4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion of radionuclides

The most important routes of intake of radionuclides are by inhalation and ingestion, for both workers and members of the public. Ingestion is a less important route for workers, but a proportion of inhaled material is escalated from the trachea and lungs and swallowed. Entry through contaminated wounds may also occur in certain industrial situations. Entry through intact skin is rare, although exposure to $^{3}H_2O$ vapour can lead to appreciable doses by this route.

Entry of radionuclides into the body by inhalation and ingestion leads to irradiation of the respiratory and gastrointestinal tracts. Entry through contaminated wounds also results in local irradiation of tissues. After intake by any route, the subsequent behaviour of the radionuclide depends on the element concerned and its chemical form during exposure. These factors determine its solubility and the extent to which it is dissolved and absorbed into the blood. On reaching the blood, the distribution to and retention in body tissues depend on the chemical nature of the element. If a radionuclide that enters the bloodstream is an isotope of an element that is normally present (e.g. sodium, potassium, chlorine), it will behave like the stable element. If it has similar chemical properties to an element normally present, it will tend to follow the metabolic pathways of that element (e.g. ^{90}Sr and ^{226}Ra behave similarly to calcium and ^{137}Cs and ^{86}Rb similarly to potassium), although the rate of transfer between the various compartments in the body may be different. For other radionuclides, the behaviour in the body depends on their affinity for biological ligands and other transport systems. Radionuclides entering the bloodstream may be distributed throughout the body (e.g. ^{3}H, ^{42}K, ^{137}Cs), be deposited selectively in a particular tissue (e.g. ^{131}I in the thyroid, ^{90}Sr in bone) or be deposited in significant quantities in a number of different tissues (e.g. ^{239}Pu, ^{241}Am, ^{144}Ce).

While dissolved radionuclides are distributed in the body directly via the blood, insoluble materials may be transported slowly as small particles through the lymphatic system, which can lead to accumulation of radionuclides in regional lymph nodes (e.g. tracheobronchial lymph nodes after transport from the lungs; axillary lymph nodes after transfer from a wound in the hand). Particles may reach the bloodstream slowly and then be removed rapidly from the circulation by phagocytic cells of the reticulo-endothelial system in the liver, spleen and bone marrow.

The ICRP has reviewed the data on the biokinetics of radionuclides in humans and animals after intake by inhalation and ingestion and has developed biokinetics and

dosimetric models for calculating organ and tissue doses (ICRP, 1979, 1980, 1981, 1986, 1989, 1993c, 1995a,b). The dose coefficients of ICRP for intake of radionuclides (dose per unit intake) have been incorporated into legislation in Europe (EURATOM, 1996) and other parts of the world.

The following sections describe the biokinetics of individual radionuclides after intake by inhalation and ingestion, including their distribution after absorption into the blood, the duration of retention in organs and tissues and the routes of excretion. Information on placental transfer is also provided. For each radionuclide, the data on humans and animals are considered together. In a final section, the use of biokinetics and dosimetric models to estimate organ and tissue doses is described, and examples are given of doses from intakes of the radionuclides considered.

4.1.1 *Hydrogen-3*

(*a*) *Inhalation*

Tritium (^3H) may be inhaled as elemental tritium gas ($^3H_2$), as ^3H-labelled water (^3H_2O), in the form of organic compounds or in particulate aerosols (reviewed by Hill & Johnson, 1993). $^3H_2$ is relatively insoluble, and < 0.1% of an inhaled dose was taken up to the blood in one study in humans. ^3H_2O vapour is rapidly absorbed from the lungs to blood. Small amounts of volatile, highly soluble organic acids may be released as effluent from tritium facilities and, because of their chemical characteristics, it is reasonable to assume that absorption to the blood will be high. Studies of the absorption of ^3H to blood after intratracheal installation of 1-μm (count median diameter) titanium tritide particles in rats indicated intermediate solubility (ICRP, 1995b).

(*b*) *Dermal intake*

Exposure to an atmosphere contaminated by ^3H_2O results in absorption through intact skin as well as from the lungs. In an adult person at rest, about 1% of the radioactivity in 1 m^3 of air was absorbed through the intact skin per minute. The ICRP (1995b) concluded that this route contributes about one-third to the total absorption of ^3H_2O to blood. An increase in physical activity results in an increase in the proportion of ^3H_2O absorbed through the lungs.

(*c*) *Ingestion*

In volunteers, ingested ^3H_2O was rapidly and virtually completely absorbed from the gastrointestinal tract. A large proportion of organically bound ^3H may be broken down in the gastrointestinal tract, producing ^3H_2O. For example, studies in rodents indicated that 80–90% of ingested [^3H]thymidine is catabolized before reaching the blood, and only a small proportion (< 5%) is incorporated into DNA in body tissues. The absorption of intact molecules of other forms of organically bound ^3H, including ^3H-labelled amino acids, and transfer to body tissues may be substantially greater. Although a proportion of

ingested organically bound ^3H may be unavailable for absorption, such as that in indigestible cellulose, it is generally assumed that absorption will be complete, either as organically bound ^3H or after catabolism to ^3H_2O (ICRP, 1979, 1989).

(*d*) *Systemic distribution, retention and excretion*

The radiobiology of tritium has been reviewed (Straume & Carsten, 1993). Studies of the urinary excretion of ^3H_2O in humans after exposure by inhalation or ingestion indicate rapid mixing of ^3H_2O with body water after absorption into the blood (see ICRP, 1979, 1989; Hill & Johnson, 1993). Since the body water is distributed fairly uniformly, it is generally assumed that all organs and tissues receive the same dose. The half-time of retention of ^3H_2O corresponds to the loss of body water, with an average value of about 10 days for adult humans. In animals, however, a small proportion of ^3H absorbed as ^3H_2O is incorporated into organic molecules and retained for longer periods (ICRP, 1979, 1989). The half-times of retention attributable to organically bound ^3H have not been characterized with precision in humans, but the range appears to be 20–80 days for the major component and 280–550 days for a smaller component (Etnier *et al*., 1984). It has been estimated that total incorporation of ^3H from ^3H_2O into organic molecules adds little (< 10%) to the total integrated activity and hence the dose (ICRP, 1979).

Comparisons in animals of the relative incorporation of ^3H into organic molecules after intake of ^3H_2O and organically bound ^3H indicated that 3–30 times more organically bound ^3H was present after intake of the radionuclide in this form (Rochalska & Szot, 1977; ICRP, 1989; Komatsu *et al*., 1990; Takeda, 1991). Takeda (1991) gave rats ^3H_2O or ^3H-labelled leucine, lysine, glucose, glucosamine, thymidine or uridine in the drinking-water for 22 days. At the end of this period, the highest concentrations of organically bound ^3H were found in rats exposed to ^3H-labelled amino acids (four to nine times higher than those in rats exposed to ^3H_2O), with intermediate concentrations after exposure to ^3H-labelled DNA/RNA precursors. Rochalska and Szot (1977) fed ^3H-labelled food or ^3H_2O to rats for five days and determined organically bound ^3H in the tissues on day 6. Incorporation, measured in dried tissues, was highest after intake of organically bound ^3H by factors ranging from three for brain tissue to 15–17 for liver and small intestine. Takeda (1991) and Komatsu *et al*. (1990) concluded that intake as organic molecules rather than ^3H_2O may increase the radiation dose by about a factor of 2. Although the distribution of organically bound ^3H between body organs and tissues has been shown to be less uniform than that of ^3H_2O in body water, greater uptake in certain organs is associated with greater metabolic activity in those organs (e.g. the liver and intestinal wall), and with shorter retention times. The doses from organically bound ^3H are therefore generally calculated on the basis of uniform distribution in the body, as for ^3H_2O (ICRP, 1979).

Because of the low energy and short range of β-particle emissions from ^3H (average energy, 5.7 keV; mean range, 0.7 μm), the doses to cell nuclei and DNA from ^3H-labelled DNA precursors have been investigated. The National Council on Radiation

Protection and Measurements (1979) compared the calculated doses to human haematopoietic and spermatogonial stem-cell nuclei after ingestion of ^3H_2O and [^{3}H]thymidine. After a single intake, [^{3}H]thymidine was estimated to give the greater dose, by a factor of about 8. In studies with mice, administration of [^{3}H]thymidine during gestation resulted in estimated doses to the offspring that were several times greater than those from administration of ^3H_2O (Saito & Ishida, 1985).

Both the loss of ^3H_2O in body water and the turnover of organically bound ^{3}H are more rapid in children than in adults. The approach adopted by ICRP (1989) for the ^3H_2O component was to relate daily water balance to energy expenditure at different ages. Similarly, shorter retention times for organically bound ^{3}H were based on estimates of body content and loss of carbon.

(*e*) *Placental transfer*

^3H_2O crosses the placenta rapidly and equilibrates between maternal and fetal tissues. The composition of the human fetus undergoes marked changes throughout gestation, with a general trend to a progressive decrease in the proportion of body water and increases in body protein, fat and minerals. The body water content is about 920 mL/kg bw in a 10-week-old fetus and declines to about 700 mL/kg bw at birth; the average value throughout the fetal period is about 800 mL/kg bw. For reference, the body water content of a nongravid female is about 500 mL/kg bw (ICRP, 1975).

The amount of non-exchangeable ^{3}H in rat dams and neonates was compared after oral administration of ^3H_2O or lyophilized ^{3}H-labelled rabbit meat from three weeks before conception to term. The ^{3}H-labelled meat was obtained by repeated intraperitoneal injection of rabbits with ^3H_2O. After administration of ^3H_2O, the specific activity of non-exchangeable ^{3}H in neonatal tissues was about 20% higher than that in maternal tissues, whereas after administration of labelled meat, no difference in specific activities was found between fetal and maternal tissues. Administration of labelled meat led to three- to fivefold greater concentrations of non-exchangeable ^{3}H in both fetal and maternal tissues (Pietrzak-Flis *et al.*, 1982). The maternal and fetal concentrations of ^{3}H were also compared 24 h after oral administration of ^3H_2O, [^{3}H]thymidine or [^{3}H]lysine to rats on day 13 or day 17 of pregnancy. After administration on day 17, transfer to the fetuses represented about 8, 9 and 19% of the administered dose, respectively, per litter. The fetal doses after administration of [^{3}H]lysine were estimated to be 1.5–3 times higher than those after ingestion of ^3H_2O or [^{3}H]thymidine (Takeda *et al.*, 1994).

4.1.2 *Carbon-14*

(*a*) *Inhalation*

Three main classes of carbon compounds may be inhaled: gases such as carbon monoxide (CO) and carbon dioxide (CO_2), organic compounds and aerosols of carbon-containing compounds such as carbonates and carbides (see ICRP, 1981, 1995b).

Extensive data are available on the retention of inhaled CO in body tissues. The gas is relatively insoluble in water and the doses are dominated by retention of CO bound to haemoglobin and, to a lesser extent, other iron–haem-containing compounds, including cytochrome oxidase. The results of a study of the formation and dissociation of carboxyhaemoglobin in individuals taking light exercise indicated that about 40% of inhaled CO was retained, with a half-time of about 200 min. Because CO is bound predominantly to circulating haemoglobin, it is reasonable to assume that doses of ^{14}C-labelled CO are delivered uniformly throughout the body (ICRP, 1981).

As CO_2 is transferred rapidly across the alveolar membrane, it is generally assumed that absorption of ^{14}C-labelled CO_2 is complete. CO_2 exists in the blood mainly as the bicarbonate. A study of the whole-body retention of ^{14}C in 13 normal persons after an intravenous injection of [^{14}C]bicarbonate showed retention of two components, about 18% with a half-time of 5 min and 82% with a half-time of 1 h. In mice, the presence of a third component was reported, with a retention half-time of 10 days or more, consistent with incorporation into organic molecules (ICRP, 1981).

Most organic compounds are not very volatile under normal circumstances and the probability of inhalation as vapours is low. In the absence of information, it seems reasonable to assume that volatile compounds are soluble when inhaled and readily absorbed into the blood.

The results of studies in which rats inhaled ^{14}C-labelled diesel exhaust particles indicated intermediate solubility (Lee *et al*., 1987; ICRP, 1995b).

(*b*) *Ingestion*

The fractional absorption of dietary carbon is usually in excess of 0.9 although some carbon compounds may be less completely absorbed, including cholesterol, fat-soluble vitamins and cellulose (ICRP, 1981).

(*c*) *Systemic distribution, retention and excretion*

The distribution and retention of ^{14}C in organs and tissues depends strongly on the chemical form in which it enters the systemic circulation. Because information is not available for the majority of these compounds, it has generally been assumed that all ^{14}C-labelled compounds are distributed rapidly and uniformly in all body organs and are retained with a half-time of 40 days. This biological half-time was derived from the average daily carbon intake (~ 0.3 kg/day) and the mass of carbon in the body of a ‘reference man’ (16 kg), according to the equation:

$$t_{½} = \ln 2 \times [\text{total body carbon/daily carbon intake}] = 0.693 \times 16/0.3 = 37 \text{ days}$$

or about 40 days (ICRP, 1975, 1981, 1989).

The retention of carbon incorporated in various metabolites in organs and tissues of the body shows wide variation. For example, studies on autopsy samples from people exposed to ^{14}C in fall-out (Stenhouse & Baxter, 1977a,b) suggest that bone collagen and bone mineral retain ^{14}C with a biological half-time that may exceed 20 years. The

retention of ^{14}C after intravenous injection of labelled glycine or acetate into patients decreased in various phases, with biological half-times between 0.1 and 17 days (ICRP, 1981). In a study with accelerator mass spectrometry of the retention of ^{14}C in three healthy men after ingestion of [^{14}C]triolein, measured as ^{14}C in exhaled CO_2, 30% of the administered ^{14}C was eliminated in the first 24 h; the remainder was retained with a half-time of at least several hundred days (Stenström *et al.*, 1996).

The rate of metabolism of ^{14}C-labelled compounds is age-dependent. For example, in rats, metabolic incorporation and release of ^{14}C from glycine or stearic acid was more rapid in younger animals (ICRP, 1989). As for organically bound ^{3}H, ICRP (1989) applied shorter retention half-times for ^{3}H and ^{14}C in organic form in children on the basis of differences in carbon balance by age.

(*d*) *Placental transfer*

CO_2 entering the blood distributes rapidly throughout the bicarbonate pool of the body water. No information on bicarbonate transfer to the embryo or fetus was found, but it may be assumed to diffuse readily through the placenta and into the fetus.

Most amino acids are actively transported across the placenta. In the human placenta, transport proteins for many amino acids are located in the microvilli and basal membranes of the syncytiotrophoblast (Moe, 1995). Few quantitative data are available, although Moe (1995) reported fetal:maternal blood concentration ratios of ~ 3 for lysine, ~ 2 for alanine and ~ 1 for glutamate and aspartate. In a study of the placental transfer of ^{11}C-labelled sugars, amino acids, adenine, adenosyl-methionine, fluorodeoxyuridine and coenzyme Q_{10} in rats, when the data allowed direct comparison of maternal and fetal tissue concentrations, the fetal values were generally similar to or lower than the corresponding maternal values, the exception being for amino acids, for which the fetus-to-placenta ratios were consistently > 1 (Ishiwata *et al.*, 1985).

4.1.3 *Phosphorus-32*

(*a*) *Inhalation*

The ICRP Task Group on Lung Dynamics (ICRP, 1966) reviewed the behaviour of materials in the lung and classified most compounds of phosphorus as soluble, assuming rapid absorption to blood. The exceptions were phosphates of zinc, tin, magnesium, iron and bismuth and the lanthanides, which were considered to be less soluble and to be retained in the pulmonary region of the lungs with a half-time of about three months.

(*b*) *Ingestion*

Dietary phosphorus is generally well absorbed, as are inorganic forms of phosphorus (see ICRP, 1979). Measurements in seven volunteers who ingested fish contaminated with ^{32}P from reactor coolant water demonstrated complete absorption of the radionuclide (Honstead & Brady, 1967). The fractional absorption of radiophosphorus of

unspecified form administered to patients was approximately 0.2–0.3, as shown by the difference in skeletal uptake of subjects after oral or intravenous administration (Castle *et al*., 1964). However, less than 5% of the phosphorus-containing compound diphosphonate etidronate was absorbed in normal subjects (Fogelman *et al*., 1986).

(*c*) *Systemic distribution, retention and excretion*

The retention of phosphorus in the body has been reviewed (Jackson & Dolphin, 1966). It was concluded that retention as phosphate in humans is well described by four terms corresponding to blood plasma, intracellular fluids, soft tissues and bone mineral. Initially, 15% of absorbed phosphorus is excreted rapidly directly from blood, with a half-time of 0.5 days, followed by loss of a further 15% from intracellular fluids with a half-time of two days. Retention in soft tissues with a half-time of 19 days was reported to account for 40%, and the remaining 30% was assumed to be permanently retained in the bone. Studies of the dietary balance and body content of phosphorus (ICRP, 1975) suggest a half-time of retention in bone of about four years. Since the radioactive half-life of ^{32}P is 14.3 days, the assumption of infinite retention in bone is appropriate for the purposes of estimating doses. Because of the short half-life of ^{32}P, ICRP (1979) calculated the doses to bone surfaces and red bone marrow, assuming that the nuclide is retained on bone surfaces. In addition, a small amount of radioactive phosphate entering the body is incorporated into cellular DNA, especially in rapidly dividing tissues such as red bone marrow and the lining of the small intestine. This fraction is larger if the radioactive phosphorus enters the blood in the form of a DNA-precursor nucleotide.

