

3. Studies of Cancer in Experimental Animals

3.1 Studies reviewed previously

The conclusions with regard to carcinogenicity in experimental animals for oestrogens used in post-menopausal oestrogen therapy in the previous monograph (IARC, 1979) are summarized below.

Conjugated oestrogens (Premarin®) were tested in only one experiment in rats by oral administration. The data were insufficient to evaluate the carcinogenicity of this compound.

Oestradiol and its esters were tested in mice, rats, hamsters, guinea-pigs and monkeys by subcutaneous injection or implantation and in mice by oral administration. Subcutaneous administration of oestradiol resulted in increased incidences of mammary, pituitary, uterine, cervical, vaginal and lymphoid tumours and interstitial-cell tumours of the testis in mice. In rats, there was an increased incidence of mammary and/or pituitary tumours. In hamsters, a high incidence of malignant kidney tumours occurred in intact and castrated males and in ovariectomized females, but not in intact females. In guinea-pigs, diffuse fibromyomatous uterine and abdominal lesions were observed. Oral administration of oestradiol to mice led to an increased incidence of mammary tumours. Subcutaneous injections to neonatal mice resulted in precancerous and cancerous cervical and vaginal lesions in later life and an increased incidence of mammary tumours.

Oestriol was tested by subcutaneous implantation in castrated mice and in rats and hamsters. It increased the incidence and accelerated the appearance of mammary tumours in both male and female mice and produced kidney tumours in hamsters.

Oestrone was tested in mice by oral administration; in mice, rats and hamsters by subcutaneous injection and implantation and in mice by skin painting. Its administration resulted in an increased incidence of mammary tumours in mice; in pituitary, adrenal and mammary tumours, as well as bladder tumours in association with stones, in rats and in renal tumours in both castrated and intact male hamsters.

Oestrone benzoate increased the incidence of mammary tumours in mice following its subcutaneous injection.

3.2 New studies of oestrogens used in post-menopausal oestrogen therapy

3.2.1 Conjugated oestrogens

(a) Subcutaneous implantation

Hamster: Groups of eight or nine adult, castrated, male Syrian golden hamsters, 50–55 days of age, were administered equilin or d-equilenin by subcutaneous implantation of a pure pellet (20 ± 1.4 mg) in the shoulder region; to maintain constant levels, the pellets were reimplanted at three-month intervals. The mean daily absorption of equilin and d-equilenin was 147 ± 22 μ g and 145 ± 15 μ g, respectively. After nine months of treatment, renal adenocarcinomas were detected microscopically in frozen serial sections (at least 25–30 sections from each kidney) stained histochemically for esterase. Equilin produced renal carcinoma in 6/8 hamsters (number of tumours per animal, 5.5 ± 0.9), whereas no detectable tumours were found in nine hamsters after d-equilenin treatment (Li *et al.*, 1983). [The Working Group noted the small number of animals, that only a single dose was used and that only the kidney was examined microscopically.]

Groups of six to eight adult, castrated, male Syrian golden hamsters (weighing 85–95 g) were implanted with pellets containing deconjugated hormones designed to provide absorption of 111 ± 11 μ g oestrogen per day. Additional pellets were implanted every 2.5 months. The duration of treatment was nine months. Renal tumours were detected in

frozen sections stained for nonspecific esterase activity (Li *et al.*, 1995). The incidence in untreated controls was not reported; historically, it was 0 under these experimental conditions (Liehr *et al.*, 1986a). All animals developed microscopic renal carcinomas. The numbers of tumours in the two kidneys combined were 15 ± 3 in animals given oestrone, 18 ± 1 in those given equilin plus d-equilenin and 16 ± 2 in those given Premarin®.

(b) *Administration with known carcinogens*

Rat: In a study reported in more detail in the monograph on 'Post-menopausal oestrogen-progestogen therapy', one group of seven ovariectomized rats treated with 7,12-dimethylbenz[*a*]anthracene (DMBA) received Premarin® at a concentration of 18.75 mg/kg diet (ppm) for 285 days. Mammary tumours occurred in 0/7 ovariectomized controls given DMBA, 6/7 intact controls given DMBA and 5/7 ovariectomized rats given both DMBA and Premarin® (Sakamoto *et al.*, 1997).

