., 2003) or most (Moreno *et al*., 2002) cases were compared with HPV-positive controls should theoretically be the best design to attempt to determine such an alteration in women with an HPV infection.

2.3.6 *Studies conducted to determine whether oral contraceptives alter the risk for progression of cervical lesions*

In a prospective study in Brazil (Cavalcanti *et al*., 2000), 280 women who were initially diagnosed with intraepithelial lesions were tested for HPV types by in-situ hybridization. The risk for progression to carcinoma *in situ* or invasive carcinoma was 1.4 (95% CI, 0.4–5.6) in users of oral contraceptives compared with non-users. [The methods used in this study could not be evaluated adequately from the published report.]

Two studies have been conducted in which cases of invasive cancer were directly compared with cases of carcinoma *in situ*. Relative risk estimates from such studies can be interpreted as risks for progression from in-situ to invasive disease.

Thomas *et al*. (2002) analysed data from five centres in Chile, Mexico and Thailand that participated in the WHO Collaborative Study of Neoplasia and Steroid Contraceptives. Based on data from more than 1300 women who had squamous-cell carcinoma *in situ* and more than 2000 women who had invasive squamous-cell carcinoma, those who had ever used oral contraceptives and those who had used them for 1–12, 13–60 and > 60 months had relative risks for invasive versus in-situ disease of 1.0 (95% CI, 0.8–1.2), 1.0 (95% CI, 0.8–1.3), 0.9 (95% CI, 0.7–1.1) and 1.0 (95% CI, 0.8–1.3), respectively, compared with non-users. [Although HPV assays were not performed in this study, since HPV is a necessary cause of cervical carcinoma, this would only be needed in the improbable event that oral contraceptive use was related differently to various types of high-risk HPVs with different potentials to cause progression to invasive disease.]

In a study in Thailand (Thomas *et al*., 2001b), HPV assays were performed on 190 women who had invasive squamous-cell cervical carcinoma and 75 women who had carcinoma *in situ*. After controlling for HPV types, the relative risk in women who had ever used oral contraceptives was 1.3 (95% CI, 0.8–2.0) for HPV-16 and 1.4 (95% CI, 0.7–2.7) for HPV 18 positivity.

2.4 Ovarian cancer

This section reviews studies on the use of combined oral contraceptives and ovarian cancer that have been published or updated since the last evaluation (IARC, 1999). On the basis of four cohort studies and 21 case–control studies, the previous Working Group

(IARC, 1999) found a substantially reduced incidence of ovarian cancer among combined oral contraceptive users, with a consistent inverse duration–risk relationship.

2.4.1 *Descriptive studies*

Analyses of mortality trends in several areas of Europe (Adami *et al.*, 1990; La Vecchia *et al.*, 1998; Levi *et al*., 2004; Bray *et al*., 2005) and in the USA (Gnagy *et al*., 2000; Tarone & Chu, 2000) showed that women born after 1920 — i.e. the generations who had used oral contraceptives — experienced reduced rates of ovarian cancer. The downward trends were larger in countries where oral contraceptives had been used more widely (Bray *et al*., 2005).

2.4.2 *Cohort studies*

Two cohort studies have been updated (Beral *et al*., 1999; Vessey *et al*., 2003) and an additional study has been published (Kumle *et al*., 2004). The main findings from these are given in Table 12.

The Royal College of General Practitioners' study was based on 46 000 women recruited in 1968 from 1400 British general practices (Beral *et al.*, 1988); 30 cases of ovarian cancer were observed up to 1987, which corresponded to multivariate relative risks of 0.6 (95% CI, 0.3–1.4) for women who had ever used oral contraceptives and of 0.3 for those who had used them for 10 years or longer. Adjustment was made for age, parity,

Table 12. Cohort studies of oral use of combined contraceptives and ovarian cancer, 1998–2004

Reference, country	No. of cases	Relative risk (95% CI)	
		Ever use	Longest use
Ramcharan *et al.* (1981), USA	16	0.4 (0.1–1.0)	–
Beral *et al*. (1988, 1999), United Kingdom	55	0.6 (0.3–1.0)	0.2 (0.1–1.3) (≥ 10 years)
Hankinson *et al.* (1995), USA	260	1.1 (0.8–1.4)	0.7 (0.4–1.1) (≥ 5 years)
Vessey & Painter (1995), Vessey *et al.* (2003), United Kingdom	61	0.4 (0.2–0.7)	0.2 (0.1–0.6) (> 8 years)
Kumle *et al*. (2004), Norway and Sweden	214[a]	0.6 (0.5–0.7)	0.1 (0.01–0.6) (> 15 years)

CI, confidence interval
[a] 135 invasive, 79 borderline

smoking and social class. At the 25-year follow-up for mortality (Beral *et al*., 1999), 55 deaths from ovarian cancer were observed, which corresponded to relative risks of 0.6 (95% CI, 0.3–1.0) for women who had ever used oral contraceptives and 0.2 (95% CI, 0.1–1.3) for ≥ 10 years of use, based on one death. The protection persisted for ≥ 20 years since cessation of use (relative risk, 0.7; 95% CI, 0.4–1.4).

The Oxford Family Planning Association study was based on 17 032 women who were enrolled between 1968 and 1976 from various family planning clinics in the United Kingdom (Vessey & Painter, 1995). Adjustment was made for age and parity. At the 32-year follow-up of the same cohort at 31 December 2000, 61 deaths from ovarian cancer were observed. The relative risks for oral use of combined hormonal contraceptives were 0.4 (95% CI, 0.2–0.7) for ever use, 1.1 (95% CI, 0.6–2.0; 17 deaths) for a duration of ≤ 48 months, 0.2 (95% CI, 0.0–0.5; three deaths) for 49–96 months and 0.2 (95% CI, 0.1–0.6; five deaths) for ≥ 97 months (Vessey *et al*., 2003).

The Norwegian-Swedish Women's Lifestyle and Health cohort included 103 551 women aged 30–49 years in 1991–92 (Kumle *et al*., 2004). During the follow-up through to 2000, 214 incident cases of ovarian epithelial neoplasms were observed (135 invasive, 79 borderline). Relative risks were adjusted for country, age, parity, menopausal status and use of menopausal hormonal therapy. Compared with women who had never used oral contraceptives, the overall relative risk for those who ever had was 0.6 (95% CI, 0.5–0.7); the relative risk for progestogen-only preparations was 0.5 (95% CI, 0.2–1.2). Ever use was associated with relative risks of 0.6 (95% CI, 0.4–0.8) for invasive cancers and 0.7 (95% CI, 0.5–1.2) for borderline cases. For duration of use, the relative risks were 0.9 for < 1 year of use, 0.5 for 1–4 years, 0.6 for 5–9 years, 0.5 for 10–14 years and 0.1 for ≥ 15 years. The trend in risk with duration of use was significant, and the relative risk per year of use was 0.91 (95% CI, 0.85–0.96). Corresponding values per year of use were 0.89 (95% CI, 0.84–0.94) for invasive and 0.96 (95% CI, 0.91–1.0) for borderline tumours.

2.4.3 *Case–control studies*

Studies that include information on oral contraceptives and ovarian cancer that have been published or updated since the previous evaluation (IARC, 1999) are summarized in Table 13.

In a population-based study of 824 cases and 860 controls diagnosed between 1990 and 1993 in three Australian states (New South Wales, Victoria and Queensland), Purdie *et al*. (1995) found a relative risk of 0.5 (95% CI, 0.4–0.7) for any use of oral contraceptives and 0.3 (95% CI, 0.2–0.4) for ≥ 10 years of use. The response rate was 90% for cases and 73% for controls. Adjustment was made for sociodemographic factors, family history of cancer, use of talc, smoking and reproductive and hormonal factors. A subsequent analysis of the same dataset (Siskind *et al*., 2000) indicated that the reduction in risk was 7% (95% CI, 4–9%) per year of use, that the protection was observed in various strata of age at first use and that there was little evidence that the effect waned with time since last use. When this dataset was analysed separately by histological type, the relative

risk was 0.62 (95% CI, 0.37–1.04) for mucinous and 0.52 (95% CI, 0.39–0.68) for non-mucinous ovarian cancers (Purdie *et al*., 2001).

Table 13. Case–control studies of oral use of combined contraceptives and ovarian cancer

Reference, country	Type of study	Relative risk (95% CI)[a]			
		No. of cases (age in years)	Ever use	Longest use	Duration (years)
Hildreth *et al.* (1981), USA	Hospital-based	62 (45–74)	0.5 (0.1–1.7)	NR	
Weiss *et al.* (1981a), USA	Population-based	112 (36–55)	0.6 (NR)	0.5 (0.2–1.3)	≥ 9
Willett *et al.* (1981), USA	Nested in a cohort	47 (< 60)	0.8 (0.4–1.5)	0.8 (0.3–2.1)	> 3
Cramer *et al.* (1982), USA	Population-based	144 (< 60)	0.4 (0.2–1.0)	0.6	> 5
Franceschi *et al.* (1982), Italy	Hospital-based	161 (19–69)	0.7 (0.4–1.1)	NR	
Rosenberg *et al.* (1982), USA	Hospital-based	136 (< 60)	0.6 (0.4–0.9)	0.3 (0.1–0.8)	≥ 5
Risch *et al*. (1983), USA	Population-based	284 (20–74)	[0.5] (NR)	NR	
Tzonou *et al.* (1984), Greece	Hospital-based	150 (NR)	0.4 (0.1–1.1)	NR	
CASH (1987b), USA	Population-based	492 (20–54)	0.6 (0.5–0.7)	0.2 (0.1–0.4)	≥ 10
Harlow *et al.* (1988), USA	Population-based	116 (20–79)	0.4 (0.2–0.9)	0.4 (0.2–1.0)	> 4
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
Booth *et al.* (1989), United Kingdom	Hospital-based	235 (< 65)	0.5 (0.3–0.9)	0.1 (0.01–1.0)	> 10
Hartge *et al.* (1989), USA	Hospital-based	296 (20–79)	1.0 (0.7–1.7)	0.8 (0.4–1.5)	> 5
Shu *et al*. (1989), China	Population-based	229 (18–70)	1.8 (0.8–4.1)	1.9 (0.4–9.3)	> 5
WHO Collaborative Study (1989a), 7 countries	Hospital-based	368 (< 62)	0.8 (0.6–1.0)	0.5 (0.3–1.0)	> 5
Parazzini *et al.* (1991a), Italy	Hospital-based	505 (22–59)	0.7 (0.5–1.0)	0.5 (0.3–0.9)	≥ 2
Parazzini *et al.* (1991b), Italy	Hospital-based	91 (23–64)	0.3 (0.2–0.6)	0.2 (0.1–0.6)	≥ 2

Table 13 (contd)

Reference, country	Type of study	Relative risk (95% CI)[a]			
		No. of cases (age in years)	Ever use	Longest use	Duration (years)
Polychronopoulou *et al.* (1993), Greece	Hospital-based	189 (< 75)	0.8 (0.2–3.7)	NR	
Risch *et al.* (1994, 1996), Canada	Population-based	450 (35–79)	0.5 (0.4–0.7)	0.3 (0.2–0.6)	≥ 10
Rosenberg *et al.* (1994), USA	Hospital-based	441 (< 65)	0.8 (0.6–1.0)	0.5 (0.2–0.9)	≥ 10
Purdie *et al.* (1995), Australia	Population-based	824 (18–79)	0.5 (0.4–0.7)	0.3 (0.2–0.4)	≥ 1
Godard *et al.* (1998), Canada	Population-based	170 (20–84)	p = 0.038	0.33 (0.13–0.82)	> 10
Salazar-Martinez *et al.* (1999), Mexico	Hospital-based (outpatient controls)	84 (mean, 54.6)	0.4 (0.2–0.8)	0.4 (0.2–0.8)	> 1
Wittenberg *et al.* (1999), USA	Population-based	322 (20–79)	0.9 (0.4–2.1)[b] 0.8 (0.6–1.3)[c]	0.4 (0.1–1.4)[b] 0.6 (0.4–1.0)[c]	≥ 5
Beard *et al.* (2000), USA	Population-based	103 (15–85+)	1.1 (0.6–2.3)	0.8 (0.4–1.1)	≥ 0.5
Cramer *et al.* (2000), USA	Population-based	563 (mean, 51)	0.7 (0.5–0.8)	–	–
Greggi *et al.* (2000), Italy	Hospital-based	440 (≤ 80)	0.4 (0.3–0.6)	0.3 (0.2–0.5)	≥ 2
Ness *et al.* (2000), USA	Population-based	767 (< 70)	0.6 (0.3–0.8)	0.3 (0.1–0.5)	≥ 10
Chiaffarino *et al.* (2001), Italy	Hospital-based	1031 (< 80)	0.9 (0.7–1.2)	0.5 (0.3–0.9)	≥ 5
Riman *et al.* (2001), Sweden	Population-based	193 (50–74) borderline	1.2 (0.9–1.8)	1.2 (0.6–2.1)	≥ 10
Royar *et al.* (2001), Germany	Population-based	282 (< 75)	0.5 (0.3–0.7)	0.4 (0.3–0.6)	> 5
Riman *et al.* (2002), Sweden	Population-based	655 (50–74)	0.7 (0.6–0.9)	0.4 (0.2–0.6)	≥ 10
Schildkraut *et al.* (2002), USA	Population-based	390 (20–54)	1.0 referent[d] 0.0[e] 2.1 (1.2–3.7)[f] 1.6 (0.9–3.0)[g] 2.9 (1.8–4.5)[h]		

Table 13 (contd)

Reference, country	Type of study	Relative risk (95% CI)[a]			
		No. of cases (age in years)	Ever use	Longest use	Duration (years)
Tung *et al*. (2003), USA (Hawaii and California)	Population-based	558 (mean, borderline, 48; invasive, 58)	0.6 (0.4–0.8)	0.4 (0.3–0.6)	> 5
Mills *et al*. (2004), USA	Population-based	256 (mean, 56.6)		0.4 (0.2–0.7)	> 10
Pike *et al*. (2004), USA	Population-based	477 (18–74)		0.5 (0.2–0.8)	≥ 10
Pooled analyses					
Franceschi *et al.* (1991a), Greece, Italy, United Kingdom	Three hospital-based studies	971 (< 65)	0.6 (0.4–0.8)	0.4 (0.2–0.7)	≥ 5
Harris *et al*. (1992), USA	Pooled analysis of 12 US population- and hospital-based case–control studies	327 (NR)	0.8 (0.6–1.1)	0.6 (0.4–0.9)	> 5
Whittemore *et al.* (1992), USA	Same pooled analysis as Harris *et al*. (1992)	2197 (NR)	0.7 (0.6–0.8)	0.3 (0.2–0.4)	≥ 6
John *et al.* (1993), USA	Pooled analysis of 7 of the 12 studies in the pooled analysis of Whittemore *et al.* (1992)	110 (mean, invasive, 53.3; borderline, 37.1)	0.7 (0.4–1.2)	0.6 (0.2–1.6)	≥ 6
Bosetti *et al.* (2002), Greece, Italy, United Kingdom	Re-analysis of 6 studies	2768 (< 40–69)	0.7 (0.6–0.8)	0.4 (0.3–0.6)	≥ 5

CI, confidence interval; NR, not reported
[a] Whenever available
[b] Mucinous
[c] Non-mucinous
[d] Potency progestogen/estrogen high/high
[e] Potency progestogen/estrogen high/low
[f] Potency progestogen/estrogen low/high
[h] Non-users, potency progestogen/estrogen low/low

A case–control study was conducted in 1995–96 in Montréal, Canada, on 170 women aged 20–84 years with invasive or borderline ovarian cancer and 170 population controls (Godard *et al*., 1998). Fifty-eight cases were familial (i.e. with a history of breast or ovarian cancer in first-degree relatives) and 111 were non-familial. Overall, 50% of cases versus 61.8% of controls reported ever having used oral contraceptives (p = 0.038). The multivariate model was based on 152 cases (101 sporadic, 51 familial) and 152 controls. Compared with women who had used oral contraceptives for < 1 year (66 cases, 88 controls), the relative risk was 0.77 (95% CI, 0.44–1.36) for use of 1–5 years, 0.49 (95% CI, 0.27–0.91) for use of 6–10 years and 0.33 (95% CI, 0.13–0.82) for use of 11–25 years.

A case–control study was conducted in Mexico city in 1995–97 on 84 cases of ovarian epithelial cancer and 668 outpatient controls (Salazar-Martinez *et al*., 1999). The response rate was 100% for cases and 93% for controls. Overall, 13 (15.4%) cases versus 195 (29.2%) controls had ever used oral contraceptives (odds ratio, 0.36; 95% CI, 0.15–0.83). Compared with never users of oral contraceptives, the multivariate relative risks, adjusted for age, parity, breast-feeding, smoking, diabetes mellitus, physical activity, menopausal status and body mass index, were 0.56 (95% CI, 0.22–1.3) for ≤ 1 year of use and 0.36 (95% CI, 0.15–0.83) for > 1 year of use.

An extraction from a previously published case–control study from western Washington State, USA, included a separate analysis of 43 mucinous and 279 non-mucinous ovarian epithelial cancers compared with 426 population controls aged 20–79 years (Wittenberg *et al*., 1999). The participation rate was approximately 64% for cases and 68% for controls. After adjustment for age and parity, the relative risk for ever use of oral contraceptives was 0.9 (95% CI, 0.4–2.1) for mucinous and 0.8 (95% CI, 0.6–1.3) for non-mucinous cancers. Corresponding values for duration of use of ≥ 5 years were 0.4 (95% CI, 0.1–1.4) and 0.6 (95% CI, 0.4–1.0) and for time since last use ≥ 15 years were 1.2 (95% CI, 0.5–3.0) and 1.0 (95% CI, 0.7–1.7), respectively.

In a population-based case–control study of 103 incident cases of ovarian cancer diagnosed between 1975 and 1991 and 103 controls from Olmsted County, MN, USA, the age-matched relative risk was 1.1 (95% CI, 0.6–2.3) for ever having used oral contraceptives and 0.8 (95% CI, 0.4–1.1) for ≥ 6 months of use (Beard *et al*., 2000).

A population-based case–control study conducted between 1992 and 1997 in Massachusetts and New Hampshire, USA, included 563 incident cases and 523 controls (Cramer *et al*., 2000). The overall participation rate was about 55% for both cases and controls. Ever (≥ 3 months) use of oral contraceptives was reported by 226 (40%) cases and 276 (53%) controls [which corresponded to a crude odds ratio of 0.66 (95% CI, 0.52–0.84)].

A hospital-based case–control study of ovarian cancer was conducted between 1992 and 1997 in the Rome area, Italy, and included 440 cases and 868 hospital controls, with a response rate over 97% for both cases and controls (Greggi *et al*., 2000). The multivariate odds ratio (adjusted for age, education, parity and family history of ovarian cancer) for ever having used oral contraceptives was 0.4 (95% CI, 0.3–0.6) and that for long-term use (≥ 2 years) was 0.3.

A study was conducted in Delaware Valley, USA, which included contiguous counties in Pennsylvania, New Jersey and Delaware, between 1994 and 1998 (Ness *et al*., 2000) on 767 cases and 1367 community controls under the age of 70 years; the response rate was 88% for incident cases and 72% for controls. A relative risk of 0.6 (95% CI, 0.3–0.8) for ever having used oral contraceptives and 0.3 (95% CI, 0.1–0.5) for ≥ 10 years of use was found after adjustment for age, parity and family history of ovarian cancer. The protection was similar for use of low-estrogen/low-progestogen and for high-estrogen (≥ 50 μg ethinylestradiol)/high-progestogen (≥ 0.5 mg norgestrel) formulations (relative risk ranged between 0.5 and 0.7 for women who had ever used various combinations). The reduced risk was similar across strata of gravidity and when hormonal preparations were used for contraceptive or non-contraceptive uses (e.g. endometriosis) (Ness *et al*., 2001). Another report from the same dataset considered 616 invasive and 151 borderline tumours and various histotypes separately (Modugno *et al*., 2001). The relative risk per year of oral contraceptive use was 0.94 (95% CI, 0.91–0.96) for all invasive cancers, 0.93 (95% CI, 0.90–0.97) for serous (218 cases), 0.93 (95% CI, 0.85–1.02) for mucinous (52 cases), 0.93 (95% CI, 0.88–0.97) for endometrioid (136 cases) and 0.98 for other invasive tumours (150 cases), 0.92 (95% CI, 0.88–0.98) for all borderline cancers, 0.91 (95% CI, 0.85–0.98) for serous (79 cases) and 0.94 (95% CI, 0.88–1.01) for mucinous (60 cases) ovarian borderline tumours. In this dataset, information on androgenicity of oral contraceptives was available for 568 cases and 1026 controls (Greer *et al*., 2005). The relative risk was 0.52 (95% CI, 0.35–0.76) for use of androgenic oral contraceptives and 0.58 (95% CI, 0.45–0.78) for use of non-androgenic oral contraceptives. No difference in risk between androgenic and non-androgenic formulations was observed in relation to duration of use, age at first use or time since last use. Another report (Walker *et al*., 2002) showed a protective effect in both women with and those without a family history of ovarian cancer, although the number of women with a family history was small (three cases, nine controls).

In a case–control study conducted in Italy between 1983 and 1991 on 971 incident cases under 75 years of age and 2758 hospital controls that was included in the previous evaluation (IARC, 1999), no appreciable difference in the relation between oral use of hormonal contraceptives and the risk for ovarian cancer was observed between women with and those without family history of ovarian and breast cancer (Tavani *et al*., 2000). The response rate was over 95% for both cases and controls. The relative risk for women who had ever used oral contraceptives was 0.7 in those with and 0.8 in those without a family history. Adjustment was made for age and area of residence. When different histological types of ovarian cancer were considered in the same dataset, the relative risk for ever use of oral contraceptives was 0.7 (95% CI, 0.5–1.0) for serous, 1.4 (95% CI, 0.6–3.4) for mucinous and 0.8 (95% CI, 0.2–2.9) for endometrioid neoplasms (Parazzini *et al*., 2004).

A multicentric study was conducted between in 1992 and 1997 in four areas of northern, central and southern Italy and included 1031 cases of ovarian cancer and 2441 hospital controls under the age of 80 years (Chiaffarino *et al*., 2001). The response rate was over 95% for both cases and controls. Adjustment was made for age, centre, education, parity and family history of ovarian and breast cancer. The multivariate relative risk was

0.9 for ever having used hormonal contraceptives and 0.5 for ≥ 5 years of use. In the same dataset, the relative risk for ≥ 5 years of oral contraceptive use was 0.9 for women with a family history of breast or ovarian cancer in first-degree relatives and 0.5 for those without (Tavani *et al*., 2004).

A case–control study was conducted between 1993 and 1996 in two regions of Germany and included 282 patients with ovarian cancer (invasive and borderline) aged 20–75 years and 533 population controls (Royar *et al*., 2001). [The overall response rate was approximately 58% for cases and 53% for controls.] The multivariate relative risk for ever having used oral contraceptives was 0.5, after adjusting for parity, breast-feeding, family history of ovarian cancer, tubal ligation and hysterectomy; the decrease in risk was 7% per year of use (95% CI, 4–10%). The reduced risk was observed for oral contraceptives that contained < 35 μg (relative risk, 0.14; 95% CI, 0.06–0.36), 35–45 μg (relative risk, 0.33; 95% CI, 0.15–0.72) and ≥ 50 μg (relative risk, 0.57; 95% CI, 0.34–0.89) ethinylestradiol.

The potency of progestogen and estrogen in oral contraceptives in relation to the risk for ovarian cancer was also considered in a re-analysis of the CASH Study that was conducted between 1980 and 1982 in eight population-based cancer registries of the US SEER Program and included 390 cases and 2869 controls. This highest risk was found for non-users compared with users of high-potency oral contraceptives (odds ratio, 2.9; 95% CI, 1.8–4.5) (Schildkraut *et al*., 2002). Compared with women who did not use oral contraceptives, the relative risk for long-term (≥ 5 years) use was 0.2 (95% CI, 0.1–0.5) for high-potency progestogen, 0.4 (95% CI, 0.2–0.6) for high-potency estrogen, 0.4 (95% CI, 0.2–0.6) for low-potency progestogen and 0.3 (95% CI, 0.1–0.6) for low-potency estrogen formulations.

In a population-based case–control study of 655 incident cases of ovarian cancer and 3899 controls aged 50–74 years conducted between 1993 and 1995 in Sweden (Riman *et al*., 2002), the relative risk for ever having used oral contraceptives was 0.73 (95% CI, 0.59–0.90) and that for longest use (≥ 10 years) was 0.36 (95% CI, 0.22–0.59). The response rate was 76% for cases and 83% for controls. Adjustment was made for age, parity, body mass index and age at menopause. The inverse association with oral use of hormonal contraceptives tended to decrease with time since last use, although the risk remained below unity 25 years or more after last use. The inverse association was observed for serous (odds ratio, 0.56; 95% CI, 0.42–0.71 for ever use), endometrioid (odds ratio, 0.71; 95% CI, 0.49–1.03) and clear-cell (odds ratio, 0.66; 95% CI, 0.31–1.43) cancers but not for mucinous tumours (relative risk, 1.96; 95% CI, 1.04–3.67). The same group of 3899 controls was used in a case–control study of 193 cases of borderline ovarian tumours (including 110 serous and 81 mucinous) aged 50–74 years (Riman *et al*., 2001). The refusal rate for cases was less than 25%. Oral use of hormonal contraceptives conferred no protection against borderline ovarian cancers. The multivariate risks for ever having used oral contraceptives, adjusted for age, parity, body mass index, age at menopause and use of various types of hormonal therapy, were 1.23 (95% CI, 0.9–1.8) overall, 1.40 (95% CI, 0.87–2.26) for serous borderline tumours and 1.04 (95% CI, 0.61–1.79) for mucinous borderline tumours. No relation was observed with duration of or time since last

use of oral contraceptives; the relative risk for ≥ 10 years of use was 1.16 (95% CI, 0.61–2.10). [The apparent difference from other studies on borderline cancers may be related to the older age of these users.]

