# HORMONAL CONTRACEPTIVES, PROGESTOGENS ONLY

## 1. Exposure

'Progestogen-only' contraceptives are available as injections, implants, oral preparations, hormone-releasing intrauterine devices and emergency contraceptives. These compounds can be used by women who are breast feeding or have other contra-indications to oestrogen therapy, such as immediately *post partum*, those with thalassaemia, sickle-cell disease, gall-bladder disease, past or present thrombo-embolic disorders, valvular heart disease, ischaemic heart disease, recent surgery, migraine or hypertension, and older women, particularly those over 35 who smoke (WHO Family Planning and Population Unit, 1996; see Annex 1 for guidelines for use). Parenteral methods of administration generally result in more effective contraception than oral routes, as they provide more constant concentrations of the hormone in the blood. Use of progestogen-only oral contraceptives leads to the peaks and troughs in concentration characteristic of oral medication but involves greater potential errors by the users, as placebo is given during seven days of the cycle.

The progestogens that are or have been used in 'progestogen-only' contraceptives are chlormadinone acetate, desogestrel, ethynodiol diacetate, levonorgestrel, lynoestrenol, medroxyprogesterone acetate, norethisterone, norethisterone acetate, norethisterone oenanthate, norgestrel, norgestrienone and progesterone. Of these, medroxyprogesterone acetate, norethisterone oenanthate and progesterone are used only in this way; the remaining progestogens are also used in combination with oestrogens. Thus, information on the progestogens used only in 'progestogen-only' hormonal contraceptives is given in this monograph, and studies on other progestogens are summarized in the monograph on 'Oral contraceptives, combined'.

### 1.1 Historical overview

The development of injectable progestogen-only contraceptives resulted from a growing understanding of steroid hormones and from the research that eventually led to the development of combined oral contraceptives. In 1953, Karl Junkman and colleagues synthesized the first injectable progestogens and then developed the first injectable contraceptive, norethisterone oenanthate, in 1957. This compound is now approved for contraceptive use in over 60 countries. Medroxyprogesterone acetate was synthesized in the late 1950s, and its depot form was subjected to clinical trials in 1963, before being released onto the international market. It has been approved for use as a contraceptive in

a steadily increasing number of countries over the last 30 years and is now available in over 100 countries worldwide. Concern about an association with cancers of the breast, endometrium and cervix and other possible side-effects meant that depot medroxyprogesterone acetate was approved as a contraceptive in the United States only in 1992, some 25 years after the manufacturer's first application (Lande, 1995); however, it had already been approved for the treatment of conditions such as endometrial cancer, and legislation in the United States does not prohibit the use of approved drugs for non-approved indications. Nevertheless, there are still concerns in the international community about issues of informed consent for the use of these long-acting methods and the potential abuse of their administration to poorly educated groups (Kleinman, 1990).

Although the very first oral contraceptive, which was tested in Puerto Rico in 1955, contained only norethynodrel and was, technically speaking, a progestogen-only oral contraceptive (McLaughlin, 1982), it was superseded by the combination of mestranol and norethynodrel during development, as the combination was shown to prevent ovulation consistently. Progestogen-only oral contraceptives were developed in response to concern raised in the late 1960s about the side-effects of oestrogens in combined oral contraceptives. The prototype progestogen-only oral contraceptive contained chlormadinone acetate and was introduced in 1969. It was withdrawn in 1970 because of evidence that it induced breast nodules in laboratory animals. Other progestogen-only oral contraceptives were developed subsequently, containing progestogens of the norethisterone and levonorgestrel groups (Kleinman, 1990).

Subcutaneous progestogen implants were developed in the late 1960s and 1970s and were approved in Finland in 1983, in Sweden in 1985, the Dominican Republic, Ecuador, Indonesia and Thailand in 1986, China, Colombia, Peru and Venezuela in 1987, Chile and Sri Lanka in 1988 and the United States in 1990 (McCauley & Geller, 1992).

A device that releases progesterone into the uterus was developed in the early 1970s and has been available since 1976. This had the disadvantage of a high rate of hormone release, necessitating annual replacement (Kleinman, 1990; Treiman *et al*., 1995). An intrauterine device that releases effective concentrations of levonorgestrel over a five-year period was approved in Finland in 1990 and in Sweden in 1992 (Chi, 1995); it has since been approved in Belgium, Denmark, France, Iceland, Norway, Singapore, Switzerland and the United Kingdom (Treiman *et al.*, 1995).

### 1.2 Injectable progestogens

Two progestogen-only injectable contraceptives are available worldwide, and their formulations have remained unchanged since their development in the late 1950s and early 1960s (Table 1).

Norethisterone oenanthate is a long-chain ester of norethisterone which is formulated in a solution of castor oil and benzyl benzoate and given intramuscularly into the gluteal or deltoid muscle. The ester is then distributed to adipose tissue throughout the body and is slowly released back into the bloodstream. It then undergoes hydrolysis in the liver to produce norethisterone, the active progestogen (Kleinman, 1990). It is most commonly

**Table 1. Formulation and availability of injectable progestogen-only contraceptives**

| Brand name | Composition | Dose (mg) and schedule | No. of countries in which registered |
|---|---|---|---|
| Depo-Provera[a] | Medroxyprogesterone acetate | 150, every 3 months | 100 |
| Dugen | Medroxyprogesterone acetate | 150, every 3 months | 100 |
| Megestron | Medroxyprogesterone acetate | 150, every 3 months | 100 |
| Noristerat, Norigest | Norethisterone oenanthate | 200, every 2 months | 60 |
| Doryxus | Norethisterone oenanthate | 200, every 2 months | 60 |

From Kleinman (1990); Lande (1995)

[a] Other names include Depo-Cliovir, Depocon, Depo-Gestin, Depo-Geston, Depo-Prodasone, Depo-Progesta, Depo-Progestin, Depo-Progevera, Medroksiprogesteron

used as a 200-mg dose given every eight weeks or two months, although in some programmes it is given on a two-month schedule for the first six months and then every three months (Lande, 1995).

Depot medroxyprogesterone acetate is administered in an aqueous microcrystalline suspension by deep intramuscular injection into the gluteal or deltoid muscle. This depot results in a high plasma concentration of medroxyprogesterone acetate initially, which declines exponentially thereafter. It is given at a dose of 150 mg every 90 days or three months (Lande, 1995).

Menstrual disturbances are common in women using these compounds and may take the form of amenorrhoea or frequent and/or irregular bleeding. Weight gain is also a common side-effect.

### 1.2.1 *Patterns of use*

About 12 million women worldwide currently use injectable contraceptives, and the vast majority of these are progestogen-only preparations (Lande, 1995). Table 2 shows the percentage of married women or women in union, aged 15–49, currently using any method of contraception (including traditional methods) and the percentage currently using injectable contraceptives. The overall proportion of women using injectable contraceptives is low in most regions, except in Indonesia, Jamaica, Kenya, Namibia, New Zealand, Rwanda, South Africa and Thailand.

In a survey conducted in New Zealand between 1983 and 1987, 14% of women aged 25–54 reported ever having used depot medroxyprogesterone acetate; however, 26% of these had only ever received one injection (Paul *et al*., 1997). In 1994, the Planned Parenthood Federation of America supplied depot medroxyprogesterone acetate to 141 000 women, representing around 7% of their clients (Lande, 1995).

**Table 2. Use of injectable contraceptives by married women or women in union aged 15–49, by country**

| Country | Year | Any contraceptive (%) | Injectable contraceptives (%) |
|---|---|---|---|
| **Africa** | | | |
| Algeria | 1992 | 51 | 0.1 |
| Benin | 1996 | 16 | 0.7 |
| Botswana | 1988 | 33 | 5.4 |
| Burkina Faso | 1993 | 8 | 0.1 |
| Cameroon | 1991 | 16 | 0.4 |
| Côte d'Ivoire | 1994 | 11 | 0.8 |
| Egypt | 1995 | 48 | 2.4 |
| Eritrea | 1995 | 8 | 0.8 |
| Ghana | 1993 | 20 | 1.6 |
| Kenya | 1993 | 33 | 7.2 |
| Madagascar | 1992 | 17 | 1.6 |
| Malawi | 1992 | 13 | 1.5 |
| Mali | 1995–96 | 7 | 0.2 |
| Mauritius | 1985 | 75 | 6 |
| Morocco | 1995 | 50 | 0.1 |
| Namibia | 1992 | 29 | 7.7 |
| Niger | 1992 | 4 | 0.5 |
| Nigeria | 1990 | 6 | 0.7 |
| Rwanda | 1992 | 21 | 8.4 |
| Senegal | 1992 | 7 | 0.2 |
| South Africa | 1987–89 | 50 | 23 |
| Black | | 49 | 27 |
| White | | 79 | 3 |
| Sudan | 1992–93 | 10 | 0.2 |
| Swaziland | 1988 | 20 | 4 |
| Tunisia | 1988 | 50 | 1 |
| Uganda | 1995 | 15 | 2.5 |
| Zimbabwe | 1994 | 48 | 3.2 |
| **Europe** | | | |
| Austria | 1981–82 | 71 | 0 |
| Belgium | 1982 | 81 | 0 |
| Hungary | 1986 | 73 | 0 |
| Italy | 1979 | 78 | 0 |
| Portugal | 1979–80 | 66 | 2 |
| United Kingdom | 1983 | 83 | 0 |
| England | 1995 | Not reported | 1.2 |
| **North America** | | | |
| Canada | 1984 | 73 | 0 |
| United States | 1988 | 74 | 0 |

**Table 2 (contd)**

| Country | Year | Any contraceptive (%) | Injectable contraceptives (%) |
|---|---|---|---|
| **Latin America and the Caribbean** | | | |
| Bolivia | 1994 | 45 | 0.8 |
| Brazil | 1996 | 77 | 1.2 |
| Colombia | 1995 | 72 | 2.5 |
| Costa Rica | 1981 | 65 | 2 |
| Dominican Republic | 1991 | 56 | < 1 |
| Ecuador | 1987 | 44 | < 1 |
| El Salvador | 1985 | 47 | 1 |
| Guatemala | 1995 | 31 | 2.5 |
| Haiti | 1994 | 18 | 2.7 |
| Jamaica | 1993 | 62 | 8 |
| Mexico | 1987 | 53 | 1 |
| Nicaragua | 1981 | 27 | 1 |
| Panama | 1984 | 58 | 1 |
| Paraguay | 1990 | 48 | 5.2 |
| Peru | 1996 | 64 | 8 |
| Trinidad and Tobago | 1987 | 53 | 0.8 |
| **Asia and Pacific** | | | |
| Bangladesh | 1993 | 45 | 4.5 |
| China | 1988 | 71 | < 1 |
| Hong Kong | 1987 | 81 | 3 |
| Indonesia | 1994 | 55 | 15 |
| Nepal | 1991 | 25 | 2 |
| Pakistan | 1990–91 | 12 | 0.1 |
| Philippines | 1993 | 40 | 0.1 |
| Sri Lanka | 1987 | 62 | 3 |
| Syria | 1993 | 40 | 0 |
| Thailand | 1991 | 69 | 12 |
| Turkey | 1993 | 63 | 0.1 |
| Yemen | 1991–92 | 7 | 0.6 |

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

### 1.2.2 *Action*

Injectable progestogen-only contraceptives prevent ovulation (Lande, 1995) by inhibiting follicle-stimulating hormone and luteinizing hormone in a similar way to combined oral contraceptives. They also thicken the cervical mucus, making it relatively impenetrable to sperm, and make the endometrium less receptive to implantation (Kleinman, 1990). They are very effective contraceptives, with 0.3 pregnancies per 100 women per

year for depot medroxyprogesterone acetate and 0.4 pregnancies per 100 women per year for norethisterone oenanthate, in the first year of use (Lande, 1995).

## 1.3 Progestogen implants

Subdermal implants release progestogen slowly over a long period and provide long-term, reversible contraception. The prototype is Norplant, which consists of six silicone rubber (Silastic) capsules 2.4 mm in diameter and 3.4 cm long which are inserted under the skin of the forearm or upper arm and provide contraception for five years. The capsules are each packed with 36 mg crystalline levonorgestrel, which is released at a rate of 85 µg/day initially, falling to 50 µg/day by nine months of use, to 35 µg/day by 18 months and then 30 µg/day during the third, fourth and fifth year of use (McCauley & Geller, 1992). They are non-biodegradable and must be removed in a minor surgical procedure. Implants consisting of two Silastic rods with similar release rates are effective for three years (Reynolds, 1996), and biodegradable implants that do not require removal are being developed.

Like the injectable progestogen-only contraceptives, progestogen implants cause amenorrhoea or frequent or irregular bleeding in most users. Implants are also more costly than many other methods (McCauley & Geller, 1992).

### 1.3.1 *Patterns of use*

Although implants are approved as a contraceptive in many countries, their use is not widespread. The country with the largest number of users is Indonesia, where over 1 million women had used them by 1992, and in 1994 they were currently being used by around 5% of married women aged 15–49 (United States Census Bureau, 1998). In the two years after their approval by the United States Food and Drug Administration in 1990, about 500 000 women in the United States obtained implants (McCauley & Geller, 1992). By mid-1992, 150 000 women in Thailand had used Norplant; in a survey in 1994, 1.2% of married women or women in union aged 15–49 in Haiti were reported to be currently using it (McCauley & Geller, 1992; United States Census Bureau, 1998).

### 1.3.2 *Action*

Implants suppress ovulation in up to 50% of women and have progestogenic effects on the cervical mucus and the endometrium (Kleinman, 1990; McCauley & Geller, 1992). The pregnancy rate is less than one per 100 women per year, averaged over five years of use (McCauley & Geller, 1992).

## 1.4 Progestogen-only oral contraceptives

Progestogen-only oral contraceptives generally contain a progestogen of the norethisterone or levonorgestrel group, given at a constant dose, to be taken at the same time every day, without a break. They are also called ‘mini-pills’. Annex 2 (Table 2) lists the common brand names of progestogen-only oral contraceptives with their compositions. Typical pills contain 0.3–0.35 mg norethisterone or 30–37.5 µg levonorgestrel (Kleinman, 1996).

### 1.4.1 *Patterns of use*

Few systematic data are available on the prevalence of use of progestogen-only oral contraceptives worldwide, as in most surveys women are asked about their use of 'oral contraceptive pills' with no distinction between combined and progestogen-only oral contraceptive pills. Use is probably more common in Australia–New Zealand, Scandinavia and the United Kingdom than it is in the United States and other parts of Europe, but use has been increasing over the last 20 years. In the United Kingdom, progestogen-only oral contraceptives represented 0.9% of all oral contraceptives used in 1973 and 8.8% in 1987 (Thorogood & Villard-Mackintosh, 1993). The *Health Survey for England 1995* showed that 4% of English women aged 16–54 were currently using progestogen-only oral contraceptives and 19% were using combined oral contraceptives; in the age group 35–44 years, 4% of women were using progestogen-only oral contraceptives and 9% were using combined oral contraceptives (Bost *et al*., 1997). In the United States, progestogen-only oral contraceptives accounted for less than 1% of oral contraceptive sales in 1984 (Piper & Kennedy, 1987). Table 3 indicates the percentages of women among the population-based controls in studies of oral contraceptives and breast cancer in Denmark, New Zealand, Sweden and the United Kingdom who reported any use of progestogen-only oral contraceptives (Collaborative Group on Hormonal Factors in Breast Cancer, 1996). In 1987, about 2% of oral contraceptives bought by pharmacies in 'developed' countries were progesterone-only pills, while they accounted for less than 1% of such sales in 'developing' countries (Wharton & Blackburn, 1988) .

**Table 3. Percentages of women reporting any use of progestogen-only oral contraceptives in selected studies**

| Country | Study | Any use of progestogen-only oral contraceptives (%) |
|---|---|---|
| Denmark | Ewertz (1992) | 5 |
| Sweden | Meirik *et al*. (1986) | 13 |
| New Zealand | Paul *et al*. (1990) | 9 |
| United Kingdom | UK National Case–Control Study Group (1989) | 15 |

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

### 1.4.2 *Action*

Progestogen-only oral contraceptives have variable effects on ovulation, suppressing it in about 40% of users. Their main contraceptive action is through a progestogenic effect on cervical mucus and, to a lesser extent, the endometrium. As the effect on cervical mucus lessens 20–22 h after administration of a pill, the user must be careful to take it regularly at a time that maximizes its effectiveness. The pregnancy rate is 0.3–5

per 100 women per year of use, with lower failure rates among older women, probably because of their lower overall fertility (Kleinman, 1990).

### 1.5 Other sources of exposure to progestogen-only contraceptives

The hormone-releasing intrauterine device 'Progestasert' contains 38 mg progesterone, which is released at a rate of 65 μg/day for one year, after which time it should be replaced (Treiman *et al*., 1995). The more recently developed LNG-20 intrauterine device contains 52 mg levonorgestrel which is released at a rate of 20–30 μg/day and lasts for at least five years (Treiman *et al*., 1995; Kleinman, 1996). The progestogen enhances the contraceptive efficacy of the intrauterine device and also reduces menstrual loss. Although worldwide use of intrauterine devices is high, with at least 72 million users in China alone (Treiman *et al*., 1995), only a small proportion of these contain progestogen. Hormone-impregnated contraceptive vaginal rings which release levonorgestrel into the systemic circulation have been developed but are not widely used (Kleinman, 1990).

Progestogen-only emergency contraception involves the administration of two doses of 750 μg levonorgestrel orally 12 h apart within 48 h of unprotected intercourse (Cullins & Garcia, 1997).

## 2. Studies of Cancer in Humans

### 2.1 Breast cancer

#### 2.1.1 *Results of published studies*

Eight studies have been published on the relationship between the incidence of breast cancer and use of progestogen-only hormonal contraceptives, i.e. progestogen-only pills or injectable progestogen (depot medroxyprogesterone acetate). They are described in Table 4. The studies were similar in that all were case–control studies of breast cancer in relation to oral contraceptive use; information was obtained on contraceptive use and other factors through interviews, with the exception of the study of Ewertz (1992), in which self-administered questionnaires were used; they confirmed the cancer diagnosis through medical records or cancer registry data; and important risk factors for breast cancer were controlled for in the analyses.

(*a*) *Mini-pills*

A case–control study in the United Kingdom (Vessey *et al*., 1983), in which 1176 cases and 1176 controls aged 16–50 years in 1968–80 were enrolled, showed no association between the risk for breast cancer and use of progestogen-only pills. Such use was reported by 2.8% of the cases and 2.5% of the controls; the relative risk estimate was not given.

A population-based study (Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development,

**Table 4. Case–control studies of use of progestogen-only contraceptives and breast cancer**

| Reference | Country | Years of case diagnosis | Age (years) | No. of cases | No. of controls | Participation rates (%) (cases/ controls) | Type of progestogen assessed | Any use of progestogen-only contraceptives (%) (cases/ controls) | RR (95% CI) for any versus no use |
|---|---|---|---|---|---|---|---|---|---|
| Vessey *et al*. (1983) | United Kingdom | 1968–80 | 16–50 | 1 176 | 1 176 Hospital-based | Not given | Pill | 2.8/2.5 | Not given |
| Cancer and Steroid Hormone Study (1986) | United States | 1980–82 | 20–54 | 4 711 Population-based | 4 676 Random digit-dialling | 80/83 | Pill | Not given | 1.3 (CI not given) |
| Paul *et al*. (1989) (New Zealand National Study) | New Zealand | 1983–87 | 25–54 | 891 Population-based | 1 864 Electoral rolls | 79/82 | Injectable (DMPA) | 12/14 | 1.0 (0.8–1.3) |
| UK National Case–Control Study Group (1989) | United Kingdom | 1982–85 | < 36 | 775 Population-based | 755 General practice | 72/89 | Pill | 16/15 | 0.85 (per year of use) |
| Clavel *et al*. (1991) | France | 1983–87 | 25–56 | 464 | 542 Hospital-based | 99/99 | Pill | 1.9/1.9 | 1.1 (0.4–2.7) |
| WHO Collaborative Study (1991a) | Kenya, Mexico, Thailand | 1979–88 | < 65 | 869 | 11 890 Hospital-based | 97/98 | Injectable (DMPA) | 13/12 | 1.2 (0.96–1.5) |
| Ewertz (1992) | Denmark | 1983–84 | < 40<br>40–59 | 203<br>856 | 212<br>778 Population-based | 90/88<br>89/80 | Pill | Not given | 0.99 (0.57–1.7) |
| Skegg *et al*. (1996) (New Zealand National Study) | New Zealand | 1983–87 | 25–54 | 891 Population-based | 1 864 Electoral rolls | 79/82 | Pill | 5.6/8.7 | 1.1 (0.73–1.5) |

RR, relative risk; CI, confidence interval; DMPA, depot medroxyprogesterone acetate

1986), conducted between 1980 and 1982 in the United States, involved 4711 cases and 4676 controls 20–54 years of age. The investigators reported a relative risk estimate of 1.3 for use of progestogen-only pills; the confidence interval and numbers of case and control women who had used the formulations were not given.