3.2.2 *Oestradiol*

(a) *Oral administration*

Mouse: Groups of 200–227 female C3H/HeJ mice, six weeks of age, with a high titre of antibodies to the mouse mammary tumour virus (MTV⁺) factor were fed diets containing 0, 100, 1000 or 5000 µg/kg diet (ppb) oestradiol for 104 weeks. Interim kills were carried out at 26, 52 and 78 weeks, and all surviving animals were killed at 104 weeks. At that time, the incidence of cervical adenosis was increased in 8/20 mice at 1000 ppb and 3/6 at 5000 ppb, and the incidence of uterine adenocarcinomas was increased in the latter group (5/207 compared with 0/227 controls). Mammary hyperplastic alveolar nodules were increased by this dose, from 0/57 in controls to 5/78 at weeks 40–65, 3/29 in controls to 5/19 at weeks 66–91 and 6/50 in controls to 6/17 at weeks 92–105; the time to development of mammary adenocarcinomas was also shortened, the tumour incidences being 4/91 in controls and 5/93 at the high dose at weeks 0–39, 15/57 in controls and 34/78 at the high dose at weeks 40–65, 13/29 in controls and 11/19 at the high dose at weeks 66–91 and 19/50 in controls and 8/17 at the high dose at weeks 92–105 (Highman *et al.*, 1980).

(b) *Subcutaneous and/or intramuscular administration*

Rat: Groups of 2–16 female Fischer 344 rats, seven weeks of age, were each injected subcutaneously with 5 mg oestradiol dipropionate once every two weeks for 13 weeks. Treated animals were killed at two-week intervals during the study. Ten untreated female rats were used as controls, five rats being killed at week 7 and at week 13. No pituitary tumour was observed in control animals, but pituitary adenomas were observed in 1/2 treated animals killed at week 5 and 11/12 killed at week 7, and carcinomas were observed in 1/12 rats killed at week 7, 6/6 at week 9, 4/4 at week 11 and 16/16 at week 13 (Satoh *et al.*, 1997).

(c) *Subcutaneous implantation*

Rat: A group of 21 intact ACI rats, 61–63 days of age, received subcutaneous implants of Silastic tubing containing 27.5 mg crystalline oestradiol. A group of three untreated females served as controls. Treatment with oestradiol resulted in rapid development of palpable mammary tumours, which were first observed 99 days after treatment; 100% of the treated group developed tumours within 197 days. The mean time to appearance of the first palpable tumour was 145 ± 26 days. All of the mammary tumours were classified as carcinomas, and invasive features were observed. The average concentration of circulating oestradiol in the serum of treated animals at the time of killing was 185 pg/mL. Mammary tumours were not observed in intact controls or in 11 ovariectomized female rats treated at 45 days of age with oestradiol for 140 days. Intact and ovariectomized rats had similar incidences of oestradiol-induced pituitary tumours (Shull *et al.*, 1997).

Hamster: Male Syrian golden hamsters were orchietomized at seven weeks of age; then, four weeks later, they received implants every three months of pellets containing 20 mg oestradiol. After 5.3 months, renal-cell dysplasia and infiltrating and non-infiltrating renal carcinoma were observed in 5/5 oestradiol-treated animals. No tumour was observed in untreated control hamsters (Goldfarb & Pugh, 1990).

Li *et al.* (1983) reported renal carcinomas in 6/6 castrated male hamsters treated similarly with pellets of 20 mg oestradiol for 8.3 months.

(d) *Administration with known carcinogens*

Mouse: Groups of virgin female Swiss mice, 12–13 weeks of age, receive no treatment (10 mice), beeswax-impregnated cotton threads inserted into the cervix (10 mice), 0.1 mL olive oil weekly by injection (4 mice), an intracervical insertion of beeswax-impregnated threads containing approximately 600 μ g 3-methylcholanthrene (MCA) and weekly injections of 0, 0.01, 0.1, 5 or 50 μ g oestradiol for 16 weeks (18–25 mice), insertion of beeswax-impregnated threads and weekly injections of 0.01, 0.1, 5 and 50 μ g oestradiol throughout the period of observation (6–9 mice) or weekly injections of 0.01, 0.1, 5 and 50 μ g oestradiol alone (5–8 mice). Placement of thread containing MCA resulted in the emergence of precancerous and cancerous lesions in the cervical epithelium. Weekly administration of oestradiol resulted in incidences of cervical squamous-cell carcinomas of 16/24, 16/26, 10/18 and 8/19 at the four doses, respectively, as compared with 16/21 mice given only MCA by the same regimen. The decrease in the incidence of carcinomas was significant ($p < 0.05$) with the high dose of oestradiol. The occurrence of hyperplastic and dysplastic changes was not correlated with treatment (Das *et al.*, 1988). [The Working Group noted that the effect could have been due to interference with the metabolism of MCA.]