(*d*) *Placental transfer*

Studies in rats have shown that phosphate readily crosses the placenta but that the transfer of phospholipid is much slower (National Council on Radiation Protection and Measurements, 1998). Experiments in guinea-pigs, sows and rats yielded consistent results. The overall pattern is of an increase in the relative concentrations in embryos and fetuses as ^{32}P is administered at progressively later stages of gestation; the rapidity in reaching maximum activity and retention followed the same pattern.

4.1.4 *Sulfur-35*

(*a*) *Inhalation*

Information is available on the behaviour of inhaled sulfur dioxide (SO_2) gas and carbon disulfide (CS_2) vapour. In a study of volunteers, virtually all of the inhaled non-radioactive SO_2 was absorbed by the nasal mucosa (Speizer & Frank, 1966). Studies in dogs showed rapid absorption into blood after deposition of SO_2 in the respiratory tract. Studies of inhaled CS_2 have shown deposition and absorption into the blood in various animal species, including rats and humans. Deposition and

absorption were not quantified in these studies, but the absorption in rats was rapid, indicating high solubility (ICRP, 1995b).

No detailed information is available on the rate of absorption of ^{35}S after inhalation of particulate materials, but two cases of accidental exposure, one to elemental sulfur and another to an unknown form, indicate high solubility in the lungs (ICRP, 1995b).

(*b*) *Ingestion*

As reviewed by ICRP (1993c), studies of sulfur balance indicated that 68–90% of dietary sulfur is absorbed by pre-adolescent girls, apparently depending on dietary nitrogen levels. Studies in rats treated by oral or intraperitoneal administration of ^{35}S as the sulfate or as [^{35}S]L-methionine (the most abundant organic form of sulfur in the diet) indicated that the fractional absorption was 0.9 or higher. Elemental ^{35}S was less well absorbed in rats, with a value around 10%.

(*c*) *Systemic distribution, retention and excretion*

Measurements of the concentration of stable sulfur in human organs and tissues have been reported. The concentrations in most tissues, including testes, were 1–2 g/kg, although the value for cartilage was higher (5.5 g/kg) and that for bone marrow was lower (0.7 g/kg). The distribution and retention of ^{35}S in rats were compared up to 130 days after intravenous injection of [^{35}S]methionine and up to eight days after injection of [^{35}S]sodium sulfate. The distribution of ^{35}S from the organic compound between tissues was fairly uniform, all the concentrations being within a factor of 3 except for a lower concentration in bone. After administration as the sulfate, the ^{35}S concentrations varied by about a factor of 20, the highest values being found for cartilage and the lowest for muscle (ICRP, 1993c).

Approximately 70–90% of ^{35}S injected intravenously as sodium sulfate in humans was excreted in the urine within the first three days. Similar results were reported for ^{35}S as sulfate in rats, while excretion of ^{35}S administered as methionine was about 10 times slower. The data on dietary intake and whole-body content in adult humans (ICRP, 1975) are consistent with a half-time of retention for organic sulfur in the diet of about 140 days, assuming complete absorption in the blood (ICRP, 1993c).

(*d*) *Placental transfer*

Little information is available on the transfer of inorganic sulfur to the fetus, although placental transfer and fetal incorporation of ^{35}S as sulfate has been demonstrated (Hagerman & Villee, 1960). Active transport of methionine and other sulfur-containing organic molecules was shown (Ishiwata *et al.*, 1985). The placental transfer of the sulfur-containing amino acids cysteine and methionine was studied in pregnant women about to undergo abortions in weeks 16–22 of pregnancy. About 15–45 min after injection of 0.5 mmol of non-radioactive L-methionine, the fetal:maternal blood concentration ratio was ~ 2. In contrast, 45–60 min after injection of 2.5 mmol L-cystine

or L-cysteine, the fetal blood concentration of the amino acids was only about half that found in the maternal blood (Gaull *et al.*, 1973).

4.1.5 *Gallium-67*

(*a*) *Inhalation and ingestion*

On the basis of studies in rats that showed little absorption of gallium chloride from the gastrointestinal tract, ICRP (1981) used a fractional absorption value of 10^{-3} for ingested gallium. However, for most forms of inhaled gallium, intermediate absorption into blood was assumed to apply, with a half-time of up to three months.

(*b*) *Systemic distribution, retention and excretion*

^{67}Ga is usually used in carrier-free form. ^{67}Ga injected intravenously to rats was initially distributed fairly uniformly throughout all tissues and organs, but by 24 h after injection greater concentrations were found in the skeleton, liver, kidneys and spleen. Human autopsy samples and γ-ray spectroscopy showed a similar distribution, with accumulation also in the adrenal glands and in the placenta during pregnancy. Studies in animals and humans indicate a biphasic release of gallium from the body, with biological half-lives of about one day and 50 days (ICRP, 1981). In volunteers given ^{67}Ga citrate by intravenous injection, more than half of the injected dose was still present in the body after 21 days. The liver and the skeleton were the major sites of deposition after 24 h, and the principal route of excretion was via the kidneys, with about 10% excretion in the faeces. This nuclide is cleared slowly from the blood, indicating protein binding (Priest *et al.*, 1995a). Studies of whole-body retention in cancer patients indicated that 10–20% of injected ^{67}Ga (as citrate) was lost from the body with a half-time of approximately one day and the remainder with a half-time of about 26 days (Saunders *et al.*, 1973). Excretion via the urine is the major excretory pathway of ^{67}Ga in humans, about 10% of the radioactivity in the systemic circulation being excreted within the first 24 h. However, there was a wide range (3–35%) among the 29 patients studied (Zivanovic *et al.*, 1979).

4.1.6 *Strontium-89 and strontium-90*

(*a*) *Inhalation*

Measurements following accidental inhalation of strontium carbonate ($^{90}SrCO_3$) by humans showed high solubility. Similarly, experiments in animals have shown that strontium in simple ionic compounds (chloride and sulfate) is cleared rapidly from the lungs, consistent with high solubility. A study *in vitro* on strontium-containing airborne fission products released during the Three Mile Island reactor accident confirmed these results (ICRP, 1995b).

(*b*) *Ingestion*

Owing to the presence of strontium isotopes in fall-out material and its long-term retention in bone as a calcium analogue, the metabolism of strontium has been the subject of a number of studies in volunteers. Similar absorption values were obtained in studies in which inorganic forms of radiostrontium were administered orally in solution and in experiments in which known quantities of radiostrontium incorporated in food were ingested (reviewed by ICRP, 1989). The mean values were between 0.15 and 0.45. In a study of the absorption of strontium from real and simulated fall-out and after administration of $^{85}SrCl_2$, 10 volunteers ingested samples of local fall-out, largely comprising silicaceous soil constituents (40–700-μm particles). The estimated absorption rate was 3%, with a range of 0–9%, while that for simulated fall-out prepared as glass microspheres (30–40 μm) was 16% (range, 6–25%), with a value of 17% (8–34%) after administration as $^{85}SrCl_2$ (LeRoy *et al.*, 1966).

A number of factors have been found to increase the absorption of strontium, including fasting and low dietary levels of calcium, magnesium and phosphorus; milk diets and vitamin D may also increase absorption. Overnight fasting increased the fractional absorption from about 0.25 to 0.55 in one study, and an average fractional absorption of 0.55 (0.38–0.72) was seen in four volunteers after an overnight fast compared with 0.11 in a single volunteer who ingested strontium after breakfast. A decrease in the dietary intake of calcium from 30–40 to 0–10 mg/kg per day increased the fractional absorption of strontium from an average of 0.2 to 0.4. The results of animal studies are generally similar to those for volunteers (ICRP, 1989).

The results of measurements of the absorption of strontium in seven-day-old infants fed cows' milk suggested values > 73%. Similar levels of absorption have been reported for 5–15-year-old children and adults. However, studies in beagles and rats have shown that the period of increased absorption of strontium extends beyond the time of weaning. In beagles, the retention values for strontium 3–9 days after ingestion were 20%, 15% and 8% in 48-, 80- and 140-day-old animals, respectively. The absorption of strontium was estimated to be 70–90% in 35- and 75-day-old rats and 12% in 270-day-old rats (ICRP, 1989).

(*c*) *Systemic distribution, retention and excretion*

The behaviour of strontium in the body is similar to that of calcium, but there are quantitative differences that reflect discrimination against strontium and result in less effective incorporation and retention in bone and more rapid urinary excretion. Long-term retention of strontium, like that of calcium, is confined to the skeleton, and doses from ^{90}Sr absorbed into blood are delivered largely to bone surfaces and red bone marrow (see Leggett *et al.*, 1982; Leggett, 1992a; ICRP, 1993c).

A great deal of information is available on the age-specific behaviour of strontium in humans. ^{90}Sr from fall-out has been measured in bones from persons of various

ages. Biokinetics models have been developed in which the body's discrimination between calcium and strontium is taken into account (Leggett *et al*., 1982).

The fraction of activity from ^{90}Sr that leaves the plasma and goes to the urine and faeces is 0.15, the assumed ratio of cumulative urinary to faecal excretion being 3.3. Strontium that enters the skeleton is assumed to deposit initially on bone surfaces but to migrate to exchangeable bone volume within a few days. About half of the strontium leaving this compartment, with a half-time of about 80 days, binds to non-exchangeable sites in bone crystal. It is assumed that roughly 1% of total body strontium is present in soft tissues after many years of exposure (ICRP, 1993c).

Measurements in human bone of ^{90}Sr from fall-out and information on dietary intake during the appropriate exposure period (Leggett *et al*., 1982) indicate that strontium is less efficiently retained in bone than calcium, consistent with discrimination against inclusion of strontium in hydroxyapatite crystals (Leggett, 1992a).

There is considerable evidence in humans and laboratory animals that the initial uptake of strontium and calcium by the skeleton is much greater in growing than in mature individuals. The available data are consistent with changes in the rate of addition of calcium to the skeleton with age. Bone turnover is also greater at younger ages, as indicated by human histomorphometric measurements and turnover rates inferred from studies on radionuclide retention in humans (Leggett *et al*., 1982; Leggett, 1992a; ICRP, 1993c).

(*d*) *Placental transfer*

Studies of human tissues and animals in which direct comparisons have been made show that strontium is transferred to the fetus less efficiently than calcium (Rivera, 1963; Mays & Lloyd, 1966; Twardock, 1967; Kawamura *et al*., 1986; Taylor & Bligh, 1992). The available data suggest a placental discrimination factor in humans of about 0.6.

The concentrations of ^{90}Sr were measured in six stillborn fetuses and their mothers who had drunk water from the Techa River at various times before pregnancy. The skeletal concentration ratios (fetus:mother) were 0.01–0.03 when maximum intake of ^{90}Sr had occurred largely during the childhood of the mother (< 15 years) and 0.19–0.24 when maximum intake had taken place during adulthood (> 25 years) (Tolstykh *et al.*, 1998).

$^{85}SrCl_2$ was administered intraperitoneally to rats at a dose of 20 kBq/kg bw one month before conception or on day 2, 13 or 19 of gestation. Neonates retained an average 0.002%, 0.03%, 0.1% and 3% of the injected activity, respectively, corresponding to fetal:dam concentration ratios of 0.03, 0.06, 0.2 and 5 (Stather *et al.*, 1987).

4.1.7 *Technetium-99m*

(*a*) *Inhalation*

Studies of ^{99m}Tc as pertechnetate ($^{99m}TcO_4$) showed that absorption by the lung after administration to humans, dogs and rats was rapid; total absorption occurred within a few hours. [^{99m}Tc]Diethylenetriaminepentaacetic acid (DTPA), used to study the permeability of the lung, was rapidly absorbed, with a half-time of about 1 h. The use of ^{99m}Tc-labelled materials, such as albumin, erythrocytes, ferric oxide, polystyrene, resin and teflon, to study mucociliary clearance from the bronchial tree relies on the fact that relatively little is absorbed from the lungs to the blood in the first day or so after deposition (ICRP, 1995b).

(*b*) *Ingestion*

Technetium administered as [^{99m}Tc]pertechnetate is well absorbed in humans. An extensive study demonstrated that the absorption was about 0.7 in most cases, although the individual values varied from 0.03 to 1, the variation occurring between subjects and between measurements in a single subject. In rats, the fractional absorption of technetium chloride was reported to be about 0.5. Incorporation into food appeared to reduce the absorption of technetium. The uptake from soya beans and animal tissues by rats and guinea-pigs was about half that seen after administration as the pertechnetate. Similar results have been reported in rats and sheep fed technetium either as pertechnetate or incorporated into maize (ICRP, 1993c).

Hunt (1998) and Hunt *et al*. (2001) measured the absorption of ^{99}Tc from cockles and lobster from the Irish Sea in a group of six volunteers. In the first study, absorption from cockles was estimated by the relatively insensitive method of comparing the ingested activity and total faecal elimination over seven days. The results indicated a 'gut-transfer factor' of about 0.6 for four subjects, the other two showing 4.6-fold lower values. In the second study, absorption from lobster was estimated by comparing urinary excretion of ^{99}Tc over seven days with the results obtained by Beasley *et al*. (1966) for excretion after intravenous injection of [^{99m}Tc]pertechnetate. The results indicated that the absorption rate was in the range 0.02–0.1.

(*c*) *Systemic distribution, retention and excretion*

The whole-body retention of technetium in humans after intravenous administration as pertechnetate can be described by three components with half-times of 1.6 days (77%), 3.7 days (19%) and 22 days (4%). Retention appeared to be fairly uniform in various tissues, except that greater concentrations were measured in the thyroid, salivary glands, stomach wall, colon wall and liver (Beasley *et al.*, 1966).

(*d*) *Placental transfer*

Studies of the placental transfer of technetium in humans and animals have been carried out almost exclusively with radiopharmaceuticals. Roedler (1987) used the available data to estimate whole-body fetus:mother concentration ratios of 0.04–4.8 for technetium as pertechnetate, 0–0.05 for technetium DTPA, 0.002–0.11 for technetium colloids, 0.006–0.018 for technetium polyphosphate and 0.004–0.05 for technetium pyrophosphate.

4.1.8 *Iodine-123, iodine-125 and iodine-131*

(*a*) *Inhalation*

Studies of the human biokinetics of iodine inhaled as elemental iodine vapour showed almost complete absorption in the conducting airways. Inhaled methyl iodide was less well retained (average, 70%). Much of the activity, however, was swallowed and readily absorbed from the gastrointestinal tract. In animals, iodine administered as silver iodide or sodium iodide was also rapidly absorbed into the blood (ICRP, 1995b).

(*b*) *Ingestion*

The absorption of iodide from the human gastrointestinal tract is virtually complete, with reported values of 0.9 and greater (see ICRP, 1979, 1989). A rate of absorption of about 5%/min was reported in fasted individuals, with complete absorption within 2 h. The rate of absorption of iodide ingested with food was slower, but absorption was nevertheless virtually complete after about 3 h (Keating & Albert, 1949). Iodide is absorbed in both the stomach and the small intestine, although the latter predominates. Absorption of other chemical forms may not be complete, but there is little direct evidence.

(*c*) *Systemic distribution, retention and excretion*

The average normal adult thyroid gland contains about 10 mg of stable iodine with a daily turnover of about 70 μg per day, corresponding to a retention half-time in the thyroid of 80 days (see ICRP, 1989). Fractional uptake by the thyroid of iodine absorbed to blood is about 0.3 at a dietary intake of about 200 μg/day. Since dietary intake varies considerably between individuals and between population groups, fractional uptake by the thyroid also varies considerably. In areas with low iodine intake, the thyroid absorbs a greater proportion of the iodine in the blood. As a result, the gland becomes enlarged, and the concentrations of iodine and hence radioiodine do not vary substantially.

Thyroxine and tri-iodothyronine released from the thyroid gland are metabolized in body tissues, and inorganic iodide is released into the circulation, allowing recycling to the thyroid. About 20% of organically bound iodine is excreted from the liver in bile, mainly as thyroxine conjugated with glucuronic acid; the conjugated hormone is not readily reabsorbed from the intestine. The amount of organically bound iodine in the

body has been reported to be 500–1200 μg, with an average value of about 800 μg, consistent with reports in the literature of a turnover rate about 10 times that in the thyroid; that is, a half-time of about eight days. In adults, there is no net increase in the body content of iodine, and the total daily urinary and faecal excretion balances the intake. At average levels of intake, > 90% of the iodine that enters the circulation is excreted in urine, mostly in inorganic forms (ICRP, 1989).

