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

4.1 Absorption, distribution, metabolism and excretion

4.1.1 Humans

The pharmacokinetics of the newer progestogens, desogestrel, norgestimate and gestodene, has been reviewed (Fotherby, 1996). There are only a few reports of studies on the disposition of these progestogens, mostly in combination with ethinyloestradiol, and these are discussed in detail in the monograph on ‘Oral contraceptives, combined’.

After oral administration of crystalline progesterone, the progestogen undergoes a first-pass effect due to extensive metabolism in the gut and liver; it thus has minimal systemic bioavailability. Micronized progesterone is rapidly absorbed and provides an adequate
concentration in the blood (Whitehead et al., 1980; Otoson et al., 1984; Kuhl, 1990; Simon et al., 1993). After oral intake of 100 or 200 mg micronized progesterone, maximal serum levels of 10–15 and 20 ng/mL, respectively, are achieved within 1–4 h; these decrease thereafter (Whitehead et al., 1980; Morville et al., 1982; Maxson & Hargrove, 1985). After oral intake, absorption is enhanced about twofold by the presence of food, but the bioavailability appears to be low, the integrated area under the curve of concentration versus time (area under the curve) after intramuscular injection of progesterone being about 10 times larger than after oral intake (Simon et al., 1993).

Both single and multiple treatments with progesterone or with most modified progesterone derivatives result in rapid absorption and maximum blood level within 1–2 h. Accumulation occurs in blood after multiple treatments as a result of binding to sex hormone-binding globulin until a steady-state concentration is reached. These progesterogens are largely stored in fat tissues (Kuhl, 1990; Fotherby, 1996).

Gestodene, levonorgestrel, cyproterone acetate and chlormadinone acetate taken orally in combination with ethinyl estradiol do not undergo first-pass metabolism and consequently have a bioavailability of almost 100%, whereas norethisterone, desogestrel and norgestimate in combination with ethinyl estradiol are rapidly and extensively metabolized in the gastrointestinal tract, with a bioavailability of 50–75% (Kuhl, 1990; Fotherby, 1996). Specific examples of the disposition of some progestogens are given below. There are large interindividual variations in the pharmacokinetic parameters.

When gestodene is taken alone, a high serum concentration is found; the mean absorption time is 0.8–1.9 h. Subjects vary widely in the area under the curve for gestodene. When combined with ethinyl estradiol, daily treatment with gestodene or 3-keto-desogestrel results in accumulation of these progestogens in serum. Poor elimination as a result of binding to sex hormone-binding globulin and inactivation of metabolizing enzymes are considered to be a likely explanation for this effect (Fotherby, 1994).

Cyproterone acetate at 5 mg taken orally with 50 μg ethinyl estradiol is rapidly absorbed, and its bioavailability is 100%. The maximum serum concentration is reached within 1–2 h after both single and multiple doses. It is largely stored in fat tissue. An increase from 11 ng/mL after a single intake to 17 ng/mL within a week of multiple intakes suggests that long-term intake leads to accumulation. After multiple oral doses, the elimination half-life remains unchanged at 2.5 days (Schlesusener et al., 1980; Kuhl, 1990).

In most of the pharmacokinetic studies of orally administered medroxyprogesterone acetate, high doses have been used. Absorption of orally administered compound is rapid, and the time to reach the maximum serum concentration is 1–3 h (Pannuti et al., 1982; Johansson et al., 1986).

Norethisterone is rapidly absorbed, and peak serum concentrations occur within 2–4 h. The bioavailability is about 60%, because of first-pass metabolism. Micronized norethisterone is quickly absorbed and results in a higher serum concentration within a shorter time (Shi et al., 1987; Kuhl, 1990; Fotherby, 1996).
4.1.2 Experimental systems

Most of the experimental data relate to the disposition of progesterone; very limited information is available on oestrogen and progestogen combinations. In ovariectomized rats, the distribution and elimination half-lives of progesterone after a single intravenous administration of 500 μg/kg bw were 0.13 and 1.21 h, respectively. Progesterone was eliminated rapidly, with a total clearance of 2.75 L/h per kg bw (Gangrade et al., 1992).

