5. Summary of Data Reported and Evaluation

5.1 Exposure

The numbers of women who have used post-menopausal oestrogen therapy vary between countries and within regions of individual countries. The prevalence of use has been greater in the United States than in most other countries; use of oestrogen therapy after the menopause is rare in developing countries but is increasing. Conjugated equine

oestrogens are the most widely prescribed preparation for oestrogen therapy for women in the United States, but oestradiol and its esters have greater use in most of Europe. Oral administration is the most popular route, but percutaneous methods are becoming commoner; use of injections, the first form of post-menopausal oestrogen therapy, has been declining.

5.2 Human carcinogenicity

Breast cancer

Information on the relationship between post-menopausal oestrogen therapy and risk for breast cancer is available from many epidemiological studies. A pooled analysis of the original data from 51 of those studies and a review of data from 15 cohort and 23 case–control studies showed that in the majority of the studies there is a small increase in risk with longer duration of use (five years or more) in current and recent users. Although there is far less information about women who used post-menopausal oestrogen therapy and then ceased use, the increase in risk appears to cease several years after use has stopped. The increase in risk is predominantly for small localized carcinomas of the breast. There are insufficient data to determine whether the risk varies with type of compound or dose.

Endometrial cancer

Three cohort and more than 30 case–control studies consistently showed an association between use of post-menopausal oestrogen therapy and an increased risk for endometrial cancer. The risk increases with increasing duration of use. It decreases with time since last use but remains higher than that of untreated women for at least 10 years.

Cervical cancer

Only one cohort and two case–control studies were available on the relationship between use of post-menopausal oestrogen therapy and the risk for invasive cervical cancer; in none of them were the possible confounding effects of oncogenic human papillomaviruses considered. On balance, the limited evidence available suggests that post-menopausal oestrogen therapy is not associated with an increased risk for invasive cervical carcinoma. The results provide some suggestion that post-menopausal oestrogen therapy is associated with a reduced risk for cervical cancer, but the finding could be due to more active screening for pre-invasive disease among women who have received post-menopausal oestrogen therapy.

Ovarian cancer

The four cohort and 12 case–control studies that addressed the risk for ovarian cancer (largely epithelial) among women undergoing post-menopausal oestrogen therapy gave mixed results. One cohort study and one large case–control study showed a significant excess risk for ovarian cancer in women who used this therapy, but a pooled analysis of the individual data from case–control studies showed no excess risk. There is therefore no clear association between post-menopausal oestrogen therapy and the risk for ovarian cancer.

Cancers of the liver and gall-bladder

The two cohort and two case–control studies that addressed the association between use of post-menopausal oestrogen therapy and the risk for cancers of the liver or biliary tract showed no alteration in risk.

Colorectal cancer

Seven cohort and 12 case–control studies have provided information on use of post-menopausal oestrogen therapy and the risk for colorectal cancer. The risk was not increased and appeared to be reduced in one-half of the studies. The reduced risk tended to be observed among recent users and did not appear to be related to duration of use.

Cutaneous malignant melanoma

One cohort and nine case–control studies addressed the risk for cutaneous malignant melanoma in relation to use of post-menopausal oestrogen therapy. Most suggested no alteration in risk.

Thyroid cancer

Seven case–control studies that provided information on thyroid cancer and use of post-menopausal oestrogen therapy suggested no effect on risk.

5.3 Carcinogenicity in experimental animals

Conjugated oestrogens

Hydrolysed conjugated equine oestrogens, equilin and d-equilenin were tested in male hamsters by subcutaneous implantation. The hydrolysed oestrogens and equilin induced microscopic renal carcinomas, whereas d-equilenin was inactive.

Oestradiol

Oestradiol and its esters were tested in mice by oral administration, in mice, rats, hamsters, guinea-pigs and monkeys by subcutaneous injection or implantation and in mice by neonatal exposure.

Oral administration of oestradiol to mice bearing murine mammary tumour virus increased the incidences of uterine (endometrial and cervical) adenocarcinomas and mammary tumours. Its subcutaneous administration to mice resulted in increased incidences of mammary, pituitary, uterine, cervical, vaginal and lymphoid tumours and interstitial-cell tumours of the testis.

Invasive pituitary tumours were induced in rats treated with oestradiol dipropionate. In hamsters, a high incidence of malignant kidney tumours occurred in intact and castrated males and in ovariectomized females treated with oestradiol, but not in intact females. In guinea-pigs, diffuse fibromyomatous uterine and abdominal lesions were observed. Subcutaneous injections to neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life and an increased incidence of mammary tumours. The 4-hydroxy metabolite of oestradiol induced renal-cell carcinomas in castrated male hamsters.