Uptake of radioiodine by the thyroid gland is enhanced in newborns (ICRP, 1989). In one study, the uptake was about 70% (range, 46–94%) in seven newborn infants given an intramuscular injection of ^{131}I; another study showed values of 50–70% for a group of 19 newborn infants. Uptake of ^{131}I by the thyroid was highest in infants two days after birth and reached adult values or less by five days of age. After the first days or weeks of life, the uptake of iodine by the thyroid appears to vary little with age at intake. Information on age-related changes in the retention of iodine in the thyroid has been reviewed (Dunning & Schwarz, 1981; Stather & Greenhalgh, 1983). Although the available data show a wide range, they indicate that the turnover rate of iodine in the thyroid is higher at younger ages.

(*d*) *Placental transfer*

Measurements of ^{131}I activity in the fetal thyroid after diagnostic administration before therapeutic abortions or after exposure to nuclear weapons fall-out have been reported. The concentration of iodine in the fetal thyroid increases progressively during fetal life and can exceed the concentrations in the maternal thyroid by factors of about 3–10 for intakes towards the end of gestation (Book & Goldman, 1975; Berkovski, 1999).

4.1.9 *Caesium-137*

(*a*) *Inhalation*

After accidental inhalation of caesium sulfate, caesium was transferred rapidly to the blood. In animals, simple ionic compounds (chloride, nitrate) were rapidly and completely absorbed. Studies of exposure to caesium associated with irradiated fuel fragments, including particles released after the Chernobyl accident, indicate that much of the caesium was rapidly absorbed within days but a fraction was retained within the particle matrix and absorbed over a period of months. High solubility and rapid uptake were also reported in a study of caesium absorption in rats exposed to a suspension of residues from a reactor fuel-cooling pond (ICRP, 1995b).

(*b*) *Ingestion*

In volunteers who ingested various radioisotopes of caesium in soluble inorganic form, absorption was virtually complete (ICRP, 1979, 1989). For example, Rundo (1964) measured an average fractional absorption of 0.99 for 10 normal subjects after ingestion of ^{137}CsCl.

Radioactive caesium incorporated into insoluble particles may be less readily available for absorption. LeRoy *et al*. (1966) reported values of < 0.1 for the uptake of ^{134}Cs from real and simulated fall-out in a study of 102 volunteers.

Measurements of uptake by volunteers of ^{137}Cs from meat (venison, mutton, caribou) contaminated as a result of the Chernobyl accident gave values in the range 0.6–0.99 (Henrichs *et al*., 1989; Talbot *et al*., 1993).

(*c*) *Systemic distribution, retention and excretion*

The monovalent alkali metal caesium behaves similarly to potassium after absorption to blood and is accumulated in all cells. Higher concentrations of caesium have been reported in muscle than in other tissues, but the differences are small. It is generally assumed that caesium is distributed uniformly between and within body organs and tissues (see ICRP, 1989).

There is a large body of data on the retention of ^{137}Cs in humans (ICRP, 1989). After entering the blood, it is cleared rapidly and deposited more or less uniformly in the tissues. Muscle contains the largest fraction of the total body burden of caesium, and slightly higher concentrations have been reported in muscle in comparison with other tissues. Studies of the whole-body retention of ^{137}Cs in several hundred persons across the world established that retention in adults can be expressed adequately by a two-component exponential function, with a fast component, amounting to ~ 10% of the total systemic burden, being cleared with a half-time of approximately two days and the remainder being lost with a half-time of the order of 100 days. It is now well established that the rate of clearance is faster in women than in men and that clearance may be further accelerated during pregnancy (Schwartz & Dunning, 1982; Rundo & Turner, 1992; Melo *et al*., 1997; Thornberg & Mattsson, 2000). The mean equivalent biological half-time of caesium ranges from about 47 to 152 days in men and from about 30 to 141 days in women. The rate of clearance of caesium is faster in children. The major excretory pathway is via the kidneys and urine (ICRP, 1989).

(*d*) *Placental transfer*

The transfer of caesium isotopes to the fetus has been followed in both humans and animals. The concentrations of ^{137}Cs arising from exposure to fall-out from nuclear weapons were measured in nine newborn children within three days of birth and in their mothers. The concentrations were similar (Wilson & Spiers, 1967). After an accident in Brazil in which a woman in her fourth month of pregnancy was contaminated with ^{137}Cs, both the mother and her newborn child were monitored one week after birth. The concentration of ^{137}Cs in the mother (0.91 kBq/kg bw) was similar to that in her newborn child (0.97 kBq/kg bw), and the concentration in the placenta was the same as that in the whole maternal body and the fetus (Bertelli *et al.*, 1992).

4.1.10 *Cerium-141 and cerium-144*

(*a*) *Inhalation and ingestion*

In a case of accidental human exposure to a ^{144}Ce–praseodymium-contaminated atmosphere, measurement of lung clearance showed intermediate solubility (see ICRP, 1995b). Studies in animals have also indicated intermediate levels of solubility in the lung for a number of forms of cerium, including ^{144}Ce chloride in dogs, ^{144}Ce hydroxide in rats, ^{144}Ce oxide in rats and hamsters and ^{144}Ce in irradiated fuel fragments in rats (ICRP, 1989).

The fractional absorption of ingested cerium was reported to be $< 1 \times 10^{-3}$ in humans (after accidental intake), and a similar value was found in rats, pigs, goats and other species (ICRP, 1979).

(*b*) *Systemic distribution, retention and excretion*

In adult rats and dogs, about 50% of cerium entering the blood is deposited in the liver, 30% in the skeleton and 20% in other tissues. The half-times of retention in liver and bone in dogs were both about 10 years. In animals, deposition in the skeleton is greater at younger ages, and liver uptake increases with age (ICRP, 1989).

(*c*) *Placental transfer*

In mice given ^{144}Ce citrate by intraperitoneal injection at 3 μCi (111 kBq) two or one months before mating or just before mating, placental transfer resulted in maximum retention in the litters (average of 10 individuals per litter) of 0.65, 0.67 and 1.4%, respectively, of the mother's body burden (Naharin *et al.*, 1969).

When ^{144}Ce chloride was administered intravenously to rats on day 15 or 19 of gestation, the ^{144}Ce concentration in the fetus one day later was about 0.02% of the administered dose per gram of tissue at both times. A considerably higher concentration was measured in the placenta and in the fetal membranes, indicating that ^{144}Ce does not freely pass from the maternal circulation to the fetus (Mahlum & Sikov, 1968). ^{141}Ce chloride was given to rats by intravenous injection before conception or at various stages during gestation and to guinea-pigs in late gestation. The retention in rat fetuses measured three days after administration was 4×10^{-5}% of the injected activity per fetus on day 13, increasing to 0.01% shortly before birth, the fetus:dam concentration ratios increasing from 0.004 to 0.02. Retention in guinea-pigs seven days after administration was about 0.05% of the injected activity per fetus, corresponding to a fetus:dam concentration ratio of about 0.01 (Levack *et al.*, 1994).

4.1.11 *Rhenium-186 and rhenium-188*

Limited data are available on the systemic distribution of rhenium in rats, but they suggest that its behaviour is similar to that of technetium. In the absence of more extensive data, ICRP (1980) assumed that the absorption of inhaled or ingested rhenium

and its systemic kinetics are similar to those for technetium. For all rhenium compounds, an absorbed fraction of 0.8 is assumed for uptake from the gastrointestinal tract.

4.1.12 *Bismuth-212*

(*a*) *Inhalation and ingestion*

Data from dietary balance studies (ICRP, 1975) suggest that the fractional absorption of bismuth is about 0.08. In the absence of relevant data, bismuth compounds were assumed to be of high or intermediate solubility in the lungs (ICRP, 1980).

(*b*) *Systemic distribution, retention and excretion*

The short-lived radioisotopes of bismuth, ^{212}Bi and ^{210}Bi, arise as products of the decay of ^{232}Th and ^{238}U. They have limited use in research, but use of ^{212}Bi-labelled antibodies for tumour treatment has been suggested (Hassfjell *et al.*, 1997). After entry of inorganic bismuth into the systemic circulation, about 40% becomes deposited in the kidneys, another 30% becomes more or less uniformly deposited in the other tissues, and about 30% is excreted rapidly. The rate of loss of bismuth from all tissues appears to be quite rapid, 60% being eliminated with a half-time of about 14 h and the remainder with a half-time of approximately six days (ICRP, 1980).

4.1.13 *Polonium-210*

(*a*) *Inhalation*

Studies of the absorption of polonium in humans after inhalation of a ^{210}Po source, which probably comprised small oxide particles, and after inhalation of ^{210}Po in tobacco smoke, indicated intermediate solubility. Studies in rats have also shown intermediate solubility after intratracheal instillation of ^{210}Po chloride in a sodium chloride aerosol. Similar treatment of rabbits with a ^{210}Po hydroxide colloid confirmed these results (ICRP, 1995b).

(*b*) *Ingestion*

ICRP (1993c) reviewed the studies in humans, including a measurement of polonium uptake in a patient being treated for chronic myelogenous leukaemia. The blood concentrations and urinary excretion after oral administration of ^{210}Po chloride suggested a fractional absorption of 0.1. Absorption of biologically incorporated ^{210}Po was reported to be significantly higher, as studies of persons who ate meat from reindeer exposed to ^{210}Po indicated a fractional absorption of 0.3–0.5 (Ladinskaya *et al.*, 1973; ICRP, 1993c). In a study in six volunteers of the absorption of ^{210}Po from crabmeat, the absorbed fraction was estimated to be about 0.8 (Hunt & Allington, 1993). The absorption of ^{210}Po by rats was reported to be 0.03–0.06 for an unspecified chemical form and 0.06 for the chloride (ICRP, 1993c). In rats, the fractional absorption was 0.05

for ^{210}Po administered as the nitrate and 0.13 for ^{210}Po incorporated into liver obtained from rats given intravenous injections of ^{210}Po citrate. For ^{210}Po administered as the citrate, absorption was reported to be 0.07–0.09 in adult rats and guinea-pigs (Haines *et al.*, 1993).

(*c*) *Systemic distribution, retention and excretion*

^{210}Po is ubiquitous in the environment as a decay product of ^{226}Ra. It has been used to a certain extent in experimental radiation science over the past century (McKay, 1971). Although polonium belongs to Group VI of the periodic table, with sulfur and selenium, it is more metallic than either of the latter two elements and does not appear to be incorporated into organic compounds, such as the amino acids methionine and selenomethionine. There is a large body of information on the biodistribution and biokinetics of polonium in animals and a considerable amount of data on the biokinetics in humans after inhalation and ingestion of ^{210}Po. In rabbits injected with ^{210}Po nitrate, the main organ of deposition was the kidney (1.3–1.5%/g), followed by the blood, spleen, lung and liver; uptake in the skeleton was relatively low (< 0.01%/g; Parfenov & Poluboyarinova, 1973; see Table 2 in General Remarks). Polonium appears to be lost from the body relatively rapidly, predominantly in the urine. Analysis of the data on human excretion suggested that the half-time of whole-body retention of ^{210}Po ranges from 30 to 50 days (ICRP, 1993c).

In a person suffering from acute leukaemia, measurements six days after injection of ^{210}Po chloride showed a retention of 40% in the liver, 5% in kidneys and 4% in spleen (see ICRP, 1993c). These organs were also the main sites of deposition in rats. A study of the retention of ^{210}Po in marmoset tissues one week after intravenous injection as the citrate showed that the liver accounted for 26% of total body retention; the kidneys retained 21%, and < 1% was retained in the spleen and testes. The femora contained 1.5% of the retained activity, corresponding to a skeletal deposit of about 15%. Autoradiographs of marmoset and rat bone showed that the retained ^{210}Po was not associated with bone surfaces but was distributed throughout the bone marrow. On the basis of the data on excretion, the average retention half-time of polonium in the body was estimated to be about 50 days (ICRP, 1993c).

Two studies of ^{210}Po retention in children aged 6–15 years showed a half-time of about 40 days, which is not significantly different from the values for adults (ICRP, 1993c).

(*d*) *Placental transfer*

The fetal transfer of polonium has been studied mainly in rodents and baboons, showing low transfer of polonium to the fetus. These results are in accord with the limited data on humans.

Accumulation of ^{210}Po in the yolk sac and placenta of rats and guinea-pigs was demonstrated autoradiographically after administration of ^{210}Po citrate on various days during gestation (Haines *et al.*, 1995). Seven days after intravenous injection of ^{210}Po

citrate to two baboons in late pregnancy (five months after conception), the retention in the fetus was about 1% of the injected activity. The concentrations in fetal and maternal bone were similar (fetus:mother, 0.6–0.7), but those in fetal liver, kidneys and spleen were an order of magnitude lower than the corresponding values for maternal tissues. The overall fetal:maternal concentration ratio was estimated to be 0.3 (Paquet *et al*., 1998).

Elevated concentrations of ^{210}Po were reported in placentae of women in northern Canada who ate reindeer and caribou meat (Hill, 1966).

4.1.14 *Astatine-211*

Astatine is the fifth and heaviest element of the halogen series. Its chemical properties resemble those of iodine more closely than those of the lighter elements, fluorine, chlorine and bromine. Like iodine, astatine can readily form a number of organic compounds. No data are available on the biokinetics of astatine in humans, and few are available for animals.

Studies of the distribution and retention of ^{211}At in mice indicated that the radioactivity is distributed rapidly throughout the body after injection. The biological half-time of clearance from blood was 8 h. Astatine deposited in the thyroid appeared to be released to the systemic circulation with a biological half-time of about 36 h. The astatine deposited in liver, kidneys, spleen, stomach, small intestine and lungs appeared to be removed with biological half-times ranging from about 6 h (spleen) to about 21 h (stomach) (Garg *et al*., 1990).

During the development of ^{211}At-containing radiopharmaceuticals for endoradiotherapy, the biokinetics of a number of organic compounds of astatine were investigated in mice (see Garg *et al*., 1995; Foulon et al., 1998; Reist *et al*., 1999). In addition, a number of ^{211}At-labelled antibodies or antibody fragments have been investigated (Garg *et al*., 1990; Foulon *et al*. 1998). In almost all these studies, the paired-label technique was used in order to provide data that were directly comparable with those for the corresponding ^{131}I-labelled analogues. The studies indicated that the biological half-times of retention of ^{211}At from organoastatine compounds are generally longer than those of ^{131}I from the equivalent iodine compound, by factors up to about 2.5. Although most of the compounds investigated contained an astatine-substituted benzene moiety, the rates of dehalogenation appeared to be variable, and the fractional uptake in the different organs varied widely.

4.1.15 *Radon-222*

Data on the inhalation, dermal absorption, ingestion, systemic distribution, retention and excretion of ^{222}Rn were reviewed previously (IARC, 1988). More recent data on placental transfer of ^{222}Rn are presented below.

It is generally accepted that radon crosses the placenta, resulting in similar concentrations in fetal and maternal tissues. The specific activities of ^{214}Pb (a decay product of ^{222}Rn) were measured in tissues of pregnant rats after 13 consecutive 18-h-per-day exposures to radon on days 7–19 of gestation, and the corresponding dose equivalent rates were calculated. Because radon progeny were inhaled with radon gas, the lung had the highest activity concentrations, followed by the kidney, which showed concentrations more than an order of magnitude lower. The concentrations in other tissues, such as femur and liver, were several-fold lower than those in the kidney. The concentration of ^{214}Pb in the placenta was similar to that in the femur and liver of the pregnant rats, and the amount measured in the fetus was about one-fifth of that in the placenta. The difference in concentration between components of the fetoplacental unit on the last day of exposure (day 19 of gestation) contrasted with the results of the same authors for ^{85}Kr in rats, in which the concentrations were more uniform and the fetoplacental radiation doses were similar to those received by other tissues in the pregnant rats (Sikov *et al.*, 1992). Thus, while radon can be assumed to cross the placental barrier freely, all the short-lived decay products do not.

4.1.16 *Radium-224, radium-226 and radium-228*

(*a*) *Inhalation*

Radium has been shown to be highly soluble in studies in which $^{232}UO_2(NO_3)_2$ with its decay products and radium nitrate, with or without thorium nitrate, were administered to rats by intratracheal injection. Less solubility was reported in a person who accidentally inhaled a mixture of radium and barium sulfate and in a person who inhaled an unidentified radium-containing compound. Lung clearance half-time values of 90 and 120 days, respectively, were reported (ICRP, 1995b).

(*b*) *Ingestion*

Data from balance studies reviewed by the ICRP Task Group on Alkaline Earth Metabolism in Adult Man (ICRP, 1973) indicated that the fraction of radium absorbed from food or drinking-water is 0.15–0.21. The results of a study of a single volunteer who ingested a known quantity of radium suggested a higher fractional absorption value of around 0.5, while elderly subjects ingesting mock radium-dial paint containing $^{224}RaSO_4$ absorbed an average of about 20% (Maletskos *et al.*, 1969).

(*c*) *Systemic distribution, retention and excretion*

The alkaline earth elements strontium, barium and radium follow the migration and deposition of calcium in the body, but quantitative differences in their retention in tissues are found due to discrimination by biological membranes and bone minerals. In general, strontium is a better quantitative tracer for calcium than the heavier elements barium and radium. Discrimination against radium, relative to calcium, results in less

effective incorporation and retention in bone and more rapid urinary excretion. Long-term retention of radium, like that of calcium, is confined to the skeleton; the doses from radium isotopes absorbed in the blood are delivered largely to bone surfaces and red bone marrow (Leggett, 1992a; ICRP, 1993c).