A multicentric case–control study conducted between 1993 and 1999 in Hawaii, HI, and Los Angeles, CA, USA, included 558 histologically confirmed ovarian epithelial cancers and 601 population controls (Tung *et al*., 2003). The participation rate was 62% for cases and 67% for controls. Adjustment was made for age, ethnicity, study site, education, pregnancy and tubal ligation. The relative risk for ever having used oral contraceptives was 0.6 (95% CI, 0.4–0.8) and that for > 5 years of use was 0.4 (95% CI, 0.3–0.6). The inverse relation was similar for mucinous (relative risk, 0.5; 95% CI, 0.3–0.9) and non-mucinous (relative risk, 0.6; 95% CI, 0.4–0.8) neoplasms, as well as for invasive (relative risk, 0.6; 95% CI, 0.4–0.8) and borderline ovarian tumours (relative risk, 0.6; 95% CI, 0.4–1.0). The protection appeared to level off with time since last use, and no protective effect was evident 10 years or more after last use.

A population-based case–control study was conducted in 2000–01 in 22 counties of central California and included 256 ovarian cancer cases (182 invasive, 74 borderline) and 1122 controls who were frequency-matched on age and ethnicity (Mills *et al*., 2004). Compared with women who had never used oral contraceptives, the relative risks adjusted for age, race or ethnicity and breast-feeding were 0.89 (95% CI, 0.59–1.36) for ≤ 1 year of use, 0.82 (95% CI, 0.55–1.21) for 2–5 years of use, 0.62 (95% CI, 0.38–1.00) for 6–10 years of use and 0.37 (95% CI, 0.20–0.68) for > 10 years of use. The results did not differ materially for invasive and borderline tumours, but the numbers were small.

A case–control study was conducted between 1992 and 1998 in Los Angeles County, CA, USA, on 477 cases of invasive ovarian epithelial cancer and 660 population controls aged 18–74 years (Pike *et al*., 2004). The participation rate was approximately 80% of cases and 70% of controls approached. Multivariate relative risks were adjusted for age, ethnicity, socioeconomic status, education, family history of ovarian cancer, tubal ligation, use of talc, nulliparity, age at last birth, menopausal status, age at menopause and type of hormonal therapy. Compared with women who had never used oral contraceptives, the relative risks were 1.00 (95% CI, 0.72–1.39) for < 5 years of use, 0.72 (95% CI, 0.46–1.13) for 5–9 years of use and 0.48 (95% CI, 0.23–0.78) for ≥ 10 years of use. The relative risk per year of use was 0.94 (95% CI, 0.91–0.97) overall and 0.88 (95% CI, 0.81–0.97) for high-estrogen/high-progestogen, 0.94 (95% CI, 0.88–1.00) for high-estrogen/low-progestogen, 0.66 (95% CI, 0.36–1.21) for low-estrogen/high-progestogen and 0.95 (95% CI, 0.92–0.99) for low-estrogen/low-progestogen preparations.

A collaborative re-analysis (Bosetti *et al*., 2002) of use of oral contraceptives and risk for ovarian cancer was based on 2768 cases and 6274 controls from six studies conducted in three European countries (Greece, Italy and the United Kingdom; Tzonou *et al*., 1984; Booth *et al*., 1989; Polychronopoulou *et al*., 1993; Parazzini *et al*., 1994; Greggi *et al*., 2000; Chiaffarino *et al*., 2001). Adjustment was made for age and other sociodemographic factors, calendar year of interview, menopausal status and parity. The multivariate relative risk was 0.7 (95% CI, 0.6–0.8) for ever use and 0.4 (95% CI, 0.3–0.6) for longest use

(≥ 5 years), which corresponded to a decrease in risk of approximately 5% per year of use. The protective effect appeared to persist for at least 20 years after last use of oral contraceptives in the absence of any significant trend of decreasing risk with time since cessation of use.

Oral hormonal contraceptives are commonly used in the treatment of endometriosis. Data from four population-based case–control studies conducted in the USA between 1993 and 2001 were pooled to analyse risk factors for ovarian cancer in women with no endometriosis (Modugno *et al*., 2004). These included 2759 cases of ovarian cancer with no endometriosis, 184 cases with endometriosis, 1972 controls with no endometriosis and 177 controls with endometriosis. Multivariate relative risks were computed with adjustment for study centre, age and family history of ovarian cancer. Compared with women who had never used oral contraceptives, the relative risk was 0.58 (95% CI, 0.33–1.03) for < 10 years and 0.21 (95% CI, 0.08–0.58) for ≥ 10 years of use among women with endometriosis and 0.70 (95% CI, 0.60–0.80) for < 10 years and 0.47 (95% CI, 0.37–0.61) for ≥ 10 years of use among women with no endometriosis.

2.4.4 *Case–control studies among breast cancer gene (BRCA1/2) carriers* (Table 14)

A study conducted in North America and Europe on 207 susceptible women with hereditary ovarian cancer (179 with *BRCA1* and 28 with *BRCA2* mutations) and 161 sister controls found a relative risk of 0.5 (95% CI, 0.3–0.8) for ever use of oral contraceptives; the risk decreased with increasing duration of use (relative risk, 0.4; 95% CI, 0.2–0.7 for > 6 years). Adjustment was made for geographical area of residence, year of birth, parity and age at first birth. The results were similar (relative risk, 0.4 for ever use and 0.3 for > 6 years of use) when the comparison was made with control carriers of *BRCA1* or *BRCA2* only (Narod *et al.*, 1998).

In a population-based case–control study from Israel (Modan *et al*., 2001), 240 cases of ovarian cancer with *BRCA1* or *BRCA2* mutations and 592 cases with no mutations were compared with 2257 controls. Oral use of hormonal contraceptives and duration of use were inversely related to the risk for ovarian cancer in women with no mutations, but not in those with *BRCA1* or *BRCA2* mutations. The relative risk for ≥ 5 years of use was 1.07 (95% CI, 0.63–1.83) in mutation carriers and 0.53 (95% CI, 0.34–0.84) in non-carriers.

In a case–control study of 36 *BRCA1*-carrier cases of ovarian cancer, 381 non-carrier cases and 568 population controls conducted between 1997 and 2001 in the San Francisco Bay Area, CA, USA (McGuire *et al*., 2004), the relative risk for ever having used oral contraceptives was 0.54 (95% CI, 0.26–1.13) among *BRCA1* mutation carriers and 0.55 (95% CI, 0.41–0.75) among non-carriers. The relative risk for use of ≥ 7 years was 0.22 (95% CI, 0.07–0.71) among *BRCA1* carriers and 0.43 (95% CI, 0.30–0.63) among non-carriers. The response rate was 75% among cases and 72% among controls. Adjustment was made for age, ethnicity and parity.

Table 14. Case–control studies on combined oral contraceptives and ovarian cancer among *BRCA1/2* carrier cases

Reference, country	Type of study	Relative risk (95% CI)		
		No. of cases (age in years)	Ever use	Longest use (duration)
Narod *et al*. (1998), USA	Hereditary cancers	207 (< 75) with *BRCA1* (179) or *BRCA2* (28) mutations	0.5 (0.3–0.8)	0.4 (0.2–0.7) (> 6 years)
Modan *et al*. (2001), Israel	Population-based	240 with *BRCA1/2* mutations	1.1 (0.7–1.9) (0.1–1.9 years) 0.8 (0.4–1.4) (2.0–4.9 years)	1.1 (0.6–1.8) (≥ 5 years)
McGuire *et al*. (2004), USA	Population-based	36 *BRCA1* carriers 381 *BRCA1* non-carriers	0.5 (0.3–1.1) 0.6 (0.4–0.8)	0.2 (0.1–0.7) (≥ 7 years) 0.4 (0.3–0.6) (≥ 7 years)
Whittemore *et al*. (2004), Australia and United Kingdom	Registry-based *BRCA1/2* carriers	147 with *BRCA1* or *BRCA2* mutations	0.9 (0.5–1.4)	0.6 (0.4–1.1) (≥ 6 years)

CI, confidence interval

A study based on registers of women with *BRCA1* or *BRCA2* germline mutations from Australia and the United Kingdom included 147 cases of ovarian cancer and 304 controls. The multivariate relative risk for ever having used oral contraceptives was 0.85 (95% CI, 0.53–1.4) and that for ≥ 6 years of use was 0.62 (95% CI, 0.35–1.1). Adjustment was made for study centre, parity and age. The continuous relative risk per year of use among users was 0.95 (95% CI, 0.91–0.99) (Whittemore *et al*., 2004).

2.5 Liver cancer

The majority of primary liver cancers are hepatocellular carcinomas. The major risk factor for these cancers in areas of high incidence is chronic infection with hepatitis B (HBV) virus, but the continuing increase seen in low-risk western populations is due at least in part to the increasing prevalence of hepatitis C virus, which also causes hepatocellular carcinoma (IARC, 1994). Aflatoxin on mouldy food, liver cirrhosis, consumption of alcoholic beverages (IARC, 1988; Baan *et al*., 2007) and tobacco smoking (IARC, 2004) are also important causes of this disease. Cholangiocarcinoma is less common than hepatocellular carcinoma, although it frequently occurs 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 years 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 years and among men were small and were not statistically significant. The authors suggested that the change was consistent with the idea that combined oral contraceptives caused some cases of liver cancer, but noted that no such trend was apparent in Australia, western Germany, the Netherlands or the USA — other countries where the use of combined oral contraceptives 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 age groups of women who had had major exposure to oral contraceptives, and Waetjen and Grimes (1996) found no evidence for an effect of the oral use of hormonal contraceptives on secular trends in death rates from liver cancer in Sweden or the USA.

2.5.2 *Cohort studies*

In the Nurses' Health Study in the USA, Colditz *et al*. (1994) studied a cohort of 121 700 female registered nurses aged 30–55 years in 1976 who were followed up for deaths until 1988. Women who reported angina, myocardial infarct, stroke or cancer at baseline were excluded, which left 116 755 women for follow-up. Of these, 55% reported having used combined oral contraceptives and 5% reported current use. Incidence rates with person–months of follow-up were used as the denominator and oral contraceptive use at recruitment as the exposure. The risks associated with any use of oral contraceptives relative to no use, adjusted for age, tobacco smoking, body mass index and follow-up interval, was 0.9 (95% CI, 0.8–1.0) for death from any cancer. Ten deaths from primary liver or biliary-tract cancer occurred 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 conducted 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. Five cases of liver cancer were observed, 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, five deaths from liver cancer occurred among women who had used combined oral contraceptives and one in a woman who had never used them,

to give a relative risk of 5.0 (95% CI, 0.6–43) (Beral *et al*., 1999). In the study of the Oxford Family Planning Association, 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, CA, USA, between 1955 and 1976. One age-matched friend control was interviewed for each case. Twenty-eight of the 34 (82%) cases and 19 of 34 (56%) controls had used oral contraceptives for more than 12 months. The risks relative to use of combined oral contraceptives for less than 12 months were 1.3 for 13–36 months of use, 5.0 for 61–84 months of use, 7.5 for 85–108 months of use and 25 for 109 months of use and longer.

Rooks *et al.* (1979) interviewed 79 of 89 eligible women aged 16–50 years in whom hepatocellular adenoma had been diagnosed between 1960 and 1976 at the Armed Forces Institute of Pathology, Washington DC, USA. Three age-matched neighbourhood controls were sought for each case, and 220 were interviewed. Seventy-two of the 79 (91%) cases and 99 of 220 (45%) controls had used oral contraceptives for more than 12 months. The risks relative to use of oral contraceptives for less than 12 months were 0.9 for 13–36 months of use, 1.16 for 37–60 months of use, 1.29 for 61–84 months of use and 5.03 for 85 months of use and longer.

Gemer *et al.* (2004) conducted a case–control study of liver haemangioma in women that included 40 cases diagnosed between 1995 and 2002 at the Barzilai Medical Centre, Ashkelon, Israel, and 109 control women with normal liver scans. The odds ratio for liver lesions was 1.2 (95% CI, 0.5–2.6) for women who had ever used oral contraceptives and 0.7 (95% CI, 0.2–3.0) for use within the last 2 years.

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

The studies on malignant tumours of the liver described below are summarized in Table 15.

Henderson *et al.* (1983b) studied women in Los Angeles County, CA, USA, 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 of 22 identified controls refused to be interviewed and were replaced, to give 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

Table 15. Case–control studies of use of combined oral contraceptives and liver cancer

Reference, study area	Age (years)	No. of cases	No. of controls	Odds ratio[a] (95% CI)	Comments
Henderson *et al.* (1983b), California, USA	18–39	11	22	[7.0 (0.7–71)]	
Forman *et al.* (1986), England and Wales	20–44	30	147	3.8 (1.0–14.6)	Adjusted for age, year of birth
Neuberger *et al.* (1986), United Kingdom	< 50	26	1333	1.0 (0.4–2.4)	Not adjusted for tobacco smoking or alcoholic beverage consumption. Three cases are also included in Forman *et al.* (1986).
Palmer *et al.* (1989), USA	19–54	12	60	[15 (1.7–126)]	No information on tobacco smoking
WHO Collaborative Study (1989b), Chile, China, Colombia, Israel, Kenya, Nigeria, Philippines, Thailand	15–56 (mean, 41.8)	122	802	0.7 (0.4–1.2)	Adjusted for alcoholic beverage consumption, number of live births, occupation
Kew *et al.* (1990), South Africa	15–54	46	92	1.9 (0.6–5.6)	No effect of alcoholic beverage or tobacco consumption on risk estimates
Vall Mayans *et al.* (1990), Catalonia region, Spain	No age limits	29	57	[4.7 (1.1–20)]	86.5 % of cases had liver cirrhosis. Tobacco and alcohol adjustment did not alter risk estimates.
Yu *et al.* (1991), California, USA	18–74	25	58	3.0 (1.0–9.0)	Adjustment for tobacco and alcohol did not alter risk estimates.
Hsing *et al.* (1992), USA	25–49	72	549	1.6 (0.6–2.6)	
Tavani *et al.* (1993), Italy	28–60	43	194	2.6 (1.0–7.0)	Adjusted for age, education, parity
Collaborative MILTS (1997), France, Germany, Greece, Italy, Spain, United Kingdom	< 65	293	1779	0.8 (0.5–1.0)	No association for duration of use, type of formulation; significantly increased risk for > 6 years of use in individuals with no hepatitis infection or liver cirrhosis
Yu *et al.* (2003), Taiwan (China)	≥ 35	218	729	0.75 (0.44–1.28)	No association for ≥ 2 years' duration of use

CI, confidence interval; MILTS, Multicentre International Liver Tumour Study

[a] Odds ratios are given for never versus ever use of oral contraceptives.

obtained for 4.3% of the houses surveyed. None of the patients or controls reported a prior history of hepatitis or jaundice; none of the four cases had HBV surface antigens (HBsAg); none of the patients reported exposure to any known hepatotoxin, and there was no difference in the frequency of alcoholic beverage consumption between cases and controls. Tobacco smoking histories were not reported. Ten of the 11 patients (including seven of the eight cases of hepatocellular carcinoma) had used oral contraceptives, and the 11th 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 (95% CI, 0.7–64) for all liver cancers (unmatched analyses).]

Forman *et al.* (1986) identified all women certified to have died from primary liver cancer at the age of 20–44 years in England and Wales between 1979 and 1982. 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 for 46 of 85 (54.1%) potential cases and for 147 of 233 (63.1%) eligible controls. Eighteen of 30 (60%) cases had used oral contraceptives compared with 79 of 147 (54%) controls. Information on tobacco smoking and alcoholic beverage consumption 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 < 4 years, 4.0 for 4–7 years and 20.1 for ≥ 8 years of use; for cholangiocarcinoma, 0.3 for any use, 0.1 for < 4 years and 0.9 for ≥ 4 years of use. [The published risks were adjusted for age and year of birth, but CIs were not given. The unadjusted relative risks and 95% CIs, calculated from the published data, were: hepatocellular carcinoma, any use, 3.2 (95% CI, 1.0–10); < 4 years, 2.4 (95% CI, 0.7–8.5); 4–7 years, 3.6 (95% CI, 0.8–16); and ≥ 8 years, 13 (95% CI, 2.1–78); cholangiocarcinoma, any use, 0.3 (95% CI, 0.1–1.3); < 4 years, 0.2 (95% CI, 0.0–1.3); and ≥ 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 below.

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 years. The cases were referred from all over the United Kingdom to the Liver Unit at King's College School of Medicine and Dentistry, London, between 1976 and 1985. The controls were 1333 women who had been hospital controls in a case–control study of breast cancer and had been interviewed during 1976–80. The results were not adjusted for tobacco smoking or alcoholic beverage use. Eighteen of the 26 (69%) cases had taken hormonal contraceptives orally. The controls were used to calculate the expected numbers of cases for each duration of oral contraceptive use, within age and calendar groups. The expected number of women who had ever used oral contraceptives was 18.7, which gave a relative risk of 1.0 (95% CI, 0.4–2.4). The relative risks by duration of use

were 0.3 (95% CI, 0.1–1.1) for < 4 years, 0.9 (95% CI, 0.3–3.4) for 4–7 years and 4.4 (95% CI, 1.5–13) for ≥ 8 years. None of the cases 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 (95% CI, 0.1–1.9) for < 4 years, 1.5 (95% CI, 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 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 cities in the USA 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 cases 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. Tobacco smoking status was not reported, but alcoholic beverage intake was similar in cases and controls. Eleven of the 12 cases (including 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 (95% CI, 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 (1989) was a hospital-based case–control study conducted in eight countries between 1979 and 1986. 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 not specified. Controls were selected from among individuals admitted to the same hospitals as the cases with conditions not thought to be related to the use of oral contraceptives. The overall response rate of controls was 94.3%. Information on tobacco smoking was not collected; there was no statistically significant difference in alcoholic beverage consumption between cases and controls (17.2% of the cases and 26% of the controls had ever drunk alcoholic beverages). The finding that 25 of 122 cases (20.5%) and 216 of 802 controls (26.9%) had used oral contraceptives gave odds ratios, adjusted for number of live births and occupation, of 0.7 (95% CI, 0.4–1.2) for any use, 0.8 (95% CI, 0.4–1.5) for use of 1–12 months, 0.7 (95% CI, 0.3–1.7) for use of 13–36 months and 0.7 (95% CI, 0.3–1.7) for use of ≥ 37 months. The odds ratios for any use by histological subtype were 0.6 (95% CI, 0.2–1.6) for hepatocellular carcinoma, 1.2 (95% CI, 0.5–3.1) for cholangiocarcinoma and 0.5 (95% CI, 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 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 had been diagnosed when they were aged 19–54 years. Two controls per case were selected and matched by 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. Tobacco smoking and alcoholic beverage 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 (15.2%) cases and eight of 92 (8.7%) controls had ever used oral contraceptives, to give 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 of < 4 years, 2.0 (95% CI, 0.1–33.1) for use of 4–8 years and 1.5 (95% CI, 0.3–7.2) for use of > 8 years. Nineteen of 46 cases were HBsAg-positive, 25 had evidence of past infection with HBV and two had never been infected. The odds ratio 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 the Catalonia region of northeastern 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 female cases, two controls were selected per case and matched on sex, age, hospital and time of admission. Patients with diagnoses related to the 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. Tobacco smoking was not associated with risk, and adjustment for alcoholic beverage intake did not alter the results. Six of 29 female cases (20.7%) and three of 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 aged 18–74 years resident in Los Angeles County, CA, USA, between 1984 and 1990. Two neighbourhood controls were sought for each case and matched on sex, year of birth and race. Adjustment for tobacco smoking and alcoholic beverage consumption did not alter the results. Thirteen of 25 (52%) cases and 18 of 58 (31%) controls had used oral contraceptives. The odds ratios were 3.0 (95% CI, 1.0–9.0) for any use, 2.3 (95% CI, 0.5–11) for use of ≤ 12 months, 1.7 (95% CI, 0.3–9.1) for use of 13–60 months and 5.5 (95% CI, 1.2–25) for use of ≥ 61 months. For the 11 cases who had formerly used oral contraceptives, the mean time since last use was 14.5 years. Seven cases had antibodies to one or more markers of hepatitis viral infection; when these cases were excluded, the association between the 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 years in the USA (except the State of Oregon) in 1985 and in the National Mortality Followback Survey in 1986. The study included 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, which left 629 controls for analysis. The odds ratios were adjusted for tobacco smoking and alcoholic beverage use. For all subjects with complete data, 39 of 72 (54.2%) cases and 243 of 549 (44.3%) controls had ever used oral contraceptives; the odds ratios were 1.6 (95% CI, 0.9–2.6) for any use, 1.2 (95% CI, 0.6–2.4) for use of < 5 years, 2.0 (95% CI, 1.0–4.4) for use of 5–9 years and 2.0 (95% CI, 0.8–4.8) for use of ≥ 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 (95% CI, 0.9–4.6) for use of < 5 years, 3.9 (95% CI, 1.6–9.6) for use of 5–9 years and 4.8 (95% CI, 1.7–14) for use of ≥ 10 years. When the four Asian cases and 10 controls from populations who were 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; 95% CI, 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 (95% CI, 0.1–2.7) for < 5 years of use, 0.6 (95% CI, 0.1–5.4) for 5–9 years of use and 3.3 (95% CI, 0.7–16) for ≥ 10 years of use.

Tavani *et al.* (1993) conducted a hospital-based case–control study of women who had histologically or serologically confirmed hepatocellular carcinoma diagnosed at the age of 28–73 years in the greater Milan area, Italy, between 1984 and 1992. Controls were women admitted to hospital for acute non-neoplastic diseases (37% traumas, 13% other orthopaedic disorders, 40% acute surgical conditions, 10% other). Since none of the women aged 60 years or over had ever used oral contraceptives, the analysis was restricted to women under that age. Nine of 43 (20.9%) cases and 21 of 194 (10.8%) controls had ever used oral contraceptives. The odds ratios, adjusted for age, education and parity, were 2.6 (95% CI, 1.0–7.0) for any use, 1.5 (95% CI, 0.5–5.0) for use of ≤ 5 years and 3.9 (95% CI, 0.6–25) for use of > 5 years. In relation to time since oral contraceptives were last used, the odds ratios were 1.1 (95% CI, 0.3–4.6) for ≤ 10 years and 4.3 (95% CI, 1.0–18) for > 10 years. No information was available on infection with hepatitis viruses.

The Multicentre International Liver Tumour Study (Collaborative MILTS Project Team, 1997) included women who had hepatocellular carcinoma and were diagnosed before the age of 65 years 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 a diagnosis of an eligible tumour 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. Oral contraceptive use was

reported for 148 of the 293 (50.5%) cases and 1086 of the 1779 (61.0%) controls. The odds ratio for any use of oral contraceptives was 0.8 (95% CI, 0.5–1.0); those by duration of use were 0.8 (95% CI, 0.5–1.3) for 1–2 years, 0.6 (95% CI, 0.3–1.1) for 3–5 years and 0.8 (95% CI, 0.5–1.1) for ≥ 6 years of use. For use of oral contraceptives that contained cyproterone acetate, the odds ratios were 0.9 (95% CI, 0.5–1.6) for any use, 0.9 (95% CI, 0.4–2.4) for use of 1–2 years, 0.9 (95% CI, 0.3–2.4) for use of 3–5 years and 0.9 (95% CI, 0.4–2.0) for use of ≥ 6 years. When the analysis was restricted to the 51 cases who had no liver cirrhosis or evidence of infection with hepatitis viruses, the odds ratios increased to 1.3 (95% CI, 0.4–4.0) for use of any oral contraceptives of 1–2 years, 1.8 (95% CI, 0.5–6.0) for use of 3–5 years and 2.8 (95% CI, 1.3–6.3) for use of ≥ 6 years.

Yu *et al.* (2003) conducted a multicentre case–control study on reproductive risk factors for hepatocellular carcinoma in women in Taiwan, China, where this disease is common. Cases were 218 women aged 35 years or over who had hepatocellular carcinoma and were recruited through four large hospitals; 729 controls were selected from first-degree or non-biological relatives. Twenty cases (9.2%) and 110 (15%) controls had used oral contraceptives, to give an adjusted odds ratio of 0.75 (95% CI, 0.44–1.28) for ever having used and 0.38 (95% CI, 0.13–1.09) for more than 2 years of use of oral contraceptives.

2.6 Colorectal cancer

Several studies have provided information on the use of combined oral contraceptives and the risk for colorectal cancer. The previous evaluation of exogenous hormones and risk for cancer reviewed four cohort and 10 case–control studies, none of which showed significantly elevated risks in women who used these preparations for any length of time (Tables 16 and 17). The relative risks were below unity for nine studies, and statistically significant in two (IARC, 1999).

Some aspects, however, remain undefined, including the risk related to duration and recency of use and more adequate allowance for confounding, which left the issue of a causal inference for the observed association open to discussion. This section reviews data on oral contraceptives and colorectal cancer that have been published since the last evaluation (IARC, 1999).

2.6.1 *Cohort studies*

In addition to the four cohort studies reviewed previously (IARC, 1999), four cohort studies have provided new data on the potential association between oral contraceptives and colorectal cancer (Table 16).

van Wayenburg *et al*. (2000) analysed the mortality from colorectal cancer according to several reproductive variables in the Diagnostisch Onderzoek Mammacarcinoom (DOM) cohort study, a population-based breast cancer screening programme in Utrecht, The Netherlands. Between 1974 and 1977, 14 697 women who lived in Utrecht were enrolled in the DOM study, and 12 239 women who attended the second screening visit

Table 16. Cohort studies of oral use of contraceptives and colorectal cancer

Reference	Country and study	Population (follow-up); no. of cases/ deaths	Relative risk (95% CI) (ever versus never users)			Comments
			Colorectal	Colon	Rectum	
Chute *et al.* (1991); Martinez *et al.* (1997)	USA Nurses' Health Study	89 448 (12 years); 501 cases	0.84 (0.69–1.02)	0.64 (0.40–1.02)	0.76 (0.49–1.18)	Adjusted for age, body mass index, exercise, family history of cancer, aspirin, alcohol, meat intake, menstrual factors; significant inverse trend with duration of use
Bostick *et al.* (1994)	Iowa State, USA	35 215 (4 years); 212 cases	–	1.0 (0.7–1.4)	–	Adjusted for age, height, parity, caloric intake, vitamin intake
Troisi *et al.* (1997)	USA BCDDP	57 528 (10 years); 95 cases	1.0 (0.7–1.4)	–	–	Adjusted for age only; adjustment for education, body mass index did not alter relative risk; no significant effect with duration of use.
Beral *et al.* (1999); Hannaford & Elliot (2005)[a]	United Kingdom RCGP OCS	46 000 (25 years); 146 cases, 438 controls	0.84 (0.56–1.24) < 5 years: 0.85 (0.52–1.38) 5–9 years: 0.75 (0.44–1.30) ≥ 10 years: 0.97 (0.52–1.80)	–	–	Adjusted for social class, smoking, parity, hormonal menopausal therapy (age and length of follow-up by matching)
van Wayenburg *et al.* (2000)	Netherlands DOM Study	10 671 (18 years); 95 deaths	0.68 (0.21–2.21)	–	–	Adjusted for age at entry, age at first birth, smoking, type of menopause, socioeconomic status, body mass index
Vessey *et al.* (2003)	United Kingdom OPFA Study	17 032 (30 years); 46 deaths	0.9 (0.4–2.1)	–	–	Relative risk of death for use < 24 months versus never use; no trend with duration of use; adjusted for age, parity, social class, smoking
Rosenblatt *et al.* (2004)	Shanghai, China	267 400 (10 years); 655 cases	–	1.09 (0.86–1.37)		No trend with duration of use; adjustment for age, parity

BCDDP, Breast Cancer Detection Demonstration Project; CI, confidence interval; DOM, Diagnostich Onderzoek Mammacarcinoom; OFPA, Oxford Family Planning Association; RCGP OCS, Royal College of General Practitioners Oral Contraceptive Study

[a] Nested case-control study within the RCGP OCS

were followed up over a median of 18 years. Few women in the cohort (5%) had ever used oral contraceptives and 95 women in the cohort died of colorectal cancer [number of deaths among exposed and unexposed not provided]. The relative risk for death from colorectal cancer was 0.68 (95% CI, 0.21–2.21), after adjustment for age at entry, age at first birth, tobacco smoking habits, natural or artificial menopause, socioeconomic status and body mass index (analysis according to duration of use not presented).