In a multicentre study in the United Kingdom (UK National Case–Control Study Group, 1989), cases in women under the age of 36 years in 1982–85 were ascertained and matched to a control from the general practice in which the case was treated. Replies about contraceptive use obtained at interview were supplemented by the general practitioner for 90% of the 755 pairs. Progestogen-only pills had been used in 16% of cases and 15% of controls, but only 2.9% of the controls had used them for more than eight years. The relative risk estimate for use of progestogen-only pills was 1.35 for less than four years of use (90 cases and 67 controls), 0.73 for > 4–8 years of use (19 cases and 27 controls) and 0.59 for > 8 years of use (14 cases and 22 controls). The trend for the relative risk to decrease with increasing duration of use was of borderline statistical significance ($p = 0.05$).

Clavel *et al*. (1991) carried out a hospital-based case–control study in France between 1983 and 1987. Among 464 cases and 542 controls aged 25–56 years, nine cases (1.9%) and 10 controls (1.9%) had use of progestogen-only pills, yielding a multivariate relative risk estimate of 1.1 (95% confidence interval [CI], 0.4–2.7).

Ewertz (1992) carried out a population-based case–control study in Denmark of cases notified in 1983–84 and obtained data on contraceptive use from self-administered questionnaires. A total of 1059 cases and 990 controls were included. Among the 377 cases and 364 controls for which data on the type of preparation used were available, 28 cases and 29 controls had used a progestogen-only pill, yielding a relative risk estimate of 0.99 (95% CI, 0.57–1.71). For five or more years' use of progestogen-only pills, the estimate was 0.65 (95% CI, 0.28–1.5), based on nine case and 14 control users.

Skegg *et al*. (1996) assessed use of progestogen-only pills in data from the New Zealand National Study. On the basis of 50 cases (5.6%) and 163 controls (8.7%) with use of progestogen-only pills, the relative risk estimate for breast cancer was 1.1 (95% CI, 0.73–1.5) after adjustment for a number of factors, including age. There was a statistically significant increased risk (2.3; 95% CI, 1.2–4.3) among women aged 25–34, on the basis of 18 case and 70 control users. The corresponding estimates were 0.97 (95% CI, 0.6–1.6) for women aged 35–44, on the basis of 28 case and 80 control users, and 0.37 (95% CI, 0.12–1.2) for women aged 45–54, on the basis of four case and 13 control users; neither estimate was statistically significant. Virtually all of the women had used the preparations for fewer than six years; the estimates for fewer than two and two to five years of use were similar. In further analyses of women of all ages together, the relative risk estimate was increased for use that had begun in the previous 10 years (1.6; 95% CI, 1.0–2.4; 40 case and 111 control users) and reduced for use that had begun 10 or more years previously (0.44; 95% CI, 0.22–0.90; 10 case and 52 control users). When time since last use was assessed, the relative risk estimate was 1.4 (95% CI, 0.86–2.2) for last use fewer than five years previously (29 case and 91 control users), 1.0 (95% CI, 0.56–1.9) for use that had

ceased five to nine years previously (16 case and 48 case users) and 0.44 (95% CI, 0.16–1.2) for use that had ceased at least 10 years previously (five case and 24 control users). There was no clear evidence of an effect of the age at which use began or the timing with respect to the first pregnancy.

(*b*) *Depot medroxyprogesterone acetate*

Paul *et al*. (1989) reported on the use of the injectable progestogen, depot medroxyprogesterone acetate, in a population-based case–control study of women aged 25–54 conducted in New Zealand between 1983 and 1987. A total of 110 (12%) of 891 cases and 252 (14%) of 1864 controls had used this preparation. There was no increase in risk overall (relative risk, 1.0; 95% CI, 0.8–1.3), but the relative risk estimate was increased among women aged 25–34 (2.0; 95% CI, 1.0–3.8; 16 case and 55 control users). The estimate was not increased in women aged 35–44 (0.94; 95% CI, 0.45–3.3; 48 case and 133 control users) or 45–54 (0.95; 95% CI, 0.63–1.4; 46 case and 64 control users). There was no trend in the overall data for an increase in risk with increasing duration of use, but only 1.5% of controls had used depot medroxyprogesterone acetate for six years or more. The relative risk estimates, although based on small numbers, were higher for women with two to five years of use before the age of 25 or before the first pregnancy than among women with less than two years of use. The relative risk estimate tended to be increased for recent users: it was 1.7 (95% CI, 0.88–3.4) for women who had begun use in the previous five years (16 case and 24 control users) and declined to 1.2 (95% CI, 0.76–1.9) five to nine years after first use, 0.92 (95% CI, 0.64–1.3) 10–14 years after first use and 0.73 (95% CI, 0.39–1.4) 15 or more years after first use; none of these estimates was statistically significant. A similar trend was seen with time since last use: the relative risk was 1.6 (95% CI, 1.0–2.5) for use within the previous five years, 0.99 (95% CI, 0.65–1.5) five to nine years after last use and 0.78 (95% CI, 0.53–1.2) 10 years or more after last use.

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991a) assessed use of depot medroxyprogesterone acetate in centres in Kenya, Mexico and Thailand in a hospital-based study conducted between 1979 and 1988 among women under 65 years of age. Among the 869 cases of breast cancer and 11 890 controls, 109 cases (13%) and 1452 controls (12%) reported use of depot medroxyprogesterone acetate, yielding an overall multivariate relative risk estimate of 1.2 (95% CI, 0.96–1.5). The relative risk estimate was 1.4 (95% CI, 0.88–2.2) for breast cancer at age < 35, 1.1 (95% CI, 0.75–1.55) at age 35–44 and 1.0 (95% CI, 0.68–1.5) at age 45 or older; none of these estimates was statistically significant. There was no trend for the risk to increase with duration of use; indeed, the largest relative risk estimate was for the shortest duration of use; however, only 3.6% of controls had been exposed for more than three years. The relative risk estimates tended to be highest among recent users: 2.0 (95% CI, 1.4–3.0) for women whose use had begun in the previous two years (31 case and 342 control users) and 1.6 (95% CI, 1.1–2.5) for current users (27 case and 291 control users).

2.1.2 *Pooled analysis of individual data*

The Collaborative Group on Hormonal Factors in Breast Cancer (1996) carried out a combined analysis of data on use of progestrogen-only oral contraceptives from 27 studies that provided information on these preparations to the investigators in 1995. On the basis of 725 of 27 054 cases and 528 of 25 551 controls with any use of these preparations, the relative risk estimate was 1.1 (95% CI, 0.99–1.2) (Figure 1). There was no significant trend with duration of use, time since first use or time since last use (Figures 2–4), although there was some suggestion that the risk was slightly elevated in current and recent users (1.2; 95% CI, 1.0–1.3) (Figure 4).

Skegg *et al*. (1995) published the results of a pooled analysis of individual data from two studies (Paul *et al*., 1989; WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1991a) on depot medroxyprogesterone acetate. As had been observed in the separate studies, there was no association between use and overall risk, but an increased risk (not statistically significant) was found for women under 35 years of age and an increased risk (statistically significant) for women who had last used the preparation during the previous five years. The age-specific results for time since first use suggested an increased risk for use begun in the previous year in each age group: 2.0 (95% CI, 1.2–3.3) for < 35 years of age, 1.5 (95% CI, 0.9–2.4) for 35–44 years of age and 1.8 (95% CI, 0.81–4.0) for 45 years of age and older, although only the estimate for women < 35 years of age was statistically significant.

The Collaborative Group on Hormonal Factors in Breast Cancer (1996) also carried out a combined analysis of use of injectable progestogens. On the basis of any use in 339 of 17 639 cases and 1935 of 38 248 controls, the relative risk estimate was 1.0 (95% CI, 0.89–1.2) (Figure 5). There was no significant trend with duration of use (Figure 6). There was some evidence of an increased risk for users of depot progestogens (Figures 7 and 8), with a significant trend of decreasing risk with time since first use (Figure 7).

## 2.2 Endometrial cancer

2.2.1 *Cohort studies*

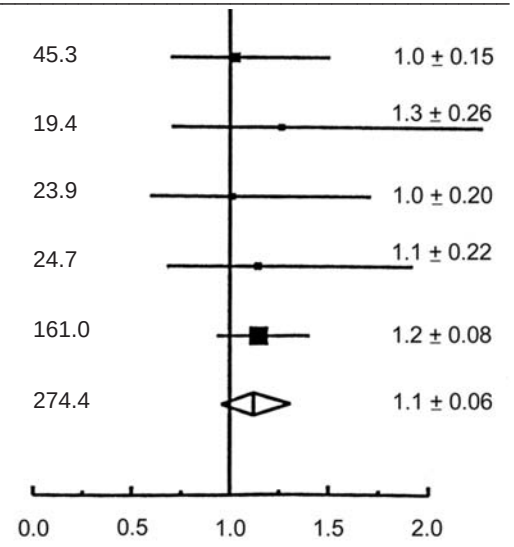
In a study at a family planning clinic in Atlanta, United States, one case of uterine cancer was found among 5000 African–American women aged 50 in 1967–76 who were receiving injections of depot medroxyprogesterone acetate, with 0.83 expected (relative risk, 1.2; 95% CI, 0.1–6.7) on the basis of the rates from the national Surveillance, Epidemiology and End Results programme (Liang *et al*., 1983).

2.2.2 *Case–control studies* (Table 5)

In a multi-centre case–control study among women under 55 years of age in the United States, only one of the 433 women with endometrial cancer and six of the 3191 control women reported use of a progestogen-only oral contraceptive (odds ratio, 0.6; 95% CI, 0.1–5.0) in personal interviews (Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study, 1987).

**Figure 1. Relative risks for breast cancer among women with any versus no use of progestogen-only oral contraceptives**

| Reference, study | Any use (no. of cases/ no. of controls) | No use (no. of cases/ no. of controls) | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|---|
| | | | O-E | var (O-E) | RR & 99% CI | RR ± SD |
| UK National case–control study group (1989) | 123/116 | 632/639 | 1.1 | 45.3 | | 1.0 ± 0.15 |
| Meirik *et al.* (1986) | 59/62 | 363/465 | 4.5 | 19.4 | | 1.3 ± 0.26 |
| Paul *et al.* (1989, 1990); Skegg *et al.* (1996) | 50/163 | 841/1 701 | 0.2 | 23.9 | | 1.0 ± 0.20 |
| Vessey *et al.* (1983) | 68/58 | 2 315/2 333 | 3.3 | 24.7 | | 1.1 ± 0.22 |
| Other | 425/129 | 22 178/19 885 | 22.0 | 161.0 | | 1.2 ± 0.08 |
| All studies | 725/528 | 26 329/25 023 | 31.1 | 274.4 | | 1.1 ± 0.06 |




Test for heterogeneity between studies: $\chi^2$ (4 d.f.) = 1.0; NS

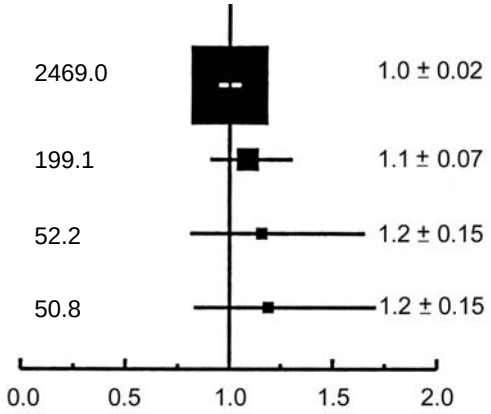
Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)

O, observed; E, expected; RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degrees of freedom; NS, not significant

[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk of conception ceased

**Figure 2. Relative risk for breast cancer by duration of use of progestogen-only oral contraceptives**

| Duration of use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | InRR / var(InRR) | 1 / var(InRR) | RR (99% CI) | RR ± SD |
| Never | 29 625/50 515 | 0.0 | 2469.0 | | 1.0 ± 0.02 |
| < 2 | 467/641 | 16.8 | 199.1 | | 1.1 ± 0.07 |
| 2–3 | 120/150 | 7.6 | 52.2 | | 1.2 ± 0.15 |
| ≥ 4 | 125/145 | 8.8 | 50.8 | | 1.2 ± 0.15 |




Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with duration of use $\chi^2$ (1 d.f.) = 0.4; NS
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

A study in Bangkok and Chiang Mai, Thailand, found that the incidence of endometrial cancer was approximately 80% lower (odds ratio, 0.2; 95% CI, 0.1–0.8) among women (three cases and 84 controls) who reported using depot medroxyprogesterone acetate than among those who reported no use (119 cases and 855 controls) in personal interviews (WHO Collaborative Study of Neoplasia and Steroid Contraceptives, 1991b). All three case women who had used this preparation had also used pre-menopausal oestrogens.

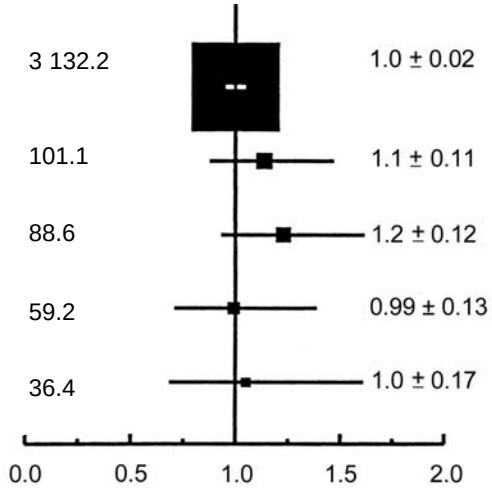
## 2.3 Cervical cancer

### 2.3.1 *Methodological considerations*

The same methodological issues as are described in section 2.3 of the monograph on 'Oral contraceptives, combined' must be considered when assessing associations between use of injectable contraceptives and cervical carcinoma.

**Figure 3. Relative risk for breast cancer by time since first use of progestogen-only oral contraceptives**

| Time since first use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | InRR | 1 | RR (99% CI) | RR ± SD |
| | | var(InRR) | var(InRR) | | |
| Never | 29 625/50 515 | 0.0 | 3 132.2 | | 1.0 ± 0.02 |
| < 5 | 250/335 | 12.9 | 101.1 | | 1.1 ± 0.11 |
| 5–9 | 218/271 | 18.2 | 88.6 | | 1.2 ± 0.12 |
| 10–14 | 129/195 | –0.5 | 59.2 | | 0.99 ± 0.13 |
| ≥ 15 | 84/103 | 1.8 | 36.4 | | 1.0 ± 0.17 |




Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with time since first use: $\chi^2$ (1 d.f.) = 0.6; NS
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

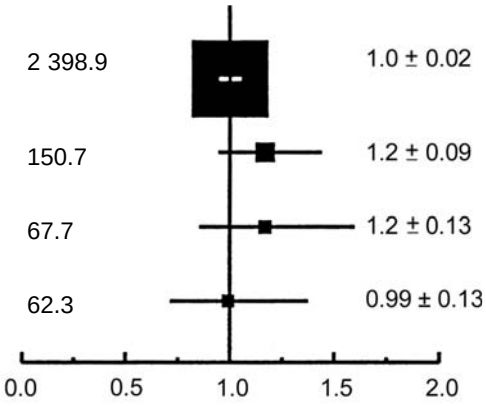
2.3.2 *Cervical dysplasia and carcinoma* in situ

The New Zealand Contraception and Health Study Group (1994) followed a cohort of 7199 women for about five years. All of the women had two normal cervical smears at entry into the cohort and were using either oral contraceptives, an intrauterine device or depot medroxyprogesterone acetate as their method of contraception. The risk for dysplasia per 1000 women was 58.7 for the users of depot medroxyprogesterone acetate and 44.4 for those with an intrauterine device. This difference was not statistically significant. The incidence rate of more severe dysplasia or carcinoma *in situ* was 0.9/1000 in both groups. After control for multiple confounding factors, including the number of sexual partners, the risk of the progestogen users relative to that of women with an intrauterine device was 1.2.

From data on women included in the WHO Collaborative Study of Neoplasia and Steroid Contraceptives in Mexico and Thailand, Thomas *et al*. (1995a) estimated that the

**Figure 4. Relative risk for breast cancer by time since last use of progestogen-only oral contraceptives**

| Time since last use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | InRR | 1 | RR (99% CI) | RR ± SD |
| | | var(InRR) | var(InRR) | | |
| Never | 29 625/50 515 | 0.0 | 2 398.9 | | 1.0 ± 0.02 |
| < 5 | 375/492 | 23.4 | 150.7 | | 1.2 ± 0.09 |
| 5–9 | 162/210 | 10.6 | 67.7 | | 1.2 ± 0.13 |
| ≥ 10 | 134/186 | –0.5 | 62.3 | | 0.99 ± 0.13 |




Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with time since last use: $\chi^2$ (1 d.f.) = 1.0; NS
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

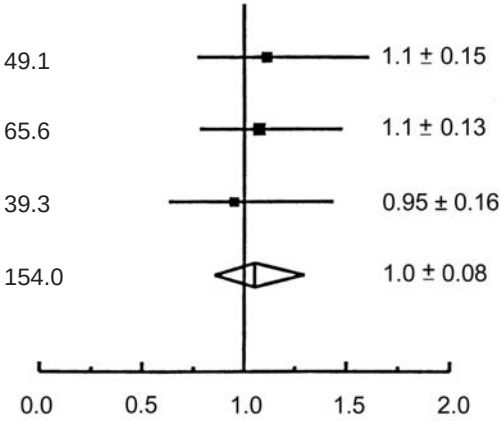
relative risk for cervical carcinoma *in situ* of women who had ever used depot medroxyprogesterone acetate was 1.4 (95% CI, 1.2–1.7); however, when the analyses were restricted to women with symptoms of vaginal bleeding or discharge, to minimize the possibility of bias due to selective screening of women on this preparation, the relative risk estimate was 1.2 (95% CI, 1.0–1.5). Nonetheless, women with symptoms had a significant trend ($p = 0.017$) in risk with duration of use: women who had used depot medroxyprogesterone acetate for more than five years had a relative risk of 1.8 (95% CI, 1.2–2.6). There was no trend in risk with time since first or last use. When considering women who had used this preparation for more than five years, the risk was increased for those who had last used it within the previous 10 years but not for those who had used it before that time.