The initial deposition of calcium, strontium, barium and radium from blood onto bone surfaces is similar in rats and rabbits, as determined from the skeletal content over the first few hours after injection. Limited data on humans also support the use of a single value for initial uptake of these elements by bone. These conclusions are derived from autoradiographic studies on bone samples taken after injection of ^{45}Ca 14 h or more before death, measurements of calcium and strontium in autopsy samples of bone from subjects injected with radioisotopes 3 h or more before death, and from external measurements of ^{45}Ca uptake and retention in the bones of the foot. The findings suggest that the initial uptake of the elements by bone in adults is about 25%. Measurements of the retention of radium in the human skeleton and in the whole body indicate that radium is less efficiently retained in bone than calcium, consistent with discrimination against inclusion of radium in hydroxyapatite crystals (Leggett, 1992a).

There is considerable experimental evidence that the initial uptake of radium and calcium by the skeleton is much greater in growing than in mature individuals (Leggett, 1992a; ICRP, 1993c). In mice, the uptake of injected ^{224}Ra by the skeleton decreased from about 50% of the total in young animals to 25% in mature animals, as measured two days after injection. Skeletal retention of ^{226}Ra in beagles 1–2 weeks after injection accounted for 85% of the injected amount in newborn animals, 65% in juveniles and 30–50% in adults. The available data are consistent with changes in the calcium addition rate to the skeleton with age. Bone turnover is also higher at younger ages, as indicated by histomorphometric measurements and turnover rates inferred from human studies on radionuclide retention (Leggett *et al*., 1982; Leggett, 1992a).

(*d*) *Placental transfer*

The normal radium content in various human tissues was measured in autopsy material from 12–56 persons of an average age > 60 years. The average amount of ^{226}Ra in the whole body was 4.8 Bq, of which approximately 60% was found in muscular tissue, 27% in the skeleton and the remainder in other tissues and organs. Analysis of eight placentae and nine samples of fetal tissue showed ^{226}Ra concentrations of 30 and 22 μBq/g of wet tissue, respectively (Muth *et al.*, 1960). The ^{226}Ra concentrations in the bone of about 200 human fetuses were similar to those in adult bone and were independent of the stage of gestation (Rajewsky *et al.*, 1965). The radium content was measured in a woman who had been a radium-dial painter for 5–7 years from the age of 16 and had died at the age of 26 in February 1928, on the day of delivery of her stillborn child. The reported ^{226}Ra activities in total mineral bone, measured in 1969, corresponded to concentrations at the time of death of 103 kBq and 64 Bq in the mother and infant, respectively. It was concluded that 0.06% of the mother's ^{226}Ra had been absorbed by the fetal skeleton, and 0.12% of the mother's

radium was absorbed by the whole fetus during the 8–9 months of fetal life (Schlenker & Keane, 1987).

4.1.17 *Thorium-232*

(*a*) *Inhalation*

Thorium decay products were measured in the chest and in exhaled air of former thorium refinery workers three or more years after the end of exposure to a range of compounds, from monazite ore to thorium nitrate; at least some of the material was highly insoluble in the lung (reviewed by ICRP, 1995b). Analysis of tissues from one worker at autopsy 30 years after exposure still showed retention in lung tissue and associated lymph nodes, consistent with low solubility. Concentration of thorium in lung tissue and lymph nodes was also reported in a study on tissues from former uranium miners. Measurements of thorium in autopsy tissues from members of the public showed that the proportion of total retained thorium accounted for by retention in the lungs (~ 25%) was greater than that for plutonium (~ 5%), indicating long-term retention in the lungs with a half-time of 1–8 years.

Studies in rats indicated that some compounds of thorium, including the citrate, chloride, nitrate and hydroxide, have intermediate solubility (ICRP, 1995b).

(*b*) *Ingestion*

Measurements of the absorption of ^{234}Th ingested as the sulfate in a mock 'dial' paint by six 63–83-year-old persons (reviewed by ICRP, 1995a) gave fractional absorption values in the range 10^{-4} to 6×10^{-4}, with a mean of 2×10^{-4}. Thorium absorption has also been estimated from data on skeletal content, dietary intake, estimated inhalation rates and excretion data, giving values of 0.001–0.01. The concentrations of thorium in tissues, body fluids and the daily diet of urban Indian populations suggested absorption values lower than 10^{-3}; it was concluded that inhalation was the dominant route of intake and that the contribution from ingestion was negligible.

Several reports on the absorption of inorganic thorium provided values of 5×10^{-5} to 0.006 for rats and about 6×10^{-4} for mice (ICRP, 1995a).

In a study of the effect of age on absorption of radionuclides, ^{228}Th nitrate was administered to two-day-old rats and adult mice. One week later, ^{228}Th was measured in various organs and in the skeleton. The amount absorbed in the neonatal rats, 1.1% of the activity administered, was nearly 20 times higher than the absorbed fraction in the mice. In parallel experiments, ^{233}Pa (protactinium) nitrate was given to two-day-old rats and adult rats. After one week, the absorbed fraction in the neonatal animals was 100-fold higher than that in the adults (Sullivan *et al.*, 1983).

(*c*) *Systemic distribution, retention and excretion*

A study was reported of the clearance of thorium from blood and its retention and excretion after intravenous injection of ^{234}Th citrate into normal human subjects (three men, two women) aged 63–83 years (reviewed by ICRP, 1995a). Whole-body retention was > 90% after three weeks in all five subjects. Cumulative urinary excretion was about 5% over the first five days and 2–3% over the following 19 days. The ratio of urinary to faecal excretion was approximately 12:1 for the men and about 25:1 for the women. External monitoring showed no significant reduction in body content over the following four months. Long-term measurements of ^{224}Th and ^{228}Th in the bodies and excreta of occupationally exposed persons suggested a half-time for clearance of thorium of at least 10–15 years and probably much longer (ICRP, 1995a).

When thorium isotopes were measured in autopsy samples from non-occupationally exposed subjects, the highest concentration was found in the lymph nodes, followed by bone, thyroid and lung (Ibrahim *et al.*, 1983).

In general, the behaviour of thorium in the body appears to be similar to that of the more frequently studied element, plutonium (ICRP, 1995a). Their patterns of distribution in bone are similar, with deposition on bone surfaces, burial in bone volume and removal by resorption. In persons with long-term exposure to environmental levels of thorium and plutonium, the content of the gonads as a proportion of the total systemic content appears to be similar for the two elements. There are, however, established differences: uptake by the liver is lower for thorium than for plutonium, and the urinary excretion and retention in the kidneys are higher.

(*d*) *Placental transfer*

In a preliminary report on the concentrations of environmental thorium in human fetoplacental tissues in the first trimester of pregnancy, they were found to be at the limit of detection. No thorium could be detected in fetuses obtained during the second or early third trimester (Weiner *et al.*, 1985). In rats, there was little transfer from the placenta to the fetus, and a strong decrease in the specific activity in the fetus (% of injected dose per g tissue) during the second half of gestation (Maurer *et al*., 1950).

4.1.18 *Uranium-234, uranium-235 and uranium-238*

(*a*) *Inhalation*

Accidental exposures of humans to UF_6 and UO_2F_2 resulted in rapid urinary excretion of radioactivity, consistent with high solubility in the lungs. Experiments in beagle dogs showed that most of the initial lung burden was rapidly absorbed to blood. High solubility has also been reported after intratracheal instillation of $UO_2(NO_3)_2$ in rats (ICRP, 1995b).

The reported behaviour of UF_4 is complex. Measurements of urinary excretion after inhalation by humans, rats and baboons showed that a large fraction of the lung deposit

was rapidly absorbed; however, the degree of solubility varied considerably between experiments (ICRP, 1995b).

The available data on UO_3, ammonium diuranate and U_3O_8 from cases of accidental intake by humans, occupational monitoring, studies in rats, dogs and monkeys and from studies *in vitro* show mainly intermediate levels of solubility, although U_3O_8 was sparsely soluble in some cases (ICRP, 1995b).

Studies in humans, rats, dogs and primates have shown that UO_2 is very insoluble (ICRP, 1995b).

(*b*) *Ingestion*

The absorption of uranium has been reviewed by Wrenn *et al*. (1985), Leggett and Harrison (1995) and ICRP (1995a).

In the first controlled study involving more than one subject, $UO_2(NO_3)_2$ was administered to four hospital patients. The results obtained were taken to suggest fractional absorption in the range 0.005–0.05 (Hursh *et al.*, 1969). Leggett and Harrison (1995) interpreted the data as suggesting absorption of 0.004, 0.01, 0.02 and 0.06 for the four subjects. A study of the absorption of uranium in 12 normal healthy adult volunteers was reported in which drinking-water with high concentrations of uranium was consumed (Wrenn *et al.*, 1989). As 40–60% of absorbed uranium was excreted in the urine within the first three days, Leggett and Harrison (1995) concluded that the mean fractional absorption was 0.01–0.015, maximum absorption was in the range 0.02–0.04, and that six subjects absorbed less than 0.25% of the ingested uranium. Results have also been reported (Harduin *et al.*, 1994) for the absorption of uranium from mineral water administered either on one day or over 15 days, resulting in fractional absorption of 0.005–0.05 with an average value of 0.015–0.02. After administration over 15 days, the fractional absorption was 0.003–0.02 (average, 0.01–0.015) (ICRP, 1995a; Leggett & Harrison, 1995).

A number of dietary balance studies have indicated mean absorption values for uranium in the range 0.004–0.04 (Leggett & Harrison, 1995).

Uranium absorption has been measured in rats, hamsters, rabbits, dogs and baboons (Wrenn *et al*., 1985; Leggett & Harrison, 1995), and these data provide the only quantitative information on the relative uptake of uranium ingested in different chemical forms. Absorption appears to be greatest when uranium is ingested as $UO_2(NO_3)_2$, UO_2F_2 or $Na_2U_2O_7$, roughly half as great for UO_4 or UO_3, and one to two orders of magnitude lower for UCl_4, U_3O_8, UO_2 and UF_4. A number of studies have shown that absorption is substantially greater in fasted than in fed animals. For example, uptake was increased by one order of magnitude in mice and baboons deprived of food for 24 h before administration of uranium (Leggett & Harrison, 1995).

The limited data available on the absorption of uranium in children over five years of age suggest that uptake does not vary substantially with age. However, the estimates were based on the assumption that the subjects were in uranium balance and could be underestimates of absorption in rapidly growing children, who may be expected to

show net retention of uranium. Increased absorption of uranium was demonstrated in neonatal rats and pigs; absorption in two-day-old rats was about two orders of magnitude greater than that in adult animals (Leggett & Harrison, 1995).

(*c*) *Systemic distribution, retention and excretion*

In experimental studies in humans, about two-thirds of uranium injected intravenously as the nitrate was excreted in urine within the first 24 h and a further 10% over the following five days (see ICRP, 1995a). Studies in humans and animals indicate that most of the remaining uranium is excreted over a few months but a small proportion is retained for years. Faecal excretion of uranium is low, accounting for less than 1% of total excretion in human subjects over the first few days after intravenous injection of $UO_2(NO_3)_2$.

Measurements of the systemic distribution of uranium at autopsy after injection of $UO_2(NO_3)_2$ showed that the skeleton, kidneys and other soft tissues accounted for 10%, 14% and 6%, respectively, of the injected activity in a person who died after 2.5 days, 4–13%, 6% and 4%, respectively, in a person who died after 18 days and 1.4%, 0.3% and 0.3%, respectively, for a third person who died 566 days after the injection. In animals, most of the retained uranium was confined to the kidneys and skeleton a few days after absorption into blood. For example, the kidneys and skeleton of beagles accounted for 90% of the retained uranium 2–6 days after injection (ICRP, 1995a).

A substantial proportion of the uranium filtered by the kidneys is retained temporarily in the renal tubules before being excreted in the urine. In humans and animals, 92–95% of the renal content is lost rapidly, but the remainder has a half-time of 30–340 days. Retention of uranium by the kidneys appears to increase with the mass absorbed into blood, complicating the interpretation of experimental studies, many of which involved administration of relatively large amounts of uranium.

The behaviour of uranium in the skeleton shows some qualitative similarities to that of the alkaline earth elements. It has been shown that uranyl ions exchange with Ca^{2+} on the surface of bone mineral crystals, although they do not participate in crystal formation or enter existing crystals. The early distribution of uranium in the skeleton is similar to that of calcium. Studies in dogs indicate that uranium may enter bone mineral by diffusion from the bone surface as well as by burial, but this has not been observed in rats or mice. As is the case for calcium, a substantial proportion of uranium deposited in bone is lost to the circulation by processes that occur more rapidly than bone resorption (ICRP, 1995a).

(*d*) *Placental transfer*

Significant concentrations of uranium were found in three of seven samples from first-trimester human abortuses and in 12 of 16 samples from second-trimester abortuses. The concentration range for all fetal tissues was 2–5 mBq/kg, whereas 5–9 mBq/kg were found in placenta and about 60 mBq/kg in umbilical cord (Weiner *et al.*, 1985). In rats, the fetal:dam concentration ratios were 2.5 for liver, ~ 1 for femur

and 0.01 for kidney 24 h after administration of ^{233}U citrate on day 19 of gestation, but the concentrations measured in the fetal tissues were highly variable (Sikov & Mahlum, 1968).

4.1.19 *Neptunium-237*

(*a*) *Inhalation*

Qualitative data on ^{237}Np in human lung tissue after exposure to fall-out suggested that its retention is lower and its absorption into blood higher than for ^{239}Pu. Studies in rats have shown intermediate levels of solubility for ^{237}Np inhaled as nitrate and oxalate aerosols (ICRP, 1995b).

(*b*) *Ingestion*

The absorption of ^{239}Np and ^{242}Cm was measured in five adult male volunteers who received solutions of the citrate complexes with a midday meal. The mean fractional absorption value, as measured in urine, was 2×10^{-4} for both radionuclides, with a range of $1–3 \times 10^{-4}$ in both cases (Popplewell *et al.*, 1991).

The results of studies of the absorption of neptunium in animals have been reviewed. After administration of milligram quantities of ^{237}Np to rats, the fractional absorption was about 0.01. Subsequent experiments established that absorption in a number of animal species after low doses is an order of magnitude or more lower. For example, in baboons, the absorbed fraction was about 0.001 in two animals given 11 ng of ^{239}Np as the nitrate and about 0.01 in those given 5 mg of ^{237}Np. Administration in a milk-supplemented diet reduced the fractional absorption of the low dose of ^{239}Np by a factor of five, to $1–2 \times 10^{-4}$, while administration in a potato diet increased absorption by a similar factor (ICRP, 1986; Métivier *et al.*, 1986; Harrison, 1991).

The effect of age on the absorption of neptunium and plutonium was also studied in baboons (ICRP, 1989; Harrison & Fritsch, 1992). In animals at four days of age, the maximum absorption of neptunium, administered as the nitrate, was about 2×10^{-2}, which was about 40 times higher than that in adult animals. At one week and four weeks after birth, absorption had decreased to 1.5×10^{-3} and 10^{-3}, respectively, which were three to four times the values found in adult animals.

(*c*) *Systemic distribution, retention and excretion*

Studies in adult animals, including baboons and cynomolgus monkeys, indicated that about half of the absorbed neptunium is deposited in the skeleton, 2–10% in the liver and about 5% in other soft tissues; the rest is excreted within a few days, primarily in urine (ICRP, 1989). The limited data on humans show a generally similar pattern (Popplewell *et al.*, 1991).

Like other actinide elements, neptunium is deposited on bone surfaces. Its distribution on various bone surfaces appears to be similar to that of americium, although

it also shows some similarities to that of strontium. Formation of aggregates in bone marrow after bone resorption has been observed (ICRP, 1989).

In rodents and primates, rapid loss of neptunium from the liver was seen with half-times of a few months or less (ICRP, 1989). In human autopsy samples, ^{237}Np was removed from the liver at least 15 times more rapidly than ^{239}Pu (Efurd *et al.*, 1986).

Studies of the uptake and retention of ^{237}Np in the gonads of rats have been reviewed (Thompson, 1982). It was concluded that the behaviour of neptunium can be assumed to be similar to that of plutonium.

(*d*) *Placental transfer*

A study of the placental transfer of neptunium and other radionuclides in two baboons in late pregnancy (five months after conception) showed that, seven days after intravenous injection of ^{237}Np citrate, the retention in the fetus represented 1–2% of the injected activity. Retention in the placenta accounted for 0.7–1.2%. The overall fetus:mother concentration ratio was estimated to be 0.6 (Paquet *et al.* 1998). When rats were given ^{237}Np citrate intravenously on day 15 or 19 of gestation, the amounts measured in the fetus (per g wet weight) 24 h later represented 0.01 and 0.02% of the injected activity, respectively (Sikov & Mahlum, 1968).

