3. Studies of Cancer in Experimental Animals

The ability of X-rays and γ-rays to induce neoplasms in experimental animals has been known for many years. The types and frequencies of radiation-induced tumours observed in experimental studies depend on the strain and species used, the total dose of radiation and whether the radiation is delivered as a single dose or over a longer time as either fractionated or low doses. Because the carcinogenic effects of X-rays and γ-rays are well recognized, most reports have emphasized the quantitative aspects of radiation carcinogenesis in experimental animals. This section is not meant to be comprehensive; the studies summarized are those that provide both qualitative and quantitative information and address critical issues in radiation carcinogenesis.

3.1 Carcinogenicity in adult animals

3.1.1 *Mice*

In a large series of studies, Upton *et al*. (1970) examined the induction of neoplasms in male and female RF/Un mice after irradiation with 250-kVp X-rays or ^{60}Co γ-rays over a range of doses and dose rates. Whole-body irradiation was initiated when the animals were 10 weeks of age, and the animals were allowed to live out their lifespan or were killed when moribund. All animals were fully necropsied, but only selected lesions were examined histopathologically, as needed to confirm diagnoses. A total of 4100 female and 2901 male mice were used, with 554 female and 623 male controls. The doses ranged from 0.25 to 4.5 Gy for acute X-irradiation and from ~1 Gy to 98.75 Gy for chronic ^{60}Co γ-irradiation. An increased frequency of all neoplasms was observed even at the lowest acute dose. The specific tumour types found included myeloid leukaemia and thymic lymphoma in both males and females, and an increased incidence of ovarian tumours was observed in females. As shown in Table 29, male mice exposed to X-rays were more sensitive to the induction of myeloid leukaemia than to thymic lymphoma, whereas females exposed to γ-rays were more sensitive to the induction of thymic lymphoma. Under conditions of a continuous low dose rate of ^{60}Co γ-irradiation for 23 h daily, the incidences of all neoplasms, myeloid leukaemia, thymic lymphoma and ovarian cancer were reduced when compared with acute X-irradiation. [The Working Group noted that the comparison of dose rate effects of X-rays and ^{60}Co γ-rays is complicated by the fact

Table 29. Incidences of leukaemia and lymphoma in male mice exposed to γ- and X-radiation

Exposure	Mean accumulated dose (Gy)	Average dose rate (mGy/min)	Myeloid leukaemia			Thymic lymphoma		
			Incidence		Mean age at death (days)	Incidence		Mean age at death (days)
			%	SE		%	SE	
Control	0	–	4	1	463	4	1	502
X-rays	0.25	800	11	2	481	4	1	436
	0.5	800	12	2	481	6	2	334
	0.75	800	12	2	468	5	2	365
	1.0	800	22	3	407	5	2	363
	1.5	800	32	2	428	6	1	357
	3.0	800	42	3	370	15	2	309
	4.5	800	27	3	346	16	2	317
γ-rays	1.48	0.038	4	2	560	3	2	542
	1.53	0.106	6	2	582	4	2	408
	1.55	0.560	6	3	622	10	3	575
	3.29	0.038	10	3	597	3	2	598
	3.03	0.101	14	3	536	9	3	417
	3.08	0.159	12	4	473	10	4	433
	3.05	0.221	15	3	597	6	2	326
	3.15	0.570	10	3	487	5	3	472
	6.03	0.037	6	2	490	10	3	454
	6.21	0.098	10	4	533	12	4	401
	6.24	0.565	9	3	382	12	4	423
	58.1	0.115	5	4	679	26	7	382

From Upton *et al.* (1970); SE, standard error

that X-rays are slightly more effective than ^{60}Co γ-rays at low doses (relative biological effectiveness = 2). The frequency of myeloid leukaemia was reduced after exposure to a low dose rate, by a factor substantially greater than 2; it is therefore clear that the decreased effect is due to the lowering of the dose rate.]

Sensitivity to induction to myeloid leukaemia varies as a function not only of the sex of the animal but also of a number of other host factors, including genetic background, hormonal status, age, proliferative state of the bone marrow and the conditions under which the animals are maintained (Upton, 1968; Walburg & Cosgrove, 1969; Ullrich & Storer, 1979a).

One of the most comprehensive series of studies on the induction of cancer by γ-rays was reported by Ullrich and Storer (1979a,b,c). The induction of neoplastic disease was studied in male and female RFM/Un mice and in female BALB/c mice exposed to a range of doses of ^{137}Cs γ-rays at acute (0.4 Gy min^{-1}) and low dose rates (0.08 Gy per 20-h day). A total of 17 610 female and 1602 male RFM mice and 5659