Groups of 30 or 31 female ICR mice, 10 weeks of age, were given 10 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) by intravaginal instillation once a week for three weeks and then fed a diet containing 0 or 5 ppm oestradiol for 20 weeks, starting one week after the last exposure to MNU; a third group of 31 mice was given oestradiol in the diet, and a fourth group of 15 mice received basal diet. At the termination of the experiment at

week 23, the incidence of endometrial adenocarcinomas in the groups receiving both MNU and oestradiol (15/31) was significantly higher ($p = 0.001$) than that in the group given MNU alone (2/29) and significantly higher ($p = 0.001$) than that given oestradiol alone (7/31). The incidence of endometrial preneoplastic lesions in the group receiving oestradiol alone was 48%. No endometrial lesions or carcinomas were observed in the 15 controls on basal diet. Small numbers of squamous-cell carcinomas and preneoplastic lesions (dysplasia and hyperplasia) were also seen in the uterine cervix of mice given MNU alone or MNU plus oestradiol (Niwa *et al.*, 1991). [The Working Group noted that no statistical comparison was presented between the untreated controls and the group receiving oestradiol alone.]

Groups of 30 female ICR mice, 10 weeks of age, were given 10 mg/kg bw MNU into the left uterine corpus and normal saline into the right. One week later, the animals received a diet containing 0 or 5 ppm oestradiol for 30 weeks. At that time, the incidence of endometrial adenocarcinomas in the group given MNU plus oestradiol (8/24) was higher than that in mice given MNU alone (3/26), but the difference was not statistically significant. The incidence of preneoplastic endometrial lesions (atypical and adenomatous hyperplasia) was somewhat increased in the group given MNU plus oestradiol in comparison with those given MNU alone (Niwa *et al.*, 1993).

Groups of 24–73 female ICR mice, 10 weeks of age, were fed a diet containing 0 or 5 ppm oestradiol from the beginning of the experiment up to 16 weeks and an intravaginal instillation of 10 mg/kg bw MNU once a week for three weeks from week 4 (73 mice), oestradiol only (41 mice), MNU only (41 mice) or were untreated (24 mice). Mice from each group were killed and necropsied at weeks 8, 12, 16, 23 and 30. Oestradiol induced cystic glandular hyperplasia and adenomatous and atypical hyperplasia of the endometrium, and MNU induced adenomatous and atypical hyperplasia. Oestradiol did not induce endometrial carcinoma. Data presented in bar graphs indicate that the incidence of endometrial adenocarcinoma was approximately 10% in the mice given MNU alone and approximately 30% in those given MNU plus oestradiol [no statistics specified] (Niwa *et al.*, 1996).

Three groups of 25–29 CD-1 mice, 10 weeks of age and in persistent oestrous, were given either a single intrauterine administration of polyethylene glycol, 12.5 mg/kg bw *N*-ethyl-*N*-nitrosourea (ENU) dissolved in polyethylene glycol or ENU in the same manner plus subcutaneously implanted oestradiol pellets one week before ENU administration, the pellets being renewed after eight weeks of the experiment. At termination of the experiment at week 15 after ENU treatment, all surviving mice were killed for assessment of proliferative uterine lesions. All groups had endometrial hyperplasia, the severity being greatest in mice given oestradiol plus ENU. The incidence of adenocarcinomas in this group (20/29) was significantly greater ($p < 0.01$) than that in mice given the vehicle (0/25) or in mice given ENU alone (0/29) (Takahashi *et al.*, 1996).

Rat: Groups of 19 female Sprague-Dawley rats were ovariectomized at 60 days of age and given a single dose of 0 or 0.25 mg MNU by vaginal instillation, followed one week later by subcutaneous implantation of long-term release Silastic pellets containing

5 mg/mL oestradiol in sesame oil. After 16 months, an increase in the incidence of benign vaginal stromal polyps (4/19) was found in the MNU plus oestradiol group. No vaginal polyps were seen in groups given either MNU alone (0/19) or oestradiol alone (0/17). A number of non-neoplastic changes also seen in the vagina and uterus were due to oestradiol treatment either with or without MNU (Sheehan *et al.*, 1982).