The Oxford Family Planning Association study was based on 17 032 women, aged 25–39 years at entry, who were recruited between 1968 and 1974 from various family planning clinics in the United Kingdom (Vessey & Painter, 1995) and followed up for mortality until the end of 2000. A total of 889 deaths were noted during 479 400 woman–years of observation. Only 8% of the woman–years related to women aged 60 years or more; 16% represented current or recent (within 1 year) users of oral contraceptive and 33% related to women who had not used such contraceptives in the preceding 96 months. From the total mortality observed, 46 women died from colorectal cancer, 18 of whom had never and 28 had ever used oral contraceptives. The multivariate relative risks for mortality from colorectal cancer were 0.9 (95% CI, 0.3–2.3) for < 4 years of oral contraceptive use, 1.1 (95% CI, 0.5–2.5) for 4–8 years of use and 0.8 (95% CI, 0.4–1.9) for > 8 years of use compared with no use. Adjustment was made for age, parity, social class and tobacco smoking (Vessey *et al*., 2003). [The relative risk for mortality from colorectal cancer was 0.90 (95% CI, 0.38–2.11) for ever versus never use, as computed by the Working Group.]

Rosenblatt *et al.* (2004) reported on a 10-year follow-up of 267 400 female textile workers at 519 factories in Shanghai, China, who were administered a questionnaire at enrolment into a randomized trial of breast self-examination between 1989 and 1991 and who were followed up until 2000. At the end of follow-up, 655 women had been diagnosed with incident colon cancer (563 who had never and 92 who had ever used oral contraceptives). The relative risk for colon cancer was 1.09 (95% CI, 0.86–1.37) for women who had ever used oral contraceptives (adjusted for age and parity), 0.97 (95% CI, 0.64–1.47) for < 6 months of oral contraceptive use, 0.96 (95% CI, 0.67–1.38) for 7–24 months of use, 1.13 (95% CI, 0.65–1.97) for 25–36 months of use and 1.56 (95% CI, 1.01–2.40) for > 36 months of use (p for trend = 0.16).

Hannaford and Elliot (2005) conducted a nested case–control study within the Royal College of General Practitioners' Oral Contraceptive Cohort Study. This cohort included 46 000 women who were recruited in 1968–69 by general practitioners throughout the United Kingdom and were followed up for 25 years. This nested case–control study updated data from a previous report (Beral *et al*., 1999). In this analysis, 146 cases of fatal and non-fatal colorectal cancer [separate number of colon and rectal cases not given] and 438 controls matched by age and length of follow-up (three controls for each case) were identified. Of these, 76 cases and 247 controls had used oral contraceptives. The odds ratio for colorectal cancer, adjusted for social class, tobacco smoking, parity and use of hormonal therapy, was 0.84 (95% CI, 0.56–1.24). The reduction in risk was greater but not significant among current users (odds ratio, 0.38; 95% CI, 0.11–1.32) than among

former users (odds ratio, 0.89; 95% CI, 0.59–1.31). The trend in risk by duration of use was not significant and no clear trend with time since last or first use was observed.

2.6.2 *Case–control studies*

In addition to the 10 case–control studies reviewed in the last evaluation (IARC, 1999), three case–control studies on the use of oral contraceptives and colorectal cancer have been published (Table 17).

Kampman *et al*. (1994) conducted a population-based case–control study of 102 women who had incident colon cancer and 123 controls in the Netherlands. Of these, 46 cases and 58 controls had ever used oral contraceptives, which gave an odds ratio for colon cancer of 0.97 (95% CI, 0.46–2.03). Adjustment was made for age, urbanization grade, energy intake, energy-adjusted intake of fat, carbohydrate, dietary fibre and vitamin C, cholecystectomy, family history of colon cancer and socioeconomic level. [Estimates for duration of use and time since first and last use were not provided.]

Levi *et al*. (2003) conducted a hospital-based case–control study of 131 women who had incident colorectal cancer (71 colon cancers, 60 rectal cancers) and 373 control women in the Swiss Canton of Vaud. Of these, 14 cases and 65 controls had ever used oral contraceptives, to give an odds ratio of 0.8 (95% CI, 0.4–1.7) [separate odds ratios for colon and rectal cancers were not given]. Adjustment was made for age, education, family history of colorectal cancer, parity, fibre intake and physical activity. There was no consistent relation with duration of or time since first or last use (most odds ratios were non-significantly below unity).

Nichols *et al*. (2005) conducted a population-based case–control study in the State of Wisconsin, USA, of 1488 women aged 20–74 years who had incident colorectal (1122 colon, 366 rectal) cancer and were enrolled in either 1988–91 or 1997–2001. Of these women, 426 cases and 1968 controls had ever used combined oral contraceptives, which gave an odds ratio for colorectal cancer of 0.89 (95% CI, 0.75–1.06). The odds ratio was conditional on age and date of enrolment and was adjusted for family history of colorectal cancer, body mass index, education, screening, tobacco smoking, use of hormonal therapy and age at first birth. The odds ratio for colon cancer was 0.87 (95% CI, 0.72–1.06), conditional on age and date of enrolment, and adjusted for family history of colorectal cancer, body mass index, education, screening, tobacco smoking, use of hormonal therapy, age at first birth, alcoholic beverage consumption and menopausal status; that for rectal cancer was 0.87 (95% CI, 0.65–1.17), conditional on age and date of enrolment, and adjusted for family history of colorectal cancer, physical activity, education, screening, smoking and use of hormonal therapy. No pattern in risk was seen according to duration of use or age at starting use. Recency of use was not related to risk for colon cancer. Among women who had rectal cancer, a reduction in risk was seen (odds ratio, 0.53; 95% CI, 0.28–1.00) in the category of most recent (i.e. < 14 years) oral contraceptive use.

Table 17. Case–control studies of use of oral contraceptives and colorectal cancer

Reference	Country and study	Cases: controls	Relative risk (95% CI) (ever versus never users)			Comments
			Colorectal	Colon	Rectum	
Weiss *et al.* (1981b)	Washington State, USA	143:707	≤ 5 years: 1.3 (0.5–3.1) > 5 years: 2.0 (0.7–5.2)	1.0	2.6 (p = 0.09)	Adjusted for age; no significant trends with duration of use
Potter & McMichael (1983)	Adelaide, Australia	155:311		0.5 (0.3–1.2)	0.7 (0.3–1.8)	Adjusted for reproductive variables; inverse trend with duration of use
Furner *et al.* (1989)	Chicago, USA	90:208	0.6 (0.3–1.3)			Unadjusted
Kune *et al.* (1990)	Melbourne, Australia	190:200	–	1.2 (0.6–2.6)	2.04 (1.0–4.1)	Adjusted for age, parity, age at first child; no significant trend with duration of use
Peters *et al.* (1990)	Los Angeles, USA	327:327	–	< 5 years: 1.0 (0.6–1.8) ≥ 5 years: 1.1 (0.4–2.9)	–	Family history of cancer, parity, exercise, fat, alcohol, calcium intake; no effect of duration of use
Franceschi *et al.* (1991b)	Northeastern Italy	89:148	0.2 (0.0–2.0)			Unadjusted; only 1 case and 9 controls had ever used oral contraceptives.
Wu-Williams *et al.* (1991)	North America (NAm) and China (Ch)	395:1112	–	NAm: 1.2 (p = 0.67) Ch: 0.55 (p = 0.27)	NAm: 0.4 (p = 0.04) Ch: 0.7 (p = 0.34)	Unadjusted (but unaltered by exercise, saturated fat, duration of residence in NAm); no trend with duration of use
Jacobs *et al.* (1994)	Seattle, USA	193:194	–	1.2 (0.70–1.90)	–	Adjusted for age, age at birth of first birth, vitamin intake; no trend with duration of use

Table 17 (contd)

Reference	Country and study	Cases: controls	Relative risk (95% CI) (ever versus never users)			Comments
			Colorectal	Colon	Rectum	
Kampman *et al.* (1994)	The Netherlands	102:123	–	0.97 (0.46–2.03)	–	Adjusted for age, urbanization, cholecystectomy, socio-economic level, colon cancer, family history, dietary habits
Kampman *et al.* (1997)	USA, KPMCP	894:1120	–	0.86 (0.67–1.10)	–	Adjusted for age, cancer family history, aspirin, caloric intake, hormonal menopausal therapy, exercise
Fernandez *et al.* (1998)	Italy	1232:2793	0.6 (0.5–0.9)	0.7 (0.5–0.9)	0.7 (0.5–1.1)	Adjusted for age, education, family history of cancer, body mass index, estrogen replacement therapy, energy intake; no effect with duration of use; stronger protection in recent users
Levi *et al.* (2003)	Canton of Vaud, Switzerland	131:373		0.8 (0.4–1.7) ≤ 5 years: 0.7 (0.2–2.4) > 5 years: 0.9 (0.4–2.0)		Adjusted for age, education, family history of colorectal cancer, parity, fibre intake, physical activity; no trend with duration, time since first or last use
Nichols *et al.* (2005)	Wisconsin State, USA	1488:4297	0.89 (0.75–1.06)	0.87 (0.72–1.06)	0.87 (0.65–1.17)	Adjusted for age, study enrollment, family history of colorectal cancer, body mass index, education, screening, smoking, hormonal menopausal therapy, alcohol, age at first birth; no effect with duration of use; greater reduced risk in recent users (rectal)

CI, confidence interval; KPMCP, Kaiser Permanente Medical Care Program

2.7 Cutaneous malignant melanoma

The previous evaluation (IARC, 1999) omitted several studies and contained inaccuracies in the reporting of some results. The four cohort and 18 case–control studies of oral contraceptive use and cutaneous melanoma have therefore been re-assessed.

2.7.1 *Cohort studies* (Table 18)

Beral *et al.* (1977) and Ramcharan *et al.* (1981) first reported on a study of oral contraceptive use and cutaneous melanoma that comprised a cohort and a case–control component. Cohort data were derived from a prospective study on non-contraceptive effects of oral contraceptive use among 17 942 women aged 17–59 years at baseline, who were members of the Kaiser Permanente Health Plan, California, USA. Between 1968 and 1976, 22 cases of melanoma were found; eight had never used oral contraceptives, eight had used oral contraceptives for less than 4 years and six had used them for 4 years or more.

In the United Kingdom, 17 032 white married women aged 25–39 years were recruited between 1968 and 1974 at 17 family planning clinics within 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 each woman's oral contraceptive status could change during the course of the study, users of these preparations may have contributed periods of observation as 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 occurred 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 tobacco smoking, were 0.6 (95% CI, 0.2–1.6) for < 5 years of use, 1.0 (95% CI, 0.4–2.6) for 5–9 years of use and 1.0 (95% CI, 0.2–3.1) for ≥ 10 years of use. There was no relationship between time since cessation of use of oral contraceptives and the risk for cutaneous malignant melanoma, and none of the formulations resulted in a specific pattern of risk.

In the United Kingdom, 1400 general practitioners recruited 23 000 women who were using oral contraceptives and an equal number of age-matched women who had never used them between 1968 and 1969 within 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 occurred among women who had ever used combined oral contraceptives; the relative risk, adjusted for age, parity, social class and tobacco smoking, was 0.9 (95% CI, 0.6–1.5). No significant trend of increasing risk with duration of use was seen, with a relative risk for 10 years or more of use of 1.8 (95% CI, 0.8–3.9). Relative risks did not vary according to recency of use, estrogen or progestogen content of the contraceptives or the site of cutaneous malignant melanoma.

Table 18. Cohort studies of the use of combined oral contraceptives and the risk for cutaneous malignant melanoma

Reference, location	Population cohort	Age (years)	No. of cases	Type of exposure	No. of cases	Relative risk (95% CI)	Comments
Beral *et al.* (1977); Ramcharan *et al.* (1981), USA	17 942 white women	17–59	22	Never used	8	NR	Walnut Creek Contraceptive Drug Study; hospital-based cases diagnosed in 1968–76; interviews based on postal, telephone and direct interviews; median follow-up, 6 years
				Ever used for < 4 years	8	NR	
				Ever used for ≥ 4 years	6	NR	
Hannaford *et al.* (1991), United Kingdom	17 032 married white women	25–39	32	Never used	15	1.0	Oxford Family Planning Association (1968–74); interviews based on postal questionnaire, telephone and home visits; maximum follow-up, 21 years; adjusted for age, parity, social class, tobacco smoking
				Ever use	17	0.8 (0.4–1.8)	
				Duration of use			
				< 5 years	5	0.6 (0.2–1.6)	
				5–9 years	8	1.0 (0.4–2.6)	
				≥ 10 years	4	1.0 (0.2–3.1)	
Hannaford *et al.* (1991), United Kingdom	46 000 women	NR	58	Never used	27	1.0	Royal College of General Practitioners; based on questionnaires provided by physicians; maximum follow-up, 21 years; adjusted for age, parity, social class, tobacco smoking
				Ever use	31	0.9 (0.6–1.5)	
				Duration of use			
				< 5 years	15	0.8 (0.4–1.5)	
				5–9 years	8	0.7 (0.3–1.5)	
				≥ 10 years	8	1.8 (0.8–3.9)	
Feskanish *et al.* (1999), USA	183 693 pre-menopausal white women	25–55	252	Never used	64	1.0	Nurses' Health Study I and II; self-reported cases by nurses; adjusted for age, skin reaction to sun exposure; history of sunburn, mole counts, hair colour, family history of melanoma, parity, height, body mass index
				Ever use	374	1.4 (0.8–1.6)	
				Current use	23	2.0 (1.2–3.4)	
				< 10 years	9	1.0 (0.4–2.8)	
				≥ 10 years	14	3.4 (1.7–7.0)	
				Past use	165	1.1 (0.8–1.5)	
				< 5 years	98	1.0 (0.7–1.4)	
				5–9 years	47	1.2 (0.8–1.9)	
				≥ 10 years	18	1.4 (0.8–2.5)	

CI, confidence interval; NR, not reported

The Nurses' Health study in the USA (Feskanich *et al.*, 1999) included two cohorts of 79 571 and 104 122 pre-menopausal white women. Response rates were 90% in both cohorts. Two hundred and fifty-two cases of melanoma were confirmed in both cohorts (146 in the first cohort from 1976 to 1994 and 106 in the second cohort from 1989 to 1995). All relative risks were adjusted for age, skin reaction to sun exposure, history of sunburn, mole counts, hair colour, family history of melanoma, parity, height and body mass index. The risk for cutaneous melanoma was 2.0 (95% CI, 1.2–3.4) among current users of oral contraceptives compared with women who had never used them. The increase in risk for melanoma was concentrated in the subgroup of current oral contraceptive users with 10 or more years of use, in whom 14 cutaneous melanomas occurred during the follow-up period (5.5% of all verified cases), which led to an adjusted relative risk of 3.4 (95% CI, 1.7–7.0). A higher estrogen dose did not appear to be associated with a higher risk for melanoma (assessed only in the second cohort). Risk did not appear to be elevated among past oral contraceptive users, even with longer duration of use. In women who had stopped taking oral contraceptives, no progressive decline in risk was observed with time since last use. No significant increase in risk was found in users who began taking oral contraceptives at 20 years of age or less.

2.7.2 *Case–control studies* (Table 19)

Beral *et al.* (1977) reported on oral contraceptive use and cutaneous melanoma in a study that was developed at a medical centre for the Kaiser Permanente Health Plan, California, USA. Thirty-seven cases aged 20–59 years at the time of diagnosis were registered at the medical centre. Two age-matched controls per case were recruited from administrative records of the plan. The crude risk for cutaneous melanoma for ever having used versus never having used oral contraceptives was 1.8 (95% CI, 0.7–4.6).

Adam *et al*. (1981) investigated 169 cases of cutaneous malignant melanoma in women aged 15–49 years who had been recorded at the cancer registries of southwestern England during 1971–76 and 342 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 from postal questionnaires for approximately 70% of the study women. The risk for cutaneous malignant melanoma was 1.1 (95% CI, 0.7–1.8) for ever having used combined oral contraceptives and [1.1 (95% CI, 0.4–2.8)] for current use. There was no increase in risk with duration of past or current use. Cases were moderately more sensitive to the sun and more likely to engage in outdoor tanning activities; 8% of cases had ever used sunlamps compared with 3% of controls ($p < 0.05$). No adjusted risks were presented, but the authors stated that adjustment did not affect the estimated risks.

In a case–control study of cutaneous melanoma in Seattle, USA (Holly *et al*., 1983), use of combined oral contraceptives for 5 years or longer was more common among cases than controls, which gave a relative risk of 3.1 (95% CI, 1.3–7.3) for duration of use of 10 years or more, with a highly significant trend ($p = 0.004$) with duration of use. The risk for melanoma increased steeply in women who had taken oral contraceptives for 5 years or

Table 19. Case–control studies of the use of combined oral contraceptives and malignant melanoma

Reference, location	No. of cases	Age (years)	No. of controls	Exposure	No. of cases/no. of controls	Odds ratios (95% CI)	Adjustment/comments
Beral *et al.* (1977), USA	37 from hospital-based cancer register	20–59	74	Never used Ever used No information	22/33 13/36 2/5	1.0 1.8 [0.7–4.6] –	Walnut Creek Contraceptive Drug Study; review of medical records
Adam *et al*. (1981), United Kingdom	169	15–49	342	Never used Ever used *Current or past use* 1 month–4 years ≥ 5 years No information	66/214 44/126 22/72 17/35 5/19	1.0 [1.1 (0.7–1.8)] [1.0 (0.6–1.8)] 1.6 (0.8–3.1)	Unadjusted; cases were moderately more sensitive to sun and more likely to engage in outdoor tanning activities; 8% of cases and 3% of controls had ever used sunlamps ($p < 0.05$); postal questionnaire
Holly *et al*. (1983), Seattle, USA	87	35–74	863	Never used *Current or past use* 1–4 years 5–9 years ≥ 10 years *For SSM only, use for ≥ 5 years, and* Current use 1–4 years since last use ≥ 5 years since last use	38/621 6/118 9/78 9/47 NR NR NR	1.0 [0.8 (0.3–2.2)] [1.9 (0.8–4.2)] [3.1 (1.3–7.3)] 0.9 (0.1–9.7) 2.5 (0.8–7.0) 5.1 (2.0–12.8)	Age; no data on exposure to sun
Lew *et al*. (1983), Massachusetts State, USA	111	23–81	107	–	–	–	No data reported but authors stated that there was no difference in combined oral contraceptive use between cases and controls.

Table 19 (contd)

Reference, location	No. of cases	Age (years)	No. of controls	Exposure	No. of cases/no. of controls	Odds ratios (95% CI)	Adjustment/comments
Beral *et al.* (1984), Sydney, Australia	287	15–24	574	Never used	79/159	1.0	No adjustment made, but authors stated that education, phenotype, history of sunburn and exposure to sun did not alter results; no difference by body location, thickness or histological type of melanoma
				Current or past use			
				1–4 years	124/274	[0.9 (0.6–1.3)]	
				5–9 years	56/103	[1.1 (0.7–1.7)]	
				≥ 10 years	28/36	[1.6 (0.9–2.9)]	
Helmrich *et al.* (1984), Canada and USA	160	20–59	640	Never used	97/370	1.0	Adjusted for age, area, religion, education, hormone-related variables
				Ever used	63/270	0.9 (0.6–1.3)	
				Use during year before study	8/52	0.5 (0.2–1.3)	
				Use for 5 years before study	4/18	0.9 (0.3–2.9)	
				Current or past use			
				< 1 year	15/82	0.7 (0.4–1.3)	
				1–4 years	23/106	0.8 (0.5–1.4)	
				5–9 years	11/49	0.8 (0.4–1.7)	
				≥ 10 years	5/21	1.0 (0.4–2.9)	
				Unknown	9/12		
				Use for ≥ 5 years, starting 10 years before study	12/46	1.0 (0.5–2.1)	
Holman *et al*. (1984), Western Australia	276	18–79	276	Never used	NR	1.0	Adjusted for sensitivity to sun, migration status, sun exposure; no difference in risk estimates for the different histological types; no association with time since last use; home interviews
				Ever used	NR	1.0 (0.6–1.6)	
				Current or past use			
				< 2 years	NR	0.8 (0.3–2.0)	
				2–4 years	NR	2.2 (0.7–6.8)	
				≥ 5 years	NR	1.6 (0.5–4.9)	

Table 19 (contd)

Reference, location	No. of cases	Age (years)	No. of controls	Exposure	No. of cases/no. of controls	Odds ratios (95% CI)	Adjustment/comments
Gallagher *et al*. (1985), Canada	361	20–79	361	Never used *Current or past use* < 1 year 1–4 years ≥ 5 years	NR NR NR NR	1.0 1.0 (NR) 0.9 (NR) 0.8 (NR) Trend NS	Adjusted for age, education, phenotype, freckling; no difference in risk estimates for the different histological types; home interviews
Green & Bain (1985), Queensland, Australia	91	15–81	91	Never used Ever used *Current and past use* 1 month–4 years > 4 years	48/42 43/49 31/30 12/19	1.0 0.7 (0.4–1.5) [0.9 (0.5–1.8)] 0.4 (0.2–1.1)	Adjustment for phenotypic characteristics, solar exposure did not change results; no trend with time since last use and age at last use
Østerlind *et al*. (1988), Denmark	280	20–79	536	Never used Ever used *Current or past use* < 2 years 2–4 years 5–9 years ≥ 10 years	167/299 111/237 24/58 30/68 27/59 30/52	1.0 0.8 (0.5–1.2) 0.8 (0.4–1.4) 0.8 (0.4–1.3) 0.8 (0.4–1.4) 1.0 (0.6–1.7)	Adjusted for age, sensitivity to sun, sunbathing; no difference according to type and potency of combined oral contraceptives
Zanetti *et al*. (1990), Province of Turin, Italy	186	19–92	205	Never used Ever used *Current or past use* < 3 years ≥ 3 years	83/88 NR 14/18 13/17	1.0 1.0 (0.5–1.9) 0.9 (0.4–1.8) 0.9 (0.3–2.0)	Analysed only in women aged 60 or younger; adjusted for age, education, phenotype, sunbathing; risk did not change according to type or location of melanoma, age or potency of combined oral contraceptive; hospital and home interviews.

Table 19 (contd)

Reference, location	No. of cases	Age (years)	No. of controls	Exposure	No. of cases/no. of controls	Odds ratios (95% CI)	Adjustment/comments
Lê *et al*. (1992), France	91	18–44	149	Never used	24/38	1.0	Adjusted for sensitivity, exposure to sun for a subgroup of cases and controls with no substantial changes in risk estimates for duration of use; no association with time since first use, age at first use or combined duration of use and time since first use
				Current or past use			
				1–9 years	54/97	1.1 (0.6–2.0)	
				≥ 10 years	13/14	2.1 (0.7–5.9)	
Palmer *et al*. (1992), New York and Philadelphia, USA	615	18–64	2107	Never used	313/800	1.0	Adjusted for age, education, body mass index, menopause, phenotype; elevated risk among non-severe cases of melanoma was attributed to surveillance bias; similar relative risk for different types
				Current or past use			
				< 3 years	201/447	[1.2 (0.9–1.4)]	
				≥ 3 years	73/193	[1.0 (0.7–1.3)]	
				Unknown	23/57		
				Severe cases with			
				5–9 years of use	12/80	1.0 (0.5–2.0)	
				≥ 10 years of use	29/187	1.1 (0.6–2.1)	
				Non-severe cases with			
				5–9 years of use	11/79	1.5 (0.8–2.6)	
				≥ 10 years of use	6/80	2.0 (0.9–4.3)	
Zaridze *et al*. (1992), Moscow, Russian Federation	54	NR	54	Never used	53/47	1.0	Adjusted for phenotype, naevi and sunbathing
				Ever used	1/7	0.04 (0.00–0.5)	

Table 19 (contd)

Reference, location	No. of cases	Age (years)	No. of controls	Exposure	No. of cases/no. of controls	Odds ratios (95% CI)	Adjustment/comments
Holly *et al*. (1995), San Francisco, USA	452	25–59	930	**CMM**			Adjusted for age; authors stated that risk estimates were unaltered by education, phenotype or exposure to sun.
				Never used	NR	1.0	
				Ever used	NR	0.7 (0.5–1.0)	
				Current or past use			
				< 5 years	NR	0.6 (0.4–0.9)	
				5–9 years	NR	0.9 (0.6–1.4)	
				≥ 10 years	NR	1.0 (0.6–1.6)	
				SMM			
				Ever used			
				Current or past use	NR	0.7 (0.5–0.9)	
				< 5 years	NR	0.6 (0.4–0.8)	
				5–9 years	NR	0.8 (0.6–1.1)	
				≥ 10 years	NR	0.8 (0.5–1.3)	
Westerdahl *et al*. (1996), Sweden	180	15–75	292	Never used	108/182	1.0	Adjusted for phenotype, naevi, sunburn; age at use and timing of use in relation to first child did not influence risk.
				Ever used	65/78	1.6 (0.9–2.8)	
				Current or past use			
				< 4 years	26/30	2.2 (0.9–4.6)	
				4–7 years	20/28	1.5 (0.7–3.5)	
				≥ 8 years	19/40	1.0 (0.5–2.0)	
Smith *et al.* (1998), Connecticut State, USA	308	≥ 18	233	Never used	170/131	1.0	Adjusted for age, marital status, hair colour, number of arm naevi, sun exposure index; no trend with duration of use and no association with age at first use
				Ever used	138/72	1.1 (0.6–1.7)	
				Current or past use			
				≤ 2 years	60/40	1.3 (0.7–2.3)	
				> 2–5 years	29/7	0.6 (0.3–1.2)	
				> 5 years	49/25	1.4 (0.7–2.8)	
Naldi *et al.* (2005), Italy	316	≥ 18	308	Never used	266/258	1.0	Adjusted for age, education, body mass index, number of melanocytic naevi, pigmentary traits, history of sunburn and reaction to sun exposure
				Ever used	50/60	1.1 (0.6–1.7)	

CI, confidence interval; CMM, cutaneous malignant melanoma; NR, not reported; NS, not significant; SMM, superficial spreading melanoma

more and had stopped since 1–4 or 5 years or more. There was no increase in risk among current users. The highest risk was found for superficial spreading melanoma. No adjustment was made for sensitivity or exposure to the sun. [Category-specific risks were not presented in the publication and were calculated by the Working Group.]