Oestradiol was tested in two-stage carcinogenesis models in mice with the known carcinogens *N*-methyl-*N*-nitrosourea, *N*-ethyl-*N*-nitrosourea or 3-methylcholanthrene and in two-stage carcinogenesis models in rats with *N*-methyl-*N*-nitrosourea, 2-acetylaminofluorene, *N*-nitrosodiethylamine, 7,12-dimethylbenz[*a*]anthracene or *N*-butyl-*N*-nitrosourea. In mice, oestradiol enhanced the incidences of endometrial adenomatous hyperplasia, atypical hyperplasia and adenocarcinomas induced by *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea. A continuously high serum concentration of oestradiol and a low concentration of progesterone appeared to be important for the development of endometrial adenocarcinomas in mice. Oestradiol suppressed the development of uterine cervical carcinomas induced by 3-methylcholanthrene. In rats, large doses of oestradiol alone or oestradiol with progesterone suppressed the development of mammary carcinomas induced by *N*-methyl-*N*-nitrosourea. Combined treatment of ovariectomized rats with oestradiol and *N*-methyl-*N*-nitrosourea induced vaginal polyps. In a two-stage model of liver carcinogenesis in rats, oestradiol showed no initiating activity. It did not show promoting effects in the livers of rats initiated with *N*-nitrosodiethylamine. In one study pretreatment with oestradiol increased the number of liver foci positive for γ-glutamyl transferase induced by *N*-nitrosodiethylamine. Oestradiol did not affect mammary tumour development in intact or ovariectomized female rats treated with 7,12-dimethylbenz[*a*]-anthracene. Oestradiol benzoate enhanced the incidence of mammary tumours in rats treated with γ-rays.

Oestriol

Oestriol was tested for carcinogenicity by subcutaneous implantation in one study in castrated mice and in one study in hamsters. In mice, oestriol increased the incidence and accelerated the appearance of mammary tumours in both male and female mice. In hamsters, oestriol produced kidney tumours.

In female mice, oestriol slightly increased the incidence of *N*-methyl-*N*-nitrosourea-induced endometrial adenocarcinomas. In several studies in female rats, oestriol inhibited the induction of mammary tumours by 7,12-dimethylbenz[*a*]anthracene when administered before the carcinogen; continuous treatment with oestriol resulted in a decreased incidence of mammary tumours. In one study in female rats, oestriol inhibited the induction of mammary carcinomas when administered 13–15 days after irradiation with γ-rays.

Oestrone

Oestrone was tested for carcinogenicity by oral administration in two studies in castrated male mice. The incidence of mammary tumours was increased. In one study in which oestrone was administered by skin application to mice, the incidence of mammary tumours was increased in males and that of pituitary tumours in animals of each sex. In studies in which oestrone was tested by subcutaneous and/or intramuscular administration, mammary tumours were induced in male mice, and the average age at the time of appearance of mammary tumours in female mice was reduced. In castrated male and female rats, subcutaneous injection of oestrone resulted in mammary tumours.

In three studies of subcutaneous or intramuscular administration, oestrone benzoate induced mammary tumours in male mice. In one study in rats, subcutaneous injection of oestrone benzoate induced mammary and pituitary tumours in animals of each sex. In several studies involving subcutaneous implantation of oestrone, the incidences of mammary and lymphoid tumours were increased in mice, and those of mammary and pituitary tumours were increased in rats. In one study in rats, implantation of low-dose oestrone pellets induced adrenal cortical tumours, but high-dose pellets reduced the incidence. In intact and castrated male hamsters, implantation of oestrone resulted in malignant kidney tumours. The oestrone metabolite, 4-hydroxyoestrone, induced kidney tumours at a low incidence in castrated male hamsters.

Oestrone-3,4-quinone, a metabolite of oestrone, was tested for carcinogenicity by direct injection into the mammary glands of rats fed a high-fat diet. There were no significant differences in mammary tumour incidence or multiplicity in comparison with controls that did not receive the metabolite.

The incidence of endometrial adenocarcinomas induced by *N*-methyl-*N*-nitrosourea in the uterine corpus of mice was significantly increased in those receiving an oestrone-containing diet; furthermore, the incidences of preneoplastic endometrial lesions in the *N*-methyl-*N*-nitrosourea-treated and untreated uterine corpora were significantly increased in mice receiving the oestrone-containing diet. In one study in female toads, subcutaneous administration of oestrone enhanced the incidence of hepatocellular carcinomas induced by subcutaneous injection of *N*-nitrosodimethylamine.

5.4 Other relevant data

Oestrogens administered orally are absorbed rapidly and achieve maximum serum levels quickly. Although the major route of metabolism for oestrogens inactivates them and facilitates their excretion, a minor metabolic pathway activates a small proportion of oestrogen to catechol intermediates, with significant potential for damaging DNA, and may also yield reactive oxygen species that damage DNA. Some oestrogens, including conjugated oestrogens, have been reported to have genotoxic activity in experimental systems. At higher concentrations, which may or may not involve receptor mediation, oestrogens have been reported to induce changes in DNA and chromosomes. Oestradiol binds to oestrogen receptors with higher affinity than oestriol or oestrone. Oestrogens can increase the number of proliferating cells in the human endometrium *in vivo*. It has been reported that oestrogens increase cell proliferation in normal breast cells in monkeys and in cultured human breast cancer cells. At higher concentrations, oestrogens stimulated cell proliferation in rat liver *in vivo* and in cultured rat hepatocytes *in vitro*. No information was available on whether the effect of oestrogens on the mammary gland is modified by body weight or by the recency or duration of exposure to oestrogens in experimental systems. Similarly, no information was available on the possible relationship between exposure to oestrogens and the degree of malignancy of breast tumours.

5.5 Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of post-menopausal oestrogen therapy.

There is *sufficient evidence* in experimental animals for the carcinogenicity of oestradiol and oestrone.

There is *limited evidence* in experimental animals for the carcinogenicity of conjugated equine oestrogens, equilin and oestriol.

There is *inadequate evidence* in experimental animals for the carcinogenicity of d-equilenin.

Overall evaluation

Post-menopausal oestrogen therapy is *carcinogenic to humans (Group 1)*.