4.1.20 *Plutonium-238 and plutonium-239*

(*a*) *Inhalation*

Intermediate solubility has been reported for plutonium nitrate inhaled by rats and dogs and plutonium tributyl-phosphate inhaled by rats and baboons (reviewed by ICRP, 1995b).

The oxides of plutonium are the most thoroughly studied of the actinide aerosols. Generally, two phases of absorption from the respiratory tract to blood can be distinguished. A small fraction, typically less than 1%, is absorbed within about one day, and the remainder is cleared with half-times of the order of years. The solubility has been shown to be highly dependent on how the aerosol is formed. For example, $^{239}PuO_2$, formed by complete oxidation of the metal or a salt at about 1000 °C (high-fired), is sparsely soluble, while material formed at lower temperatures is more soluble, reflecting incomplete oxidation. The sparse solubility of high-fired $^{239}PuO_2$ has been demonstrated in studies in dogs and primates, and supporting data on lung retention have been reported for exposed workers (ICRP, 1995b).

The lung clearance characteristics of plutonium are also different when it is inhaled as a mixed metal oxide. Greater absorption was observed in rats exposed to oxides containing plutonium mixed with sodium, potassium, calcium or magnesium. In contrast, studies in which rats, dogs and primates inhaled mixed UO_2/PuO_2 aerosols showed the same sparse solubility as for PuO_2 (ICRP, 1995b).

Measurements in human autopsy material of ^{239}Pu resulting from the atmospheric testing of nuclear weapons show that a relatively high proportion of ^{239}Pu is retained in

lung tissue and tracheobronchial lymph nodes, consistent with low solubility (McInroy *et al.*, 1991). More soluble forms of ^{239}Pu have also been reported. For example, ^{239}Pu discharged into the sea from the Sellafield reprocessing plant and attached to sediments was of intermediate solubility when administered to rats by intratracheal instillation (Morgan *et al.*, 1990).

(*b*) *Ingestion*

The fractional absorption of ^{244}Pu administered in citrate solution with a midday meal to three volunteers was in the range 3×10^{-4}–9×10^{-4} (Popplewell *et al.*, 1994). Measurements on two further volunteers increased the range to 10^{-4}–10^{-3}, with an average of 6×10^{-4} (Ham & Harrison, 2000). In volunteers who ate winkles collected on the Cumbrian coast near to the nuclear-fuel reprocessing plant at Sellafield, the average fractional absorption of plutonium was 1.7×10^{-4}, with a range of 2×10^{-5}–5×10^{-4} (Hunt *et al.*, 1990; ICRP, 1993c). The fractional absorption from the gut of fall-out plutonium in reindeer meat was determined by comparing the ratio of body content to dietary intake of $^{239/240}$Pu in persons who had lived in Lapland or in the urban areas of southern Finland. The absorption was estimated to be 8×10^{-4}, but the estimate is uncertain (Mussalo-Rauhamaa *et al.*, 1984).

Studies on the absorption of plutonium in rodents, pigs, dogs and primates have been reviewed extensively (ICRP, 1986; Harrison, 1991). The lowest fractional absorption values were reported for the oxide, ranging from about 2×10^{-4} in rats to about 3×10^{-8} in pigs. This wide range probably reflects the solubility of the oxide preparation, which is affected by the temperature of production, the proportion of small particles present and the specific activity of the isotope. Mixed Pu–sodium oxides contain a higher proportion of very small particles (about 1 nm in diameter) than the pure oxides. Furthermore, because of their much higher specific radioactivity, suspensions of ^{238}Pu oxide (6.27×10^{8} kBq/g) are more prone than those of ^{239}Pu oxide (2.25×10^{6} kBq/g) to radiolytic breakdown to small particles (Fleischer & Raabe, 1977).

The fractional absorption values after uptake of plutonium administered to animals as the nitrate, chloride or bicarbonate are in the range 10^{-5}–10^{-4}. Fasting has been shown to increase absorption by up to an order of magnitude. Values of 10^{-3}–2×10^{-3} were reported for uptake of ^{237}Pu nitrate given as a single, low dose to rats and mice (Sullivan, 1980; Sullivan *et al.*, 1983). These results were taken as evidence of increased absorption at low masses; however, in experiments to determine the effect of long-term ingestion of low doses, a value of 3×10^{-5} was obtained for the nitrate in rats and 10^{-5} for the bicarbonate in hamsters (ICRP, 1986). In general, the ingested mass and valence appear to have little effect on absorption; however, when large masses of pentavalent plutonium are ingested, absorption may be increased by an order of magnitude, as demonstrated by Métivier *et al*. (1986) in baboons.

The fractional absorption of plutonium administered to animals as organic complexes or incorporated into food is generally greater than that of inorganic forms.

For example, most of the values reported for Pu citrate are in the range 6×10^{-5}–6×10^{-4}, while those for the nitrate are 10^{-5}–10^{-4}. An organic form of importance in fuel reprocessing is Pu tributylphosphate, for which the absorption in rats has been reported to be about 10^{-4}–2×10^{-4} (ICRP, 1986).

The absorption of plutonium in newborn hamsters and guinea-pigs has been shown to be greatest on the first day of life, with values of about 0.02–0.03, and to decrease progressively during the period of suckling to reach adult values by about the time of weaning at 21–22 days (Harrison & Fritsch, 1992; ICRP, 1986). In a study of the absorption of plutonium given as the nitrate to different animal species, a value of 2×10^{-2} was obtained for one-day-old rats, 6×10^{-2} for two-day-old dogs and 10^{-1} for one-day-old piglets. The values for the absorption of plutonium in baboons after administration of ^{239}Pu citrate were 2×10^{-2} at four days of age and 5×10^{-4} for adult animals (Sullivan & Gorham, 1982).

Increased absorption of plutonium has been shown to be associated with high levels of intestinal retention in rats and pigs but not in guinea-pigs or primates (Harrison & Fritsch, 1992). Autoradiographic studies have shown that the high levels of retention of plutonium in rats and pigs are confined mainly to epithelial cells. The kinetics of loss has been considered to involve the normal migration and sloughing of epithelial cells from the tips of villi. In guinea-pigs, baboons and macaques, low levels of plutonium were retained, mainly in macrophages in the lacteal region in the tips of villi (ICRP, 1986).

(*c*) *Systemic distribution, retention and excretion*

Studies on the distribution of plutonium in humans and experimental animals show that the main sites of deposition and retention are the liver and skeleton, with small fractions retained in the gonads (Leggett, 1985; ICRP, 1989, 1993c). In animals of all ages, about 90% of plutonium absorbed into blood is initially deposited in the liver and skeleton. In experiments with beagles, the distribution between liver and skeleton varied with age, skeletal uptake being almost 70% in juveniles and 40–60% in adults. In persons given intravenous injections of ^{239}Pu citrate, about 50% was retained in the skeleton and 30% in the liver 4–457 days after injection (Durbin, 1972; ICRP, 1986, 1989).

Within the skeleton, plutonium is deposited initially on bone surfaces, the highest concentrations being found at sites with red haematopoietic marrow and the lowest at sites with yellow fatty marrow. In adults, nearly all the red bone marrow is in trabecular bone, and deposition in these bones is likely to be greater than that in cortical bone. In children, some or all of the marrow in cortical bone is active, and a more uniform distribution in cortical and trabecular regions might be expected. Bone surfaces labelled with plutonium may remain unchanged, or they may be buried by the formation of new bone or resorbed by osteoclasts. The rate of removal from surfaces by burial or resorption depends on the age of the individual and on the bone surface type (trabecular or cortical). Plutonium resorbed by osteoclasts may be released and

concentrated by macrophages in bone marrow. In beagles injected with ^{239}Pu, the peak labelling of macrophages in bone marrow was found two years after injection, and no labelled macrophages were seen after four years. There is autoradiographic evidence that resorbed plutonium can be re-deposited on bone surfaces either locally or after entry into blood (ICRP, 1989).

Studies in rats and beagles indicate that hepatocytes are responsible for the initial uptake of plutonium by the liver, leading to association with the iron-storage protein, ferritin. Later, retained plutonium becomes associated with subcellular structures, including lysosomes, microsomes and mitochondria, and the proportion contained within reticuloendothelial cells increases with time. Studies of human autopsy samples show that plutonium may be retained for many years in the liver. Estimates of the ratio of total retained plutonium in liver and skeleton indicate that the ratio depends on the age at which exposure occurred, being near 1 for older subjects and 0.5 or less for younger individuals. The dominant factor in this age-related difference is the lower initial deposition in the liver at younger ages, although it is also possible that retention times may be shorter in children (ICRP, 1989).

Studies of actinide retention in human and animal gonads have been reviewed (Thomas *et al*., 1989) and show fractional uptake equivalent to about 10^{-5} per g of gonadal tissue for both testes and ovaries in humans and no evidence of loss with time in most of the animal species studied. Studies in beagles showed that the initial uptake of ^{239}Pu in testes and ovaries is greater in younger than in older animals (ICRP, 1993c).

(*d*) *Placental transfer*

Most of the data on the transfer of plutonium across the placenta have been obtained in rats, although some information is available for baboons, mice and guinea-pigs. Data on human placental transfer of plutonium are limited.

^{239}Pu was measured in human fetal tissue, obtained from second-trimester pregnancy terminations in the United Kingdom, by α-particle spectrometry and thermal ionization mass spectrometry. The typical concentration was < 50 μBq/kg. The whole-body concentration of ^{239}Pu of the mothers in this study was estimated to be approximately 0.3 mBq/kg (Prosser *et al.*, 1994).

A study of the placental transfer of ^{239}Pu in two baboons in late gestation (five months after conception; total gestation time, six months) showed that, seven days after intravenous injection of ^{239}Pu citrate, the fetus had retained about 4% of the amount injected. Retention in the placenta accounted for 8–12% of the injected activity. The concentrations of ^{239}Pu in fetal and maternal bone were similar, but those in fetal liver and other soft tissues were considerably lower than the corresponding values for maternal tissues. The overall fetal:maternal concentration ratio was about 1, while the concentration in the placenta was about four to five times the average maternal tissue concentration (Paquet *et al.*, 1998).

Less plutonium is transferred to the fetus in rodents than in baboons. When ^{238}Pu nitrate was administered to pregnant rats and guinea-pigs on various days of gestation

and the embryos or fetuses were analysed three days later, retention by a single rat embryo or fetus accounted for about 0.00004% of the injected activity on day 10.5, rising to 0.02% on day 17.5. In guinea-pigs, retention increased from 0.00001% of the injected activity for a day-17 embryo to 0.2% for a highly developed 57-day-old fetus (Morgan *et al.*, 1991).

4.1.21 *Americium-241*

(*a*) *Inhalation*

Most of the cases of human exposure for which adequate data from bioassays were available involved inhalation of oxides of americium. In these studies, a large proportion of the lung deposit (> 80%) was cleared with a half-time of tens of days, and the remainder had retention half-times of months or years. In general, the results of experiments in various animal species support the findings in humans *in vivo* (ICRP, 1995b).

^{241}Am inhaled or instilled as the nitrate in rats and dogs and as the chloride, citrate or hydroxide polymers in rats was highly soluble. Although various chemical forms of ^{241}Am have been shown to be more soluble than equivalent forms of ^{239}Pu, this appears not to be the case when both nuclides are present as minor components in an insoluble matrix. Similar levels of transfer of ^{241}Am and ^{239}Pu to blood were observed in rats, dogs and monkeys after inhalation of a mixed UO_2/PuO_2 aerosol. The rates of dissolution of ^{241}Am and ^{239}Pu from a number of industrial dusts were similar after intratracheal instillation in rats, but there was a trend towards greater absorption of ^{241}Am than ^{239}Pu from more soluble dusts (ICRP, 1995b).

(*b*) *Ingestion*

The only data on the absorption of americium in humans are from two studies of the absorption of plutonium and americium by volunteers who ate winkles collected on the Cumbrian coast near to the nuclear-fuel reprocessing plant at Sellafield. The average fractional absorption value obtained for americium was 1×10^{-4}, with a range of 4×10^{-5}–3×10^{-4} (Hunt *et al.*, 1990; ICRP, 1993c).

Studies on the absorption of americium in animals have been reviewed (ICRP, 1986; Harrison, 1991). Absorption after administration of americium to rodents as the nitrate or chloride was 2×10^{-4}–10^{-3}, while that of ^{241}Am oxide in fresh suspension was about 10^{-4} in rats and 6×10^{-5} in hamsters. The results for rodents and primates suggest that the absorption of americium, unlike plutonium, is not increased by binding to organic ligands (Harrison, 1991).

The absorption of americium in newborn hamsters and guinea-pigs was shown to be highest on the first day of life, with values of about 0.01–0.05, and to decrease progressively over the period of suckling to reach adult values by about the time of weaning at 21–22 days (Harrison & Fritsch, 1992).

(*c*) *Systemic distribution, retention and excretion*

In animals of all ages, most absorbed americium is deposited in the skeleton and liver within a few days of injection (ICRP, 1993c; Leggett, 1992b). Data on accidentally exposed humans and experimental data on baboons, monkeys and beagles indicate that the liver contains a major part of the systemic deposit soon after exposure, and a considerable proportion of the initial liver deposit is transferred to the skeleton within a few years. The skeletons of immature animals generally accumulate a greater proportion of injected americium than do mature animals. For example, the initial skeletal uptake accounted for 76–84% of injected ^{241}Am in newborn beagles and about 30% in mature animals (Stevens *et al.*, 1977).

In the skeleton, americium is deposited predominantly on bone surfaces, including resorbing and forming surfaces, in a more uniform manner than that of plutonium. Removal from bone surfaces occurs, as for plutonium, by burial and resorption, the burial rates probably being determined by age-specific bone formation rates, as for plutonium. Studies in experimental animals indicate that americium resorbed from bone is retained in the bone marrow to a lesser extent than plutonium (Leggett, 1992b).

Retention of americium in the liver has been shown to involve binding to ferritin and association with lysosomes, as for plutonium (Stover *et al.*, 1970). Transfer to liver reticuloendothelial cells was demonstrated after high doses. Data on humans and experiments in beagles indicate a removal half-time from liver of about one year (compared with about 10 years for plutonium). When account is taken of recycling of americium to the liver, this rate of loss corresponds to an apparent half-time of 2–3 years (Griffith *et al.*, 1983).

In humans, the testicular retention of the total systemic burden of ^{241}Am is about 0.03% long after exposure. This finding is consistent with results obtained in beagles. In adult baboons, the ovaries retained 4.8 and 1.5% of the injected dose per kilogram wet weight when measured 1 and 27 months after injection of ^{241}Am, respectively (Guilmette *et al.*, 1980; ICRP, 1986).

(*d*) *Placental transfer*

The transfer of americium to the embryo and fetus has been measured in mice, rats, guinea-pigs and baboons. The results obtained show that the levels of transfer are consistently lower than the corresponding values for plutonium.

A study of the placental transfer of ^{241}Am in two baboons in late gestation (five months after conception) showed that, seven days after intravenous injection of ^{241}Am citrate, retention by the fetus represented 0.3–0.4% of the amount injected. Retention in the placenta accounted for 2–3% of the injected activity. The concentrations of ^{241}Am in fetal bone and soft tissues were an order of magnitude or more lower than the corresponding values for maternal tissues. The overall fetus:mother concentration ratio was about 0.1 (Paquet *et al.*, 1998).

In rodents, the levels of transfer of americium to the fetus were lower than in baboons. The concentrations of ^{241}Am in guinea-pig fetuses in late gestation were two orders of magnitude higher when the mother had been treated one week previously than when they had been treated one month before conception (Levack *et al*., 1994).

4.1.22 *Curium-244*

(*a*) *Inhalation*

Follow-up of cases of accidental inhalation of ^{244}Cm oxides and a mixture of the nitrate, chloride and oxide, and the results of studies in rats and dogs exposed by inhalation show that curium isotopes generally behave similarly to americium. No large differences in solubility were observed between oxides and other chemical forms (ICRP, 1995b).

(*b*) *Ingestion*

The absorption of ^{239}Np and ^{242}Cm was measured in five adult male volunteers who received solutions of the citrate complexes with a midday meal. The mean fractional absorption value, as measured in urine, was 2×10^{-4} for both radionuclides, with a range of $1–3 \times 10^{-4}$ in both cases (Popplewell *et al.*, 1991).

The fractional absorption of curium in rats was in the range $3 \times 10^{-5}–7 \times 10^{-4}$. In guinea-pigs given ^{242}Cm prepared in the same way as for the study of Popplewell *et al*. (1991), described above, the fractional absorption was about 10^{-4}. High values have been reported for the absorption of curium in neonatal rats and pigs, similar to those for plutonium and americium. Leaving ^{244}Cm oxide in water for four days before administration to rats increased the absorption from 4×10^{-5} to 5×10^{-4} (ICRP, 1995b).

(*c*) *Systemic distribution, retention and excretion*

The biological behaviour of curium is similar to that of americium, particularly after absorption to blood, as shown in studies in rats and in more limited studies in dogs, baboons and sheep (ICRP, 1995b).