In a study in Sydney, Australia (Beral *et al*., 1984), increasing duration of oral contraceptive use was not associated with increased risk for cutaneous malignant melanoma. An increased risk was found for a subgroup of women who had used these formulations for 5 years or longer 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.

A case–control study carried out in several parts of the USA and Canada between 1976 and 1982 (Helmrich *et al*., 1984) included 160 women aged 20–59 years who had a recent histological diagnosis of cutaneous malignant melanoma and 640 control women aged 20–59 years who were admitted to hospital for trauma or orthopaedic and surgical conditions. The age-adjusted relative risk for women who had ever used combined oral contraceptives was 0.9 (95% CI, 0.6–1.3). There was no trend in risk with increasing duration of use, and the relative risk for ≥ 10 years of use was 1.0 (95% CI, 0.4–2.9). For the 40 cases and 140 controls who had first used combined oral contraceptives at least 10 years previously, the relative risk was 1.1 (95% CI, 0.7–1.8). 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 Australia (Holman *et al.*, 1984), a study was conducted in 276 women with melanoma and age-matched controls. The risk for melanoma for ever having used oral contraceptives was 1.0 (95% CI, 0.6–1.6). Extensive adjustement for sensitivity and exposure to the sun and migration status was made. For all melanoma and for the different types of melanoma, no association was observed with duration of use or with time since last use.

In a Canadian study (Gallagher *et al*., 1985), no association was found between the risk for cutaneous malignant melanoma and the use of combined oral contraceptives in 361 cases and an equal number of controls aged 20–69 years. The relative risks for < 1, 1–4 and ≥ 5 years of use, adjusted for age, phenotypic characteristics and freckling, were 1.0, 0.9 and 0.8, respectively. No association was seen between the histological 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 for 10 or more years before diagnosis of cutaneous malignant melanoma was 1.0.

A study in Queensland, Australia, in 1979–80 (Green & Bain, 1985) included 91 women aged 15–81 years who had melanoma and 91 age-matched controls chosen at random from the population. No increased risk for cutaneous malignant melanoma was found in relation to ever having used combined oral contraceptives (age-adjusted odds ratio, 0.7; 95% CI, 0.4–1.5), and no trend in risk was found with increasing duration of use,

age at last use or time since last use. Adjustment for sensitivity and exposure to the sun did not affect the risk estimates.

In a study from Denmark (Østerlind *et al*., 1988), all risk estimates were adjusted for age, phenotypic characteristics and sunbathing. The risk from ever having used oral contraceptives was 0.8 (95% CI, 0.5–1.2) for all melanoma and 0.9 (95% CI, 0.6–1.3) for superficial spreading melanoma. There was no evidence of an increased risk for cumulative exposure; the relative risk for ≥ 10 years of use was 1.0 (95% CI, 0.6–1.7). No specific pattern of risk was seen with the type of oral contraceptive, such as sequential progestogen-only or high-potency combined oral contraceptives, when these were assessed separately, but there were few women in each group.

Zanetti *et al*. (1990) carried out a case–control study in the Province of Turin, Italy, of 186 of 211 women aged 19–92 years who had histologically confirmed cutaneous malignant melanoma and were identified from the Turin Cancer Registry between 1984 and 1987 and 205 control women aged 17–92 years drawn from the National Health Service Registry. Use of combined oral contraceptives was analysed only in women aged 60 years or younger. Adjustment was made for age, education, phenotypic characteristics and sunbathing. The risk for cutaneous malignant melanoma of ever having used combined oral contraceptives was 1.0 (95% CI, 0.5–1.9) for all melanoma and 1.3 (95% CI, 0.4–4.5) for superficial spreading melanoma. No association was observed with duration of use. The longest duration of use (≥ 3 years) that began 10 or more years before the diagnosis of cutaneous malignant melanoma was not associated with an increased risk [risk estimates not reported]. The relative risks were identical for use of combined oral contraceptives that contained high estrogen doses (≥ 50 μg) and low estrogen doses.

Lê *et al*. (1992) assessed the effect of the use of combined oral contraceptives on the risk for cutaneous malignant melanoma in France between 1982 and 1987. The 91 cases from five hospitals were women under 45 years of age who had newly diagnosed histologically confirmed melanomas. Controls were 149 age-matched women who consulted in the same hospital for diagnosis or treatment of diseases that were unrelated to the use of combined oral contraceptives, including skin diseases. The risk for cutaneous malignant melanoma for ≥ 10 years of use of oral contraceptives was 2.1 (95% CI, 0.7–5.9). No association was found with time since first use (relative risk for 15–20 years since first use, 1.9; 95% CI, 0.8–4.5). No difference was found between superficial spreading melanoma and other types of cutaneous malignant melanoma. In the subgroup of 49 cases and 78 controls who were aged 30–40 years, a risk for melanoma of 4.4 (95% CI, 1.1–17) was found, based on 10 cases and eight controls who had used oral contraceptives for 10 years or more.

A case–control study of cutaneous malignant melanoma was carried out between 1979 and 1991 in Philadelphia and New York, USA (Palmer *et al*., 1992); the cases were 615 women under the age of 70 years (median age, 40 years) 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 (median age, 41 years) with other malignancies (610 patients) or non-malignant illnesses (1497 patients) that were judged to be unrelated to the 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 and 1.1 (95% CI, 0.6–2.1) for ≥ 10 years of use. For non-severe cases, duration of use was not associated with the risk.

Zaridze *et al*. (1992) evaluated risk factors in 96 cases of cutaneous malignant melanoma in Moscow, Russian Federation. Controls were recruited from among persons who were visiting cancer patients and matched by age. Use of combined oral contraceptives was 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), based on one case and seven controls who had ever used combined oral contraceptives.

In the study of Holly *et al*. (1995), 72% of cases of cutaneous malignant melanoma and 79% of control subjects in San Francisco, USA, reported ever having used combined oral contraceptives. The age-adjusted relative risk was 0.7 (95% CI, 0.5–1.0) for all cutaneous malignant melanoma and 0.7 (95% CI, 0.5–0.9) for superficial spreading melanoma. Examination by latency and duration of use showed no significant trend. The relative risk for ≥ 10 years of 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 all 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), 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 sunburn. No trend in risk was seen with duration of use (relative risk for > 8 years of use, 1.0; 95% CI, 0.5–2.0), age at first use or age at last use.

In Connecticut State, USA, Smith *et al.* (1998) investigated 308 women with melanoma aged ≥ 18 years and 233 control women in 1987–89. Cases were drawn from hospital-based records and controls were chosen from the general population by random-digit dialling. The risk for cutaneous melanoma among women who had ever used oral contraceptives was 1.1 (95% CI, 0.7–1.8) after adjustment for age, hair colour, marital status, number of arm naevi and sun exposure index. No association was found with duration of oral contraceptive use or with age at first use.

In Italy, Naldi *et al.* (2005) investigated 316 cases of melanoma in women of all ages and 308 control women in 1992–94. Cases were drawn from hospital-based records and controls were chosen from among non-dermatological and non-oncological patients who attended the same hospitals. The participation rate for cases and controls was 99%. The risk for cutaneous melanoma among women who had ever used oral contraceptives was 1.1 (95% CI, 0.6–1.7) after adjustment for age, education, body mass index, number of melanocytic naevi, pigmentary traits, history of sunburn and reaction to sun exposure.

2.7.3 *Meta- and pooled analyses*

A meta-analysis of 18 published case–control studies of cutaneous malignant melanoma and the use of combined oral contraceptives showed a pooled relative risk of 1.0 (95% CI, 0.9–1.0) (Gefeller *et al.*, 1998). The data for 3796 cases and 9442 controls showed no significant variation in 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.

In 2002, the investigators of case–control studies of cutaneous melanoma agreed to pool their original data in order to perform a new analysis of associations between melanoma and oral contraceptive use, using the same categories for exposure (Karagas *et al.*, 2002). The analyses were limited to studies that ascertained data on major risk factors for melanoma including pigmentary characteristics and exposure to sunlight. Analysis was further restricted to studies that involved a personal interview because questions designed for postal surveys may have been phrased differently or have been less complex. Studies that were limited to hospitalized cases were also excluded since these cases might have been biased by over-representation of advanced lesions. Finally, only studies that included at least 100 cases and 100 controls were retained, as smaller studies would have required a similar analytical effort, but would have contributed little to the overall analysis. Eleven case–control studies met the analytical criteria (Beral *et al.*, 1984; Holman *et al.*, 1984; Gallagher *et al.*, 1985; Green & Bain, 1985; Østerlind *et al.*, 1988; Swerdlow *et al.*, 1986; Elwood *et al.*, 1990; Zanetti *et al.*, 1990; Kirkpatrick *et al.*, 1994; Holly *et al.*, 1995; Langholz *et al.*, 2000) and data were available for all but one of these (Beral *et al.*, 1984). Two studies had never published their results on oral contraceptive use (Kirkpatrick *et al.*, 1994; Langholz *et al.*, 2000). The 10 pooled studies totalled 2110 women with melanoma and 3178 control women. Overall, no excess risk was associated with oral contraceptive use for 1 year or longer compared with never use or use for less than 1 year (pooled odds ratio, 0.86; 95% CI, 0.74–1.01) and there was no evidence of variation between studies. No relation was found between incidence of melanoma and duration of oral contraceptive use, age at starting use, year of use, years since first use or last use or specifically current oral contraceptive use.

2.8 Thyroid cancer

The results of 13 case–control studies of thyroid cancer and the use of oral contraceptives, 10 of which were reviewed in the previous evaluation (IARC, 1999), were pooled by La Vecchia *et al.* (1999) (see Table 20). The overall odds ratio was 1.5 (95% CI, 1.0–2.1) for current users, and declined to 1.1 over 10 years after cessation of oral contraceptive use.

Six subsequent studies are also summarized in Table 20. The largest (Sakoda & Horn-Ross, 2002), in which 544 cases and 558 population controls from the San Francisco Bay area, USA, were interviewed, yielded a slightly reduced risk for ever users (odds ratio, 0.7; 95% CI, 0.5–1.0). A hospital-based case–control study in Serbia of 204 matched case–control pairs reported ever use of oral contraceptives in 52 cases and 25 controls,

Table 20. Studies of the use of combined oral contraceptives and thyroid cancer

Reference, location	Age (years)	Cancer type	Oral contraceptive use	Cases	Controls	Odds ratio (95% CI)	Comments
Rossing *et al.* (1998), Washington State, USA	18–64	Papillary thyroid	*Age < 45 years*				
			Never	48	40	1.0	
			Ever	247	341	0.6 (0.4–0.9)	
			Age 45–64 years				
			Never	34	62	1.0	
			Ever	81	131	1.2 (0.7–2.2)	
La Vecchia *et al.* (1999), North America, Europe and Asia	All ages	Thyroid	Never	1324	2011	1.0	Pooled data from 13 studies
			Ever	808	1290	1.2 (1.0–1.4)	
			Current	91	118	1.5 (1.0–2.1)	
Mack *et al.* (1999), Los Angeles County, USA	15–54	Thyroid	Never	81	90	1.0	
			Ever	211	202	1.0 (0.6–1.6)	
Iribarren *et al*. (2001), San Francisco Bay area, USA	10–89	Thyroid	Use in last year	NR	NR	1.07 (0.69–1.67)	Kaiser Permanente cohort
Sakoda & Horn-Ross (2002), San Francisco Bay Area, USA	20–74	Papillary thyroid	Never	204	177	1.0	
			Ever	337	380	0.7 (0.5–1.0)	
			Current	79	83	0.7 (0.5–1.1)	
Haselkorn *et al.* (2003), San Francisco Bay Area, USA	20–74	Thyroid	*Age < 50 years*				No effect of duration; cases were Caucasian and Asian.
			Never	121	97	1.0	
			Ever	246	239	0.8 (0.6–1.2)	
			Age ≥ 50 years				
			Never	79	62	1.0	
			Ever	69	87	0.5 (0.3–0.8)	
Zivaljevic *et al.* (2003), Serbia	14–87	Thyroid	Never	152	179	1.0	
			Ever	52	25	2.5 (1.4–4.2)	

CI, confidence interval; NR, not reported

which gave a significant excess risk for ever users (odds ratio, 2.5; 95% CI, 1.4–4.2) (Zivaljevic *et al.*, 2003). The remaining four studies gave odds ratio estimates for ever use of oral contraceptives of between 0.6 and 1.2 (Rossing *et al.*, 1998; Mack *et al.*, 1999; Iribarren *et al.*, 2001; Haselkorn *et al.*, 2003).

2.9 Other cancers

Twenty-one studies of cancers at other sites (lung, gallbladder, pancreas, lymphomas, gestational trophoblastic diseases, neuroblastoma, oesophagus and kidney) are summarized in Table 21. Marginally significant reductions in risk among ever users of oral contraceptives were reported in two studies of lung cancer and in single studies for cancer of the pancreas, B-cell non-Hodgkin lymphoma and oesophageal cancer. All overall confidence intervals for other studies included unity.

3. Studies of Cancer in Experimental Animals

In this section, only relevant studies on estrogens and progestogens alone and in combination that were published subsequent to or were not included in the previous evaluation (IARC, 1999) are reviewed in detail. Studies that were reviewed previously are summarized briefly.

3.1 Estrogen–progestogen combinations

The results of studies reviewed previously (IARC, 1979, 1999) on the carcinogenicity of combinations of estrogens and progestogens that are used in combined oral contraceptives are summarized below (see Tables 22 and 23).

The incidence of pituitary adenomas in female and male mice was increased by administration of mestranol plus chlormadinone acetate, mestranol plus ethynodiol diacetate, ethinylestradiol plus ethynodiol diacetate, mestranol plus norethisterone, ethinylestradiol plus norethisterone (females only) and mestranol plus norethynodrel. The latter combination also increased the incidence of pituitary adenomas in female rats.

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

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

Table 21. Association between oral contraceptive use and the risk for other cancers

Reference, location	Age (years)	Cancer type	Oral contraceptive use	Cases	Controls	Odds ratio (95% CI)	Comments
Nelson *et al.* (2001), Los Angeles, USA	18–75	Intermediate or high-grade B-cell non-Hodgkin lymphoma	Never	111	93	1.00	Matched, fitting education, place of birth
			Ever	66	84	0.47 (0.26–0.86)	
			< 5 years	43	53	0.50 (0.26–0.95)	
			≥ 5 years	21	29	0.35 (0.15–0.82)	
Glaser *et al.* (2003), San Francisco Bay Area, USA	19–79	Hodgkin lymphoma	Never	87	91	1.0	Multivariate parsimonious model that includes variables significant at $p < 0.10$ (reproductive history, socio-economic status)
			≤ 2.2 years	91	79	1.2 (0.8–1.9)	
			2.3–5.3 years	72	73	0.9 (0.6–1.5)	
			> 5.3 years	62	82	0.8 (0.5–1.3)	
Vessey *et al.* (2003), England and Scotland	25–39	Lymphoma/ haemopoietic cancer	Never	16	–	1.0	OFPA cohort of 17 032 Caucasian women; adjusted for parity, social class, smoking
			< 4 years	6	–	0.9 (0.3–2.5)	
			4–8 years	8	–	1.0 (0.4–2.5)	
			> 8 years	11	–	1.2 (0.5–2.7)	
Schiff *et al.* (1998), Rochester, MN, USA	≥ 20	Central nervous system lymphoma	Never	35	52	1.0	
			Ever	3	19	0.3	
Gago-Dominguez *et al*. (1999), Los Angeles, USA	25–74	Renal-cell cancer	Never	258	255	1.0	Adjusted for age, education, hysterectomy
			Ever	164	167	1.0 (0.7–1.4)	
Olshan *et al.* (1999), Canada and USA	< 20	Neuroblastoma	*Maternal use during first trimester*				Odds ratios also unity for oral contraceptive use in previous year or ever
			No	442	444	1.0	
			Yes	17	15	1.0 (0.5–2.1)	

Table 21 (contd)

Reference, location	Age (years)	Cancer type	Oral contraceptive use	Cases	Controls	Odds ratio (95% CI)	Comments
Schüz *et al.* (2001), Germany	≤ 7	Neuroblastoma	*Maternal use during pregnancy*				
			No	159	1671	1.0	
			Yes	4	26	5.7 (1.5–23)	
			Unspecified	16	70	NR	
Palmer *et al.* (1999), USA	≥ 18	GTD	Never	36	98	1.0	Matched analysis; $p = 0.03$ for trend with duration
			Ever	199	315	1.8 (1.2–2.8)	
Parazzini *et al.* (2002), Greater Milan area, Italy	13–56	GTD	Never	164	306	1.0	Risk increased with duration
			Ever	104	130	1.5 (1.1–2.1)	
Taioli & Wynder (1994), USA	20–89	Lung adeno-carcinoma	Never	134	229	1.0	
			Ever	46	74	0.8 (0.5–1.5)	
Beral *et al.* (1999), United Kingdom	16–79	Lung	Never	40	–	1.0	RCGP cohort of 46 000 women
			Ever	75	–	1.2 (0.8–1.8)	
Kreuzer *et al.* (2003), Germany	≤ 75	Lung	Never	528	557	1.00	Adjusted for age, region, education, smoking variables
			Ever	279	354	0.69 (0.51–0.92)	
			< 5 years	86	105	0.69 (0.46–1.03)	
			5–11 years	87	130	0.65 (0.44–0.95)	
			≥ 12 years	102	115	0.69 (0.47–1.02)	
			Nonsmokers				Adjusted for age, region, education, time since stopped smoking
			Never	–	–	1.00	
			Ever	–	–	1.18 (0.78–1.79)	
			Smokers				
			Never	–	–	1.00	
			Ever	–	–	0.50 (0.34-0.74)	

Table 21 (contd)

Reference, location	Age (years)	Cancer type	Oral contraceptive use	Cases	Controls	Odds ratio (95% CI)	Comments
Vessey *et al.* (2003), England and Scotland	25–39	Lung	Never < 4 years 4–8 years > 8 years	15 9 12 18	– – – –	1.0 1.4 (0.6–3.5) 1.2 (0.5–2.6) 1.3 (0.6–2.8)	OFPA cohort of 17 032 Caucasian women; adjusted for parity, social class, smoking
Kreiger *et al.* (2001), Ontario, Canada	20–74	Pancreas	< 6 months ≥ 6 months	41 9	160 64	1.00 0.36 (0.13–0.96)	Multivariate, fitting age, oral contraceptives, hormonal menopausal therapy, obstetric history, body mass index, diet, smoking
Skinner *et al.* (2003), USA	30–55	Pancreas	Never Ever < 1 year 1–2.9 years 3–7.9 years ≥ 8 years	159 83 26 13 27 17	– – – – – –	1.00 1.21 (0.91–1.61) 1.45 (0.94–2.21) 0.78 (0.44–1.39) 1.38 (0.85–1.99) 1.26 (0.76–2.10)	Nurses Health Study; multivariate, fitting age, period, smoking, diabetes, body mass index, parity; age at baseline, 30–55 and followed up to 1998
Duell & Holly (2005), San Francisco Bay Area, USA	21–85	Pancreas	Never Ever < 1 year 1–2 years 3–7 years ≥ 8 years	135 102 18 25 18 41	402 394 70 140 73 103	1.00 0.95 (0.65–1.4) 0.85 (0.47–1.5) 0.67 (0.39–1.2) 0.92 (0.50–1.7) 1.4 (0.89–2.3)	Adjusted for age, education, smoking
Yen *et al.* (1987), Rhode Island State, USA	< 60	Gallbladder	Never Ever	6 4	70 6	1.0 7.8 (2.0–30)	

Table 21 (contd)

Reference, location	Age (years)	Cancer type	Oral contraceptive use	Cases	Controls	Odds ratio (95% CI)	Comments
WHO Collaborative Study (1989c), Chile, China, Columbia, Israel, Kenya, Mexico	NR	Gallbladder	Never Ever Current	49 9 1	269 86 8	1.0 0.6 (0.3–1.3) 0.9 (0.1–7.4)	
Moerman *et al.* (1994), Netherlands	35–79	Gallbladder and biliary tract	Never Ever	61 14	203 49	1.0 1.1 (0.5–2.4)	
Zatonski *et al.* (1997), Australia, Canada, Netherlands, Poland	64.9 (mean)	Gallbladder	Never Ever	132 20	558 142	1.0 1.0 (0.5–2.0)	
Gallus *et al.* (2001), Italy and Switzerland	< 79	Squamous-cell oesophageal cancer	Never Ever	110 4	392 33	1.0 0.24 (0.06–0.96)	Three hospital-based case–control studies pooled; adjusted for age, education, body mass index, energy intake, tobacco, alcoholic beverages

CI, confidence interval; GTD, gestational trophoblastic diseases; OFPA, Oxford Family Planning Association; RCGP, Royal College of General Practitioners

Table 22. Effects of combinations of various progestogens and estrogens 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 + ethinylestradiol			+/c				
Ethynodiol diacetate + mestranol	+	+					
Ethynodiol diacetate + ethinylestradiol	+	+				+	
Lynestranol + mestranol					+/–		
Lynestranol + ethinylestradiol + 3-methylcholanthrene							+[a]
Megestrol acetate + ethinylestradiol				+	+		
Norethisterone acetate + ethinylestradiol	+/?	+/?					
Norethisterone + ethinylestradiol		+					
Norethisterone + mestranol	+	+					
Norethynodrel + mestranol	+	+	c		+/?		+
Norethynodrel + mestranol + 3-methylcholanthrene						+	–
Norgestrel + ethinylestradiol + 3-methylcholanthrene							+[a]

From IARC (1979, 1999)

+, 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 estrogen or progestogen alone

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

Table 23. Effects of combinations of various progestogens and estrogens 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 + ethinylestradiol				+	+					
Ethynodiol diacetate + mestranol				?	?					
Megestrol acetate + ethinylestradiol			+/–	+/–	+/–	+/?	+/?			
Norethisterone acetate + ethinylestradiol			+			+		–	+	
Norethisterone + mestranol					+	+	–			
Norethynodrel + mestranol	+/?	+	+/?	+/?	+	+/?	–	–	–	+
Norethynodrel + mestranol + *N*-nitroso-diethylamine							–		–	+
Norgestrel + ethinylestradiol			+/–							

From IARC (1979, 1999)

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

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

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

Subcutaneous implantation and oral administration in rabbits

Virgin female New Zealand white rabbits, about 6 months of age and weighing 3.2–4.7 kg, were randomized into groups of five. Steroids (estradiol plus levonorgestrel or ethinylestradiol plus levonorgestrel) were delivered by subdermal implants in the neck or, in one case, by oral administration. The estimated dose of levonorgestrel was based on its loss from implants of the same type in women. Estimates of the doses of estradiol or ethinylestradiol delivered were based on measurements of in-vitro release from the implants used. Because of the high release and early exhaustion of steroid supply, the progestogen implants were replaced at 20-day intervals. The steroids administered orally were the commercially available combination pill Lo Femenal®; each tablet contained 30 μg ethinylestradiol and 300 μg norgestrel. Norgestrel contained equal amounts of levonogestrel and its inactive isomer. The pills were dispersed in 2 mL water and were administered intragastrically, but evaluation of the effects was confounded by the very low bioavailability of ethinylestradiol in this species when given orally. Hence, a new experiment was conducted in which levonorgestrel was delivered by oral pill and 30 μg of ethinylestradiol per day was delivered by implant for 8 weeks. At necropsy, lesions were noted and the weights of the uterus, liver and spleen were determined. Samples for tissue block preparation were taken from all lesions identified in organs or tissues, and samples were routinely taken from the uterus, spleen, lung, liver and bone marrow. Tissues were stained with haematoxylin and eosin and were studied microscopically. The animals were killed after 2 or 4.5 months. The incidences of tumours among the groups treated subcutaneously with a combination of estradiol and levonorgestrel are shown in Table 24. Estradiol alone resulted in decidualization, but did not induce deciduosarcoma, nor did tumours develop when estradiol was supplemented with lower doses of levonorgestrel, with the exception of an early atypical deciduosarcoma in one group. All animals in the groups treated with 66 or 233 μg/day levonorgestrel for 2 or 4.5 months had multiple deciduosarcomas. Neoplastic decidual

cells were found in the uterine blood vessels of many animals of these groups. In the other experiments by subcutaneous administration, ethinylestradiol was substituted for estradiol. The implants were in place for 6 months before the animals were killed. The experimental design and histological findings are summarized in Table 25. Ethinylestradiol alone induced deciduosarcomas in the spleen and ovary of one animal. In combination with a high dose of levonorgestrel (150 μg/day), even 10 μg/day ethinylestradiol produced many

Table 24. Incidence of malignant tumours in female New Zealand white rabbits treated subcutaneously with estradiol (E_2) and levonorgestrel (LNG)

Treatment time	E_2 (μg/day)	LNG (μg/day)	% of animals with deciduosarcomas (n = 4–5)				
			Uterus	Spleen	Liver	Ovary	Lung
2 months	0	0	0	0	0	0	0
	60	0	0	0	0	0	0
	60	8	0	25[a]	0	0	0
	60	25	0	0	0	0	0
	60	66	80	100	60	40	40[b]
	60	233	100	100	40	20	0
4.5 months	0	0	0	0	0	0	0
	6	233	20	0	0	0	0
	20	233	100	40	20	0	0
	60	233	100	75	25	0	25
	200	233	100	100	40	40	20

From Jänne *et al.* (2001)
[a] Atypical deciduosarcoma
[b] Metastasis

Table 25. Incidence of malignant tumours in female New Zeland white rabbits treated subcutaneously with ethinylestradiol (EE) and levonorgestrel (LNG)

Treatment time	EE (μg/day)	LNG (μg/day)	Proliferation of hepatic bile ductules (%)	% of animals with deciduosarcomas (n = 5)				
				Uterus	Spleen	Liver	Ovary	Lung
6 months	0	0	0	0	0	0	0	0
	30	0	80	0	20	0	20	0
	10	150	20	100	100	20	0	0
	30	150	20	100	100	100	20	20[a]

From Jänne *et al.* (2001)
[a] Metastasis

deciduosarcomas. As in the experiments cited earlier, hyperplasia of splenic mesenchymal cells was seen in most animals in all groups that received estrogen. Proliferation of hepatic bile ductules was observed in 40% of rabbits that received ethinylestradiol. This lesion was not seen in any other test or control rabbits. In the experiment in which levonorgestrel was delivered by an oral pill and 30 µg/day ethinylestradiol were delivered by implant, one of five animals developed a spleen deciduosarcoma (Jänne *et al*., 2001). [This study clearly shows the carcinogenic effects of combinations of levonorgestrel with ethinylestradiol as well as with estradiol. It is interesting to note that, although the study is well designed, there is no human counterpart for deciduosarcomas. Hepatic bile duct proliferation is a more relevant lesion in this context.]