female BALB/c mice were used; groups of 4762 female and 430 male RFM mice and 865 female BALB/c mice served as controls. The doses ranged from 0.1 to 3 Gy for the RFM mice and 0.5 to 2 Gy for BALB/c mice. As shown in Table 30, male and female RFM/Un mice showed dose-dependent increases in the frequencies of myeloid leukaemia and thymic lymphoma; females were more sensitive to the induction of thymic lymphoma. Significantly increased frequencies of thymic lymphomas were observed at doses as low as 0.25 Gy in both male and female RFM mice. Dose-dependent increased frequencies of ovarian, pituitary and Harderian gland tumours were observed in female RFM mice (Table 31), with an almost threefold increase in the frequency of ovarian cancer at 0.25 Gy. Higher doses were required to increase the frequencies of tumours at other sites. In male RFM mice, only the frequency of Harderian gland tumours was clearly increased in a dose-dependent manner, and males and females were equally sensitive to the induction of these tumours. Lowering the dose rate reduced the carcinogenic effectiveness of the radiation (Ullrich, 1983; Ullrich *et al.*, 1987). In the same study, female BALB/c mice were not sensitive to the induction of leukaemia or lymphoma over the dose range used (0.5–2.0 Gy), but dose-dependent increased frequencies of ovarian tumours and significant increases in the frequencies of lung and mammary adenocarcinomas were observed even at the lowest dose. Again, lowering the dose rate markedly reduced the carcinogenic effect.

Table 30. Incidences of thymic lymphoma and myeloid leukaemia in γ-irradiated RFM/Un mice

Dose (Gy)	Incidence (% ± SE)							
	Thymic lymphoma				Myeloid leukaemia			
	Male		Female		Male		Female	
	Obs	Adj	Obs	Adj	Obs	Adj	Obs	Adj
0	6.6	6.6 ± 1.3	13.4	13.4 ± 0.6	1.3	1.3 ± 0.59	0.77	0.77 ± 0.14
0.1	6.5	6.5 ± 1.7	14.2	14.2 ± 0.63	0.86	0.8 ± 0.56	0.80	0.72 ± 0.15
0.25	9.6	9.6 ± 3.4	20.8	20.8 ± 1.3	1.2	1.2 ± 0.92	0.85	0.84 ± 0.30
0.5	12.9	9.1 ± 2.8	27.6	27.6 ± 1.2	3.6	4.5 ± 1.5	1.1	1.1 ± 0.32
1.0	9.2	15.9 ± 2.2	30.3	30.3 ± 1.3	9.2	9.1 ± 2.2	1.4	1.6 ± 0.41
1.5	20.2	20.3 ± 3.6	38.3	38.3 ± 1.2	9.5	10.2 ± 2.7	2.5	3.6 ± 0.76
2.0	NT	NT	44.4	44.4 ± 3.1	NT	NT	3.0	3.5 ± 0.78
3.0	25.8	25.9 ± 2.6	52.4	52.4 ± 1.3	17.7	19.5 ± 2.4	3.0	5.2 ± 0.56

From Ullrich & Storer (1979a). Obs, observed incidence; Adj, age-adjusted incidence; NT, not tested; SE, standard error

Table 31. Incidences of solid tumours in γ-irradiated female RFM/Un mice

Dose (Gy)	No. of animals	Incidence (% ± SE)					
		Ovarian tumours		Pituitary tumours		Harderian gland tumours	
		Obs	Adj	Obs	Adj	Obs	Adj
0	4014	2.4	2.4 ± 0.55	6.6	6.6 ± 0.87	1.2	1.2 ± 0.38
0.1	2827	2.2	2.0 ± 0.61	6.0	5.8 ± 1.0	1.5	1.3 ± 0.45
0.25	965	7.0	6.4 ± 1.7	6.2	5.5 ± 1.5	1.2	1.6 ± 0.88
0.5	1143	33.3	35.5 ± 2.8	8.0	9.1 ± 1.8	1.8	2.3 ± 1.0
1.0	1100	31.7	35.1 ± 1.9	8.2	9.5 ± 1.9	6.0	6.6 ± 1.6
0.15	1043	32.2	42.4 ± 3.0	6.5	9.4 ± 2.1	3.5	5.3 ± 1.7
0.2	333	28.8	43.9 ± 6.8	6.7	10.2 ± 4.1	8.5	15.4 ± 2.4
0.3	4133	27.2	47.8 ± 1.9	7.7	20.9 ± 1.8	7.5	16.2 ± 1.6

From Ullrich & Storer (1979b); Obs, observed incidence; Adj, age-adjusted incidence; SE, standard error

Subsequent studies by Ullrich and co-workers (Ullrich, 1983; Ullrich *et al*., 1987) provided extensive data on the dose–response and time–dose relationships of ^{137}Cs γ-rays in the induction of both lung and mammary adenocarcinomas in female BALB/c mice at doses as low as 0.1 Gy. For mammary adenocarcinoma, a linear–quadratic dose–response relationship ($I = 7.7 + 0.035\ D + 0.015\ D^2$; where I = tumour incidence and D = dose) was observed over the 0–0.5-Gy dose range, while the response tended to flatten over the 0.5–2-Gy dose range. [The Working Group noted that the flattening is probably related to the effects of radiation on the ovary, since this organ is essentially ablated at doses ≥ 0.5 Gy.] Chronic exposure at a low dose rate (0.08 Gy day^{-1}) reduced the risk, while the effects of fractionated doses depended on the fraction size. In mice exposed chronically to ^{137}Cs γ-rays delivered at a dose rate of 0.01 Gy day^{-1} up to a total dose of 2 Gy, a linear dose–response relationship ($I = 7.7 + 0.035\ D$) was seen for mammary tumours. [The Working Group noted that the linear term of this response was consistent with the linear–quadratic model for acute exposure.] When multiple small acute daily fractions of 0.01 Gy were given, the results were similar to those with the low dose rate, whereas the cancer incidence after the same total doses were delivered as 0.05-Gy daily fractions was similar to that after single acute doses. For lung adenocarcinomas, single exposure to ^{137}Cs γ-rays over a 0–2-Gy dose range showed a linear–quadratic dose–response relationship ($I = 11.8 + 0.041\ D + 0.00043\ D^2$). Delivery of γ-rays at a dose rate of 0.08 Gy day^{-1} resulted in a diminution of the D^2 portion of the dose–response curve, such that it was linear over the entire dose range ($I = 12.5 + 0.043\ D$). When the doses were fractionated, the