### 2.3.3 *Invasive cervical carcinoma*

Two case–control studies have been conducted of the risk for invasive cervical cancer and use of injectable contraceptives. Herrero *et al*. (1990) recruited cases from six hospitals in Colombia, Costa Rica, Mexico and Panama. Controls were selected from the

**Figure 5. Relative risk for breast cancer among women with any use of depot progestogens versus those who had never used them**

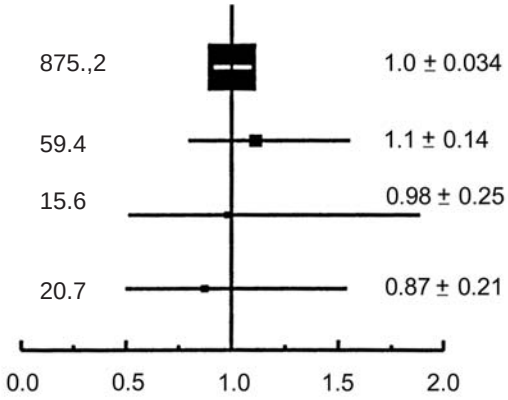
| Reference, study | Any use (no. of cases/ no. of controls) | No use (no. of cases/ no. of controls) | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|---|
| | | | O-E | var (O-E) | RR & 99% CI | RR ± SD |
| Paul *et al.* (1989, 1990); Skegg *et al.* (1996) | 110/252 | 781/1 612 | 5.2 | 49.1 | | 1.1 ± 0.15 |
| WHO Collaborative Study (1991a) | 138/1 525 | 3 156/17 577 | 4.7 | 65.6 | | 1.1 ± 0.13 |
| Other | 91/158 | 13 363/17 124 | –2.0 | 39.3 | | 0.95 ± 0.16 |
| All studies | 339/1 935 | 17 300/36 313 | 7.9 | 154.0 | | 1.0 ± 0.08 |




Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for heterogeneity between studies: $\chi^2$ (2 d.f.) = 0.6; NS
O, observed; E, expected; RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

**Figure 6. Relative risk for breast cancer by duration of use of depot progestogens**

| Duration of use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | InRR | 1 | RR (99% CI) | RR ± SD |
| | | var(InRR) | var(InRR) | | |
| Never | 25 612/45 437 | 0.0 | 875.,2 | | 1.0 ± 0.034 |
| < 2 | 129/257 | 6.3 | 59.4 | | 1.1 ± 0.14 |
| 2–3 | 28/65 | –0.3 | 15.6 | | 0.98 ± 0.25 |
| ≥ 4 | 37/88 | –2.8 | 20.7 | | 0.87 ± 0.21 |

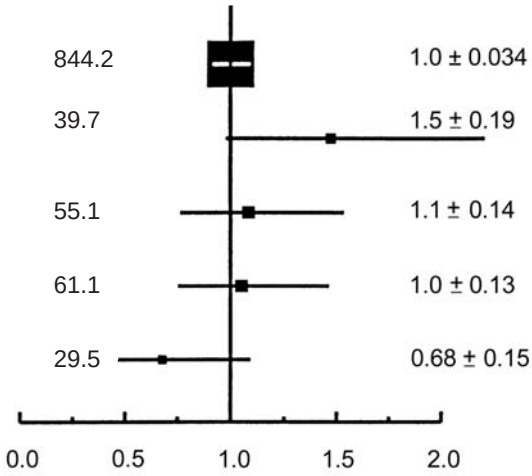



Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with duration of use $\chi^2$ (1 d.f.) = 0.4; NS
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

same hospitals from which the cases were recruited; in Costa Rica and Panama, community controls were also selected. The results were reported for use of all injectable contraceptives combined and not separately for specific agents. Of the users, 55% reported using injectable contraceptives monthly and 45% reported using them every three months. The preparation used more frequently than every three months was probably norethisterone oenanthate and that used every three months was probably depot medroxyprogesterone acetate. Cervical swabs were taken from the study subjects and tested for type-specific human papillomavirus DNA by filter in-situ hybridization. After control for age, age at first intercourse, number of sexual partners, number of pregnancies, detected presence of human papillomavirus type-16/-18 DNA, interval since last Papanicolaou (Pap) smear and socioeconomic status, the risk of women who had ever used injectable contraceptives for six or more months, relative to non-users, was estimated to be 0.8 (95% CI, 0.5–1.2). The risk was not increased for women who had used these products for fewer than five years (0.5; 95% CI, 0.3–0.9) but was 2.4 (95% CI, 1.0–5.7) for women who had used them for five or more years. There were no significant trends in risk with time since first or last use;

**Figure 7. Relative risk for breast cancer by time since first use of depot progestogens**

| Time since first use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | lnRR | 1 | RR (99% CI) | RR ± SD |
| | | var(lnRR) | var(lnRR) | | |
| Never | 25 612/45 437 | 0.0 | 844.2 | | 1.0 ± 0.034 |
| < 5 | 84/516 | 15.4 | 39.7 | | 1.5 ± 0.19 |
| 5–9 | 94/592 | 4.4 | 55.1 | | 1.1 ± 0.14 |
| 10–14 | 110/534 | 3.0 | 61.1 | | 1.0 ± 0.13 |
| ≥ 15 | 44/281 | –11.4 | 29.5 | | 0.68 ± 0.15 |



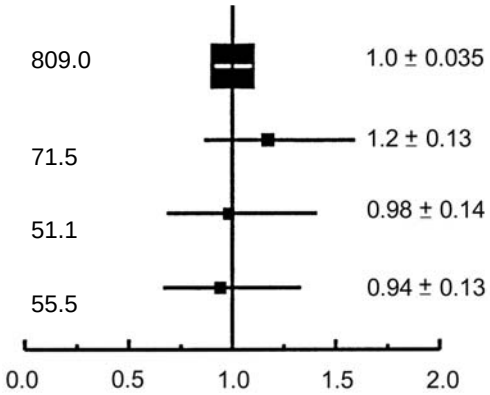

Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with duration of use $\chi^2$ (1 d.f.) = 8.8; $p$ = 0.003
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

however, the highest relative risks were observed for women who had used these products for more than five years and who had first used them more than 10 years previously (relative risk, 3.4; 95% CI, 1.1–25) and for women who had used the products for more than five years and who had last used them more than five years previously (relative risk, 5.3; 95% CI, 1.1–10). These increased risks must be interpreted with caution, however, because significantly reduced risks were observed for women who had used the products for fewer than five years and had used them for the first time within the past 10 years (relative risk, 0.4; 95% CI, 0.2–0.8) or within the past five years (relative risk, 0.4; 95% CI, 0.2–0.8). The reduced risks in relatively recent users could be due to more intensive screening in women receiving depot medroxyprogesterone acetate, so that earlier stages of disease are detected before progression to invasive disease.

In the WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1992), described in section 2.1.1(b), 2009 women with invasive cervical cancer were compared

**Figure 8. Relative risk for breast cancer by time since last use of depot progestogens**

| Time since last use (years) | No. of cases/ no. of controls | Statistics | | Relative risk[a] | |
|---|---|---|---|---|---|
| | | InRR | 1 | RR (99% CI) | RR ± SD |
| | | var(InRR) | var(InRR) | | |
| Never | 25 612/45 437 | 0.0 | 809.0 | | 1.0 ± 0.035 |
| < 5 | 137/921 | 11.4 | 71.5 | | 1.2 ± 0.13 |
| 5–9 | 82/514 | –0.9 | 51.1 | | 0.98 ± 0.14 |
| ≥ 10 | 101/467 | –3.3 | 55.5 | | 0.94 ± 0.13 |




Adapted from Collaborative Group on Hormonal Factors in Breast Cancer (1996)
Test for trend with duration of use $\chi^2$ (1 d.f.) = 1.6; NS
RR, relative risk; CI, confidence interval; SD, standard deviation; d.f., degree of freedom; NS, not significant
[a] Relative to no use, stratified by study, age at diagnosis, parity, age at first birth and age at which risk for conception ceased

with 9583 hospital controls. After taking into consideration age, total number of pregnancies, number of prior Pap smears, use of oral contraceptives and centre, the relative risk for women who had ever used depot medroxyprogesterone acetate was estimated to be 1.1 (95% CI, 1.0–1.3).

Using data from this study, Thomas *et al*. (1995b) also assessed risks for adenocarcinoma and adenosquamous carcinoma in relation to use of depot medroxyprogesterone acetate. On the basis of 239 women with adenocarcinoma and 85 with adenosquamous carcinoma, the risks of women who had ever used this preparation relative to 2534 age-matched hospital controls were estimated to be 0.8 (95% CI, 0.5–1.3) for adenocarcinomas and 0.7 (95% CI, 0.3–1.7) for adenosquamous carcinoma. All of the relative risks in this study were assessed for possible confounding by variables including numbers of pregnancies, live births and sexual partners, history of abortion and stillbirths, age at first live birth, age at first sexual intercourse, marital status, history of a variety of sexually transmitted diseases, serological evidence of herpes simplex virus infection, prior Pap smears, level of education and use of other methods of contraception. Because the relative

**Table 5. Case–control studies of use of progestogen-only contraceptives and endometrial cancer**

| Reference | Location/period/ ages | Source of controls | Ascertain-ment | Participation (%) | | Type/measure of therapy | No. of subjects | | OR (95% CI) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Cases | Controls | | Cases | Controls | |
| Centers for Disease Control (1987) | Eight US SEER areas/Dec. 1980–Dec. 1982/ 20–54 years | General population | Personal interviews | 73 | 84 | Never used POC<br>Any use of POC | 250<br>1 | 1 147<br>6 | Referent<br>0.6 (0.1–5.0) |
| WHO Collaborative Study (1991b) | Bangkok, Chiang Mai, Thailand/ Jan. 1979–Feb. 1986/< 60 years | Hospital patients | Personal interviews | 98 | 96 | Never used DMPA<br>Ever used DMPA | 119<br>3 | 855<br>85 | Referent<br>0.2 (0.1–0.81) |

OR, odds ratio; CI, confidence interval; POC, progestogen-only contraceptives; DMPA, depot medroxyprogesterone acetate

risk estimates for the two histological types were similar, the data for the two types were combined, giving a relative risk of 0.8 (95% CI, 0.5–1.1) for women who had ever used depot medroxyprogesterone acetate. No trends in relative risk with length of use or time since first or last use were observed. The relative risk of women who had used the product for more than four years was estimated to be 0.7 (95% CI, 0.4–1.4).

## 2.4 Ovarian cancer

### 2.4.1 *Cohort studies*

In the study of Liang *et al.* (1983) described in section 2.2, one case of ovarian cancer was observed with 1.2 expected, corresponding to a relative risk of 0.8 (95% CI, 0.1–4.6).

### 2.4.2 *Case–control studies*

Within the framework of the WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991c), hospital-based data from Mexico and Thailand were analysed with reference to use of depot medroxyprogesterone acetate and the risk for epithelial ovarian cancer. A total of 224 cases and 1781 hospital controls were collected between 1979 and 1988. The multivariate relative risk for any use was 1.1 (95% CI, 0.6–1.8) in the absence of any duration–risk relationship (relative risk, 1.1 for ≥ 5 years of use; 95% CI, 0.4–3.2).

Little information is available on progestogen-only oral contraceptives. In a hospital-based case–control study of 441 cases and 2065 controls recruited between 1977 and 1991 from various areas of the United States (Rosenberg *et al.*, 1994), 1% of cases and 3% of controls had ever used such preparations. [The unadjusted odds ratio was 0.3.]

## 2.5 Liver cancer

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991d) also addressed the association between use of depot medroxyprogesterone acetate and the risk for cancer of the liver. Cases were women diagnosed with cancer in three centres in Thailand in 1979–88 and one centre in Kenya in 1979–86. Of the 94 eligible cases, 71 (75.5%) were interviewed. About two controls were identified for each case, chosen from the same hospital but not otherwise matched; women were not eligible as potential controls if they had been admitted to the hospital for conditions that might have altered their use of steroid contraceptives. Of 10 796 eligible controls that were identified, 10 382 (96.2%) were interviewed. Eight controls per case of liver cancer were randomly selected from the pool, resulting in the inclusion of 530 controls, matched on hospital, age and date of diagnosis. Information on smoking was not collected. As alcohol intake was not associated with the risk for liver cancer in these women, the relative risks were not adjusted for alcohol intake. Subjects were not tested for evidence of infection with hepatitis B virus, but both countries are endemic for this infection. The relative risks were adjusted for age, centre, date of diagnosis and number of live births and were presented separately for Kenya and Thailand. In Kenya, four out of 22 cases (18.2%) had used

depot medroxyprogesterone acetate; the relative risks were 1.6 (95% CI, 0.4–6.6) for any use, 0.7 (95% CI, 0.1–6.8) for use for 1–26 months and 2.9 (95% CI, 0.5–15.2) for use for more than 26 months. Fifteen of the 22 cases in Kenya were diagnosed only on clinical grounds. In Thailand, four out of 49 cases (8.2%) had used depot medroxyprogesterone acetate; the relative risks were 0.3 (95% CI, 0.1–1.0) for any use, 0.2 (95% CI, 0.0–1.2) for use for 1–26 months, 0.3 (95% CI, 0.0–2.5) for 27–58 months and 0.7 (95% CI, 0.2–3.2) for more than 58 months.

Kew *et al.* (1990) conducted a hospital-based case–control study in Johannesburg, South Africa. The cases were those of patients with histologically confirmed hepatocellular carcinoma which had been diagnosed when they were aged 19–54. Two controls per case were selected, matched on age, race, tribe, rural or urban birth, hospital and ward; patients with diseases in which contraceptive steroids might be causally implicated were not eligible as controls. The response rates were not given. Smoking and alcohol intake were associated with the risk for liver cancer, but inclusion of these variables in the analysis did not alter the results. Five of 46 cases (11%) and 21 of 92 controls (23%) had used injectable progestogens, giving an overall relative risk of 0.4 (95% CI, 0.1–1.2). Nineteen of the 46 cases had antibodies to hepatitis B surface antigen, 25 had evidence of past infection with hepatitis B virus, and two had no evidence of infection.

### 2.6 Malignant melanoma

One Danish case–control study of malignant melanoma (Østerlind *et al.*, 1988), described in detail in the monograph on 'Post-menopausal oestrogen therapy', provided data on the use of progestogens alone. These preparations were used as oral contraceptives by 14 cases and 23 controls (relative risk, 1.2; 95% CI, 0.6–2.6) and as post-menopausal therapy by three cases and four controls (crude relative risk, 1.5; 95% CI, 0.3–8.1).

## 3. Studies of Cancer in Experimental Animals

In the only study evaluated previously (IARC, 1979) on the carcinogenicity of progestogen-only contraceptives in experimental animals, medroxyprogesterone acetate, tested by intramuscular injection in dogs, produced malignant mammary tumours. No information was available at that time on levonorgestrel. The results of relevant studies published since that time are described below. Except where indicated, tumour development in tissues other than those mentioned was not reported.

### 3.1 Medroxyprogesterone acetate

3.1.1 *Subcutaneous implantation*

(*a*) *Mouse*

A group of virgin female BALB/c mice, eight weeks of age, was divided into three subgroups: 44 received 60 mg progesterone, as 40 mg in a Silastic pellet implanted subcutaneously initially and 20 mg six months later; 45 received 60 mg medroxyprogesterone

acetate, as a 40-mg pellet initially and 20 mg six months later; and 47 received 160 mg of the progestogen, 40 mg subcutaneously every three months for one year, representing the protocol used in the development of this model (Lanari *et al*., 1986). The incidence of mammary adenocarcinoma and the numbers and latency of the tumours are shown in Table 6. The carcinomas induced by medroxyprogesterone acetate were predominantly ductal but included some lobular carcinomas. The incidence of mammary carcinomas in untreated controls was reported previously by Lanari *et al*. (1986) to be 0/42 at 80–90 weeks of age (Kordon *et al*., 1993).

**Table 6. Mammary tumour incidence, number and latency in BALB/c mice treated with medroxyprogesterone acetate (MPA)**

| Treatment | Dose (mg) | Mammary tumour incidence | | No. of tumours | Latency (weeks) |
|---|---|---|---|---|---|
| | | No. | % | | |
| Progesterone | 60 | 9/44 | 28 | 10 | 46.2 |
| MPA | 60 | 18/45 | 58[a] | 30 | 51.3 |
| MPA | 160 | 34/38 | 98[b] | 38 | 50.1 |

From Kordon *et al.* (1993)
[a] Significantly greater than with progesterone ($p < 0.05$)
[b] Significantly greater than with 60 mg MPA ($p < 0.0001$)

Female BALB/c mice, two months of age, were either left intact or sialoadenectomized. One month after sialoadenectomy, all mice were injected subcutaneously with 40 mg depot medroxyprogesterone acetate, and the same treatment was given every three months for one year. The incidence of ductal and lobular mammary adenocarcinomas in the intact mice was 34/47, and that in the sialoadenectomized group was significantly less (11/48; $p < 0.001$). The tumour latency was similar: $52.5 \pm 3.8$ and $50.1 \pm 2.1$ weeks, respectively (Kordon *et al*., 1994).

(*b*) *Dog*

Groups of 20 virgin beagle bitches were hysterectomized at four to six months of age and given medroxyprogesterone acetate intramuscularly as an aqueous suspension, at a dose of 0 (control), 30, 180 or 690 mg every three months for 48 months, corresponding to one, six and 23 times the human contraceptive dose. As shown in Table 7, the incidence of mammary tumour nodules was increased in treated animals. Histopathological examination of the nodules revealed the presence of hyperplasia, including 13 animals at the high dose with complex lobular hyperplasias. At that dose, the tumour type was predominantly (12/14) complex adenoma. No carcinomas were detected (Frank *et al*., 1979).

**Table 7. Mammary tumour nodules in beagle bitches treated with medroxyprogesterone acetate (MPA)**

| Dose of MPA (mg/kg bw) | No. of surviving bitches | No. of bitches with nodules | No. of nodules |
|---|---|---|---|
| 0 (vehicle) | 17 | 2 | 2 |
| 3 | 19 | 13 | 29 |
| 30 | 18 | 15 | 93 |
| 75 | 14 | 12 | 105 |

From Frank *et al*. (1979)

Data on mammary tumour incidence in dogs treated therapeutically with medroxyprogesterone acetate for the prevention of oestrus were obtained from 10 veterinary practices in the Netherlands (van Os *et al.*, 1981) for 341 bitches; 339 untreated bitches were included as controls. The minimum age was two years, but most were older. The practitioners had used the recommended dose, which was 50–100 mg per bitch, with an interval of six months between doses, except at the start when dosing was more frequent. Putative mammary tumours were generally not examined histologically and are thus referred to as 'nodules', which were classed by size as < 1, 1–< 2, 2–< 3 and ≥ 3 cm. The first two sizes were combined and referred to as 'small' nodules and the last two were combined and referred to as 'large' nodules. The appearance of nodules was reported as a function of age, and the data were stratified in ranges of 2–< 4, 4–< 6, 6–< 9 and ≥ 9 years. Table 8 shows the incidence of mammary nodules by age in the treated and untreated groups. Treatment with medroxyprogesterone acetate increased the incidence of mammary nodules of all sizes in comparison with controls, and the tumour incidence increased with time, although treatment caused a significant increase even when given for less than four years.

**Table 8. Mammary nodules in bitches treated with medroxyprogesterone acetate (MPA)**

| Age (years) | Controls (% with nodules) | | MPA (% with nodules) | |
|---|---|---|---|---|
| | All sizes | 2–≥ 3 cm | All sizes | 2– ≥ 3 cm |
| 2–< 4 | 0 | 0 | 5 | 2 |
| 4–< 6 | 5 | 5 | 19[a] | 14[a] |
| 6–< 9 | 21 | 13 | 50[a] | 39[a] |
| ≥ 9 | 53 | 43 | 71[a] | 56[a] |

From van Os *et al*. (1981)
[a] Significantly greater than untreated controls by $\chi^2$ test

Beagle bitches, one to six years of age, were used to determine the effect of medroxyprogesterone acetate on mammary tumour development. In one study, the progestogen was given as a single intramuscular injection into alternate rear legs every three months at a dose of 2 or 10 mg/kg bw, measured at the start of the experiment. Half of the animals in each group received seven injections and were killed at 20–22 months; the other half received six injections and were then maintained for 19 months without further treatment. In the second study, the protocol was similar except that the amount of progestogen administered was based on body weight at the time of treatment. The doses injected were 0.2, 0.8 and 1.2 mg/kg bw, made by diluting Depo-Provera in vehicle. A total dose of 75 mg/kg bw was given at two to three intramuscular sites in the hind legs. Controls received the vehicle alone. The incidences of gross mammary gland nodules observed at necropsy in bitches treated with medroxyprogesterone acetate are shown in Table 9. The nodular lesions consisted of simple or complex lobular hyperplasia, simple adenomas, complex adenomas and benign mixed tumours; no malignant tumours were observed. In similar groups of bitches given 75 mg/kg bw medroxyprogesterone acetate, prior ovariectomy did not significantly affect the induced mammary gland enlargement or nodule development, and prior hypophysectomy did not affect the induced mammary gland enlargement but significantly reduced the incidence of nodules (Concannon *et al*., 1981).

