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, α_1-antitrypsin, α_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 bio-degradable 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 5×10^{-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, intraperitoneal

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.