Groups of 29–30 female Sprague-Dawley rats, 50 days of age, received an intravenous injection of 50 mg/kg bw MNU and, 10 days later, subcutaneous injections of 20 µg oestradiol, 4 mg progesterone, 20 µg oestradiol plus 4 mg progesterone or sesame oil on five days a week for 40 days. A further group were ovariectomized at 60 days of age and received no further treatment. Administration of oestradiol delayed the appearance of mammary carcinomas, reduced the incidence (13/30 compared with 27/30 with MNU alone) and decreased the number of tumours per rat (0.6 versus 3.5). Concomitant administration of oestradiol and progesterone after initiation with MNU was as effective as ovariectomy in inhibiting mammary carcinogenesis after initiation with MNU: 4/29 and 4/29, respectively, compared with 27/30 with MNU alone (Grubbs *et al.*, 1983).

Groups of 10 male Fischer 344 rats, weighing 130–150 g, were given 0 or 0.05 mg/kg bw oestradiol after partial hepatectomy. After a 13-day recovery phase, all animals received 0.02% 2-acetylaminofluorene in the diet for two weeks, with a further growth stimulus in the form of 2 mL/kg bw carbon tetrachloride given by intragastric instillation on day 7 of the feeding period. There was no significant difference in the incidence of γ -glutamyltranspeptidase-positive foci [putative preneoplastic lesions] in oestradiol and control groups (Schuppler *et al.*, 1983).

Groups of 12 or 14 male Wistar Furth rats were castrated at 40 days of age and received subcutaneous implants of pellets containing 5 mg oestradiol, which were replaced every two months throughout the 12-month experiment. Twelve rats at 50–55 days of age were further given 5 mg/kg bw *N*-butyl-*N*-nitrosourea. None of the 14 castrated rats given oestradiol alone developed hepatic tumours. Treatment with the nitrosourea plus oestradiol did not elicit any hepatic tumours; however, oestradiol alone or in combination with the nitrosourea resulted in high incidences of pituitary adenomas (9/14 and 8/11, respectively). No control data were reported (Sumi *et al.*, 1984).

Groups of 12 or 13 female Sprague-Dawley rats, seven weeks of age, were subjected to partial hepatectomy; 24 h later, they received 5 mg/kg bw *N*-nitrosodiethylamine (NDEA) and then 0 or 0.6 ppm oestradiol in the diet for nine months. In NDEA-initiated rats, oestradiol did not increase the number of γ -glutamyltranspeptidase-positive lesions per liver or the incidence of hepatic nodules or hepatocellular carcinoma (Yager *et al.*, 1984).

A group of 30 female Sprague-Dawley rats, 50–55 days of age, received implants of 3 mg oestradiol-containing silicone wafers; 48 h later, all animals were given 20 mg DMBA by oral gavage. The animals were palpated for mammary tumours after one month and twice weekly thereafter. Oestradiol treatment was continued for 160 days in 15 animals, and the implants from 15 rats were removed after 14 days. After 160 days, 90% of the surviving animals treated continuously with oestradiol had developed palpable

mammary tumours; this incidence was similar to that in rats from which the implant had been removed at 14 days (Wotiz *et al.*, 1984).

Groups of 24–31 female Sprague-Dawley rats, 40 days of age, were given 0 or 20 µg oestradiol and/or 4 mg progesterone by subcutaneous injection on five days a week for five weeks. A dose of 50 mg/kg bw MNU was administered at 96 and 103 days of age, three and four weeks, respectively, after the last hormone injection. The incidence of mammary adenocarcinomas was 48% in the MNU plus oestradiol group, 42% in the MNU plus progesterone group, 13% in the MNU plus oestradiol plus progesterone group ($p < 0.05$) and 61% in the group given MNU alone (Grubbs *et al.*, 1985).

Groups of six female Sprague-Dawley rats, weighing 200–250 g, received 5 mg/kg bw oestradiol 1 or 24 h before an intraperitoneal injection of 0 or 50 mg/kg bw NDEA and were killed after eight weeks. The numbers of γ -glutamyltranspeptidase-positive foci per cm³ of liver increased from 364 ± 57 in animals given only NDEA to 1149 ± 186 in those receiving oestradiol 24 h before NDEA ($p < 0.01$); the number was increased to 3779 ± 280 ($p < 0.001$) when the hormone was injected 1 h before the carcinogen, i.e. about 25% of the number of foci scored in control rats receiving NDEA 24 h after partial hepatectomy (Taton *et al.*, 1990).

