3. Studies of Cancer in Experimental Animals

During the past 60 years, many studies have been conducted on the biological disposition and lifetime health effects of internally deposited radionuclides in laboratory animals. These studies were conducted to (a) increase knowledge about radionuclides for which some human data are available (see section 2); (b) extend the knowledge base to other radionuclides and routes of exposure for which no human data are available; and (c) examine the underlying processes and mechanisms of the behaviour and effects of radionuclides in the body. In this section, studies of carcinogenicity of a broad range of α- and β-particle-emitting radionuclides are summarized, most of which were large and were conducted over many years in specialized facilities equipped for safe work with relatively large amounts of radioactive materials. The results of these long, expensive studies are uniquely valuable international resources.

Readers interested in obtaining more information are encouraged to consult the files of the International Radiobiology Archives of Long-term Animal Studies, described by Gerber *et al*. (1999). These archives were created by American, European and Japanese scientists to safeguard the data and make them available for use by other scientists now and in the future.

3.1 α-Particle-emitting radionuclides

3.1.1 *Pure α-particle emitters*

(*a*) *Radon-222*

^{222}Rn and its decay products were evaluated previously for carcinogenicity (IARC, 1988), and there was judged to be *sufficient evidence* for their carcinogenicity in experimental animals. After exposure by inhalation, respiratory tract tumours were induced in rats and dogs.

(*b*) *Polonium-210*

Hamster: The relative carcinogenicity of ^{210}Po was studied after intratracheal injection of a soluble form, resulting in relatively uniform distribution of radioactivity in the lung, in contrast to the non-uniform distribution of ^{210}Po adsorbed onto Fe_2O_3 carrier particles. Female Syrian golden hamsters, eight weeks of age, received multiple intratracheal instillations of ^{210}Po and/or other materials, as described below, and were observed for life. In the first study, the hamsters were divided into three

groups that received two instillations every week for seven weeks. Group 1 received separate instillations of ^{210}Po alone and Fe_2O_3 (3 mg); group 2 received an instillation of ^{210}Po plus Fe_2O_3 (3 mg) and an instillation of saline; and group 3 received the same treatment as group 2, except that the ^{210}Po was adsorbed onto 0.3 mg Fe_2O_3. The doses given to these animals were calculated from data on distribution and retention in hamsters that were killed periodically. In each group, 34–38 hamsters were examined histologically. The doses and lung tumour incidences in the three groups were: group 1, 1500 rad [15 Gy], 22/38; group 2, 2700 rad [27 Gy], 24/37; and group 3, 1700 rad [17 Gy], 15/34. The ultimate tumour incidence was not significantly different between groups 1 and 2, but the incidence in group 3 was slightly lower. These experiments at relatively high doses showed that 'hot spot' radiation is not more carcinogenic than a diffuse pattern of α-particles when the absorbed dose is taken into account. This point was examined further in an experiment with larger numbers of hamsters and lower doses. One group received ^{210}Po in saline, another received ^{210}Po on Fe_2O_3 (3 mg) particles, and the controls were either instilled with Fe_2O_3 or unexposed. The ^{210}Po was given as 15 weekly instillations of 1.25 nCi [46 Bq] each. Necropsy of 99 animals that received ^{210}Po in saline, resulting in an average dose to the lung of 55 rad [0.55 Gy], showed nine lung tumours. With the combined ^{210}Po–Fe_2O_3 treatment, the α-particle dose was 75 rad [0.75 Gy], and the lung tumour incidence was 10/82. The authors found no evidence to support the 'hot particle' hypothesis in these studies. They did note a difference in the histological features of the lung cancers in the two studies: most of the tumours found at the high dose were classified as combined epidermoid and adenocarcinomas because both histological features were often present in the same tumour, whereas all the tumours found at the low dose were combined tumours (Little *et al.*, 1978a). [The Working Group noted that these results raise an important, unexplained point: why high incidences of lung tumours were produced in hamsters by intratracheal instillation of ^{210}Po, while hamsters exposed by inhalation to aerosols of the α-particle emitter $^{239}PuO_2$ (Sanders & McDonald, 1992) or the β-particle emitter ^{144}Ce with fused aluminosilicate particles (Lundgren *et al.*, 1982) developed few lung tumours.]

The same group studied intratracheal instillation of ^{210}Po and benzo[*a*]pyrene into male Syrian golden hamsters from 11 weeks of age in order to examine the possible synergistic effects of combined exposures to these two compounds, both of which are present in tobacco smoke. Various exposure strategies were used, including simultaneous administration of the two agents and sequential administration of one agent before the other. In preliminary studies of simultaneous administration, an additive but not a synergistic lung cancer response was seen. In the study of sequential administration, 312 hamsters each received a single intratracheal instillation of 40 nCi of ^{210}Po [1480 Bq] either in saline or on Fe_2O_3 particles at 11 weeks of age. Eighteen weeks later, half of the animals were given a series of seven weekly intratracheal instillations of 0.3 mg benzo[*a*]pyrene adsorbed on 3 mg Fe_2O_3 carrier particles. No lung tumours occurred in 65 animals given ^{210}Po alone on Fe_2O_3 particles and one occurred in

74 animals given ^{210}Po in 0.9% saline. Addition of the seven instillations of benzo[*a*]-pyrene raised these numbers to 13/72 and 10/63 tumours, respectively, which the authors interpreted as a synergistic effect. Subsequent studies showed that multiple instillations of 0.9% saline alone could also significantly increase the number of ^{210}Po-induced lung tumours, even when no chemical carcinogen was present. The authors suggested that their results showed a minimal interaction between ^{210}Po and benzo[*a*]-pyrene. The effect of repeated saline injections after instillations of ^{210}Po was considered to mimic the effect of chronic lung irritation, such as might be produced by cigarette smoke acting as a potentiating factor (Little *et al.*, 1978b).

3.1.2 *Mixed α-particle emitters*

(*a*) *Radium-224*

Radium-224 is a short lived α-particle-emitting isotope (half-life, 3.6 days) which deposits most of its energy on the bone surface. It has been studied because of its medical use and to determine the relative carcinogenicity of surface- and volume-seeking isotopes.

Mouse: In a study to compare the occurrence, location and characteristics of osteogenic sarcomas induced by ^{224}Ra and by ^{226}Ra, 500 female ICR mice, 10 weeks of age, were given an intraperitoneal injection of ^{226}Ra [vehicle not specified] at a dose of 8.8 (200 mice), 24.6 (200 mice) or 70.5 μCi/kg bw (100 mice) [326, 910 and 2610 kBq/kg bw, respectively]. Radium-224 was administered by fractionated intraperitoneal injections at three- or four-day intervals for 75 weeks to another three groups of 100 mice [presumed to be female], to give total activities of 0.65, 1.8 and 5.22 μCi/mouse [24, 67 and 193 kBq/mouse]. Administration of ^{224}Ra was intended to provide a cumulative skeletal dose corresponding to that of ^{226}Ra. Skeletal tumours were identified by radiological examination and later confirmed by histological examination. The authors quantified the radiographic lesions in an attempt to differentiate tumours induced by the two nuclides. Seventy-four tumours were identified in the 500 animals exposed to ^{226}Ra and 89 tumours in the 300 animals exposed to ^{224}Ra. The authors noted that the ^{224}Ra-induced tumours tended to be diagnosed sooner than those induced by ^{226}Ra, that there appeared to be differences in their anatomical location and that the larger tumours (measured in the radiographs) were generally seen more frequently in ^{224}Ra-exposed animals (Svoboda *et al.*, 1977).

Several life-span studies on the comparative effects of single and protracted exposures to ^{224}Ra and ^{226}Ra have been conducted. In a large series of experiments, fractionated injections of short-lived bone-seeking radioisotopes were shown to cause a remarkable increase in the incidence of osteosarcomas in NMRI mice when compared with a single administration of the same skeletal dose. This effect was observed with both α- and β-particle emitters (^{224}Ra, ^{227}Th, ^{177}Lu). In addition, the latency was shortened by protracting the dose (Müller *et al*., 1983).

A study of the effect of age on osteosarcoma induction by the short-lived bone-seeking isotopes, ^{224}Ra and ^{227}Th was described. Weanling female NMRI mice, 36 days of age, and adult mice, 152–167 days of age (mean, 159 days), were given an intraperitoneal injection of 25 μCi/kg bw ^{224}Ra [925 kBq/kg bw], and the tumour incidence was compared with the spontaneous tumour incidence in 2000 control NMRI mice in the same colony. The mean skeletal dose was 750 rad [7.5 Gy]. The incidences of osteosarcoma were 17/94 (18%) in weanling rats and 21/246 (9%) in adults, after average latencies of 553 and 476 days, respectively, a statistically significant difference. The authors reported an additional five osteosarcomas of the jaw in adults, but these were considered to be a special 'feature' of rodents. [The Working Group noted that jaw necrosis is observed in humans, however; indeed 'radium jaw' was described in the 1920s.] Of animals injected with 5 μCi/kg bw ^{227}Th [185 kBq/kg bw], 21/50 (42%) weanling animals and 29/150 (19%) adult mice developed an osteosarcoma. The latencies were again significantly different, as seen with ^{224}Ra, that for the weanling animals being longer (average, 545 days) than that for adults (average, 432 days) (Luz *et al.*, 1979).

The same group later described another study with ^{224}Ra in mice. Approximately 300 female NMRI mice, four weeks of age, received a single injection of ^{224}Ra at a dose of 18.5 kBq/kg bw, corresponding to a mean skeletal dose of about 0.15 Gy. A second group of approximately 300 mice received the same total amount of ^{224}Ra in 72 injections given twice a week. Complete necropsy was carried out on all dead or moribund animals and included a radiographical examination. All diagnoses were confirmed by histopathology. A high incidence (13%) of early malignant lymphoma was observed soon after the injection period in the group given fractionated doses, and this was followed later by a 7% incidence of osteosarcomas. The group given the single injection did not develop the early lymphomas, but 5.8% developed osteosarcomas. After 800 days, no new osteosarcomas were observed in the group given the single injection, but about one-third of the osteosarcomas in the group given the protracted dose occurred after this time (Müller *et al.*, 1990).

Four groups of 400 male CBA/H mice, 12 weeks of age, were given an intraperitoneal injection of 69, 139, 280 or 550 kBq/kg bw ^{224}Ra. Another group of 400 mice was given the vehicle and served as controls. The mice were then permitted to live out their lifespan until they either died or were killed when moribund. If anaemia was detected, blood was taken and examined for leukaemia or other blood disorders. Organs and tissues were prepared for histopathology, and animals suspected of having a bone tumour were radiographed. As shown in Table 69, a dose–response relationship was apparent for both myeloid leukaemia and osteosarcomas. The authors concluded that the mice were at greater risk for myeloid leukaemia than for osteosarcomas at the doses studied (Humphreys *et al.*, 1993).

Dog: A lifetime study on the effects of ^{224}Ra in beagle dogs was started at the University of Utah but completed at the Inhalation Toxicology Research Institute (ITRI), Albuquerque, New Mexico, USA (Muggenburg *et al.*, 1995, 1996a). Two

Table 69. Leukaemia and osteosarcoma in groups of 400 male CBA/H mice injected with ^{224}Ra

^{224}Ra (kBq/kg bw)	No. of mice excluded	Median survival (days)	No. of mice with myeloid leukaemia	No. of mice with osteosarcoma
0	6	692	1	1
69	6	702	6	1
139	1	663	11	4
280	5	673	17	6
550	1	669	18	10

From Humphreys *et al.* (1993)

groups of 18 male and 18 female dogs received 10 or 50 weekly intravenous injections and a further group of 19 male and 19 female dogs received a single injection of ^{224}Ra citrate at one of four activity levels to give a mean skeletal dose to bone of approximately 0.1, 0.3, 1.0 or 3 Gy. A control group of 18 dogs of each sex was injected with citrate buffer. Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected at clinical examination or at necropsy and classified by histopathology. The main late effect was the development of bone tumours, and the next most frequent was tumours of the nasal mucosa. Three dogs that received the highest single dose by injection died after 9–16 days from severe haematological dyscrasia. Eighteen dogs developed bone tumours: 15 had a single tumour, two had two tumours, and one had three tumours. Sixteen of the tumours were classified histologically as osteoblastic osteosarcomas, one as a chondroblastic osteosarcoma, one as a fibroblastic osteosarcoma, two as fibrosarcomas and one as a myxosarcoma. Kaplan-Meier methods indicated that tumours occurred sooner in the animals given protracted doses. When the number of tumours per Gy of skeletal dose was calculated and then summed across the years, there were 0.84 tumours per Gy in the dogs receiving 50 injections, 0.20 tumours per Gy in the dogs receiving 10 injections and 0.23 tumours per Gy in the dogs receiving one injection. The authors also reported (Muggenburg *et al*., 1996a) that the age-specific incidence rate for mammary tumours was increased in all three groups treated by injection and was related to dose. They concluded that the frequency of haematological dyscrasia was amplified by delivery of a relatively high dose at a high rate, whereas that of bone tumours was amplified by delivery of relatively high doses at a lower rate. Lloyd *et al.* (1997a) calculated that the ratio of toxicity of ^{224}Ra given in 50 weekly injections relative to a single injection of ^{226}Ra was 16 ± 5. This value was nearly identical to the toxicity ratio of a single injection of ^{239}Pu. Muggenburg *et al.* (1996a) estimated that the risk for developing a bone tumour was about 40 times higher in dogs than that reported in humans.

(*b*) *Radium-226*

Mouse: A large study in female CF1 mice was conducted by Mays and Finkel (1980), and the results were summarized by C.W. Mays in the discussion section of a paper by Raabe *et al*. (1983). The animals were given one intraperitoneal injection of ^{226}Ra, and the 3174 mice that lived at least 150 days were included in the analyses. The amounts injected ranged from 0 to 120 μCi/kg bw [0–4440 kBq/kg bw], and dose-related increases in the incidence of skeletal tumours were found, associated with a decrease in the average number of days after injection to the appearance of the skeletal tumours (Table 70).

Table 70. Bone sarcomas in female CF1 mice injected with ^{226}Ra

Average injected dose (μCi/kg bw)	No. of mice 150 days after injection	No. of mice with bone sarcomas	Average time from injection to appearance of bone tumour (days)	Average skeletal dose (Gy) 100 days before appearance of tumour
0	521	6	730	0
0.05	254	11	710	0.26
0.10	252	5	853	0.62
0.25	247	19	580	1.09
0.50	683	80	655	2.44
0.75	504	94	686	3.83
1.00	239	56	643	4.80
1.25	104	22	657	6.14
2.5	104	45	639	11.90
5	45	28	544	20.40
10	43	34	484	36.40
20	44	38	428	64.20
40	45	33	394	118.00
80	44	31	359	213.00
120	45	14	328	289.00

From C.W. Mays in discussion following paper by Raabe *et al*. (1983)

Five hundred female ICR mice were given an intraperitoneal injection of ^{226}Ra [carrier not reported], at 8.8 μCi/kg bw for 200 mice, 24.6 μCi/kg bw for 200 mice and 70.5 μCi/kg bw for 100 mice [326, 910 and 2610 kBq/kg bw]. An additional 300 mice were given graded doses of ^{224}Ra, and this report is described in that section (Svoboda *et al.*, 1977). While the authors did not provide a detailed report on the incidence of skeletal tumours, they reported a comparative radiological analysis of the tumours in mice exposed to ^{224}Ra and ^{226}Ra.

C57BL/Do black and C57BL/Do albino mice of each sex were given graded amounts of ^{239}Pu, ^{226}Ra, ^{241}Am, ^{239}Cf and ^{252}Cf by intraperitoneal injection in order to

determine the relative effectiveness of the nuclides, known as the 'toxicity ratio', in inducing skeletal cancers and the relative effectiveness of fission fragments (^{252}Cf) versus α-particles in inducing bone sarcoma. Groups of 12–18 mice received the nuclides at about 10 weeks of age, and 94 males and 87 females served as controls. The animals were then permitted to live out their natural life-span. The average activity of ^{226}Ra ranged from 0.057 to 9.88 μCi/kg bw [2.1–366 kBq/kg bw]. The results are summarized in Table 71 (Taylor *et al.*, 1983).

Table 71. Bone sarcomas in male and female C57BL/Do (black) mice and C57BL/Do (albino) mice injected with ^{226}Ra

Average dose injected (μCi/kg bw)	No. of mice	No. of bone sarcomas	Average time from injection to death (days)	Average skeletal dose (Gy) 140 days before death
Male, C57BL/Do (black)				
0	94	0	759	0
0.057	12	0	780	0.4
0.344	12	0	838	2.56
1.03	12	1	801	7.35
3.10	11	0	711	19.7
9.25	12	6	572	46.8
Female, C57BL/Do (black)				
0	87	1	741	0
0.057	12	0	743	0.38
0.344	12	0	761	2.32
1.03	12	0	699	6.4
3.10	12	3	675	18.6
9.25	14	4	498	39.6
Male, C57BL/Do (albino)				
0	60	0	608	0
0.058	10	0	768	0.39
0.344	15	0	758	2.33
1.08	18	0	700	6.77
3.23	14	0	635	18.1
9.88	14	1	527	45.5
Female, C57BL/Do (albino)				
0	58	0	626	0
0.058	15	0	727	0.37
0.344	12	0	752	2.31
1.08	11	0	766	7.43
3.23	14	1	752	17.6
9.88	10	4	727	56.5

From Taylor *et al*. (1983)

Rabbit: When rabbits [strain not specified] were injected with radium chloride [amount and dose is not clear], early changes were seen in lymphatic and haematopoietic tissues and cells. The authors also noted that two of the seven rabbits that survived 11–19 months developed osteogenic sarcomas (Sabin *et al.*, 1932).

Dog: Lifespan studies on the effects of ^{226}Ra given as a single injection to beagle dogs were started in 1952 in parallel with studies on ^{239}Pu, such that the results could be used to derive a 'toxicity ratio' for Pu:Ra that would serve as a basis for extrapolating data on the toxicity of Pu and other nuclides to humans.

A group of 120 young adult (17–20 months) beagle dogs equally divided by sex were given a single intravenous injection of ^{226}Ra at doses of about 0.2–440 kBq/kg bw (Lloyd *et al.*, 1991, 1993). An additional 132 animals served as unexposed controls. Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. One tumour was observed in the control group (Lloyd *et al.*, 1993). The distribution of ^{226}Ra in the bones was determined and expressed as a percentage of total activity by bone weight and percentage of total activity (Lloyd *et al.*, 1991). Fifty-seven primary skeletal malignancies were observed in 43 animals, of which 35 were found in the appendicular skeleton and 22 in the axial skeleton. The doses, average time to death, average skeletal dose to one year before death and the incidence of bone sarcomas are presented in Table 72. The authors concluded that, with few exceptions, the distribution of radium-induced skeletal malignancies follows the distribution of radium throughout the skeleton. Most tumours were observed in the tibia. The authors reported that the distribution of radium-induced skeletal cancers was similar in humans, with a preponderance of tumours in the appendicular versus the axial skeleton, and reflected skeletal mass and skeletal radium. One of the exceptions noted was a relatively greater number of tumours in the femur and pelvis of humans.

The location of selected ^{226}Ra-induced skeletal tumours was correlated with the relative amounts of cortical and trabecular bone determined by dissection and neutron activation analysis. The per cent tumour occurrence was linearly related to the corresponding percentage of cortical bone at these sites, resulting in a high correlation coefficient. A negative linear relationship between tumour location and trabecular calcium and trabecular surface was established (Jee *et al.*, 1986).

While skeletal cancers have been the predominant malignancy associated with exposure to ^{226}Ra in dogs exposed for life, the incidence of soft-tissue cancer has also been reported to be increased. Exposure to ^{226}Ra increased the incidence of eye tumours in dogs (Taylor *et al.*, 1972a; Lloyd *et al.*, 1994a; Taylor *et al.*, 2000), but tumours at this site have not been reported in humans exposed to radium. In dogs, radium is concentrated in the tapetum, a structure that is absent from the human eye.

In a detailed analysis of the soft-tissue tumours found among beagle dogs in these lifespan studies, it was suggested that the occurrence of skeletal tumours with increasing dose may have precluded the development of some soft-tissue lesions. In

Table 72. Dose–response relationship for skeletal malignancies in beagle dogs given a single intravenous injection of ^{226}Ra as young adults (17–20 months)

Dose injected (kBq/kg bw)	No. of dogs	No. of bone sarcomas	Average skeletal dose (Gy ± SD) 1 year before death	Age (years ± SD) at death with bone cancer	Age (years ± SD) at death without bone cancer
0	132	1	0	16.1	13.1 ± 2.6
0.275	10	0	0.28 ± 0.07	–	12.3 ± 1.9
0.651	25	2	0.80 ± 0.12	12.7 ± 1.7	13.4 ± 1.5
2.31	23	2	1.66 ± 0.77	10.8 ± 1.2	12.2 ± 3.5
6.13	14	2	3.57 ± 1.69	10.1 ± 1.6	10.8 ± 4.0
12.5	12	5	8.95 ± 1.98	10.3 ± 1.5	11.3 ± 4.0
39.6	12	11	19.1 ± 4.0	6.3 ± 1.1	5.8
119	12	12	43.3 ± 15.1	4.4 ± 0.5	2.4
383	9	9	101 ± 36	3.0 ± 0.5	2.4

From Lloyd *et al*. (1993)

this analysis, only the eye tumours were found to occur at a statistically significantly greater frequency in radium-exposed dogs than in the controls (Lloyd *et al.*, 1994a). In another analysis, no significant difference was found in the occurrence of the first or only mammary tumour, but a subsequent analysis indicated a significant relationship between exposure to ^{226}Ra and the number of mammary tumours and the age at which they occurred. With Cox regression statistics, an increase in risk for mammary tumours was associated with increasing dose (Bruenger *et al*., 1994).

In a lifespan study, 243 young adult beagle dogs, 435 days of age, were given eight fortnightly intravenous injections of graded doses of ^{226}Ra dissolved in a 0.1 N nitric acid–saline solution. A group of 78 control dogs was similarly injected, but the entire control group used in the comparisons included 159 animals. Skeletal tumours were detected by periodic radiographic and clinical examination and confirmed by histopathological evaluation after necropsy or amputation. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. Several papers were published while the study was in progress. Raabe *et al*. (1981, 1983) summarized the data up to 1978 and reported that more deaths from bone tumour occurred in dogs exposed to ^{226}Ra than to ^{90}Sr (see section 3.2.1(*c*)) on the basis of average dose rate to bone. The dose–response relationship for bone cancer was represented by a log-normal curve.

A total of 155 primary bone sarcomas were identified in 131 of the 246 exposed animals (Table 73). The limbs of dogs with single skeletal tumours that were free of metastasis or other debilitating disease were amputated; after amputation, the animal was removed from the main study but was monitored separately. The authors reported that 31 additional primary bone sarcomas developed in the 44 dogs that were removed from the study after amputation. Five primary bone sarcomas were found in four of the

Table 73. Dose–response relationship for primary bone sarcoma in beagle dogs given eight fortnightly injections of ^{226}Ra at 435–540 days of age

Total dose injected (kBq/kg bw)	No. of dogs		No. of dogs with sarcomas		Skeletal dose (Gy ± SD)	Median age at death (years)
	Male	Female	Male	Female		
0	80	78	3	1	0	14.6
0.789	21	25	0	0	0.9 ± 0.2	14.5
2.37	19	19	3	1	3.0 ± 1.1	13.8
13.9	19	22	12	14	13.9 ± 3.5	10.9
41.4	20	19	19	15	31.6 ± 6.5	7.4
124	19	22	19	22	77.6 ± 22.9	5.1
370	22	19	13	12	167 ± 44	4.3

From White *et al*. (1994)

158 unexposed controls (incidence, 2.5%). The osteosarcomas in the exposed animals were relatively evenly distributed between the males and females (72:74), and the ratio of those occurring in the appendicular skeleton versus the axial skeleton was 108:38. Non-osteosarcomas predominated in the controls and the animals at the two lower doses, while osteosarcomas predominated at the higher doses. The authors reported that the incidence of osteosarcomas tended to increase with dose (White *et al.*, 1994).

To study possible age-related effects on the induction of skeletal tumours, life-span studies were conducted on beagle dogs exposed as juveniles (three months), as young adults (17–18 months) or when mature (five years). Ten juvenile dogs, 12 young adults and nine mature dogs were given a single intravenous injection of 41 kBq/kg bw ^{226}Ra in a citrate solution. Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. The incidences of dogs with bone tumours were 7/10, 11/12 and 5/9, respectively (Bruenger *et al.*, 1991a). In a final analysis, Lloyd *et al*. (1999a) reported that the dogs exposed as juveniles or when mature had fewer bone tumours per Gy of average skeletal dose than those exposed as young adults. The relative radiation sensitivities were 0.66 ± 0.12 for the juveniles, 0.53 ± 0.09 for the mature animals and 1.0 for the animals exposed as young adults. The authors noted that some of the mature dogs died prematurely and thus may not have lived long enough to develop skeletal tumours. With this caveat, the authors concluded that young adult dogs appear to be at greater risk for developing a skeletal tumour than dogs exposed to an equivalent amount of ^{226}Ra as juveniles or when mature.