response was dependent on the dose per fraction. When the dose per fraction was < 0.5 Gy, the response was similar to that with low dose rates; when the dose per fraction was > 0.5 Gy, the tumour incidences were similar to those after acute exposure.

Grahn *et al.* (1992) reported the results of a large series of experiments with more than 8000 male and female $B6CF_1$ (C57BL/6JAn1 × BALB/CJAn1) hybrid mice, which were irradiated with ^{60}Co γ-rays at 0.225–7.88 Gy at high dose rates, at 0.225–24.6 Gy at low dose rates or in fractionation regimens. Increased frequencies of lymphoreticular tumours, tumours of the lung and Harderian gland and all epithelial tumours were observed in male mice, which appeared to increase as a linear function of dose. In addition, increased frequencies of ovarian tumours were observed in female mice [frequencies at each dose not reported]. Protraction or fractionation of the dose reduced the carcinogenic effects of the radiation.

Maisin *et al*. (1983) exposed 1267 male BALB/c mice to single doses of ^{137}Cs γ-rays at doses of 0.25–6 Gy. The incidences of thymic lymphoma were increased at 4 and 6 Gy. Maisin *et al.* (1988) examined the effects of acute and 8 × 3 h or 10 × 4 h fractionated doses of ^{137}Cs γ-rays over the same dose range in male C57BL/6 mice. While the greatest effect was to cause early death [considered by the Working Group to be due mainly to death from cancers], increased frequencies of leukaemia and all malignancies were found after acute doses of 4 and 6 Gy. Fractionation resulted in an earlier and more frequent appearance of tumours at 1–2 Gy, but the results were not statistically significant.

The induction of myeloid leukaemia in 951 male CBA/H mice exposed to 250-kVp X-rays at 0.25–6 Gy was compared with that in 800 controls. The frequency of myeloid leukaemia increased with increasing doses up to 3 Gy and then decreased at higher doses (Mole *et al*., 1983).

Di Majo *et al*. (1996) examined the influence of sex on tumour induction by irradiation with 250-kVp X-rays (half-value layer, 1.5 mm Cu). After irradiation of 289 male and 259 female three-month-old CBA/Cne mice with doses of 1–7 Gy, increased incidences of myeloid leukaemia and malignant lymphomas were observed in males, and the incidence of Harderian gland tumours was increased in a dose-dependent manner and to a similar degree in males and females.

In 153 female RFM mice given a single localized thoracic X-irradiation at doses of 1–9 Gy, the incidence of pulmonary tumours at nine months increased as a linear–quadratic function of dose, but significant increases in the frequency of lung tumours over that in 88 controls and in the numbers of lung tumours per mouse were observed only at 6.5 and 9 Gy. While no data were available on low dose rates, experiments in which the doses were fractionated into two equal portions and given at intervals of 24 h or 30 days were conducted in 311 female RFM mice. A reduction in the carcinogenic effect was observed in animals given the high doses (6.5 and 9 Gy) at a 24-h interval, but no significant difference was observed with an interval of 30 days (Ullrich *et al*., 1979; Ullrich, 1980).

Lung tumours developed in male and female SAS/4 mice after local exposure to thoracic X-rays at doses of 0.25–7.5 Gy (Coggle, 1988). A total of 557 male and 551 female mice were irradiated, and the animals were killed 12 months after irradiation and their lungs examined for tumours. As shown in Table 32, a dose-dependent increase in the frequency of lung tumours was found in both males and females with increasing frequencies over the range of 0.25–5 Gy.