The systemic distribution and retention of ^{241}Am citrate, ^{241}Am nitrate and ^{244}Cm nitrate were virtually identical in rats during the first week after intubation into various regions of the respiratory tract. Similar behaviour of ^{241}Am nitrate and ^{242}Cm nitrate was also shown in rats after inhalation or intramuscular injection in a comparison of urinary excretion, uptake and retention in bone and sites of binding on bone. The long-term retention of ^{244}Cm in rat skeleton was similar to that of ^{241}Am. The distributions of americium and curium in beagles were also similar, although the initial liver:skeleton concentration ratio and the rate of faecal excretion appeared to be slightly higher for curium. Studies in a small number of baboons injected with ^{241}Am citrate or $^{243/244}$Cm citrate indicated similar patterns of distribution and retention. Studies of the transfer of

radionuclides to the milk of lactating ewes showed similar levels of transfer for ^{241}Am and ^{244}Cm (ICRP, 1995b).

(*d*) *Placental transfer*

^{244}Cm citrate was administered to a baboon by intravenous injection about four months after conception, and transfer to the fetus was measured after 45 days. The amount retained in the fetus represented 0.45% of total maternal retention, with 95% in the fetal skeleton. Placental retention represented 1% of total maternal retention. The average fetus:mother concentration ratio was estimated to be about 0.2 (Neton *et al.*, 1979). Studies of the transfer of americium and curium to rat fetuses gave similar results (Sikov & Mahlum, 1975; Sikov, 1987b).

4.1.23 *Biokinetics models and dose coefficients*

ICRP (1979, 1980, 1981) considered the behaviour of a large number of radioactive elements and calculated dose coefficients and limits of intake of their radioisotopes for workers by inhalation or ingestion. General models were proposed for the organs of intake, the respiratory and gastrointestinal tracts, and element-specific models were presented for the systemic distribution and retention of radionuclides after their entry into the blood. A model for the behaviour of bone-seeking radionuclides and dose delivery within the skeleton was also included. The doses to members of the public, including children, were also considered and models and dose coefficients have been published for isotopes of 31 elements (ICRP, 1989, 1993c, 1995a,b). When sufficient information was available, physiologically realistic, age-dependent models were developed, for example, taking account of the effects of bone remodelling on the distribution and retention of actinide and alkaline earth elements. A revised respiratory tract model has been developed (ICRP, 1994b), and revision of the model of the gastrointestinal tract is in progress. A CD-ROM has become available which gives the organ and tissue doses for intakes of radionuclides by workers and members of the public (ICRP, 1999). A report is in preparation on doses to the fetus after intake by the mother.

(*a*) *Respiratory tract model*

In the ICRP model (ICRP, 1994b), the respiratory tract is represented by five regions: the extrathoracic (ET) airways are divided into ET_1, the anterior nasal passages, and ET_2, which comprises the posterior nasal and oral passages, the pharynx and larynx. The thoracic regions are bronchial (BB: trachea, generation 0; bronchi, airway generations 1–8), bronchiolar (bb: airway generations 9–15) and alveolar–interstitial (AI: the gas exchange region). Lymphatic tissue is associated with the extrathoracic and thoracic airways (LN_{ET} and LN_{TH}, respectively). Reference values of dimensions and scaling factors for subjects of different ages are specified.

The deposition of radionuclides is calculated for each region of the respiratory tract, taking account of both inhalation and exhalation. This is done as a function of particle

size, breathing parameters and/or work load, according to the age and sex of the subject. Deposition parameters are given for a particle size range of 0.006–100 μm activity median aerodynamic diameter. Default deposition parameters for individuals are based on average daily patterns of activity. Table 102 shows values for the regional deposition of aerosols in a reference man who breathes through his nose and is engaged in light work, taking into account default aerosol particle sizes for environmental exposure (1 μm) and occupational exposure (5 μm). In general, deposition in the ET region is greater for larger particles, whereas smaller particles are more likely to reach the AI region of the lung.

Table 102. Deposition of inhaled aerosols in a reference man (%)

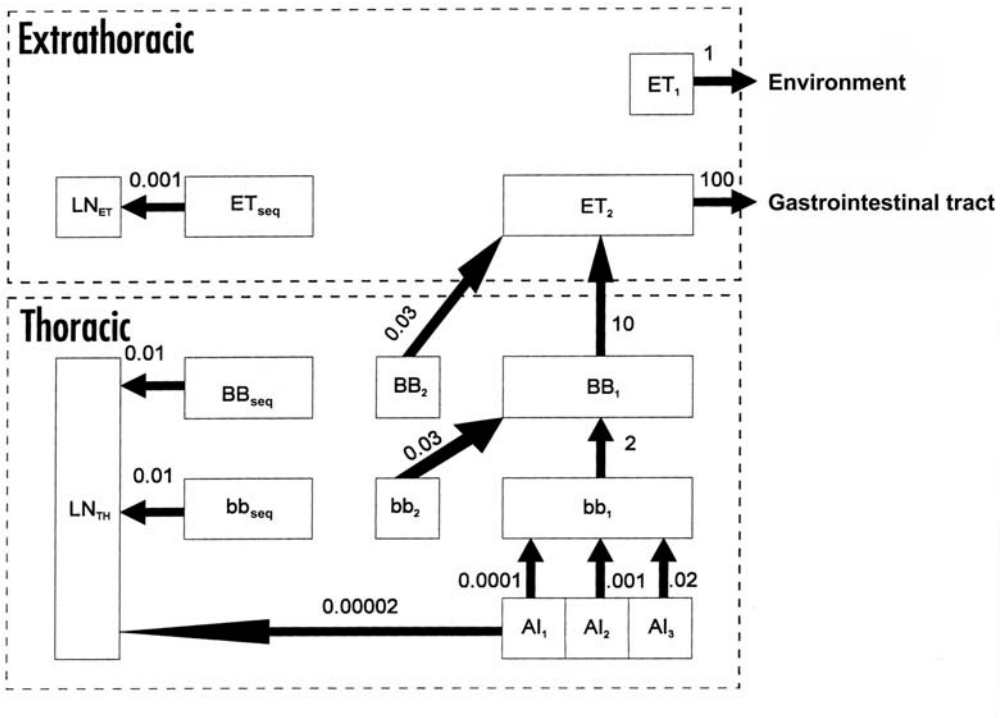
Region	Activity median aerodynamic diameter (μm)	
	1	5
ET_1	16.5	33.9
ET_2	21.1	39.9
BB	1.24	1.78
bb	1.65	1.10
AI	10.7	5.3
Total	51.2	82.0

From ICRP (1994b). ET_1, anterior nasal passages; ET_2, posterior nasal and oral passages; BB, trachea, generation 0; and bronchi, airway generations 1–8; bb, bronchioli, airway generations 9–15; AI, alveolar–interstitial gas exchange region

The model describes three clearance pathways. Material deposited in ET_1 is removed by extrinsic means such as nose-blowing. In other regions, clearance occurs either by particle transport to the gastrointestinal tract and lymphatic system or by absorption into blood. Transport to the gastrointestinal tract occurs by mucociliary clearance. It is assumed that the particle transport rates are the same for all materials. Absorption into blood is material-specific and is assumed to occur at the same rate in all regions except ET_1, where none occurs. Fractional clearance rates vary with time, but to facilitate calculations they are represented by combinations of compartments cleared at constant rates. It is assumed that the clearance rates by both processes are independent of age and sex.

Figure 10 shows the compartment model for particle transport. The rate constants shown are reference values (per day), which were derived, when possible, from studies in humans, since particle transport rates are known to vary greatly among mammalian

Figure 10. Particle transport in the respiratory tract



From ICRP (1994b)

ET_1, anterior nasal passages; LN_{ET}, lymphatic tissue associated with extrathoracic airways; ET_{seq}, extrathoracic walls; ET_2, posterior nasal and oral passages; BB_{seq}, trachea, generation 0; and bronchi, airway generations 1–8, fraction retained on walls; BB_2, fraction of deposit in BB that moves slowly; BB_1, fraction of deposit in BB that moves rapidly; LN_{TH}, lymphatic tissue associated with thoracic airways; bb_{seq}, airway generations 9–15, fraction retained on walls; bb_2, fraction of the deposit in bb that moves slowly; bb_1, fraction of the deposit in bb that moves rapidly; AI_1, AI_2, AI_3, deposits in three areas of the gas exchange region

species. It is assumed that the AI deposit is divided between AI_1, AI_2 and AI_3 in the ratio 0.3:0.6:1, with half-times of about 30, 700 and 7000 days, respectively. The fraction of the deposit in bb and BB that moves slowly (bb_2 and BB_2) depends on particle size (up to 50% for particles < 2.5 μm), 0.7% being retained in the walls (bb_{seq} and BB_{seq}).

Absorption of radionuclides into blood depends on the chemical nature of the element and the chemical form deposited. The use of material-specific dissolution rates is encouraged, but default values are given for types F (fast), M (medium) and S (slow). Expressed as approximate half-times for one or two components of clearance, these absorption rates correspond to: type F, 10 min (100%); type M, 10 min (10%), 140 days (90%); and type S, 10 min (0.1%), 7000 days (99.9%). The amounts of 1-μm activity median aerodynamic diameter aerosol that reach the blood from the respiratory tract can

be calculated as 24% (F), 9% (M) and 1% (S). In each case, a further fraction of the inhaled material will reach the blood by absorption from the gut after mucociliary clearance from the respiratory system and swallowing; this fraction will be greatest for type F materials (most soluble) and least for type S materials. The recommended default dissolution rate for most of the elements considered in this monograph is type M; the exceptions are iodine and caesium (type F) and thorium (type S).

Doses are calculated for each region of the respiratory tract, taking account of the location of target cells for induction of cancer. In the ET region, the target is taken to be the basal cells of the epithelial layer. In the bronchial epithelium, both secretory cells and basal cells are included as targets, while in the bronchiolar epithelium only secretory (Clara) cells are considered to be a target. In the AI region, Clara cells, type II epithelial cells and epithelial cells of blood capillaries are considered tissues from which tumours can arise.

(*b*) *Gastrointestinal tract model*

The model of the gastrointestinal tract used by ICRP (1979) is that developed for calculation of doses to workers. It has since been applied to members of the public, including children (ICRP, 1989, 1993c, 1995a,b), taking into account the smaller tissue masses in children.

The model has four compartments: the stomach, small intestine, upper large intestine and lower large intestine. Material is transferred successively between the four compartments of the model in an exponential manner, with half-times taken to be 0.69, 2.8, 9.2 and 17 h (relative clearance rates of 24, 6, 1.8 and 1 per day), respectively; immediate mixing within the contents of the different compartments is assumed, and doses are calculated separately for the mucosal wall of each compartment. As specific information on the age-dependence of the half-time in the different compartments is not available, these rates are taken to apply to all age groups. Since the total transit time in children is shorter than in adults, the dose to the gastrointestinal tract in children may be overestimated.

Radionuclides are absorbed mainly in the small intestine. Absorption is usually expressed as the fraction of the ingested radionuclide reaching the blood. ICRP has specified values for the intake of each radioactive element by workers and has recommended intake limits by children for 31 radionuclides. The recommended absorption values for the radioisotopes included in this monograph for intakes by members of the public are given in Table 103. Absorption by infants refers to intakes at three months of age.

The colon (large intestine) receives doses from radionuclides passing unabsorbed through the gut and from radionuclides excreted into the gut. Systemic models for the behaviour of radionuclides after their entry to blood specify the proportions excreted in urine and faeces. For mathematical convenience in the current model, excretion into the gut is generally assumed to occur in the upper large intestine, whereas absorption takes place in the small intestine.

Table 103. Fractional absorption of radionuclides from the gastrointestinal tract by members of the public

Element	Adult	Infant
H, C, Cs, S, I, At	1	1
P, Re	0.8	1
Tc, Po	0.5	1
Sr[a]	0.3	0.6
Ra[a]	0.2	0.6
Bi	0.05	0.1
U	0.02	0.04
Ga	0.001	0.01
Th, Np, Pu, Am, Cm	0.0005	0.005

From ICRP (1989, 1993c, 1995a,b)

[a] Intermediate values for 1-, 5- 10- and 15-year-old children: Sr, 0.4; Ra, 0.3

For penetrating radiations, the average dose to the wall of each region is used as a measure of the dose to the mucosal layer. For non-penetrating radiations, the fraction absorbed by the mucosal layer is taken to be equal to 0.5 v/M where M is the mass of the contents of that section of the gastrointestinal tract and v is a factor between 0 and 1 representing the proportion of radiation energy reaching sensitive cells. The factor 0.5 is introduced because the radiation dose from non-penetrating radiations near the intestinal wall is approximately half that within the contents. For β-particles, v is taken to be 1; for α-particles, an arbitrary value of 0.01 is used. The cells sensitive to cancer induction are thought to be the stem cells in the base of the crypts, which in this model are likely to receive negligible doses from α-emitting nuclides in the gut lumen.

(*c*) *Bone models*

In ICRP Publication 30 (ICRP, 1979), radionuclides deposited in bone were classified as either volume or surface seekers, and the initial distribution was assumed to persist throughout the retention of the radionuclide in the bone. For example, plutonium and related actinide elements were classified as surface seekers, while the alkaline earth elements calcium, strontium and radium were classified as volume seekers. The target regions were taken to be a 10-μm layer of marrow adjacent to endosteal bone surfaces for induction of solid tumours and of red bone marrow for induction of leukaemia. Table 104 shows the proportion of energy from decay of α- and β-particle-emitting radionuclides deposited in these two target regions.

In practice, all the radionuclide deposited in bone is initially on bone surfaces, and then migrates within the bone. Dynamic models for the skeleton have been adopted by ICRP (1993c) for the main groups of bone-seeking radionuclides, those of the alkaline earth elements, calcium, barium, radium and strontium, and the actinides, plutonium,

Table 104. Absorbed fractions of energy from radionuclides deposited in bone

Source organ	Target organ	Class of radionuclide				
		α-emitter		β-emitter		
		Uniform in volume	On bone surfaces	Uniform in volume	On bone surfaces	
					$\bar{E}_\beta \geq 0.2$ MeV	$\bar{E}_\beta < 0.2$ MeV
Trabecular bone	Bone surface	0.025	0.25	0.025	0.025	0.25
Cortical bone	Bone surface	0.01	0.25	0.015	0.015	0.25
Trabecular bone	Red bone marrow	0.05	0.5	0.35	0.5	0.5
Cortical bone	Red bone marrow	0.0	0.0	0.0	0.0	0.0

From ICRP (1979); $\bar{E}_\beta$, average energy of β-particles

americium, neptunium and thorium. The alkaline earth model is also used for lead and uranium. These models take account of the burial of surface deposits, transfer of radioactivity to marrow and recycling to the circulation and between organs.

(*d*) *Assumptions concerning elements*

The following sections describe some assumptions made in the ICRP model for ^{3}H, ^{131}I, ^{137}Cs and for radionuclides of the alkaline earth elements and the actinide elements.

(i) *Hydrogen*

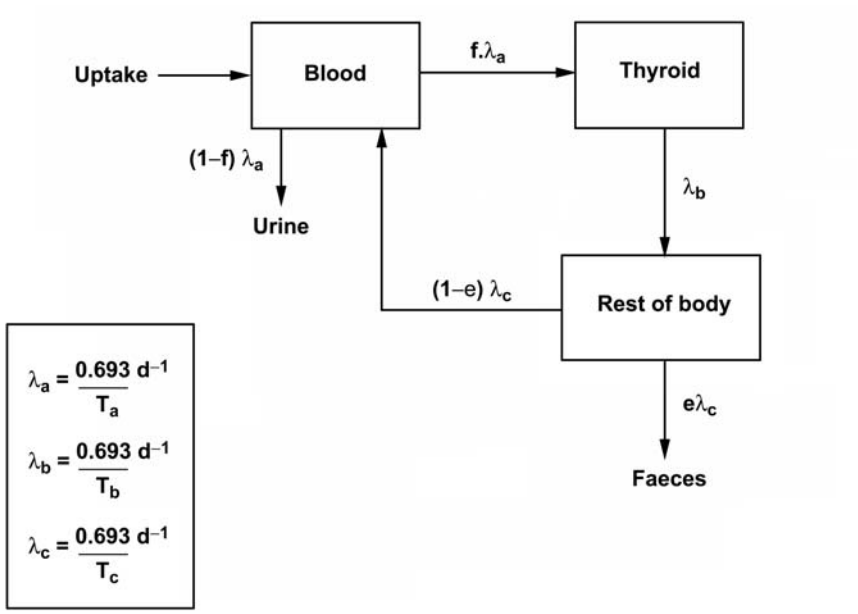
For ^{3}H entering body fluids as $^{3}H_2O$, the doses to tissues are calculated by assuming that 97% equilibrates with body water and is retained with a half-time of 10 days in adults (range, 4–18 days). On the basis of studies in animals, the remaining 3% is assumed to be incorporated into organic molecules and retained with a half-time of 40 days. For ^{3}H absorbed after ingestion of organically bound ^{3}H, it is assumed that 50% is readily exchangeable with hydrogen from the body water pool and will therefore be retained with a half-time of 10 days in adults. A half-time of 40 days is applied to the remaining 50%. On the basis of these assumptions and given a uniform distribution of retained ^{3}H, the dose to all body tissues is the same and is about 2.5 times higher for organically bound ^{3}H than for $^{3}H_2O$.