3.2 Estrogens used in combined oral contraceptives

The results of studies reviewed previously (IARC, 1979, 1999) on the carcinogenicity of estrogens used in combined oral contraceptives are summarized below (see Tables 26 and 27).

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

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

Ethinylestradiol increased the incidence of cervical tumours in female mice.

In female and male mice, ethinylestradiol increased the incidence of hepatocellular adenomas. In female rats, ethinylestradiol and mestranol increased the numbers of altered hepatic foci. In rats, ethinylestradiol increased the incidence of hepatocellular adenomas in females and males and of hepatocellular carcinomas in females, whereas mestranol increased the incidence of hepatic nodules and carcinomas combined in females.

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

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

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

Table 26. Effects of ethinylestradiol and mestranol alone and with known carcinogens on tumour incidence in mice

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

From IARC (1979, 1999)
+, increased tumour incidence; –, no effect

Table 27. Effects of ethinylestradiol and mestranol alone and with known carcinogens on tumour incidence in rats

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

From IARC (1979, 1999)
+, increased tumour incidence; –, no effect; +/–, slightly increased tumour incidence
[a] In one of three studies, ethinylestradiol initiated hepatocarcinogenesis.

3.2.1 *Subcutaneous implantation*

(*a*) *Mouse*

A total of 272 female CD-1 (ICR) mice, 9 weeks of age, were divided equally into 17 groups and received subcutaneous implants into the back of cholesterol pellets (31.84 mg) that contained 0 or 0.16 mg estrone, estradiol, estriol, 2-hydroxyestrone, 2-hydroxyestradiol, 2-hydroxyestriol, 2-methoxyestrone, 2-methoxyestradiol, 2-methoxyestriol, 4-hydroxyestrone, 4-hydroxyestradiol, 16β-hydroxyestrone diacetate, 16-epiestriol, 16,17-epiestriol, 16α-hydroxyestrone or 17-epiestriol. The pellets were renewed every 7 weeks throughout the experiment. At 10 weeks of age, each mouse received a single injection of 12.5 mg/kg bw *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine (ENNG) dissolved at a concentration of 1.5% (w/v) in polyethylene glycol into one of the uterine cavities via the vagina. The experiment was terminated at 30 weeks of age, when all surviving animals were autopsied to obtain reproductive organs, and the uterus and ovaries were weighed and processed for histological examination. Endometrial proliferative lesions were classified histologically into two categories — hyperplasia and adenocarcinoma — and the incidence is given in Table 28. The results indicated that estrogens and their metabolites affect endometrial carcinogenesis in mice initiated with ENNG in a manner that is dependent on their metabolic attributes. Estrogens (estrone, estradiol and estriol) and their metabolites that belong to the 16α-hydroxylation pathway (16α-hydroxyestrone and 17-epiestriol) and the upstream of the 16β-hydroxylation pathway (16β-hydroxyestrone diacetate) exerted both promoting and additive co-carcinogenic effects on endometrial carcinogenesis in ENNG-initiated mice as shown by the development of invasive adenocarcinomas. Estrogen metabolites that belong to the downstream of the 16β-hydroxylation pathway exerted mainly promoting effects in this experimental model, as shown by the enhanced development of endometrial hyperplasia (16-epiestrol), or exerted no effect (16,17-epiestriol) (Takahashi *et al.*, 2004).

(*b*) *Hamster*

Groups of 10 male Syrian hamsters, 4–6 weeks of age, were implanted subcutaneously with 25-mg pellets of estradiol, 17α-estradiol, ethinylestradiol, menadione, a combination of 17α-estradiol and ethinylestradiol or a combination of ethinylestradiol and menadione [no effective dose provided]. The hamsters received a second identical pellet 3 months after the initial treatment. A control group of 10 animals was sham-operated and left untreated. The animals were killed after 7 months and inspected macroscopically for tumour nodules on the surface of each kidney (see Table 29). No tumour nodules were detected in untreated hamsters or in groups of hamsters that were treated with 17α-estradiol, ethinylestradiol, menadione or a combination of 17α-estradiol and ethinylestradiol for 7 months. In contrast, hamsters treated with either estradiol or a combination of ethinylestradiol and menadione for 7 months developed renal tumours. The tumour incidence was 90% in the group treated with estradiol (versus 0% in untreated controls; $p < 0.05$, Fisher's exact test) and 30% in the group treated with a combination of ethinylestradiol and menadione. The mean number of tumour nodules per hamster was higher in the group treated with estradiol compared

Table 28. Incidence of endometrial proliferative lesions in female CD-1 mice treated with a single dose of ENNG and various estrogens

Treatment	Effective no. of animals	Endometrial hyperplasias[a,b]				Adeno-carcinomas[b]	Total[b]
		+	++	+++	Total		
Control	16	2 (13)	6 (38)	1 (6)	9 (56)	0	9 (56)
Estrone	16	1 (6)	1 (6)	2 (13)	4 (25)	12 (75)*	16 (100)*
Estradiol	16	0	2 (13)	1 (6)	3 (19)	13 (81)*	16 (100)*
Estriol	16	1 (6)	7 (44)	2 (13)	10 (63)	6 (38)*	16 (100)*
2-Hydroxyestrone	16	5 (31)	6 (38)	2 (13)	13 (81)	0	13 (81)
2-Hydroxyestradiol	16	7 (44)	4 (25)	1 (6)	12 (75)	0	12 (75)
2-Hydroxyestriol	16	6 (38)	8 (50)	1 (6)	15 (93)*	0	15 (93)*
2-Methoxyestrone	16	7 (44)	3 (19)	0	10 (62)	0	10 (62)
2-Methoxyestradiol	16	7 (44)	7 (44)	1 (6)	15 (93)*	0	15 (93)*
2-Methoxyestriol	16	8 (50)	8 (50)	0	16 (100)*	0	16 (100)*
4-Hydroxyestrone	15	4 (27)	5 (33)	1 (7)	10 (67)	0	10 (67)
4-Hydroxyestradiol	16	6 (38)	4 (25)	2 (13)	12 (75)	0	12 (75)
16β-Hydroxyestrone diacetate	15	2 (13)	7 (47)	2 (13)	11 (73)	4 (27)*	15 (100)*
16-Epiestriol	16	9 (56)*	5 (31)	2 (13)	16 (100)*	0	16 (100)*
16,17-Epiestriol	16	5 (31)	6 (38)	1 (6)	12 (75)	0	12 (75)
16α-Hydroxyestrone	15	1 (7)	6 (40)	4 (27)	11 (73)	4 (27)*	15 (100)*
17-Epiestriol	15	1 (7)	2 (13)	1 (7)	4 (27)	11 (73)*	15 (100)*

From Takahashi *et al*. (2004)
ENNG, *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine
[a] Hyperplasias are classified based on the degree of cellular atypism into three subcategories: +, slight; ++, moderate; +++, severe
[b] Numbers of animals (percentage incidence)
* Significantly different from control value ($p < 0.05$, Fisher's exact test)

Table 29. Influence of different estrogens and menadione on renal carcinogenesis in male Syrian hamsters

Treatment	No. of animals with tumours/ no. of animals	Mean no. of tumour nodules per hamster
Estradiol	9/10[a]	7.0 ± 3.16[b]
17α-Estradiol	0/10	0
Ethinylestradiol	0/10	0
17α-Estradiol + ethinylestradiol	0/10	0
Menadione	0/10	0
Menadione + ethinylestradiol	3/10[c]	1.6 ± 2.67
Untreated	0/10	0

From Bhat *et al*. (2003)
[a] $p < 0.05$ compared with untreated controls and the menadione + ethinylestradiol-treated group by Fisher's exact test
[b] $p < 0.05$ compared with untreated controls by the $\div^2$ test; $p < 0.05$ compared with the menadione + ethinylestradiol-treated group by using the unpaired *t* test
[c] $p < 0.10$ compared with untreated controls by Fisher's exact test

with the group treated with a combination of ethinylestradiol and menadione or the untreated control group (Bhat *et al*., 2003).

3.2.2 *Subcutaneous injection*

Mouse

Groups of outbred female CD-1 mice [initial number of animals per group not specified] were given daily subcutaneous injections of 2 μg estrogen (2- or 4-hydroxyestradiol, estradiol or ethinylestradiol) dissolved in corn oil or corn oil alone (as a control) on days 1–5 of life. Mice were killed at 12 or 18 months of age. Tissue sections were stained and evaluated by light microscopy. The number of mice with reproductive tract tumours was determined. The incidence of uterine adenocarcinomas was compared by Fisher's exact tests (see Table 30). Both of the catechol estrogens examined (2- and 4-hydroxyestradiol) induced tumours in CD-1 mice. 2-Hydroxyestradiol was more carcinogenic than estradiol: 14 and 11% of the rodents developed uterine adenocarcinomas at 12 and 18 months, respectively, after neonatal administration of catechol estrogen. In contrast, 4-hydroxyestradiol was considerably more carcinogenic than 2-hydroxyestradiol and produced a 74% (12 months) and 56% (18 months) tumour incidence after treatment during the neonatal period. Ethinylestradiol also induced more tumours (38% at 12 months and 50% at 18 months) than estradiol. This study, which was designed to demonstrate the carcinogenic properties of catechol estrogens, also included a group that was treated with ethinylestradiol in which uterine adenocarcinomas were induced at a high incidence (Newbold & Liehr, 2000). [It should be

Table 30. Uterine adenocarcinomas in groups of female CD-1 mice injected subcutaneously with various estrogens during the neonatal period

Compound	Incidence of uterine adenocarcinoma No. of animals with tumour/total no. of animals (%)		
	12 months	18 months	Total
Control (corn oil)	0/12 (0)	0/22 (0)	0/34 (0)
2-Hydroxyestradiol	3/21 (14)	2/19 (11)	5/40 (12)*
4-Hydroxyestradiol	14/19 (74)	9/16 (56)	23/35 (66)**
Estradiol	0/5[a]	1/10 (10)	1/15 (7)
Ethinylestradiol	9/24 (38)	9/18 (50)	18/42 (43)**

From Newbold & Liehr (2000)
* $p < 0.05$ versus corn oil controls (Fisher's exact test)
** $p < 0.01$ versus corn oil controls (Fisher's exact test)
[a] These data were published previously (Newbold *et al*., 1990).

noted that studies in neonatal mice could have additional limitations for the extrapolation of the effects of hormones in adult women who take oral contraceptives.]

3.2.3 *Oral administration to transgenic mice*

Sixty female *p53* (+/–) mice (heterozygous female *p53*-deficient CBA mice, in which exon 2 of the lateral *p53* allele was inactivated, were the F_1 offspring of heterozygous *p53*-deficient male C57 BL/6J mice that had been back-crossed with female CBA mice (Tsukada *et al*., 1993)) and 60 female wild-type *p53* (+/+) litter mates, 6 weeks of age, were each divided into four groups of 15 animals. [The Working Group noted the small number of animals.] All mice received an intraperitoneal injection of 120 mg/kg bw *N*-ethyl-*N*-nitrosourea (ENU) in physiological saline, followed by no further treatment (Group 1) or were fed *ad libitum* diets that contained 1 ppm ethinylestradiol (Group 2), 2.5 ppm ethinylestradiol (5 ppm for the first 4 weeks reduced to 2.5 ppm thereafter because of marked body weight depression; Group 3) or 2000 ppm methoxychlor [a weakly estrogenic pesticide] (Group 4) for 26 weeks. Individual body weights in each group were measured every week. One of the 15 *p53* (+/–) mice in Group 2 died during the early period of the experiment. After the end of the 26-week experiment, surviving animals were killed and autopsied. The uterine tissues were sectioned and stained for microscopic examination. The differences in the incidence of uterine proliferative lesions were assessed by the Fisher's exact test. Multiple nodules (5–20 mm in diameter) of the uterine horn suggestive of uterine tumours were observed in seven, nine, 12 and seven *p53* (+/–) mice in Groups 1, 2, 3 and 4, respectively. The absolute uterine weights and uterine weight/body weight ratios of *p53* (+/–) and *p53* (+/+) mice in Groups 2, 3 and 4 were significantly higher than those in the Group 1 mice. The uterine weights of *p53* (+/–) mice, especially in Group 3, were considerably

increased because of marked growth of uterine tumours. Uterine proliferative lesions were classified into endometrial stromal polyps, endometrial stromal sarcomas, adenocarcinomas, atypical hyperplasias and endometrial glandular hyperplasias. Atypical hyperplasias were classified into two cell types — clear and basophilic — characterized by small proliferative foci of endometrial glandular epithelia with atypia. Non-atypical endometrial glandular hyperplasias were composed of increased numbers of endometrial glands with occasional cysts. The incidence of uterine stromal tumours in *p53* (+/–) mice was 87% (47% stromal sarcomas, 40% polyps), 85% (64% stromal sarcomas, 21% polyps), 87% (stromal sarcomas) and 53% (stromal sarcomas) in Groups 1, 2, 3 and 4, respectively; there was a significant difference in the incidence of stromal sarcomas between Groups 1 and 3 (see Table 31). In *p53* (+/+) mice, only stromal polyps were seen at an incidence of 20, 13, 0 and 0% in Groups 1, 2, 3 and 4, respectively; these values displayed a clear decrease compared with the incidence of stromal tumours in the groups of *p53* (+/–) mice. The incidence of clear-cell type atypical hyperplasias in *p53* (+/–) mice was 0, 14, 60 and 0% in Groups 1, 2, 3 and 4, respectively; that in *p53* (+/+) mice was 0, 7, 53, and 0%; the difference between Groups 1 and 3 was significant in both cases ($p < 0.05$, Fisher's exact test). For atypical hyperplasias of the basophilic cell type, there were no significant differences among the groups [incidence not specified]. One *p53* (+/–) mouse in Group 3 developed a clear-cell adenocarcinoma. The incidence of glandular hyperplasias in *p53* (+/–) mice was 60, 79, 60 and 27% in Groups 1, 2, 3 and 4, respectively, whereas that in *p53* (+/+) mice was 60, 80, 100 and 100%; the incidence in Group 3 and Group 4 *p53* (+/+) mice showed significant differences from values in Group 1 (Mitsumori *et al.*, 2000). [This study suggests that ethinylestradiol possibly exerts tumour-promoting (co-carcinogenic) effects on stromal and epithelial proliferative lesions of the uterus in *p53*-deficient mice initiated with ENU.]

3.3 Progestogens used in combined oral contraceptives

The results of studies that were reviewed previously (IARC, 1979, 1999) on the carcinogenicity of progestogens used in combined oral contraceptives are summarized below (see Tables 32, 33 and 34).

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

The incidence of malignant mammary tumours was increased in female mice by lynestrenol, megestrol acetate and norethynodrel. In female rats, lynestrenol and norethisterone slightly increased the incidence of malignant mammary tumours. In male rats, norethisterone also slightly increased the incidence of malignant mammary tumours, while norethynodrel increased the incidence of both benign and malignant mammary tumours. In female dogs, chlormadinone acetate, lynestrenol and megestrol acetate increased the incidence of benign and malignant mammary tumours; however, lynestrenol 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.

Table 31. Incidence of lesions (percentage) in female *p53* (+/+) and (+/–) CBA mice injected intraperitoneally with ENU and given EE or MXC in the diet

Treatment	*p53* (+/–) Mice (n = 15)[a]					*p53* (+/+) Mice (n = 15)				
	Uterine stromal tumours		Atypical hyperplasias		Glandular hyperplasias	Uterine stromal tumours		Atypical hyperplasias		Glandular hyperplasias
	Polyps	Stromal sarcomas	Clear-cell type	Basophilic cell type[b]		Polyps	Stromal sarcomas	Clear-cell type	Basophilic cell type[b]	
ENU	40	47	0	NR	60	20	0	0	NR	60
ENU + 1 ppm EE	21	64	14	NR	79	13	0	7	NR	80
ENU + 2.5 ppm EE	0	87*	60*[c]	NR	60	0	0	53*	NR	100*
ENU + MXC	0	53	0	NR	27	0	0	0	NR	100*

From Mitsumori *et al*. (2000)
ENU, *N*-ethyl-*N*-nitrosourea; EE, ethinylestradiol; MXC, methoxychlor; NR, not reported
* Significantly different from the ENU group ($p < 0.05$, Fisher's exact test)
[a] ENU + 1 ppm EE group (n = 14)
[b] Crude number not specified in the paper, but the authors state that there were no significant differences among treatment groups.
[c] One animal in this group developed a clear-cell adenocarcinoma.

Table 32. Effects of various progestogens alone or 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				+/–			
Lynestrenol				+			+			
Megestrol acetate				+				+		
Norethisterone acetate							+/–			
Norethisterone		+					+/–			
Norethynodrel	+	+	c	+				+/–		
Norethynodrel + 3-methyl-cholanthrene					+	–				

From IARC (1979, 1999)

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

[a] Dose exceeded the maximum tolerated daily dose

Table 33. Effects of various progestogens alone or 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		+							
Lynestrenol				+/–					
Norethisterone acetate					+	+		+	+ or –[c]
Norethisterone		+/–	+/–	+/–	+				
Norethynodrel	+	+	+		+	+	+		–[c]
Norethynodrel + *N*-nitrosodiethylamine									+

From IARC (1979, 1999)
+, 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 5 days and negative when it was administered as a single dose.
[c] Tested as a single dose for initiating activity

Table 34. Effects of various progestogens on mammary tumour incidence in female dogs

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

From IARC (1979, 1989)
+, increased tumour incidence
[a] In this study, lynestrenol 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.

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, lynestrenol, 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 incidence of liver adenomas and that of hepatocellular carcinomas in male and female mice, but at levels that exceeded the maximum tolerated dose. In rats, the incidence 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 female 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 increase the incidence of renal dysplastic lesions or tumours in female hamsters.

Oral administration to dogs

Groups of three female beagle dogs, 9–10 months old, were treated orally for 91 days with 0.03, 0.3 or 3 mg/kg bw per day dienogest. Three control animals were given the vehicle (0.5% carboxymethylcellulose) alone. On the day after the last treatment, the animals were killed and their mammary glands and pituitary glands were removed. The mammary glands from dogs treated with dienogest for 91 days showed dose-dependent proliferation. Dogs given 3 mg/kg bw per day showed severe alveoli hyperplasia and a large number of vacuoles in the alveolar cells. The pituitary glands from dienogest-treated dogs showed slight hypertrophy compared with those from control dogs (Ishikawa *et al.*, 2000). [Dienogest has progestational activity and caused proliferation of mammary gland epithelial cells in dogs, although no conclusive evidence of a carcinogenic effect was provided. As part of this same study, and with a similar design, dienogest showed no effects in female rats or monkeys.]

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

4.1 Absorption, distribution, metabolism and excretion in humans

The metabolism and disposition of various formulations of oral contraceptives used in humans differ. After entering the small intestine, estrogenic and progestogenic compounds in combined oral contraceptives undergo metabolism by bacterial enzymes and enzymes in the intestinal mucosa to varying extents. The mixture of metabolized and unmetabolized compounds then undergoes intestinal absorption, and thus enters the portal vein blood, which perfuses the liver. In the liver, the compounds can be metabolized extensively, which leads to variations in the amount of active drug. A fraction of the absorbed dose of ethinylestradiol and some progestogens is also excreted in the bile during its first transit through the liver. Although some of these compounds are partially reabsorbed via the enterohepatic circulation, a fraction may also be excreted in this 'first pass', which reduces overall bioavailability.

Since steroids penetrate normal skin easily, various systems have also been developed that deliver estrogens and progestogens parenterally, e.g. transdermal patches, nasal sprays, subcutaneous implants, vaginal rings and intrauterine devices (Fanchin *et al.*, 1997; Dezarnaulds & Fraser, 2002; Meirik *et al.*, 2003; Sarkar, 2003; Wildemeersch *et al.*, 2003; Sturdee *et al.*, 2004). These different modes of administration have been described previously (IARC, 1999). In general, all parenteral routes avoid loss of the drug by hepatic first-pass metabolism and minimally affect hepatic protein metabolism.

The absorption rates of orally administered estrogens and progestogens are usually rapid; peak serum values are observed between 0.5 and 4 h after intake. Serum concentrations rise faster with multiple treatments than with 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 estrogens and progestogens on cytochrome P450 (CYP) metabolic enzyme activities. Alternatively, estrogens may induce the production of sex hormone-binding globulin (SHBG), which may increase the capacity of the blood to carry progestogens. The metabolism of progestogens and ethinylestradiol typically involves reduction, hydroxylation and conjugation. In some cases, metabolism converts an inactive pro-drug into a hormonally active compound. Hydroxylated metabolites are typically conjugated as glucuronides or sulfates and most are eliminated rapidly, with half-lives of 8–24 h (IARC, 1999).

Little is known about the effect of hormonal therapy on aromatase (CYP 19), which is responsible for the synthesis of estrogens. Aromatase is expressed in both normal and malignant breast tissues and its activity in the breast varies widely. However, the mechanisms and extent of regulation of aromatase in breast tissues have not been fully established

and, to investigate the potential role of estrogen in this regulation, studies were carried out in an in-vitro model (Yue *et al.*, 2001) in which MCF-7 cells were cultured in long-term estrogen-deprived medium (LTED cells). It was found that long-term estrogen deprivation enhanced aromatase activity by three- to fourfold compared with that in wild-type MCF-7 cells. Re-exposure of LTED cells to estrogen reduced aromatase activity to the levels of wild-type MCF-7 cells. The authors also measured aromatase activity in 101 frozen breast carcinoma specimens and compared tumour aromatase activities in premenopausal patients with those in postmenopausal patients and in postmenopausal patients who did or did not take hormonal therapy. Although not statistically significant, a trend was observed that paralleled that in the in-vitro studies. Aromatase activity was higher in breast cancer tissues from patients who had lower levels of circulating estrogen. These data suggest that estrogen may be involved in the regulation of aromatase activity in breast tissues.

Two epidemiological studies examined the association of a common aromatase polymorphism (intron 4 TTTA repeat) and osteoporosis in postmenopausal women who did or did not take hormonal menopausal therapy. The Danish Osteoporosis Prevention Study showed an increase in bone mineral density in women who had long TTTA repeats and who received therapy (Tofteng *et al.*, 2004). In untreated women, no association was observed between bone mass or bone loss and the TTTA polymorphism. In contrast, a Finnish study found that the TTTA polymorphism did not influence bone mineral density, which is a risk for fracture, or circulating levels of estradiol in treated or untreated women (Salmen *et al.*, 2003).

4.1.1 *Ethinylestradiol and mestranol*

Structural modification of the estradiol molecule by insertion of an ethinyl group at carbon 17 yields ethinylestradiol, which is considerably more potent than estradiol and has high activity following oral administration. This compound is used frequently as the estrogenic component of oral contraceptives.

Modification of ethinylestradiol by formation of a methyl ether at carbon 3 gives rise to mestranol, which was widely used in the early years of oral contraception, but is now rarely employed. Mestranol binds poorly to the estrogen receptor and its estrogenic effect is due to its rapid demethylation in the liver to form ethinylestradiol; however, demethylation is not complete and more mestranol must be administered than ethinylestradiol to achieve similar effects.

Goldzieher and Brody (1990) studied the pharmacokinetics of doses of 35 μg ethinylestradiol (24 women) and 50 μg mestranol (27 women) in combination with 1 mg norethisterone. Serum concentrations of ethinylestradiol were measured after treatment with either estrogen, each of which produced an average serum concentration of approximately 175 pg/mL ethinylestradiol, with wide inter-individual variation. The maximal serum concentrations were achieved within about 1–2 h, and the half-life for elimination ranged from 13 to 27 h. The oral bioavailability of ethinylestradiol was 38–48%, and a 50-μg dose of mestranol was shown to be bioequivalent to a 35-μg dose of ethinylestradiol.

Hümpel *et al*. (1990) obtained serum samples from one group of 30 women who were taking a cycle of a combined oral contraceptive that contained ethinylestradiol and desogestrel and from another group of 39 women who were taking ethinylestradiol and gestodene. The serum concentrations of ethinylestradiol reached mean maximal levels of 106–129 pg/mL 1.6–1.8 h after intake. The mean serum concentrations of SHBG were 186–226 nmol/L, those of cortisol-binding globulin were 89–93 mg/L and those of cortisol were 280–281 μg/L.

Kuhnz *et al*. (1990a) compared the pharmacokinetics of ethinylestradiol administered as a single dose in combination with either gestodene or desogestrel to 18 women. In contrast to previous reports (Goldzieher & Brody, 1990; Hümpel *et al.*, 1990), which showed that the bioavailability of ethinylestradiol differed according to the associated progestogen, this study showed no significant difference.