Table 32. Incidences of primary lung tumours in SAS/4 mice given 200-kVp X-irradiation at 0.6 Gy/min

Sex	Dose (Gy)	No. of mice exposed	No. of mice with tumours	Incidence ± SE
Males	0	291	48	16.5 ± 2.2
	0.25	61	12	19.7 ± 5.1
	0.5	62	11	17.7 ± 4.8
	1.0	67	13	19.4 ± 4.8
	2.0	56	15	26.8 ± 5.9
	2.5	69	23	33.3 ± 5.7
	3.0	32	12	37.5 ± 8.6
	4.0	45	17	37.7 ± 7.2
	5.0	45	22	48.9 ± 7.5
	6.0	48	18	37.5 ± 7.0
	7.5	72	16	22.2 ± 4.9
Females	0	210	19	9.0 ± 2.0
	0.5	62	7	11.3 ± 4.0
	1.0	61	6	9.8 ± 3.8
	1.5	64	8	12.5 ± 4.1
	2.0	63	10	15.9 ± 4.1
	3.0	60	16	26.7 ± 5.7
	4.0	61	23	37.7 ± 6.2
	5.0	59	21	35.6 ± 6.2
	6.0	60	15	25.0 ± 5.6
	7.5	61	9	14.8 ± 4.5

From Coggle (1988); SE, standard error

3.1.2 *Genetically engineered mice*

Genetically engineered mice are used in radiation carcinogenesis mainly to study the genes that may affect susceptibility and as a means of elucidating mechanisms. Mice lacking the *p53* gene are useful because of the role of *p53* in damage recognition

and response mechanisms. In addition, *p53* is known to be mutated in Li–Fraumeni syndrome, a genetic syndrome that affects sensitivity to radiation.

Thirty-three *p53* heterozygous (+/–) and 28 *p53* wild-type (+/+) mice were exposed by whole-body irradiation to 4 Gy of ^{60}Co γ-rays at 7–12 weeks of age and observed until they were moribund, when they were killed and autopsied. Eighteen null (–/–) and 14 heterozygous mice served as controls. None of the irradiated wild-type mice developed tumours within 80 weeks, but radiation significantly reduced the latency for tumour development (mainly lymphomas and sarcomas) in *p53* heterozygous mice. Approximately 90% of the heterozygous mice developed tumours with a mean latency of 40 weeks, before any of the unirradiated heterozygous mice developed tumours (mean latency, > 70 weeks). In the same study, a dose of 1 Gy of γ-rays given to two-day-old *p53* null (–/–) mice also decreased the latency for tumour development (Kemp *et al*., 1994).

Radiation-induced thymic lymphoma has also been studied in Eμ-*pim*-1 mice. The *pim*-1 gene was discovered as a preferential proviral integration site in murine leukaemia virus-induced T-cell lymphomas (Cuypers *et al.*, 1984) and can act as an oncogene in mice (Van Lohuizen *et al.*, 1989). The transgenic mice have a low incidence of spontaneous T-cell lymphomas before the age of seven months but are highly susceptible to genotoxic carcinogens. In this study, groups of 12 female and 14 male heterozygous Eμ-*pim*-1 transgenic mice and 15 female and 11 male non-transgenic littermates, four to seven weeks of age, were irradiated with four fractions of 1.5 Gy of X-rays. The fractions were given one week apart for four weeks. Groups of 15 female and 11 male Eμ-*pim*-1 transgenic mice and 15 female and 16 male non-transgenic mice were irradiated with four fractions of 1 Gy of X-rays. Groups of 32 female and 31 male Eμ-*pim*-1 and 25 female and 38 male littermates were irradiated with four fractions of 0.5 Gy. Thirteen female and 12 male transgenic and 13 female and 11 male non-transgenic mice served as controls. The animals were monitored for lymphoma development for 250 days after the last exposure. All 26 Eμ-*pim*-1 mice exposed to four fractions of 1.5 Gy of X-rays developed lymphomas within 250 days. At the lower doses per fraction, 20/22 effective mice developed lymphomas after exposure to four fractions of 1.0 Gy and 17/61 after exposure to four fractions of 0.5 Gy. In the non-transgenic littermates, 12/31, 6/31 and 0/62 irradiated mice developed lymphomas (Van der Houven van Oordt *et al*., 1998).

3.1.3 *Rats*

A total of 398 female adult Sprague-Dawley rats were divided into seven groups and exposed to γ-rays at different ages: to single doses of 5 Gy at 40 days of age or 160 days of age or to four fractionated doses of 1.25 Gy; to eight fractions of 0.62 Gy; to 16 fractions of 0.3 Gy or to 32 fractions of 0.15 Gy at 40 days of age. One group was sham-irradiated. All of the fractionated doses of ^{60}Co γ-rays were delivered twice weekly at a dose rate of 0.40 Gy/min. The incidence of mammary tumours (adeno-

carcinomas, adenofibromas and fibroadenomas) was determined histologically up to the age of 1000 days. An increased frequency of mammary fibroadenomas and, to a lesser extent, adenocarcinomas, was observed, with 64 in controls and 92, 90, 96, 89, 85 and 87% with the different regimes, respectively. No significant difference between single and fractionated exposures was reported (Shellabarger *et al*., 1966).

A total of 191 female adult Sprague-Dawley rats, 61–63 days of age, were given single whole-body doses of 0.28, 0.56 or 0.85 Gy of 250-kVp X-rays at a dose rate of 0.30 Gy min^{-1}. A group of 167 controls was available. The animals were observed over their lifespan (1033–1053 days) for the induction of mammary tumours, and the neoplasms were identified histopathologically as adenocarcinomas or fibroadenomas. The incidences of mammary tumours were 67% in controls and 72, 77 and 79% in the irradiated groups, showing a dose-dependent increase in all mammary tumours and in particular in fibroadenomas. The principal effect of the irradiation was to cause an earlier time of onset of fibroadenomas, which was dose-dependent (Shellabarger *et al*., 1980).