The half-times of retention are lower in children than in adults when account is taken of their smaller mass of total body water, the values for daily water balance based on their energy expenditure and, for the organically bound component, carbon content and balance. For example, half-times of three and eight days are applied for a three-month-old-infant and 10- and 40-day values for adults (see above). Despite the more rapid loss of ^{3}H from the body of children, the doses per unit intake are higher than for adults because of the dominating effect of smaller body mass (ICRP, 1989).

(ii) *Iodine*

The systemic model used for radioiodines is shown in Figure 11, and the parameters used are listed in Table 105. For children, there is no evidence that the level of uptake of iodine into the thyroid varies with age, although it may be higher in the first few days of life. As there is some evidence, however, that the half-time of retention in the thyroid is reduced in children, shorter half-times were used for children in the model (ICRP, 1989).

Figure 11. Model for biokinetics of iodine



From ICRP (1989). *f*, fractional uptake by thyroid; *e*, fractional excretion in faeces; λ, coefficient of transport from blood to thyroid (λ_a), thyroid to rest of body (λ_b), rest of body to faeces (λ_c); T_a, biological half-time in blood; T_b, biological half-time in thyroid; T_c, biological half-time in rest of body

(iii) *Caesium*

Human retention of ^{137}Cs has two components. The first accounts for about 10% of the administered activity and is excreted mainly in the urine with a half-time of about two days in adults. The second component has a half-time of about 110 days in men (range, 50–150 days) and is shorter in women (mean, 60–65 days). The use of the ICRP value of 110 days is therefore likely to be conservative for women (ICRP, 1989).

The rate of loss of caesium from the body appears to be higher in children than in adults, and, in newborns, the short-term component accounts for an increasing proportion of the activity entering the blood with decreasing age (Table 106). The dose

Table 105. Parameters used for model of biokinetics of iodine

Age	Uptake by thyroid (%)	Faecal excretion (%)	Biological half-time (days)			'Apparent half- time' (days)
			Blood (T_a)	Thyroid (T_b)	Rest of body (T_c)	Thyroid[a]
3 months	30	20	0.25	11.2	1.12	15
1 year	30	20	0.25	15	1.5	20
5 years	30	20	0.25	23	2.3	30
10 years	30	20	0.25	58	5.8	70
15 years	30	20	0.25	67	6.7	80
Adult	30[b]	20	0.25[b]	80[b]	12[b]	91

From ICRP (1989)
[a] 2–16 days after intake
[b] From ICRP (1979)

Table 106. Biokinetics of caesium

Age	Total body distribution (%)		Total body biological half-time (days)	
	A	B	A	B
3 months	–	100	–	16
1 year	–	100	–	13
5 years	45	55	9.1	30
10 years	30	70	5.8	50
15 years	13	87	2.2	93
Adult[a]	10	90	2	110

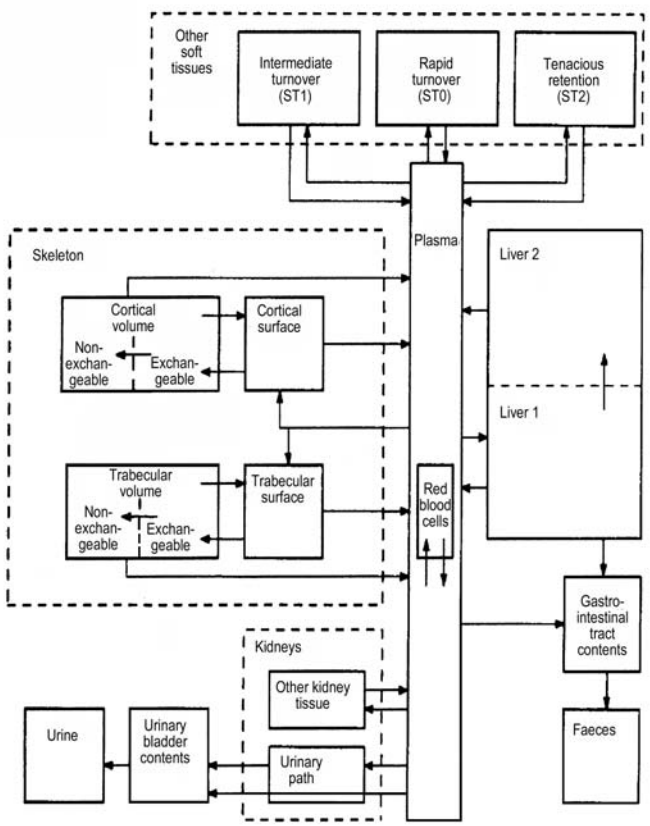
From ICRP (1989). A, short-term component; B, long-term component
[a] From ICRP (1979); the half-time of 110 days would yield a conservative estimate for women

coefficients for ^{137}Cs are largely independent of age, the maximum difference being less than a factor of two, because the shorter biological half-times at younger ages counteract the effect of smaller body mass (ICRP, 1989).

(iv) *Alkaline earth elements*

The model shown in Figure 12 was applied by ICRP (1989, 1993c, 1995a,b) to the alkaline earth elements calcium, strontium, barium and radium and to lead and uranium. Some features added to the model to enable its extension to the latter two elements do not apply to the alkaline earth elements. Thus, retention in red blood cells and kidneys is not considered for the alkaline earth elements, and the liver is included with other soft tissues.

Figure 12. Model for the biokinetics of alkaline earth elements (and Pb and U)



From ICRP (1993c)

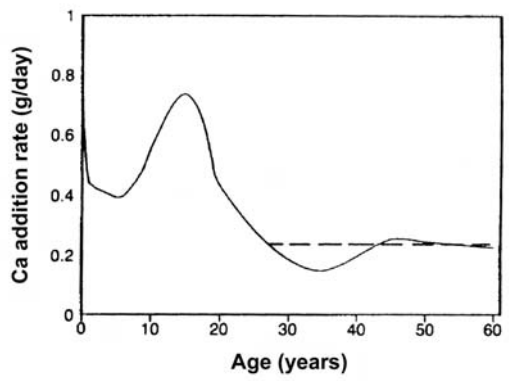
From blood, 25% of each alkaline earth element is assumed to deposit on bone surfaces in adults. A large proportion (60% for strontium, 68% for calcium) is deposited in soft tissues, mostly with a rapid turnover (ST0) with a half-time of retention of a few hours (representative of tissue fluids), and the remainder is excreted in the urine and faeces. About three times more strontium is excreted in the urine than calcium, as a result of less efficient reabsorption by kidney tubules; that is, the kidneys discriminate against strontium. The faecal excretion of calcium and strontium is similar.

Much of the bone surface deposit returns to blood plasma over the next few days, but a fraction is transferred inside the bone. Radionuclides returning to plasma from bone surfaces or soft tissues are available for uptake by these tissues again or for excretion. Cortical and trabecular regions of bone are distinguished, although many of the parameters used for these compartments are the same. Bone volume is separated into

'exchangeable' and 'non-exchangeable' regions, and discrimination among the alkaline earth elements by bone is accounted for by fractional transfer of activity from exchangeable to non-exchangeable bone volume. It is assumed in effect that the elements are equally likely to become temporarily incorporated into bone mineral but that the likelihood of reaching non-exchangeable sites in bone crystal decreases in the order calcium > strontium > barium > radium (fractional transfers exchangeable → non-exchangeable of 0.6, 0.5, 0.3 and 0.2, respectively). Release from non-exchangeable bone volume is assumed to result from the relatively slow process of bone turnover, involving resorption and remodelling. In adults, the bone turnover rates are assumed to be 3% per year for cortical bone and 18% per year for trabecular bone (ICRP, 1995b).

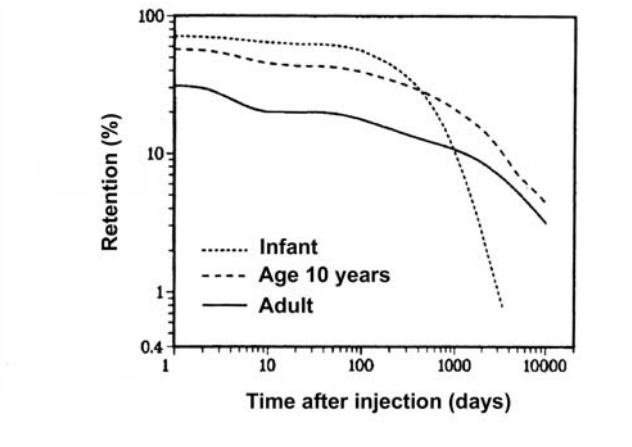
There are extensive data for humans and animals indicating that there is greater early retention of alkaline earth elements in growing than in mature individuals and that much of the variation with age is due to increased uptake by the immature skeleton. However, individuals exposed at younger ages also have a higher rate of loss of these elements from the skeleton than do mature individuals, owing to their higher rate of bone turnover. Accordingly, the most important changes to the model parameters to account for intake by infants and children are increased initial deposition in the skeleton in relation to the calcium addition rate (Figure 13) and increased release of elements from the non-exchangeable bone volume, proportional to the bone turnover rate. Figure 14 illustrates the resulting retention of strontium in the skeleton after intake at different ages, showing the higher initial uptake but more rapid subsequent loss after intake at younger ages.

Figure 13. Estimated calcium addition rate to the human skeleton as a function of age



From Leggett (1992a)

Figure 14. Model predictions for the retention of strontium in the skeleton as a function of time after entry into blood (% total entering blood)



From ICRP (1993c)

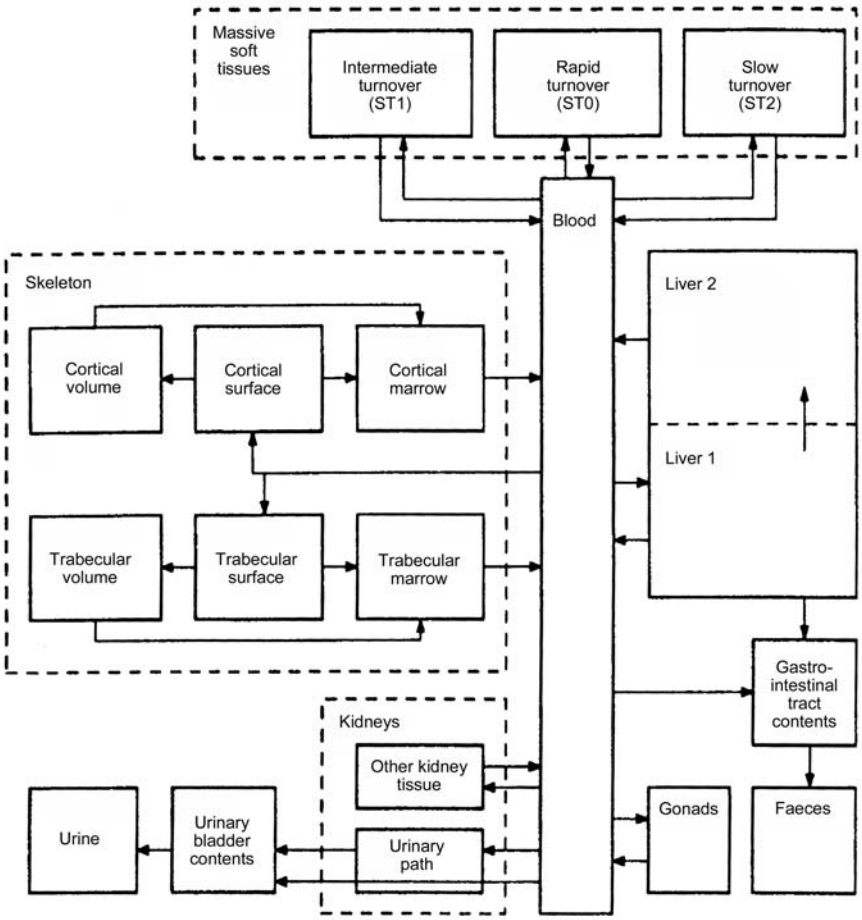
(v) *Actinide elements*

After absorption to blood, the principal sites of deposition of plutonium and related elements are the skeleton and liver. ICRP (1979) assumed that, for adults, 45% of the absorbed dose from blood is deposited in skeleton and in the liver, a small amount is deposited in gonads (0.042% in testis, 0.01% in ovary), and the remainder is promptly excreted. The half-times of retention were assumed to be 100 years for the skeleton and 40 years for the liver; no loss was considered to occur from gonads. In the skeleton, plutonium and related elements were treated as bone-surface seekers; that is, they remain on bone surfaces throughout their retention in the skeleton.

ICRP (1989, 1993c) later developed a physiologically realistic, age-dependent model and applied it to plutonium, americium and neptunium and subsequently to thorium and curium (ICRP, 1995a,b). The model (Figure 15) is similar to that for the alkaline earth elements with respect to the compartments considered, the main difference being in the migration of elements within the skeleton. The model is based on and validated against data in humans and animals for the behaviour of the actinide elements.

The main differences between plutonium and the other elements with regards to the parameters used in the model are in the rates of urinary and faecal excretion (e.g. plutonium < americium < neptunium for urinary excretion), the proportions deposited in the skeleton and liver and retention by the liver. The main age-dependent differences are in the relative proportions deposited in the skeleton and liver and the rates of migration in the skeleton (and hence skeletal retention). Table 107 illustrates the age dependence of uptake of plutonium and americium by the skeleton and liver.

Figure 15. Model for the biokinetics of actinide elements, Pu, Am, Cm, Th and Np



From ICRP (1993c)

Table 107. Age-dependent uptake of Pu and Am by the skeleton and liver (% reaching blood), excluding 4% Pu and 7% Am excreted

Tissue	Infant (3 months)		Child (1–15 years)		Adult (20 years)	
	Pu	Am	Pu	Am	Pu	Am
Liver	10	10	20	30	30	50
Skeleton	70	70	60	50	50	30

From ICRP (1993c)

As in the model for the alkaline earth elements, cortical and trabecular regions of the skeleton are treated separately, with initial deposition on bone surfaces. While subsequent migration of the alkaline earth elements to the interior of the bone is related to the chemical processes involved in the transfer of calcium to bone crystal, incorporation of plutonium and related elements into bone volume depends on burial by surface deposition of new bone. Different regions of bone surfaces may be growing by the deposition of new bone (by osteoblasts), losing mineral by resorption (by osteoclasts) or resting.

There is evidence that plutonium removed from the bone surfaces may be retained in bone marrow. Accordingly, the model considers transfer of actinide elements to bone volume and to bone marrow. It also includes transfer from bone volume to bone marrow due to resorption. Activity is eventually released from bone marrow to blood and becomes available for recycling to all tissues, including the skeleton. For simplicity, the rates of bone growth and resorption are taken to be the same and to show the same age-dependence.

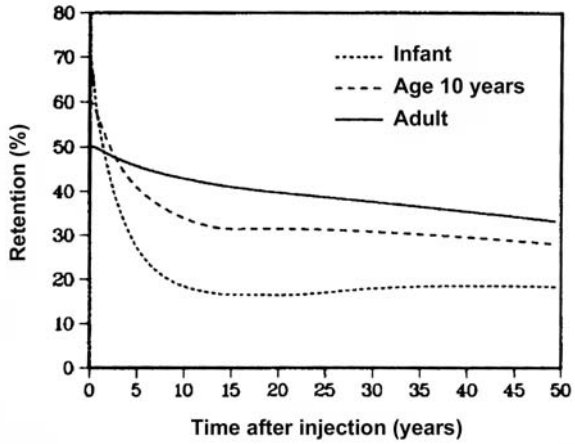
The liver has two compartments in the model, for all elements except americium and curium, for which one compartment is used. Activity is taken up by Liver 1 and transferred to Liver 2, except for a small proportion that is removed to blood or excreted in bile to the gastrointestinal tract. Material leaving Liver 2 is released to blood. Uptake and retention in the gonads is similar to that assumed in the previous models of ICRP (1979). Figures 16 and 17 illustrate the retention of Pu in the skeleton and liver predicted by the model for different ages at intake. The predictions of the model are consistent with data for autopsy samples from occupationally exposed persons. The model is also consistent with the data on urinary and faecal excretion (ICRP, 1993c).

(*e*) *Dose coefficients*

Having established the distribution of radionuclides between body organs and tissues and the time course of their retention, the resulting distribution of the absorbed energy and absorbed dose, defined as absorbed energy per unit mass (expressed in J/kg or Gy), can be calculated. For non-penetrating radiations, the energy is usually deposited largely in the region in which the radionuclide is located. For penetrating radiations, however, it is necessary to take account of 'cross-fire' between tissues. This is done by using a 'mathematical phantom' (i.e. a phantom that can be described with simple mathematical equations), which describes the geometric relationship between the different tissues and organs of the body. Such phantoms have been developed for various age groups (ICRP, 1989). Tissue doses are commonly integrated over a 50-year period for adults or up to age 70 years for children, and the resulting values are referred to as 'committed doses'.