The major pathway of ethinylestradiol metabolism in the liver of humans and animals is 2-hydroxylation, which is presumably catalysed by CYP 3A4 (Guengerich, 1988; see Section 4.1 of the monograph on Combined estrogen–progestogen menopausal therapy).

4.1.2 *Norethisterone*

Most of the data that pertain to the pharmacokinetics of norethisterone derive from a study (Back *et al.*, 1978) in which l mg norethisterone acetate and 0.05 mg ethinylestradiol were administered orally and intraveneously as a single dose or at 4-weekly intervals to a group of six premenopausal women. The results show that the absolute bioavailability of norethisterone after administration ranged from 47 to 73% (mean, 64%) compared with intravenous administration. The half-life [of the β phase of a two-component model] of elimination ranged from 4.8 to 12.8 h (mean, 7.6 h) with no significant differences between oral and intravenous administration.

Data from two different studies (Odlind *et al.*, 1979; Stanczyk *et al*., 1983; Stanczyk, 2003) showed a dose–response in circulating levels of norethisterone following oral administration to premenopausal women of 5 mg, 1 mg (combined with 0.12 mg ethinylestradiol), 0.5 mg and 0.3 mg norethisterone. Mean peak plasma levels were approximately 23, 16, 6 and 4 ng/mL, respectively, within 1–2 h after treatment.

Norethisterone undergoes extensive ring A reduction to form dihydro- and tetrahydronorethisterone metabolites that undergo conjugation; it can also be aromatized. Low serum levels of ethinylestradiol have been measured in postmenopausal women following oral administration of relatively large doses of norethisterone acetate or norethisterone (Kuhnz *et al.*, 1997). On the basis of the area-under-the-curve (AUC) values that were determined for ethinylestradiol and norethisterone, it was shown that the mean conversion ratio of norethisterone to ethinylestradiol was 0.7 and 1.0% at doses of 5 and 10 mg, respectively. The authors calculated that this corresponds to an oral dose equivalent of about 6 μg ethinylestradiol/mg of norethisterone acetate. Similarly, it was shown that a dose of 5 mg norethisterone administered orally was equivalent to about 4 μg ethinylestradiol/mg norethisterone. On the basis of these calculations, it was estimated that lower doses of norethiste-

rone or its acetate (e.g. 0.5–1.0 mg) in combination with ethinylestradiol would add between 0.002 and 0.01 mg ethinylestradiol to that already present. [The estimations for these lower doses were extrapolated from high doses of these compounds, which were not combined with ethinylestradiol. Nevertheless, it appears that significant amounts of ethinylestradiol are formed from norethisterone, and that the amount formed appears to be highly variable.]

No information on the pharmacokinetics of ethinylestradiol or mestranol via the dermal route (patch) was available to the Working Group.

4.1.3 *Norethisterone acetate, ethynodiol diacetate, norethynodrel and lynestrenol*

It is generally considered that progestogens that are structurally related to norethisterone are pro-drugs and that their progestational activity is due to their conversion to norethisterone. After oral administration, norethisterone acetate and ethynodiol diacetate are rapidly converted to norethisterone by esterases during hepatic first-pass metabolism. Although less is known about the transformation of lynestrenol and norethynodrel (Stanczyk & Roy, 1990), it appears that lynestrenol first undergoes hydroxylation at carbon 3 and then oxidation of the hydroxyl group to form norethisterone. Although there is no convincing evidence for the in-vivo transformation of norethynodrel to norethisterone, data from receptor-binding tests and bioassays suggest that norethynodrel is also a pro-drug.

4.1.4 *Levonorgestrel*

Stanczyk and Roy (1990) reviewed the metabolism of levonorgestrel in women treated orally with the 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). Approximately 45% of radioactively labelled levonorgestrel was excreted via the urine and about 32% via the faeces. The major urinary metabolites were glucuronides (the most abundant was 3α,5β-tetrahydrolevonorgestrel glucuronide) and smaller quantities of sulfates were found (Sisenwine *et al*., 1975b).

A dose–response has been demonstrated for circulating levels of levonorgestrel (Stanczyk, 2003) following administration of single oral doses of 0.25, 0.15 and 0.075 mg levonorgestrel to six, 24 and 24 women, respectively (Humpel *et al.*, 1977; Goebelsmann *et al.*, 1986; Stanczyk *et al.*, 1990). When the three doses were combined with 30–50 μg ethinylestradiol, mean peak levonorgestrel levels of 6.0, 3.5 and 2.5 ng/mL were attained at 1–3 h with the decreasing order of doses. At 24 h, the mean levonorgestrel level was 1.2 ng/mL with the highest dose and less than 0.5 ng/mL with the other two doses.

The bioavailability of levonorgestrel is generally accepted to be 100%. This generalization is based on two studies that used only a small number of women (Humpel *et al.*, 1978; Back *et al.*, 1981). In one of the studies (Back *et al.*, 1981), absolute bioavailabilities were determined for doses of 0.25 and 0.15 mg levonorgestrel, each of which was administered to five women in combination with ethinylestradiol (0.05 mg). The results show that the bioavailability for the 0.15-mg dose of levonorgestrel ranged from 72 to 125% (mean, 99%); that for the 0.15-mg dose ranged from 63 to 108% (mean, 89%). When the individual bioavailabilities for the 0.25-mg dose were examined, it was noted that 60% of the subjects had bioavailabilities greater than 100%. [This demonstrates that, for each of these subjects, the AUC for levonorgestrel obtained by the oral route was greater than that obtained intravenously, and implies that there was a methodological problem in the study.] In the same study, the mean half-life of elimination was found to be 13.2 h and 9.9 h for the 0.15-mg and 0.25-mg doses of levonorgestrel, respectively, when administered intravenously. These values were similar after oral dosing.

Carol *et al*. (1992) evaluated the pharmacokinetics of levonorgestrel in groups of 11–20 women who were given single or multiple treatments with combined oral contraceptive preparations that contained 0.125 mg levonorgestrel plus 0.03 or 0.05 mg ethinylestradiol. The serum concentrations of levonorgestrel reached a maximum of about 4 ng/mL at 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 SHBG increased after treatment with both contraceptives (but to a greater extent with the contraceptive containing 0.05 mg ethinylestradiol), which indicates the important role of estrogen in the induction of this protein.

Kuhnz *et al*. (1992) treated a group of nine women with a single dose of a combined oral contraceptive that contained 0.15 mg levonorgestrel plus 0.03 mg ethinylestradiol; eight of these women received the same regimen for 3 months after an abstention period of 3 months. The peak concentrations of levonorgestrel were found 1 h after single or multiple treatments. The peak serum concentrations of levonorgestrel were 3.1 and 5.9 ng/mL, respectively. The AUC concentration for total and free levonorgestrel increased by two- to fourfold after a single dose compared with multiple treatments. The distribution of free, albumin-bound and SHBG-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.

Kuhnz *et al*. (1994a) treated 14 women with a combined oral contraceptive that contained 0.125 mg levonorgestrel plus 0.03 mg ethinylestradiol as a single dose and for 3 months as a triphasic regimen (Triquilar®) after an abstention period of 1 week. The serum concentration of free levonorgestrel reached a peak of 0.06–0.08 ng/mL about 1 h after treatment with a single dose or on day 1 of the first or third cycle. In contrast, the calculated values of the AUC more than doubled, from 0.32 (single dose) to 0.75 (multiple treatment, first cycle)–0.77 (multiple treatment, third cycle) ng × h/mL. The serum concentrations of cortisol-binding globulin and SHBG more than doubled after multiple treatments with the contraceptive. After a single dose, 1.4% of the levonorgestrel in serum was free, 43% was

bound to albumin and 55% was bound to SHBG. After multiple treatments, only 0.9–1.0% levonorgestrel in serum was free and 25–30% was bound to albumin and the amount that was bound to SHBG 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 and increased again to 2 and 420 pg/mL, respectively, by the 1st day of the third cycle. Thus, the treatment-free interval of 1 week was sufficient to restore pretreatment values for testosterone.

4.1.5 *Desogestrel*

Desogestrel is a pro-drug and its progestational activity is mediated by one of its metabolites, 3-ketodesogestrel. In a study in which 10 women ingested single doses of 0.15 mg desogestrel combined with 0.03 mg ethinylestradiol and another group of women ingested single doses of 0.15 mg 3-ketodesogestrel combined with 0.03 mg ethinylestradiol (Hasenack *et al.*, 1986), serum 3-ketodesogestrel levels were essentially the same in both groups of women, whereas desogestrel was not found in significant amounts.

Following administration of a single oral dose of 0.15 mg desogestrel combined with 0.03 mg ethinylestradiol to a group of 25 women, the mean maximum concentration of 3-ketodesogestrel was 3.69 ng/mL, which was reached at a mean time of 1.6 ± 1.0 h (Bergink *et al.*, 1990). The mean bioavailability of 3-ketodesogestrel in two cross-over studies, in which women received either a single oral or intravenous dose of 0.15 mg desogestrel combined with 0.03 mg ethinylestradiol, was reported to be 76 and 62%, respectively (Back *et al.*, 1987; Orme *et al.*, 1991). The mean half-life of elimination for 3-ketodesogestrel, calculated from two studies (Back *et al.*, 1987; Bergink *et al.*, 1990) in which women were given a single oral dose of 0.15 mg desogestrel combined with 0.03 mg ethinylestradiol, was not consistent (23.8 h versus 11.9 h); the longer half-life was calculated from serum 3-ketodesogestrel levels that were obtained up to 72 h (Bergink *et al.*, 1990) whereas frequent blood sampling was carried out only up to 24 h in the other study. [The lack of data beyond 24 h is a deficiency of most studies of progestogen pharmacokinetics in which the half-life of elimination is calculated.]

A multiple dosing study (Kuhl *et al.*, 1988a) was carried out in 11 women who ingested 0.15 mg desogestrel in combination with 0.03 mg ethinylestradiol daily for 12 continuous treatment cycles and whose blood was sampled at frequent intervals on days 1, 10 and 21 of cycles 1, 3, 6 and 12. The results showed that 3-ketodesogestrel levels were relatively low on day 1 but rose progressively and were higher on day 21 of the treatment cycles. This increase was attributed to the elevated serum levels of SHBG induced by the estrogenic component of the pill.

A group of 19 women were given three cycles of a triphasic oral contraceptive that contained combinations of desogestrel and ethinylestradiol at doses of 0.15, 0.05 and 0.035 mg for the first 7 days, 0.10 and 0.03 mg for days 8–14 and 0.15 and 0.03 mg for days 15–21, respectively, followed by 7 days without hormone. Multiple blood samples were taken from the women throughout this interval, and serum concentrations of 3-keto-

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

4.1.6 *Gestodene*

Gestodene is metabolized primarily in the liver by CYP 3A4 and is a strong inducer of this enzyme. Although ethinylestradiol 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.

Gestodene is not a pro-drug. Following administration of a single oral dose of 0.025, 0.075 or 0.125 mg gestodene in combination with or without 0.03 mg ethinylestradiol to six women, a dose–response was observed. The half-life of elimination of gestodene was shown to range from 12 to 14 h for the three doses studied (Tauber *et al.*, 1989). Mean maximum concentration values of 1.0, 3.6 and 7.0 ng/mL gestodene were attained between 1.4 and 1.9 h, respectively. The mean absolute bioavailability was calculated to be 99% (range, 86–112%) for the commonly prescribed dose of 0.075 mg gestodene. In another similar study (Orme *et al.*, 1991), the mean absolute bioavailability was reported to be 87% (range, 64–126%).

The same experimental design that was used for the multiple dosing studies with desogestrel (Kuhl *et al.*, 1988a) was also used for gestodene (Kuhl *et al.*, 1988b). The results showed a dramatic rise in mean gestodene levels between day 1 and day 10, and a further rise between day 10 and day 21 in all study cycles. These findings were attributed to increased levels of SHBG and were similar to those obtained when multiple dosing was performed with desogestrel.

Following oral administration of 0.075 mg gestodene combined with 0.03 mg ethinylestradiol, either as a single dose or as multiple doses, the circulating levels of gestodene were relatively high compared with similar treatments with other progestogens in combination with ethinylestradiol (Fotherby, 1990). [This finding was surprising because the 0.075-mg dose of gestodene is the lowest dose of any progestogen used in a combined oral contraceptive pill. The major factor responsible for the elevated levels of gestodene appears to be the high affinity of SHBG for gestodene, which results in a lower metabolic clearance and consequently a higher concentration of this progestogen in the blood. It has been reported that approximately 75% of gestodene is bound to SHBG after oral treatment with gestodene/ethinylestradiol, which is considerably higher than that observed with other progestogens combined with ethinylestradiol.]

Kuhnz *et al*. (1990b) studied the binding of gestodene to serum proteins in 37 women who had taken a combined oral contraceptive that contained gestodene plus ethinyl-

estradiol for at least 3 months: 0.6% was free, 24% was bound to albumin and 75% was bound to SHBG.

Kuhnz *et al*. (1991) examined the effects of a single administration followed by multiple administrations over one cycle (after an abstention period of 1 week) of a triphasic combined oral contraceptive that contained gestodene and ethinylestradiol on the concentrations of ethinylestradiol and testosterone in 10 women. After a single oral dose of 0.10 mg gestodene plus 0.03 mg ethinylestradiol, the serum ethinylestradiol 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 intake. In comparison with day 21 after the single dose treatment, the levels of total and free testosterone were reduced by about 60% on day 21 of the treatment cycle.

Kuhnz *et al*. (1993) treated 14 women with a combined oral contraceptive that contained 0.10 mg gestodene plus 0.03 mg ethinylestradiol as a single dose and for 3 months as a triphasic regimen after an abstention of 1 week. The maximum serum concentrations of gestodene 30 min after dosing 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. A half-life for clearance of 18 h was observed after a single treatment, with a volume of distribution of 84 L. Multiple treatments increased the clearance half-life to 20–22 h and reduced the distribution volume to about 19 L. The serum concentration of SHBG increased with multiple treatments, presumably as an effect of ethinylestradiol, which is thought to account for the observed change in the distribution of gestodene (from 1.3% free, 69% bound to SHBG and 29% bound to albumin after a single treatment to 0.6% free, 81% bound to SHBG and 18% bound to albumin after multiple treatments).

Heuner *et al*. (1995) treated 14 women with a combined oral contraceptive that contained 0.075 mg gestodene plus 0.02 mg ethinylestradiol by a single administration or for 3 months as a triphasic regimen. The serum concentrations of gestodene, ethinylestradiol, cortisol-binding globulin, SHBG and testosterone were followed after the single treatment and through cycles 1 and 3. The concentration of gestodene reached a maximum of 3.5 ng/mL within 0.9 h after a single dose and 8.7 ng/mL within 0.7 h after multiple doses. The clearance half-time for a single dose of gestodene also increased from 12.6 h to nearly 20 h after multiple treatments. There was a large increase in the concentration of SHBG with time after multiple treatments. After a single dose, 1.3% of gestodene in serum was free, 30% was bound to albumin and 68% was bound to SHBG.

4.1.7 *Norgestimate*

Very little is known about the pharmacokinetics of orally administered norgestimate except that it is a relatively complex pro-drug. After its oral administration, the acetate group at carbon 17 is rapidly removed during hepatic first-pass metabolism. The product formed — levonorgestrel-3-oxime — has progestational activity. It has also been referred to as deacetylated norgestimate and, more recently, has been assigned the common name

norelgestromin. Rapid formation of norelgestromin from norgestimate was demonstrated by McGuire *et al.* (1990); serum levels of norelgestromin were measured after administration of single and multiple oral doses of 0.36 mg norgestimate combined with 0.07 mg ethinylestradiol to 10 women. Mean peak serum levels of 17-deacetylnorgestimate (norelgestromin) were approximately 4 ng/mL and were attained after about 1.4 h; the levels remained elevated up to 36 h after treatment. In contrast, peak levels of norgestimate were only ~100 pg/mL 1 h after treatment; the concentration declined rapidly thereafter and none was detectable 5 h after treatment.

Norgestimate is converted to levonorgestrel. In a randomized, comparative pharmacokinetic study by Kuhnz *et al.* (1994b), 12 women received single oral doses of 0.25 mg norgestimate combined with 0.035 mg ethinylestradiol and 0.25 mg levonorgestrel combined with 0.05 mg ethinylestradiol. The levonorgestrel AUC ratios were determined after administration of both formulations and were used to calculate the bioavailability of norgestimate-derived levonorgestrel: on average, about 22% of the administered dose of norgestimate became systemically available as levonorgestrel.

In addition to norelgestromin and levonorgestrel, a third progestationally active metabolite of orally administered norgestimate is formed, which is probably levonorgestrel-17-acetate (Kuhnz *et al*., 1994b).

4.1.8 *Newly developed progestogens*

In recent years, new progestogens have been synthesized that may improve the performance of combined hormones. Two of these that are currently in use (Sitruk-Ware, 2004a) are discussed below. Other members of this group, e.g. nesterone, nomegestrol acetate and trimegestone, are used much less frequently, although their pharmacological profile is similar with respect to receptor binding (see Tables 17 and 18 in Section 4.2 of the monograph on Combined estrogen–progestogen menopausal therapy) (Kuhl, 1996; Couthino *et al*., 1999; Kumar *et al.*, 2000; Tuba *et al.*, 2000; Lepescheux *et al.*, 2001; Shields-Botella *et al.*, 2003).

(*a*) *Dienogest*

Dienogest (17α-cyanomethyl-17β-hydroxyestra-4,9-dien-3-one) is a derivative of 19-nortestosterone. It has progestational activity but no androgenic, estrogenic, anti-estrogenic or corticoid activity. It strongly suppresses endometrial proliferation and does not antagonize the beneficial effects of estrogens. Dienogest binds highly selectively to the progesterone receptor, but does not bind to SHBG. As a result, it does not compete with testosterone for binding, and thereby helps to minimize the free serum levels of the androgen. Relatively high levels of the compound (approximately 9%) are free in the serum. After oral intake, a maximum serum concentration of dienogest is reached after about 1 h and does not accumulate after repeated dosing. The compound has an elimination half-life of 9.1 h. Studies of receptor binding have shown that the anti-androgenic activity of dienogest is similar to that of cyproterone acetate and progesterone (Teichmann, 2003).

Studies on the pharmacokinetics of dienogest have been carried out following oral and intravenous administration of different doses (Oettel *et al.*, 1995). A dose–response in serum dienogest levels was observed in 12 women after oral administration of four single doses (1, 2, 4 and 8 mg) in randomized order during four consecutive menstrual cycles. Following administration of the 1-mg dose, the mean maximum concentration was 23.4 ng/mL and the time to reach this level was 2.2 ± 1.1 h; half-life of elimination was 6.5 h. The absolute bioavailability of dienogest was determined in 16 healthy male volunteers who ingested a single dose of two tablets, each of which contained 2 mg dienogest and 0.03 mg ethinylestradiol; the average bioavailability value was 96.2%. In the same study, the average terminal half-life was reported to be 10.8 and 11.6 h after oral and intravenous doses, respectively.

From the two studies described above and other related studies by Oettel *et al.* (1995), it can be concluded that circulating levels of dienogest are relatively high compared with those found with similar doses of other progestogens. The clearance of dienogest appears to be lower than that of other progestogens, although most of it is weakly bound to albumin in the blood (Oettel *et al.*, 1995). No significant accumulation of dienogest was observed in serum during its daily intake (Oettel *et al.*, 1995).

(*b*) *Drospirenone*

The pharmacokinetic characteristics of drospirenone (3 mg) combined with ethinylestradiol (0.03 mg) were assessed in 13 women during 13 continuous cycles, each of which consisted of 21 continuous days of treatment followed by a 7-day treatment-free interval (Blode *et al.*, 2000). Frequent blood sampling was carried out on day 21 of treatment cycles 1, 6, 9 and 13. After administration of the first tablet, the mean maximum concentration of drospirenone was 36.9 ng/mL, which rose to 87.5 ng/mL on day 21 of the first cycle and ranged from 78.7 to 84.2 ng/mL on day 21 of the next three sampling cycles. The corresponding time to reach peak levels ranged from 1.6 to 1.8 h, and the half-life of elimination values were 31.1–32.5 h.

Other pharmacokinetic characteristics of drospirenone, based on data obtained by the manufacturer of an oral contraceptive that contained 3 mg drospirenone combined with 0.03 mg ethinylestradiol, have been reviewed (Krattenmacher, 2000). It was reported that a steady-state in circulating drospirenone levels is achieved after 1 week of treatment, and a dose–response in circulating drospirenone levels is obtained following oral administration of doses ranging from 1 to 10 mg. In addition, the absolute bioavailability was reported to be on average 76%.

4.1.9 *Interactions of other drugs with oral contraceptives*

Kopera (1985) reviewed the drug interactions associated with the administration of progestogens to patients who received other medications. Progestogens adversely affect the metabolism of certain drugs and, in turn, the metabolism of progestogens is affected by other drugs. These effects presumably occur as a consequence of the induction of

metabolic enzymes, or competition for metabolic pathways or for binding to serum carrier proteins (Shenfield *et al*., 1993).

Data on the effects of some oral contraceptive estrogens and progestrogens in animals were reviewed previously (IARC, 1999) (see also Section 4.2 of this monograph and Sections 4.1 and 4.2 of the monograph on Combined estrogen–progestogen menopausal therapy in this volume).

4.2 Receptor-mediated effects

There is evidence that not all the effects of estrogens and progestogens that are used in combined hormonal (oral) contraceptives are mediated through nuclear or other receptors. In addition, the effects of these steroids probably involve several molecular pathways and cross-talk between receptor- and/or non-receptor-mediated pathways. During the past decade, research on the mechanisms of hormonal action and on hormones and cancer has grown immensely. Two different subtypes of the progesterone receptor, subtypes A and B (Kazmi *et al*., 1993; Vegeto *et al*., 1993), and several estrogen receptors that have different functions — the nuclear estrogen receptors-α and -β and their subtypes — have been identified (Kuiper *et al*., 1996; Mosselman *et al*., 1996; Kuiper *et al*., 1997). In addition, estrogen receptors-α and other estrogen-binding proteins that are located in the plasma membrane appear to be responsible for rapid non-genomic estrogen responses (Pietras *et al*., 2001; Song *et al*., 2002; Razandi *et al*., 2003) and may activate signal transduction pathways by estrogens (Razandi *et al*., 2003; Song *et al*., 2004). There is also some evidence to suggest that a non-genomically acting progesterone receptor is responsible for rapid progestogen responses (Castoria *et al*., 1999; Sutter-Dub, 2002; Sager *et al*., 2003). However, the literature on specific interactions of constituents of combined oral contraceptive preparations with these receptor subtypes is still limited.

Increased attention to the various components of combined oral contraceptives in recent years has resulted in the availability of more information on the progestogens used with respect to their hormonal activities and binding to various receptors and other binding proteins. This information is summarized in Tables 35 and 36.

4.2.1 *Combined oral contraceptives*

(*a*) *Humans*

(i) *Breast epithelial cell proliferation*

It was concluded previously that exposure to combined oral contraceptives increases breast epithelial cell proliferation and that, when ethinylestradiol is the estrogen component, this effect is dose-dependent (IARC, 1999). Increased breast epithelial cell proliferation may be associated with an increased risk for breast cancer (Russo & Russo, 1996; Pihan *et al*., 1998). Isaksson *et al*. (2001) confirmed and extended these conclusions which are consistent with an increase in risk for breast cancer: all 53 women who had taken combined

Table 35. Overview of the spectrum of hormonal activities of progestogens used in combined oral contraceptives[a]

Progestogen	Progesto-genic	Anti-estrogenic	Estrogenic	Androgenic	Anti-androgenic	Gluco-corticoid	Antimineralo-corticoid
Chlormadinone acetate	+	+	–	–	+	+	–
Cyproterone acetate	+	+	–	–	+, +	+	–
Desogestrel	+	+	–	+	–	±, –	–
Dienogest	+	+, ±	–, ±	–	+	–	–
Drospirenone	+, +	+	–	–	+	?, –	+
Gestodene	+	+	–	+	–	±, +	+
Levonorgestrel	+	+	–	+	–	–	–
Norethisterone acetate	+, +	+	+	+	–	–	–

Adapted from Wiegratz & Kuhl (2004); second value for progestogenic activity only from Sitruk-Ware (2002); second value, except for progestogenic activity from Schindler *et al*. (2003)

+, effective; ±, weakly effective; –, ineffective; ?, unknown

[a] Data are based mainly on animal experiments. The clinical effects of progestogens are dependent on their tissue concentrations. No comparable data were available for ethynodiol diacetate or lynestrenol.

Note: This information should be viewed as only an indication of the hormonal activity (and its order of magnitude) of the various progestogens.

Table 36. Relative binding affinities of progestogens used in combined oral contraceptives to steroid receptors and serum binding globulins[a]

Progestogen	PR	AR	ER	GR	MR	SHBG	CBG
Chlormadinone acetate	134	5	0	8	0	0	0
Cyproterone acetate	180	6	0	6	8	0	0
Desogestrel (as 3-ketodesogestrel)	300	20	0	14	0	15	0
Dienogest	10	10	0	1	0	0	0
Drospirenone	70, 19	65, 2	0, < 0.5	6, 3	230, 500	0	0
Gestodene	180, 864	85, 71	0, < 0.02	27, 38	290, 97	40	0
Levonorgestrel	300, 323	45, 58	0	1, 7.5	75, 17	50	0
Norethisterone acetate	150, 134	15, 55	0.015	0, 1.4	0, 2.7	16	0
Reference compounds (100%)	Progesterone	Metribolone R1881	Estradiol-17β	Dexamethasone (or cortisol)	Aldosterone	5α-dihydro-testosterone	Cortisol

Adapted from Wiegrazt & Kuhl (2004); second value from Sitruk-Ware (2004)

AR, androgen receptor; CBG, corticoid-binding globulin; ER, estrogen receptor; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; PR, progesterone receptor; SHBG, sex hormone-binding globulin

[a] Values were compiled by these authors by cross-comparison of the literature. Because the results of the various in-vitro experiments depend largely on the incubation conditions and biological materials used, the published values are inconsistent. These values do not reflect the biological effectiveness, but should be viewed as only an indication of the order of magnitude of the binding affinities of the various progestogens. No comparable data were available for ethinodiol acetate or lynestrenol.

oral contraceptives had used preparations that contained ethinylestradiol combined with levonorgestrel, desogestrel, lynestrenol or norethisterone; however, the actual dose levels were not provided. Data from these women were compared with those from 54 women who had not used hormonal contraception. Fine needle aspirates were obtained between days 16 and 21 on the first cycle of treatment or, for control women, during the second half of the menstrual cycle. The mean percentage of breast epithelial cells that stained for Ki-67 (using the MIB-1 antibody that reacts with a human nuclear antigen present in proliferating cells) was 4.8% (median, 3.0%; range, 0–50%) in women exposed to combined oral contraceptives and 2.2% (median, 1.5%; range, 0–8%) in control women, a difference that was statistically significant. In 37 women who had taken ethinylestradiol plus levonorgestrel, the correlation between serum levonorgestrel levels and breast epithelial cell proliferation was found to be statistically significant in a positive direction (Spearman $r = 0.43$; $p = 0.02$). For 16 women who had taken ethinylestradiol plus levonorgestrel, fine needle aspirates were obtained both before the start of the oral contraceptive treatment and 2 months afterwards. The mean percentage of breast epithelial cells that stained for Ki-67 was 1.4% (median, 0.5%; range, 0–5%) before treatment and 5.8% (median, 0.8%; range, 0–50%) after 2 months of treatment; this change was of borderline significance ($p = 0.055$).