Female Wistar MS rats were ovariectomized at 23 days of age and, at 60 days of age, were divided into groups of 20–25 rats. The animals were given subcutaneous injections of 50 µg oestradiol benzoate in 0.2 mL olive oil, 5 mg progesterone in 0.2 mL olive oil or 50 µg oestradiol benzoate plus 5 mg progesterone daily for 14 days. At this time, rats were irradiated with 260 cGy γ -rays, followed 30 days later by subcutaneous implantation of pellets containing diethylstilboestrol (estimated release rate, 0.38 µg per day). The rats were observed for the appearance of palpable mammary tumours for up to one year. The tumour incidences were 6/23 in the controls, 12/21 in rats given oestradiol benzoate alone ($p < 0.05$), 8/25 in rats given progesterone alone and 9/23 in those given oestradiol plus progesterone. These increases were accompanied by significant increases in DNA synthesis in the mammary gland, as determined on the final day of oestrogen or progestogen at the time of radiation treatment (Inano *et al.*, 1995).

(e) *Carcinogenicity of metabolites*

Hamster: In two studies, castrated male Syrian golden hamsters were given the 2-hydroxy- and 4-hydroxy metabolites of oestradiol. In the first study, the oestrogen-containing pellets (25 mg) were implanted at 0 and three months and left for six months. In the second study, the oestrogen-containing pellets were implanted every three months and left for 9–10 months. Oestradiol produced renal-cell carcinomas in 4/5 and 6/6 hamsters, respectively; 2-hydroxyoestradiol in 0/5 and 0/6 hamsters, respectively; and 4-hydroxyoestradiol in 4/5 and 5/5 hamsters, respectively (Liehr *et al.*, 1986a; Li & Li, 1987).

3.2.3 *Oestriol*

Mouse: Groups of 30 female ICR mice, 10 weeks of age, received 10 mg/kg bw MNU solution into the left uterine corpus and saline into the right. One week later, animals

received a diet containing 0 or 25 mg/kg diet (ppm) oestriol for 30 weeks. At that time, all surviving mice were necropsied and underwent histological examination. Endometrial adenocarcinomas developed in both groups: the incidence was 7/25 in mice given MNU plus oestriol and 3/26 in controls, but the difference was not statistically significant (Niwa *et al.*, 1993).

Rat: Two groups of 30 female Sprague-Dawley rats, 55 days old, received subcutaneous implants of Silastic wafers containing 0 or 5 mg oestriol; 48 h later, all rats were given 20 mg DMBA by oral gavage. The animals were examined one month after DMBA treatment and thereafter once weekly. Seven weeks after the onset of the first mammary tumour (day 42 after DMBA treatment), palpable mammary tumours were found in all of the 28 surviving animals given DMBA alone and in 6/26 given DMBA plus oestriol; 180 days after the onset of the first mammary tumour, 13/26 given DMBA plus oestriol had palpable mammary tumours (Wotiz *et al.*, 1984).

Thirty female Sprague-Dawley rats, 50–55 days old, received subcutaneous implants of Silastic wafers containing 5 mg oestriol; 48 h later, all animals received 20 mg DMBA by oral gavage. The implants were removed from 15 animals after 14 days. At the termination of the experiment at 180 days, the incidence of mammary tumours was 60% after two weeks of oestriol treatment and 20% with continuous oestriol treatment (Wotiz *et al.*, 1984).

Groups of 19 female Sprague-Dawley rats, 35–50 days of age, received 20 mg DMBA in 1.5 mL sesame oil by oral gavage; two weeks later, one group received subcutaneous implants of crystalline pellets containing 638 µg oestriol each month for 10 months. The incidences of mammary carcinomas at one year were 12/19 in the group receiving DMBA plus oestriol and 18/19 in those given DMBA alone ($p < 0.05$, χ^2 test) (Lemon, 1987).