Groups of 40 control and low-dose and 20 mid- and high-dose female WAG/Rij, BN/Bi and Sprague-Dawley rats, eight weeks of age, were exposed by whole-body irradiation to a single dose of 300-kVp X-rays (Sprague-Dawley rats, 0.1, 0.3, 1 or 2 Gy; WAG/Rij and BN/bi rats, 0.5, 1 and 4 Gy [dose rate not given]). In another experiment, the numbers of animals in these groups were increased to 40 and 60, respectively. The animals were observed for life, and the mammary tumour incidences were determined by gross and histopathological observations. A dose-dependent increase in the incidence of all mammary tumours was observed: Sprague-Dawley rats, 30 (control), 70, 72, 75 and 86%; WAG/Rij rats, 27 (control), 26, 35 and 76%; and BN/Bi rats, 8 (control), 15, 86 and 88% (Broerse *et al*., 1986, 1987).

Groups of 40 female WAG/Rij inbred rats were exposed to a single dose of 1 or 2 Gy of ^{137}Cs γ-radiation at 8, 12, 16, 22, 36 or 64 weeks of age at a dose rate of 0.75 Gy min^{-1} to study the effect of age at exposure. A group of 120 controls was available. The animals were observed for life, and tumours of the mammary gland were classified histologically as fibroadenoma or carcinoma. No statistically significant difference in the incidence of mammary tumours was found by age on the basis of crude incidences, but examination of normalized excess risk demonstrated a reduced risk after exposure at 64 weeks of age (Barstra *et al*., 1998).

Lee *et al.* (1982) studied the induction of thyroid tumours in young, female Long-Evans rats after localized external irradiation of the thyroid glands with X-rays (250 kVp; half-value layer, 0.55 mm Cu) at estimated doses of 0.94, 4.1 or 10.6 Gy. The incidences of both follicular thyroid adenomas and carcinomas were increased with dose: 9/281 (control), 11/275, 35/282 and 74/267.

In 115 Sprague-Dawley rats, eight weeks of age, that received nerve isografts on the right posterior tibial nerve, exposure of the thigh region to 0 (control), 46, 66, 86 or 106 Gy ^{60}Co-γ radiation as 2-Gy fractions at a dose rate of 73 cGy/min, resulted in

osteosarcomas and/or fibrous histiocytomas in 0/7 (controls), 0/20, 2/27, 2/20 and 8/41 rats in the respective groups (Tinkey *et al*., 1998).

3.1.4 *Rabbits*

A group of 21 male and female Dutch rabbits were irradiated with 4.4–14.1 Gy of 2.5-MeV γ-rays at a dose rate of 17.6 Gy h^{-1}; a control group of 17 unirradiated rabbits was available. The animals were allowed to die naturally, and selected tissues were examined histologically. Tumours were found in 24% of controls, 75% at 4.4 Gy, 88% at 8.8–10.6 Gy and 56% at 11.5–14.1 Gy. The tumours included four osteosarcomas of the jaw, five fibrosarcomas of the dermis and six basal-cell tumours of the skin (Hulse, 1980).

3.1.5 *Dogs*

Groups of 120 male and female beagle dogs, aged 2 or 70 days, were exposed by whole-body irradiation to 0.88 or 0.83 Gy of ^{60}Co γ-rays, and a further group of 240 dogs received 0.81 Gy at 365 days of age; 360 controls were available. The animals were allowed to die naturally or were killed because of terminal illness. In 1343 dogs allowed to live out their life span, heritable lymphocytic thyroiditis with hypothyroidism was a major contributor to mortality. Of 86 dogs irradiated at 70 days of age, 25/86 had thyroid follicular adenomas and 10/86 had carcinomas, which represented a significant increase ($p < 0.01$) over the 40/231 controls with adenomas and 16/231 with carcinomas. No significant increase in the incidence of thyroid tumours was found in dogs irradiated at 2 or 365 days of age. The irradiated dogs showed a consistent trend for a lower incidence of hypothyroidism when compared with controls. Hypothyroidal dogs had a significantly increased risk for thyroid neoplasia, including a greater risk for carcinomas, but no evidence was found in this group of a greater sensitivity to radiation-induced tumours (Benjamin *et al*., 1991, 1997).

3.1.6 *Rhesus monkeys*

Twenty rhesus monkeys (*Macaca mulatta*), three years of age, were exposed by whole-body irradiation to doses of 4–8.6 Gy of X-rays (300 kVp; half-value layer, 3 mm Cu) at a dose rate of 0.3 Gy min^{-1}. A few hours after irradiation, most of the animals received intravenous grafts of $2–4 \times 10^8$ autologous bone-marrow cells. Between 7.5 and 15.5 years later, eight animals developed malignant tumours, comprising five adenocarcinomas of the kidney, two follicular carcinomas of the thyroid, two osteocarcinomas and one glomus tumour of the subcutaneous tissues. No malignant tumours occurred in 21 controls within 18 years (Broerse *et al*., 1981).