Data were collected from eight veterinary practices around Amsterdam, the Netherlands, on 2031 bitches, comprising 576 with mammary tumours and 1455 control animals. Of the animals studied, 441 had been ovariectomized (most were ovariohysterectomized); 350 of these were controls. Medroxyprogesterone acetate was used in seven practices and proligestone in one [the data were not stratified for progestogen type]. Three groups were formed: animals in which tumours were diagnosed in 1976–79, animals in which tumours were diagnosed in 1980 and a control group formed in 1980. The groups were subdivided into age strata of 0–3, 4–5, 6–7, 8–9, 10–11 and 12 years and older. A com-

**Table 9. Mammary gland nodules in beagle bitches treated with medroxyprogesterone acetate (MPA)**

| Dose of MPA (mg/kg bw) | No. of animals | Bitches with nodules (%) | | |
|---|---|---|---|---|
| | | 5–9 mm | 10–14 mm | ≥ 15 mm |
| 0 | 24 | 25 | 4 | 0 |
| 1.2[a,b] | 6 | 7 | 0 | 0 |
| 2[b] | 6 | 0 | 0 | 0 |
| 10[b] | 7 | 57 | 14 | 14 |
| 75[a,c] | 12 | 92 | 58 | 75 |

From Concannon et al. (1981)
[a] Data from second study
[b] Killed at 20–22 months
[c] Combination of animals killed at 20–22 months and 24 months

parison of the two tumour groups with the controls showed that the progestogen-treated bitches had a somewhat greater risk for developing benign and malignant mammary tumours combined. The calculated relative risks for the most recent tumour group were 1.5 ($p < 0.05$) for regular progestogen treatment and 1.3 ($p < 0.05$) for irregular treatment. The proportions of malignant mammary tumours were similar after regular and irregular treatment; however, the author reported that progestogen treatment caused an earlier appearance of both benign and malignant mammary tumours (Misdorp, 1988, 1991).

Two groups of seven elderly beagle bitches weighing 10–15 kg (median ages, 7 and 6.8 years) that had not previously been treated with progestogens were subjected to surgical ovariohysterectomy to eliminate endogenous progesterone. Four to six weeks later, depot medroxyprogesterone acetate (10 mg/kg bw) or proligestone (50 mg/kg bw) was administered subcutaneously at three-week intervals for a total of eight injections. Four to eight weeks after the last injection, three dogs per group were killed for analysis of tissues, and the remaining four per group were maintained for six months without additional progestogen treatment. After this time, treatment was resumed at the same intervals, for a total of five more injections. The dogs were killed five to eight weeks later. Four dogs served as untreated controls; no abnormalities were found in any organ. The most frequent changes in the progestogen-treated dogs were adrenal atrophy (6/7 receiving medroxyprogesterone acetate and 7/7 receiving proligestone) and benign mammary tumours (5/7 receiving medroxyprogesterone acetate and 5/7 receiving proligestone). Some hepatic and pancreatic toxicity was also observed (Selman *et al*., 1995).

(*c*) *Cat*

Misdorp (1991) obtained data on 735 cats from the same veterinary practices as those from which data were obtained on dogs; 154 of the cats had mammary carcinomas, 35 had benign tumours, and 546 were used as controls. Medroxyprogesterone acetate was the commonest progestogen used, but some cats had been treated with megestrol acetate and some with proligestone. The type of progestogen was not taken into account in the analysis. Regular progestogen treatment was associated with a significantly increased relative risk (2.8; $p < 0.001$) for developing mammary carcinoma and a significantly increased risk (5.3; $p < 0.001$) for developing benign mammary tumours. Irregular treatment was not associated with an increased risk.

(*d*) *Monkey*

[The Working Group was aware of an unpublished study on female rhesus monkeys reviewed by Jordan (1994). In this 10-year study, treatment with doses 50 times the human contraceptive dose of depot medroxyprogesterone acetate increased the incidence of endometrial carcinomas, two tumours appearing in the treated monkeys and none in controls.]

3.1.2 *Administration with known carcinogens*

(*a*) *Mouse*

Two groups of 20 female BALB/c mice, two to three months of age, received medroxyprogesterone acetate as a subcutaneous implant of 40-mg Silastic pellets followed after four months with 20 mg, and a further group received pellets without medroxyprogesterone acetate. One week later, mice in one of the treated groups and the controls were injected intraperitoneally three times at monthly intervals with 50 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU). The mammary tumour incidences and latencies were increased in the group given the combined treatment (Table 10) after seven months, before mammary tumours induced by medroxyprogesterone acetate would have appeared. The differences in tumour incidence and latency between the groups receiving MNU and without the progestogen were significant ($p < 0.01$ and $p < 0.05$, respectively) (Pazos *et al*., 1991).

**Table 10. Mammary tumour incidence and latency in BALB/c mice treated with medroxyprogesterone acetate (MPA) followed by *N*-methyl-*N*-nitrosourea (MNU)**

| Treatment | Tumour incidence | | Latency (days) |
|---|---|---|---|
| | No. | % | |
| MPA + MNU | 15/19 | 79 | 154 ± 19 |
| MNU | 3/20 | 15 | 179 ± 7 |
| MPA | 0/20 | 0 | > 180 |

From Pazos *et al*. (1991)

Adult virgin female Swiss albino mice, eight to nine weeks of age, were given about 300 μg 3-methylcholanthrene intracervically in beeswax-impregnated threads. Medroxyprogesterone acetate was given intramuscularly at a dose of 50 μg/mouse every fifth day for 30, 60 or 90 days, with or without 3-methylcholanthrene, and mice were killed after 30, 60 and 90 days and observed for cervical lesions. The incidences of cervical invasive squamous-cell carcinomas in mice given the carcinogen plus medroxyprogesterone acetate were 0/30 after 30 days, 4/30 after 60 days and 22/38 after 90 days ($p < 0.05$). 3-Methylcholanthrene alone caused small increases in tumour incidence after 60 days (2/20) and 90 days (8/26) in comparison with the wax thread alone. Cervical dysplasia, but no cervical tumours, was observed in mice receiving medroxyprogesterone acetate alone (Hussain & Rao, 1991).

Groups of 35, 30 and 30 female ICR mice, 10 weeks of age, were treated with 10 mg/kg bw MNU after laparotomy by injection into the left uterine tube; the right uterine tube received saline. A group of 20 mice did not receive MNU. Two of the groups receiving MNU were fed a diet containing 5 ppm oestradiol and the other group and those not receiving MNU were fed basal diet. One group given both MNU and oestradiol and those not given MNU received subcutaneous injections of 2 mg/mouse medroxyprogesterone

acetate every four weeks from week 7 after MNU or no treatment. The duration of the experiment was 30 weeks. As shown in Table 11, adenocarcinomas and preneoplastic lesions developed in the uteri of mice in all groups treated with MNU. Medroxyprogesterone acetate significantly decreased the incidence of endometrial adenocarcinomas. In addition, while it caused a reduction in uterine weight, it had no effect on body weight. Medroxyprogesterone acetate alone did not induce either uterine or mammary tumours (Niwa *et al*., 1995).

**Table 11. Uterine tumour incidence in ICR mice treated with *N*-methyl-*N*-nitrosourea (MNU) followed by medroxyprogesterone acetate (MPA), with or without oestradiol**

| Treatment | Atypical hyperplasia | Adenocarcinoma |
|---|---|---|
| MNU + oestradiol + MPA | 4/30* | 2/30** |
| MNU + oestradiol | 16/24 | 8/24 |
| MNU alone | 7/26 | 3/26 |
| MPA alone | 0/20 | 0/20 |

From Niwa *et al*. (1993)
* Significantly less than with MNU plus oestradiol ($p < 0.001$)
**Significantly less than with MNU plus oestradiol ($p < 0.05$)

Four groups of 40 virgin female CD2$F_1$ (BALB/c × DBA/2) mice, six weeks of age, received six doses of 1 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) by gavage at 6, 9, 10, 11, 12 and 13 weeks; four doses of DMBA at 9, 10, 12 and 13 weeks; a subcutaneous implant of a 20-mg pellet of medroxyprogesterone acetate at six weeks plus DMBA at 9 and 10 weeks; or an implant of medroxyprogesterone acetate at six weeks plus DMBA at 9, 10, 12 and 13 weeks. A control group of 20 mice received a subcutaneous implant of medroxyprogesterone acetate at six weeks. The experiment was terminated at 56 weeks. The incidences of mammary adencarcinoma and the latencies are shown in Table 12 (Aldaz *et al*., 1996). Medroxyprogesterone acetate shortened the latency and enhanced the incidences of mammary adenocarcinomas. [The Working Group noted that it is not possible to assess whether medroxyprogesterone acetate alone produces mammary adenocarcinomas in this strain of mice, since the latency for mammary tumour induction in BALB/c mice by this compound alone is > 50 weeks (Lanari *et al*., 1986).]

Virgin female BALB/c mice, two months of age, were injected subcutaneously with 40 mg depot medroxyprogesterone acetate once or twice at three-month intervals with and without one dose of 50 mg/kg bw MNU administered either one week before or one week after the first injection of medroxyprogesterone acetate. The experiment was terminated at nine months to avoid detection of tumours induced by medroxyprogesterone acetate alone, which have a latency of 52 weeks (Lanari *et al*., 1986). No mammary tumours developed in 43 mice given MNU only or in the 22 given the progestogen only.

**Table 12. Incidence, number and latency of mammary tumours in CD2F$_1$ mice treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) with and without medroxyprogesterone acetate (MPA)**

| Treatment | No. of mice | Mammary adenocarcinoma incidence | Total no. of mammary tumours | Latency (days) |
|---|---|---|---|---|
| DMBA × 6 | 32 | 5/32 | 8 | 152 ± 75 |
| DMBA × 4 | 35 | 15/35 | 24 | 218 ± 72 |
| MPA + DMBA × 2 | 36 | 21/36 | 28 | 210 ± 65 |
| MPA + DMBA × 4 | 30 | 21/30 | 35 | 99 ± 51[a] |
| MPA | 20 | 0/20 | 0 | – |

From Aldaz *et al.* (1996)
[a] Significantly less than the other groups ($p < 0.0001$)

A significant increase in the incidence of lobular adenocarcinomas was observed in the groups treated with MNU plus two injections of medroxyprogesterone acetate. When the first of the two progestogen treatments preceded MNU by one week, the incidence was 16/44 with a latency of 223 ± 34 days; a total of 23 tumours developed. When the first of the two progestogen treatments followed MNU by one week, the incidence was 9/43 with a latency of 211 ± 38 days; a total of 10 tumours was observed. The difference in the number of tumours between these two groups was significant ($p < 0.01$), but the difference in tumour incidence was not. When medroxyprogesterone acetate was given once one week after MNU and then withdrawn two months later, the tumour incidence was significantly ($p < 0.01$) reduced to 3/42 (Pazos *et al.*, 1998).

(*b*) *Rat*

Groups of 75 female Sprague-Dawley rats, 45, 55, 65 and 75 days of age at the start of treatment, respectively, were further subdivided into three groups of 25 rats each: one control and the two others implanted with a 21-day time-release pellet that contained 0.5 or 5 mg medroxyprogesterone acetate. The low dose corresponded to doses of 3.1, 2.8, 2.7 and 2.5 mg/kg bw, respectively, estimated to be equivalent to the amount of hormone administered to women weighing 50–60 kg and receiving an injection of 140 mg Depo-Provera every 90 days. At the end of 21 days, the remains of the pellets were removed. After a further 21 days, 20 rats per group were treated with 8 mg/kg bw DMBA by gavage. Mammary tumour development was monitored twice a week, and all animals were killed after 24 weeks. DMBA induced mammary tumours, including adenocarcinomas, in both control and progestogen-treated rats. The results are summarized in Table 13. Susceptibility to DMBA-induced mammary carcinogenesis declined and the latency increased with increasing age at the start of treatment. The low dose of medroxyprogesterone acetate did not alter the probability of mammary tumour development in younger rats; however, both

**Table 13. Mammary tumour formation in Sprague-Dawley rats treated with medroxyprogesterone acetate (MPA) followed by 7,12-dimethylbenz-[*a*]anthracene (DMBA)**

| Treatment | No. of rats evaluated for tumours | Rats with tumours | | Rats with adenocarcinomas | | No. of tumours/ rat | Latency (days) |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | | |
| 45 days | | | | | | | |
| Control[a] | 12 | 9 | 75 | 5 | 42 | 1.8 | 82 |
| MPA, low dose | 11 | 9 | 82 | 5 | 46 | 1.6 | 115 |
| MPA, high dose | 8 | 7 | 88 | 4 | 50 | 1.8 | 116 |
| 55 days | | | | | | | |
| Control[a] | 15 | 7 | 47 | 6 | 40 | 1.3 | 121 |
| MPA, low dose | 18 | 5 | 28 | 1 | 6 | 0.5 | 73 |
| MPA, high dose | 17 | 12 | 71 | 6 | 35 | 2.9 | 41 |
| 65 days | | | | | | | |
| Control[a] | 12 | 6 | 50 | 5 | 42 | 1.6 | 110 |
| MPA, low dose | 15 | 7 | 47 | 4 | 27 | 0.6 | 60 |
| MPA, high dose | 16 | 10 | 63 | 6 | 38 | 1 | 90 |
| 75 days | | | | | | | |
| Control[a] | 18 | 8 | 44 | 4 | 22 | 1.2 | 177 |
| MPA, low dose | 16 | 10 | 63 | 7 | 44 | 1.9 | 95 |
| MPA, high dose | 16 | 11 | 69 | 7 | 44 | 2.3 | 120 |

From Russo *et al*. (1989a)

[a] Controls received 8 mg/kg bw DMBA and cholesterol pellets

the low and the high dose caused a twofold increase in the incidence of adenocarcinoma in older animals over that with DMBA alone (Russo *et al*., 1989a). [The Working Group noted that statistical analysis of the data did not allow an evaluation of the effects of medroxyprogesterone acetate on DMBA-induced mammary tumorigenesis.]

### 3.2 Levonorgestrel

*Rabbit*: One hundred and fourteen does, approximately 2.5 years old, were subjected to laparotomy and cross-sectional endomyometrial biopsy. Randomly selected rabbits then received a levonorgestrel-containing or an inert intrauterine implant in the right uterine horn. The implants consisted of a $0.3 \times 2$ cm core of either polydimethylsiloxane or 50% polydimethylsiloxane and 50% levonorgestrel. The rabbits then underwent a second cross-sectional endomometrial biopsy at six, 12 and 24 months. Of the 55 rabbits that received levonorgestrel and the 53 rabbits that received inert implants, 29 given levonorgestrel and 33 given inert implants survived 24 months. After 24 months, the incidence of endometrial carcinomas in rabbits receiving levonorgestrel (17%) was significantly lower ($p < 0.05$)

than that developing spontaneously in rabbits receiving the inert implant (42%) (Nisker *et al*., 1988).

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

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

### 4.1 Absorption, distribution, metabolism and excretion

#### 4.1.1 *Medroxyprogesterone acetate*

(*a*) *Humans*

Three women received intramuscular injections of 150 mg medroxyprogesterone acetate, and blood was obtained several times on the first day after injection, then daily for two weeks, then less frequently. The serum concentration of medroxyprogesterone acetate was measured by a sensitive radioimmunoassay. The concentrations rose rapidly after injection, reaching 0.26–0.47 ng/mL within 0.5 h and increasing to 0.97–2.66 ng/mL by 24 h; the concentrations remained in the range of 1.0–1.5 ng/mL for the first two to three months (Ortiz *et al*., 1977).

Depot medroxyprogesterone acetate was administered intramuscularly to four groups of five healthy ovulating women at a dose of 25, 50, 100 or 150 mg. Medroxyprogesterone acetate was measured by radioimmunoassay in serum samples obtained periodically over the course of six months. All four doses initially produced serum concentrations that were above the sensitivity limit of the assay, ranging from 0.1 to 0.3 ng/mL. The concentration decreased with time, and the two lower doses reached the sensitivity limit more rapidly than the higher doses (Bassol *et al*., 1984).

Medroxyprogesterone acetate was administered orally at a daily dose of 5 or 10 mg to groups of five women, and blood samples were obtained several times on the first day of treatment and daily 12 h after intake of the tablets. The serum concentrations were measured by radioimmunoassay. The concentrations rose rapidly within 1–3 h to peak values of 1.2–5.3 nmol/L [0.46–2.05 ng/mL] after the first 5-mg dose and to about 4.2–6.7 nmol/L [1.62–0.84 ng/mL] after the 10-mg dose (Wikström *et al*., 1984).

Twenty women were given 150 mg medroxyprogesterone acetate intramuscularly every 90 days for 12 months. They were subjected to an oral glucose tolerance test before

and 3, 6 and 12 months after the start of treatment, and fasting and post-oral glucose load (2-h) measurements were made of glucose, insulin, growth hormone, glucagon, pyruvate and cortisol. Significant increases in mean blood glucose, blood pyruvate, serum insulin, growth hormone and serum glucagon concentrations were seen after three months, which progressed to their highest concentrations at 12 months (Fahmy *et al*., 1991).

Groups of 22 women recruited in roughly equal proportions from medical centres in Hungary, Mexico and Thailand were treated with 12.5 or 25 mg medroxyprogesterone acetate by intramuscular injection at 28-day intervals for three consecutive months. Blood samples were obtained for measurement of serum medroxyprogesterone acetate, oestradiol and progesterone before treatment, three times per week after treatment and for two months after the end of the last treatment. Ovulation was inhibited in all women. Restoration of ovulation after cessation of treatment occurred more slowly in the group given the high dose. The pharmacokinetic profiles differed between the three medical centres: dose-dependent differences in the serum concentration of medroxyprogesterone acetate were observed in the Thai women but not in Mexican women (Garza-Flores *et al*., 1987).

(*b*) *Experimental systems*

No data were available to the Working Group.

4.1.2 *Levonorgestrel* (see also the monograph on 'Oral contraceptives, combined', section 4.1.7)

(*a*) *Humans*

Ball *et al*. (1991) reported on 16 women who were treated with 30 μg/day levonorgestrel for six months. At the end of the treatment period, plasma cholesterol, lipoprotein, triglyceride and glucose concentrations, and fibrinogen, plasminogen, factor VII, factor X and antithrombin III activities were compared with pre-treatment values and with those of a group of 23 women treated with 350 μg/day norethisterone for six months. There were no significant differences between the two groups.

A group of 47 healthy women received subdermal implants in the arm of Norplant®, from which levonorgestrel alone is released at a rate of about 30 μg/day. The women were compared with two groups given combined oral contraceptives: 25 received 1 mg norethisterone plus 50 μg mestranol per day for 21 days of a 28-day cycle, and 30 women received 150 μg levonorgestrel plus 30 μg ethinyloestradiol in a similar regimen. Blood samples were taken at admission and after one, three and six months. Coagulation parameters were measured, including platelet count, prothrombin time, thrombin time, partial thromboplastin time with kaolin, clotting factors I, II, V, VII–XIII, plasminogen, antithrombin III, $\alpha_1$-antitrypsin, $\alpha_2$-macroglobulin and fibrinogen degradation products. In contrast to the group receiving combined oral contraceptives, the Norplant® users had little alteration in coagulation parameters; the only significant changes were an increase in factor VII and a decrease in antithrombin III six months after implantation (Shaaban *et al*., 1984).

(*b*) *Experimental systems*

See the monograph on 'Oral contraceptives, combined'.