Groups of 8–26 virgin female Sprague-Dawley rats, 40–50 days of age, were irradiated from a cobalt-60 gamma source delivering 3.5 Gy to the dorsal area of the rats. Crystalline sodium chloride pellets containing oestriol (638 ± 175 µg per month) were implanted subcutaneously into the anterior dorsal area each month for life. Control rats were irradiated without oestriol treatment. Oestriol treatment was begun one to three days before irradiation or 5, 13 or 15 days after irradiation. The rats were weighed and examined every 10–14 days during their natural life span after irradiation. Biopsies were performed on persistent and growing tumours within two to four weeks of discovery, and biopsy tissues were examined histopathologically. Tumour-free rats were observed until death, at which time they underwent necropsy. Of 142 irradiated controls, 93 developed mammary carcinomas; two-thirds of the tumours appeared more than 300 days after irradiation. When oestriol administration was begun one to three days before or five days after irradiation, no significant reduction in mammary carcinoma incidence (29/54 controls versus 50/113 oestriol-treated) was observed. When oestriol administration was further delayed, a significant reduction in mammary carcinogenesis was observed: 7/12 controls versus 6/14 given oestriol 15 days after irradiation ($p < 0.07$) and 14/20 controls versus 6/18 rats given oestriol 13 days after irradiation ($p < 0.02$). This inhibition was stated to be associated with the rapid differentiation of the mammary gland (Lemon *et al.*, 1989).

3.2.4 Oestrone

(a) Subcutaneous implantation

Hamster: Implantation of 20-mg pellets of oestrone resulted in microscopic renal carcinomas in 8/10 male castrated Syrian hamsters after 8.5 months of treatment (Li *et al.*, 1983).

(b) Administration with known carcinogens

Mouse: Groups of 30 female ICR mice, 10 weeks of age, were given 10 mg/kg bw MNU into the left uterine corpus and normal saline into the right. One week later, the mice received a diet containing 0 or 25 mg/kg diet (ppm) oestrone for 29 weeks. At that time, the incidence of adenocarcinoma in the group given MNU plus oestrone (9/23) was significantly higher ($p < 0.05$) than that in mice given MNU alone (3/26). In addition, the incidences of preneoplastic endometrial lesions (atypical and adenomatous glandular hyperplasia) in mice receiving oestrone with or without MNU were higher than that in controls (Niwa *et al.*, 1993).

Toad: Groups of 100 female toads (*Bufo regularis*), weighing approximately 50 g, received either subcutaneous injections of 1 mL amphibian saline containing 3 mg *N*-nitrosodimethylamine (NDMA) into the dorsal lymph sac once a week, subcutaneous injections of 0.1 mg oestrone dissolved in 1 mL corn oil once a week or 3 mg NDMA followed by direct injection of 0.1 mg oestrone in 1 mL corn oil once a week. The duration of the experiment was 14 weeks. The incidence of hepatocellular carcinomas was 17/99 in toads given NDMA alone, the first tumour appearing at week 8. In toads treated with oestrone alone, the incidence was 4/97, the first tumour appearing at week 12 after the first injection. The incidence of liver tumours was 23/94 in toads treated with NDMA plus oestrone, the first tumour appearing at week 6 after initiation (Sakr *et al.*, 1989).

(c) Carcinogenicity of metabolites

Rat: Groups of 20 female CrI:CD(SD)BR rats, 30 days of age, were given 100 μ L dimethyl sulfoxide (DMSO) containing 30 μ mol/rat oestrone-3,4-quinone [purity not specified], DMSO alone or 1.2 μ mol/rat *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrobenzo[*c*]phenanthrene (purity, > 99%), which were used as vehicle and positive controls, respectively. One-sixth of the total dose was injected under each of six nipples on the left side of each rat, whereas DMSO only was injected under the nipples on the right side. The thoracic mammary glands of the rats were treated at 30 days of age, and those located in the inguinal area were treated on the following day. Rats were fed a high-fat AIN76A diet (23.5% corn oil) throughout the course of the experiment. The experiment was terminated 44 weeks after treatment. The positive control induced mammary tumours in 20/20 rats, but there was no difference in tumour incidence or multiplicity among rats receiving DMSO (3/20) and those treated with oestrone-3,4-quinone (4/20) (El-Bayoumy *et al.*, 1996).

Hamster: Li and Li (1987) investigated the carcinogenicity of the 2-hydroxy and 4-hydroxy metabolites of oestrone in castrated male Syrian golden hamsters given implants

of oestrogen-containing pellets every three months for 9–10 months. Renal tumours were found in 8/10 hamsters given oestrone, 0/6 given 2-hydroxyoestrone and 2/6 given 4-hydroxyoestrone.