Some giant breed dogs have a higher spontaneous incidence of skeletal malignancies than smaller dogs. To determine whether such differences influence sensitivity to bone-

seeking nuclides and thus present a bias for extrapolating risk to humans, a limited lifespan study was conducted to compare the radiosensitivity of beagle and St Bernard dogs. ^{226}Ra in a citrate solution was given at a dose of about 0.2 to 40 kBq/kg bw by a single intravenous injection to 91 beagle and 23 St Bernard dogs of each sex, aged 554 ± 39 days. St Bernard dogs tended to have a shorter induction time for bone tumours than beagle dogs, but there was no proportional relationship between size and the incidence of tumours. The incidence of tumours is shown in Table 81 in section 3.1.2(*e*) (Taylor *et al.*, 1997).

(*c*) *Thorium-227, thorium-228, thorium-230 and thorium-232*

Mouse: ^{227}Th is a short-lived α-particle emitter (half-life, 18.7 days). The occurrence of osteosarcoma was compared in groups of 50 female BALB/c, C57BL and NMRI mice after a single intraperitoneal injection of 5 μCi/kg bw [185 kBq/kg bw]. At day 672 after exposure, the incidence of osteosarcoma (corrected for competing risks by the Kaplan-Meier statistical method) was 51%, 41% and 50% in the three strains, respectively. While tumours tended to occur earlier in the BALB/c mice, there were no significant differences among the three strains in the incidence of osteosarcomas. No osteosarcomas were observed in any of the controls (Luz *et al.*, 1982).

A study in which Thorotrast (colloidal $^{232}ThO_2$) was compared with ^{241}Am (Taylor *et al*., 1986) is described in the section on americium.

Rat: In a study to determine whether the tumour-inducing properties of Thorotrast were due to radiation, foreign body reactions or both, 20 groups of 96 Wistar rats, 12 weeks of age, were given various volumes and doses of colloidal $^{232}ThO_2$, some of which was enriched with ^{230}Th to achieve the desired radiation dose. In the volume experiments, the animals received 60, 120 or 300 μL of colloidal suspension; in the radiation experiments, the groups were given preparations in which the total α-particle emissions varied by factors of 1, 2, 5 and 10 relative to standard Thorotrast. The animals lived 8–41 months after injection. The occurrence of liver tumours was linearly related to dose, but no association with volume was established. The liver carcinomas, intrahepatic bile-duct carcinomas and haemangiosarcomas that developed in the exposed animals had similar histopathological features to those observed in humans; in addition, some benign tumours were noted, including liver-cell adenomas and intrahepatic bile-duct adenomas (Wegener *et al.*, 1988; Wesch *et al.*, 1983).

In another experiment in Wistar rats, a colloid with properties similar to Thorotrast, called Zirconotrast (ZrO_2, non-radioactive), was made 'radioactive' by the addition of various amounts of ^{228}Th/^{230}Th during its preparation. Various volumes and doses were injected. The results again demonstrated that the frequency of hepatic or splenic tumour-bearing animals depended on the dose rate and was not correlated with the number of injected particles. The pure non-radioactive colloid did not induce primary hepatic or splenic tumours in excess (Wesch *et al.*, 1986).

The livers of female Wistar rats, 3–4 months of age, were exposed to fractionated neutron irradiation at 14-day intervals (0.2 Gy per fraction) over two years to a total

dose of 10 Gy to simulate α-particles with no foreign-body effect. Before the start of irradiation, half of the animals received non-radioactive Zirconotrast. At the end of the lifespan study, about 40% of the irradiated animals had liver tumours. In the animals treated additionally with Zirconotrast, the incidence, time of onset and overall number of liver tumours were nearly equal, indicating that fractionated neutron irradiation was the only cause of the tumours (Spiethoff *et al*., 1992).

Hamster: Colloidal $^{232}ThO_2$ or ^{239}Pu citrate was administered to Chinese hamsters in order to compare the liver carcinogenicity of uniform (^{239}Pu) and non-uniform (^{232}Th) exposure. Hamsters of each sex, aged 90–120 days, were given intravenous injections of $^{232}ThO_2$ at 0.3, 1.5 or 7.4 kBq/kg bw or monomeric ^{239}Pu citrate at 7.4 kBq/kg bw. Some animals were killed for cytogenetic analysis (see section 4.4), while others were held for lifetime observation. In a Cox proportional hazard statistical model, dose-related increases in the incidences of hepatocellular carcinomas and hyperplastic lesions in the liver were found with $^{232}ThO_2$ (Guilmette *et al*., 1989).

Dog: Groups of 4–13 beagle dogs, approximately equally divided between males and females, were given a single intravenous injection of ^{228}Th in a citrate solution at the age of 482–559 days. Twelve dogs were given an injection of the citrate carrier and served as contemporary controls (age 557 days). In later analyses, additional controls were added for statistical analyses. The injected dose ranged from 0.063 to 31.7 kBq/kg bw (see Table 74). Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. The occurrence of skeletal tumours (Table 74) showed a dose–response relationship. In a linear regression analysis, the lifetime risk for developing a bone tumour was about 39% per Gy of average skeletal dose. It was concluded that the

Table 74. Bone sarcomas in young adult beagle dogs given a single intravenous injection of ^{228}Th

Injected dose (kBq/kg bw)	Total no. of dogs	No. of bone sarcomas	Skeletal dose (Gy) 1 year before death	Age at death (days)
0	12	0	0	4763 ± 346
0.063	13	0	0.13	4770 ± 316
0.192	12	2	0.39	4309 ± 392
0.562	12	5	1.13	4006 ± 308
1.12	12	11	2.14	2943 ± 136
3.41	12	12	4.44	1708 ± 47
10.7	12	12	10.28	1353 ± 48
31.7	4	2	26.30	1259 ± 58

From Mays *et al*. (1987)

toxicity ratio for skeletal cancers in comparison with ^{226}Ra was 8.5 ± 2.3 (Mays *et al*., 1987; Lloyd *et al*., 1997a).

(*d*) *Uranium (natural)*

Mouse: In a study of the relative effectiveness of ^{239}Pu, ^{241}Am and ^{233}U in producing osteosarcomas or leukaemias, groups of 50–100 CBA/H mice, 12 weeks of age, were injected intraperitoneally with one of three activity concentrations of ^{239}Pu, ^{241}Am or ^{233}U in the citrate form; a group of 100 controls were injected with non-radioactive citrate. The three doses were selected to provide average doses to the skeleton of 0.2–0.3 Gy, 0.5–1.0 Gy and 1.3–1.6 Gy on the basis of calculations from a parallel serial sacrifice study. The mice were held for lifetime observation and were necropsied and examined histologically. A total of 42 mice developed osteosarcomas. The incidences of osteosarcoma in the three dose groups were 2, 11 and 15%, respectively, with ^{239}Pu, 0, 3 and 21% with ^{241}Am and about 2% at each of the three doses of ^{233}U. The incidence of osteosarcomas in control mice was also about 2%. The relative risks for osteosarcoma were 4.2 with ^{239}Pu, 2.3 with ^{241}Am and 1.1 with ^{233}U. The relative risks with ^{239}Pu and ^{241}Am, but not with ^{233}U, were statistically significant at the 95% confidence level. Myeloid leukaemia was found in 47 radionuclide-injected mice but in none of the controls. The incidences were 4, 6 and 9%, respectively, at the three doses of ^{239}Pu, 4, 8 and 10% with ^{241}Am and 4% at each dose of ^{233}U. The relative risks for myeloid leukaemia were 1.8 with ^{239}Pu, 2.0 with ^{241}Am and 1.5 with ^{233}U. The relative risks with ^{239}Pu and ^{241}Am, but not with ^{233}U, were statistically significant at the 95% confidence level (Ellender *et al*., 2001).

Rat: Four groups of 63 male Sprague-Dawley rats, six weeks of age, were acclimatized to the exposure process and then, at nine weeks of age, were exposed daily by inhalation in nose-only inhalation chambers to aerosols of uranium ore dust at a concentration of 0 (control), 19 or 50 mg/m^3. The ore used contained 44% elemental uranium, and about 75% of the mass of airborne uranium was in particles < 5 μm. Exposure was for 4.2 h per day on five days per week for 65 weeks. When the exposure regimen was completed, the rats were held for lifetime observation. At death, each animal was necropsied and examined grossly and histologically, and the lung burdens of U were determined radiochemically for use in calculating the total absorbed dose of α-particles. The average doses were 0.87 and 1.64 Gy. Survival data were plotted but not analysed statistically. The numbers of primary malignant lung tumours and the numbers of rats examined were: controls, 1/63; 19 mg/m^3, 22/126 and 50 mg/m^3, 20/61. The corresponding numbers of primary benign lung tumours were: controls, 1/63; 19 mg/m^3, 17/126; and 50 mg/m^3, 8/61. All but one of the benign lung tumours were bronchioloalveolar adenomas. Of the malignant lung cancers, bronchioloalveolar carcinomas were most prevalent, followed by bronchiolar carcinomas and squamous-cell carcinomas (Mitchel *et al.*, 1999).

(*e*) *Plutonium-238 and plutonium-239*

(i) *Inhalation*

Mouse: The effects of protracted and single exposures to $^{239}PuO_2$ were compared in C57BL/6J mice. [The Working Group noted that most of the ^{238}Pu and ^{239}Pu source material also contained small amounts of ^{240}Pu.] Two groups of mice were exposed once at either 84 or 460 days of age to achieve an initial lung burden of 20, 90, 460 or 2300 Bq. The groups given protracted exposure from the age of 84 days were exposed every other month for up to six exposures in 10 months in order to establish a lung burden of 20, 90 or 460 Bq. The mice were permitted to live out their lifespans and were then necropsied. The groups of mice with protracted exposure to similar cumulative doses to the lung had a 3.4–4.4 times greater incidence of pulmonary tumours (identified as adenomas and adenocarcinomas) than those exposed once. The excess number of pulmonary tumours per unit dose to the lung was also greater in the groups given protracted exposures than in those exposed once (Lundgren *et al.*, 1987).

Groups of 15 male and 15 female heterozygous $p53^{+/-}$ knock-out mice and wild-type $p53^{+/+}$ mice were exposed to 500 Bq of $^{239}PuO_2$ by inhalation. Some of the mice were killed at six months, and the remainder were permitted to live their lifespan. Four of 29 knock-out $p53^{+/-}$ mice developed lung tumours, and the latency of these tumours was significantly shorter than that of the seven lung tumours that developed in 30 of the wild-type $p53^{+/+}$ mice. The data indicate that the *p53* allele plays a role in the expression of radiation-induced cancers in mice (Finch *et al.*, 1998).

Rat: The effect of neonatal thymectomy on lung cancer induction after inhalation of $^{239}PuO_2$ was studied to examine the influence of cell-mediated immunity, which is suppressed after neonatal thymectomy. Thymectomy was carried out within the first 24 h of birth of male and female Wistar rats. At 90 days of age, the animals were exposed to $^{239}PuO_2$ by inhalation (count median aerodynamic diameter, about 2.06 μm). A total of 258 rats were used, but some were eliminated during the study for various reasons. One group underwent sham surgery and was not exposed, one group was thymectomized and not exposed, while a third group was thymectomized and exposed. The lung dosimetry indicated a broad range of doses, such that the groups were divided into four subgroups: 101–300, 301–1000, 1001–3000 and 3001–15 000 rad [1–3, 3–10, 10–30 and 30–150 Gy]. Significant numbers of lung tumours were induced in the exposed animals, but there were no differences between the intact and thymectomized groups (total, 64/122 compared with 38/70). An increase in the incidence of extrapulmonary neoplasms was attributed to the effects of thymectomy (5/122 compared with 12/70). While thymectomy had no significant effect on the frequency of lung tumours, differences were noted in the staging of the cancers that did develop, including an increase in tumour size, enhanced tumour invasion and a greater frequency of regional metastases (Nolibe *et al.*, 1981).

A series of papers have been published on a lifespan study in which female Wistar rats were exposed to ^{239}Pu aerosols. In the first paper in a series (Sanders, 1992a), the

experimental design and lung dosimetry methods and calculations were reported. The study consisted of 2105 exposed and 1052 sham-exposed rats. The body weights at the time of exposure were about 250 g. The rats were exposed by nose inhalation only to a high-fired mixture of $^{169}Yb_2O_3$–$^{239}PuO_2$ with an activity median aerodynamic diameter of 1.6 ± 0.11 μm. The average activity ratio of Yb:Pu was 0.4. Doses to the lung were calculated for the 2105 exposed rats, whereas the doses of the controls were assumed to be at background or below 0.002 Gy. The animals were permitted to live out their lifespan, and an extensive range of tissues were examined histopathologically. Survival was reduced only for rats exposed to doses > 30 Gy. Except for pulmonary tumours, no significant difference was found in tumour location or type between the controls and the exposed rats; 90% of the non-pulmonary tumours were in the pituitary gland, mammary gland, uterus and thyroid. Although twofold greater incidences of tumours of the Zymbal gland, bladder, brain and liver were observed in exposed rats, the tumour incidence in each of these organs was < 1%. Ninety-nine primary lung tumours were identified, of which 92% were classified as malignant and 80% were carcinomas. Of the malignant tumours, 49 were squamous-cell carcinomas, 23 adenocarcinomas, nine haemangiosarcomas, seven adenosquamous carcinomas and three fibrosarcomas. In the controls, one adenocarcinoma was observed. Animals exposed to < 1.5 Gy developed only four adenomas. The lowest doses at which the various types of tumours developed were 1.5 Gy for squamous-cell carcinoma, 3.1 Gy for adenocarcinoma, 4.1 Gy for haemangiosarcoma and about 9 Gy for adenosquamous carcinoma and fibrosarcoma. The incidences of all lung tumours were 0.095% in the control rats, 0.21% in the 1877 rats that received lung doses < 1 Gy and 41% in the 228 rats that received > 1 Gy. The average absolute risk for malignant lung tumours was 270 lung tumours per 10^4 rat–Gy above a dose of 1 Gy (Sanders *et al.*, 1993a,b).

Five pleural mesotheliomas were observed among the 2105 exposed rats. In a comparison of the results of this study with those of previous studies in which $^{239}PuO_2$ was given by intraperitoneal injection, the $^{239}PuO_2$ particles were found aggregated on mesothelial surfaces after injection, resulting in a higher relative incidence of these tumours (four mesotheliomas in 527 exposed rats) (Sanders, 1992b).

A total of 2272 female and 138 male Wistar rats were exposed by inhalation to $^{239}PuO_2$, and the incidence of brain tumours was compared with that in 1058 female and 60 male controls. Survival was compared by a life-table approach and the incidence of tumours by a Mantel-Haenzel statistic. In the females, six brain tumours were found in the 1058 controls and 24 brain tumours in 2134 exposed rats (survival-adjusted $p = 0.29$). In the males, two tumours were found in the 60 controls and seven tumours in the 138 exposed rats (survival-adjusted $p = 0.33$). The incidence of brain tumours in males was thus about five times greater than that in females ($p = 0.0001$), but this was not related to the exposure. The tumour types were distributed similarly among the control and exposed animals, and the mean lifespans of control and exposed rats with brain tumours were not significantly different (Sanders *et al.*, 1992).

To determine whether the strain of rat determines pulmonary carcinogenesis after exposure to ^{239}Pu, the results for Wistar rats were compared with those for Fischer 344 rats. Two hundred female Fischer 344/nTac rats were exposed at 70 days of age to an aerosol of $^{239}PuO_2$ at 0.8–1.0 Gy (lower doses) or 34–37 Gy (higher doses), and 60 rats served as sham-exposed controls. The protocol was similar to that described above for Wistar rats. The median survival times were similar in the control and low-dose groups of both strains but were significantly decreased in the high-dose groups when compared with controls. Squamous metaplasia was observed in 62–65% of the high-dose groups of both strains but not in the controls. The incidence of adenomatous metaplasia was significantly higher in the controls and low-dose groups of Fischer 344 rats than Wistar rats. The incidences of lung tumours in Fischer 344 rats were 1.7% in the controls, 20% at the low doses and 82% at the high doses, whereas the incidences of lung tumours in Wistar rats were 0.1% in the controls, 0% at the low doses and 68% at the high doses. Rats of both strains at the high doses that died with lung tumours had longer median survival times than rats at these doses that died without lung tumours. These differences were not observed at the low doses, in which the absolute risk for lung tumours was 1900 per 10^4 rat–Gy for Fischer 344 rats and 0 for Wistar rats; the absolute risk in the high-dose groups of both strains was about 210 tumours per 10^4 rat–Gy. The adenomatous tumour types predominated in Fischer 344 rats, while squamous tumours dominated in Wistar rats. The authors concluded that overall, Fischer 344 rats are more 'sensitive' than the Wistar strain (Sanders & Lundgren, 1995).

Five hundred female Fischer 344/N rats, aged 13 ± 2 weeks, were exposed to a nebulized ^{239}Pu aerosol, and the dosimetry was determined with a ^{169}Yb tracer on days 7, 14 and 21 after exposure. Six rats were killed after 7, 14, 30, 60, 120, 240 and 360 days and the tissues prepared for histological and morphometric evaluation. For determination of cytokinetics, five exposed and five control rats were each given ^{3}H-thymidine as a marker of cell proliferation, and autoradiographs were prepared. The measured retention of ^{239}Pu was calculated to commit an average dose to the lung of 16 Gy 500 days after exposure. Maximal increases in alveolar and bronchiolar epithelial cell labelling were seen 30 days after exposure and then decreased. Focal proliferative epithelial lesions had developed in the lung by 180 days and preceded the onset of lung neoplasms. Neoplasms, primarily adenocarcinomas and squamous-cell carcinomas, were initially observed at 308 days. It was concluded that Pu-induced pulmonary neoplasms develop through a sequence of focal proliferative lesions that represent developmental preneoplastic lesions (Herbert *et al.*, 1993).

In another study in Fischer 344 rats, the measured retention of inhaled ^{239}Pu was calculated to result in an average dose to the lung of 16 Gy 500 days after exposure. Immunohistochemical, histological and ultrastructural methods were used to study each histological type of lesion. The epithelial cells of the proliferative lesions and neoplasms had ultrastructural features consistent with type II pneumocytes, and the authors concluded that most ^{239}Pu- induced proliferative lesions and neoplasms in rats originate from alveolar type II pneumocytes (Herbert *et al.*, 1994).

The long-term effects of single and repeated exposure to $^{239}PuO_2$ was studied in 84-day-old Fischer 344/Crl rats. For repeated exposure, rats were exposed by inhalation to aerosols of $^{239}PuO_2$ seven times at two-month intervals: 123 rats were exposed 130 ± 52 Bq, 71 rats to 410 ± 140 Bq and 105 rats to 1500 ± 590 Bq; 98 rats were sham-exposed and held for lifetime observation. The single exposures consisted of 146 rats at 11 ± 7 Bq, 119 rats at 140 ± 81 Bq, 101 rats at 370 ± 210 Bq and 40 rats at 1400 ± 560 Bq; 82 sham-exposed rats were held for lifetime observation. Animals that died or were killed when moribund were necropsied and examined for tumours by histopathology. The incidences of lung tumours were not significantly different in the groups of rats with similar lifetime mean doses to the lungs of 0.9 ± 0.39 to 4.4 ± 1.8 Gy, whether exposed once or repeatedly. Rats that received a mean dose of 12 ± 2.4 Gy in a single exposure had a significantly higher crude incidence of lung tumours than animals that received 10 ± 2.1 Gy to the lung by repeated exposure. The crude incidence rates of benign and malignant tumours in rats that inhaled a single or repeated doses of $^{239}PuO_2$ as a function of dose to the lung could be described by a curve from a single-parameter Weibull model. The results for lung tumours in young adult rats that inhaled an insoluble form of ^{144}Ce, $^{144}CeO_2$, once or at repeated doses (see section 3.2.2(*c*)) might also be represented by this type of curve. The two curves of the crude incidence of lung tumours versus dose to lung for $^{239}PuO_2$ and $^{144}CeO_2$ were separated along the dose axis by a factor of about 21, reflecting the greater effectiveness of α-irradiation from $^{239}PuO_2$ than the β-irradiation from $^{144}CeO_2$ (Lundgren *et al.*, 1995).

The incidence of ^{239}Pu-induced pulmonary tumours was studied in rats with bleomycin-induced pulmonary fibrosis in order to identify possible modifying factors for radiation-induced carcinogenesis. Equal numbers of male and female Fischer 344/Cr1 rats, 14–16 weeks of age, received bleomycin by intratracheal instillation at a dose of 8.5 IU/kg bw to induce pulmonary fibrosis; 45–49 days later, the animals were exposed to an aerosol of ^{239}Pu at an initial lung burden of 85 or 850 Bq with a ^{169}Yb tracer for dosimetry. A group without induced fibrosis was included. Retention of ^{239}Pu in the lungs was studied in rats killed 2 h and 8, 16, 32, 64, 128, 192, 359 and 541 days after exposure. A cross-sectional study of lung function was conducted in 4–11 females in each exposed group (control, low and high dose) and in the two groups with and without bleomycin. Clearance of ^{239}Pu from the lungs was significantly decreased and the incidence of non-neoplastic lung lesions was significantly increased in the rats with fibrosis than in controls. Groups of rats that received similar doses of α-particles showed no significant effects of pre-existing pulmonary fibrosis on the incidence of neoplastic lesions in the lung, time to death of rats with lung neoplasms or the risk for lung tumours per unit dose of α-particles (Lundgren *et al.*, 1991).

A total of 310 female Wistar rats, eight weeks of age, were exposed once by inhalation to an aerosol of $^{239}PuO_2$ and then classified into one of seven groups on the basis of the mean initial lung deposition and cumulative lung dose, ranging from 0.71 to 8.5 Gy. An unexposed group of 130 controls was available. The study design and

results are shown in Table 75. At death, the animals were necropsied, their tissues were assessed by routine histopathology, and sections from all epithelial tumours were stained immunocytochemically for intranuclear *p53* protein. Primary lung tumours were found in 2.3% of the unexposed controls, in about 44% of those at a mean lung dose of 0.71 Gy and in 97% at 5.4 Gy. The dose-related appearance differed by histological type of tumour: the maximum incidence of adenomas was seen at 0.71 Gy, adenocarcinomas at 2.9 Gy and adenosquamous and squamous-cell carcinomas at 5.4–8.5 Gy (Oghiso *et al.*, 1994a, 1998).

Table 75. Primary lung tumours in female Wistar rats exposed to an aerosol of $^{239}PuO_2$

Initial lung deposition (Bq)	Number of animals	Lung dose (Gy)	Length of survival (days)	No. of primary lung tumours	Crude incidence (%)
0	130	0	790 ± 144	3	2.3
97 ± 27	43	0.71 ± 0.19	871 ± 105	19	44.2
225 ± 48	75	1.52 ± 0.28	712 ± 162	45	60.0
461 ± 118	60	2.88 ± 0.51	631 ± 158	46	76.7
787 ± 79	40	4.67 ± 118	675 ± 98	37	92.5
948 ± 76	31	5.43 ± 0.29	622 ± 105	30	96.7
1147 ± 114	31	6.61 ± 0.28	550 ± 82	29	93.5
1672 ± 261	30	8.52 ± 0.67	458 ± 95	27	90.0

[a] From Oghiso *et al.* (1998)

In studies of the possible synergistic effect of $^{239}PuO_2$ with the environmental chemical carcinogen benzo[*a*]pyrene, eight groups of male Wistar rats, two months of age, received $^{239}PuO_2$ at various doses with or without benzo[*a*]pyrene given as two intratracheal instillations of 5 mg/animal. The results are shown in Table 76. Survival decreased with increasing dose of plutonium and exposure to benzo[*a*]pyrene. Some of the data fit a multiplicative relative risk model. The incidence of malignant lung tumours, adjusted for differences in survival, increased in a dose-related manner with dose of $^{239}PuO_2$ and was further increased in the presence of benzo[*a*]pyrene (Métivier *et al.*, 1984).

Hamster: Plutonium microspheres injected intravenously into Syrian hamsters were reported to lodge in the lung capillaries and to produce a low incidence of lung tumours (Anderson *et al.*, 1975). In a study to determine whether this response is unique to the hamster or whether a dose delivered by inhalation rather than injection would cause tumours in these animals, 10 groups of 14–34 hamsters of each sex were given microspheres of ^{239}Pu- or ^{238}Pu-laden ZrO_2 ceramic particles about 10 μm in diameter by intravenous administration. These particles were large enough to lodge in the capillaries of

Table 76. Lung tumours in Wistar rats given ^{239}Pu and benzo[*a*]pyrene

Initial lung deposition (Bq)	Benzo[*a*]-pyrene (mg)	No. of animals	Median survival (days)	Median life-time dose (Gy)	No. of pulmonary malignancies
0	0	89	864	0	0
220	0	89	820	3.3	17
630	0	30	798	9.4	14
6300	0	19	345	76.3	6
0	2 × 5	38	675	0	10[a]
220	2 × 5	29	444	2.9	17[b]
630	2 × 5	22	480	8.5	16
6300	2 × 5	19	330	75.4	19

From Métivier *et al*. (1984)
[a] Also two fatal benign tumours
[b] Also 10 fatal benign tumours

the lung. One week later, six groups were additionally exposed by inhalation to the same plutonium-laden ZrO_2 particles, which were generally spheroidal and had an activity median aerodynamic diameter of approximately 1.5–2.0 μm. An untreated control group was available. The mean initial lung burdens were 8–143 nCi [296–5291 Bq] of ^{239}Pu in three groups and ^{238}Pu in the other three groups. The particles were tagged with a ^{57}Co tracer to permit whole-body counting. The animals were permitted to live out their life-spans, and moribund animals were killed. All animals were necropsied, and pathological diagnoses were confirmed by histopathology. Males in both control and treated groups lived longer than females. A plot of the survival data showed that animals that received the highest burden by inhalation had shorter lifespans than the controls, but this was not seen at lower doses. The results suggested a decreased induction time and an increased total incidence of lung tumours with increasing lung burden, particularly with ^{238}Pu. Lung tumours developed in 5–50% of animals exposed by inhalation. Inhalation of plutonium particles was also associated with lung fibrosis. Concomitant intravenous administration of particles with plutonium had little effect on the incidence of respiratory and non-neoplastic, degenerative changes in the respiratory tract. No pulmonary tumours were seen in animals treated only by intravenous injection (Thomas & Smith, 1979).