Intravenous administration of 3H-progesterone to cynomolgus monkeys (Macaca fascicularis) resulted in the total disappearance of the hormone from the circulation within 3 h; 0.5–1.75 h later, about 5% of the initial maximal concentration of the hormone reappeared, perhaps as a result of delayed release from tissue stores (Kowalski et al., 1996). In female cynomolgus monkeys (Macaca fascicularis), progesterone has a volume of distribution of 1.75 L/kg bw and a plasma clearance of 0.06 L/kg bw per min. In comparison with humans, plasma progesterone binding is greater and progesterone clearance is slower in cynomolgus monkeys (Braasch et al., 1988). In baboons (Papio anubis), the bioavailability of chlormadinone acetate was 100%, and the peak serum concentration was reached within 1–2 h (Honjo et al., 1976).

4.2 Receptor-mediated effects

4.2.1 Humans

A group of 14 post-menopausal women was given ethinyloestradiol for one month at a daily dose of 50 μg, followed by a dose escalation of 50 μg per day over four days to a final dose of 200 μg; half of the women received 5 mg per day chlormadinone acetate during the four-day period. Endometrial biopsy samples were taken at the end of the first month and at the end of the four-day dose escalation period. The addition of chlormadinone decreased the twofold increase in uterine progesterone receptor concentration induced by the ethinyloestradiol dose escalation to that observed after the first month of ethinyloestradiol treatment (approximately 2150 fmol/mg DNA). The uterine oestrogen receptor concentration was not affected by the chlormadinone treatment (Kreitmann et al., 1979).

Post-menopausal women continuously receiving either 2 mg/day oestradiol valerate, 1.5 mg/day oestropipate, 0.625 or 1.25 mg/day conjugated equine oestrogens (Premarin®), 50 mg oestradiol implants or 5 g/day of a skin cream which contained 3 mg oestradiol received an oral progestogen during the last 7–10 days of each month. In endometrial biopsy samples, the soluble progesterone receptor content was found to be elevated by approximately 40% as compared with proliferative-phase endometrium from 12 unexposed women; this increase occurred only in the nine women receiving oestradiol implants and the seven women receiving oestrone sulfate. The nuclear content of oestrogen receptor was increased (by about 30%) only in the endometrial samples from the 12–15 women who had received the high dose of Premarin® before progestogen as compared with proliferative-phase endometrium from 16 unexposed women. Progestogen treatment reduced the oestrogen receptor concentration to that found in secretory-phase endometrium within six days, regardless of the type of progestogen or dose. The percentage of endometrial glandular cells
from five women receiving 1.25 mg/day Premarin® that incorporated tritiated thymidine in vitro was similar to that of proliferative-phase endometrium of 12 unexposed women. After the start of progestogen treatment, the labelling index decreased to the very low levels found in secretory-phase endometrium within six days. This effect of progestogens was also found with norethisterone at doses of 1, 2.5 and 5 mg/day and with norgestrel at doses of 150 and 500 μg/day. At a dose of 10 mg/day, norethisterone showed less inhibition of cell proliferation than at lower doses (Whitehead et al., 1981). Similar effects of norgestrel and norethisterone were observed in a separate study of the same design, confirming the absence of a dose–response relationship at the doses tested. Medroxyprogesterone (at 2.5, 5 and 10 mg/day), dydrogesterone (5, 10 and 20 mg/day) and progesterone (at 100, 200 and 300 μg/day) also inhibited the oestrogen-induced increase in labelling index, but these effects were dose-related, reaching a maximal effect at doses of 10 mg, 20 mg and 200 μg, respectively. All of these progestogens decreased the nuclear oestrogen receptor content and had clear progestational effects on endometrial morphology (King & Whitehead, 1986).