3.2 Prenatal exposure

3.2.1 *Mice*

C57BL/6 female mice, 10–14 weeks of age, were mated with WHT/Ht males of the same age overnight and removed next morning for timed pregnancies. Subsequently, 19 pregnant females were irradiated with approximately 2 Gy of X-rays (180 kVp, 20 mA with a filter of 0.7 mm Cu) at a dose rate of ~0.86 Gy min^{-1} on days 12 or 16–18 *post coitum*. A total of 573 male and female offspring were delivered and observed for life, and all suspected lesions or tumours were examined histopathologically. The control group consisted of 141 unirradiated C57BL/6 × WHT/Ht offspring of 19 mice. Significant increases were found in the incidences of tumours of the lung (both sexes), the pituitary gland (females) and the ovary of the offspring that had been irradiated on days 16–18 *post coitum* [statistical methods not given], whereas X-irradiation at day 12 *post coitum* did not increase the incidence of tumours in the offspring (Sasaki *et al*., 1978a). In a study of 167 $B6WF_1$ (C57BL/6 × WHT/Ht) female mice irradiated 17 days *post coitum* with approximately 1.5 or 3 Gy of X-rays (200 kVp, 20 mA with a filter of 0.5 mm Al + 0.5 mm Cu) at a dose rate of 0.5–0.6 Gy min^{-1}, the offspring were allowed to die naturally. Significant increases were observed in the incidences of hepatocellular tumours in both male and female offspring in a dose-dependent manner (Table 33) [statistical method not given] (Sasaki *et al*., 1978b).

A total of 410 C57BL/6 female × DBA/2 male fetuses were exposed to 0.2, 0.5, 1.0 or 2.0 Gy of ^{60}Co γ-rays on day 18 of gestation and were killed and autopsied when moribund or at two years of age. Tissues showing macroscopic alterations were submitted to histopathological examination. A group of 1009 historical controls was available. Tumours were found mainly in the lung, uterus and lymphoid tissues, and the total tumour incidence was significantly increased at 0.5, 1.0 and 2.0 Gy (Pearson's χ^2 test) (Lumniczky *et al*., 1998).

In order to mimic human exposure to various carcinogenic and promoting agents in the diet and the environment, carcinogenic and/or promoting agents were given in some experiments postnatally after prenatal exposure to radiation. A total of 79 pregnant ICR mice, 9–11 weeks of age, were irradiated with 0.36 Gy of X-rays (180 kVp, 20 mA with a filter of 0.5 mm Cu) at the dose rate of 0.72 Gy min^{-1} on days 0, 2, 4, 6, 8, 10, 12, 14 or 16 of gestation. Then, 496 live offspring were treated with 5 μmol $(g\ bw)^{-1}$ of urethane, while 237 received distilled water, at 21 days of age. The mice were killed five months after the postnatal treatment, and tumour nodules in the lung were counted. As controls, 78 and 181 offspring of 26 unirradiated mice were similarly treated with urethane and water, respectively. No increase in the incidence of tumours was observed after prenatal X-irradiation alone, but both the incidence and the number of lung tumours per mouse were significantly increased when prenatal irradiation was coupled with postnatal urethane treatment on days 0–14 (except day 6) of gestation (χ^2 and Student's *t* test) (Nomura, 1984).

Table 33. Incidences of tumours in B6WF$_1$ (C57BL/6 × WHT/Ht) mice after prenatal exposure to X-radiation

Treated stage (dpc)	Sex	Dose (Gy)	No. of mice	Incidence (%)					Reference
				Total incidence	Lung tumour	Liver tumour	Ovarian tumour	Pituitary tumour	
12	Male	2	44	11**	5*	0	–	0	Sasaki *et al.* (1978a)
	Female		53	15**	4	0	0	0	
16–18	Male	2	126	73**	56**	17	–	1	
	Female		140	77	39**	10	14*	9*	
Control	Male	0	55	46	24	7	–	0	
	Female		77	65	17	7	1	1	
17	Male	3	22	–	–	46**	–	–	Sasaki *et al.* (1978b)
	Female		53	–	–	13*	–	–	
17	Male	1.5	39	–	–	28**	–	–	
	Female		53	–	–	8	–	–	
Control	Male		84	–	–	7	–	–	
	Female		129	–	–	1	–	–	

dpc, days *post coitum*. Significantly different from controls at *$p < 0.05$ and **$p < 0.01$

In a further study from the same laboratory, 289 fetuses of coat colour-mutant strains of PT and HT mice were exposed to 0, 0.3 or 1.03 Gy of X-rays at a dose rate of 0.54 Gy min^{-1} on day 10.5 of gestation. Offspring were examined for somatic mutations at six weeks of age, and then 139 offspring were treated with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) and 150 with the acetone solvent. The mice were killed at 12 months of age, and the induced tumours were diagnosed histopathologically. Although a significant, linear dose-dependent increase in the incidence of somatic mutations was detected, no increase in tumour frequency was observed after prenatal irradiation alone. The incidences of skin tumours and hepatomas were increased in male offspring after prenatal irradiation and postnatal treatment with TPA (Table 34). When 59 $PTHTF_1$ fetuses were exposed to 1.03 Gy of X-rays at the low dose rate of 4.3 mGy min^{-1}, the mutant spot sizes and tumour incidences were about one-fifth of those produced by the dose rate of 0.54 Gy min^{-1} (Nomura *et al*., 1990).