Groups of 25 female hamsters, four months of age, were exposed to a high-fired $^{239}PuO_2$ with an activity median aerodynamic diameter of 1.64 ± 0.13 μm. The initial lung burden 10 days after exposure was 2.4 ± 1.7 kBq, and, in animals killed 5–14 months after inhalation, the lung burdens were calculated to be 0.1–0.5 kBq. The dose to the lung one year after exposure was estimated to be about 12 Gy. Groups exposed to benzo[*a*]pyrene (positive control) and saline (negative control) were included. At scheduled necropsy 5–14 months after exposure, the lungs were dissected and the 10

most 'suspicious' 1-mm^3 areas were removed. Half of each sample was prepared for histology, and the other was transplanted into hamster cheek pouches for one month and then removed for histopathological evaluation. None of the lung transplants from the hamsters exposed to $^{239}PuO_2$ or the negative controls grew in the recipient cheek pouches, but 14% of the pulmonary lesions from the benzo[*a*]pyrene-exposed positive controls grew when transplanted (Sanders & McDonald, 1992). [The Working Group noted that these and other authors found that the hamster does not appear to be particularly sensitive to the development of plutonium-induced lung tumours when compared with other species].

Dog: Lifespan studies of beagle dogs have been conducted at several laboratories. The results have been reported both independently and combined for statistical strength. Studies in which dogs were given a single exposure to $^{238}PuO_2$ with a count median diameter of 0.1 μm were started in 1967 at the Pacific Northwest Laboratories in the USA, and in 1972 a study was begun with a single exposure to $^{238}PuO_2$ with an activity median aerodynamic diameter of 1.8 μm. At the Inhalation Toxicology Research Institute (ITRI), also in the USA, a study in which $^{238}PuO_2$ with an activity median aerodynamic diameter of 3.0 μm was given was started in 1973 and a study with $^{238}PuO_2$ with an activity median aerodynamic diameter of 1.5 μm was started in 1974.

The studies conducted at the Pacific Northwest Laboratories involved 136 beagle dogs in groups of 13–22 animals each, approximately equally divided by sex. A total of 116 dogs were exposed once to $^{238}PuO_2$ aerosols, resulting in lung depositions of 0 (controls), 0.13, 0.68, 3.1, 13, 52 and 210 kBq, and were observed for life. Interim reports were published as the study progressed, the early reports presenting clinical findings that had appeared at the higher doses. Some dogs developed respiratory insufficiency due to plutonium-induced pneumonitis within three years of exposure (Park *et al*., 1976). Nine of the first 11 dogs that were killed 4–6 years after exposure had developed osteosarcomas, and 30–55% of the terminal plutonium body burden was in the skeleton. Park *et al*. (1997) summarized the completed study. In the tissues of the 30 dogs that survived the longest (mean, about 14 years), about 1% of the final body burden was found in lung, 46% in the skeleton, 42% in the liver and 6% in the thoracic lymph nodes. Of the 116 exposed dogs, 34 (29%) developed skeletal tumours, 31 (27%) developed lung tumours and eight (7%) developed liver tumours. Bone tumours were the primary cause of death at the three higher doses, and the survival of these animals was significantly decreased. The total accumulated doses of radiation to the lung were higher than the average skeletal dose, but more deaths were attributed to skeletal than to lung tumours. Some of the deterministic effects observed included radiation pneumonitis, osteodystrophy, hepatic nodular hyperplasia, lymphopenia, neutropenia and sclerosing tracheobronchial lymphadenitis. Hypoadrenocorticism was also observed in a few dogs.

In the study conducted at the ITRI, 72 beagle dogs were exposed once to monodisperse aerosols of $^{238}PuO_2$ with an activity median aerodynamic diameter of 1.5 ± 1.2 μm; 72 dogs were exposed once to $^{238}PuO_2$ particles with a diameter of

3.0 ± 1.1 μm, and 24 dogs served as sham controls. Equal numbers of female and male dogs were entered into the study. The dogs were observed for life, with periodic examinations. A detailed post-mortem examination was done on all animals, and their tissues were processed for histopathology. Gillett *et al*. (1988) summarized the study to date. Significant translocation of ^{238}Pu from the lung to other tissues, especially the liver and bone, was observed. Of the 144 dogs that had been exposed, 112 had died by 4000 days after exposure. Of these dogs, 100 had developed an osteosarcoma and 28 had developed a lung cancer. Liver lesions were observed with greater frequency with increasing time after exposure. Ten primary liver cancers were diagnosed in animals that survived to 4000 days, and an additional five tumours were found in three of the nine animals that were killed after 4000 days. Most of the liver tumours were classified as fibrosarcomas and were generally not the cause of death.

Muggenburg *et al*. (1996b) summarized some of the additional findings from this study and reported that bone tumours had developed in 93 of the 144 dogs, lung tumours in 46 and liver tumours in 20. Some dogs had tumours in all three of these organs. Liver tumours occurred later than bone and lung tumours. The skeletal distribution of the bone tumours in dogs that inhaled $^{238}PuO_2$ was reported to be similar to that seen in dogs injected with ^{239}Pu citrate (Lloyd *et al*., 1994b).

A statistical analysis of the combined data from these studies involved use of age-specific risk (hazard functions) to evaluate the relationships between lung, liver and bone tumours and cumulative dose of radiation and to estimate lifetime risks. For the lung tumours, a linear–quadratic function provided an adequate fit to the data from both laboratories, and linear functions were adequate for doses < 20 Gy. Some significant differences were found between the data from the two laboratories, the estimated risk coefficients for these functions being larger when based on the data from ITRI than when based on those from the Pacific Northwest Laboratories. Furthermore, the bone tumour response functions appeared to differ between the two laboratories, but mainly at higher dose rates. The authors attributed the possible differences to 'dosimetry biases'. Both studies provided evidence of radiation-induced bone tumours at doses < 0.5 Gy. The risk for liver tumours was similar in the two laboratories, and linear functions provided an adequate fit to these data (Gilbert *et al.,* 1998b).

In dogs that inhaled $^{239}PuO_2$, significant lymphopenia was observed in 58% of the animals at the five higher doses (0.69–213.3 kBq), and lymphoid atropy, sclerosis of the thoracic lymph nodes and lymphopenia were observed at the four higher doses (> 2.5 kBq). Using a linear regression analysis, the authors found a moderate correlation between the reduction in lymphocyte values and initial lung deposition, in both magnitude and the time of appearance after exposure. No primary tumours were identified in the thoracic lymph nodes in this study, but lung tumours were found in 70% of the dogs with lymphopenia (Weller *et al.,* 1995a).

The effects on the liver of dogs exposed to $^{239}Pu(NO_3)_4$ are summarized in Table 77. At the end of the study, the liver contained $40 \pm 1\%$ of the plutonium, but this was less than the amount found in the skeleton. Autoradiographs indicated that the

Table 77. Incidences of liver tumours in dogs exposed to $^{239}Pu(NO_3)_4$ by inhalation

Initial lung burden (kBq/kg bw)	No. of animals exposed	Median survival (months) after exposure	No. of dogs with liver tumours	Liver dose (mGy)
0	20	139	1	0
0 (vehicle)	20	154	0	0
0.98 ± 0.18	20	150	3	17 ± 3
2.6 ± 0.3	20	157	3	42 ± 5
19 ± 2	20	135	3	310 ± 40
91 ± 7	20	114	5	1110 ± 70
518 ± 51	20	62	0	2960 ± 2420

From Dagle *et al*. (1996)

parenchymal cells received a higher dose rate than would have been calculated if the distribution of plutonium were considered uniform (e.g. radiochemical analysis of the whole organ). Liver tumours, primarily bile-duct epithelial tumours, occurred late and were observed at doses at which the lifespan was not shortened by lung or bone tumours (Dagle *et al.*, 1996).

The incidence of pulmonary cancers among 108 beagle dogs exposed to $^{239}PuO_2$ at the age of 12–18 months is shown in Table 78. The lung was the sole target organ for neoplasia. An increased incidence of lung carcinomas was observed in animals that received doses ≥ 2 Gy; 178 neoplasms were found, almost all of which were carcinomas (47% carcinoma, 40% adenocarcinoma, 27% bronchioloalveolar carcinoma, 12% adenosquamous carcinoma and the remainder bronchial gland carcinoma, carcinosarcoma and sarcoma) (Hahn *et al.*, 1999).

The occurrence of testicular tumours in dogs exposed to $^{238}PuO_2$, $^{239}PuO_2$ and $^{239}Pu(NO_3)_4$ in these studies was analysed separately. A statistical correlation was found between the initial lung burden and the final concentration of plutonium in the testes, the per cent of the initial lung burden in the testes ranging from 0.0001 to 0.03%, depending on the solubility of the compound. There was, however, no statistically significant difference among the three plutonium-exposed groups or the control group in the cumulative proportion of dogs with testicular tumours, the distribution of tumour types and the mean time to first tumour appearance (Weller *et al.*, 1995b).

Monkey: Twenty male cynomolgus monkeys were exposed to aerosols of $^{239}Pu(NO_3)_4$ resulting in lung burdens of about 4, 10 and 40 kBq. Six mature and six immature monkeys were exposed to 40 kBq and two mature and two immature monkeys to 10 or 4 kBq; two mature and two immature monkeys were exposed to the carrier aerosols and served as controls. The animals were killed or died 0.1, 1, 12, 40 or 99 months after exposure. The concentration of ^{239}Pu in the liver increased to a maximum, one year after exposure, but had decreased to about 10% of this value by

Table 78. Incidences of lung tumours in dogs exposed to $^{239}PuO_2$ by inhalation

Initial lung burden (kBq/kg bw)	No. of animals exposed		Median survival (days) after exposure	Incidence of lung tumours
	Males	Females		
0 (control)	18	18	4846	0
0.19	11	11	4871	7/22
0.63	17	19	4058	15/22
1.6	25	21	3080	32/36
3.7	16	16	2022	28/32
6.3	12	17	1422	19/29
14	14	13	737	4/27
30	13	11	387	0/24

From Hahn *et al*. (1999)

99 months after exposure. ^{239}Pu was efficiently cleared from the lungs, so that by 99 months < 0.05 kBq of the initial 40 kBq remained. The skeletal content of plutonium increased during the first year, but the total skeletal activity remained relatively constant for the remainder of the study; the relative fraction of ^{239}Pu in the skeleton increased during this time, however, because of clearance of ^{239}Pu from other organs. Three of the animals at the highest dose died of radiation-related pulmonary pneumonitis or fibrosis. One primary papillary adenocarcinoma of the lung was observed at the high dose at 99 months (Brooks *et al.*, 1992). [The Working Group noted the short duration of the study and the fact that it was designed to investigate toxicity and chromosomal effects (see section 4.4).]

(ii) *Injection*

Mouse: In the study of Taylor *et al*. (1983), described in section 3.1.2(*b*), groups of 11–13 mice of each sex of two strains (C57BL/Do black and C57BL/Do albino) were given an intraperitoneal injection of ^{239}Pu at 0.016–2.92 μCi/kg bw [0.6–108 kBq/kg bw] at about 10 weeks of age. The frequency of induction of skeletal cancers was about four times greater in female than in male mice of both strains, a relationship that was not observed in life-span studies in dogs (Lloyd *et al*., 1999b). When the data for both sexes and strains were combined, the relative effectiveness of ^{239}Pu in inducing skeletal cancers relative to a defined relative effectiveness of ^{226}Ra of 1, was 15.3 ± 3.9. In another study, a similar incidence of ^{239}Pu-induced bone tumours was seen in castrated male and female C57BL/Do (albino) mice (Taylor *et al*., 1981).

The effects of a single injection of monomeric ^{239}Pu-citrate were studied in adult grasshopper mice (*Onychomys leukogaster*), which are known to retain plutonium in the liver longer than strains of mice used routinely in the laboratory. The purpose of the study was to compare the responses to ^{239}Pu with those to ^{241}Am and Thorotrast.

Two groups of 10 male and 10 female mice aged 130 ± 31 days were given a single intraperitoneal injection of ^{239}Pu at a dose of 44 or 129 kBq/kg bw and permitted to live for their lifespan. They were compared with 49 controls. Eighteen primary liver tumours were reported, which included adenomas, carcinomas and sarcomas. The lowest average dose to the liver among the mice given ^{239}Pu was about 9 Gy. The risk coefficient was similar to that observed with similar doses in other strains of mice (Taylor *et al.*, 1993).

Groups of 100 female ICR mice, 10 weeks of age, were given an intravenous injection of ^{239}Pu at a dose of 0 (control), 3.0, 6.0 or 12.3 kBq/kg bw. The animals were killed when moribund or when found dead, and complete necropsies were performed. ^{239}Pu retention was measured in a separate experiment. The survival rates and the incidences of haematopoietic tumours and osteosarcomas are shown in Table 79. The increased incidence of osteosarcomas was dose-related, and the length of survival was significantly shorter for mice that died of ^{239}Pu-induced myeloid and lymphocytic neoplasms. The haematopoietic tumours were categorized into myeloid leukaemia, lymphocytic leukaemia, lymphosarcoma and reticulum-cell sarcomas. When the haematopoietic tumours were taken together, their occurrence was independent of the dose of radiation, but the frequencies of myeloid and lymphocytic leukaemia and lymphosarcoma were associated moderately strongly with exposure (Svoboda & Bubeníková, 1990). Studies on the occurrence of myloid leukaemias and osteosarcomas in mice after exposure to ^{239}Pu had been reported previously by this group (Svoboda *et al.*, 1981), and some morphological aspects of ^{239}Pu-induced granulocytic leukaemias were discussed in another paper (Svoboda *et al.*, 1982).

Female C3H mice, which have low spontaneous incidence of skeletal tumours and myeloid leukaemias, received ^{239}Pu citrate by injection at doses of 10–11 600 Bq/animal. A total of 260 exposed and 100 control animals were entered into the experiment. The survival time was significantly reduced at mean skeletal doses > 2.9 Gy, due to the appearance of bone and lymphoid tumours. The incidence of osteosarcomas was dose-dependent, reaching a maximum of 70% in animals that received a mean skeletal dose

Table 79. Incidences of haematopoietic and bone tumours in groups of 100 mice injected with ^{239}Pu

Dose injected (kBq/kg bw)	Mean survival (days ± SE)	No. of haematopoietic tumours	No. of osteosarcomas
0 (control)	617 ± 15	43	0
3.0 ± 0.1	599 ± 16	45	2
6.0 ± 0.1	569 ± 16	44	3
12.3 ± 0.1	590 ± 10	43	10

From Svoboda & Bubeníková (1990). SE, standard error

< 10 Gy; no osteosarcomas were observed in the controls. An increased incidence of non-thymic, mostly pre-B cell-type leukaemic lymphomas occurred early after ^{239}Pu exposure, whereas thymic, lymphocytic or histocytic lymphomas occurred in the controls only later. Myeloid leukaemias and myelogenous neoplasms were not observed in exposed or control animals (Oghiso *et al.*, 1994b, 1997).

These results differ from those of an earlier study in groups of 36–45 female CBA mice, 12 weeks of age, which were given ^{239}Pu in either a single injection or in 16 injections at 3–5-day intervals at doses of 1.85–18.5 kBq/kg bw. A significant, dose-related increase in the incidence of osteosarcomas was seen with both single and repeated injections. Myeloid leukaemias were found in five animals given multiple injections, whereas none had been observed in 900 controls in that laboratory (Humphreys *et al.*, 1987).

A short communication summarized the occurrence of skeletal and lymphoid cancers induced by ^{239}Pu given by intraperitoneal injection to C3H, C57BL/6 and hybrid $BC3F_1$ mice. Similar dose–response relationships were seen for both bone and lymphoid tumours in the three strains of mice, but the frequency of various types of lymphoid tumour appeared to differ. The incidence of bone tumours was significantly increased at skeletal doses from about 0.6–0.7 Gy. Pre-B-cell leukaemic lymphomas were induced preferentially soon after ^{239}Pu exposure, whereas myeloid leukaemias and other myelogenous neoplasms were either rare or not observed, depending on the strain. The authors concluded that the ^{239}Pu-induced tumours were different from those observed in studies after exposure to external low-LET radiation (Oghiso & Yamada, 1999).

Hamster: A total of 145 male and female Chinese hamsters, 100–130 days of age, were injected in five groups with ^{239}Pu citrate at activity levels of 0.074–740 kBq/kg bw, and 190 animals in six groups were injected with $^{239}PuO_2$ particles. Three groups were given a dose of 0.74, 7.4 or 74 kBq/kg bw in a particle size of 0.24 μm; one group was given 74 kBq/kg bw in a particle size of 0.84 μm, one group was given 74 kBq/kg bw in a particle size of 0.60 μm, and one group was given 74 kBq/kg in a particle size of 0.17 μm. A control group consisted of 38 animals injected with either the sodium citrate or sodium chloride solvents, and an additional 55 colony controls were not injected. ^{239}Pu citrate was distributed relatively uniformly in the liver, whereas the $^{239}PuO_2$ particles were localized primarily in the Kupffer cells, as determined by autoradiography. The animals were permitted to live for their lifespans or were killed when moribund. Histopathological results were not obtained for animals that were autolysed. The cumulative liver tumour incidence in animals that received 14 Gy to the liver from ^{239}Pu citrate was 39%, and the bone tumour incidence was 26%; the incidences of liver tumours at 2.7, 0.3 and 0.04 Gy were 32, 5 and 0, respectively. Animals that were injected with 0.24-μm $^{239}PuO_2$ particles received doses to the liver of 0.8, 7.2 and 42 Gy and had tumour incidences of 5, 26 and 34%, respectively. The histological distribution of liver tumour types was 30 hepatocellular carcinomas, 28 bile-duct adenomas, nine hepatocellular carcinomas, five haemangiosarcomas and one

classified as 'other'. The time to 50% survival of the animals injected with 740, 74, 7.4, 0.74 and 0.074 kBq/kg bw ^{239}Pu citrate was 180, 530, 1000, 860 and 1060 days, respectively. The time to 50% survival of the animals injected with 74 kBq/kg bw ^{239}Pu oxide in particle sizes of 0.17, 0.60, 0.84 and 0.24 μm was 810, 580, 980 and 680 days, respectively. The animals given 74, 7.4 or 0.74 kBq/kg bw in a particle size of 0.24 μm had 50% survival times of 680, 1030 and 950 days, respectively; the corresponding 50% survival time in the controls was 1060 days. The authors concluded that the more uniform irradiation from ^{239}Pu citrate was more effective in causing liver cancer than the non-uniform irradiation from $^{239}PuO_2$ particles. They also concluded that the local distribution of radiation dose was less important in altering tumour incidence than injected activity or average dose (Brooks *et al.*, 1983).

Dog: Lifespan studies on the effects of monomeric ^{239}Pu given as a single injection to beagle dogs were started in 1952 in parallel with studies on ^{226}Ra, such that the results could be used to derive a 'toxicity ratio' for Pu:Ra that would serve as a basis for extrapolating data on the toxicity of Pu to humans.

A group of 234 young adult beagle dogs, about 18 months of age, were given a single intravenous injection of monomeric ^{239}Pu citrate at doses of about 0.02–106 kBq/kg bw. An additional 132 dogs were given the citrate buffer and served as unexposed controls (see Table 80). Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. There were 84 radiographically identified bone tumours in 76 ^{239}Pu-injected dogs and one tumour in the control group (Lloyd *et al*., 1993). No significant difference in sensitivity to bone tumour induction was found between males and females (Lloyd *et al*., 1999b). The relationship between the percentage of dogs with tumours at any dose and the average skeletal dose calculated at one year before death (the assumed time of tumour initiation) was approximately linear below an average skeletal dose of about 1.3 Gy (Lloyd *et al*., 1993; Taylor *et al*., 1997). When these data were compared with those for ^{226}Ra (see section 3.1.2(*b*)), the relative effectiveness of bone cancer induction by ^{239}Pu was about 16 ± 5 (Lloyd *et al.*, 1993).

The skeletal tumours in plutonium-treated animals consisted of seven chondrosarcomas, one liposarcoma and one plasma-cell tumour, the remainder being osteosarcomas. The distribution of the skeletal tumours generally followed the distribution of skeletal mass and skeletal ^{239}Pu content (Lloyd *et al.*, 1994b). The tumours were approximately equally distributed between the axial and appendicular skeleton, but there was some correlation between the site of tumour occurrence and the presence of red (haematopoietic) bone marrow, indicative of greater vascularization, and increased bone turnover, indicative of greater bone surface cell activity (Lloyd *et al.*, 1997b). Similar relationships were described for other bone surface-seeking radionuclides, including ^{241}Am, ^{228}Th, ^{249,252}Cf and ^{224}Ra, but not for the bone volume-seeking nuclide ^{226}Ra.

The occurrence of soft-tissue tumours in young adult beagle dogs given a single injection of monomeric ^{239}Pu was also reported. A significant correlation was found

Table 80. Dose–response relationships for bone cancer induction in beagle dogs given a single intravenous injection of ^{239}Pu citrate as young adults

Dose injected (kBq/kg bw)	Total no. of dogs	No. of bone sarcomas	Average. skeletal dose (Gy ± SD) 1 year before death	Age (years ± SD) at death with bone cancer	Age (years ± SD) at death without bone cancer
0 (control)	132	1	0	16.1	13.1 ± 2.6
0.026	28	1	0.02 ± 0.01	13.6	12.3 ± 2.3
0.067	46	2	0.05 ± 0.01	11.8 ± 1.0	12.8 ± 2.4
0.201	38	4	0.15 ± 0.03	12.5 ± 0.9	12.3 ± 2.6
0.382	38	8	0.29 ± 0.06	12.8 ± 1.2	12.7 ± 2.6
0.576	26	10	0.42 ± 0.09	12.2 ± 2.3	12.6 ± 2.6
1.77	14	10	0.99 ± 0.31	10.6 ± 1.7	8.4 ± 3.5
3.52	12	10	1.70 ± 0.32	7.8 ± 2.4	6.5 ± 0.8
11.0	12	12	4.26 ± 0.73	5.9 ± 0.6	–
33.6	12	12	10.3 ± 1.9	5.1 ± 0.5	–
106	8	7	38.4 ± 11.8	5.7 ± 1.3	3.7

From Lloyd *et al.* (1993)

between liver tumours and increasing dose of radiation, and a significant difference was seen in the relative numbers of malignant liver tumours (22 observed, 3.2 expected) and benign liver tumours (66 observed, 18.1 expected). No other soft-tissue or haematopoietic malignancies were found in significantly increased incidence as a result of exposure to ^{239}Pu (Taylor *et al.*, 1991; Lloyd *et al.*, 1995).

To determine whether differences in the spontaneous incidence of skeletal cancers among canine species might affect their sensitivity to bone-seeking nuclides, and thus present a bias for extrapolating risk to humans, a limited lifespan study was conducted to compare the radiosensitivity of beagle and St Bernard dogs, which have a higher spontaneous incidence of skeletal cancer than beagles. Twenty-six St Bernard dogs, aged 554 ± 39 days and weighing 48.5 ± 6.3 kg, were given an intravenous injection of ^{239}Pu at doses of 0.025–11.0 kBq/kg bw, and 30 animals were given one injection of ^{226}Ra at doses of about 0.7–40 kBq/kg bw. Animals of each sex were used, but the relative numbers were not indicated. Young adult beagle dogs were treated similarly. St Bernard dogs tended to have a shorter latency for radiation-induced bone tumours (see Table 81). No radiation-induced bone tumours were observed in the distal radius, which is the site of the highest incidence of naturally occurring tumours. When these results were compared with those obtained in the group of St Bernards exposed to ^{226}Ra, the toxicity ratio for the St Bernard dogs was approximately equal to that of the beagle dogs (Taylor *et al.*, 1997).