4.1.3 *Norethisterone* (see also the monograph on 'Oral contraceptives, combined', section 4.1.9)

(*a*) *Humans*

In the study of Ball *et al*. (1991) described in section 4.1.2, 23 women were treated with 350 μg/day norethisterone for six months. No significant differences in the end-points measured were seen in comparison with pre-treatment values or with those of 16 women treated with 30 μg/day levonorgestrel for six months.

In the study of Fahmy *et al*. (1991) described in section 4.1.1, 20 women were treated with 200 mg norethisterone oenanthate intramuscularly every 60 days for six months, then with 200 mg every 84 days for another six months. Significant increases in mean blood glucose, pyruvate, serum insulin, growth hormone and glucagon concentrations were seen after three months, which reached a peak at six months. The concentrations reverted to normal at 12 months after the frequency of treatments was reduced.

(*b*) *Experimental systems*

Three adult female baboons were injected intramuscularly with conventional biodegradable microspheres containing 75 mg norethisterone which was released continuously, while three other baboons received 75 mg norethisterone in encapsulated microspheres. The animals with the encapsulated microspheres showed two peaks of the blood concentration of norethisterone, while those given conventional microspheres showed a single peak. In addition, norethisterone was released for about 40–50 days longer from the encapsulated microspheres (Cong & Beck, 1991).

## 4.2 Receptor-mediated effects

4.2.1 *Medroxyprogesterone acetate*

(*a*) *Humans*

Zalanyi *et al*. (1986) gave groups of women 5 or 10 mg medroxyprogesterone acetate orally on days 7–10 of the menstrual cycle and took endometrial biopsy samples on the 11th day before treatment and on the 11th day after the last dose of medroxyprogesterone acetate. Medroxyprogesterone acetate reduced the numbers of glandular and stromal mitoses, reduced the epithelial height, increased glandular diameter and increased the numbers of vacuolated cells in the endometrium.

Tiltman (1985) examined archived specimens from hysterectomies and determined the number of mitotic figures in uterine fibromyomas from 61 women who had received unknown oral or subcutaneous doses of medroxyprogesterone acetate and 71 women who had not received any hormonal treatment. The mitotic activity was significantly higher in fibromyomas from the progestogen-exposed women than in the control samples or in 63 samples from women treated with a combined oestrogen–progestogen oral contraceptive.

(*b*) *Experimental systems*

Medroxyprogesterone acetate bound with high affinity to the progesterone receptor of human endometrium (Briggs, 1975), human MCF-7 breast cancer cells (Schoonen *et al*., 1995a) and canine uterus (Selman *et al*., 1996), its relative binding affinity exceeding that of progesterone by 2.5-fold in uterine tissue (Shapiro *et al*., 1978; Selman *et al*., 1996) and 10-fold in MCF-7 cells (Schoonen *et al*., 1995a). Medroxyprogesterone acetate down-regulated the mRNA and protein expression of both progesterone receptor-A and -B isoforms in primary cultures of isolated human endometrial epithelial cells, but surprisingly up-regulated these two receptor isoforms in human endometrial stromal cells, an effect that was inhibited by the anti-progestogen RU486 (Tseng & Zhu, 1997).

Medroxyprogesterone acetate had clear progestational activity *in vivo*, as measured by inhibition of ovulation and endometrial stimulation in rabbits, indicating that its activity is similar to that of progesterone (Phillips *et al*., 1987).

Progestogen-specific stimulation of alkaline phosphatase activity in T47D human breast cancer cells indicated that medroxyprogesterone acetate has agonist activity, which was equal to that of progesterone (Markiewicz & Gurpide, 1994). It was eightfold more potent than progesterone in increasing glycogen levels in human endometrial explant cultures (Shapiro *et al*., 1978).

Medroxyprogesterone acetate bound with much lower affinity than the natural ligand to the oestrogen receptor in whole rat uterine homogenate (van Kordelaar *et al*., 1975); no binding occurred in MCF-7 human breast cancer cells (Schoonen *et al*., 1995a). It had no oestrogenic activity at concentrations of $10^{-7}$–$10^{-6}$ mol/L, as demonstrated by oestrogen-stimulated alkaline phosphatase activity in Ishikawa-Var I human endometrial cancer cells, which is an oestrogen-specific response inhibited by 4-hydroxytamoxifen (Markiewicz *et al*., 1992; Markiewicz & Gurpide, 1994; Botella *et al*., 1995); however, the binding of oestradiol to rat uterine cytoplasmic oestrogen receptor was reduced by medroxyprogesterone acetate both *in vivo* and *in vitro* (Di Carlo *et al*., 1983). Medroxyprogesterone acetate also slightly reduced the hyperplastic response in the endometrium of oestrogen-primed ovariectomized rats treated with conjugated equine oestrogen; tamoxifen did not have a similar effect (Kumasaka *et al*., 1994). In addition, medroxyprogesterone acetate inhibited the up-regulation of mRNA expression of fibroblast growth factors-1 and -2 by oestradiol in Ishikawa human endometrial cancer cells; the effect was similar to that of the anti-oestrogen tamoxifen (Fujimoto *et al*., 1997).

Medroxyprogesterone acetate did not affect the growth of most oestrogen-sensitive human mammary cancer cell lines tested, at concentrations of $10^{-8}$–$10^{-6}$ mol/L (Jeng & Jordan, 1991; Jeng *et al*., 1992; Catherino & Jordan, 1995; Schoonen *et al*., 1995a,b). It stimulated cell proliferation only in two human breast cancer cell sub-lines (MCF-7 sub-line M and T47D sub-line A) at concentrations of $10^{-6}$ mol/L and $10^{-8}$–$10^{-6}$ mol/L, respectively (Schoonen *et al*., 1995a,b). Cappelletti *et al*. (1995) also found stimulation of proliferation of an MCF-7 line by medroxyprogesterone acetate at concentrations of $10^{-7}$–$10^{-6}$ mol/L. The latter effects were not changed by addition of tamoxifen or RU486, but both anti-progestogens and anti-oestrogens by themselves strongly counteracted oestradiol-

stimulated cell proliferation in T47D cells (Schoonen *et al*., 1995a,b). All of these experiments were performed with breast cancer cell lines grown in phenol red-free medium which contained steroid-free (dextran-coated charcoal-stripped) fetal bovine serum (Jeng *et al*., 1992; Cappelletti *et al*., 1995; Schoonen *et al*., 1995a,b). Sutherland *et al*. (1988) found a high degree of variability in the inhibitory effects of medroxyprogesterone acetate on various human breast cancer cell lines, with about 50% inhibition at concentrations of $10^{-10}$ mol/L in T47D cells and $10^{-6}$ mol/L in MCF-7 cells and no effect in ZR75-1 cells; these studies were performed in the presence of phenol red and serum. Musgrove *et al*. (1991) reported that progestogens, including medroxyprogesterone acetate at $10^{-9}$ mol/L, could both stimulate and inhibit the cell cycle progression of the same human breast cancer cell line; they demonstrated an initial growth acceleration, increasing the number of cells in S-phase, followed later by growth inhibition due to $G_1$ arrest. The discrepancies in the response of different breast cancer cell sub-lines to medroxyprogesterone acetate may be related to differences in the time course of the biphasic effect of progestogens on their growth.

Medroxyprogesterone acetate did not *trans*-activate oestradiol-responsive reporter constructs containing oestrogen response elements in oestrogen receptor-positive cells (Jeng *et al*., 1992; Catherino & Jordan, 1995), and did not alter the mRNA expression of transforming growth factors (TGF)-β1, -β2 and -β3 (Jeng & Jordan, 1991).

Oestradiol at concentrations of $10^{-10}$–$10^{-8}$ mol/L strongly induced the growth of MCF-7 and T47D cell lines, regardless of the sub-line used (Cappelletti *et al*., 1995; Schoonen *et al*., 1995a,b). Medroxyprogesterone acetate did not affect the growth stimulation of MCF-7 sub-lines L and M or T47D sub-line A by oestrogen (at $10^{-10}$ mol/L), but it inhibited stimulation of the growth of sub-line B MCF-7 cells and sub-line S T47D cells in a dose-dependent fashion at concentrations of $10^{-11}$–$10^{-6}$ mol/L and $10^{-10}$–$10^{-6}$ mol/L, respectively. These inhibitory effects at $10^{-8}$ mol/L were not blocked by the anti-progestogen RU486 at a concentration of $10^{-6}$ mol/L (Schoonen *et al*., 1995a,b). Cappelletti *et al*. (1995), Botella *et al*. (1994) and Sutherland *et al*. (1988) also reported inhibition of oestradiol stimulation of growth of MCF-7 and T47D cell sub-lines by medroxyprogesterone acetate at concentrations of $10^{-8}$–$10^{-6}$ mol/L. Cappelletti *et al*. (1995) found that medroxyprogesterone acetate inhibited stimulation of MCF-7 breast cancer cell growth by TGF-α, but not by insulin-like growth factor-I and -II.

Medroxyprogesterone acetate increased the reductive activity of 17β-hydroxysteroid dehydrogenase in an oestrogen- and progestogen-stimulated MCF-7 cell line in phenol red-free medium (Coldham & James, 1990), indicating a possible mechanism for its stimulating effects on the growth of breast cancer cells *in vivo*, by increasing the formation of oestradiol. Medroxyprogesterone acetate also inhibited the activity of microsomal oestrone sulfatase in human breast carcinoma tissue, however, suggesting that it could reduce the intracellular formation of biologically active oestrogen in human breast cancer cells via the sulfatase pathway (Prost-Avallet *et al*., 1991).

Administration of medroxyprogesterone acetate to 50-day-old virgin female Sprague-Dawley rats at a dose of 0.5 or 5 mg/rat per day for 21 days reduced the tritiated thymi-

dine labelling index (an indicator of cell proliferation) in the terminal ducts and alveolar buds, but not in the terminal end-buds (Russo & Russo, 1991). This effect protected against the induction of mammary cancer by DMBA in a similar study with a norethynodrel–mestranol combination (Russo *et al*., 1989b).

Subcutaneous administration of medroxyprogesterone acetate at doses of 1–1.5 mg/rat twice daily for 18 days inhibited stimulation by oestrone (1 μg/rat subcutaneously twice daily) of the growth of mammary gland carcinomas induced by DMBA in female Sprague-Dawley rats which were ovariectomized after tumours had developed; the effect of medroxyprogesterone acetate and the tumour growth inhibition caused by treatment with the anti-oestrogens EM-219 and EM-800 were additive (Li *et al*., 1995; Luo *et al*., 1997). Uterine weight was increased by medroxyprogesterone acetate in ovariectomized animals, while adrenal weights were decreased; the anti-oestrogens EM-219 and EM-800 did not have similar effects and did not alter the effects of the medroxyprogesterone acetate. The reductive activity of 17β-hydroxysteroid dehydrogenase in mammary tumour tissue was altered by medroxyprogesterone acetate in such a way that the formation of oestradiol in tumours of the ovariectomized oestrone-treated animals was reduced by more than 50%, while anti-oestrogens had no significant effect. In the uterus, medroxyprogesterone acetate caused 48% inhibition of the stimulatory effect of oestrone on 17β-hydroxysteroid dehydrogenase activity in the ovariectomized animals, while the anti-oestrogens reduced this enzymic activity to the levels found in ovariectomized animals.

Detectable but variable levels of either oestrogen or progesterone receptors were found in four of seven mammary adenocarcinomas induced by medroxyprogesterone acetate in BALB/c mice, while only three tumours contained both receptor types (Molinolo *et al*., 1987).

Medroxyprogesterone acetate bound to the glucocorticoid receptor in canine liver cytosol (Selman *et al*., 1996) and human mononuclear leukocytes and induced glucocorticoid-like effects in these cells, including reduced proliferative responses to mitogenic stimuli (Kontula *et al*., 1983).

In studies with ovariohysterectomized bitches, administration of depot medroxyprogesterone acetate at three-week intervals for a total of eight subcutaneous injections of 10 mg/kg bw increased the concentrations of circulating growth hormones. This effect was reversed within 2 h after surgical removal of all mammary tissue, which contained the highest levels of growth hormone; there was also a distinct arterio-venous gradient of growth hormone across the mammary glands. This study provides evidence for local production of growth hormone in the canine mammary gland in response to medroxyprogesterone acetate treatment (Selman *et al*., 1994). Further evidence for local production came from the demonstration by reverse transcriptase polymerase chain reaction (Mol *et al*., 1995a,b) of the induction of growth hormone mRNA in canine, feline and human tumours. As growth hormone has been shown to stimulate human breast cancer cells (Biswas & Vonderhaar, 1987; Bonneterre *et al*., 1990), the induction of mammary growth hormone production may be a major mechanism for the development of proliferative lesions in canine and perhaps human mammary gland (Mol *et al*., 1996). It should be noted, however,

that medroxyprogesterone acetate did not increase circulating growth hormone levels in men and women given a dose of 150 mg per day for three weeks to six months (Dhall *et al*., 1977; Meyer *et al*., 1977).

Medroxyprogesterone acetate stimulated the growth of androgen-sensitive mouse mammary carcinoma Shionogi cells with a reduction in the doubling time of approximately 75% at a concentration of $10^{-6}$ mol/L. This effect could be counteracted by blocking the androgen receptor with $5 \times 10^{-6}$ mol/L of the anti-androgen hydroxyflutamide, which itself did not stimulate the growth of these cells (Luthy *et al*., 1988). Consistent with these observations, medroxyprogesterone acetate weakly bound to the rat ventral prostate androgen receptor (Botella *et al*., 1987); however, it has been shown to be a strong competitor for binding of 5α-dihydrotestosterone to the androgen receptor in human foreskin fibroblasts, with activity similar to that of testosterone (Breiner *et al*., 1986). Medroxyprogesterone acetate inhibited the growth of an oestrogen and progesterone receptor-negative human breast cancer cell line (MFM-223), the growth of which is inhibited by androgens (Hackenberg & Schulz, 1996).

Subcutaneous injection of medroxyprogesterone acetate to castrated male rats at a dose of 0.15 mg/rat twice daily for 14 days increased the ventral prostate weight by about 50% and stimulated the activity of the cell proliferation-related enzyme ornithine decarboxylase in the ventral prostate by almost 20-fold. Effects of similar magnitude were found with a dose of 0.15 mg 5α-dihydrotestosterone twice daily. No evidence for any anti-androgenic activity of medroxyprogesterone acetate was detected in these studies (Labrie *et al*., 1987). Phillips *et al*. (1987), however, found no androgenic activity of medroxyprogesterone acetate in immature, castrated rats. The compound also increased the activity of 5α-reductase and decreased the activity of hepatic 3α- and 3β-hydroxysteroid dehydrogenase in male and female rats, which could lead to increased circulating levels of 5α-reduced androgens. These effects were blocked by flutamide or oestradiol, suggesting that androgen receptor mediation was involved (Lax *et al*., 1984).

In dogs, medroxyprogesterone acetate induced cystic endometrial hyperplasia when administered subcutaneously at 10 mg/kg bw 5–13 times at intervals of three weeks. Although the presence of growth hormone was demonstrated in glandular epithelial cells by immunohistochemistry, no evidence could be found for local production in this tissue (Kooistra *et al*. 1997), in contrast to the canine mammary gland (Mol *et al*., 1995a). A role for the elevated levels of circulating growth hormone in medroxyprogesterone acetate-induced canine endometrial hyperplasia has not been determined.

In cultured human endometrial stromal cells, medroxyprogesterone acetate and progesterone were equally effective in markedly stimulating protein and mRNA expression of insulin-like growth factor binding protein-2. This response was inhibited by RU486 (Giudice *et al*., 1991).

Medroxyprogesterone acetate and progesterone increased secretion of vascular endothelial growth factor by the human breast cancer cell line T47D to a similar extent (three- to fourfold over basal levels). This effect, which was progestogen-specific and did not

occur in MCF-7, ZR-75 or MDA-MB-231 cells, suggests an angiogenic response of this cell line to medroxyprogesterone acetate (Hyder *et al*., 1998).

Treatment of isolated primary normal human endometrial cells with medroxyprogesterone acetate, oestradiol or their combination *in vitro* increased mRNA expression of vascular endothelial growth factor in the cells by 3.1-, 2.8- and 4.7-fold, respectively, over control values (Shifren *et al*., 1996). Intramuscular injection of medroxyprogesterone acetate at 2 mg/mouse one to three times at weekly intervals did not alter the expression of vascular endothelial growth factor in the tumour tissue of oestradiol-treated ovariectomized nude mice carrying a human endometrial carcinoma xenograft line (Kim *et al*., 1996).

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

Medroxyprogesterone acetate at concentrations of 0.5–5.0 ng/mL did not affect the growth of decidual endothelial cells derived from human endometrium, which was stimulated by exposure to 5 ng/mL oestradiol (Peek *et al*., 1995).

Using migration and invasion assays which involve cell growth along a fibronectin gradient, Ueda *et al*. (1996) demonstrated the inhibitory activity of $10^{-7}$–$10^{-5}$ mol/L medroxyprogesterone acetate on endometrial adenocarcinoma SNG-M cells in both systems. At these concentrations, medroxyprogesterone acetate did not affect the growth of these cells but inhibited cell locomotion, as determined in a monolayer wounding model *in vitro*. The secretion by these cells of matrix metalloproteinases and stromelysin was not affected. Fujimoto *et al*. (1996a,b) demonstrated, however, that medroxyprogesterone acetate does not affect the migration of human endometrial cancer-derived cells (Ishikawa, HEC-1 or HHUA cell lines) through an artificial basement membrane or their expression of cell adhesion-related molecules such as E-cadherin and α- and β-catenins. Oestradiol increased the migration of these cells and their expression of the cell adhesion-related molecules.

Medroxyprogesterone acetate given to female Wistar rats at an oral dose of 15 mg/kg per day for seven days increased the liver weight and increased oxidation of aminopyrine and ethyl morphine but had no significant effect on the liver DNA content (Schulte-Hermann *et al*., 1988). This perhaps reflects the hepatic enzyme-inducing activity of medroxyprogesterone acetate (Lax *et al*., 1984).

### 4.2.2 *Levonorgestrel*

#### (*a*) *Humans*

Anderson *et al*. (1989) obtained breast biopsy samples from 347 pre-menopausal women and determined the tritiated thymidine labelling index for epithelial cells. The 14 women in this group who used progestogen-only contraceptives [not specified] had a mean labelling index of 1.55% (95% CI, 0.87–2.75), whereas the labelling index in 83 unexposed women was 0.66% (95% CI, 0.52–0.85). This study indicates that progestogen-only contraceptive use is associated with increased epithelial breast cell proliferation.

The mRNA expression of progesterone receptor in endometrial biopsy samples from 39 women using Norplant® (subcutaneous implants of levonorgestrel) was examined by in-situ hybridization and compared with that in 53 unexposed women (Lau *et al*., 1996a). Exposure to levonorgestrel resulted in a signal intensity in endometrial glands that was comparable with that observed in control women during the menstrual and early proliferative phase, which was lower than that found during the early to mid-proliferative and secretory phases. The expression of progesterone receptor in endometrial stromal cells of levonorgestrel-exposed women was reduced by approximately 20–25% as compared with control tissue. The expression of cathepsin D, an indirect marker of the functional status of progesterone receptors, was examined in 46 women using Norplant® and 45 unexposed women (Lau *et al*., 1996b). No differences were detected between these two groups, and no differences were found between phases of the menstrual cycle.

