3. Studies of Cancer in Experimental Animals

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

3.1 Medroxyprogesterone acetate

3.1.1 *Subcutaneous implantation*

(*a*) *Mouse*

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

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

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

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

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

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

(*b*) *Dog*

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

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

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

From Frank *et al*. (1979)

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

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

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

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

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

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

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

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

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

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

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

(*c*) *Cat*

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

(*d*) *Monkey*

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

3.1.2 *Administration with known carcinogens*

(*a*) *Mouse*

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

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

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

From Pazos *et al*. (1991)

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

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

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

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

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

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

Four groups of 40 virgin female CD2F$_1$ (BALB/c × DBA/2) mice, six weeks of age, received six doses of 1 mg 7,12-dimethylbenz[*a*]anthracene (DMBA) by gavage at 6, 9, 10, 11, 12 and 13 weeks; four doses of DMBA at 9, 10, 12 and 13 weeks; a subcutaneous implant of a 20-mg pellet of medroxyprogesterone acetate at six weeks plus DMBA at 9 and 10 weeks; or an implant of medroxyprogesterone acetate at six weeks plus DMBA at 9, 10, 12 and 13 weeks. A control group of 20 mice received a subcutaneous implant of medroxyprogesterone acetate at six weeks. The experiment was terminated at 56 weeks. The incidences of mammary adencarcinoma and the latencies are shown in Table 12 (Aldaz *et al*., 1996). Medroxyprogesterone acetate shortened the latency and enhanced the incidences of mammary adenocarcinomas. [The Working Group noted that it is not possible to assess whether medroxyprogesterone acetate alone produces mammary adenocarcinomas in this strain of mice, since the latency for mammary tumour induction in BALB/c mice by this compound alone is > 50 weeks (Lanari *et al*., 1986).]

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

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

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

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

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

(*b*) *Rat*

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

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

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

From Russo *et al.* (1989a)
[a] Controls received 8 mg/kg bw DMBA and cholesterol pellets

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

3.2 Levonorgestrel

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

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

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