In lifespan studies, 12 beagle dogs were exposed as juveniles (three months) and 10 when mature (60 months) to ^{239}Pu by injection of 11 kBq/kg bw and were observed for life. A group of young adult dogs that had received a similar amount of ^{239}Pu at about 18 months of age was also included. Skeletal tumours were detected by periodic

Table 81. Incidences of bone tumours in St Bernard dogs given a single intravenous injection of ^{226}Ra or ^{239}Pu

Dose injected (kBq/kg bw)	Total no. of dogs	No. of bone sarcomas	Time between injection and death (days ± SD)	Average skeletal dose (Gy ± SD) 1 year before death
Radium-226				
0 (control)	8	0	1803 ± 776[a]	0
0.71 ± 0.007	5	0	3301 ± 41	0.75 ± 0.06
2.23 ± 0.093	5	0	2371 ± 816	1.54 ± 0.35
12.54 ± 0.15	3	3	1639 ± 77	6.86 ± 1.24
39.96 ± 1.48	2	1	739 ± 305	9.30 ± 7.12
Plutonium-239				
0 (control)	8	0	1803 ± 776[a]	0
0.025 ± 0.004	3	0	2841 ± 797	0.01 ± 0.01
0.063 ± 0.007	3	1	3597 ± 187	0.04 ± 0.01
0.19 ± 0.004	6	0	3052 ± 317	0.11 ± 0.01
0.58 ± 0.015	4	2	3026 ± 433	0.33 ± 0.04
4.33 ± 1.26	3	3	1456 ± 212	1.24 ± 0.16
11.04 ± 0.52	3	3	891 ± 97	1.91 ± 0.25

From Taylor *et al*. (1997)
[a] Five dogs were killed at 2424 ± 496 days of age.

radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology (Bruenger *et al.*, 1991a). Dogs exposed as juveniles or when mature had fewer bone tumours per Gy of average skeletal dose than did those exposed as young adults. The relative radiation sensitivities were 0.27 ± 0.09 for the juveniles, 0.41 ± 0.13 for the mature animals and 1.0 for the animals exposed as young adults. When the average skeletal dose rate was considered, the juvenile and mature animals appeared to be about 0.2 times as sensitive to the induction of skeletal malignancies as young adults (Lloyd *et al*., 1999a).

(*f*) *Neptunium-237*

Rat: Groups of 40 female albino Sprague-Dawley rats, 10–12 weeks old, were given ^{237}Np at a dose of 5.2 or 26 kBq/kg bw, and 77 control rats received unspecified treatment without the radionuclide. Lifetime observations were made on 28 rats in each exposed group and the 77 controls. At death, all rats were necropsied and examined histologically. The median survival times were: controls, 800 days; 5.2 kBq/kg bw, 754 days; and 26 kBq/kg bw, 644 days. Control rats developed mainly mammary tumours (56/77) and pituitary tumours (40/77). In the treated rats, mammary tumours were removed surgically to increase the opportunity of observing radiation-induced effects,

which occurred significantly in the skeleton as osteosarcomas: controls, 1/77; 5.2 kBq/kg bw, 1/28; and 28 kBq/kg bw, 10/28. These results reflect the preferential distribution and retention of ^{237}Np on bone surfaces (Sontag *et al.*, 1997).

A group of 106 male Sprague-Dawley rats, eight weeks of age, received a single, nose-only exposure to an aerosol of $^{237}NpO_2$ (activity median aerodynamic diameter, 2.6 μm; geometric standard deviation, 2.17). The initial lung burdens of ^{237}Np in individual rats ranged from 0.1 to about 7 kBq. The exposed rats and 785 controls [treatment unspecified] were held for lifetime observation. Animals were necropsied at death, and the tissues were examined histologically. When the ^{237}Np-exposed rats were divided into four groups on the basis of mean initial lung burdens of 0.2, 0.5, 2 and 4 kBq, the mean length of survival of rats at the highest dose, 653 days, was significantly shorter than that of the unexposed controls (828 days). For the analysis of lung tumours, the exposed rats were divided into six groups on the basis of doses ranging from 0.6 ± 0.1 Gy (SD) to 26 ± 7 Gy. The numbers of rats with malignant lung tumours in these six groups, ranked from lowest to highest dose, were 2/19, 2/20, 5/18, 11/20, 11/14 and 14/15. The tumours were primarily adenocarcinomas and squamous-cell carcinomas. The incidence of adenocarcinomas versus dose fitted a linear–quadratic relationship, with a threshold for the quadratic component at doses < 2 Gy. No squamous-cell carcinomas were seen at doses < 2 Gy, and no adenosquamous carcinomas at doses < 8 Gy (Dudoignon *et al.*, 1999).

(*g*) *Americium-241*

Mouse: A total of 314 male CBA mice were given an intraperitoneal injection of ^{241}Am at 0.04, 0.2, 0.4, 8 or 16 μCi/kg bw [1.48, 7.4, 14.8, 296 or 592 kBq/kg bw]; 50 animals served as controls. Skeletal tumours were identified from radiographs, and many soft tissues were examined histologically. Higher doses were almost always associated with severe effects on the haematopoietic and lymphatic systems and osteolysis and osteonecrosis. The highest frequencies of ^{241}Am-induced tumours of the skeleton (45%) and lymphoreticular system (10%) were found among 100 animals injected with 8 μCi/kg bw. In a comparison with ^{90}Sr, ^{241}Am was considered to be more carcinogenic (Nilsson & Broomé-Karlsson, 1976).

In the study of Taylor *et al.* (1983), described in the section on ^{226}Ra (section 3.1.2(b)), the relative effectiveness of ^{241}Am at doses of 0.02–3.48 μCi/kg bw [0.74–129 kBq/kg bw] in inducing skeletal cancers was 4.9 ± 1.4 in comparison with ^{226}Ra.

The toxicity of ^{241}Am was compared with that of ^{226}Ra in male C57BL mice in order to define a 'toxicity ratio'. ^{241}Am in citrate buffer was given by intraperitoneal injection to groups of 57–107 mice aged 11–14 weeks at a dose of 0 (control), 22, 58, 190, 373 or 1197 kBq/kg bw. An additional group was given 937 kBq/kg bw ^{226}Ra. At the end of the study, histopathology was completed for 49–97 mice in each group. A dose-related decrease in mean survival time was seen: 593, 594, 475, 245, 168 and 135 days in the six groups, respectively. The early deaths at the higher doses may have

been associated with lesions of the haematopoietic and immune systems. Increased incidences of liver carcinomas, lymphomas and lymphoreticulosarcomas were associated with increasing doses of ^{241}Am. In a comparison of the regression coefficients in a proportional hazards model, the rate of death from a bone tumour was 12.9 ± 5.2 times higher with ^{241}Am than with ^{226}Ra when the regression covariate was the average skeletal dose and 3.5 ± 1.7 when the covariate was the amount of injected radioactivity. The overall mortality rate with ^{241}Am was 20.4 ± 3.6 times higher than that with ^{226}Ra when the covariate was average skeletal dose (Schoeters *et al.*, 1991).

Four groups of 15–62 adult female BALB/c mice were given ^{241}Am at 0, 0.8, 1.6 or 3.8 kBq/mouse, and two groups of males were given 0 or 2.5 kBq/mouse by intravenous injection; all animals were followed for their lifespan. Exposure significantly decreased the length of survival and increased the incidence of osteosarcomas. Female mice were more susceptible to the induction of osteosarcoma (0, 44, 43 and 39%) than males (0 and 8.6%) (Van den Heuvel *et al.*, 1995).

The effects of ^{241}Am were compared with those of Thorotrast in producing liver cancer in deer mice (*Peromyscus maniculatus*) and grasshopper mice (*Onychomys leucogaster*), which retain americium in the liver longer than conventional strains of laboratory mice. ^{241}Am citrate was given as a single intraperitoneal injection at a dose of 0–3.9 μCi/kg bw [0–144 kBq/kg bw] to 87 deer mice and 83 grasshopper mice. Thorotrast was administered by intravenous injection at doses resulting in a dose range of 0–4 Gy to the liver. Survival decreased with increasing dose of ^{241}Am in both strains. The numbers of liver cancers were increased in exposed animals of both strains, beginning at the lowest dose used (0.1 μCi/kg bw [3.7 kBq/kg bw]) for both ^{241}Am and Thorotrast. The histological spectrum of tumours (bile-duct adenomas and carcinomas, fibrosarcomas and haemangiosarcomas) was similar in animals of both strains given ^{241}Am and Thorotrast (Taylor *et al.*, 1986).

Rat: The carcinogenicity of ^{241}Am, ^{239}Pu and ^{244}Cm was compared in male August/Marshall hybrid rats given the nuclides by intravenous injection of doses of 109 kBq/kg bw of ^{239}Pu, 92 or 260 kBq/kg bw of ^{241}Am and 150 kBq/kg bw of ^{244}Cm. The rats were held for life and necropsied at death, and tissues were examined histologically. The most frequently observed late effect of all three nuclides was osteosarcoma. One leukaemia of myelogenous origin was seen in each treated group. The numbers of osteosarcomas per rat necropsied were: ^{239}Pu, 17/22; ^{241}Am (92 kBq/kg bw), 4/22; ^{241}Am (260 kBq/kg bw), 15/32; and ^{244}Cm, 10/42. The risk coefficient for osteosarcoma in ^{241}Am- or ^{244}Cm-injected rats was about one-third that with ^{239}Pu (Taylor, 1986).

Dog: Young mature dogs of each sex were exposed once by inhalation to a monodisperse ^{241}Am aerosol with an activity median aerodynamic diameter of 0.75, 1.5 or 3.0 μm or a polydisperse aerosol (1.8 μm). The animals were killed serially 8, 32, 64, 128, 256, 365 and 730 days and four, six and eight years after exposure. Osteoblastic osteosarcomas developed in four of the 15 animals that were scheduled for sacrifice at times after 1000 days (Gillett *et al.*, 1985).

Studies of the effects of single injections of ^{241}Am, ^{226}Ra and ^{239}Pu in beagle dogs are described in the sections on ^{226}Ra and ^{239}Pu above. The relative toxicity of ^{241}Am for inducing bone tumour compared with ^{226}Ra was 8.5 ± 2.3 (Lloyd *et al.*, 1997b).

(*h*) *Curium-242*

Mouse: Four groups of approximately 160 female CBA/Ca mice, 10 weeks of age, received graded exposures to ^{242}Cm fused aluminosilicate particle aerosols, resulting in initial alveolar deposits of 16.5 ± 1.3 (SE), 48.7 ± 4.0, 80.9 ± 3.8 and 142 ± 7.2 Bq per group. An additional 372 control mice inhaled unlabelled fused aluminosilicate particles, and 124 were unexposed. Fifty mice were withdrawn from each group for radiochemical analysis, and the remainder were held for lifetime observation. The overall median survival time was 910 days, with no significant differences in survival among the treated groups. At death, the animals were necropsied and examined both grossly and histologically. Bronchioalveolar adenomas were the most frequently encountered type of lung tumour, although some bronchioalveolar adenocarcinomas were observed. The numbers of mice with lung tumours were 108/372 in controls given fused aluminosilicate particles, 35/124 in untreated controls and 45/111 (low-deposit group), 49/113, 56/101 and 59/112 (high-deposit group) in the ^{242}Cm-treated mice. Four other groups of mice of the same type were exposed to the β-emitter ^{45}Ca fused aluminosilicate particles to compare the relative effectiveness of α- and β-particles in producing lung tumours. ^{242}Cm and ^{45}Ca have similar radioactive half-lives, and the common use of the fused aluminosilicate particle vector reduced possible temporal and spatial differences in comparing the effectiveness of α- and β-particles in producing tumours. Under these experimental conditions, the α-particles were approximately twice as effective as the β-particles (Kellington *et al.*, 1998). This result differs from those of others; e.g. $^{239}PuO_2$ was found to be 21 times more effective than $^{144}CeO_2$ in rats by Lundgren *et al*. (1995) (see section 3.1.2(*e*)).

Rat: Randomly bred, female Wistar rats, 70 days of age, were exposed by inhalation once for 30 min by nose only to $^{244}CmO_2$ prepared by calcination of curium oxalate at 750 °C for 2 h. The initial alveolar burdens were 0.54 nCi for 57, 4.4 ± 1.3 nCi (± SD) for 60, 48.0 ± 36.0 nCi for 55, 450 ± 300 nCi for 44 and 1800 ± 540 nCi for 24 rats [0.02, 0.163, 1.78, 16.7 and 66.6 kBq]. The tissue distribution and data on retention in other rats were used to calculate the absorbed doses of α-particles. The mean dose to the lung in the five groups ranged from 6.6 to 2100 rad [0.066–21 Gy]; the corresponding values for the skeleton were 0.8–950 rad [0.008–9.5 Gy]. Decreased survival times were observed at 450 and 1800 nCi ($p < 0.05$). Twenty-two primary lung tumours were found, all but one of which were adenocarcinomas; the other was a squamous-cell carcinoma. There were also 12 bone tumours (osteosarcomas) and two liver tumours. The increased incidences of lung and bone tumours ($p < 0.05$) were found in groups with initial alveolar burdens of 48 and 450 nCi. A significant increase in the incidence of mammary tumours was found at 4.4, 48, and 450 nCi, most of which were benign fibrosarcomas. No lung, liver or bone tumours were found in the 188 rats used as controls (Sanders &

Mahaffey, 1978). The same group compared the dose–response relationship for lung cancers due to inhaled $^{244}CmO_2$ with similar data for tumours from $^{238}PuO_2$ and $^{239}PuO_2$ inhaled by Wistar rats. Although some differences were seen in the total doses of α-particles above several Gy, the response was similar for doses below that level (Sanders *et al.*, 1976, 1977).

Because both the spatial and the temporal distribution of dose to the lung and skeleton can influence dose–response relationships, four groups of female Wistar rats, 70 days of age, were exposed once (38 rats) or 10 times separated by about 21 days (64 rats) to $^{244}CmO_2$. The activity median aerodynamic diameters were 0.70 μm for the single exposure and 0.39 ± 0.06 μm for the repeated exposure, with geometric standard deviations of 1.9–2.0. A group of 31 rats was sham exposed, and 18 were unexposed. Ten rats from each group were used for dosimetry, and the remainder were held for lifetime observation. One lung tumour and no bone tumours were observed among 49 control rats. The rats given a single exposure received a mean dose of 1.4 ± 1.3 (SD) Gy to the lung and 0.74 ± 1.1 Gy to the bone. In this group, three rats had a lung tumour and four had a bone tumour. The doses received by the rats exposed repeatedly were not statistically significantly different from those of rats exposed once, but the numbers of rats with lung tumours increased to 12 and those with tumours of the bone to 14. Overall, 15 of the 92 exposed rats had lung tumours, comprising eight squamous-cell carcinomas, five adenocarcinomas, one haemangiosarcoma and one fibrosarcoma. The one lung tumour in a control was an adenocarcinoma. The bone tumours were osteogenic sarcomas. After translocation from the lung, the ^{244}Cm was deposited in the liver and skeleton, but the retention time was much longer in the latter, which may account for the observation of more bone tumours than liver tumours (Sanders & Mahaffey, 1990). When these results were compared with those in rats exposed repeatedly to aerosols of $^{239}PuO_2$, the authors concluded that protraction of the inhaled dose of α-emitting radionuclides did not significantly influence the incidence of either lung or bone tumours in rats (Sanders & Mahaffey, 1981).

Seven groups of 84-day-old Fischer 344/CRl rats (totals of 637 males and 645 females) were exposed once to $^{244}Cm_2O_3$ or were sham-exposed to filtered air. The sizes of the groups exposed to $^{244}Cm_2O_3$ ranged from 70 to 211 rats, and the group mean initial lung burdens ranged from 0.50 to 240 kBq/kg bw; there were 157 control rats. The median survival times of male and female rats with mean initial lung burdens ≥ 27 kBq/kg bw were significantly shorter than those of the sham-exposed rats. There was a general increase in the prevalence of benign and malignant lung neoplasms with increasing initial lung burden, except at the highest exposure where survival was much shorter. The results are shown in Table 82 with data for liver and skeletal tumours. Most of the tumours were adenocarcinomas, and the next most frequent were squamous-cell carcinomas and adenosquamous carcinomas, the latter two types occurring more frequently at higher exposure. The incidence of liver tumours did not increase with dose, but a dose-related increase in the incidence of bone neoplasms was observed at 26 and 98 kBq/kg bw (Fisher's exact test, $p < 0.05$). The doses of α-particles to the bone in these

Table 82. Incidences of primary lung, liver and skeletal neoplasms in rats given aerosols of $^{244}Cm_2O_3$ by inhalation

Initial lung burden (kBq/kg bw ± SD)	Mean survival time (days)	No. of rats examined	Lung neoplasms (%)			Liver neoplasms (%)	Bone neoplasms (%)
			Benign	Malignant	Both combined		
Sham	805	157	0.64	1.9	2.5	0	0.6
0.50 ± 0.18	819	168	1.2	4.1	5.3	1.8	0.6
1.2 ± 0.32	802	210	2.9	3.3	6.2	2.9*	1.0
2.7 ± 0.82	798	130	1.5	6.9*	8.5*	1.5	1.5
9.1 ± 2.4	762	114	4.4*	18*	22*	0	1.8
26 ± 6.4	707	116	1.7	44*	46*	1.7	12*
98 ± 18	539	66	1.5	32*	33*	3.0	23*
240 ± 77	63	122	0	4.9	4.9	0	3.3

From Lundgren *et al.* (1997)
*Incidence greater than in sham-exposed rats (Fisher's exact test, two-tailed; $p < 0.05$)

two groups were 0.83 ± 0.30 and 2.3 ± 1.0 Gy, respectively. When the results for lung cancer were expressed as excess numbers of rats with lung tumours per 10^4 Gy and compared with similar values for Fischer 344 rats that inhaled $^{239}PuO_2$ aerosols (Lundgren *et al.*, 1995), $^{239}PuO_2$ was found to produce about twice as many lung tumours at a given dose. This finding is contrary to the 'hot particle' hypothesis, in which higher local doses around particles of high specific activity such as $^{244}Cm_2O_3$ should result in a higher incidence of lung cancer than particles of lower specific activity such as $^{239}PuO_2$ (Lundgren *et al.*, 1997).

(i) *Californium-249 and californium-252*

The patterns of retention, disposition and distribution of californium are similar to those of americium. ^{252}Cf is unique in that it decays by emission of an α-particle and a fission fragment, the latter accounting for about half its total decay energy. ^{249}Cf decays by emission of α-particles, with some accompanying γ-radiation. Thus, the decay of ^{252}Cf has about twice as much energy per disintegration as that of ^{249}Cf.

Mouse: In the study of Taylor *et al.* (1983) described in section 3.1.2(*b*), ^{249}Cf and ^{252}Cf were given to groups of 10–12 mice of each strain (C57BL/Do black and albino) and each sex at doses ranging from 0.013 to 3.30 μCi/kg bw [0.48–122 KBq/kg bw], starting at about 10 weeks of age. The original hypothesis had been that the more energetic decay of ^{252}Cf would induce more tumours than ^{249}Cf, but this was not the case: 26 bone sarcomas were observed in the ^{252}Cf-exposed animals and 33 in mice exposed to ^{249}Cf. The effectiveness of ^{249}Cf and ^{252}Cf in inducing bone cancers relative to ^{226}Ra was 5.0 ± 1.4 for ^{249}Cf and 2.6 ± 0.8 for ^{252}Cf. The calculated relative effectiveness of fission fragment irradiation from ^{252}Cf in inducing cancer in comparison with α-radiation was 0.02 ± 0.28.

Dog: Five or six young adult beagle dogs received intravenous injections of graded doses of ^{249}Cf or ^{252}Cf. All six animals given 10.7 kBq/kg bw ^{249}Cf and five of six given 3.42 kBq/kg bw ^{249}Cf developed an osteosarcoma. The risk coefficient for bone sarcoma was 0.28 per Gy, and the toxicity ratio relative to ^{226}Ra was 6. All six dogs given ^{252}Cf at 10.7 kBq/kg bw also developed a bone sarcoma. The authors concluded that the effectiveness of ^{252}Cf fission fragments for bone sarcoma induction was essentially negligible compared with the α-radiation from ^{249}Cf. The risk coefficient for bone sarcomas from ^{252}Cf was 0.21 per Gy, and the toxicity ratio relative to ^{226}Ra was 4 ± 2 (Lloyd *et al.*, 1994c).

3.2 β-Particle-emitting radionuclides

3.2.1 *Pure β-particle emitters*

(*a*) *Hydrogen-3*

When tritium (^{3}H) is administered as ^3H_2O, it is distributed relatively uniformly throughout the body, resulting in whole-body exposure to low-energy β-particles similar to whole-body exposure from external X- or γ-radiation (see section 4.1).

Mouse: A common theme of the lifetime studies has been to compare the biological effects of long-term β-irradiation with ^{3}H with acute or chronic irradiation from external X- or γ-rays. In a lifetime study, seven groups of about 765 male CBE/H mice, about 99 days of age, received a dose of 1, 2 or 3 Gy from injected ^{3}H or whole-body X-rays. The three ^{3}H-exposed groups received a single intraperitoneal injection of 0.9, 1.8 or 2.7×10^8 Bq per mouse, resulting in mean total absorbed doses of β-particles of 0.85, 1.86 and 3.04 Gy. Three other groups received a single exposure to 200 kVp or 150 kVp [X-ray generator maximum applied voltage; see IARC, 2000] of X-rays, resulting in average whole-body doses of 1.06, 1.98 and 2.64 Gy. The average age at death in the six groups exposed to radiation was 714–737 days, while that in controls was 767 days. During the study, 11–36 mice in the various groups were lost to follow-up, resulting in final group sizes of 732–754 mice. At death, the animals were necropsied and their tissues examined histologically. The numbers of myeloid leukaemias were 33, 47 and 45 in the three X-ray-exposed groups and 43, 48 and 60 in the three ^{3}H-exposed groups, with one case in controls. The calculated effectiveness (± SD) of β-particles from ^{3}H for inducing myeloid leukaemia relative to X-rays ranged from 1.0 ± 0.5 to 1.3 ± 0.3 with a best estimate of 1.2 ± 0.3 (Johnson *et al.*, 1995).

A series of studies was carried out in female mice given ^3H_2O throughout their lifetime. In all studies, 10-week-old female (C57BL/6N × CH3/He)F_1 mice were placed in closed exposure chambers divided into compartments to separate the dose groups. At death, each mouse was necropsied and examined histologically (Yamamoto *et al.*, 1990, 1995, 1998).

In the first study, the dose of ^{3}H in the drinking-water ranged from 3.7×10^9 to 5.92×10^{11} Bq/L. Mice that received ^3H_2O at 1.48×10^{11} to 5.92×10^{11} Bq/L died within two weeks from haematopoietic failure. This information was used to design the subsequent studies, which were conducted at lower doses. In the second study, the mice were provided with drinking-water containing one of five concentrations of ^{3}H, resulting in the mean cumulative organ doses and survival times shown in Table 83. The mean survival time increased with decreasing ^{3}H concentration. The type of tumours differed with dose: at the two highest doses (0.24 and 0.096 Gy/day), the main cause of death was thymic lymphoma, with incidences of 29/45 and 22/38; at a dose rate of 0.048 Gy/day, the incidence of thymic lymphomas decreased sharply (15/60), and the tumours observed were more diverse. At the two lower doses, more reticular-cell

Table 83. Experimental design of studies of liftime ingestion of ^{3}H by female mice

No. of mice	$^{3}H_2O$ concentration in drinking-water (Bq/L)	Estimated dose rate (Gy/day)	Mean dose ± SD (Gy)	Median survival time (days ± SD)
67	0	0	0	811 ± 134
53	3.70×10^8	0.010	5.9 ± 1.1	622 ± 121
60	9.25×10^8	0.024	11.5 ± 2.7	481 ± 112
60	1.85×10^9	0.048	19.7 ± 3.1	414 ± 66
38	3.70×10^9	0.096	24.6 ± 4.9	259 ± 52
45	9.25×10^9	0.24	38.8 ± 8.5	165 ± 36

From Yamamoto *et al.* (1995)

neoplasms, ovarian tumours, fibrosarcomas and lung tumours were seen. The third series of mice were exposed to ^{3}H in the drinking-water at a dose of 1.39×10^8, 3.48×10^7 or 8.69×10^6 Bq/L, with associated dose rates of β-particles of 3.6, 0.9 and 0.2 mGy/day and cumulative doses of 2.62 ± 0.41 Gy, 0.71 ± 0.13 Gy and 0.17 ± 0.03 Gy, respectively. The numbers of mice used were 120 at 3.6 mGy/day, 58 at 0.9 mGy/day, 55 at 0.2 mGy/day and 51 controls. The survival of mice exposed to 3.6 mGy/day was shortened, but that of mice at 0.9 or 0.2 mGy/day was indistinguishable from that of controls. The frequency of thymic lymphomas was 5% at 3.6 mGy/day and 0% at the two lower dose rates and in controls.

Rat: The relative effectiveness of β-particles from ^{3}H and 200-kVp X-rays was studied in 11 groups of about 129 female Sprague-Dawley rats aged 45–59 days. Four groups were exposed continuously to X-rays over 10 days, resulting in a total dose of 0.29, 0.57, 1.10 or 2.00 Gy. Two groups received a single exposure for about 1 h, resulting in doses of 0.57 and 1.78 Gy. The four ^{3}H-exposed groups received a single intraperitoneal injection of $^{3}H_2O$ in normal saline at a dose of 440, 890, 1780 or 3700 MBq/kg bw. Analyses of tissue and excreta indicated that the total doses of β-particles to the mammary glands were 0.46, 0.92, 1.63 and 3.85 Gy, respectively. The relative effectiveness was estimated on the basis of various criteria, including the mammary tumour incidence per Gy 450 days after irradiation and the time required to induce mammary tumours in 50% of the animals at risk. The authors calculated that β-particle irradiation from ^{3}H was 1.1–1.3 times more effective than 200-kVp X-rays (Gragtmans *et al.*, 1984).