The effects of levonorgestrel administered from an intrauterine device on the expression of insulin growth factor (IGF)-I and IGF-II and those of IGF-binding protein I in the human endometrium were examined by Pekonen *et al*. (1992) and Rutanen *et al*. (1997). Endometrial tissue was obtained from surgical hysterectomy specimens and uterine biopsies taken from women who had carried intrauterine devices releasing levonorgestrel at a rate of 20 μg/day for 6–36 months ($n$ = 60) (Rutanen *et al*., 1997) or four months to seven years ($n$ = 11) (Pekonen *et al*., 1992). Control tissue was taken from 49 women carrying copper-releasing intrauterine devices (Pekonen *et al*., 1992) or 13 untreated women (Rutanen *et al*., 1997). Levonorgestrel induced expression of endometrial IGF-binding protein I (detected by immunohistochemistry and western blot) in 58/60 women, whereas none of 49 control women had detectable expression of this protein (Pekonen *et al*., 1992). This finding was confirmed at the mRNA level by northern hybridization and reverse transcriptase polymerase chain reaction (Rutanen *et al*., 1997); no expression occurred in either normal proliferative or secretory-phase endometrium, except for a very low level in late secretory-phase endometrium. IGF-I and IGF-II transcripts were found in all endometria, but expression was markedly higher for IGF-I in proliferative-phase endometrium and for IGF-II in endometrium from levonorgestrel-exposed women. IGF-binding protein-I was also expressed consistently in the latter group. Pekonen *et al*. (1992) also studied a group of six women with subcutaneous Norplant® capsules releasing 30–70 μg/day levonorgestrel. This treatment, in contrast to the effect of local progestogen, induced IGF-binding protein-I expression in the endometrium of only one of the women. None of the treatments resulted in increased serum concentrations of IGF-binding protein-I.

Using a cell migration assay for endothelial cells taken from endometrial biopsy samples, Subakir *et al*. (1995, 1996) showed that levonorgestrel reduced the mobility of these cells. Migration of human umbilical vein endothelial cells towards endometrial explants occurred in only 16/46 (35%) explant samples taken from women using Norplant® but in 22/30 (73%) explant samples from unexposed women. Furthermore, the median migratory scores were higher in the latter group.

(*b*) *Experimental systems*

See the monograph on 'Oral contraceptives, combined', section 4.2.9.

## 4.3 Genetic and related effects

4.3.1 *Humans*

See the monograph on 'Oral contraceptives, combined', section 4.3.1.

4.3.2 *Experimental systems*

Progesterone did not induce DNA repair in female rat liver cells *in vitro*. It induced cell transformation in Syrian hamster embryo cells *in vitro* at a dose that did not induce chromosomal aberrations. Progesterone also induced cell transformation in baby rat kidney cells infected with human papillomavirus-16 carrying the Ha-*ras*-1 oncogene (Table 14).

See also the monograph on 'Oral contraceptives, combined', section 4.3.2.

## 4.4 Reproductive and prenatal effects

4.4.1 *Medroxyprogesterone acetate*

(*a*) *Humans*

When used as a contraceptive agent, medroxyprogesterone acetate at intramuscular doses of 100–150 mg reaches serum concentrations of 0.1–1 ng/mL, which inhibit ovulation for several months (Ortiz *et al*., 1977; Bassol *et al*., 1984). Oral administration of 5–10 mg per day results in serum concentrations of 0.4–1.7 nmol/L [0.125–0.531 ng/mL] 12 h after dosing; this also inhibits ovulation but less reliably (Wikström *et al*., 1984). The steroid hormone profiles in early pregnancy are not affected by large doses of medroxyprogesterone given for the treatment of threatened abortion, although increased plasma concentrations of progesterone and decreased plasma concentrations of oestrogen were observed after the 20th week of gestation (Willcox *et al*., 1985; Yovich *et al*., 1985).

A number of early studies reported associations between the use of medroxyprogesterone acetate during pregnancy and the induction of a variety of congenital malformations in offspring (reviewed by Schardein, 1980). It was concluded that the evidence for malformations, such as cardiac, limb or central nervous system defects, was unconvincing. In a well-controlled study of 1608 infants born to women treated for genital bleeding during the first trimester of pregnancy with medroxyprogesterone acetate or other progestogens and 1147 infants born to women who had no treatment, the prevalence of congenital malformations, including genital malformations, was similar in the two groups (Katz *et al*., 1985). A group of 449 subfertile pregnant women with high rates of recurrent abortion or who suffered a threatened abortion were treated with medroxyprogesterone (80–120 mg per day orally) from week 5 after the last menstrual period until at least the 18th week of pregnancy and were compared with a matched group of 464 women from the same clinic who were untreated. No difference was found in the prevalence of congenital malformations between the two groups: there were 15/366 (4.1%) infants with malformations in the treated group and 15/428 (3.5%) in the control group. In particular,

**Table 14. Genetic and related effects of progesterone**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98, reverse mutation | – | NT | 40000 µg/plate | Ansari *et al*. (1982) |
| *Salmonella typhimurium* TA100, TA98, reverse mutation | – | – | 500 µg/plate | Bokkenheuser *et al*. (1983) |
| *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, TA98, reverse mutation | – | – | 333 µg/plate | Dunkel *et al*. (1984) |
| *Escherichia coli* WP2 *uvrA*, reverse mutation | – | – | 333 µg/plate | Dunkel *et al*. (1984) |
| DNA strand breaks, cross-links and related damage, Chinese hamster V79 cells *in vitro* | – | – | 94.5 | Swenberg (1981) |
| DNA repair exclusive of unscheduled DNA synthesis, female rat hepatocytes *in vitro* | – | NT | 15.7 | Neumann *et al*. (1992) |
| Chromosomal aberrations, Chinese hamster ovary cells *in vitro* | – | – | 480 | Ishidate (1983) |
| Chromosomal aberrations, Syrian hamster embryo cells *in vitro* | – | NT | 30 | Tsutsui *et al*. (1995) |
| Cell transformation, BALB/3T3 mouse cells | (+) | NT | 0.08 | Dunkel *et al*. (1981) |
| Cell transformation, Syrian hamster embryo cells, clonal assay | – | NT | 50 | Dunkel *et al*. (1981) |
| Cell transformation, Syrian hamster embryo cells, clonal assay | + | NT | 30 | Tsutsui *et al*. (1995) |
| Cell transformation, RLV/Fischer rat embryo cells | + | NT | 2.6 | Dunkel *et al*. (1981) |
| Cell transformation, primary baby rat kidney + HPV16 + H-*ras* | + | NT | 0.31 | Pater *et al*. (1990) |
| Sister chromatid exchange, HE2144 human fibroblasts *in vitro* | – | NT | 15.7 | Sasaki *et al*. (1980) |
| Chromosomal aberrations, human lymphocytes *in vitro* | – | NT | 100 | Stenchever *et al*. (1969) |
| Chromosomal aberrations, HE2144 human fibroblasts *in vitro* | – | NT | 31.4 | Sasaki *et al*. (1980) |
| Dominant lethal mutation, mice *in vivo* | – | | 167 ip × 1 | Epstein *et al*. (1972) |
| Sperm morphology, mice *in vivo* | – | | 500 ip × 5 | Topham (1980) |

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

[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; ip, intraperitroneal

there was no suggestion of an increase in the incidence of cardiac or limb defects (Yovich *et al*., 1988).

Long-term follow-up studies have been reported on more than 2000 people exposed to medroxyprogesterone acetate prenatally; most have shown no treatment-related effects on health or development (Jaffe *et al*., 1990; Gray & Pardthaisong, 1991a; Pardthaisong & Gray, 1991; Pardthaisong *et al*., 1992). In a large study in Thailand of 1431 children of mothers who had used depot medroxyprogesterone acetate as a contraceptive (Pardthaisong & Gray, 1991), a small but significant increase in the prevalence of low-birth-weight infants was found, accompanied by an increase in perinatal and infant mortality (Gray & Pardthaisong, 1991a). The treated and control groups in this study were not well matched, as the women taking medroxyprogesterone acetate had a higher incidence of pregnancy risk factors, and the conclusions of the study have been debated (Gray & Pardthaisong, 1991b; Hogue, 1991).

As treatment of men with medroxyprogesterone acetate can reduce their testosterone levels and sperm counts, it has been tested as a male contraceptive. Testosterone must be given at the same time to counter the decreased testosterone effects (Melo & Coutinho, 1977; Soufir *et al*., 1983). In a study of 25 healthy men, who had each fathered at least two children, monthly injections of 100 mg medroxyprogesterone acetate and 250 mg testosterone oenanthate were given for 4–16 months. In 24 of the men, a marked drop in sperm count was observed one to three months after the first injection. By nine months, 11/14 men were azoospermic or had marked oligospermia (< 1 million sperm per millilitre). One subject was unresponsive, but no reason could be found (Melo & Coutinho, 1977).

Six men were given a daily oral dose of 20 mg medroxyprogesterone acetate in combination with 50 or 100 mg testosterone for one year. From the third month, the sperm count was $< 10^6$/mL. The sperm count returned to normal levels ($> 20 \times 10^6$/mL) three to six months after cessation of treatment (Soufir *et al*., 1983).

(*b*) *Experimental systems*

Groups of 8–12 male Sprague-Dawley Crl:CD(SD)Br rats were castrated and injected immediately thereafter twice daily for 14 days with one of a number of synthetic progestogens, including medroxyprogesterone acetate, used in the treatment of prostate cancer. Controls were injected with the vehicle, 1% gelatine, in 0.9% saline. Dihydrotestosterone was injected at a dose of 150 µg twice daily for 14 days as a positive control. All of the animals were killed on the morning after the last day of treatment, and the ventral prostate and adrenals were removed and weighed; furthermore, the prostatic content of ornithine decarboxylase was measured, as it is considered to be a highly specific, sensitive marker of androgenic activity in the prostate. Dihydrotestosterone increased the ventral prostate weight to 43% above that of castrated controls. Medroxyprogesterone acetate was equipotent with dihydrotestosterone and caused significant increases in prostate weight, by about 49% at 150 µg and 162% at a dose of 500 µg per injection. Whereas dihydrotestosterone caused a 14-fold increase in ornithine decarboxylase activity in the prostate,

medroxyprogesterone acetate caused a 20-fold increase at the same dose. Medroxyprogesterone acetate thus has very powerful androgenic activity in the rat ventral prostate, equal to that of the potent natural androgen dihydrotestosterone (Labrie *et al*., 1987).

Pregnant Wistar rats were given 1 or 5 mg medroxyprogesterone acetate orally for four days on days 17–20 of pregnancy, and the fetuses were removed on day 21. After fixation, histological sections of the pelvic region were examined and the urovaginal septum length measured. Very marked masculinization of female fetuses was detected, as evidenced by decreased development of the urogenital septum at both doses (Kawashima *et al*., 1977).

In a study in which Silastic intrauterine devices containing medroxyprogesterone acetate were implanted between fetal implantation sites on day 9 in groups of 16 pregnant Wistar rats, masculinization of female fetuses and feminization of males occurred, as judged from changes in anogenital distance and the morphology of the genital papilla (Barlow & Knight, 1983).

Anti-androgenic effects have also been reported. Groups of 10 albino Wistar mice were treated subcutaneously with vehicle alone or with 1.0 mg per animal per day of medroxyprogesterone acetate for seven days. The mice were killed on the eighth day and the testes removed for histological and morphometric examination. Treatment inhibited spermatogenesis and caused marked decreases in the volume, surface area and length of the seminiferous tubules (Umapathy & Rai, 1982).

In the previous monograph (IARC, 1979), it was reported that medroxyprogesterone acetate caused facial clefts in rabbits but not in rats or mice. A low incidence of facial clefts and malformations of the respiratory tract and renal system was reported in NMRI mice by Eibs *et al*. (1982) after subcutaneous injection of 30 mg/kg bw medroxyprogesterone acetate. Injection of doses up to 900 mg/kg on day 2 of gestation also increased the incidence of facial clefts and reduced fetal weight, but the effects were not dose-related. Genital anomalies, masculinization of females and feminization of males have been reported in rats (Lerner *et al*., 1962; Barlow & Knight, 1983) and non-human primates. Time-mated cynomolgus monkeys were injected once intramuscularly with 25 (11 animals) or 100 (4 animals) mg/kg bw medroxyprogesterone acetate on day 27 (± 2) of gestation (10 and 40 times the human dose). All of the fetuses were affected: the female offspring had labial fusion, clitoral hypertrophy and penile urethra, while the male offspring had short penis, reduced scrotal swelling and hypospadia. The adrenal weight was markedly reduced in animals at the high dose, but no other malformation was observed, and no genital effects were seen in animals treated with 2.5 mg/kg bw, which is equivalent to the human dose (Prahalada *et al*., 1985). Similar effects were reported in baboons at three to four times the human dose (8–10 mg/kg bw on day 30) (Tarara, 1984).

### 4.4.2 *Levonorgestrel*

#### (*a*) *Humans*

The primary contraceptive mechanism of action of levonorgestrel is inhibition of ovulation, although effects on cervical mucus and on maturation of oocytes are also involved.

In a study of 32 women using Norplant® implants, daily blood samples were obtained for hormone analysis throughout most of one menstrual cycle. Half of the women had anovulatory cycles, and the others had abnormal concentrations of hormones in comparison with women not taking contraceptives. Reduced progesterone concentrations and short luteal phases were seen. None of the women using Norplant® had increased concentrations of human chorionic gonadotrophin, indicating that early abortion is not the mechanism of contraceptive action (Faundes *et al.*, 1991; Segal *et al.*, 1991). In a study of 178 women who had requested removal of their Norplant® implant for a planned pregnancy and 91 women who had requested removal of intrauterine devices containing Norplant®, fertility was unimpaired after cessation of use. Most of the women had used their contraceptive method for two to four years (Silvin *et al.*, 1992).

(*b*) *Experimental systems*

See the monograph on 'Oral contraceptives, combined', section 4.4.5.

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure

Progestogen-only contraceptives have been available worldwide for over 40 years. Intramuscular depot injections and subcutaneous implants are the most common routes of administration in developing countries, where there is the widest use. Oral progestogen-only 'mini pills' are used primarily in Europe and North America, but fewer women use these preparations than parenterally administered progestogens and combined oral contraceptives.

### 5.2 Human carcinogenicity

*Breast cancer*

Data on injectable progestogen-only contraceptives were available from two case–control studies and a pooled analysis of original data, overall including about 350 women with breast cancer who had used these drugs. Data on oral progestogen-only contraceptives were available from a pooled analysis of original data on 725 women with breast cancer who had used these drugs. Overall, there is no evidence of an increased risk for breast cancer.

*Endometrial cancer*

One case–control study addressed the relationship between use of oral progestogen-only contraceptives and risk for endometrial cancer; less than 2% of the control women had used these preparations. Women with endometrial cancer were less likely to have used oral progestogen-only contraceptives than control women but not significantly so.

The effects of use of depot medroxyprogesterone acetate on the risk for endometrial cancer have been evaluated in one cohort and one case–control study. No reduction in risk

was seen in the cohort study, whereas a strong reduction was observed in the case–control study. Although the evidence is based on small numbers of women, the results of these studies suggest that women who use progestogen-only contraceptives have a reduced risk for endometrial cancer.

*Cervical cancer*

There is little evidence that use of depot medroxyprogesterone acetate or other progestational injectable contraceptives alters the risk for either squamous-cell carcinoma or adenocarcinoma of the uterine cervix.

*Ovarian cancer*

One case–control study addressed use of progestogen-only oral contraceptives, and one case–control study specifically addressed any use of depot medroxyprogesterone acetate. Neither showed any alteration in risk, either overall or in relation to duration of use.

*Liver cancer*

Two case–control studies have addressed the association between risk for liver cancer and use of injectable progestogen-only contraceptives. In neither study did the risk for liver cancer differ significantly between women who had ever or never used these contraceptives. Both studies were conducted in areas endemic for hepatitis viruses.

*Cutaneous malignant melanoma*

One case–control study of cutaneous malignant melanoma showed no increase in risk among users of progestogen-only contraceptives.

### 5.3 Carcinogenicity in experimental animals

Medroxyprogesterone acetate has been tested for carcinogenicity in mice by subcutaneous implantation of pellets or injection and in dogs by subcutaneous or intramuscular administration. In mice, it induced mammary adenocarcinomas; in dogs, it induced mammary hyperplasia, nodules and benign mammary tumours. Tumour development in other organs and tissues of these animals was not reported.

Medroxyprogesterone acetate was tested in combination with some known carcinogens. With 7,12-dimethylbenz[*a*]anthracene or *N*-methyl-*N*-nitrosourea, it increased the incidence of mammary adenocarcinomas in mice and shortened the latency to tumour appearance. Medroxyprogesterone acetate enhanced the incidence of cervical invasive squamous-cell carcinomas in mice treated with 3-methylcholanthrene. It decreased the incidence of endometrial adenocarcinoma in mice previously treated with *N*-methyl-*N*-nitrosourea plus oestradiol.

Two studies in dogs and one study in cats treated by veterinarians for suppression of oestrus and compared with untreated animals indicated that medroxyprogesterone acetate increases the risk for developing benign and malignant mammary tumours in both species.

Levonorgestrel was tested by implantation into the uterus of rabbits, with no indication of carcinogenicity. In combination with *N*-nitrosobis(2-oxopropyl)amine, levonorgestrel did not enhance the incidence of renal dysplastic lesions or tumours in hamsters.

### 5.4 Other relevant data

Use of depot injections of progestogens or subcutaneous implants of controlled-release devices results in sustained levels of hormone release over long periods. Progestogens used in this way vary in their spectrum of hormonal activities. In addition to progestational activity, levonorgestrel has some oestrogenic activity. In contrast, medroxyprogesterone acetate has no marked oestrogenic activity but has some androgenic activity. Both compounds can modify oestrogenic effects. Progestogen-only contraceptives have growth potentiating effects in the human mammary gland, as indicated by elevated rates of cell proliferation. No data were available on the genetic activity of these progestogens in humans, but norethisterone induced some changes in DNA and chromosomes in experimental systems. Progesterone induced cell transformation in mammalian cells *in vitro*. Early studies on use of depot medroxyprogesterone acetate during pregnancy suggested that genital malformations were induced in the fetus, but the results of later studies provided no support for that suggestion. Medroxyprogesterone acetate administered to men can reduce testosterone levels and semen production.

### 5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of progestogen-only contraceptives.

There is *sufficient evidence* in experimental animals for the carcinogenicity of medroxyprogesterone acetate.