(ii) *Effects on the endometrium*

Four independent studies have investigated the effects of combined oral contraceptives on the endometrium. Moyer and Felix (1998) obtained endometrial biopsies from two groups of nine women who were exposed for an unreported duration to a regimen that consisted of oral treatment with either 0.06 mg mestranol plus 5 or 10 mg norethisterone acetate or 0.075 mg mestranol plus 5 mg norethynodrel for 21 days, followed by 7 days with no treatment. Biopsies were taken during the 7 days with no treatment. For comparison, biopsies were also taken from 10 untreated premenopausal women between days 5 and 14 of the menstrual cycle. Mitotic counts were significantly reduced from 12.3/1000 glandular cells in untreated women to 1.6 and 0.01/1000 cells in women exposed to mestranol plus norethynodrel and norethisterone acetate, respectively. [The Working Group noted that the timing of biopsy in the treated and untreated women probably does not permit an adequate comparison.]

Archer (1999) studied 11 women who took a triphasic oral regimen of 0.02 mg ethinylestradiol plus 0.05 mg desogestrel for 21 days, placebo for 2 days and 0.01 g ethinylestradiol for the last 5 days of each 28-day cycle. Endometrial biopsies were taken after 13–14 cycles. A progestational effect on endometrial histology was observed with secretory changes in samples obtained between days 11 and 21 of the treatment cycle. However, this was benign and no endometrial hyperplasia or metaplasia was observed.

Oosterbaan (1999) reported a study of women who took an oral preparation that consisted of 0.05 mg ethinylestradiol plus 0.06 mg gestodene for 24 days followed by 4 days with no treatment. Endometrial biopsies were taken during the late luteal phase before treatment (baseline biopsies) and between days 15 and 24 of the treatment cycle during cycle 3 or 6. Histological evidence of endometrial atrophy was observed in three of nine subjects

during cycle 3 and in four of nine subjects during cycle 6, whereas 11 of 13 baseline biopsies showed a secretory endometrium.

A regimen that consisted of 21 days of 0.03 mg ethinylestradiol plus 3 mg drospirenone followed by 7 days of no treatment was studied by Lüdicke *et al.* (2001). Endometrial biopsies were taken at baseline (26 women) and after 3 (11 women), 6 (10 women) and 13 cycles (26 women); endometrial thickness was also assessed by ultrasound at these four time-points (n = 26). Morphometry and ultrasound showed endometrial atrophy following the treatment: after 3, 6 and 13 cycles, 41, 44 and 63% of subjects, respectively, had an atrophic endometrium. Glandular mitotic activity (normally 0.3/1000 cells) was also absent at these three time-points.

Collectively, these four studies indicate atrophic and anti-proliferative endometrial effects of progestogen-containing combined oral contraceptives, apparently regardless of the regimen and the actual progestogen used. This anti-proliferative effect may be associated with a reduction in the risk for endometrial cancer.

(iii) *Effects on the colon*

In addition to a range of growth factor receptors, the human colon has both estrogen and progesterone nuclear receptors and expresses known estrogen-inducible genes (*pS2* and *ERD5*) (Di Leo *et al*., 1992; Hendrickse *et al.*, 1993; Singh *et al*., 1998). However, some studies did not confirm these findings; in addition, there are conflicting results on the expression of these receptors in stromal versus epithelial cells which are perhaps related to differences in the methodology used between studies (Waliszewski *et al*., 1997; Slattery *et al*., 2000). Nevertheless, these observations suggest that there may be a link between exposures to hormones in combined oral contraceptives and menopausal therapy and colon cancer, but they do not predict the nature of such a relationship.

(iv) *Effects on hormonal systems*

Several new studies have investigated the relationship between combined oral contraceptives and hormonal parameters. In four of these, serum levels of SHBG and free testosterone were measured. Levels of SHBG were increased by two- to fourfold and free testosterone levels were reduced by 40–80%, regardless of the combined oral contraceptive regimen used (Piérard-Franchimont *et al*., 2000; Boyd *et al*., 2001; Isaksson *et al*., 2001; Wiegratz *et al*., 2003). Wiegratz *et al*. (2003) studied women who were taking four different contraceptive regimens for 21 days followed by 7 days with no treatment: 0.03 mg ethinylestradiol plus 2 mg dienogest, 0.02 mg ethinylestradiol plus 2 mg dienogest, 0.01 mg ethinylestradiol plus 2 mg estradiol valerate plus 2 mg dienogest or 0.02 mg ethinylestradiol plus 0.10 mg levonorgestrel. In addition to reducing free testosterone and increasing the levels of SHBG, all treatments reduced levels of dehydroepiandrosterone sulfate and increased those of corticoid-binding globulin by approximately twofold and of thyroxin-binding globulin by approximately 50%, while prolactin was not affected. All four regimens had comparable effects, except that ethinylestradiol plus levonorgestrel increased levels of SHBG by only 50–100% and ethinylestradiol plus estradiol valerate plus dienogest

increased levels of prolactin by up to 40%. In the study by Isaksson *et al.* (2001), levels of androstenedione and total testosterone were also reduced as well as that of serum progesterone by 10-fold; levels of insulin-like growth factor-1 in the serum were not affected. Chatterton *et al.* (2005) found an even stronger reduction in nipple aspirate fluid progesterone from the breast (98%) and in serum progesterone (96.5%) in women who took a variety of triphasic contraceptives, but observed no effect on dehydroepiandrosterone or its sulfate or androstenedione, which are the potential precursors of 17β-estradiol. Levels of 17β-estradiol and estrone sulfate were also substantially reduced. These data suggest the possible involvement of reduced androgenic and estrogenic stimulation of responsive tissue, e.g. the breast. However, all combined oral contraceptives contain estrogens and several of the progestogens used, such as levonorgestrel and norethisterone, have androgenic activity. These studies highlight the possibility of complex interactions with other hormonal systems.

(*b*) *Experimental systems*

Rodriguez *et al*. (1998, 2002) examined the effects of a triphasic oral contraceptive regimen on the ovary of cynomolgus macaque monkeys. The treatment was equivalent to human doses of 0.03 mg ethinylestradiol plus 0.05 mg levonorgestrel per day for 6 days, followed by 0.04 mg ethinylestradiol plus 0.075 mg levonorgestrel for 5 days, followed by 0.03 mg ethinylestradiol plus 0.125 mg levonorgestrel for 10 days, followed by 7 days with no treatment. Parallel groups received ethinylestradiol only, levonorgestrel only or no treatment (control). This cyclic regimen was repeated every 28 days continuously for 35 months. The percentage of ovarian epithelial (surface) cells that stained positively for a reaction indicative of apoptosis was reduced by ethinylestradiol alone to a mean value of 1.8% compared with the mean control value of 3.9%. In contrast, apoptosis was increased more than sixfold by levonorgestrel alone and almost fourfold by the contraceptive combination of ethinylestradiol and levonorgestrel (Rodriguez *et al*., 1998). The treatments also affected the protein (immunohistochemical) expression of isoforms of transforming growth factor-β (TGF-β) that are known to be associated with apoptosis. TGF-β1 expression was reduced in epithelial cells, whereas expression of TGF-β2/3 was increased (Rodriguez *et al.*, 2002). These findings may be consistent with a protective effect of combined oral contraceptives against the risk for ovarian cancer.

In cell culture, 0.01 nM, 1 nM and 100 nM gestodene, levonorgestrel and the active metabolite of gestodene, 3-ketodesogestrel, given together either for 7 days or for the last 4 days of a 7-day estrogen treatment, all inhibited the cell proliferation induced by 10^{-10}M 17β-estradiol in estrogen receptor-positive MCF-7 breast cancer cells (Seeger *et al*., 2003). The inhibition was dose-dependent for the combined 7-day treatment, but was similar regardless of dose when given on the last 4 days of estrogen treatment. A concentration of 10 μM had a lesser (continuous progestogen) or no (sequential progestogen) inhibitory effect. This study suggests that the progestogen components of combined oral contraceptives may reduce the stimulation of breast cell proliferation by the estrogen component, but no human studies are available.

4.2.2 *Oral contraception and HPV*

(*a*) *Humans*

Both estrogen and progesterone receptors are expressed in normal human uterine cervix epithelium; in many cases, estrogen receptors, but not progesterone receptors, are lost during the development of cervical carcinoma *in situ* and invasive cancer (Nonogaki *et al.*, 1990; Monsonego *et al.*, 1991). Expression of progesterone receptors is increased in carcinoma *in situ* and greatly diminished in invasive cancer (Monsonego *et al.*, 1991). Infection with HPV is an essential causative component of human cervical cancer in more than 90% of cases (see IARC, 2007). HPV has more than 100 genotypes, some of which are associated with high risk for cancer and others with lower risk (see IARC, 2007). The loss of estrogen receptors may be associated with infection with specific types of HPV (Nonogaki *et al.*, 1990). These observations suggest that estrogen and progesterone may be involved in cervical carcinogenesis (de Villiers, 2003).

(*b*) *Experimental systems*

Transcription of HPV is regulated by the long control region of the viral genome (see IARC, 2007). Expression of the E6 and E7 genes of HPV is affected by progesterone (and glucocorticoids for which there are also receptors in cervical epithelium) through hormone response elements on the long control region (Chan *et al.*, 1989). This appears to occur in both high-risk (HPV 16 and 18) and low-risk HPV (HPV 11) types. There is also evidence that E6 and E7 expression is regulated by estrogen but there are no known estrogen-response elements on the long control region. Plasmids for the expression of chloramphenicol acetyl transferase (CAT) in the HPV 16 and 18 long control regions that were transfected into HeLa cells that contain progesterone but no estrogen receptors responded differentially to different estrogen and progestogens, but combinations of these were not tested (Chen *et al.*, 1996). 17β-Estradiol and estriol, but not estrone, induced HPV 16 CAT expression (2.3- and 2.7-fold, respectively) and, to a lesser extent, HPV 18 CAT expression (1.3- and 1.5-fold, respectively) at concentrations of 100 nM. HPV 18 CAT expression was only minimally increased by some progestogens (cyproterone acetate, norethynodrel and ethynodiol diacetate), hardly increased by progesterone itself and not increased by norethisterone acetate or norgestrel (all tested at concentrations of 100 nM). In contrast, HPV 16 CAT expression was increased by all of these progestogens (except norgestrel); progesterone and norethisterone acetate were the least active (1.7- and 1.8-fold increase, respectively) and ethynodiol diacetate, cyproterone acetate and northynodrel were the most active (2.3-, 2.5- and 2.5-fold increase, respectively).

In cervical epithelial cells transfected with and immortalized by HPV 16, 17β-estradiol was metabolized to a greater extent to 16α-hydroxy metabolites than to 2-hydroxy metabolites; 4-hydroxy metabolites were not detected (Auborn *et al.*, 1991). 17β-Estradiol and 16α-hydroxyestrone stimulated cell proliferation and caused increased growth in soft agar of cervical epithelial cells transfected with and immortalized by HPV 16 (Newfield *et al.*, 1998). In both studies, human foreskin epithelial cells (which do not express estrogen recep-

tors) transfected with and immortalized by HPV 16 did not metabolize or respond to estrogen. Fifty women who had moderate or high-grade carcinoma *in situ* of the cervix and an HPV infection but not 29 women who had carcinoma *in situ* and no HPV infection had serum estrone levels twofold higher ($p < 0.05$) than those found in women who did not have cervical carcinoma *in situ* ($n = 45$) (Salazar *et al*., 2001). [The hypothesis that 16α-hydroxyestrone is a major factor in estrogen-induced carcinogenesis in general is not supported by recent data (see Sections 4.1 and 4.3 in the monograph on Combined estrogen–progestogen menopausal therapy of this volume), but it may play a role in the HPV-infected cervix. However, in the study by Salazar *et al*. (2001), only serum estrone and not 16α-hydroxyestrone was measured and other estrogen metabolites have not been considered systematically in the studies reviewed. Therefore, support for the hypothesis that 16α-hydroxyestrone is a major factor in HPV 16-induced cervical carcinogenesis (de Villiers, 2003) is uncertain.]

In transgenic mice that carry the β-galactosidase gene under the control of the HPV 18 long control region (Morales-Peza *et al*., 2002), ovariectomy suppressed gene expression in the vagina–cervix, but not in the tongue (used as a non-estrogen-responsive control). Treatment with 17β-estradiol, but not progesterone, restored gene expression in the vagina–cervix, and combined treatment with these hormones did not further increase expression. The effect of estrogen was partially blocked by the anti-estrogen tamoxifen. The anti-progestogen RU486 markedly blocked the effect of both hormones combined and also that of 17β-estradiol alone.

In transgenic mice that carry the HPV 16 early region, which contains the *E6* and *E7* genes, under the control of the human keratin 14 promotor (Arbeit *et al*., 1994), *E6*/*E7* gene expression was increased by treatment with 17β-estradiol, which ultimately resulted in the development of cervical (and vaginal) squamous-cell carcinomas (Arbeit *et al*., 1996). These genes were not shown to be estrogen-responsive (Arbeit *et al*., 1996), and a direct effect of estrogen on their expression is unlikely. In contrast with estrogen-treated wild-type mice, 17β-estradiol caused a substantial increase in proliferating cells in the cervical epithelium in the K14-HPV 16 transgenic mice, an effect that is known to be mediated by the estrogen receptor (Lubahn *et al*., 1993). When these K14-HPV 16 transgenic mice were compared with other transgenic mice that carry either HPV 16 *E6*, HPV 16 *E7* or HPV 16 *E6*/*E7* genes under the control of the keratin 14 promotor, the estrogen-induced increase in cell proliferation appeared to be confined to the HPV 16 E7 and the HPV 16 E6/E7 mice, whereas estrogen-induced up-regulation of transgene expression was confined to the HPV 16 E6 mice. After 6 months of treatment with estrogen, the HPV 16, HPV 16 E7 and HPV 16 E6/E7 mice all developed cervical cancer at a high incidence, but no such cancer occurred in the HPV 16 E6 mice (Riley *et al.*, 2003). In another study, continuous treatment with estrogen for 9 months resulted in the development of cervical cancer in 100% of HPV 16 E7 and HPV 16 E6/E7 mice (HPV 16 E6 mice were not studied). However, when the estrogen treatment was discontinued 3 months before the end of the experiment, only the HPV 16 E6/E7 mice developed cervical cancer at a high incidence, whereas the HPV 16 E7 mice had a 50% lower cancer incidence, tumours were smaller and multiplicity was lower (Brake & Lambert, 2005). Estrogen appears to act in

these models as a receptor-mediated stimulus of proliferation that is related to the neoplastic transforming activity of HPV to which genotoxic estrogen metabolites possibly contribute (Arbeit *et al*., 1996; Riley *et al.*, 2003; Brake & Lambert, 2005). [The results of the discontinuation study by Brake and Lambert (2005) and the observation that *E6* and *E7* expression is enhanced by estrogen via a mechanism that apparently does not involve the estrogen receptor (Arbeit *et al*., 1996) indicate a complex interaction between *E6* and *E7* in the estrogen-enhanced causation of cervical cancer in these mouse models that carry parts of the HPV genome. In addition to acting as a mitogen via the estrogen receptor, the effects of estrogen probably involve progesterone, progesterone receptors and/or other cellular factors that act on HPV gene expression in a manner that is poorly understood. The effects of progestogens or estrogen–progestogen combinations were not examined in any of these studies.]

4.2.3 *Individual estrogens and progestogens*

(*a*) *Humans*

Pakarinen *et al*. (1999) studied 28 premenopausal women (mean age, 31–32 years) who had used either 0.03 mg per day levonorgestrel orally, an intrauterine device that released levonorgestrel or an intrauterine device that contained copper for 3 months. The only statistically significant change from baseline was a slight reduction in serum levels of SHBG in the women who took oral levonorgestrel. Levels of serum testosterone and insulin growth factor-binding protein-1 were not changed and no changes occurred in women who used the two intrauterine devices.

(*b*) *Experimental systems*

No new studies of the estrogens ethinylestradiol or mestranol or the progestogens chlormadinone acetate, ethynodiol diacetate, lynestrenol or norethynodrel that are relevant to the evaluation of the carcinogenic risk of combined contraceptives via the oral or other routes have been published since the last evaluation (IARC, 1999).

(i) *Estrogens*

17β-Estradiol stimulates the growth of human colon cancer CaCo-2 cells directly *in vitro* via the estrogen receptor which is blocked by anti-estrogens (Di Domenico *et al.*, 1996) and by anti-sense-mediated inhibition of estrogen receptor expression in mouse colon cancer MC-26 cells (Xu & Thomas, 1994). This stimulation appears to be mediated via estrogen receptor-α, since growth is inhibited in colon cancer cells when estrogen receptor-β is expressed (Arai *et al.*, 2000; Nakayama *et al*., 2000). Moreover, estrogen receptor-β appears to be the predominant form of estrogen receptor in colon cancer and colon cancer cells (Fiorelli *et al.*, 1999; Campbell-Thompson *et al.*, 2001; Witte *et al.*, 2001). Mediation of the stimulatory action of 17β-estradiol on the proliferation of colon cancer cells may also involve a non-genomic mechanism via the protein kinase C pathway (Winter *et al*., 2000). Male rats treated with colon carcinogens develop more colon

cancers than female rats (Di Leo *et al*., 2001). Treatment of ovariectomized female rats with 17β-estradiol and the colon carcinogen dimethylhydrazine leads to a significant inhibition of the development of colon tumours, from 8.1 ± 1.9 tumours per rat in those treated with carcinogen only to 2.3 ± 1.1 tumours per rat ($p < 0.001$) (Smirnoff *et al*., 1999). Thus, the available evidence indicates that estrogens inhibit colon carcinogenesis in animal experiments, and experimental studies strongly suggest that the estrogen receptor-β plays an inhibitory role in colon carcinogenesis. These observations support the protective effect of hormonal oral contraceptives and menopausal therapy against colon cancer that has been observed in epidemiological studies (Nanda *et al*., 1999; Grodstein *et al*., 1999; Di Leo *et al*., 2001).

(ii) *Progestogens*

Cyproterone acetate stimulated the in-vitro production of growth hormone by explants of normal human breast tissue with an estrogen receptor-negative and progesterone receptor-positive phenotype and of insulin-like growth factor I by explants of normal and cancerous human breast tissue with this phenotype (Milewicz *et al*., 2002).

Recent studies have demonstrated that desogestrel activates the estrogen receptor-α at an activity of about 50% of that of 17β-estradiol but activates the estrogen receptor-β at an activity of only 20% (Rabe *et al*., 2000). Desogestrel and/or its metabolite 3-keto-desogestrel (etonogestrel) were strongly progestogenic (approximately twofold over progesterone), weakly or not androgenic in animal studies *in vivo* and in-vitro binding assays and weakly or not active on the glucocorticoid receptor (Deckers *et al*., 2000; Schoonen *et al*., 2000). The active metabolite of desogestrel, 3-ketodesogestrel, strongly bound to and activated progesterone receptor-A and, to a slightly lesser extent, progesterone receptor-B (Schoonen *et al*., 1998).

Dienogest has the same degree of progestogenic activity as progesterone; it is anti-estrogenic and anti-androgenic, and binds weakly to progesterone and androgen receptors (Kaufmann *et al*., 1983; Katsuki *et al*., 1997a). It has uterotropic effects in rabbits that are stronger than those of norethisterone, medroxyprogesterone acetate and dydrogesterone and are blocked by the anti-progestogen RU486 but does not appear to have anti-mineralocorticoid activity (Katsuki *et al*., 1997a). Mammary hyperplasia but not neoplasia was observed in preclinical toxicological studies of dienogest in dogs (Hoffmann *et al*., 1983). Dienogest can inhibit neovascularization, including tumour cell-induced angiogenesis (Nakamura *et al*., 1999), which raises the possibility that it may counteract tumour progression.

The in-vivo anti-tumour activity and anti-uterotropic activity of dienogest were studied in mice and compared with those of several progestogens. At oral doses of 0.01–1 mg/kg bw per day, dienogest significantly suppressed the 17β-estradiol benzoate-dependent tumour growth of HEC-88nu cells, which express estrogen receptors but not progesterone receptors. These cells were unresponsive to known progestogens such as medroxyprogesterone acetate (100 mg/kg bw per day orally) and norethisterone (100 mg/kg bw per day orally). The suppressive effect of dienogest on tumour growth was not diminished in the presence of excess medroxyprogesterone acetate (100 mg/kg bw per day). Dienogest also suppressed the

estradiol-dependent tumour growth of Ishikawa cells (derived from a well-differentiated human endometrial carcinoma) and MCF-7 cells (derived from a human breast carcinoma), both of which express estrogen and progesterone receptors and respond to medroxyprogesterone acetate. However, the minimal effective dose of dienogest (0.01–1 mg/kg per day) was much lower than that of medroxyprogesterone acetate (100 mg/kg per day). Thus dienogest showed potent anticancer activity against hormone-dependent cancers at doses at which other progestogens show no activity. Dienogest showed no anti-uterotropic activity at tumour-suppressive doses (Katsuki *et al*., 1997b).

Drospirenone is a relatively new progestogen that is used in combined oral contraceptives (Keam & Wagstaff, 2003). It has anti-androgenic and anti-mineralocorticoid effects; it binds strongly to mineralocorticoid receptors, but weakly or not at all to the androgen, glucocorticoid or estrogen (α) receptors (Pollow *et al*., 1992), with the potential to decrease blood pressure (for reviews see Muhn *et al*., 1995; Krattenmacher, 2000; Keam & Wagstaff, 2003; Rübig, 2003; Oelkers, 2004).

Studies on the highly progestogenic compound gestodene have demonstrated that its progestogenic activity in an in-vivo system is far lower than those of its in-vitro binding or receptor activation (Deckers *et al*., 2000; Schoonen *et al*., 2000; Garcia-Becerra *et al*., 2004); however, its androgenic activity *in vitro* has been confirmed (Garcia-Becerra *et al*., 2004) and it appears to have weak binding activity to the glucocorticoid receptor (Schoonen *et al*., 2000). Its weak estrogenic activity (transactivation of estrogen receptor-mediated gene expression in model cells) appears to derive from its metabolism to the A ring-reduced metabolites, 3β- and 3α,5α-tetrahydrogestodene, and is probably mediated by the activity of 5α-reductase (Lemus *et al*., 2000, 2001). These metabolites appeared to be selective agonists of estrogen receptor-α but not of estrogen receptor-β (Larrea *et al*., 2001). The parent compound did not activate estrogen receptors-α or -β (Rabe *et al*., 2000).

Recent studies have demonstrated that the estrogenic activity (transactivation of estrogen receptor-mediated gene expression in model cells) of levonorgestrel appears to be derived from its metabolism to the A ring-reduced metabolites, 3β- and 3α,5α-levonorgestrel, and is abolished by co-treatment with the pure steroidal anti-estrogen ICI 182,780 (Santillán *et al*., 2001). Levonorgestrel appears to activate estrogen receptor-β strongly (75–90% of the activity of 17β-estradiol) but estrogen receptor-α is only slightly activated (15–25% of the activity of 17β-estradiol) (Rabe *et al*., 2000). Its weak androgenic activity was confirmed in androgen receptor-binding and transactivation studies (Garcia-Becerra *et al*., 2004). Levonorgestrel weakly induced a decrease in insulin growth factor-I and an increase in growth hormone production in primary human breast cancer explants *in vitro* when the explants were progesterone receptor-positive and estrogen receptor-negative, but not in the presence of estrogen receptors or the absence of progesterone receptors (Milewicz *et al*., 2002).

Studies on norethisterone are summarized in the monograph on Combined estrogen–progestogen menopausal therapy.

4.3 Genetic and related effects

The extensive literature on direct genetic toxicological effects, or the lack of such effects, of the steroid hormones used in combined oral contraceptives has been reviewed previously (IARC, 1979, 1999), and the reader is referred to these tabular and textual considerations of the earlier genotoxicity data. Reports published since the previous evaluations are summarized below. Because many hormones are used in both combined oral contraceptives and hormonal menopausal therapy, synthetic hormones that are used widely in combined oral contraceptives are considered below, but several hormones relevant to this topic are listed exclusively in Section 4.4 of the monograph on Combined estrogen–progestogen menopausal therapy. New evidence has shown that, in aggregate with previous findings, there is a stronger case for the potential of some of these hormones to cause direct genetic damage that could result in genetic alterations of cells.

4.3.1 *Ethinylestradiol*

(*a*) *Humans*

Daily oral doses of 0.02 mg ethinylestradiol and 0.075 mg gestodene administered to 30 healthy women in a monthly cycle of 3 weeks with and 1 week without treatment for six consecutive menstrual cycles did not induce micronuclei in the peripheral blood lymphocytes (Loncar *et al.*, 2004).

A significant increase in the number of lymphocytes with DNA fragmentation and an increased frequency of sister chromatid exchange per metaphase was observed in 18 women who took combined oral contraceptives (daily oral doses of 0.02–0.03 mg ethinylestradiol and 0.15 mg desogestrel) for 24 months compared with age-matched untreated controls ($p < 0.005$) (Biri *et al.*, 2002).