(*b*) *Phosphorus-32*

Mouse: Thirty-six adult female BALB mice, 3–5 months of age, were given a single intraperitoneal injection of ^{32}P as Na_3PO_4: 15 mice received 40 μCi [1480 kBq], seven received 60 μCi [2220 kBq], eight received 90 μCi [3330 kBq], and six received 120 μCi [4440 kBq]. Eighty control mice received unspecified treatment without ^{32}P. All

mice were held for lifetime observation, necropsied at death and examined histologically. The authors used the term 'leukaemia' in the broad sense to include malignancies, such as lymphosarcomas and reticulum-cell sarcomas. Leukaemias were seen at all doses and in the controls, as follows: 40 μCi, 6/15; 60 μCi, 3/7; 90 μCi, 3/8; 120 μCi, 3/6; controls, 13/80. The occurrence of leukaemia in the four ^{32}P-injected groups ranged from 38 to 50%, whereas the control incidence was 16%. The authors calculated that the overall incidence of leukaemia in the ^{32}P-injected mice (42%) was significantly higher (χ^2, 7.42; $p < 0.01$) than that in controls (16%) (Holmberg *et al.*, 1964).

Rat: Adult male Wistar rats [age not specified] received one or repeated injections of ^{32}P in an unspecified form. Those injected once were the survivors of an LD_{50} dose of ^{32}P of 4.5 mCi/kg bw [167 MBq/kg bw]; the repeated injections consisted of 1.5 mCi/kg bw [55 MBq/kg bw] given every three weeks for a total of 9 or 12 mCi/kg bw [333 or 444 MBq/kg bw]. Of the 19 rats injected once, 16 were killed between six months and one year after injection, and the other three died. Five of these animals had osteogenic sarcomas and three had squamous-cell carcinomas in the soft tissues of the face. Fifteen rats received repeated injections, but seven died before they received all eight injections. Four osteogenic sarcomas were observed, three in the eight rats that survived the eight-injection regimen and one in the rat that died after six injections (Koletsky *et al.*, 1950).

(*c*) *Strontium-90*

Mouse: A series of experiments was reported on the effects of intraperitoneally injected $^{90}Sr(NO_3)_2$ in CBA mice (Nilsson, 1970, 1971; Nilsson *et al*., 1980). Groups of 120–122 male CBA mice, 75 days of age, were injected intraperitoneally with a dose of 0.2, 0.4, 0.8 or 1.6 mCi/kg bw [7.4, 14.8, 29.6 or 59.2 MBq/kg bw]. A group of 95 controls was available. Five mice per group were killed at one, two, three and four weeks and at monthly intervals thereafter until all the mice had either died or been killed. Tumours of the bone, mucous membranes of the head and haematopoietic system were found. The numbers of osteosarcomas were 0, 8, 90, 292 and 219 in controls and at the four doses, and the numbers of carcinomas of the hard palate, jaw, nose or sebaceous ear ducts were 0, 0, 3, 23 and 74, respectively. A total of 75 lymphatic or thymic lymphomas were reported in treated mice, the highest incidence occurring in mice injected with 0.4 mCi/kg bw [14.8 MBq/kg bw] (Nilsson, 1970, 1971). In a study of the effects of age and dose on the carcinogenicity of ^{90}Sr, groups of 47–51 female CBA mice were injected intraperitoneally with $^{90}Sr(NO_3)_2$ at a dose of 0.2, 0.4 or 0.8 mCi/kg bw [7.4, 14.8 or 29.6 MBq/kg bw] at 25, 75, 150 or 300 days of age. Higher incidences of osteosarcomas were seen in mice injected at 75 days of age, but no age-related effect was seen in the incidence of lymphatic tumours, which occurred more frequently at the low dose (Nilsson *et al*., 1980).

Dog: The design of a lifetime study on the effects of ^{90}Sr given intravenously to beagle dogs and early results were described by Dougherty *et al.* (1962). Groups of

12–14 pure-bred beagle dogs received a single intravenous injection of ^{90}Sr in a citric acid–sodium citrate buffer solution, and 13 control dogs received the buffer solution only. The doses and survival rates are shown in Table 84. The average dose to the skeleton was calculated from measurements made throughout the study of whole-body retention and skeletal content in dogs that died (Boecker *et al.*, 1994). Twenty-four primary bone tumours occurred in 18 dogs: 18 were osteosarcomas, five were haemangiosarcomas, and one was of undetermined histological phenotype. These tumours all occurred at a median absorbed dose of β-particles of 94 Gy (range, 18–164 Gy); none was seen in dogs that received doses to the skeleton of 0.7–18 Gy. In spite of substantial irradiation of the bone marrow, no myeloproliferative disease was observed (Gillett *et al.*, 1992).

Table 84. Experimental design of a study of beagle dogs injected with ^{90}Sr citrate at ~ 17 months of age (mean and range)

No. of dogs	Dose of ^{90}Sr injected (kBq/kg bw)	Average survival after injection (days)	Dose to skeleton (Gy)
13	0	4198 (708–5755)	0
12	21 (19–26)	4723 (2705–5902)	1.1 (0.78–2.2)
13	64 (60–75)	4129 (1973–5624)	3.5 (1.64–5.7)
12	128 (118–153)	3941 (2467–5193)	6.3 (4.6–8.5)
12	400 (370–429)	4481 (2898–5667)	23 (9.5–33)
12	1200 (1130–1500)	3645 (2093–4942)	63 (33–79)
12	2350 (2250–2380)	2125 (993–3030)	80 (51–110)
14	3620 (3430–3890)	1243 (35–2256)	94 (5.2–164)

From Boecker *et al*. (1994)

In response to concern about the possible long-term biological effects of ^{90}Sr in fall-out from atmospheric nuclear weapons tests, a second lifespan study was conducted in the USA, in which beagle dogs were exposed to ^{90}Sr *in utero* and up to 540 days of age. A detailed description of the experimental design and lifetime biological effects has been published (White *et al*., 1993) and is shown in Table 85. A total of 403 pure-bred beagle dogs (approximately equal numbers of males and females) were divided into seven groups receiving logarithmically spaced doses. The animals were derived from 125 dams fed ^{90}Sr from 40 days after breeding to 42 days after parturition when they weaned their pups. The pups received ^{90}Sr in the same ^{90}Sr:calcium ratio as the dams daily until they were 540 days of age. Eighty control dogs were fed stable strontium. Another 82 unexposed control dogs from a parallel lifetime study of ^{226}Ra were also included in the analyses when it was shown that there were no significant differences between their survival and those of the ^{90}Sr study controls. Whole-body counting and radiochemical analyses of tissue samples from the dogs at death were used to calculate the skeletal

Table 85. Experimental design of lifetime study of the toxicity of ^{90}Sr in beagles treated in the diet, from mid-gestation until 540 days of age

Dose group	No. of dogs		kBq ^{90}Sr/g dietary calcium	Ingested kBq/day	Total ingested (kBq)	Median survival age (years)	Skeletal dose (Gy) (mean ± SD)
	Males	Females					
Controls	81	81	0	0	0	14.5	0
1	38	40	0.259	0.74	370	14.2	0.4 ± 0.2
2	21	19	0.777	2.59	1 480	13.5	1.2 ± 0.3
3	33	32	4.55	16.3	8 880	14.4	6.7 ± 2.0
4[a]	38	34	13.7	48.1	25 900	14.1	22.5 ± 5.7
5[a]	30	35	41.1	148.0	81 400	12.0	50.4 ± 18.0
6[a]	32	32	123.0	444.0	241 000	5.2	80.2 ± 35.2
7[b]	12	7	370.0	1332.0	718 000	2.2	107.0 ± 32.0

Modified from White *et al*. (1993)

[a] Data include 15 dogs fed throughout life: seven in group 4, four in group 5 and four in group 6.

[b] Not in original experimental design (1961); added in 1967

doses received by individual dogs. The median survival times at the four lower doses ranged from 13.5 to 14.4 years, while that for the 162 pooled controls was 14.4 years, and those at the three higher doses were 2.2–12 years. At death, each dog was necropsied, and its organs and tissues were examined histologically. Primary sarcomas of bone were classified as osteosarcoma, chondrosarcoma, fibrosarcoma, haemangiosarcoma and undifferentiated sarcoma. In all, 66 primary bone sarcomas were found in 47 dogs, including four controls, multiple sarcomas being found in one female control, four males at the highest dose and 10 females at the two higher doses. All the bone sarcomas occurred at the four higher doses (Table 86), and 74% of these tumours were osteosarcomas; the remainder was made up of other sarcoma types. The ratio of bone sarcomas of the appendicular skeleton to those of the axial skeleton was 38:23.

Table 86. Frequency distribution by dose of bone sarcomas in beagles fed ^{90}Sr and in controls

Dose group	Incidence of sarcomas			Age at onset (days) (mean ± SD)	Skeletal dose (Gy) (mean ± SD)	Average dose-rate (mGy/day) (mean ± SD)
	Males	Females	%			
Controls	3/81	1/81	3.8	5634 ± 641	0	0
1	0/38	0/40				0.08 ± 0.03
2	0/21	0/19				0.25 ± 0.05
3	0/33	0/32				1.46 ± 0.27
4	4/38	0/34	5.6	4894 ± 898	31 ± 2	4.75 ± 1.03
5	5/30	5/35	15.4	4032 ± 875	48 ± 12	13.4 ± 2.9
6	6/32	13/32	29.7	2864 ± 466	112 ± 19	42.3 ± 8.8
7	7/12	3/7	52.6	839 ± 165	116 ± 27	133 ± 25

From White *et al*. (1993)

In a third lifetime study of the biological effects of ^{90}Sr in dogs, 33 young adult (12–14 months) male and 33 female pure-bred beagle dogs were exposed once, briefly, to an aerosol of $^{90}SrCl_2$ in a caesium chloride vector aerosol, with activity median aerodynamic diameters of 1.4–2.7 μm. The initial body burdens of ^{90}Sr were achieved by adjusting the air concentration of ^{90}Sr and the length of exposure (from 2 to 22 min). An additional 22 unexposed dogs served as controls. The dogs were housed individually in metabolism cages for the first 60 days after exposure and then transferred to the kennel facilities where they were housed, two per run, for the remainder of their lives. Whole-body counting, parallel studies of inhalation of ^{85}Sr and radiochemical analyses of the organ burden of ^{90}Sr at death were used to calculate the retained burden of ^{90}Sr in each dog and the resulting total doses received by the skeleton and other organs (Table 87). Six dogs with retained burdens of 1.7–4.1 MBq/kg bw died 18–31 days after exposure from bone-marrow hypoplasia. Bone tumours were the main biological finding, 45 tumours occurring in 31 dogs, of which 24 were osteosarcomas,

Table 87. Experimental design of lifespan study in dogs that inhaled $^{90}SrCl_2$

Group[a]	No. of dogs	Retained burden (MBq ^{90}Sr/kg bw) (range)	Median survival (days) (range)	Median skeletal dose to death (Gy) (range)	Cause of death
1	22	0	4555 (2615–5505)	0	Varied
2	12	0.067 (0.036–0.12)	4725 (2247–5948)	5.9 (4.2–13)	Varied
3	12	0.29 (0.21–0.36)	4624 (2436–5678)	32 (21–49)	Varied
4	15	1.0 (0.56–1.4)	2633 (585–4222)	100 (40–140)	Bone tumours, other
5	14	1.8 (1.4–2.5)	1386 (927–3122)	130 (76–180)	Bone tumours
6	7	3.7 (2.6–4.4)	886 (585–1142)	170 (99–220)	Bone tumours
7	6	3.7 (1.7–4.1)	18–31	8.3 (6.0–13)	Bone-marrow hypoplasia

Adapted from Gillett *et al*. (1987a,b)

[a] Groups 1–6 represent the retained burden of ^{90}Sr; group 7 consisted of six dogs that died 18–31 days after exposure.

14 were haemangiosarcomas, three were fibrosarcomas and one was a myxosarcoma. Four carcinomas of soft tissues near the bone in the nasal cavity and skull were found. Two cases of myelomonocytic leukaemia were observed in dogs, with retained burdens of 1.0 and 0.35 MBq/kg bw. Three ^{90}Sr-related tumour deaths (one bone tumour, one nasal cavity tumour and one case of myelomonocytic leukaemia) were found in 24 dogs with retained burdens of < 0.5 MBq/kg bw (Gillett *et al.*, 1987a,b).

Because of the soluble nature of the ^{90}Sr used in these three studies, the distribution and retention patterns of ^{90}Sr after inhalation can be compared with those after intravenous injection. Minor differences were found. After injection, there was rapid deposition in the skeleton, which decreased with time due to biological processes, whereas exposure by ingestion led to continuous deposition of ^{90}Sr in the skeleton up to 540 days of age and a more uniform distribution within the bones (Gillett *et al.*, 1992).

In order to compare the dosimetric and the biological results obtained in these three studies, tissue sections from all bone tumours were re-examined by one of two pathologists to ensure consistency. Table 88 shows the doses and responses in the three studies. The range of doses over which bone tumours were seen was similar after inhalation, injection and ingestion. Bone tumours appeared later in life after ingestion than in the other two studies. Differences were also noted in the distribution of tumours within the skeleton: after inhalation and injection, bone tumours were found predominantly in the axial skeleton, whereas the distribution between axial and appendicular locations was about equal after ingestion (Gillett *et al.*, 1992).

The haematological responses seen in dogs that were exposed once by inhalation or intravenous injection were compared with those in dogs or pigs (see below) that received ^{90}Sr from their mothers while *in utero* and subsequently in their daily feed. When inhaled or injected in a soluble form, ^{90}Sr was translocated quickly to the skeleton and produced effects on the bone marrow at the highest dose that were similar to acute radiation injury from external radiation. Subsequent occurrence of myeloproliferative disease in the long-term survivors in these studies was rare. The situation was reversed after long-term ingestion. Instead of a rapid decrease in blood parameters, gradual, persistent leukopenia developed due primarily to depression of neutrophils. Myeloproliferative disease was a more frequent health effect in dogs and pigs that survived for a long time than after a high single exposure. These results emphasize that differences in the patterns of dose accumulation can substantially change the types of biological effects seen (Gillett *et al.*, 1987b).

Pig: About 800 Pitman-Moore miniature pigs [sex not specified] representing three generations were fed ^{90}Sr at doses ranging from 0.037 to 115 MBq/day. Animals in the first generation were exposed from nine months of age, and those of the second and third generations were exposed *in utero*, during lactation and in their feed after weaning at a dose that was initially one-fourth that of the sow and was increased by six months to the same as that of the sow. The tumours that occurred in females in the F_1 and F_2 generations are shown in Table 89. Bone sarcomas were observed only at the

Table 88. Primary bone tumours identified in dogs exposed to ^{90}Sr by various routes and observed for lifespan

Route of exposure	No. of dogs exposed	No. of primary bone tumours	No. of dogs with tumours	Median cumulative absorbed dose to bone at time of death (Gy) (range)		Median survival (days) after exposure of dogs with bone tumours
				All exposed dogs	Dogs with bone tumours	
Inhalation	66	45	31	76 (4–220)	130 (28–220)	1702 (759–3472)[a]
Injection	83	24	18	18 (0.7–164)	94 (18–164)	1740 (960–4664)[a]
Ingestion	385	46[b]	41	9 (0.1–193)	123 (26–193)	3058 (576–5697)[c]

From Gillett *et al*. (1992)

[a] To approximate the age at death for dogs that inhaled ^{90}Sr, add 395 days (2097 [1150–3870]), and to approximate that for dogs injected with ^{90}Sr, add 550 days (2290 [1510–5210]).

[b] An additional eight tumours were documented histologically after random sampling of bone lesions identified radiographically. These tumours were not visible grossly, nor did they produce clinical signs. These eight macro-osteosarcomas were excluded from the comparison of the three studies to achieve greater uniformity in sampling. Furthermore, the slides are still in review and other tumours may be identified.

[c] Values shown are the same as the dogs' ages.

Table 89. Tumours in female miniature swine ingesting ^{90}Sr daily (combined F_1 and F_2 generations)

Dose (MBq/day)	No. of animals	Mean lifespan (years)	Cumulative skeletal dose (Gy)	Tumour site (number)					
				Bone sarcoma	Myeloid tumour	Lymphoid tumour	Liver tumour	Ovarian tumour	Uterine tumour
0	74	11	0	0	4	1	8	1 (malignant)	38
0.037	52	11	3	0	0	6	8	0	54 (4 malignant)
0.185	29	11	15	0	3	7	17	0	48 (7 malignant)
0.925	47	10	50	0	9	9	23 (2 malignant)	9 (malignant)	32 (4 malignant)
4.62	40	3.5	140	10	38	15	0	0	5
23.1	24	0.25	11[a]	8[a]	8[a]	0	0	0	0

From National Council on Radiation Protection and Measurements (1991)

[a] Two animals removed from ^{90}Sr feeding at three months of age developed bone tumours and leukaemia at three and four years of age; the remaining animals, not removed from ^{90}Sr, died at about three months of age from bone-marrow aplasia.

two higher doses. Most of the tumours occurred in the skull, including the mandible and maxilla. An increased incidence of hepatic neoplasia was seen at moderate doses, but only two of these tumours were malignant. Haematopoietic effects of irradiation of the bone marrow, including neutropenia, lymphopenia, thrombocytopenia and myeloproliferative disorders and histiocytic infiltration of organs such as the kidney, heart, testis and lung were observed among animals at the higher doses but not in controls or pigs at lower doses and rates (National Council on Radiation Protection and Measurements, 1991).

(*d*) *Yttrium-90 and yttrium-91*

Dog: A group of 46 pure-bred beagle dogs (approximately equal numbers of males and females), 12–14 months of age, received a single, brief (3–30 min) exposure by inhalation to an aerosol of relatively soluble $^{91}YCl_3$ in a non-radioactive caesium chloride vector (activity median aerodynamic diameter, 1.7 μm (1.2–2.5 μm); geometric standard deviation, 2.1 (1.5–2.4)). Another group of 12 young adult beagles served as unexposed controls, and information on control dogs in the three studies of dogs exposed to soluble forms of ^{137}Cs, ^{90}Sr and ^{144}Ce, described elsewhere in this section, was also used in analysing the results. As in the studies of inhaled $^{90}SrCl_2$ and $^{144}CeCl_3$, the exposure was expressed as the burden that remained in the body after the first, rapid clearance phase was completed soon after exposure. Whole-body counting and radiochemical analyses of tissues from a parallel dosimetric study and from dogs that died early in this study were used in dosimetry modelling and calculations. Because yttrium has metabolic characteristics of a rare-earth element, the lung, liver and skeleton were considered to be the primary target organs in this study. The experimental design and dosimetry are shown in Table 90. As in the studies with ^{90}Sr, ^{137}Cs and ^{144}Ce, several dogs at the higher doses died after 12–33 days from haematological dyscrasia. The blood-cell counts in 28 dogs that had haematological dyscrasia but survived returned to normal, and their median survival time was 4328 days, similar to that of the other seven dogs which had no early haematological dyscrasia (4392 days). None of the dogs with haematological dyscrasia that survived more than 33 days died from diseases of the bone marrow or blood-forming organs. Malignant neoplasms occurred in bone-associated nasal mucosa (three squamous-cell carcinomas), bone-associated oral mucosa (one squamous-cell carcinoma, one malignant melanoma), liver (one hepatocellular carcinoma, one lymphosarcoma) and lung (three papillary adenocarcinomas, two bronchioloalveolar carcinomas) in the exposed dogs. Five malignant lung neoplasms occurred in the 61 pooled control dogs (three papillary adenocarcinomas, two bronchioloalveolar carcinomas). Only one of these tumours occurred less than 10 years after exposure (Muggenburg *et al.*, 1998). Lung cancers occurred more frequently in aged control dogs, especially those over 10 years of age (Hahn *et al.*, 1995). [The Working Group noted that the late occurrence of lung tumours in ^{91}Y-exposed dogs may have been related to ageing.]

Table 90. Experimental design, dosimetry and survival times for dogs that inhaled $^{91}YCl_3$

No. of dogs		Retained body burden (MBq/kg bw)	Median organ dose (Gy) (range)			Survival after exposure (days) (range)
Males	Females		Lung	Liver	Skeleton	
6	6	1.5 (0.48–1.8)	6.5 (2.1–7.8)	2.0 (0.64–2.4)	5.8 (1.8–6.9)	4563 (2663–5752)
5	7	2.5 (1.9–3.6)	11 (8.0–16)	3.3 (2.5–4.8)	9.5 (7.1–14)	4563 (364–5398)
7	5	5.9 (4.0–7.6)	21 (17–31)	6.4 (2.3–9.5)	18 (5.6–27)	3125 (22–4955)
5	5	8.6 (7.7–1.9)	25 (21–38)	3.1 (2.5–11)	7.6 (6.1–32)	22 (15–4563)
6[a]	6[a]	0	0	0	0	4591 (2241–5455)
26[b]	23[b]	0	0	0	0	4738 (647–6016)

Adapted from Muggenburg *et al*. (1998)
[a] Concurrent controls
[b] Other controls

As part of a larger investigation of the carcinogenic response of the lung to chronic β-irradiation, equal numbers of young adult (12–14 months of age) male and female beagle dogs received a single, nose-only exposure by inhalation to graded activity levels of ^{90}Y or ^{91}Y in fused aluminosilicate particles and were observed for life. Use of the same insoluble vector and yttrium isotopes with different radioactive half-lives (^{90}Y, 2.7 days; ^{91}Y, 59 days) allowed a comparison of the effect of protracting the dose rate on lung carcinogenesis. The experimental design and results for lung tumours are given in Table 91. The doses were calculated from information obtained by whole-body counting, and the distribution and retention from parallel studies with serial sacrifices. All 32 dogs that inhaled ^{90}Y and had an initial lung burden ≥ 26 MBq/kg bw died within the first 500 days after exposure due to radiation pneumonitis and pulmonary fibrosis. This initial lung burden corresponded to an average absorbed dose to the lung of about 110 Gy. In the 60 dogs with lower initial lung burdens (3.0–25 MBq/kg bw

Table 91. Experimental design and incidences of lung tumours in groups of 12 young adult beagle dogs that inhaled aerosols of ^{90}Y- or ^{91}Y-fused aluminosilicate particles

Initial lung burden (MBq/kg bw)	Pulmonary injury	No. of dogs with pulmonary tumours	
		Carcinomas	Sarcomas
^{90}Y			
0	0	4	0
3.0–4.8	0	1	0
5.2–9.3	0	1	0
9.3–13	0	2	0
14–17	1	5	0
18–26	9	1	1
26–41	12	0	0
41–70	12	0	0
89–190[a]	5	0	0
^{91}Y			
0	0	0	0
0.41–0.70	0	4	0
0.85–1.3	0	5	0
1.4–2.5	1	4	0
2.6–3.3	2	10	0
3.4–4.1	4	6	0
4.1–5.5	8	4	0
5.5–7.0	12	0	0
7.0–13	12	0	0

From Boecker *et al.* (1994)
[a] Five animals

[12.7–110 Gy]), eight lung carcinomas and one lung fibrosarcoma were observed. Four carcinomas were also found in the 12 control dogs that inhaled non-radioactive fused aluminosilicate particles. In the parallel study with ^{91}Y, 96 dogs were exposed, and 12 dogs that inhaled non-radioactive fused aluminosilicate particles served as controls. All 28 dogs with an initial lung burden ≥ 4.8 MBq/kg bw (270 Gy to the lung) also died within the first 500 days from radiation pneumonitis and pulmonary fibrosis. Of the 68 dogs with lower lung burdens (0.41–4.8 MBq/kg bw with associated average absorbed doses of 23–270 Gy), 33 had lung carcinomas. No lung tumours were seen in the 12 control dogs. Comparison of the results of these two studies shows that, although lung tumours can be produced by a brief, high dose rate of ^{90}Y, the tumorigenic response is not pronounced. Increasing the lung burden only leads to early deaths from deterministic effects. In contrast, the more prolonged pattern of β-irradiation from ^{91}Y produced a substantial increase in the number of lung carcinomas at doses below those that produced early deaths from pulmonary injury (Boecker *et al.*, 1994).

(*e*) *Promethium-147*

Rat: The biological effects of ^{147}Pm inhaled in fused aluminosilicate particles were studied in 270 Fischer 344/Crl rats of each sex exposed once by inhalation to graded doses of ^{147}Pm designed to result in initial lung burdens of 0–4400 kBq. The low initial lung burdens were expected to produce early, non-stochastic pulmonary effects and pulmonary tumours. Forty rats exposed to non-radioactive fused aluminosilicate particles served as controls. The remaining 230 rats were divided into four dose groups with the following expected ranges of mortality rates one year after exposure: 60 rats, 0–2%; 80 rats, 2–25%; 50 rats, 25–95%; and 40 rats, 95–100%. All rats that died or were killed when moribund were necropsied and examined grossly and histologically. Rats at the highest doses at death (211–630 Gy) generally died during the first year from radiation pneumonitis and pulmonary fibrosis. Rats with lower lung burdens and correspondingly lower doses of β-particles lived longer, and a large proportion developed pulmonary tumours. Exposed rats developed 98 pulmonary tumours, including 43 haemangiosarcomas, 41 squamous-cell carcinomas, five lung adenocarcinomas and two lung adenomas. Only one pulmonary tumour, a carcinosarcoma, was seen in a control rat. The highest crude incidence of lung tumours (87%) was seen in rats with initial lung burdens of 2410–3280 kBq/g of lung tissue (Herbert *et al.*, 1987, 1988).