In groups of four to six post-menopausal women given Premarin® alone at 1.25 mg/day or Premarin® and norgestrel at a dose of 150 or 500 μg/day or norethisterone at a dose of 1, 2.5, 5 or 10 mg/day for three months, the progestogens reduced the high tritiated thymidine labelling index found in the epithelial cells (approximately 9%) and stromal cells (approximately 13%) in endometrial biopsy samples from Premarin®-treated women to values found in secretory-phase endometrium from unexposed women. The highest dose of norethisterone (10 mg/day) was less effective than the lower doses; the nuclear oestrogen receptor content of the endometrium was also reduced by more than 65% (Siddle et al., 1982). Identical findings were reported from a study in groups of 6–12 post-menopausal women given dydrogesterone as the progestogen. Reduction of the endometrial labelling index and oestrogen receptor content was maximal at a dose of 10 mg/day of progestogen. With dydrogesterone at a dose of 20 mg/day, the labelling index was suppressed to a lesser extent than at 10 mg/day. An apparently positive relation was observed between the dose of Premarin® and induction of the endometrial enzymes oestradiol and isocitrate dehydrogenase (Lane et al., 1986).

Oestradiol was given transdermally at a dose of 50 μg/day throughout the cycle and norethisterone acetate at a transdermal dose of 170 or 350 μg/day either for the last 14 days of each cycle or continuously. A reference group received the same transdermal dose of oestrogen, but norethisterone acetate (1 mg/day) or dydrogesterone (20 mg/day) was given orally for the last 14 days of each cycle. Each group consisted of at least 150 women who were followed for at least one year. Atrophy, presumably induced by the progestogens, was more frequent in the group receiving the progestogen transdermally in a continuous regimen (66 and 84% for high and low dose, respectively) than in the group given the progestogen orally or transdermally in a sequential regimen (32–38%). No hyperplastic changes occurred in women in any group (Johannison et al., 1997).

Post-menopausal women received norethisterone at 5 mg/day, ethinyloestradiol at 50 μg/day or their combination orally, each for one month. Cervical biopsy samples were
taken at baseline and at the end of each treatment period. Multivariate analysis indicated that the oestrogen increased the percentage of cells in S-phase (by flow cytometry) and the endometrial content of both oestrogen and progesterone receptors. There were no significant effects of progestogen, and there was no interaction between the progestogen and oestrogen treatment on these three parameters (Bhattacharya et al., 1997).

Dydrogesterone was given at 10 mg/day in combination with conjugated equine oestrogens at 0.625 mg/day orally to 12 post-menopausal women. The serum concentrations of sex hormone-binding globulin more than doubled, whereas the circulating concentrations of insulin-like growth factor-I decreased by approximately 20%. When the therapy of the women was changed after six months to an oral regimen of norethisterone at 6 mg/day and the oestrogens for three months, the increase in sex hormone-binding globulin was largely abolished and the decrease in insulin-like growth factor-I disappeared. In another six women given oestradiol at 0.05 mg/day transdermally, the combination with dydrogesterone and norethisterone did not alter these parameters, except for a small decrease in sex hormone-binding globulin concentration (Campagnoli et al., 1994).

Six post-menopausal women were given 20 μg/day ethinyloestradiol, 1.25 mg/day conjugated equine oestrogens (Premarin®) or 2 mg/day oestradiol valerate for subsequent periods of four weeks; during the last 12 days of each treatment cycle, the women also received 10 mg/day medroxyprogesterone acetate. The serum concentrations of insulin-like growth factor-I were decreased by approximately 15–25% with all three treatments when compared with the pretreatment period, while the serum concentrations of growth hormone and growth hormone-binding protein were increased by two- to threefold when compared with the pretreatment period (Kelly et al., 1993).

Women receiving transdermal oestradiol developed histological signs of progestational endometrial effects when given levonorgestrel in an intrauterine device releasing 20 μg/day of the progestogen; such effects were not seen in women receiving progesterone orally at 100 mg/day or vaginally at 100–200 mg/day (Suvanto-Luukkonen et al., 1995). Insulin-like growth factor binding protein-I was also induced by intrauterine exposure to levonorgestrel but not by the other routes of exposure. The observations for the binding protein-I were confirmed in a similar comparison of intrauterine and subcutaneous treatment with levonorgestrel (Suhonen et al., 1996).