In a population-based study of young women (< 45 years of age) in the USA, those who had started using oral contraceptives at least 20 years before the reference date had a twofold increased risk for breast cancer with cyclin D1 overexpression (odds ratio, 2.2; 95% CI, 1.2– 4.0) but not for breast cancer without cyclin D1 overexpression (odds ratio, 1.1; 95% CI, 0.7–1.8) (Terry *et al.*, 2002). The authors suggested that early oral contraceptive use may be associated with the induction of a subset of mammary tumours that overexpress cyclin D1.

Prolonged use of oral contraceptives is more strongly associated with p53-positive breast cancer (odds ratio, 3.1; 95% CI, 1.2–8.1) than p53-negative breast cancer (odds ratio, 1.3; 95% CI, 0.6–3.2) among younger women only (Furberg *et al.*, 2003).

[In the above studies, women who were administered combined oral contraceptives appear to have sustained genetic alterations. It should be recognized that the observed effects of combined oral contraceptives could have been the result of a direct genotoxic effect of the hormonal preparation or could have been an indirect effect of hormonal influences on cellular functions, most notably cell proliferation, mediated by receptor- or

non-receptor-linked mechanisms. It is therefore appropriate not to overinterpret these observations as evidence for a direct genotoxic effect.]

(*b*) *Experimental systems*

The tissue- and gender-specific patterns of DNA methylation in the promoter regions of the estrogen receptor and aromatase genes were analysed in adult male and female Japanese Medaka fish (*Oryzias latipes*) exposed to either 0 or 500 ng/L 17α-ethinylestradiol in the water for 14 days. The protein content of the estrogen receptor in exposed fish was significantly increased in all male and female tissues (liver, gonads and brain) compared with controls. Aromatase activity in the exposed fish was significantly increased in the male brain and gonads and female brain compared with controls (Contractor *et al.*, 2004). The changes in DNA methylation of the estrogen receptor and aromatase genes observed indicated that the mechanisms that control gene expression could potentially be altered, as well as gender- and tissue-specific sensitivity. While differences in patterns of DNA methylation did not parallel the changes observed in protein expression, they may impact the regulation of normal gene expression and could be genetically imprinted and transmitted to offspring.

The formation of 8-dihydroxy-2′-deoxyguanosine, an indicator of oxidative DNA damage, has been shown to be increased in the testicular cells of Wistar rats 1 h or 4 h after intraperitoneal injection of 0, 2.8 or 56 mg/kg bw ethinylestradiol *in vivo* and after exposure to 0.1–10 nM 17α-ethinylestradiol for 30 min *in vitro* (Wellejus & Loft, 2002). In the total cell population and in round haploid rat testicular cells, oxidized purines show a bell-shaped concentration–response relationship with maximally increased levels at 10 nM. No significant effects were observed in diploid, S-phase or tetraploid cells. The mRNA level of rat 8-oxo-guanine DNA glycosylase was unaffected by ethinylestradiol (Wellejus *et al.*, 2004).

Siddique *et al.* (2005) recently analysed the genotoxicity of ethinylestradiol in human lymphocytes by measuring chromosomal aberrations, mitotic index and sister chromatid exchange. Ethinylestradiol was genotoxic at 5 and 10 μM in the presence of a rat liver microsomal fraction (metabolic activation system) with nicotinamide adenine dinucleotide phosphate (NADP). Concomitant treatment with superoxide dismutase increased the frequency of chromosomal aberrations and sister chromatid exchange and decreased the mitotic index compared with levels induced by treatment with ethinylestradiol alone, whereas concomitant treatment with catalase decreased the frequencies of chromosomal aberrations and sister chromatid exchange and increased the mitotic index. Concomitant treatment with catalase in combination with superoxide dismutase also decreased the frequencies of chromosomal aberrations and sister chromatid exchange and increased the mitotic index, which suggests a possible role of reactive oxygen species in the induction of the genotoxic damage. Bukvic *et al.* (2000) reported that ethinylestradiol and norgestrel (1:5) had an aneuploidogenic effect on cultures of human fibroblasts and lymphocytes. Fluorescent in-situ hybridization (with pancentromeric alphoid probes) analysis of micronuclei from lymphocyte cultures and anaphase preparations from fibroblast cultures supported this conclusion.

In primary rat hepatocytes exposed to ethinylestradiol for 20 h at subtoxic concentrations in the range of 1–50 μM, DNA repair was induced in cells derived from both of two males and one of two females (Martelli *et al.*, 2003).

4.3.2 *Progestogens*

Most progestogens have not been tested systematically for genotoxicity (see Table 20 in the monograph on Combined estrogen–progestogen menopausal therapy and Brambilla & Martelli, 2002). [It should be noted that the negative results for progestogens obtained with the standard battery of genotoxicity tests may be the consequence of using insufficiently sensitive assays, inappropriate target cells and/or suboptimal metabolic activation systems.]

(*a*) *Cyproterone acetate and some structural analogues*

The genotoxic potential of cyproterone acetate and some of its analogues has been established. Cyproterone acetate is metabolically activated in the liver of female rats to one or more DNA-damaging intermediates that may induce the formation of DNA adducts, DNA repair and increased levels of micronuclei and gene mutations (reviewed by Kasper, 2001; Brambilla & Martelli, 2002; Joosten *et al.*, 2004). Most importantly, cyproterone acetate induced the formation of DNA adducts in primary cultures of human hepatocytes, which indicates that human liver cells can metabolically activate cyproterone acetate to genotoxic intermediates (Werner *et al*., 1996, 1997). Cyproterone acetate is activated by hepatocytes to reactive species with such a short half-life that they react only with the DNA of the cell in which they are formed. The response is similar in both men and women but is markedly greater in female than in male rats. The promutagenic character of DNA lesions in the liver of female rats is indicated by the increase in the frequency of micronucleated cells, mutations and enzyme-altered preneoplastic foci (reviewed by Brambilla & Martelli, 2002).

Two other synthetic progestogens, chlormadinone acetate and megestrol acetate, and an aldosterone antagonist, potassium canrenoate, share the 17-hydroxy-3-oxopregna-4,6-diene structure with cyproterone acetate. They all induce genotoxic effects that are qualitatively similar to those of cyproterone acetate (Brambilla & Martelli, 2002).

Chlormadinone acetate and megestrol acetate are genotoxic only in the liver of female rats and in primary human hepatocytes from male and female donors (Brambilla & Martelli, 2002). The metabolic activation of these molecules to reactive species and the consequent formation of DNA adducts occur only in intact hepatocytes.

In primary rat hepatocytes exposed for 20 h to subtoxic concentrations ranging from 1 to 50 μM, DNA repair was induced by drospirenone in both of two males and all of three females, by ethinylestradiol in both of two males and one of two females, by oxymetholone in one of two males and one of two females, by norethisterone in one of two males, by progesterone in one of four females and by methyltestosterone in one of four males (Martelli *et al.*, 2003). A few inconclusive responses were observed in rat hepatocytes exposed to progesterone, medroxyprogesterone, norethisterone, methyltestosterone and oxymetholone. The authors of this small study assert that steroid hormones differ in their ability to induce

DNA repair, and that their genotoxicity may be: (*a*) different in rat and human hepatocytes; (*b*) dependent on the sex of the donor; and (*c*) affected by interindividual variability.

In rat hepatocytes, subtoxic concentrations of potassium canrenoate ranging from 10 to 90 μM consistently induced a dose-dependent increase in DNA fragmentation (Martelli *et al*., 1999; Brambilla & Martelli, 2002). In another study with other steroids, DNA fragmentation was greater in female than in male rat hepatocytes and DNA-damaging potency was decreased in the following order: cyproterone acetate > dienogest > 1,4,6-androstatriene-17β-ol-3-one acetate > dydrogesterone (Mattioli *et al.*, 2004). Under the same experimental conditions, responses in an assay of DNA repair synthesis were positive or inconclusive in hepatocytes from female rats and were consistently negative in those from male rats.

Analysis of sister chromatid exchange and chromosomal aberrations in bone-marrow cells from mice exposed *in vivo* to chlormadinone acetate (5.62 mg/kg bw) showed that this dose is non-genotoxic (Siddique & Afzal, 2004). However, doses of 11.25 and 22.50 mg/kg bw chlormadinone acetate significantly increased the frequency of sister chromatid exchange ($p < 0.001$) and chromosomal aberrations ($p < 0.01$) compared with untreated controls (Siddique & Afzal, 2004). The authors suggested a genotoxic effect of chlormadinone acetate in mouse bone-marrow cells.

Administration of a single high dose of 100 mg/kg bw cyproterone acetate to female *lac*I-transgenic Big Blue™ rats induced a strong initial rise in mutation frequency in the liver over that in controls up to a maximum at 2 weeks after administration accompanied by a corresponding increase in cell proliferation and levels of DNA adducts (Topinka *et al.*, 2004a). The mutation frequency decreased after 2 weeks to one-third of the maximum level and this was maintained for an additional 4 weeks. The levels of DNA adducts in the liver decreased by only 15% during this time, which suggests that most adducts were lost as affected hepatocytes were eliminated. When given as a single dose, 5 mg/kg bw cyproterone acetate did not produce significantly elevated levels of mutation. However, mutation frequencies increased 2.5-fold when female *lac*I-transgenic Big Blue™ rats received repeated daily doses of 5 mg/kg bw cyproterone acetate for 3 weeks (Topinka *et al.*, 2004b).

(*b*) *Norgestrel*

A study on human lymphocytes showed that norgestrel induced chromosomal aberrations and significant levels of sister chromatid exchange and inhibited lymphocyte proliferation at concentrations of 25 and 50 μg/mL only. In the presence of a metabolic activation system, the values obtained for chromosomal aberrations, sister chromatid exchange and mitotic index were more significant. A time- and dose-dependent genotoxic effect of norgesterol was observed (Ahmad *et al.*, 2001). The authors concluded that norgestrel itself, and possibly its metabolites, are potent mutagens in human lymphocytes.

(*c*) *Norethisterone*

A study on human lymphocytes that used chromosomal aberrations, sister chromatid exchange and cell-growth kinetics as end-points showed that doses of 20, 40 and 75 μg/mL

norethisterone were non-genotoxic either in the presence or in the absence of a metabolic activation system (Ahmad *et al.*, 2001).

Gallmeier *et al.* (2005) applied a novel and particularly sensitive method to screen for DNA damage with special attention to double-strand breaks. They found that norethisterone is probably genotoxic and therefore potentially mutagenic. A *p53*-reporter assay served as a first, high-throughput screening method and was followed by the immunofluorescence detection of phosphorylated H2AX (a variant of histone H_2A protein) as a sensitive assay for the presence of double-strand breaks. Norethisterone at concentrations of 2–100 μg/mL activated p53 and induced phosphorylation of H2AX on Ser-139 in the vicinity of double-strand breaks. Phosphorylation of H2AX increased in a dose-dependent manner. Double-strand breaks were not detected with the neutral comet assay, a less sensitive method than H2AX phosphorylation. The authors suggested that, since norethisterone induced double-strand breaks in their experiments, this both complements and adds a new aspect to the existing literature on its genotoxic potential. However, they noted that, since the effective concentrations of norethisterone in these assays were approximately 100–1000-fold higher than therapeutic doses, the significance of these findings with regard to human exposure has yet to be determined.

In-vitro studies that analysed gene expression of isolated normal endometrial epithelial cells treated with estradiol and norethisterone acetate showed upregulation of the *Wnt-7a* gene; with estradiol only, *Wnt-7a* was expressed at very low levels (Oehler *et al.*, 2002). *Wnt* genes are a large family of developmental genes that are associated with cellular responses such as carcinogenesis.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

The first oral hormonal contraceptives that were found to inhibit both ovulation and implantation were developed in the 1950s and included both estrogen and progestogen. Since that time, changes in component ingredients, doses used and the temporal sequencing of exposure to hormones have occurred with emerging technologies and in an effort to reduce adverse effects. The dominant trends in recent years have been towards the use of lower doses of estrogen, the use of progestogens that are less androgenic, the multiplication of product formulations and the continuing development of novel delivery systems. In current preparations, ethinylestradiol is the most common estrogen, although a variety of other estrogens is also available. An even greater range of progestogens is used. The estrogen and progestogen components are usually given together orally in a monthly cycle, e.g. 21 days of constant or varying doses followed by 7 days without hormones. Combined hormonal contraceptives can also be administered by injection, transdermal patch and vaginal device. In addition to their regular use for contraception, other common indications for these products include emergency contraception, and the treatment of acne and menstrual dis-

orders. Some commonly used formulations, doses, routes of administration and schedules of exposure are new and their possible long-term adverse effects have not been evaluated.

Worldwide, more than 100 million women — an estimated 10% of all women of reproductive age — currently use combined hormonal contraceptives, a large majority of which are in the form of oral preparations. Current use of these drugs is greatest in developed countries (16%) and is lower in developing countries (6%). Rates of 'ever use' higher than 80% have been reported for some developed countries. In developing countries, 32% of women were estimated to have ever used hormonal contraception. Overall, the use of combined hormonal contraception is increasing, but there is extreme variability between countries. In many countries, these preparations are mainly used by women of younger age and higher level of education, and who have greater access to health care.

5.2 Human carcinogenicity data

Breast cancer

More than 10 cohort studies and 60 case–control studies that included over 60 000 women with breast cancer reported on the relationship between the oral use of combined hormonal contraceptives and the risk for this disease. The totality of the evidence suggested an increase in the relative risk for breast cancer among current and recent users. This effect was noted particularly among women under 35 years of age at diagnosis who had begun using contraceptives when young (< 20 years), whereas the increased risk declined sharply with older age at diagnosis. By 10 years after cessation of use, the risk in women who had used combined hormonal contraceptives appeared to be similar to that in women who had never used them. Important known risk factors did not appear to account for the association. The possibility that the association seen for current and recent users is due to detection bias was not ruled out, but it was considered to be unlikely that this would explain the association observed in young women.

Endometrial cancer

Four cohort studies and 21 case–control studies reported on the relationship between the oral use of combined hormonal contraceptives and the risk for endometrial cancer. The results of these studies consistently showed that the risk for endometrial cancer in women who had taken these medications is approximately halved. The reduction in risk was generally greater with longer duration of use of combined hormonal contraceptives and persisted for at least 15 years after cessation of use, although the extent of the protective effect may wane over time. Few data were available on the more recent, low-dose formulations.

Cervical cancer

Five cohort and 16 case–control studies of the oral use of combined hormonal contraceptives and invasive cervical cancer had been reviewed previously. The Working Group at that time could not rule out biases related to sexual behaviour, screening and other factors as possible explanations for the observed association with increasing duration of use.

Since then, two cohort and seven case–control studies have provided new information on invasive or in-situ carcinoma and oral use of combined hormonal contraceptives; all but the three most recent studies were summarized in a meta-analysis of published data. The totality of the evidence indicated that, overall, the risk for cervical cancer increased with increasing duration of use of combined hormonal contraceptives, and was somewhat greater for in-situ than for invasive cancer. The relative risk appeared to decline after cessation of use. The results were broadly similar regardless of adjustment for the number of sexual partners, cervical screening, tobacco smoking and the use of barrier contraceptives. The association was found in studies conducted in both developed and developing countries. The possibility that the observed association is due to detection bias was not ruled out, but it was considered to be unlikely that this would explain the increase in risk. Studies in which information on human papillomavirus infection — the main cause of cervical cancer — was available suggested that the prevalence of the infection was not increased among users of combined hormonal contraceptives, and the association with cervical cancer was also observed in analyses that were restricted to human papillomavirus-positive cases and controls.

Ovarian cancer

Data from an additional three cohort and 20 case–control studies that were new or had been updated since the last evaluation showed that women who had ever used combined hormonal contraceptives orally had an overall reduced risk for ovarian cancer and an inverse relationship was observed with duration of use. The reduced risk appeared to persist for at least 20 years after cessation of use. The effect of combined hormonal contraceptives on the reduction of risk for ovarian cancer is not confined to any particular type of oral formulation nor to any histological type of ovarian cancer, although it was less consistent for mucinous than for other types in several studies.

Liver cancer

Long-term oral use of combined hormonal contraceptives was associated with an increase in the risk for hepatocellular carcinoma in all nine case–control studies conducted in populations that had low prevalences of hepatitis B viral infection and chronic liver disease — which are major causes of liver cancer — and in analyses in which women with such infections were excluded. Three cohort studies showed no significant association between the oral use of combined hormonal contraceptives and the incidence of or mortality from liver cancer, but the expected number of cases was very small, which resulted in low statistical power. Few data were available for the more recent, low-dose formulations. In the three case–control studies conducted in populations that had a high prevalence of infection with hepatitis viruses, no statistically significant increase in the risk for hepatocellular carcinoma was associated with the oral use of combined hormonal contraceptives, but little information was available on long-term use.

Cutaneous melanoma

Four cohort and 16 case–control studies provided information on the oral use of combined hormonal contraceptives and the risk for cutaneous malignant melanoma. No consistent evidence for an association was found with respect to current use, duration of use, time since last use or age at first use. The few studies that suggested an increase in risk may reflect the possibility that women who took oral contraceptives may have had more contacts with the medical system and were thus more likely to have had pigmented lesions removed.

Colorectal cancer

Seven cohort and 13 case–control studies provided information on the oral use of combined hormonal contraceptives and the risk for colorectal cancer. Most studies did not show an increase in risk in women who had ever used contraceptives or in relation to duration of use. The results were generally similar for colon and rectal cancer when examined separately, and two case–control studies showed a significant reduction in risk.

5.3 Animal carcinogenicity data

The data evaluated in this section showed a consistent carcinogenic effect of several estrogen–progestogen combinations across different animal models in several organs. The evidence of carcinogenicity for one of the newer progestogens studied, dienogest, was not satisfactory for an evaluation.

Estrogen–progestogen combinations

In female and male mice, the incidence of pituitary adenoma was increased by administration of mestranol plus chlormadinone acetate, mestranol plus ethynodiol diacetate, ethinylestradiol plus ethynodiol diacetate, mestranol plus norethisterone, ethinylestradiol plus norethisterone (females only) and mestranol plus norethynodrel. The latter combination also increased the incidence of pituitary adenomas in female rats.

The incidence of malignant mammary tumours was increased in female and male mice by ethinylestradiol plus megestrol acetate, in female and male rats by ethinylestradiol plus ethynodiol diacetate and in female rats by mestranol plus norethisterone and mestranol plus norethynodrel. The incidence of benign mammary tumours was increased in male rats by ethinylestradiol plus norethisterone acetate, in intact and castrated male mice by ethinylestradiol plus chlormadinone acetate and in castrated male mice by mestranol plus norethynodrel. Ethinylestradiol plus norethisterone acetate did not cause tumour formation in any tissue in one study in female monkeys.

In female mice, the incidence of malignant non-epithelial uterine tumours was increased by ethinylestradiol plus ethynodiol diacetate and the incidence of vaginal or cervical tumours was increased by norethynodrel plus mestranol. In female mice treated with 3-methylcholanthrene to induce genital tumours, ethinylestradiol plus lynestrenol, ethinylestradiol plus norgestrel and mestranol plus norethynodrel increased the incidence

of uterine tumours; however, this occurred only at the highest doses of ethinylestradiol plus lynestrenol and ethinylestradiol plus norgestrel that were tested. Lower doses inhibited tumorigenesis induced by 3-methylcholanthrene alone.

In female rats, the incidence of hepatocellular carcinomas was increased by ethinylestradiol plus norethisterone acetate; this combination and mestranol plus norethisterone also increased the incidence of liver adenomas in male rats. Liver foci, which are putative preneoplastic lesions, were induced in female rats by mestranol plus norethynodrel. In female rats initiated for hepatocarcinogenesis with *N*-nitrosodiethylamine, mestranol plus norethynodrel increased the formation of altered hepatic foci.

In one study, subcutaneous administration of levonorgestrel with ethinylestradiol or estradiol to female rabbits induced deciduosarcomas in several organs (uterus, spleen, ovary, liver and lung).

Estrogens

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

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

Ethinylestradiol increased the incidence of cervical tumours in female mice.

In female and male mice, ethinylestradiol increased the incidence of hepatocellular adenomas. In female rats, ethinylestradiol and mestranol increased the numbers of altered hepatic foci. In rats, ethinylestradiol increased the incidence of adenomas in females and males and that of hepatocellular carcinomas in females, whereas mestranol increased the incidence of hepatic nodules and carcinomas combined in females.

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

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

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

In female rabbits, subcutaneous administration of ethinylestradiol alone was associated with the proliferation of hepatic bile duct cells.

In female mice, subcutaneous injection of ethinylestradiol alone was associated with the development of uterine adenocarcinomas. In male hamsters, subcutaneous implantation of estradiol alone was associated with the development of renal tumours of unspecified histology.

Oral administration of ethinylestradiol to *p53*-deficient female mice in combination with an intraperitoneal injection of the known carcinogen *N*-ethyl-*N*-nitrosourea increased the incidence of uterine atypical hyperplasias and stromal sarcomas.

Subcutaneous injection of 2-hydroxy- and 4-hydroxyestradiol induced uterine adenocarcinomas in female mice and subcutaneous implantation of estradiol induced renal tumours in male hamsters.

In female mice initiated with *N*-ethyl-*N*′-nitro-*N*-nitrosoguanidine, subcutaneous implantation of estradiol, estrone, estriol, 16β-hydroxyestrone diacetate, 16α-hydroxyestrone and 17-epiestrol increased the incidence of endometrial adenocarcinomas.

Progestogens

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

The incidence of malignant mammary tumours was increased in female mice by lynestrenol, megestrol acetate and norethynodrel. In female rats, lynestrenol 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 female dogs, chlormadinone acetate, lynestrenol and megestrol acetate increased the incidence of benign and malignant mammary tumours; however, lynestrenol 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.

Megestrol acetate increased the incidence of liver adenomas in female mice. Cyproterone acetate increased the incidence of liver adenomas and hepatocellular carcinomas in female and male mice, but at levels that exceeded the maximum tolerated dose. In rats, the incidence of liver adenomas was increased by norethisterone acetate (females and males), norethisterone (males), norethynodrel and cyproterone acetate (females and males). The numbers of altered hepatic foci in female rats were also increased by norethisterone acetate and cyproterone acetate. In male mice treated with chlormadinone acetate, ethynodiol diacetate, lynestrenol, norethisterone or norethisterone acetate, the incidence of liver adenomas was increased. In female 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 increase the incidence of renal dysplastic lesions or tumours in female hamsters.

Oral administration of dienogest induced mammary gland proliferation in female dogs but not in female rats or monkeys.

5.4 Other relevant data

Absorption, distribution, metabolism and excretion

Estrogenic and progestogenic compounds in oral contraceptives are readily absorbed and undergo metabolism to varying extents by bacterial enzymes, enzymes in the intestinal mucosa and especially enzymes in the liver. The metabolism typically involves reduction, hydroxylation and conjugation. The so-called 'first-pass' through the liver reduces the overall bioavailability of oral contraceptives. Peak concentration levels in the systemic circulation are observed between 0.5 and 4 h after intake. Hydroxylated metabolites are usually conjugated as glucuronides or sulfates and are eliminated rapidly with half-lives of 8–24 h.

The formulations of combined hormonal contraceptives continue to evolve, especially with the introduction of new progestogens. In general, the chemical structure of a progestogen determines its relative binding affinities for progesterone and other steroid receptors, as well as sex hormone-binding globulin, which determine its biological effects. The logic involved in the development of newly synthesized progestogens, such as dienogest and drospirenone, is that they be devoid of estrogenic, androgenic and antagonist effects.

Estrogens are discussed in the monograph on Combined estrogen–progestogen menopausal therapy.

Receptor-mediated effects

Exposure to combined hormonal contraceptives increases the proliferation of human breast epithelial cells, as observed in biopsies and fine-needle aspirate samples collected during small randomized studies. Combined hormonal contraceptives have atrophic and anti-proliferative effects on the endometrium that are apparently independent of the regimen and the progestogen used. Ethinylestradiol plus levonorgestrel induces ovarian epithelial cell apoptosis in intact monkeys. Estrogens or progestogens may enhance human papillomavirus gene expression in the human cervix via progesterone receptor mechanisms and hormone-response elements in the viral genome. In-vitro studies support this concept, and mechanisms other than those that are receptor-mediated may be involved. Experiments in transgenic mouse models that express human papillomavirus 16 genes in the cervix showed that estrogens can cause cervical cancer, probably via receptor-mediated processes. This effect was diminished after cessation of treatment with estrogens. Colon carcinogenesis in animal models is inhibited by estrogens and there is adequate evidence to suggest that estrogens have inhibitory effects on colon cancer cells via estrogen receptor-β. Various studies document the possibility of complex interactions of combined hormonal contraceptives with hormonal systems. No data were available to the Working Group on the effects of time since cessation of treatment or duration of treatment.

Genetic and related effects

There is additional evidence to support the conjecture that certain estrogens function as directly acting genotoxins. These findings give further credence to the hypothesis that

certain estrogens are carcinogenic through direct genotoxic effects in addition to their presumed action via a receptor-mediated mechanism. Some of the more recent genotoxicity data suggest that some progestogens used in combined hormonal contraceptives may also act as direct genotoxins. Few data were available on the effects of combined exposures to estrogens and progestogens.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of combined oral estrogen–progestogen contraceptives. This evaluation was made on the basis of increased risks for cancer of the breast among current and recent users only, for cancer of the cervix and for cancer of the liver in populations that are at low risk for hepatitis B viral infection.

There is *evidence suggesting lack of carcinogenicity* in humans for combined oral estrogen–progestogen contraceptives in the endometrium, ovary and colorectum. There is convincing evidence in humans for their protective effect against carcinogenicity in the endometrium and ovary.

There is *sufficient evidence* in experimental animals for the carcinogenicity of the combinations of ethinylestradiol plus ethynodiol diacetate, mestranol plus norethynodrel, ethinylestradiol plus levonorgestrel and estradiol plus levonorgestrel.

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

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

There is *limited evidence* in experimental animals for the carcinogenicity of the combinations of ethinylestradiol plus megestrol acetate, mestranol or ethinylestradiol plus chlormadinone acetate, mestranol plus ethynodiol diacetate, mestranol plus lynestrenol, mestranol or ethinylestradiol plus norethisterone and ethinylestradiol plus norgestrel.

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

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

Overall evaluation

Combined oral estrogen-progestogen contraceptives are *carcinogenic to humans (Group 1)*. There is also convincing evidence in humans that these agents confer a protective effect against cancer of the endometrium and ovary.

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