Hamster: As part of a research programme carried out at Los Alamos National Laboratory, USA, to study the lifetime biological effects of non-uniform α- and β-radiation in the lung, 10-μm ZrO_2 ceramic particles containing ^{147}Pm were injected into the right jugular vein of 241 Syrian golden hamsters such that the particles became permanently lodged in the capillary bed of the lung. Two activity levels were used, 70 and 450 pCi [2.6 and 16.7 Bq] per particle. The local and average doses to the lung were adjusted by the activity of the particle and the number of particles (total radioactivity) in the lung. Two initial dose rates to the lung were studied, 4.4–4.6 and 14–21 krad/year [44–46 and 140–210 Gy/year], with large numbers of 70-pCi particles or small numbers of 450-pCi

particles. The median survival times were not correlated with dose and were judged to be similar to those of the 528 concurrent controls. Of the controls, 192 were unexposed and the remainder injected with particles not containing ^{147}Pm. No lung tumours were seen in the controls. Of the 49 lung tumours observed in the 241 treated hamsters, 17 were adenomas, 20 were adenocarcinomas, and 12 were epidermoid carcinomas. The occurrence of death from lung tumour peaked 600 days after exposure to both the low and the high doses (Anderson *et al.*, 1979).

3.2.2 *Mixed β-particle emitters*

(*a*) *Iodine-131*

Mouse: The effects of irradiation by β-particles from injected ^{131}I were compared with single doses of X-rays in 4-month-old male CBA mice. Groups of animals were exposed to 0 (controls), 1.5, 3 or 4.5 μCi [0, 56, 111 or 167 kBq] of ^{131}I or 0, 500, 1000 or 1500 R [about 0, 5, 10 or 15 Gy] of X-rays, localized by shielding. A few moribund animals were killed at 580–680 days of age, but most were killed at 680–730 days. The pituitaries, thyroids and grossly observable tumours were sectioned and examined microscopically. Ancillary experiments were performed to evaluate cell survival and dosimetry after exposure to ^{131}I. Both treatments resulted in degenerative changes and reduced thyroid weights, but lower doses of X-rays than of β-irradiation were required to produce the same degree of weight reduction. No thyroid tumours occurred in controls, but administration of either type of radiation increased the incidence of thyroid tumours. In groups that received higher doses of radiation from ^{131}I than those from X-rays, the incidences were 4/93, 5/88 and 15/80, but similar incidences (2/96, 4/95 and 13/84) were obtained with X-rays. Dose-related increases in pituitary weight were seen and, at higher doses, pituitary tumours typical of cells with raised concentrations of thyroid-stimulating hormone (Walinder, 1972).

Rat: A total of 550 male and female Long-Evans rats, 6–12 weeks old, were injected [route unspecified] with carrier-free ^{131}I at a dose of 10, 25, 100, 200 or 400 μCi [370, 925, 3700, 7400 or 14 800 kBq]. There were 385 controls [treatment not specified]. More than half of the rats died of respiratory disease, but 156 controls and 198 treated rats survived for 18–36 months after injection. The survivors were killed, and the thyroid glands with trachea were removed and examined histologically. The thyroids of irradiated rats were smaller than those of controls, and no thyroid tissue was grossly visible in rats at the highest dose. Diffuse or nodular enlargement was observed in several irradiated thyroids. Microscopic changes, including follicular atrophy, epithelial degeneration and condensation of perifollicular stroma, were seen, and their severity increased with the dose of ^{131}I. Neoplastic lesions designated as alveolar carcinomas occurred in 59 of the 354 rats that survived; the incidence was similar in control rats and those injected with 10 or 25 μCi but was markedly lower at the high doses, and these tumours were found in only two rats at 200 μCi. The thyroid glands showed minimal evidence of radiation injury. An alveolar carcinoma was

found in the thyroid of one rat at 400 μCi that had injury comparable to that of rats at 100 μCi. Malignant thyroid epithelial lesions were seen in five exposed rats, consisting of four follicular carcinomas and one papillary adenocarcinoma in one of six survivors at 10 μCi, three of 20 at 25 μCi and one of 10 at 100 μCi. These results are important because they show that ^{131}I-induced tumours are cytologically and histologically different from naturally occurring tumours (Lindsay *et al.*, 1957).

Groups of 300 female Long-Evans rats, six weeks of age, received an intraperitoneal injection of Na^{131}I in normal saline at 0.48, 1.9 or 5.4 μCi [18, 70 or 200 kBq] to achieve mean doses to the thyroid of 80, 330 and 850 rad [0.8, 3.3 and 8.5 Gy] or local exposure of the thyroid to X-rays at a dose of 94, 410 or 1060 rad [0.94, 4.10 and 10.6 Gy]. Control rats were either injected with normal saline or sham-exposed to X-rays. The remaining two groups received localized X-irradiation at 410 rad [4.1 Gy] to the pituitary gland or the pituitary and thyroid glands. As the minimum latent period for radiogenic thyroid tumours was assumed to be six months, rats that died during the first six months were not included in the subsequent analyses. After 6–24 months, moribund animals were killed and necropsied, and the rats still alive at two years (62%) were also killed and necropsied. Two independent histological evaluations were made. The first thyroid adenoma was observed at 12 months and the first thyroid carcinoma 16 months after exposure. The numbers and types of thyroid carcinomas in rats injected with ^{131}I (36) were almost identical to those in rats exposed to X-rays (40), although more follicular adenomas were seen in the X-irradiated rats at higher doses (Lee *et al.*, 1982). No significant difference was seen in the ratio of thyroid carcinoma incidence due to X-rays and that due to ^{131}I at any dose (Table 92). The response ratios for thyroid adenomas at 0.8 and 3.3 Gy were close to unity; at 8.5 Gy, the X-rays may have been more effective in inducing adenomas, although this was not significant. X-irradiation of the pituitary alone did not increase the occurrence of thyroid tumours above the control level, and concomitant irradiation of the pituitary and thyroid did not increase the number of thyroid tumours above those seen with irradiation of the thyroid alone.

Because of the importance of these studies, an independent evaluation was made of all the histological slides. Close agreement was found with the original diagnosis of follicular-cell carcinoma, but the concordance was less close for adenomas, perhaps owing to differences in the histological categories used. These results support the overall conclusion that the proportion of rats that developed thyroid carcinoma was similar with internal ^{131}I irradiation and localized external X-rays within the range of doses used (Capen *et al.*, 1999). [The Working Group noted that no other study of this type has been conducted.]

In a recent review of studies in laboratory mice and rats in which the effects of internally deposited ^{131}I and external X-irradiation were compared, Royal (1999) noted that the effectiveness of ^{131}I relative to X-rays appeared to be 0.5–0.05, despite the use of high doses, older animals, relatively small numbers of animals and poor survival rates in the early studies.

Table 92. Response ratios of thyroid tumour induction in rats by X-rays and ^{131}I evaluated at mean thyroid doses of 0.8, 3.3 and 8.5 Gy

Type of tumour	Dose (Gy)	Response ratio	95% confidence interval
Thyroid carcinoma	0.8	1.3	0.46–2.7
	3.3	1.0	0.45–2.4
	8.5	0.9	0.31–2.6
Thyroid adenoma	0.8	1.1	0.42–2.9
	3.3	1.4	0.62–2.9
	8.5	2.1	0.52–8.5
Thyroid carcinoma or adenoma	0.8	1.1	0.32–3.7
	3.3	1.2	0.43–3.2
	8.5	1.4	0.24–7.6

From Lee *et al.* (1982)

(*b*) *Caesium-137*

Dog: Fifty-four pure-bred beagle dogs (equally divided by sex) received single intravenous injections of ^{137}CsCl in sterile isotonic saline at 12–14 months of age, six dogs receiving mean initial body burdens of ^{137}Cs of 141 MBq ^{137}Cs/kg bw and groups of 12 dogs receiving 104, 72, 52 and 36 MBq/kg bw. Twelve dogs injected at the same age with non-radioactive saline served as controls. After injection, the dogs were housed individually in cages for 60 days and then moved to a kennel facility where they lived, two per run, for lifetime clinical observations. The absorbed doses of β- and γ-radiation were determined for each ^{137}Cs-injected dog from data on its own whole-body retention and information on the tissue distribution and retention of ^{137}Cs injected in this form in parallel studies with serial sacrifices. Because ^{137}Cs is highly soluble in body fluids, it was distributed rapidly throughout the body. The γ-radiation component represented approximately two-thirds of the total dose; the β-particle component represented about one-third of the total dose, but differences were seen between organs because of differences in ^{137}Cs concentrations, i.e. skeletal muscle had higher and bones lower concentrations than the overall average. Because of the relatively short effective half-life of ^{137}Cs in these dogs, about 30 days, all dogs that lived more than one year after injection had received their full dose commitment. The total doses received in the six dose groups and the associated survival times were, in descending order by dose: 11.8 ± 2.0 Gy, 19–33 days; 16.4 ± 5.1 Gy, 24–4537 days; 14.0 ± 1.8 Gy, 77–5138 days; 11.2 ± 2.5 Gy, 2148–5298 days and 7.42 ± 1.2 Gy, 2471–5342 days; the controls survived 647–6015 days. The results for an additional 49 control dogs from contemporary studies were also used in the analyses. Eleven dogs that received ^{137}Cs at the highest dose died ≤ 81 days after injection, primarily due to haematopoietic-cell damage resulting in pancytopenia from irradiation of the

blood-forming organs. Two years after the injection, 42 ^{137}Cs-injected and 60 pooled control dogs were available for the study of late biological effects. As the whole body was exposed, the biological effects were distributed throughout the body instead of being localized in specific organs such as the skeleton. The incidence of malignant neoplasms was increased in the liver and biliary system (nine dogs) and in the nasal cavity and paranasal sinuses (four dogs). No leukaemias were found, in spite of the large radiation doses received by the blood-forming organs. When all malignant neoplasms were combined and female mammary neoplasms were excluded, a dose-related treatment response for the incidence of malignant neoplasms ($p < 0.001$) was observed in both male and female dogs (Nikula *et al.*, 1995).

A similar study was conducted by the same group in beagle dogs of various ages, with 15 juveniles (aged 142–151 days), 38 young adults (aged 388–427 days) and 10 middle-aged dogs (aged 1387–2060 days). An additional 17 dogs served as controls and lived for 2972–5680 days. The dosimetry was similar to that described by Nikula *et al.* (1995). The dogs that died soon after injection comprised three juvenile dogs exposed to 10.2–11.1 Gy, 10 young adult dogs exposed to 10.5–14.6 Gy and all 10 middle-aged dogs which were exposed to 9.4–12.9 Gy. As in the study described above, these deaths resulted from haematopoietic-cell damage that resulted in severe pancytopenia leading to fatal haemorrhage and/or septicaemia. Of the 40 dogs that died later in the study (> 2 years), five juvenile dogs that received total whole-body doses < 11.5 Gy had a median survival time of 3207 days (range, 1861–3517 days), and seven that received doses > 11.5 Gy had a median survival time of 3294 days (range, 2361–4815 days). The median survival of the young adult dogs was 3350 days (range, 2323–4690 days). Thirty-two of the 40 ^{137}Cs-injected dogs that survived > 2 years had malignant neoplasms: 11 dogs had carcinomas only, 10 had sarcomas only, and 11 had both carcinomas and sarcomas. Of the 17 control dogs, eight had carcinomas, one had a sarcoma and two had both a sarcoma and carcinoma. The most striking differences between the ^{137}Cs-injected dogs and the controls were the larger number of sarcomas with spindle-cell morphology (13 versus two in controls) and the occurrence of leukaemias and myeloproliferative disease (three in the ^{137}Cs-injected dogs and none in controls) (Nikula *et al.*, 1996). When the results of this study were contrasted with those of the study in young adult dogs described above, certain similarities and differences were seen. In both studies, the long-term effects included increased incidences of malignant, non-mammary neoplasms; however, splenic and thyroid tumours were seen in the study with dogs of various ages but not in the young adults. Conversely, the incidences of malignant mammary neoplasms and benign non-mammary neoplasms were increased in the young adults and not in the other study, and nasal cavity tumours were seen in the young adults and not in the other study. These differences may have been due, at least in part, to the relatively small numbers of dogs in both studies.

(*c*) *Cerium-144*

A large research programme was conducted at ITRI (New Mexico, USA) on the long-term biological effects of inhaled aerosols of ^{144}Ce over three decades, and the results are summarized below. The overall goals of the programme were to study the lifetime effects of inhaled α- and β-emitting radionuclides. Much of the research on β-particle emitters was focused on ^{144}Ce because of its relatively long radioactive half-life, prolonged retention in the body and emission of energetic β-particles from ^{144}Ce and its decay product, praseodymium (^{144}Pr).

Mouse: Female C57BL/6J mice, 10 weeks of age, were exposed once by inhalation to an aerosol of $^{144}CeO_2$, producing initial lung burdens of 1–12 μCi [37–444 kBq]. Of the initial 714 mice, 178 were held for lifetime observation, and 299 unexposed mice were maintained under the same conditions as the exposed mice. The high lung burdens led to a substantial shortening of survival, and no primary malignant pulmonary tumours were observed in the exposed mice (Lundgren *et al.*, 1974). A new study was therefore conducted at lower initial lung burdens in mice of various ages, and another was conducted on the lifetime effects of repeated exposure to $^{144}CeO_2$ by inhalation. The design of these studies in mice was also used in similar studies in rats, Syrian hamsters and beagle dogs, discussed below.

Female C57BL/6J mice, aged 70, 260 or 450 days, were exposed once to an aerosol of $^{144}CeO_2$ to achieve an initial lung burden of 0.2, 1.0 or 4.5 μCi [7.4, 37 or 167 kBq] in the 1178 animals. The $^{144}CeO_2$ aerosol, produced by heat treatment of airborne droplets of $^{144}CeCl_3$, had an activity mean aerodynamic diameter of 1.5–1.8 μm (geometric standard deviation, 1.5–1.7). A total of 674 control mice were either unexposed, sham-exposed or exposed once to an aerosol of stable CeO_2. A number of mice were withdrawn for dosimetric and other purposes during the study. The median survival times of the mice held for lifetime observation and exposed to the two lower doses were 93–106% of those of the respective controls. The survival times of mice at 4.5 μCi were shorter owing to early non-neoplastic radiation effects and bacterial infections. In the 918 mice (89%) available for histological evaluation, the incidence of lung adenomas (13, 4 and 9%) was significantly increased for all those with an initial lung burden of 1.0 μCi [37 kBq] after exposure at any age. Pulmonary adenocarcinomas and one squamous-cell carcinoma were also seen in mice exposed at 70 days of age. The authors concluded that 70-day-old mice are more sensitive to the development of late effects of $^{144}CeO_2$ than are 260- or 450-day-old mice (Lundgren *et al.*, 1980a).

In a study of repeated exposure, groups of 160 female C57BL/6J mice were exposed to a $^{144}CeO_2$ aerosol at 9–11 weeks of age to produce an initial lung burden of 0, 0.2, 1.0 or 4.5 μCi [0, 7.4, 37 or 167 kBq]. The mice were further exposed six more times at 60-day intervals to re-establish the lung burdens to the original levels. The reduction in median lifespan was 5% at 0.2 μCi, 37% at 1.0 μCi and 84% at 4.5 μCi. For the same total absorbed dose of β-particles to the lung, a single exposure resulted in a dose rate that was initially higher and eventually lower than that after re-established lung burdens.

In mice exposed to 0.1 or 1 μCi that had prolonged survival times, the incidence of benign and malignant lung tumours was correlated with cumulative dose and not with dose rate. The number of malignant pulmonary tumours was increased in mice exposed repeatedly: controls, 0.3%; 0.1 μCi, 6.3% and 0.2 μCi, 7.9% (Hahn *et al.*, 1980; Lundgren *et al.*, 1980b).

Rat: The biological effects of single and repeated exposures to $^{144}CeO_2$ by inhalation were studied in Fischer 344/Crl rats. Of a total of 968 animals (465 males and 503 females), 244 were used for dosimetry, leaving 453 exposed and 271 control rats that were observed for life. The study had three components: (i) a single exposure at 94 days of age to produce an initial lung burden of 1.9, 9.2, 46 or 230 kBq; (ii) repeated exposures beginning at 94 days of age and continuing every 60 days for a total of seven exposures with the goal of re-establishing the lung burden to 1.9, 9.2, 46 or 230 kBq at each exposure; and (iii) a single exposure at 500 days of age to produce an initial lung burden of 46 or 239 kBq. All exposures were to airborne, uniformly spherical particles of $^{144}CeO_2$ with activity median aerodynamic diameters of 0.9–2.2 μm (geometric standard deviation, 1.4–2.0). Control rats were either unexposed, sham-exposed or exposed to stable CeO_2, but, because no difference was observed among these types of controls, they were pooled for the analyses. Analyses of the dose to the lung of rats withdrawn at various times during the study indicated that the mean lifetime absorbed doses of β-particles to the lung were 0.26–46 Gy in rats exposed once at 94 days of age, 2.1–250 Gy in the rats exposed repeatedly and 8.5–36 Gy in rats exposed at 500 days of age. The survival rate of all groups of ^{144}Ce-exposed rats was 90–114% of that of the respective control groups, except for the group that was repeatedly exposed to re-establish a lung burden of 230 kBq, in which survival was only 70% that of the controls. The mean lifetime doses and the incidences of primary lung tumours in the various experimental groups are given in Table 93. The repeated exposure regimen to re-establish a given lung burden resulted in a lifetime dose to the lung that was about five times higher than that from a single exposure, although the initial lung burden was of the same magnitude. At the same total dose, the single exposure regimen resulted in an initially higher and eventually a lower dose rate than the re-established dose rate in the repeatedly exposed rats. The incidence of primary lung tumours was related to cumulative dose of β-particles rather than to the rate at which the dose was accumulated, especially at the higher doses. With doses to the lung > 10 Gy, there was a clear dose–response relationship for lung tumours; however, for rats with total doses to the lung of < 10 Gy, the incidences of primary lung tumours were not directly related to dose, perhaps because of the relatively smaller numbers of rats at the lower doses (Lundgren *et al.*, 1992a,b). Hahn and Lundgren (1992) described the types of lung tumours seen. Rats at the highest exposure level, with significantly shortened survival, had a high incidence of squamous-cell carcinomas of the lung and lower incidences of adenocarcinomas of the lung, haemangiosarcomas of the lung and pleural mesotheliomas. Neither the exposure mode (single or repeated) nor the sex of the animal influenced the lung tumour type or incidence. Because

Table 93. Incidences of primary lung tumours in Fischer 344 rats exposed once or repeatedly by inhalation to $^{144}CeO_2$

Desired initial or re-established lung burden (kBq)	No. of rats examined[a]	Mean lifetime dose (Gy) to lung	Incidence of primary lung tumours (%)	
			Benign	Malignant
94-day-old rats exposed once				
Controls	115[b]	0	0	0
1.9	35	0.26	0	2.8
9.2	49	1.2	0	2.0
46	39	6.8	2.6	2.6
230	57	46	3.5	26.3
94-day-old rats exposed repeatedly				
Controls	110	0	1.8	3.6
1.9	36	2.1	2.8	2.8
9.2	46	9.5	2.2	0
46	33	50	6.1	27.3
230	37	250	0	91.9
500-day-old rats exposed once				
Controls	37	0	2.7	0
46	38	8.5	0	2.6
230	62	36	1.6	8.1

From Lundgren *et al*. (1992b)

[a] Only rats held for lifespan observation and evaluated histologically

[b] Pooled controls

$^{144}CeO_2$ is relatively insoluble, most of the deposited material remained in the lung instead of translocating to the liver and skeleton as more soluble forms of cerium might do. Five osteosarcomas of the ribs were seen, perhaps due to β-radiation originating from the lung.

To examine the possible dose–response relationships for lung cancer caused by $^{144}CeO_2$ at lower doses, a study was conducted with larger numbers of rats (Lundgren *et al.*, 1996). This study was designed to be the β-emitter counterpart to the study of Sanders *et al.* (1993a,b), which involved relatively low doses to the lung from inhaled α-emitting $^{239}PuO_2$ in rats. A total of 2751 Fischer 344/N rats (1358 males and 1393 females) were used. Of these, 1059 received a single exposure to $^{144}CeO_2$ by inhalation to achieve an initial lung burden of 18 kBq (low level), 247 rats to achieve 60 kBq (medium level) and 381 rats to achieve 180 kBq (high level). Samples of the $^{144}CeO_2$ particles from the 36 exposures showed them to be uniformly spherical with an activity median aerodynamic diameter of 1.4 ± 0.1 μm (geometric standard deviation, 2.0 ± 0.2). The 1064 control rats were exposed for 25 min to an aerosol of stable CeO_2 to produce

lung burdens of cerium that were similar to those from ^{144}Ce. The organ doses were calculated from the distribution and retention of ^{144}Ce in rats that were killed periodically during the study. The total absorbed doses of β-particles to the lung from this relatively insoluble form of ^{144}Ce were 3.5 ± 1.3 Gy (SD) for the low-dose group, 12 ± 4.6 Gy for the medium-dose group and 40 ± 10 Gy for the high-dose group. The total dose to the liver was approximately 1/55 and the mean skeletal dose about 1/200 of the corresponding dose to the lung. A total of 2571 rats were held for lifetime observation, and 2538 (99%) were evaluated histologically. The median survival times of the rats at the three levels of exposure were 96–102% of those of controls. Table 94 shows the distribution of lung tumour types in the four experimental groups. These tumours were the cause of death in 71% of controls, 64% of rats at the low dose, 67% of those at the medium dose and 67% of those at the high dose. In decreasing order, the commonest malignant lung tumours were adenocarcinoma, squamous-cell carcinoma and adenosquamous carcinoma. The crude incidences of benign and malignant lung tumours combined were 0.57% in the controls, 2.04% at the low dose, 6.1% at the medium dose and 19% at the high dose. These crude incidence values were well described by a linear function with an intercept of 0.13 ± 0.12% (SD) and a slope of 0.51 ± 0.00054/Gy (correlation coefficient, $r^2 > 0.999$). Several other functions, such as the linear–quadratic, exponential linear–quadratic and Weibull functions, also described these results adequately, but a quadratic function did not.

Hamster: A lifetime study of the biological effects of single or repeated inhalation of aerosols of $^{144}CeO_2$ in Syrian hamsters was reported. Male Syrian hamsters [Sch:SYR] inhaled aerosols of $^{144}CeO_2$, and 222 controls were either unexposed or exposed to stable CeO_2. For the single exposure, 311 hamsters inhaled $^{144}CeO_2$ at 84

Table 94. Primary neoplasms in the lungs of rats exposed by inhalation to aerosols of $^{144}CeO_2$ and held for life

Lung neoplasm	Exposure level			
	Controls	Low	Medium	High
Total number/rats examined histologically	7/1049[a]	22/1025[a]	18/295	30/133[b]
Adenoma, alveolar or papillary (%)	14	23	39	11
Adenocarcinoma, alveolar, papillary or tubular (%)	58	41	39	53
Adenosquamous carcinoma (%)	0	9	11	17
Squamous-cell carcinoma (%)	14	23	11	17
Fibro- or osteosarcoma (%)	14	4	0	3

From Lundgren *et al*. (1996)

[a] One rat in each group had two lung neoplasms each.

[b] Two rats in each group had two lung neoplasms each.

days of age (0.4, 2 or 10 μCi [14.8, 74 or 370 kBq]), 220 days (2 or 10 μCi) or 360 days (2 or 10 μCi). Three groups of about 75 hamsters were exposed to $^{144}CeO_2$ to re-establish a lung burden at 0.4, 2 or 10 μCi at each exposure, and 75 of the controls received repeated exposures to stable CeO_2. The aerosols were produced by heat treatment of airborne droplets of $^{144}CeCl_3$ and stable $CeCl_3$; the resulting aerosols had activity median aerodynamic diameters of 0.9–2.2 μm (geometric standard deviation, 1.4–2.0). Electron micrographs showed that the particles were spherical with minimal aggregation. Information on whole-body and organ retention was used to calculate the doses to the lung and other organs and tissues. The mean dose to the lungs of hamsters exposed once was 10 ± 3.7 Gy (SD) for an initial lung burden of 0.4 μCi [14.8 kBq], up to 190 ± 59 Gy for an initial lung burden of 10 μCi [370 kBq]. The mean doses to the lung from repeated exposure ranged from 2800 ± 720 rad [28 ± 7.2 Gy] for lung burdens re-established at 0.4 μCi to 290 ± 72 Gy for a re-established lung burden of 10 μCi. The doses of β-particles to the liver were about 1/100 to 1/400 of that to the lung, and those to the skeleton were about 1/300 to 1/900 of that to the lung. Decreased survival was seen primarily in hamsters exposed once or repeatedly to a lung burden of 10 μCi. Hamsters exposed to 0.4 or 2 μCi had median survival times that were 98–113% that of the controls. Of 659 hamsters held for lifetime observation, 641 (97%) were evaluated histologically. No difference was seen between the two types of controls. The only neoplasms for which significantly (χ^2 test, $p < 0.05$) increased incidences occurred when compared with controls were primary lung tumours. Eleven carcinomas (four adenocarcinomas, six squamous-cell carcinomas and one undifferentiated carcinoma) and two alveolar adenomas were observed in the ^{144}Ce-exposed hamsters, whereas none was found in the controls. Repeated exposure to ^{144}Ce did not change the incidence of lung tumours or time to death with lung tumour when compared with hamsters exposed once (Lundgren *et al.*, 1982).