Table 34. Induction of tumours in $PTHTF_1$ mice after irradiation *in utero* and postnatal treatment with TPA

Dose (Gy)	TPA	Tumour-bearing mice		Skin tumour[a]		Hepatoma in males		
		Incidence	%	Incidence	%	Incidence	%	Tumours per liver
1.03	+	14/47	29.8**	5/47	10.6*	8/23	34.8*	0.57
	–	3/49	6.1	0/49	0.0	1/25	4.0	0.04
0.3	+	6/38	15.8	1/38	2.6	4/20	20.0	0.20
	–	2/51	3.9	0/51	0.0	1/26	3.8	0.04
0	+	4/54	7.4	0/54	0.0	1/29	3.4	0.03
	–	3/50	6.0	0/50	0.0	1/22	4.5	0.05

From Nomura *et al.* (1990). TPA, 12-*O*-tetradecanoylphorbol 13-acetate

[a] Four squamous-cell carcinomas and two pigmented basal-cell carcinomas

*$p < 0.05$, **$p < 0.01$ when compared with untreated controls

In a separate study, 2241 male and female NMRI mouse fetuses were irradiated *in utero* with 0.2, 0.4, 0.8 or 1.6 Gy of X-rays (180 kVp, 10 mA with a filter of 0.3 mm Cu) at a dose rate of 0.6 Gy min^{-1} on day 15 of gestation. After birth, one subgroup at each dose received 45 mg (kg bw)$^{-1}$ *N*-ethyl-*N*-nitrosourea (ENU) at 21 days of age while another did not. All surviving animals were killed at 22 months. No significant increase in the incidence of tumours was observed in the offspring exposed to 0.2 or 0.8 Gy of X-radiation alone [0.4 and 1.6 Gy not tested], but significantly increased incidences of tumours of the liver, intestine, uterus and ovary were observed after prenatal exposure to 0.2, 0.4 or 0.8 Gy of X-rays in combination with postnatal treatment with ENU ($p < 0.05$–0.001; χ^2 test) when compared with ENU

alone. In mice at 1.6 Gy in combination with ENU, the tumour incidences were often reduced (Schmahl, 1988).

3.2.2 *Dogs*

Groups of 60 male and 60 female beagles received mean doses of 0.16 or 0.83 Gy of ^{60}Co γ-radiation on day 8 (preimplantation), 28 (embryonic) or 55 (late fetal) *post coitum*. The offspring were allowed to die naturally, when they were examined histopathologically. As controls, 360 dogs were sham-irradiated. The tumours found predominantly in the offspring of irradiated and unirradiated bitches up to 16 years of age were malignant lymphoma, haemangiosarcoma and mammary carcinoma. Analysis of trends with increasing dose indicated that the incidences of both fatal malignancies and all neoplasms were significantly increased in the offspring of bitches irradiated on day 55 *post coitum*, while no significant increase was observed after exposure *in utero* at day 28 *post coitum*; however, the incidence of fatal haemangiosarcomas was significantly increased in the offspring of bitches exposed on day 8 *post coitum* (Peto's test) (Benjamin *et al*., 1991).

3.3 Parental exposure

Male and female ICR mice were treated with X-rays (180 kVp, 20 mA with a filter of 0.5 mm Al + 0.5 mm Cu) at 0.36, 1.08, 2.16, 3.6 or 5.04 Gy at a dose rate of 0.72 Gy min^{-1} and mated with untreated mice at various intervals of days to examine the sensitivity of germ cells at different stages. About half of the pregnant mice were killed just before delivery (day 18 of gestation), and the others were allowed to deliver live offspring. Significant increases in the frequencies of dominant lethal mutations and congenital malformations were observed in a dose-dependent manner after exposure of the spermatozoa and spermatid stages to X-rays. Groups of 1529 and 1155 live offspring of male and female exposed parental mice were killed at eight months of age, and suspected tumours were diagnosed histopathologically. The control group consisted of 548 offspring of unirradiated mice. Significant increases in the incidences of total tumours were reported after paternal (153/1529, 10.0%) and maternal exposure (101/1155, 8.7%), when compared with controls (29/548, 5.3%; $p < 0.01$–0.005; χ^2 test). About 87% of the induced tumours were in the lung. At both germ-cell stages, the tumour incidence in the offspring increased in a nearly linear, dose-dependent mode after paternal exposure, and the increase was statistically significant at the high doses (χ^2 and *t* test). The sensitivity at the spermatogonial stage was about half that at the spermatid stage. No increase in the incidence of tumours was observed in offspring after maternal exposure to up to 1.08 Gy, but the incidence increased significantly at higher doses. When male and female parental mice were treated with doses of 0.36 Gy of X-rays at 2-h intervals, fractionation significantly reduced the carcinogenic effects of irradiation in offspring exposed at the spermatogonial and mature

oocyte stages; however, no such reduction was observed when postmeiotic stages were treated. In another study, F_1 offspring of X-irradiated male mice were mated and their progeny were examined. Significantly higher incidences of tumours were observed in the F_2 generation of F_1 progeny that had tumours. The author suggested that germ-line alterations that caused tumours were transmitted to the next generation (Nomura, 1982).