**Overall evaluation**

Progestogen-only contraceptives are *possibly carcinogenic to humans (Group 2B).*

## 6. References

Aldaz, C.M., Liao, Q.Y., LaBate, M. & Johnston, D.A. (1996) Medroxyprogesterone acetate accelerates the development and increases the incidence of mouse mammary tumors induced by dimethylbenzanthracene. *Carcinogenesis*, **17**, 2069–2072

Anderson, T.J., Battersby, S., King, R.J.B., McPherson, K. & Going, J.J. (1989) Oral contraceptive use influences resting breast proliferation. *Hum. Pathol.*, **20**, 1139–1144

Ansari, G.A.S., Walker, R.D., Smart, V.B. & Smith, L.L. (1982) Further investigations of mutagenic cholesterol preparations. *Food chem. Toxicol.*, **20**, 35–41

Ball, M.J., Ashwell, E. & Gillmer, M.D.G. (1991) Progestagen-only oral contraceptives: Comparison of the metabolic effects of levonorgestrel and norethisterone. *Contraception*, **44**, 223–233

Barlow, S.M. & Knight, A.F. (1983) Teratogenic effects of Silastic intrauterine devices in the rat with or without added medroxyprogesterone acetate. *Fertil. Steril.*, **39**, 224–230

Bassol, S., Garza-Flores, J., Cravioto, M.C., Diaz-Sanchez, V., Fotherby, K., Lichtenberg, R. & Perez-Palacios, G. (1984) Ovarian function following a single administration of depo-medroxyprogesterone acetate (DMPA) at different doses. *Fertil. Steril.*, **42**, 216–222

Biswas, R. & Vonderhaar, B.K. (1987) Role of serum in the prolactin responsiveness of MCF-7 human breast cancer cells in long-term tissue culture. *Cancer Res.*, **47**, 3509–3514

Bokkenheuser, B.D., Winter, J., Mosenthal, A.C., Mosbach, E.H., McSherry, C.K., Ayengar, N.K.N., Andrews, A.W., Lebherz, W.B., III, Pienta, R.J. & Wallstein, S. (1983) Fecal steroid 21-dehydroxylase, a potential marker for colorectal cancer. *Am. J. Gastroenterol.*, **78**, 469–475

Bonneterre, J., Peyrat, J.P., Beuscart, R. & Demaille, A. (1990) Biological and clinical aspects of prolactin receptors (PRL-R) in human breast cancer. *J. Steroid Biochem. mol. Biol.*, **37**, 977–981

Bost, L., Dong, W., Hedges, B., Primatesta, P., Prior, G., Purdon, S. & di Salvo, P. (1997) *Health Survey for England 1995*, Vol. I, *Findings*, London, The Stationery Office, p. 254

Botella, J., Paris, J. & Lahlou, B. (1987) The cellular mechanism of the antiandrogenic action of nomegestrol acetate, a new 19-nor progestagen, on the rat prostate. *Acta endocrinol.*, **115**, 544–550

Botella, J., Duranti, E., Duc, I., Cognet, A.M., Delansorne, R. & Paris, J. (1994) Inhibition by nomegestrol acetate and other synthetic progestins on proliferation and progesterone receptor content of T47-D human breast cancer cells. *J. Steroid Biochem. mol. Biol.*, **50**, 41–47

Botella, J., Duranti, E., Viader, V., Duc, I., Delansorne, R. & Paris, J. (1995) Lack of estrogenic potential of progesterone- or 19-nor-progesterone-derived progestins as opposed to testosterone or 19-nortestosterone derivatives on endometrial Ishikawa cells. *J. Steroid Biochem. mol. Biol.*, **55**, 77–84

Breiner, M., Romalo, G. & Schweikert, H.U. (1986) Inhibition of androgen receptor binding by natural and synthetic steroids in cultured human genital skin fibroblasts. *Klin. Wochenschr.*, **64**, 732–737

Briggs, M.H. (1975) Contraceptive steroid binding to the human uterine progesterone-receptor. *Curr. med. Res. Opin.*, **3**, 95–98

Cancer and Steroid Hormone Study of the Centers for Disease Control and the National Institute of Child Health and Human Development (1986) Oral contraceptive use and the risk of breast cancer. *New Engl. J. Med.*, **315**, 405–411

Cappelletti, V., Miodini, P., Fioravanti, L. & DiFronzo, G. (1995) Effect of progestin treatment on estradiol- and growth factor-stimulated breast cancer cell lines. *Anticancer Res.*, **15**, 2551–2555

Catherino, W.H. & Jordan, V.C. (1995) Nomegestrol acetate, a clinically useful 19-norprogesterone derivative which lacks estrogenic activity. *J. Steroid Biochem. mol. Biol.*, **55**, 239–246

Centers for Disease Control and the National Institute of Child Health and Human Development, Cancer and Steroid Hormone Study (1987) Combination oral contraceptive use and the risk of endometrial cancer. *J. Am. med. Assoc.*, **257**, 796–800

Chi, I.-C. (1995) The progestin-only pills and the levonorgestrel-releasing IUD: Two progestin-only contraceptives. *Clin. Obstet. Gynecol*., **38**, 872–889

Clavel, F., Andrieu, N., Gairard, B., Brémand, A., Piana, L., Lansac, J., Bréart, G., Rumeau-Rouquette, C., Flamant, R. & Renaud, R. (1991) Oral contraceptives and breast cancer: A French case–control study. *Int. J. Epidemiol*., **20**, 32–38

Coezy, E., Auzan, C., Lonigro, A., Philippe, M., Menard, J. & Corvol, P. (1987) Effect of mestranol on cell proliferation and angiotensinogen production in HepG2 cells: Relation with the cell cycle and action of tamoxifen. *Endocrinology*, **120**, 133–141

Coldham, N.G. & James, V.H.T. (1990) A possible mechanism for increased breast cell proliferation by progestins through increased reductive 17β-hydroxysteroid dehydrogenase activity. *Int. J. Cancer*, **45**, 174–178

Collaborative Group on Hormonal Factors in Breast Cancer (1996) Breast cancer and hormonal contraceptives: Further results. *Contraception*, **54** (Suppl. 3), 1S–106S

Concannon, P.W., Spraker, T.R., Casey, H.W. & Hansel, W. (1981) Gross and histopathologic effects of medroxyprogesterone acetate and progesterone on the mammary glands of adult beagle bitches. *Fertil. Steril*., **36,** 373–387

Cong, H. & Beck, L.R. (1991) Preparation and pharmacokinetic evaluation of a modified long-acting injectable norethisterone microsphere. *Adv. Contracep.*, **7**, 251–256

Cullins, V.E. & Garcia, F.A.R. (1997) Implantable hormonal and emergency contraception. *Curr. Opin. Obstet. Gynecol*., **9**, 169–174

Dhall, K., Kumar, M., Rastogi, G.K. & Devi, P.K. (1977) Short-term effects of norethisterone oenanthate and medroxyprogesterone acetate on glucose, insulin, growth hormone, and lipids. *Fertil. Steril*., **28**, 154–158

Di Carlo, F., Gallo, E., Conti, G. & Racca, S. (1983) Changes in the binding of oestradiol to uterine oestrogen receptors induced by some progesterone and 19-nor-testosterone derivatives. *J. Endocrinol*., **98**, 385–389

Dunkel, V.C., Pienta, R.J., Sivak, A. & Traul, K.A. (1981) Comparative neoplastic transformation responses of Balb/3T3 cells, Syrian hamster embryo cells, and Rauscher murine leukemia virus-infected Fischer 344 rat embryo cells to chemical carcinogens. *J. natl Cancer Inst*., **67**, 1303–1315

Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S. & Simmon, V.F. (1984) Reproducibility of microbial mutagenicity assays: I. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ. Mutag*., **6** (Suppl. 2), 1–254

Eibs, H.G., Spielmann, H. and Hagele, M. (1982) Teratogenic effects of cyproterone acetate and medroxyprogesterone treatment during the pre- and postimplantation period of mouse embryos. 1. *Teratology*, **25**, 27–36

Englund, D.E. & Johansson, E.D. (1980) Endometrial effect of oral estriol treatment in postmenopausal women. *Acta obstet. gynecol. scand*., **59**, 449–451

Epstein, S.S., Arnold, E., Andrea, J., Bass, W. & Bishop, Y. (1972) Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol. appl. Pharmacol*., **23**, 288–325

Ewertz, M. (1992) Oral contraceptives and breast cancer risk in Denmark. *Eur. J. Cancer*, **28A**, 1176–1181

Fahmy, K., Abdel-Razik, M., Shaaraway, M., Al-Kholy, G., Saad, S., Wagdi, A. & Al-Azzony, M. (1991) Effect of long-acting progestagen-only injectable contraceptives on carbohydrate metabolism and its hormonal profile. *Contraception*, **44**, 419–430

Faundes, A. Brache, V., Tejada, A.S., Cochon, L. & Alvarez-Sanchez, F. (1991) Ovulatory dysfunction during continuous administration of low-dose levonorgestrel by subdermal implants. *Fertil. Steril*., **56**, 27–31

Frank, D.W., Kirton, K.T., Murchison, T.E., Quinlan, W.J., Coleman, M.E., Gilbertson, T.J., Feenstra, E.S. & Kimball, F.A. (1979) Mammary tumors and serum hormones in the bitch treated with medroxyprogesterone acetate or progesterone for four years. *Fertil. Steril*., **31,** 340–346

Fujimoto, J., Hori, M., Ichigo, S., Morishita, S. & Tamaya, T. (1996a) Estrogen activates migration potential of endometrial cancer cells through basement membrane. *Tumour Biol*. **17**, 48–57

Fujimoto, J., Ichigo, S., Hori, M., Morishita, S. & Tamaya, T. (1996b) Progestins and danazol effect on cell-to-cell adhesion, and E-cadherin and alpha- and beta-catenin mRNA expressions. *J. Steroid Biochem. med. Biol*., **57**, 275–282

Fujimoto, J., Hori, M., Ichigo, S. & Tamaya, T. (1997) Antiestrogenic compounds inhibit estrogen-induced expression of fibroblast growth factor family (FGF-1,2, and 4) mRNA in well-differentiated endometrial cancer cells. *Eur. J. Gynaecol. Oncol*., **18**, 497–501

Garza-Flores, J., Rodriguez, V., Perez-Palacios, G., Virutamasen, P., Tang-Keow, P., Konsayreepong, R., Kovacs, L., Koloszar, S. & Hall, P.E. (1987) A multicentered pharmacokinetic, pharmacodynamic study of once-a-month injectable contraceptives. I. Different doses of HRP112 and of Depoprovera (Task Force of WHO). *Contraception*, **36**, 441–457

Giudice, L.C., Milkowski, D.A., Fielder, P.J. & Irwin, J.C. (1991) Characterization of the steroid-dependence of insulin-like growth factor-binding protein-2 synthesis and mRNA expression in cultured human endometrial stromal cells. *Hum. Reprod*., **5**, 632–640

Gray, R.H. & Pardthaisong, T. (1991a) In utero exposure to steroid contraceptives and survival during infancy. *Am. J. Epidemiol*., **134**, 804–811

Gray, R.H. & Pardthaisong, T. (1991b) The authors' response to Hogue. *Am. J. Epidemiol*., **134**, 816–817

Hackenberg, R. & Schultz, K.D. (1996) Androgen receptor mediated growth control of breast cancer and endometrial cancer modulated by antiandrogen- and androgen-like steroids. *J. Steroid Biochem. mol. Biol*., **56**, 113–117

Herrero, R., Brinton, L.A., Reeves, W.C., Brenes, M.M., de Britton, R.C., Tenorio, F. & Gaitan, E. (1990) Injectable contraceptives and risk of invasive cervical cancer: Evidence of an association. *Int. J. Cancer*, **46**, 5–7

Hogue, C.J. (1991) Invited commentary: The contraceptive technology tightrope. *Am. J. Epidemiol*., **134**, 812–817

Hussain, S.P. & Rao, A.R. (1991) Modulatory influence of injectable contraceptive steroid medroxyprogesterone acetate on methylcholanthrene-induced carcinogenesis in the uterine cervix of mouse. *Cancer Lett*., **61,** 187–193

Hyder, S.M., Murthy, L. & Stancel, G.M. (1998) Progestin regulation of vascular endothelial growth factor in human breast cancer cells. *Cancer Res*., **58**, 392–395

IARC (1979) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Vol. 21, *Sex Hormones (II)*, Lyon

Ishidate, M., Jr, ed. (1983) *The Data Book of Chromosomal Aberration Tests in Vitro on 587 Chemical Substances using a Chinese Hamster Fibroblast Cell Line (CHL Cells)*, Tokyo, Realize

Jaffe, B., Shye, D., Harlap, S., Baras, M., Belmaker, E., Gordon, L., Magidor, S. & Fortney, J. (1990) Health, growth and sexual development of teenagers exposed in utero to medroxyprogesterone acetate. *Paediatr. perinat. Epidemiol*., **4**, 184–195

Jeng, M.H. & Jordan, V.C. (1991) Growth stimulation and differential regulation of transforming growth factor-β1 (TGF β1), TGF β2, and TGF β3 messenger RNA levels by norethindrone in MCF-7 human breast cancer cells. *Mol. Endocrinol*., **8**, 1120–1128

Jeng, M.H., Parker, C.J. & Jordan, V.C. (1992) Estrogenic potential of progestins in oral contraceptives to stimulate human breast cancer cell proliferation. *Cancer Res*. **52**, 6539–6546

Jordan, A. (1994) Toxicology of depot medroxyprogesterone acetate. *Contraception*, **49**, 189–201

Katz, Z., Lancet, M., Skornik, J., Chemke, J., Mogilner, B.M. & Klinberg, M. (1985) Teratogenicity of progestogens given during the first trimester of pregnancy. *Obstet. Gynecol*., **65**, 775–780

Kawashima, K., Nakaura, S., Nagao, S., Tanaka, S., Kuwamura, T. & Omori, Y. (1977) Virilizing activities of various steroids in female rat fetuses. *Endocrinol. Jpn.*, **24**, 77–81

Kew, M.C., Song, E., Mohammed, A. & Hodkinson, J. (1990) Contraceptive steroids as a risk factor for hepatocellular carcinoma: A case–control study in South African black women. *Hepatology*, **11**, 298–302

Kim, Y.B., Berek, J.S., Martinez-Maza, O. & Satyaswaroop, P.G. (1996) Vascular endothelial growth factor expression is not regulated by estradiol or medroxyprogesterone acetone in endometrial carcinoma. *Gynecol. Oncol*., **61**, 97–100

Kleinman, R.L. (1990) *Hormonal Contraception*, London, IPPF Medical Publications

Kleinman, R.L. (1996) *Directory of Hormonal Contraceptives*, London, IPPF Medical Publications

Kontula, K., Paavonen, T., Luukkainen, T. & Andersson, L.C. (1983) Binding of progestins to the glucocorticoid receptor. Correlation to their glucocorticoid-like effects on *in vitro* functions of human mononuclear leukocytes. *Biochem. Pharmacol*., **32**, 1511–1518

Kooistra, H.S., Okkens, A.C., Mol, J.A., van Garderen, E., Kirpensteijn, J. & Rijnberk, A. (1997) Lack of association of progestin-induced cystic endometrial hyperplasia with GH gene expression in the canine uterus. *J. Reprod. Fertil.*, **Suppl. 51**, 355–361

van Kordelaar, J.M.G., Vermorken, A.J.M., de Weerd, C.J.M. & van Rossum, J.M. (1975) Interaction of contraceptive progestins and related compounds with the oestrogen receptor. Part II: Effect on [$^3$H]oestradiol binding to the rat uterine receptor in vitro. *Acta endocrinol*., **78**, 165–179

Kordon, E.C., Molinolo, A.A., Pasqualini, C.D., Pazos, P., Dran, G. & Lanari, C. (1993) Progesterone induction of mammary carcinomas in female BALB/c mice. *Breast Cancer Res. Treat*., **28,** 29–39

Kordon, E.C., Guerra, F., Molinolo, A.A., Elizalde, P., Charreau, E.H., Pasqualini, C.D., Montecchia, F., Pazos, P., Dran, G. & Lanari, C. (1994) Effect of sialoadenectomy on medoxyprogesterone-acetate-induced mammary carcinogenesis in BALB/c mice. Correlation between histology and epidermal-growth-factor receptor content. *Int. J. Cancer*, **59,** 196–203

Kumasaka, T., Itoh, E., Watanabe, H., Hoshino, K., Yoshinaka, A. & Masawa, N. (1994) Effects of various forms of progestin on the endometrium of the estrogen-primed, ovariectomized rat. *Endocrine J.*, **41**, 161–169

Labrie, C., Cusan, L., Plante, M., Lapointe, S. & Labrie, F. (1987) Analysis of the androgenic activity of synthetic 'progestins' currently used for the treatment of prostate cancer. *J. Steroid Biochem.*, **28**, 379–384

Lanari, C., Molinolo, A.A. & Pasqualini, C.D. (1986) Induction of mammary adenocarcinomas by medroxyprogesterone acetate in BALB/c female mice. *Cancer Lett.*, **33,** 215–233

Lande, R.E (1995) New era for injectables. *Popul. Rep.*, **23**, 1–31

Lau, T.M., Witjaksono, J., Affandi, B. & Rogers, P.A.W. (1996a) Expression of progesterone receptor mRNA in the endometrium during the normal menstrual cycle and in Norplant users. *Hum. Reprod.*, **12**, 2629–2634

Lau, T.M., Affandi, B. & Rogers, P.A.W. (1996b) Immunohistochemical detection of cathepsin D in endometrium from long-term subdermal levonorgestrel users and during the normal menstrual cycle. *Mol. hum. Reprod.*, **4**, 233–237

Lax, E.R., Baumann, P. & Schriefers, H. (1984) Changes in the activities of microsomal enzymes involved in hepatic steroid metabolism in the rat after administration of androgenic, estrogenic, progestational, anabolic and catatoxic steroids. *Biochem. Pharmacol.*, **33**, 1235–1241

Lerner, L.J., dePhillipo, M., Yiacas, E., Brennan, D. & Borman, A. (1962) Comparison of the acetophenone derivative of 16α,17α-dihydroprogesterone with other progestational steroids for masculinisation of the rat fetus. *Endocrinology*, **71**, 448–451

Li, S., Lévesque, C., Geng, C.-S., Yan, X. & Labrie, F. (1995) Inhibitory effects of medroxyprogesterone acetate (MPA) and the pure antiestrogen EM-219 on estrone ($E_1$)-stimulated growth of dimethylbenz(a)anthracene (DMBA)-induced mammary carcinoma in the rat. *Breast Cancer Res. Treat.*, **34**, 147–159

Liang, A.P., Levenson, A.G., Layde, P.M., Shelton, J.D., Hatcher, R.A., Potts, M. & Michelson, M.J. (1983) Risk of breast, uterine corpus, and ovarian cancer in women receiving medroxyprogesterone injection. *J. Am. med. Assoc.*, **249**, 2909–2912

Luo, S., Stojanovic, M., Labrie, C. & Labrie, F. (1997) Inhibitory effect of the novel anti-estrogen EM-800 and medroxyprogesterone acetate on estrone-stimulated growth of dimethylbenz[a]-anthracene-induced mammary carcinoma in rats. *Int. J. Cancer*, **73**, 580–586

Luthy, I.A., Begin, D.J. & Labrie, F. (1988) Androgenic activity of synthetic progestins and spironolactone in androgen-sensitive mouse mammary carcinoma (Shionogi) cells in culture. *J. Steroid Biochem.*, **31**, 845–852

Markiewicz, L. & Gurpide, E. (1994) Estrogenic and progestagenic activities coexisting in steroidal drugs: Quantitative evaluation by *in vitro* bioassays with human cells. *J. Steroid Biochem. mol. Biol.*, **48**, 89–94

Markiewicz, L., Hochberg, R.B. & Gurpide, E. (1992) Intrinsic estrogenicity of some progestagenic drugs. *J. Steroid Biochem. mol. Biol*., **41**, 53–58

Maudelonde, T., Lavaud, P., Salazar, G., Laffargue, F. & Rochefort, H. (1991) Progestin treatment depresses estrogen receptor but not cathepsin D levels in needle aspirates of benign breast disease. *Breast Cancer Res. Treat.*, **19**, 95–102

McCauley, A. & Geller, J. (1992) Decisions for Norplant programs. *Popul. Rep.*, **24**, 1–31

McLaughlin, L. (1982) *The Pill, John Rock and the Church*, Toronto, Little, Brown & Co., pp. 138–145

Meirik, O., Lund, E., Adami, H.O., Bergstrom, R., Christoffersen, T. & Bergsjo, P. (1986) Oral contraceptive use and breast cancer in young women. *Lancet*, **ii**, 650–654

Melo J.F. & Coutinho, E.M. (1977) Inhibition of spermatogenesis in men with monthly injections of medroxyprogesterone acetate and testosterone enanthate. *Contraception*, **15**, 627–633

Meyer, W.J., Walker, P.A., Wideking, C., Money, J., Kowarski, A.A., Migeon, C.J. & Borgaonkar, D.S. (1977) Pituitary function in adult males receiving medroxyprogesterone acetate. *Fertil. Steril.*, **28**, 1072–1076

Misdorp, W. (1988) Canine mammary tumours: Protective effect of late ovariectomy and stimulating effect of progestins. *Vet. Q.*, **10,** 26–33

Misdorp, W. (1991) Progestagens and mammary tumors in dogs and cats. *Acta endocrinol*., **125,** 27–31

Mitsumori, K., Furukawa, F., Sato, M., Yoshimura, H., Imazawa, T., Nishikawa, A. & Takahashi, M. (1994) Promoting effects of ethinyl estradiol on development of renal proliferative lesions induced by *N*-nitrosobis(2-oxopropyl)amine in female Syrian golden hamsters. *Cancer Res. clin. Oncol*., **120**, 131–136

Mol, J.A., Henzen-Logmans, S.C., Hageman, P., Misdorp, W., Blankenstein, M.A. & Rijnberk, A. (1995a) Expression of the gene encoding growth hormone in the human mammary gland. *J. clin. Endocrinol. Metab*., **80**, 3094–3096