Dog: A lifetime study on the biological effects of an inhaled, relatively soluble form of ^{144}Ce, $^{144}CeCl_3$, was reported. This study involved 70 pure-bred beagle dogs, 55 of which (28 males and 27 females) received a single, nose-only exposure by inhalation to an aerosol of $^{144}CeCl_3$ at 12–14 months of age. Eight male and seven female animals were unexposed. The $^{144}CeCl_3$ aerosol was generated from a solution containing $^{144}CeCl_3$ (0.7–1% by weight of stable cerium and other total solids) in 0.1 or 1 M HCl. The resulting aerosol had activity median aerodynamic diameters of 1.4–2.4 μm (geometric standard deviation, 1.6–2.1). The animals were exposed for 3.6–28 min in order to obtain the desired initial body burdens. Because the first, rapidly cleared component of whole-body retention did not contribute substantially to the long-term organ burdens or doses, the tissue distribution and dosimetry were expressed as functions of the retained whole-body burden. The results of a parallel study of dogs exposed to a similar aerosol and killed in series were used to model the disposition and average organ dosimetry of ^{144}Ce inhaled in this form. The resulting total dose coefficients of β-particles (Gy/MBq retained burden of ^{144}Ce per kg bw) were: liver, 59; tracheo-bronchial lymph nodes, 50; bone-associated nasal mucosa, 30; lung, 24; bone, 18;

bone-associated oral mucosa, 18; bone marrow, 9; thyroid, 7.5; and kidneys, 7.5. All other organs that were examined received doses that were smaller by at least a factor of 2.5. Because of variation in the deposition and retention of ^{144}Ce among dogs in this study, the retained burdens ranged from 0.1 to 13 MBq/kg bw. In subsequent analyses of survival times, the animals were divided into five groups of exposed dogs and one group of controls. The median survival times for the five exposed groups ranged from 31 to 4382 days, and the median value for the control dogs was 5064. Nine dogs at the highest dose died within the first 2.5 years from haematological dyscrasia, three from radiation pneumonitis and three from hepatic degeneration. The neoplasms observed are summarized in Table 95. Neoplasms occurred relatively early, 2.2–6.8 years after exposure, in the liver, bone, bone marrow and oral mucosa closely associated with bone. Neoplasms occurred more than seven years after exposure in the liver, lung and nasal mucosa closely associated with bone. Although only one primary bone tumour was found, 11 tumours were found in bone-associated tissues (oral and nasal mucosa and bone marrow) (Hahn *et al.*, 1995, 1997).

In a study of ^{144}Ce embedded on fused aluminosilicate particles, 111 beagle dogs (58 males and 53 females) were exposed once at 12–14 months of age to an aerosol prepared by nebulizing a suspension of montmorillonite clay particles containing ^{144}Ce. The airborne droplets were passed through a tube furnace at 1150 °C to produce ^{144}Ce fused aluminosilicate particles with activity median aerodynamic diameters of 1.4–2.7 μm (geometric standard deviation, 1.5–2.3). Another eight males and seven females of the same age were exposed to an aerosol of stable cerium in fused aluminosilicate particles. The initial lung burdens achieved ranged from 0.093 to 7600 kBq/kg bw ^{144}Ce. The dogs were assigned to groups of 6–16 on the basis of their initial lung burdens. The median survival times ranged from 5264 days at the lowest dose to 177 days at the highest; the median survival time was 4652 days. Dogs with the highest lung burdens died of radiation pneumonitis and pulmonary fibrosis. In the 94 dogs that survived more than two years, neoplasia was the primary long-term biological effect: neoplasms occurred in the lung in 28 dogs, in the tracheobronchial lymph nodes in 23 dogs and in the heart in three dogs. Some of the insoluble particles were cleared from the lung via the lymphatic system to the tracheobronchial lymph nodes, which resulted in β-irradiation of the lymph nodes and adjacent heart tissue which also produced haemangiosarcomas in these organs. Haemangiosarcomas were also found in the lung in some surviving dogs at the highest dose. At lower doses, carcinomas of various kinds were observed in the lung. The percentage distribution of the 32 lung neoplasms by type was: adenoma, 6.2%; adenocarcinoma, 31%; carcinoma, 25%; bronchiolo-alveolar, 19%; adenosquamous, 3%; squamous-cell, 3%; carcinosarcoma, 9.4%; and sarcoma, 28% (Hahn *et al.*, 1999).

Table 95. Neoplasia in target organs of beagle dogs that inhaled $^{144}CeCl_3$ and in controls

Target organ	Exposed (41 dogs at risk: alive two years after exposure)		Unexposed controls (15 dogs at risk: alive two years after exposure)	
	Benign neoplasms	Malignant neoplasms	Benign neoplasms	Malignant neoplasms
Bone	None	Osteosarcoma-vertebra (1)	None	None
Bone marrow	Myelodysplasia (1)	Myeloid leukaemia (2)	None	None
Bone-associated oral mucosa	None	Squamous-cell carcinoma, maxilla (3)	None	None
Bone-associated nasal mucosa	None	Squamous-cell carcinoma (4) Haemangiosarcoma (1)	None	None
Liver	Biliary cystadenoma (4) Fibroma (1)	Haemangiosarcoma (7) Fibrosarcoma (1) Cholangiocarcinoma (1) Hepatocellular carcinoma (1)	Biliary cystadenoma (1) Biliary adenoma (1)	None
Lung	Bronchioalveolar adenoma (1)	Adenocarcinoma (3) (bronchioalveolar, papillary, mucocystic)	None	Adenocarcinoma (2) (papillary, bronchioalveolar)[a]
Thyroid	Follicular adenoma (3) Solid-follicular adenoma (1)	Solid-follicular adenocarcinoma (3)	Solid adenoma (1)	Solid adenocarcinoma (2) Solid follicular adenocarcinoma (2)

From Hahn *et al*. (1997). In parentheses, number of dogs with neoplasms; some dogs had more than one neoplasm.
[a] Three primary lung tumours in two dogs

(*d*) *Radium-228* (see Table 7 of General Remarks, footnote l)

Dog: Groups of 6–13 beagle dogs aged 511–550 days were given a single intravenous injection of ^{228}Ra in a citrate solution, and 13 dogs aged 584 days were given an injection of the citrate carrier and served as contemporary controls. In later statistical analyses, further controls were added. The animals were about equally divided between males and females, and the injected amounts ranged from about 0.65 to about 307 kBq/kg bw. Skeletal tumours were detected by periodic radiographic examination and confirmed by histopathological evaluation after necropsy. Soft-tissue tumours were detected by clinical examination or at necropsy and classified by histopathology. The occurrence of skeletal tumours is presented in Table 96, which shows a dose–response relationship. From a linear regression analysis, the authors concluded that the lifetime risk for the development of bone tumours was about 9% per Gy of average skeletal dose. The toxicity ratio (^{228}Ra /^{226}Ra) for skeletal cancers was 2.0 ± 0.5 (Mays *et al*., 1987; Lloyd *et al*., 1997a).

Table 96. Effects of ^{228}Ra given as a single intravenous injection to young adult beagle dogs

Dose injected (kBq/kg bw)	Total no. of dogs	No. of bone sarcomas	Skeletal dose (Gy) 1 year before death	Age at death (days)
0	13	0	0	4755 ± 348
0.65	12	0	0.93	4849 ± 201
1.84	13	1	2.39	4494 ± 261
5.66	12	10	6.32	3570 ± 142
11.2	11	8	8.36	2619 ± 224
34.8	11	11	18.21	2059 ± 63
92.5	6	5	27.88	1548 ± 74
307	6	1	52.73	1302 ± 63

From Mays *et al*. (1987)

3.3 Pre- and perinatal carcinogenesis

Only a limited number of radionuclides have been evaluated for carcinogenicity during the pre- and perinatal periods, although the internal and internal plus external exposures differ from those of adults. Radionuclides that emit α- or β-particles include some that deliver a dose to the fetus, resulting from internal deposition or from external β or photon emissions from maternal depositions. It is not always possible to differentiate between the two.

Perinatal carcinogenesis due to external radiation in human populations and experimental animals was considered in a previous monograph (IARC, 2000). Most of the major incidents in which pregnant women or experimental animals have been

exposed to ionizing radiation were acute or of relatively short duration and, at least in part, involved external sources (X-rays, γ-rays or neutrons). Such radiation passes through the abdomen and uterus, often with little attenuation. The resulting exposure of the conceptus does not involve placental transport or metabolism, and the energy deposited or the radiation dose is roughly uniform throughout the entire fetoplacental unit. This section addresses studies of tumour development associated with exposure of pregnant or neonatal animals to radionuclides. Studies on exposure of male parents before conception are summarized in section 3.4.

3.3.1 *α-Particle emitters*

(*a*) *Plutonium-238 and plutonium-239*

Several investigators (see also section 4.1) have shown that the placental transfer of plutonium and metabolically related actinides is limited. Actinides are initially deposited mainly in the liver and on bone surfaces in both adults and newborns. Their biological behaviour during the prenatal period is complex, as it is strongly influenced by maternal metabolic relationships and by patterns of deposition throughout the feto-placental unit, hepatic development and localization and redistribution in bone dependent on the stage of gestation.

A series of comparative long-term studies was carried out which included sequential measurements of tissue concentrations and dosimetry (Sikov, 1989).

In the initial study, young adult (3-month-old) and weanling (21-day-old) Wistar-derived rats were injected intravenously with 0.3, 1 or 3 mCi/kg bw [11.1, 37 or 111 MBq/kg bw] of ^{239}Pu prepared in a 100-fold excess of citrate, so that it was primarily a monomeric solution. Rats at 19 days of gestation were injected with 6, 20 or 60 mCi/kg bw [222, 740 or 2220 MBq/kg bw] of the same solution in order to expose the fetuses, and newborns were injected intracardially with 3, 10 or 30 mCi/kg bw [111, 370 or 1110 MBq/kg bw]. Other rats were injected with citrate solutions at the same concentration to serve as controls. The doses were selected to deliver similar doses of radiation to the femur (7, 23 or 70 rad [0.07, 0.23 or 0.7 Gy]) during the first 10 days after injection in all age groups, although the cumulative doses in the group exposed prenatally fell below the target dose. Because of dilution due to greater growth rates, the concentration decreased more rapidly in the newborn animals, and since the skull grew less than the remainder of the skeleton, it received a relatively greater dose. Most of the dose was received within the first few months after exposure, while adults continued to be exposed at a higher level throughout life. Groups of about 25 male and 25 female rats in each age group (adults, weanlings, newborns and fetuses) and each group of plutonium dose as well as controls were selected for long-term observation, and two additional cross-fostered groups of prenatally exposed and control newborns were used in an ancillary experiment. Survival decreased significantly with increasing dose in the three groups exposed postnatally. In this and subsequent studies of this series, moribund animals were killed, and all survivors were killed at 30 months of

age. Complete necropsies, with radiographs, were performed, and histopathology was carried out when not precluded by autolysis. The incidences of bone tumours in the adult and weanling rats increased progressively with dose (0, 10, 37 and 60% in adults and 2, 4, 23 and 39% in weanlings), but reached a plateau in the newborns (4, 15, 18 and 17%, not statistically significant). The incidence in the group exposed prenatally was slightly increased by the lowest dose (4%), became maximal at the intermediate dose (10%) but decreased at the highest dose (4%). When the offspring of some dams at the highest dose were cross-fostered with the litters of control mothers, they had a higher incidence (27%) of osteogenic sarcoma than offspring kept with their own mothers (3%). The pattern of relative sensitivity may be misleading when expressed on this basis, however, because the doses of radiation were not proportional to the administered dose in all four age groups (Sikov *et al.*, 1978).

In a second experiment, pregnant Sprague-Dawley rats were injected intravenously with ^{239}Pu citrate at a dose of 0.3, 3 or 30 mCi/kg bw [11, 111 or 1110 MBq/kg bw] on day 9, 15 or 19 of gestation. The cumulative dose rate of radiation and the doses to the embryo or fetus and offspring increased with prenatal age at injection. Exposure had dose-related effects on postnatal growth and survival that were more severe and/or more frequent among offspring from litters injected at 19 days than at 9 days of gestation, while they were of intermediate severity in those exposed at 15 days. The incidence of adenomatous hyperplasia of the liver was analysed only for the 46–75 pooled rats per dose group that survived beyond 800 days. The incidence progressed from 8/75 in controls to 29/70, 35/74 and 46/56, respectively, in the three exposed groups, and a numerical score for severity tended to be higher with increasing gestational age at exposure. The incidence of bone tumours was increased in the offspring of dams injected with the highest dose at 19 days. The incidence of 14% bone tumours at a cumulative femur dose of 40 rad [0.4 Gy] was compatible with the results of the first experiment (Sikov, 1982, 1983, 1989).

In a third experiment, the influence of foster-rearing of rats on the postnatal effects of prenatal exposure to plutonium was examined. At 19 days of gestation, pregnant Sprague Dawley-derived rats were injected intravenously with 60 mCi/kg bw ^{239}Pu citrate [2220 MBq/kg bw] or with a citrate (control) solution. A matrix of six experimental groups was formed by giving one-day-old offspring for fostering to control or exposed dams (see Table 97). Subgroups of offspring, each containing no more than two males and two females from each litter, were kept for long-term studies, and the other offspring were killed at intervals for dosimetry. The growth curves and body masses of prenatally exposed offspring reared by control dams were similar to those of control offspring reared by their own or control foster dams, but the curves for control offspring that were nursed by exposed dams were depressed. In contrast, the three groups of offspring that were exposed prenatally lived significantly less long than control groups, although a consistent effect of fostering was not detected. As shown in Table 97, the incidence of osteogenic sarcomas was significantly greater (about 15%) in the offspring of the three groups that were injected with ^{239}Pu citrate

Table 97. Incidences of osteosarcomas in the offspring of rats injected with ^{239}Pu on day 19 of gestation

Exposure		No. of offspring examined	No. of osteosarcomas			
Prenatal	Postnatal		Skull	Axial skeleton	Appendicular skeleton	All
Exposed	Not fostered[a]	75	10	1	2	13
Exposed	Exposed	80	8	2	3	13
Control	Exposed	75	0	0	0	0
Exposed	Control	81	8	2	2	12
Control	Control	88	0	0	0	0
Control	Not fostered[a]	78	0	0	1	1

From Sikov (1987)
[a] Kept with their dams.

than in controls injected with citrate (1%). The incidences of bone tumours were not influenced by exposure of rats that reared the offspring. The incidences of several histopathological lesions of soft tissues, including tumours of the liver and adrenal gland, were increased in the three groups that were exposed to plutonium prenatally, but the incidences were not influenced by fostering or by the exposure of the dams that reared the offspring. These patterns are in accord with measurements that showed that most of the lifetime ^{239}Pu burden was derived from placental transfer after prenatal exposure and that milk made little contribution (Sikov, 1985, 1987a, 1989).

The relationships between the absorbed doses of radiation to the skeleton and its components after injection of pregnant rats on day 19 of gestation and the resulting incidences of osteogenic sarcoma in the offspring in the three experiments show a consistent pattern. When the data on bone tumours were analysed together, the composite dose–response curve for tumour incidence showed a progressive increase with dose, as described by the equation: Incidence (%) = $1.6 + 0.5 \times$ Dose (cGy). This was followed by a sharp downwards inflection at a dose of 40 cGy (Sikov, 1989). The comparisons of the dose–response relationships suggested that the perinatal skeleton is more radiosensitive to oncogenesis than that of the adult. The actual pattern of sensitivity cannot be identified because, as indicated above, the dose rates and cumulative radiation doses to embryos, fetuses and offspring are affected by the age at injection, and temporal and spatial considerations are superimposed on the radiation doses. Within the useable range for each age group, however, the average dose that would produce a 10% increase in bone tumour incidence was estimated roughly to be 43 rad [0.43 Gy] to 19-day-old fetuses, 86 rad [0.86 Gy] to newborns, 110 rad [1.1 Gy] to weanlings and 275 rad [2.75 Gy] to adults (Sikov, 1983). Another consistent finding was that tumours of the head predominated in perinatally exposed rats in all three experiments; this pattern clearly differed from that in animals that were older when exposed, in which axial and

appendicular tumours predominated. The tumour distribution in adults was similar to that found by others, and the effect of age agrees well with the age-related spatial and temporal distributions of ^{239}Pu and the resulting radiation doses (Sikov, 1989).

In a summary of studies by the Ministry of Health of the former USSR on the carcinogenic effects of perinatal exposure to plutonium, pregnant or nursing Wistar rats were given the radionuclide ^{238}Pu or ^{239}Pu. Malignant tumours were found in the offspring in the organs in which the nuclides were deposited preferentially, specifically the skeleton and liver. After intraperitoneal injection of ^{238}Pu nitrate at 185 kBq/kg bw, liver tumours developed in 1/86 offspring injected on day 15 and 3/73 injected on day 19; no liver tumours were found among 78 control rats. Osteosarcomas were found in 2/86 offspring that received cumulative lifetime doses to the skeleton of 8–8.5 mGy after injection on day 15 and in two offspring injected on day 19 that received a lifetime absorbed dose of 3.4 mGy. No liver or bone tumours were found among 74 offspring of dams treated on day 4 with a cumulative dose to the skeleton of 164 mGy [this value may be incorrect]. The incidence of other tumours did not differ from that in controls (Moskalev *et al.*, 1989).

In another experiment, female Wistar rats were given ^{239}Pu citrate by intravenous injection one day *post partum* at a dose of 1600 kBq/kg bw, so that the offspring received plutonium in the milk. Three of 152 suckling rats (1.97%) that received an absorbed dose of 90 mGy to the skeleton developed osteosarcomas. There were no tumours in the controls [number and treatment unstated], but the spontaneous incidence in this strain was reported to be 0.015%. The authors stated that the quantitative results and the location of most osteosarcomas in the skeletal components that contained ^{239}Pu were in good agreement with those reported by others (Moskalev *et al*., 1989).

(*b*) *Americium-241*

When female rats were given ^{241}Am at a dose of 92.5 kBq/kg bw by intravenous administration on day 16 of gestation, four ostosarcomas were detected among the 78 female and 52 male offspring, while none was found in controls. One of the tumours was found in the head and three in the femur. The authors reported that tumours of the bone and liver occurred at cumulative doses of 3–8.5 mGy incorporated during the embryonic and fetal periods or during lactation (Moskalev *et al.*, 1989).

Pregnant BALB/c mice, 12 weeks of age, were injected intravenously with ^{241}Am at a dose of 100, 500 or 1500 kBq/kg bw on day 14 of gestation, and the offspring were reared by unexposed dams. Control mice were sham-injected, and their litters were raised by other dams. Radioanalyses for ^{241}Am in the femur were performed on litters on days 15 and 17 of gestation (one and three days after injection of their dams), at birth and four additional times in three months. The offspring were kept until death, when they were necropsied and radiographed, and the main organs and any others that appeared abnormal were examined histologically. The mean length of survival of exposed offspring was not reduced. As shown in Table 98, the incidences of osteosarcoma and all sarcomas were significantly increased, although the results of the statistical tests were

Table 98. Per cent of deaths from tumours in offspring of mice exposed to ^{241}Am during gestation

Tumour	Dose of ^{241}Am administered (kBq/kg bw)							
	Females				Males			
	0	100	500	1500	0	100	500	1500
(No. of mice evaluated histologically	46	45	41	49	81	46	55	77)
Osteosarcomas	0	4.4	2.4	4.1	0	2.2	1.8	0
Lung carcinomas	15	16	15	14	27	26	25	32
All malignant tumours	54	71	58	65	57	72	64	65
All leukaemias	35	38	27	39	18	30	31	29
All sarcomas	2.2	11	9.8	8.2	4.9	11	7.3	1.3

From Van den Heuvel *et al.* (1995)

not consistent. The incidences of all malignant tumours and leukaemias were significantly increased in male offspring (Van den Heuvel *et al*., 1995).

3.3.2 *β-Particle emitters*

(*a*) *Hydrogen-3*

The radioactive isotope of hydrogen (^{3}H) is distributed rapidly throughout the body water after ingestion of the inorganic form, usually as $^{3}H_2O$ (see section 4.1). As such, it freely crosses the placenta and shows no strongly preferential site of localization. This minimizes many of the potential dosimetric complications that pertain to other nuclides, and average tissue doses can be calculated by sequential measurements. As with other radionuclides, the distribution of ^{3}H differs when it is administered in different organic compounds. Several studies have addressed the disposition in pregnant animals of organic compounds of metabolic importance labelled with ^{3}H or radiocarbon. Most of these studies were of normal placental transfer and maternal–fetoplacental physiology, but some were conducted for radiological protection. Tritiated thymidine has been studied in particular to establish the mechanism of action of radiobiological compounds.

(i) *^{3}H-Labelled water*

Mouse: Groups of nine pregnant mice were injected with $^{3}H_2O$ at a dose of 0.067, 0.135 or 0.27 Ci/kg bw [2.5, 5 or 10 GBq/kg bw] on day 9 of gestation or kept as controls. The litter sizes and perinatal mortality were not affected, although more deaths ocurred before weaning among offspring at the highest dose. There was no effect on mortality rate in the period between weaning and 4–5 months of age, when many

offspring were killed and examined. Growth and survival were decreased at 0.27 Ci/kg bw, and tests of mating at two months of age showed that these offspring were not fertile; furthermore, females at 0.135 Ci/kg bw, but not males, showed reduced fertility. The weights and histological integrity of the gonads were reduced in both males and females at all doses, and the brain weight was reduced at higher doses (see section 4.3). A total of 21 females and 23 males at 0.27 Ci/kg bw and the 59 female and 80 male controls were held until 18 months of age. The incidence of ovarian tumours in the exposed offspring (67%) was markedly higher than that in the controls (14%), but the incidences of other tumours were not markedly altered (Török *et al.*, 1970).

Groups of 138–142 male and 109–120 female C57BL/6 mice received a single intraperitoneal injection of ^3H_2O at a dose of 1 μCi [37 kBq] after weaning; 1 μCi/mL in drinking-water after weaning and throughout life; a single intraperitoneal injection of 1 μCi to females after the birth of their litters; 1 μCi/mL in the drinking-water of females and their newborn litters throughout life; or 1 μCi/mL in the drinking-water of females identified as pregnant by a vaginal plug and of their offspring throughout life. The 577 male and 525 female control mice received ordinary drinking-water and were observed throughout life. An extensive array of tissues and tumours were taken for histological evaluation. Statistically significant increases in the incidences of tumours were found in all treated groups, the type of tumour depending on sex and exposure regimen. The incidences of reticulo-endothelial tumours were increased in the group given 1 μCi/mL in drinking-water after weaning and that treated from conception. Tumours were found in the liver, lung, intestine and other organs, in decreasing order of frequency. The incidences of lymphocytic lymphomas were significantly increased in mice of each sex in all groups, and the increase was most marked in mice exposed via maternal milk and those exposed from conception (Méwissen *et al.*, 1989).

Rat: Pregnant Sprague-Dawley rats, about 125 days of age, were injected intraperitoneally with ^3H_2O to produce a dose to body water of 0, 1, 50 or 100 μCi/mL [37, 1850 or 3700 kBq/mL]. These nominal equilibrium body water concentrations were maintained by providing ^3H_2O in drinking-water throughout gestation. There were 36 control rats and 24 rats at 1, 26 rats at 10, 23 rats at 50 and 36 rats at 100 μCi/mL of labelled body water. The corresponding cumulative whole-body doses of β-particles were calculated to be 0.6–660 rad [0.006–6.6 Gy] for the pregnant animals and their fetuses. All rats were allowed to give birth and to wean their litters, after which the dams were maintained for lifetime evaluation (Cahill *et al.*, 1975a). The offspring were held for long-term study, the results of which were described in a second paper. The dams were kept until death or were killed and necropsied for histological evaluation. For logistics, the study was initiated as six separate increments or replicates; analysis of variance showed no statistically significant effect of this design, but the age differences had to be addressed in some of the statistical analyses. The survival of exposed dams was reduced, but this was not attributable to tumours. In order to allow for deaths from other causes, the incidences of mammary tumours, which developed late in life, were evaluated on the basis of rat–days at risk. The incidence was found to be increased by treatment. The

increase in the incidence of fibroadenomas was significant at the two higher doses, and that of malignant mammary neoplasms was increased at all doses but statistically significantly so only at the highest dose. Offspring that survived to 30 days of age were defined as the study population, and the numbers were 242 control rats, 111 rats at 0.006 Gy, 187 rats at 0.06 Gy, 170 rats at 3.3 Gy and 207 rats at 6.6 Gy, approximately evenly divided by sex. Exposure to 6.6 Gy decreased the survival of offspring of each sex, and the microscopic appearance of the male and female gonads confirmed the previous report that rats exposed to 3.3 or 6.6 Gy were sterile. This finding is also consistent with the results of studies of prenatal exposure to external X- or γ-rays and other radionuclides under conditions that resulted in congenital ovarian hypoplasia. The incidence of tumours of the ovary was increased among female offspring at the two higher doses, but the differences were not statistically significant when adjustments were made for replicate and litter interactions. On the basis of rat offspring days at risk, there was no increase in the incidence of mammary fibroadenomas (Cahill *et al.*, 1975b).

(ii) *[^{3}H]Thymidine*

[^{3}H]Thymidine has been studied in relation to tumorigenesis because the ^{3}H labels a precursor of DNA. A fraction (roughly estimated at 10%) is incorporated into the DNA of proliferating cells, while the remainder is rapidly catabolized and excreted. The incorporated thymidine remains in the DNA until the cell divides, at which time it is distributed among its descendents or until the cell dies, when it can be re-used. Because of their short range, the weak β-particles selectively irradiate the nucleus. Estimates of the corresponding average radiation dose are of uncertain significance because of the non-homogeneity of the energy deposition within the cell.