In order to confirm these results, male mice of the N5 and LT strains were similarly treated with 5.04 Gy of X-rays at the spermatogonial or postmeiotic stage, respectively, and 229 irradiated and 244 unirradiated N5 offspring and 75 irradiated and 411 unirradiated LT offspring were killed at 12 months of age. A significant increase in the incidence of lymphocytic leukaemias was observed: N5 strain, 3.9% versus 0.4% in controls and LT strain, 5.3% versus 1.0% in controls ($p < 0.05$; χ^2 test) (Nomura, 1986, 1989).

Cattanach *et al.* (1995) used the experimental protocol of Nomura (1982) but a different strain of mice. Male BALB/cJ mice were treated with 2.5 or 5.0 Gy of X-rays (250 kVp, 14 mA, filter of 0.25 cm Cu) at a dose rate of 0.76 Gy min^{-1} and were mated with females of the same strain for one week and then new ones for a further week. All of the progeny obtained were therefore derived from irradiated spermatozoa and late spermatids. The study was carried out as a series of 21 replicate experiments over a one-year period in order to accommodate the maximum capacity of the histological laboratory (approximately 45 animals per week). The offspring of about 600 male mice at each dose were retained for examination for lung tumours at eight months of age, and offspring of 70 animals at each dose were retained for examination at 12 months. The total incidences of lung tumours were not significantly different in offspring from irradiated and unirradiated male parents. Nevertheless, the incidence of lung tumours changed significantly in all treated groups during the one-year study: adenocarcinomas were found only in the later experiments, while the incidence of benign adenomas declined over the first 8–10 replicates and then rose to yet higher rates than observed in the early series. The authors ascribed this effect to a seasonal change in the incidence of tumours in these mice.

The same group carried out a study in a different strain of mice, C3H/HeH. In a series of replicate studies over two years, male mice were exposed to 0, 2.5 or 5.0 Gy of X-rays and mated with untreated females in the same protocol as in the previous study. In 1381 offspring killed at 12 months of age, no significant increase in the incidence of lung tumours was observed. Again, a seasonal variation in tumour incidence was observed (Cattanach *et al.*, 1998).

Groups of 27–28 male N5 mice were irradiated under conditions similar to those used by Nomura (1982, 1986) with 0 (control) or 5 Gy of X-rays (160 kVp, 18 mA, with a filter of 0.5 mm Cu + 10 mm Al) [dose rate not given] and mated 3, 7, 10 or 17 days after irradiation; 312 irradiated and 305 unirradiated offspring were observed until they were killed at one year of age. All tumours were examined histopathologically. The probability of dying from leukaemia (Kaplan-Meyer product-limit

procedure) and overall survival (Cox–Mantel log-rank one-tailed test) were statistically significantly different ($p < 0.05$) in the offspring of X-ray-treated males and unirradiated controls. The incidences of leukaemia at one year of age were 11/165 (6.7%) in those exposed to X-rays and 10/305 (3.3%) in controls ($p = 0.07$, Fisher's exact test) (Daher *et al*., 1998).

A lifetime experiment in CBA/J NCrj mice was carried out to examine whether paternal exposure to X-rays increases the risk for tumours. Male mice were exposed to 1 or 2 Gy of X-rays (100 kVp, 8 mA, with a filter of 1.7 mm Al + 0.2 mm Cu) at a dose rate of about 0.65 Gy min^{-1} and mated with unirradiated females one, three or nine weeks later. The 282 and 206 offspring of mice at 1 and 2 Gy were allowed to die naturally. A group of 631 unirradiated control offspring was available. The female offspring of males that had been exposed to 2 Gy of X-radiation one week before mating (spermatozoal stage) showed a trend towards a higher incidence of tumours of the haematopoietic system when compared with unirradiated offspring, and male offspring of these males had a somewhat higher incidence of broncho-alveolar adenocarcinomas. No increase in tumour incidence was observed in the offspring of males irradiated three or nine weeks before conception (Mohr *et al*., 1999).

Further studies were carried out in which the offspring of irradiated parents were treated with chemical carcinogens or promoting agents. Significant increases in the frequencies of lung tumour nodules per mouse were observed in the offspring of X-irradiated ICR mice given urethane by subcutaneous injection postnatally (Nomura, 1983), and similar results were obtained with outbred Swiss mice given urethane intraperitoneally (Vorobtsova & Kitaev, 1988). The incidence of skin tumours was significantly increased in the offspring of parentally X-irradiated outbred SHR mice treated postnatally with TPA by dermal application (Vorobtsova *et al.*, 1993). Similar enhancing effects were not, however, observed when CBA/J male mice were irradiated and their offspring were treated postnatally with urethane by subcutaneous injection (Mohr *et al.*, 1999).