In the Postmenopausal Estrogen/Progestin Interventions Trial (1996) 875 post-menopausal women were assigned randomly to placebo, conjugated equine oestrogens (0.625 mg/day), conjugated equine oestrogens plus cyclic medroxyprogesterone acetate (10 mg/day for 12 days per month) or conjugated equine oestrogens plus cyclic micronized progesterogen (200 mg/day for 12 days per month). During the three-year study, the women assigned to oestrogen were more likely to develop simple (cystic), complex, adenomatous or atypical hyperplasia than those given placebo (27.7 versus 0.8%, 22.7 versus 0.8% and 11.8 versus 0%, respectively). The rates of hyperplasia were similar in all groups, and the occurrence of hyperplasia was distributed across the three-year trial.
4.2.2 Experimental systems

The relevant effects in experimental systems of combinations of progestogens and oestrogens used in post-menopausal hormonal therapy are summarized in detail in the monographs on ‘Oral contraceptives, combined’, section 4.2, and ‘Hormonal contraceptives, oestrogens only’, section 4.2. These effects are briefly mentioned for each of the progestogens covered in this monograph, but the effects of oestrogen–progestogen combinations in vivo at doses similar to those used for humans are described in detail.

In some but not all of the studies, cyproterone acetate inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture; it was not oestrogenic. Desogestrel, gestodene, norethisterone and levonorgestrel have oestrogenic properties but also inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture. In some but not all of the studies, medroxyprogesterone acetate inhibited the stimulatory effects of oestradiol on human breast cancer cells in culture; it was not oestrogenic.

Medroxyprogesterone acetate at 2 μg/rat per day decreased the hyperplastic effects of conjugated equine oestrogens at 50 μg/rat per day on the endometrium of ovariectomized rats, whereas dydrogesterone and cyproterone acetate at the same dose appeared to enhance these oestrogen-induced hyperplastic effects slightly (Kumasaka et al., 1994).

Subcutaneous administration of medroxyprogesterone acetate at 1–1.5 mg/rat twice daily over 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 reductive activity of 17β-hydroxysteroid oxidoreductase [dehydrogenase] on mammary tumour tissue was altered by medroxyprogesterone acetate in such a way that the formation of oestradiol in tumours of these oestrone-treated animals was reduced by more than 50%. In the uterus, however, medroxyprogesterone acetate decreased the activity of this enzyme by less than 20% (Luo et al., 1997).

The effects of a combination of dietary administration of conjugated equine oestrogens (Premarin®) and medroxyprogesterone acetate on the mammary glands of 25 adult female cynomolgus monkeys (Macaca fascicularis) were studied in comparison with 22 monkeys receiving control diet; all of the animals had been ovariectomized before the experiment. The daily dose of Premarin® was approximately 7.2 μg per animal for the first eight months of the experiment and 166 μg per animal for the subsequent duration of the 30-month study. The latter dose was stated by the authors to be equivalent to a human dose of 0.625 mg/day. The dose of medroxyprogesterone acetate was 650 μg/day throughout the experiment; this dose was stated by the authors to be equivalent to a human dose of 2.5 mg/day. The combined oestrogen–progestogen treatment increased the concentration of circulating oestradiol from 5 to 161 pg/mL; the concentration of medroxyprogesterone acetate in the blood was 116 pg/mL. Exposure to the hormones increased the thickness of the mammary tissue by 70% and significantly enlarged the estimated surface areas of lobular tissue and epithelial tissue; it also induced mammary gland hyperplasia in 18/21 animals, as compared with 41% of animals given Premarin® alone and none in the control group. The mean percentage of epithelial breast cells that
stained for Ki-67 MIB-1 antibody (a marker of cell proliferation) was increased from 2.5 to 8.0% in alveoli, from 0.6 to 1.9% in terminal ducts and from 1.2 to 5.5% in major mammary ducts. These effects on labelling were not different from those in monkeys given Premarin® alone (see the monograph on ‘Post-menopausal oestrogen therapy’, section 4.2). The mean percentage of epithelial breast cells that stained for progesterone receptor was not changed in these mammary structures, but the percentage of cells that stained for oestrogen receptor was decreased by approximately 65% in alveoli, by 40% in terminal ducts and by more than 90% in major mammary ducts (Cline et al., 1996).

4.3 Genetic and related effects
4.3.1 Humans
No data were available to the Working Group.

4.3.2 Experimental systems
Relevant data are contained in section 4.3.2 of the monographs on ‘Oral contraceptives, combined’ and ‘Post-menopausal oestrogen therapy’.