Groups of about 100 C × A hybrid mice were injected subcutaneously with 1 mCi/kg bw [37 MBq/kg bw] [^{3}H]thymidine at birth or at 2, 6 or 12–14 months of age. Another group of 85 newborn mice was injected once with 0.1 mCi/kg bw [3.7 MBq/kg bw], and two groups of 85 and 113 mice received 10 mCi/kg bw [370 MBq/kg bw] either at birth or as six injections over eight days. A group of 176 offspring exposed prenatally were obtained from dams that had been injected with a dose of 250 μCi [9250 kBq] between days 15 and 17 of gestation, which resulted in an estimated ^{3}H concentration of 1 mCi/kg bw [37 MBq/kg bw] in the fetuses. The control group was composed of 464 mice that received unlabelled thymidine or water. Another group, which was initiated later, consisted of newborns that were injected with 15 mCi/kg bw [555 MBq/kg bw] ^3H_2O; these mice were kept only until 27 months of age, although the other groups were studied for life. These regimens generally reduced the survival of the newborn mice, and the effect was significant at 1 and 10 mCi/kg bw. The incidence of 'miscellaneous' tumours, which occur infrequently in this strain, was significantly increased in newborns and offspring at 1 and 10 mCi/kg bw. The induction time for lung tumours and lymphomas was decreased in newborns at 10 mCi/kg bw, and the latency for lung tumours was decreased in the offspring of dams exposed during gestation to 1 mCi/kg bw (Baserga *et al.*, 1966).

Quantitative comparisons were made of the relationships between radiation modality, dose, carcinogenesis and tumour type in irradiated and control pathogen-free C57BL mice. In one component of the study, groups of 21–56 male and 22–47 female newborn mice were given an intraperitoneal injection of 0.3, 0.4, 0.6, 0.9, or 1.5 mCi/kg bw [11–55.5 MBq/kg bw] [^{3}H]labelled thymidine. A group of 178 male and 205 female controls was available. The treated mice and controls were observed for life and evaluated histologically. The numbers of animals available for necropsy ranged from 43 to 99 in the groups exposed to ^{3}H (total, 344) and was 383 in controls. The incidence of all tumours combined was significantly higher in the ^{3}H-exposed newborn mice than in the controls, although the group sizes were insufficient for analysis by dose (see Table 99). The differences in incidence were attributable to a significantly increased incidence of lymphosarcomas. The age-specific incidence rates for reticular-tissue tumours tended to be higher in both exposed males and females than in their corresponding controls. The tumour incidences were independent of sex, and a dependence on dose was not established, perhaps because of the small group size and the relatively narrow dose range (Méwissen *et al.*, 1978).

Table 99. Differences in crude incidence rates (%) of tumours in newborn C57BL/6 mice injected with [^{3}H]thymidine in comparison with controls

Tumour	Males	Females	Significance
Lymphosarcoma	+9.4	+9.3	$p < 0.05$
Thyroid	–1.4	–0.6	
Liver	–0.1	+0.1	
Lung	+1.7	–4.0	
Other	–3.2	–1.3	
All	+4.9	+5.2	$p < 0.05$

From Méwissen *et al*. (1978)

In-dwelling subcutaneous catheters were used to infuse [^{3}H]thymidine continuously for 12 days from day 7 of gestation through term in pregnant SAS/4 mice. The amounts infused (0.6, 1.1, 1.7 or 3.3 MBq/day) resulted in four groups of 'fully ^{3}H-labelled' neonates, which had organically bound ^{3}H concentrations of about 10, 27, 50 and 130 MBq/kg bw. The calculations were based on measurements of bound ^{3}H and ^3H_2O concentrations in serially sacrificed fetuses and offspring. The corresponding intrauterine doses of radiation were estimated to range from 15 to 172 cGy. The average dose to mice at the highest concentration during the remainder of their life was about 50 cGy. The exposed offspring and a group of controls were kept until death, and complete histopathological evaluations were performed. Growth and survival were adversely affected at the higher doses, and an increased frequency of non-neoplastic symptoms was seen and referred to as general 'ill health'. As shown in Table 100, the

Table 100. Per cent tumour incidence in groups of 200–300 offspring of SAS/4 dams that received infusions of [^{3}H]thymidine from day 7 of gestation through term

Tumour	Controls		Dose infused (MBq/day)							
	Male	Female	0.6		1.1		1.7		3.3	
			Males	Females	Males	Females	Males	Females	Males	Females
Lung	37	36	35	35	38	44	41	38	48	15
Hepatoma	5	0	10	0	12	2	14	0	15	3
Reproductive tract	1	11	0	10	0	18	0	19	0	22
Leukaemia	4	21	8	25	13	28	11	28	11	30
Harderian gland	12	6	11	5	16	9	18	13	20	15

From Lambert & Phipps (1983)

incidence of neoplasia, especially of uncommon tumours, including those of the lung and liver and leukaemias, increased with increasing ^{3}H burden. The incidence of mammary tumours decreased at the highest doses, apparently in relation to the reduced lifespan (Lambert & Phipps, 1983).

(*b*) *Carbon-14*

The experiments of Baserga *et al*. (1966) on the carcinogenicity of ^{3}H, described above, also provide information on the perinatal carcinogenicity of ^{14}C. Thymidine is incorporated into DNA irrespective of the label, but ^{14}C β-particles are more energetic, so that the entire cell is irradiated, rather than just the nucleus. Four groups of newborn mice were injected with a single dose of 0.02, 0.2 or 2 mCi/kg bw [0.74, 7.4 and 74 MBq/kg bw] [^{14}C]thymidine and with six doses of the highest concentration over eight days. The overall incidences of tumours were similar in the experimental groups and in the controls. The group that received multiple exposures, however, had an increased incidence of 'miscellaneous' tumours and a decreased incidence of lymphoma. There were no significant effects on tumour latency in any group.

(*c*) *Phosphorus-32*

When ^{32}P is injected as inorganic phosphates, it readily crosses the placenta, and the average concentrations in the embryo or fetus soon reach those of the pregnant animal. Phosphorus is incorporated preferentially into proliferating tissues and bone matrix, resulting in differences in the concentration and radiation doses in tissues, depending on gestational stage.

Mouse: Pregnant BALB mice [number unspecified] were injected intraperitoneally once between days 11 and 15 of gestation with $Na_2{}^{32}PO_4$ at a dose of 2.5, 5, 10, 20, 40, 60 or 90 μCi per animal [92.5, 185, 370, 740, 1480, 2220 and 3300 kBq]. A comparison group of 36 adult female mice were injected at 3–5 months of age with a dose of 40, 60 or 90 μCi per animal. Eighty females and 20 males were used as controls. The pregnant mice were allowed to give birth, and 108 out of 149 offspring that survived until five months of age and were not autolysed were used in the analysis. All surviving mice were killed at two years of age or when moribund and were evaluated by necropsy and histological examination. The incidences of 'leukaemias', which included reticulum-cell sarcoma and lymphosarcoma, were calculated for each dose group but were pooled without regard to gestational stage. The overall incidence among the 71 exposed female offspring (20.2%) was significantly greater than that in 80 controls (16.2%). The incidences in each group ranged from 22 to 71%. In 37 prenatally exposed males, the overall incidence was 10.8%, while that in 20 control males was 10%. Latency was unaffected in animals of either sex. The incidences of leukaemia in the female offspring were similar to those in adults that had been exposed to doses of 40–90 μCi per animal (Holmberg *et al.*, 1964).

Rat: Pregnant BD rats were injected intravenously with $Na_2{}^{32}PO_4$ at a dose of 50, 100, 400 or 800 μCi [1850, 3700, 14 800 and 29 600 kBq] from day 2 of gestation. Malignant tumours developed in 17/130 offspring, which led to death at 270–800 days. Eleven of these tumours were neurogenic (three in the brain, two in the spinal cord, four in the peripheral nervous system and two in the heart) and were histologically identical to the tumours found after exposure to alkylating agents (*N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea). Of the other six rats with tumours, two had lung carcinomas, two had skin carcinomas, one had a liver carcinoma and one had an osteosarcoma of the jaw (Druckrey, 1973).

The effects of radioactive phosphate were evaluated after intraperitoneal injection of pregnant Sprague-Dawley rats with 1 or 3 mCi/kg bw [37 or 111 MBq/kg bw] on day 16, 18 or 20 of gestation. About 18–22 offspring in each age group received 1 mCi/kg bw, and 48–57 received 3 mCi/kg bw. A total of 128 control rats [age and treatment not stated] were studied throughout the experiment, but comparisons for evaluation of carcinogenesis were made with historical controls. Other litters were used to measure milk transfer by reciprocal cross-fostering and by analysis of stomach contents. The total dose per fetus was calculated to be approximately 10 rad [0.1 Gy], with non-uniform distribution. The offspring were evaluated throughout life, sacrifice and excision of breast tumours being conducted as needed. All animals were necropsied and examined histologically. Growth was unaffected at 1 mCi/kg bw but appeared to be reduced during the first year at 3 mCi/kg bw. The mean lifespan was not affected by 1 mCi/kg bw but was substantially reduced by 3 mCi/kg bw [statistics not presented]. The curves for 'cumulative deaths not due to tumours' of animals at 3 mCi/kg bw were shifted to the left for both males and females, as were the curves for 'cumulative mortality from tumour-associated deaths' in males. The spontaneous incidence rate of leukaemia, which is low in this strain, was not affected by exposure. The incidence of bone tumours, which were selected *a priori* as the primary end-point, was not increased among offspring of exposed dams, but showed a clear tendency to appear earlier in postnatal life among exposed offspring than in controls (Berry *et al.*, 1983).

(*d*) *Strontium-90*

Strontium and its isotopes have long physical and biological half-lives. As strontium is a homologue of calcium, it displays site-specific deposition in the perinatal skeleton. Both elements are distributed throughout the bone volume, as is radium, in contrast to other actinide elements, which are deposited on surfaces. The placental transfer, gastrointestinal absorption, dosimetry and early and delayed effects of radiolabelled strontium administered to prenatal or neonatal animals have been evaluated in numerous studies, but fewer studies have been reported of late effects, especially carcinogenesis.

Mouse: Groups of five to eight CBA mice were given an intravenous injection of ^{90}Sr as the nitrate at a dose of 46.3, 92.5, 185, 370 or 740 kBq on day 19 of gestation. Three controls were available. The female offspring (15–26 per group) were housed individually with untreated CBA males when they reached adulthood, and were bred for

seven months. The mice were killed at an average age of 10 months and the ovaries prepared for histological evaluation. The ovaries of mice at the higher doses were severely depleted of follicles and oocytes, but multiple corpora lutea were seen at lower doses. Interstitial fibrosis and cysts were the primary findings at the lower doses, and the incidence reached a maximum of about 50% at 185 kBq. Hyperplasia of interstitial cells and 'down-growth' of the germinal epithelium into the ovarian parenchyma was seen at this dose and above. Ovarian tubular adenomas were found in 1/19, 5/10 and 12/21 mice treated with 185, 370 and 740 kBq, respectively (Rönnbäck & Nilsson, 1982).

Rat: ^{90}Sr as the nitrate was injected intravenously into 12-week-old Wistar rats on day 18 of gestation. One group of 13 dams was injected with 100 μCi [3700 kBq], 11 dams with 150 μCi [5555 kBq] and another 24 dams received sterile saline. The offspring (60 males and 60 females and 53 males and 47 females in the two treated groups, and 111 male and 113 female controls) were necropsied at death or at 30 months of age and examined histologically. Strontium was selectively deposited in the ossification centres of the basioccipital bones of the skull and near the sella turcica, where the dose rates were maximal 96 h after injection. The cumulative inter-surface dose was calculated to be 60–120 rad [0.6–1.2 Gy] over the lifespan; approximately one-half of the lifetime dose was received within the first week after injection. Pituitary tumours (chromophobe adenomas) were detected at necropsy in exposed animals as young as 15 months of age, but were found in the saline-injected controls only after 22 months. Among animals killed at 30 months, the incidence of pituitary tumours in exposed male offspring (about 30%) was about 10-fold that in controls (2.7%), and the rate in females (46–50%) was about three times higher than that in controls (15.9%). A dose-related increase in the incidence of mammary hyperplasia was found in exposed female offspring, while the frequency of hyperplastic nodules or adenomas of the adrenal glands was increased in animals of each sex at the lower dose but was similar to that in controls at the higher dose. About 9% of the male controls had lymphatic tumours of the thymus; this incidence rose to 16% at 100 μCi but was the same as in controls at 150 μCi. The incidence was increased to 55% and 50% in the two exposed groups of females compared with 35% in controls. The authors indicated that 'about 30% of the thymomas were of a preponderantly epitheloid cell type, where the lymphocytes often only seemed to be dispersed within the epitheloid cell bonds' and that this special type of thymoma was usually observed with a pituitary tumour (Schmahl *et al.*, 1979; Schmahl & Kollmer, 1981). Metastatic meningeal sarcomas were detected in 11 and 10 offspring from the two exposed groups (5.8 and 7.0%) but not in the controls (Schmahl & Kollmer, 1981).

(*e*) *Cerium-144*

Rat: Groups of newborn, weanling or adult Sprague-Dawley rats [group sizes not specified] were injected intracardially or intravenously with ^{144}Ce at a dose of 0.25, 0.5 or 1.0 mCi/kg bw [9.25, 18.5 and 37 MBq/kg bw]. All animals were radiographed at intervals, and some from each treated group were killed for radioanalysis and histo-

logical examination. Routine gross and radiographic examination of the rats revealed gross lesions only 5–6 months after exposure. At that time, tumours were palpable on the legs of many of the weanlings exposed to 1.0 mCi/kg bw. The radiographs showed a 50% incidence of bone tumours, which increased to 80% by nine months. These tumours were confirmed histologically as osteogenic sarcomas. The lungs of some animals contained calcified metastatic nodules, while metastases to the spinal column were found in others. At this time, about one-half the adults that received 0.5 mCi/kg bw had bone tumours, but no tumours were detected in those exposed to 0.25 or 1.0 mCi/kg bw. None of the animals exposed as newborns developed bone tumours (Mahlum & Sikov, 1969).

(*f*) *Iodine-131*

Studies with ^{131}I illustrate the important role of stage of gestation or early postnatal life on the local tissue concentration, the associated doses of radiation and both early and late effects. Injection of pregnant animals after onset of fetal thyroid function leads to retarded neonatal growth, as also seen after neonatal exposure. The thyroid glands of offspring exposed to high doses show necrosis, fibrosis and compensatory hyperplasia (see section 4.3.3).

In an experiment with CBA mice, the effects of maternal exposure to X-rays (180 rad [1.8 Gy] to the fetus), prenatal exposure to ^{131}I β-particles or exposure to both X-rays and ^{131}I on day 18 of gestation were compared. The surviving offspring were killed and evaluated after 680–750 days. The initial group sizes and the doses of ^{131}I were not specified, but the numbers of thyroids examined histologically and the calculated range of doses to the lobe centre were reported (Table 101). A total of 488 untreated controls and 95 offspring that received X-rays only were available. One goal of the study was to compare the results with those of an experiment in adults, described in section 3.2 (Walinder, 1972). A quantitative comparison was not possible because the control thyroids weighed about twice that in the previous experiment, and the pituitary weights were also greater. The morphological changes seen in ageing control thyroids were different in this study (increased number and size of hyperplastic areas) and accounted for the weight difference. In contrast to the experiment in adults, no increase in pituitary weight with increasing thyroid dose was seen. Thyroid tumours were detected in moribund mice from 558 days of age. Some thyroid tumours occurred in the control offspring, but the incidence was not increased in those that received X-rays only. As shown in Table 101, the thyroid tumour incidence was increased at the three lower doses of β-particles, and the effect appeared to be greater in males than in females. There was no further increase in the incidence of thyroid tumours at the highest dose in males, and the incidence fell slightly in females. The quantitative effect of combined exposure to X-rays and ^{131}I was not clear; however, there was a greater incidence at the higher dose from ^{131}I, with no sex difference. The overall incidence of tumours was greater than in the experiment with adults. The effective doses were lower in prenatal animals than in adults (Walinder & Sjödén, 1972).

Table 101. Incidences of thyroid tumours in offspring of mice that received ^{131}I and/or X-rays at 18 days of gestation

^{131}I median dose to lobe centre (Gy)	X-Ray dose (Gy)	No. of animals examined	Sex	% with thyroid tumours
0	0	263	Male	4.2
		225	Female	1.2
20	0	172	Male	10.2
		160	Female	3.1
38	0	89	Male	15
		58	Female	3.5
48	0	16	Male	31
		20	Female	25
70.5	0	102	Male	34
		83	Female	18
0	1.8	48	Male	4.2
		47	Female	0
16.5	1.8	79	Male	6.3
		73	Female	6.9
28	1.8	46	Male	20
		30	Female	20

From Walinder & Sjödén (1972)

In a subsequent experiment, involving exposure of both fetuses and adults, necropsies were performed at one year of age to avoid loss of animals by death, and no thyroid tumours were found among mice exposed as adults after this relatively short interval. In contrast, an increased incidence of thyroid tumours (7/109) was detected in the offspring of dams that had received the highest dose of ^{131}I, which resulted in a dose of 78 Gy to the fetal thyroid (Walinder & Sjödén, 1973).

On the basis of their comparisons of the effects of radiation on the kinetics and regeneration of the cell cycle in the thyroid, Walinder and Rönnbäck (1984) provided additional explanations of the greater frequency of thyroid tumours and shorter latent period in offspring that had been irradiated *in utero*, relative to those irradiated as adults. At equal doses of radiation, cell killing was more frequent during adulthood than prenatally, suggesting that the primary carcinogenic event is independent of age. The greater sensitivity of the prenatal thyroid to tumour development may be associated with age-related differences in cell proliferation rates and the short life of epithelial cells.

Weanling and adult Sprague-Dawley rats were exposed to ^{131}I by gavage, newborns via maternal milk and fetuses via placental transfer and maternal milk. Carrier-free ^{131}I was administered by gavage on five successive days at four activity concentrations over ranges that were adjusted to produce similar subacute effects in rats of the four ages. The total administered amounts of ^{131}I were 1, 15 and 150 μCi [37, 555 and 5555 kBq]

to adult, newborn and pregnant rats and 0.25, 3.75 and 37.5 μCi [9.25, 139 and 1390 kBq] to weanlings. A pooled control group comprised rats representative of all four ages. To establish the dosimetry and early effects, groups of rats from each age and dose group, including pregnant dams and offspring, were killed serially. The animals for long-term observation were identified at weaning and were kept until death or 30 months of age. Selected organs were preserved at necropsy, and the thyroid and pituitary glands were examined histologically. A total of 76 controls and 20–65 animals from of each age and dose group were evaluated. All slides were examined, and the tumours were classified into C-cell and follicular-cell tumours (Sikov *et al*., 1989). The predominant thyroid neoplasm found in control rats of all ages was C-cell tumours, which correspond to the alveolar carcinomas described by Lindsay *et al*. (1957). The incidence of C-cell tumours in the pooled controls (18.4%) was similar to those at the low doses, but the incidence was markedly and significantly decreased (0–5%) in all age groups at the highest dose. A single follicular tumour was seen among the controls, but these were the most frequent thyroid tumour type in exposed groups. Their incidence was increased at the two lower doses in all age groups but was decreased in adults and weanlings exposed at the highest dose. In the groups exposed prenatally or neonatally, however, the incidence was even greater at the highest dose (12/40 in newborns and 37/61 in the prenatal group). The mammary tumour incidence was increased by postnatal exposure but was not affected by prenatal exposure.

3.4 Exposure of male parents

3.4.1 *α-Particle emitter: Plutonium-239*

Mouse: An experiment was carried out to test the hypothesis that paternal irradiation might alter susceptibility of offspring to the development of defects of the lymphatic or haematopoietic system or predispose them to disease due to various insults. Groups of 20 DBA2 and 20 CBA/H male mice, 12 weeks of age, were injected intravenously with ^{239}Pu citrate at a dose of 128 or 256 kBq/kg bw, while controls received the citrate carrier. Twelve weeks later they were mated with 12-week-old female mice of the C57BL or CBA/H strain. All of the female BDF_1 offspring were injected at 10 weeks of age with 50 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU), while female CBA/H offspring were exposed to 3.3 Gy of ^{60}Co γ-rays by whole-body irradiation. The mice were killed when they showed overt signs of disease, such as weight loss, hyperventilation, languor or enlargement of the spleen or liver. The first malignancy seen in a control BDF_1 offspring was a thymic lymphoma that developed 89 days after injection of MNU. Subsequently, lymphomas and myeloid leukaemias developed, so that 50% of the MNU-treated controls were affected by 185 days. The first symptoms in the offspring of irradiated fathers were seen 28 days earlier (a significant decrease), and were expressed as thymic lymphomas, which were detected at 61 and 69 days in mice that received 128 and 256 kBq/kg bw and MNU, respectively,

while leukaemia first developed at 77 and 86 days in these groups, as compared with 92 days in the controls. Thereafter, leukaemia accumulated at approximately twice the rate of the lymphomas, so that mice in these groups had to be killed after a significantly shorter time than the controls. By 250 days, 93 and 83% of the mice at the two doses, respectively, had developed tumours, as compared with 70% in the control group. The first of the CBA/H offspring in the control group that received whole-body radiation was killed at 215 days with developing myeloid leukaemia. Pathological conditions also developed in several other tissues in all groups. A total of 69% of the irradiated mice were killed during the 635 days after exposure. Lymphatic and haematopoietic system tumours were detected in 30% of the irradiated mice and about 20% of the control group. Although the latent period for onset of lymphatic or haematopoietic disease appeared to be delayed by preconceptional paternal exposure to ^{239}Pu, the subsequent incidences were significantly increased. At 128 kBq/kg bw, the incidence of lymphoid leukaemia was more than doubled ($p < 0.001$). This increase competed with the development of myeloid leukaemia, however, so that its incidence fell (–30% compared with controls). In animals at 256 kBq/kg bw, the incidences of myeloid leukaemia and lymphoid leukaemia were increased (+ 16% and + 100%) when compared with controls (Lord *et al.*, 1998a,b).

3.4.2 *β-Particle emitter: Hydrogen-3*

Mouse: Males of a subline of inbred C57BL/6M mice, 35 days of age, received drinking-water containing ^{3}H at a concentration of 10 μCi/mL [370 kBq/mL] for 35 days. The mice were then mated with unexposed females. The offspring were separated after weaning, and the new generation of males was given ^{3}H-labelled drinking-water by the same dose regimen and then mated with their untreated female siblings. This sequence was repeated for a total of 18 generations. The number of offspring and the sex ratio were recorded. Some mice of each generation were maintained for lifetime observation, and the others were killed. The mice of the original line were maintained similarly but without ^{3}H in their water. In the 15th generation, three male and four female offspring from a litter of the exposed line were paired with mice of the opposite sex from the control line and allowed to produce further litters without restriction. The sibling pairings in the first generation (F_1) produced a second generation (F_2) of animals, which were kept for lifetime observation. This procedure was repeated through the fifth generation. Multiple adenocarcinomas of the intestine were observed in the cross between the control and ^{3}H-exposed lines. The tumours were heritable and were found in an average of 44% of the 117 males and 70% of the 88 females examined in the F_1–F_5 generations. The corresponding latent periods were 441 and 542 days, and the incidences of other tumours were 24 and 15% (Méwissen *et al.*, 1984).

On the basis of the reports of Nomura of an increased incidence of leukaemia after X-irradiation of paternal N5 mice (see IARC, 2000), the question of the carcinogenic effect of preconceptional irradiation of males was addressed in a comparison of $^{3}H_2O$

and X-irradiation. Male descendants of the same strain of mouse were exposed to internal irradiation from ^{3}H or to X-rays and mated with untreated females. $^{3}H_2O$ was given as six intraperitoneal injections at 6-day intervals to groups of 33 and 39 males at a dose of 15 or 22.5 MBq at each injection, resulting in a total dose of 1 or 1.5 Gy. Another group of 28 mice received a single exposure to X-rays at 5 Gy, and 27 control males received no exposure. The males were mated 3, 10 or 17 days after the last injection or exposure. The progeny from these groups were kept for study, but the group sizes were randomly reduced to about 300 at one month of age. Because there was an adequate number of offspring of mice given 1.5 Gy of ^{3}H, the mice at the lower dose were discarded. There were thus 305 controls, 165 mice treated with X-rays and 312 mice treated with $^{3}H_2O$. None of the offspring of mice exposed to X-rays for 17 days survived beyond one month of age. The probability of dying from leukaemia during the one-year observation period was significantly greater for offspring of X-irradiated than for those of unexposed fathers, but the difference for offspring of ^{3}H-exposed fathers was not significant ($p = 0.2$). Leukaemia occurred earlier in both the X-ray and the ^{3}H-exposed groups, and treated fathers were more likely to have more than one affected offspring than were control males. The leukaemia rate at 210 days was 8/312 in the offspring of fathers given $^{3}H_2O$ and 1/305 in controls. In the offspring of $^{3}H_2O$-treated fathers mated after 3, 10 or 17 days, the leukaemia rates were 2/100, 4/108 and 8/104, respectively. The leukaemia incidence rate was higher in female than in male offspring (Daher *et al.*, 1998).