Mol, J.A., van Darderen, E., Selman, P.J., Wolfswinkel, J., Rijnberk, A. & Rutteman, G.R. (1995b) Growth hormone mRNA in mammary gland tumors of dogs and cats. *J. clin. Invest*., **95**, 2028–2034

Mol, J.A., van Garderen, E., Rutteman, G.R. & Rijnberk, A. (1996) New insights in the molecular mechanism of progestin-induced proliferation of mammary epithelium: induction of the local biosynthesis of growth hormone (GH) in the mammary gland of dogs, cats and humans. *J. Steroid Biochem. mol. Biol*., **57**, 67–71

Molinolo, A.A., Lanari, C., Charreau, E.H., Sanjuan, N. & Dosne Pasqualini, C. (1987) Mouse mammary tumors induced by medroxyprogesterone acetate: Immunohistochemistry and hormonal receptors. *J. natl Cancer Inst*., **79**, 1341–1350

Musgrove, E.A., Lee, C.S.L. & Sutherland, R.L. (1991) Progestins both stimulate and inhibit breast cancer cell cycle progression while increasing expression of transforming growth factor α, epidermal growth factor receptor, c-*fos*, and c-*myc* genes. *Mol. cell. Biol*., **11**, 5032–5043

Neumann, I., Thierau, D., Andrae, U., Greim, H. & Schwartz, L.R. (1992) Cyproterone acetate induces DNA damage in cultured rat hepatocytes and preferentially stimulates DNA synthesis in γ-glutamyltranspeptidase-positive cells. *Carcinogenesis*, **13**, 373–378

New Zealand Contraception and Health Study Group (1994) Risk of cervical dysplasia in users of oral contraceptives, intrauterine devices, or depot-medroxyprogesterone acetate. *Contraception*, **50**, 431–441

Nisker, J.A., Kirk, M.E. & Nunez-Troconis, J.T. (1988) Reduced incidence of rabbit endometrial neoplasia with levonorgestrel implant. *Am. J. Obstet. Gynecol.*, **158,** 300–303

Niwa, K., Morishita, S., Murase, T., Itoh, N., Tanaka, T., Mori, H. & Tamaya, T. (1995) Inhibitory effects of medroxyprogesterone acetate on mouse endometrial carcinogenesis. *Jpn. J. Cancer Res.*, **86,** 724–729

Odlind, V., Weiner, E., Victor, A. & Johansson, E.D. (1980) Effects of sex hormone binding globulin of different oral contraceptives containing norethisterone and lynestrenol. *Br. J. Obstet. Gynaecol.*, **87**, 416–421

Ortiz, A., Hiroi, M., Stanczyk, F.Z., Goebelsmann, U. & Mishell, D.R., Jr (1977) Serum medroxyprogesterone acetate (MPA) concentrations and ovarian function following intramuscular injections of Depo-MPA. *J. clin. Endocrinol. Metab.*, **44**, 32–38

van Os, J.L., van Laar, P.H., Oldenkamp, E.P. & Verschoor, J.S. (1981) Oestrus control and the incidence of mammary nodules in bitches, a clinical study with two progestogens. *Vet. Q.*, **3,** 46–56

Østerlind, A., Tucker, M.A., Stone, B.J. & Jensen, O.M. (1988) The Danish case–control study of cutaneous malignant melanoma. III. Hormonal and reproductive factors in women. *Int. J. Cancer*, **42**, 821–824

Pardthaisong, T. & Gray, R.H. (1991) In utero exposure to steroid contraceptives and outcome of pregnancy. *Am. J. Epidemiol.*, **134**, 795–803

Pardthaisong, T., Yenchit, C. & Gray, R.H. (1992) The long-term growth and development of children exposed to Depo-Provera during pregnancy or lactation. *Contraception*, **45**, 313–324

Parzefall, W., Monschau, P. & Schulte-Hermann, R. (1989) Induction by cyproterone acetate of DNA synthesis and mitosis in primary cultures of adult rat hepatocytes in serum free medium. *Arch. Toxicol.*, **63**, 456–461

Pater, A., Bayatpour, M. & Pater, M.M. (1990) Oncogenic transformation by human papilloma virus type 16 deoxyribonucleic acid in the presence of progesterone or progestins from oral contraceptives. *Am. J. Obstet. Gynecol.*, **162**, 1099-1103

Paul, C., Skegg, D.C.G. & Spears, G.F.S. (1989) Depot medroxyprogesterone (Depo-Provera) and risk of breast cancer. *Br. med. J.*, **299**, 759–762

Paul, C., Skegg, D.C.G. & Spears, G.F.S. (1990) Oral contraceptives and risk of breast cancer. *Int. J. Cancer*, **46**, 366–373

Paul, C., Skegg, D.C.G. & Williams, S. (1997) Depot medroxyprogesterone acetate: Patterns of use and reasons for discontinuation. *Contraception*, **56**, 209–214

Pazos, P., Lanari, C., Meiss, R., Charreau, E.H. & Pasqualini, C.D. (1991) Mammary carcinogenesis induced by *N*-methyl-*N*-nitrosourea (MNU) and medroxyprogesterone acetate (MPA) in BALB/c mice. *Breast Cancer Res. Treat.*, **20,** 133–138

Pazos, P., Lanari, C., Eizalde, P., Montecchia, F., Charreau, E.H. & Molinolo, A.A. (1998) Promoter effect of medroxyprogesterone acetate (MPA) in *N*-methyl-*N*-nitrosourea (MNU) induced mammary tumors in BALB/c mice. *Carcinogenesis*, **19,** 529–531

Peek, M.J., Markham, R. & Fraser, I.S. (1995) The effects of natural and synthetic sex steroids on human decidual endothelial cell proliferation. *Hum. Reprod.*, **10**, 2238–2243

Pekonen, F., Nyman, T. & Rutanen, E.M. (1994) Differential expression of mRNAs for endothelin-related proteins in human endometrium, mymetrium and leiomyoma. *Mol. cell. Endocrinol*., **103**, 165–170

Phillips, A., Hahn, D.W., Klimek, S. & McGuire, J.L. (1987) A comparison of the potencies and activities of progestogens used in contraceptives. *Contraception*, **36**, 181–192

Piper, J.M. & Kennedy, D.L. (1987) Oral contraceptives in the United States: Trends in content and potency. *Int. J. Epidemiol.*, **16**, 215–221

Population Council (1994) Syria 1993: Results from the PAPCHILD Survey. *Stud. Fam. Plann.*, **25**, 248–252

Population Council (1995) Sudan 1992/93: Results from the PAPCHILD Health Survey. *Stud. Fam. Plann.*, **26**, 116–120

Population Council (1996a) Bolivia 1994: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **27**, 172–176

Population Council (1996b) Morocco 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **27**, 344–348

Population Council (1997a) Uganda 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 156–160

Population Council (1997b) Egypt 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 251–255

Population Council (1997c) Guatemala 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 151–155

Population Council (1997d) Kazakstan 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 256–260

Population Council (1997e) Eritrea 1995: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 336–340

Population Council (1997f) Mali 1995–96: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **28**, 341–345

Population Council (1998a) Benin 1996: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **29**, 83–87

Population Council (1998b) Brazil 1996: Results from the Demographic and Health Survey. *Stud. Fam. Plann.*, **29**, 88–92

Prahalada, S., Carroad, E., Cukierski, M. & Hendrickx, A.G. (1985) Embryotoxicity of a single dose of medroxyprogesterone acetate (MPA) and maternal serum MPA concentrations in cynomolgus monkey (*Macaca fascicularis*). *Teratology*, **32**, 421–432

Prost-Avallet, O., Oursin, J. & Adessi, G. (1991) *In vitro* effect of synthetic progestogens on estrone sulfatase activity in human breast carcinoma. *J. Steroid Biochem. mol. Biol*., **39**, 967–973

Reynolds, J.E.F., ed. (1996) *Martindale: The Extra Pharmacopoeia*, 31st Ed., London, The Pharmaceutical Press, pp. 1495–1496, 1500–1501

Rosenberg, L., Palmer, J.R., Zauber, A.G., Warshauer, M.E., Lewis, J.L., Jr, Strom, B.L., Harlap, S. & Shapiro, S. (1994) A case–control study of oral contraceptive use and invasive epithelial ovarian cancer. *Am. J. Epidemiol.*, **139**, 654–661

Russo, I.H. & Russo, J. (1991) Progestagens and mammary gland development: Differentiation versus carcinogenesis. *Acta endocrinol.*, **125**, 7–12

Russo, I.H., Gimotty, P., Dupuis, M. & Russo, J. (1989a) Effect of medroxyprogesterone acetate on the response of the rat mammary gland to carcinogenesis. *Br. J. Cancer*, **59,** 210–216

Russo, I.H., Frederick, J. & Russo, J. (1989b) Hormone prevention of mammary carcinogenesis by norethynodrel-mestranol. *Breast Cancer Res. Treat.*, **14**, 43–56

Rutanen, E.M., Salmi, A. & Nyman, T. (1997) mRNA expression of insulin-like growth factor-I (IGF-I) is suppressed and those of IGF-II and IGF-binding protein-1 are constantly expressed in the endometrium during use of an intrauterine levonorgestrel system. *Mol. hum. Reprod.*, **9**, 749–754

Sasaki, M., Sugimura, K., Yoshida, M.A. & Aber, S. (1980) Cytogenetic effects of 60 chemicals on cultured human and Chinese hamster cells. *Kromosomo*, **20**, 574–584

Schardein, J.L. (1980) Congenital abnormalities and hormones during pregnancy: A clinical review. *Teratology*, **22**, 251–270

Schoonen, W.G.E.J., Joosten, J.W.H. & Kloosterboer, H.J. (1995a) Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: I. MCF-7 cell lines. *J. Steroid Biochem. mol. Biol.*, **55**, 423–437

Schoonen, W.G.E.J., Joosten, J.W.H. & Kloosterboer, H.J. (1995b) Effects of two classes of progestagens, pregnane and 19-nortestosterone derivatives, on cell growth of human breast tumor cells: II. T47D cell lines. *J. Steroid Biochem. mol. Biol.*, **55**, 439–444

Schulte-Hermann, R., Ochs, H., Bursch, W. & Parzefall, W. (1988) Quantitative structure–activity studies on effects of sixteen different steroids on growth and monooxygenases of rat liver. *Cancer Res.*, **48**, 2462–2468

Segal, S.J., Alvarez-Sanchez, F., Brache, V., Faundes, A., Vilja, P. & Tuohimaa, P. (1991) Norplant® implants: The mechanism of contraceptive action. *Fertil. Steril.*, **56**, 273–277

Selman, P.J., Mol, J.A., Rutteman, G.R., van Garderen, E. & Rijnberk, A.D. (1994) Progestin-induced growth hormone excess in the dog originates in the mammary gland. *Endocrinology*, **134**, 287–292

Selman, P.J., van Garderen, E., Mol, J.A. & van den Ingh, T.S. (1995) Comparison of the histological changes in the dog after treatment with the progestins medroxyprogesterone acetate and proligestone. *Veter. Q.*, **17,** 128–133

Selman, P.J., Wolfswinkel, J. & Mol, J.A. (1996) Binding specificity of medroxyprogesterone acetate and proligestone for the progesterone and glucocorticoid receptor in the dog. *Steroids*, **61**, 133–137

Shaaban, M.M., Elwan, S.I., El-Kabsh, M.Y., Farghaly, S.A. & Thabet, N. (1984) Effect of levonorgestrel contraceptive implants, Norplant®, on blood coagulation. *Contraception*, **30**, 421–450

Shapiro, S.S., Dyer, R.D. & Colas, A.E. (1978) Synthetic progestins: In vitro potency on human endometrium and specific binding to cytosol receptor. *Am. J. Obstet. Gynecol.*, **132**, 549–554

Shifren, J.L., Tseng, J.F., Zaloudek, C.J., Ryan, I.P., Meng, Y.G., Ferrara, N., Jaffe, R.B. & Taylor, R.N. (1996) Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: Implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. *J. clin. Endocrinol. Metab.*, **8**, 3112–3118

Silvin, I., Stern, J., Diaz, S., Pavéz, M., Alvarez-Sanchez, F., Brache, V., Mishell, D.R., Macarra, M., McCarthy, T., Holma, P., Darney, P., Klaisle, C., Olsson, S.E. & Odlind, V. (1992) Rates and outcomes of planned pregnancy after use of Norplant capsules, Norplant II rods, or levonorgestrel-releasing or copper TCu 380Ag intrauterine contraceptive devices. *Am. J. Obstet. Gynecol.*, **166**, 1208–1213

Skegg, D.C., Noonan, E.A., Paul, C., Spears, G.F.S., Meirik, O. & Thomas, D.B. (1995) Depot medroxyprogesterone acetate and breast cancer. A pooled analysis of the World Health Organization and New Zealand studies. *J. Am. med. Assoc.*, **273**, 799–804

Skegg, D.C., Paul, C., Spears, G.F.S. & Williams, S.M. (1996) Progestogen-only oral contraceptives and risk of breast cancer in New Zealand. *Cancer Causes Control*, **7**, 513–519

Soufir, J.-C., Jouannet, P., Marson, J. & Soumah, A. (1983) Reversible inhibition of sperm production and gonadotrophin secretion in men following combined oral medroxyprogesterone acetate and percutanoeus testosterone treatment. *Endocrinologia*, **102**, 625–632

Stenchever, M.A., Jarvis, J.A. & Kreger, N.K. (1969) Effect of selected estrogens and progestins on human chromosomes in vitro. *Obstet. Gynecol.*, **34**, 249–252

Subakir, S.B., Hadisaputra, W., Siregar, B., Irawati, D., Santoso, D.I., Cornain, S. & Affandi, B. (1995) Reduced endothelial cell migratory signal production by endometrial explants from women using Norplant contraception. *Hum. Reprod.*, **10**, 2579–2583

Subakir, S.B., Hadisaputra, W., Handoyo, A.E. & Affandi, B. (1996) Endometrial angiogenic response in Norplant users. *Hum. Reprod.*, **11**, 51–55

Sutherland, R.L., Hall, R.E., Pang, G., Sutherland, R.L., Hall, R.E., Pang, G.Y., Musgrove, E.A. & Clarke, C.L. (1988) Effect of medroxyprogesterone acetate on proliferation and cell cycle kinetics of human mammary carcinoma cells. *Cancer Res.*, **48**, 5084–5091

Swenberg, J.A. (1981) Utilization of the alkaline elution assay as a short-term test for chemical carcinogens. In: Stich, H.F. & San, R.H.C., eds, *Short-term Tests for Chemical Carcinogens*, New York, Springer-Verlag, pp. 48–58

Tarara, R. (1984) The effect of medroxyprogesterone acetate (Depo-provera) on prenatal development in the baboon (*Papio anubis*): A preliminary study. *Teratology*, **30**, 181–185

Thomas, D.B., Ye, Z., Ray, R.M. & the WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1995a) Cervical carcinoma in situ and use of depot-medroxyprogesterone acetate (DMPA). *Contraception*, **51**, 25–31

Thomas, D.B., Ray, R.M. & the WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1995b) Depot-medroxyprogesterone acetate (DMPA) and risk of invasive adenocarcinomas and adenosquamous carcinomas of the uterine cervix. *Contraception*, **52**, 307–312

Thorogood, M. & Villard-Mackintosh, L. (1993) Combined oral contraceptives: Risks and benefits. *Br. med. Bull.*, **49**, 124–139

Tiltman, A.J. (1985) The effect of progestins on the mitotic activity of uterine fibromyomas. *Int. J. Gynecol. Pathol.*, **4**, 89–96

Topham, J.C. (1980) Do induced sperm-head abnormalities in mice specifically identify mammalian mutagens rather than carcinogens? *Mutat. Res.*, **74**, 379–387

Treiman, K., Liskin, L., Kols, A. & Ward, R. (1995) IUDs—An update. *Popul. Rep.*, **23**, 1–35

Tseng, L. & Zhu, H.H. (1997) Regulation of progesterone receptor messenger ribonucleic acid by progestin in human endometrial stromal cells. *Biol. Reprod.*, **57**, 1360–1366

Tsutsui, T., Komine, A., Huff, J. & Barrett, J.D. (1995) Effects of testosterone, testosterone propionate, 17β-trenbolone and progesterone on cell formation and mutagenesis in Syrian hamster embryo cells. *Carcinogenesis*, **16**, 1329–1333

Ueda, M., Fujii, H., Yoshizawa, K., Abe, F. & Ueki, M. (1996) Effects of sex steroids and growth factors on migration and invasion of endometrial adenocarcinoma SNG-M cells *in vitro*. *Jpn. J. Cancer Res.*, **87**, 524–533

UK National Case–Control Study Group (1989) Oral contraceptive use and breast cancer risk in young women. *Lancet*, **i**, 973–982

Umapathy, E. & Rai, U.C. (1982) Effect of antiandrogens and medroxyprogesterone acetate on testicular morphometry in mice. *Acta morphol. acad. sci. hung.*, **30**, 99–108

United States Census Bureau (1998) Int. Data Base http://www.census.gov/ipc/www/idbacc.html.

Vanderboom, R.J. & Sheffield, L.G. (1993) Estrogen enhances epidermal growth factor-induced DNA synthesis in mammary epithelial cells. *J. Cell Physiol.*, **156**, 367–372

Vessey, M., Buron, J., Doll, R., McPherson, K. & Yeates, D. (1983) Oral contraceptives and breast cancer: Final report of an epidemiologic study. *Br. J. Cancer*, **47**, 455–462

Wharton, C. & Blackburn, R. (1988) Lower dose pills. *Popul. Rep.*, **16**, 1–31

WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991a) Breast cancer and depot medroxyprogesterone acetate: A national study. *Lancet*, **338**, 833–838

WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991b) Depot medroxyprogesterone acetate (DMPA) and risk of endometrial cancer. *Int. J. Cancer*, **49**, 186–190

WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991c) Depot-medroxyprogesterone acetate (DMPA) and risk of epithelial ovarian cancer. *Int. J. Cancer*, **49**, 191–195

WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1991d) Depot-medroxyprogesterone acetate (DMPA) and risk of liver cancer. *Int. J. Cancer*, **49**, 182–185

WHO Collaborative Study of Neoplasia and Steroid Contraceptives (1992) Depot-medroxyprogesterone acetate (DMPA) and risk of invasive squamous cell cervical cancer. *Contraception*, **45**, 299–312

WHO Family Planning and Population Unit (1996) Family planning methods: New guidance. *Popul. Rep.*, **24**, 1–48

Wikström, A., Green, B. & Johansson, E.D.B. (1984) The plasma concentration of medroxyprogesterone acetate and ovarian function during treatment with medroxyprogesterone acetate in 5 and 10 mg doses. *Acta obstet. gynecol. scand.*, **63**, 163–168

Willcox, D.L., Yovich, J.L., McColm, S.C. & Schmitt, L.H. (1985) Changes in total and free concentrations of steroid hormones in the plasma of women throughout pregnancy: Effects of medroxyprogesterone acetate in the first trimester. *J. Endocrinol.*, **107**, 293–300

Yamamoto, T., Terada, N., Nishizawa, Y. & Petrow, V. (1994) Angiostatic activities of medroxyprogesterone acetate and its analogues. *Int. J. Cancer*, **56**, 393–399

Yovich, J.L., Willcox, D.L., Wilkinson, S.P., Poletti, V.M. & Hähnel, R. (1985) Medroxyprogesterone acetate does not perturb the profile of steroid metabolites in urine during pregnancy. *J. Endocrinol*., **104**, 453–459

Yovich, J.L., Turner, S.R. & Draper, R. (1988) Medroxyprogesterone acetate therapy in early pregnancy has no apparent fetal effects. *Teratology*, **38**, 135–144

Zalanyi, S., Jr, Aedo, A.R., Johannisson, E., Landgren, B.M. & Diczfalusy, E. (1986) Pituitary, ovarian and endometrial effects of graded doses of medroxyprogesterone acetate administered on cycle days 7 to 10. *Contraception*, **33**, 567–578