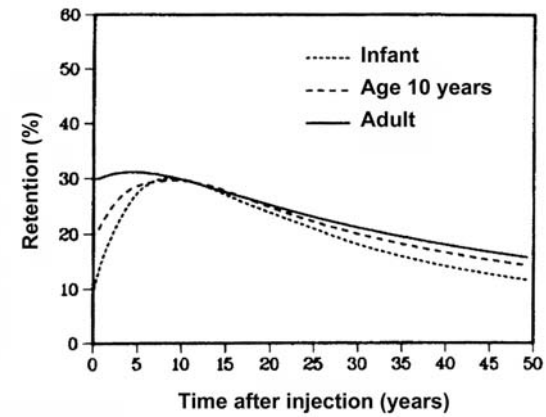
Tables 108–113 give the committed doses to selected tissues (in Gy per Bq of radioactivity) for intakes by adult members of the public by inhalation or ingestion. ICRP (1995a,b) specified default assumptions for the solubility of inhaled radionuclides: type M is assumed for all the radionuclides considered here except isotopes of caesium and iodine (type F) and thorium (type S). The values used for the fractional absorption of ingested radionuclides are as shown in Table 103.

Figure 16. Model predictions for the retention of plutonium in the skeleton as a function of time after entry into blood (% total entering blood)



From ICRP (1993c)

Figure 17. Model predictions for the retention of plutonium in the liver as a function of time after entry into blood (% total entering blood)



From ICRP (1993c)

The doses received vary from a low value of 1.8×10^{-11} Gy/Bq from inhaled or ingested $^{3}H_2O$, with uniform dose to all tissues, to a high value of about 8×10^{-5} Gy/Bq to bone surfaces from inhaled ^{239}Pu or ^{241}Am.

Table 108. Committed doses from inhalation of β- and γ-emitting radionuclides by adults (Gy/Bq)

Nuclide	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{3}H								
$^{3}H_2O$	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}
Organically bound	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}	4.1×10^{-11}
^{14}C	1.6×10^{-8}	8.2×10^{-11}	4.1×10^{-10}	6.8×10^{-11}	6.8×10^{-11}	6.8×10^{-11}	6.8×10^{-11}	6.8×10^{-11}
^{32}P	2.4×10^{-8}	3.4×10^{-10}	1.2×10^{-9}	1.7×10^{-10}	2.1×10^{-9}	2.1×10^{-9}	1.7×10^{-10}	1.7×10^{-10}
^{35}S	1.2×10^{-8}	2.1×10^{-11}	3.4×10^{-11}	7.7×10^{-12}	7.7×10^{-12}	7.7×10^{-12}	7.7×10^{-12}	7.7×10^{-12}
^{67}Ga	1.6×10^{-9}	2.6×10^{-11}	2.5×10^{-10}	1.8×10^{-11}	1.7×10^{-11}	4.5×10^{-11}	3.8×10^{-12}	8.1×10^{-12}
^{89}Sr	4.5×10^{-8}	2.0×10^{-10}	3.9×10^{-9}	4.6×10^{-11}	1.1×10^{-9}	1.4×10^{-9}	4.6×10^{-11}	4.6×10^{-11}
^{90}Sr	2.1×10^{-7}	3.8×10^{-10}	5.2×10^{-9}	2.8×10^{-10}	7.0×10^{-8}	1.6×10^{-7}	2.8×10^{-10}	2.8×10^{-10}
^{99}Tc	3.2×10^{-8}	5.2×10^{-10}	8.9×10^{-10}	1.2×10^{-11}	9.2×10^{-12}	9.2×10^{-12}	9.2×10^{-12}	2.4×10^{-10}
^{125}I	1.5×10^{-11}	1.1×10^{-11}	1.6×10^{-11}	8.9×10^{-12}	1.2×10^{-11}	6.0×10^{-11}	7.9×10^{-12}	1.0×10^{-7}
^{131}I	6.0×10^{-11}	4.0×10^{-11}	2.5×10^{-11}	1.7×10^{-11}	3.7×10^{-11}	4.6×10^{-11}	1.4×10^{-11}	1.5×10^{-7}
^{137}Cs	4.3×10^{-9}	4.4×10^{-9}	5.1×10^{-9}	4.6×10^{-9}	4.4×10^{-9}	4.6×10^{-9}	4.2×10^{-9}	4.4×10^{-9}
^{141}Ce	2.4×10^{-8}	9.8×10^{-11}	1.2×10^{-9}	1.4×10^{-9}	2.9×10^{-10}	2.9×10^{-9}	2.1×10^{-11}	3.8×10^{-11}
^{144}Ce	1.9×10^{-7}	2.1×10^{-9}	1.2×10^{-8}	1.4×10^{-7}	2.8×10^{-8}	4.9×10^{-8}	1.7×10^{-9}	1.8×10^{-9}
^{186}Re	6.4×10^{-9}	1.2×10^{-9}	9.1×10^{-10}	3.2×10^{-11}	2.4×10^{-11}	2.6×10^{-11}	2.3×10^{-11}	1.1×10^{-9}
^{188}Re	2.0×10^{-9}	1.0×10^{-9}	6.4×10^{-10}	2.4×10^{-11}	1.9×10^{-11}	1.9×10^{-11}	1.8×10^{-11}	1.5×10^{-9}

Default assumptions for members of the public: H is vapour and completely absorbed; 1-μm activity mean aerodynamic diameter aerosol for others; type F for Cs and I; type M for all others

Table 109. Committed doses from ingestion of β- and γ-emitting radionuclides by adults (Gy/Bq)

Nuclide	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{3}H								
$^{3}H_2O$	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}	1.8×10^{-11}
Organically bound	4.1×10^{-11}	4.8×10^{-11}	4.3×10^{-11}	4.8×10^{-11}	4.8×10^{-11}	4.8×10^{-11}	4.8×10^{-11}	4.8×10^{-11}
^{14}C	5.7×10^{-11}	6.3×10^{-11}	5.9×10^{-11}	5.7×10^{-11}	5.7×10^{-11}	5.7×10^{-11}	5.7×10^{-11}	5.7×10^{-11}
^{32}P	6.7×10^{-10}	1.5×10^{-9}	5.6×10^{-9}	6.7×10^{-10}	8.2×10^{-9}	8.2×10^{-9}	6.7×10^{-10}	6.7×10^{-10}
^{35}S								
Inorganic	9.6×10^{-11}	1.5×10^{-10}	2.5×10^{-10}	9.6×10^{-11}	9.6×10^{-11}	9.6×10^{-11}	9.6×10^{-11}	9.6×10^{-11}
Organic	7.6×10^{-10}	8.1×10^{-10}	8.3×10^{-10}	7.6×10^{-10}	7.6×10^{-10}	7.6×10^{-10}	7.6×10^{-10}	7.6×10^{-10}
^{67}Ga	2.2×10^{-12}	8.9×10^{-11}	1.2×10^{-9}	1.3×10^{-11}	2.6×10^{-11}	1.9×10^{-11}	1.2×10^{-11}	1.9×10^{-13}
^{89}Sr	2.0×10^{-10}	8.8×10^{-10}	1.4×10^{-8}	2.0×10^{-10}	4.8×10^{-9}	6.0×10^{-9}	2.0×10^{-10}	2.0×10^{-10}
^{90}Sr	6.6×10^{-10}	9.1×10^{-10}	1.3×10^{-8}	6.6×10^{-10}	1.8×10^{-7}	4.0×10^{-7}	6.6×10^{-10}	6.6×10^{-10}
^{99}Tc	3.9×10^{-11}	2.2×10^{-9}	2.5×10^{-9}	5.2×10^{-11}	3.9×10^{-11}	3.9×10^{-11}	3.9×10^{-11}	1.0×10^{-9}
^{125}I	4.1×10^{-11}	6.3×10^{-11}	5.5×10^{-11}	2.6×10^{-11}	3.3×10^{-11}	1.7×10^{-10}	2.3×10^{-11}	3.1×10^{-7}
^{131}I	1.0×10^{-10}	3.1×10^{-10}	1.2×10^{-10}	4.9×10^{-11}	1.0×10^{-10}	1.3×10^{-10}	4.0×10^{-11}	4.3×10^{-7}
^{137}Cs	1.3×10^{-8}	1.3×10^{-8}	1.5×10^{-8}	1.4×10^{-8}	1.3×10^{-8}	1.4×10^{-8}	1.3×10^{-8}	1.3×10^{-8}
^{141}Ce	1.4×10^{-12}	2.3×10^{-10}	5.5×10^{-9}	2.4×10^{-11}	1.9×10^{-11}	4.9×10^{-11}	7.9×10^{-12}	2.9×10^{-13}
^{144}Ce	1.3×10^{-11}	1.1×10^{-9}	4.2×10^{-8}	9.6×10^{-10}	1.9×10^{-10}	3.3×10^{-10}	1.7×10^{-11}	1.2×10^{-11}
^{186}Re	5.4×10^{-9}	5.4×10^{-9}	4.2×10^{-9}	1.3×10^{-10}	9.9×10^{-11}	1.0×10^{-10}	9.8×10^{-11}	4.8×10^{-9}
^{188}Re	7.9×10^{-11}	4.8×10^{-9}	3.1×10^{-9}	1.1×10^{-10}	8.0×10^{-11}	8.1×10^{-11}	7.9×10^{-11}	6.6×10^{-9}

Table 110. Committed doses from inhalation of α-emitting radionuclides by adults (Gy/Bq)

Nuclide	LET[a]	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{210}Po	High	1.3×10^{-6}	2.5×10^{-9}	2.9×10^{-9}	5.8×10^{-8}	2.3×10^{-8}	1.4×10^{-8}	2.4×10^{-9}	2.4×10^{-9}
^{211}At	High	4.4×10^{-8}	1.3×10^{-10}	1.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}	1.1×10^{-10}
^{212}Bi	High	9.8×10^{-9}	9.3×10^{-12}	3.3×10^{-12}	9.4×10^{-13}	9.4×10^{-13}	9.4×10^{-13}	9.4×10^{-13}	9.4×10^{-13}
	Low	1.5×10^{-10}	8.7×10^{-11}	2.7×10^{-11}	2.5×10^{-12}	2.5×10^{-12}	1.9×10^{-12}	4.3×10^{-13}	1.9×10^{-12}
^{224}Ra	High	1.2×10^{-6}	1.7×10^{-10}	1.4×10^{-9}	6.7×10^{-10}	2.0×10^{-9}	2.1×10^{-8}	1.2×10^{-10}	1.3×10^{-10}
	Low	1.3×10^{-8}	1.1×10^{-10}	3.8×10^{-9}	1.4×10^{-10}	1.8×10^{-10}	2.6×10^{-10}	2.9×10^{-11}	6.1×10^{-11}
^{226}Ra	High	1.4×10^{-6}	1.2×10^{-9}	2.0×10^{-9}	5.3×10^{-9}	2.6×10^{-8}	3.7×10^{-7}	1.2×10^{-9}	1.2×10^{-9}
	Low	2.1×10^{-9}	3.8×10^{-10}	2.6×10^{-9}	4.7×10^{-10}	6.5×10^{-9}	1.6×10^{-8}	3.2×10^{-10}	5.7×10^{-10}
^{228}Ra	High	4.8×10^{-7}	1.0×10^{-8}	1.5×10^{-8}	1.2×10^{-7}	2.3×10^{-7}	2.8×10^{-6}	2.5×10^{-8}	1.0×10^{-8}
	Low	1.2×10^{-7}	2.6×10^{-9}	1.9×10^{-8}	7.7×10^{-9}	2.1×10^{-8}	3.9×10^{-8}	1.4×10^{-9}	2.4×10^{-9}

Default assumptions for members of the public: 1-μm activity mean aerodynamic diameter aerosol; type M for each nuclide. LET, linear energy transfer

[a] Doses from high-LET α- and low-LET β- or γ-radiation given separately; low LET not shown when < 1%

Table 111. Committed doses from ingestion of α-emitting radionuclides by adults (Gy/Bq)

Nuclide	LET[a]	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{210}Po	High	1.4×10^{-8}	1.4×10^{-8}	1.5×10^{-8}	3.3×10^{-7}	1.3×10^{-7}	8.0×10^{-8}	1.4×10^{-8}	1.4×10^{-8}
^{211}At	High	5.3×10^{-10}	6.0×10^{-10}	5.4×10^{-10}	5.3×10^{-10}	5.3×10^{-10}	5.3×10^{-10}	5.3×10^{-10}	5.3×10^{-10}
^{212}Bi	High	3.4×10^{-13}	5.4×10^{-11}	1.4×10^{-11}	3.4×10^{-13}	3.4×10^{-13}	3.4×10^{-13}	3.4×10^{-13}	3.4×10^{-13}
	Low	2.7×10^{-12}	5.3×10^{-10}	1.6×10^{-10}	6.2×10^{-12}	6.1×10^{-12}	2.9×10^{-12}	1.5×10^{-12}	3.6×10^{-13}
^{224}Ra	High	5.4×10^{-10}	7.5×10^{-10}	6.3×10^{-9}	2.8×10^{-9}	8.3×10^{-9}	8.7×10^{-8}	5.2×10^{-10}	5.4×10^{-10}
	Low	6.0×10^{-11}	2.0×10^{-10}	1.6×10^{-8}	2.4×10^{-10}	5.4×10^{-10}	8.7×10^{-10}	1.2×10^{-10}	4.6×10^{-11}
^{226}Ra	High	2.0×10^{-9}	2.0×10^{-9}	4.6×10^{-9}	8.9×10^{-9}	4.3×10^{-8}	6.2×10^{-7}	2.0×10^{-9}	2.0×10^{-9}
	Low	9.3×10^{-10}	6.4×10^{-10}	7.1×10^{-10}	7.7×10^{-10}	1.1×10^{-8}	2.7×10^{-8}	5.5×10^{-10}	9.5×10^{-10}
^{228}Ra	High	7.4×10^{-9}	7.4×10^{-9}	8.6×10^{-9}	5.2×10^{-8}	1.1×10^{-7}	1.1×10^{-6}	1.0×10^{-8}	7.4×10^{-9}
	Low	1.9×10^{-9}	1.7×10^{-9}	2.0×10^{-8}	4.1×10^{-9}	2.4×10^{-8}	4.0×10^{-8}	1.3×10^{-9}	1.8×10^{-9}

[a] Doses from high-LET α- and low-LET β- and γ-radiation given separately; low LET not shown when < 1%

Table 112. Committed doses from inhalation of α-emitting actinide radionuclides by adults (Gy/Bq)

Nuclide	LET[a]	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{232}Th	High	8.0×10^{-6}	4.0×10^{-8}	4.4×10^{-8}	2.5×10^{-7}	6.2×10^{-7}	1.4×10^{-5}	1.3×10^{-7}	4.0×10^{-8}
	Low	5.5×10^{-7}	1.1×10^{-8}	1.5×10^{-8}	1.8×10^{-8}	3.8×10^{-8}	1.1×10^{-7}	3.2×10^{-9}	9.9×10^{-9}
^{234}U	High	1.4×10^{-6}	6.8×10^{-9}	7.2×10^{-9}	2.7×10^{-8}	2.0×10^{-8}	1.9×10^{-7}	6.8×10^{-9}	6.8×10^{-9}
^{235}U	High	1.2×10^{-6}	6.3×10^{-9}	6.6×10^{-9}	2.5×10^{-8}	1.9×10^{-8}	1.8×10^{-7}	6.3×10^{-9}	6.3×10^{-9}
	Low	5.5×10^{-8}	7.7×10^{-10}	1.9×10^{-9}	2.0×10^{-9}	4.8×10^{-9}	1.3×10^{-8}	5.0×10^{-10}	7.4×10^{-10}
^{238}U	High	1.1×10^{-6}	6.0×10^{-9}	6.3×10^{-9}	2.4×10^{-8}	1.8×10^{-8}	1.7×10^{-7}	6.0×10^{-9}	6.0×10^{-9}
	Low	1.2×10^{-7}	1.4×10^{-9}	2.5×10^{-9}	5.6×10^{-9}	1.9×10^{-8}	4.6×10^{-8}	1.6×10^{-9}	1.4×10^{-9}
^{237}Np	High	1.4×10^{-6}	6.7×10^{-8}	6.7×10^{-8}	8.2×10^{-7}	2.0×10^{-6}	5.1×10^{-5}	6.9×10^{-7}	6.7×10^{-8}
	Low	6.0×10^{-8}	9.3×10^{-9}	1.3×10^{-8}	5.9×10^{-8}	1.6×10^{-7}	2.6×10^{-6}	4.5×10^{-8}	1.1×10^{-8}
^{238}Pu	High	1.8×10^{-6}	1.2×10^{-7}	1.2×10^{-6}	1.5×10^{-5}	3.4×10^{-6}	7.0×10^{-5}	9.3×10^{-7}	1.2×10^{-7}
^{239}Pu	High	1.7×10^{-6}	1.4×10^{-7}	1.4×10^{-7}	1.6×10^{-5}	3.7×10^{-6}	7.7×10^{-5}	1.0×10^{-6}	1.4×10^{-7}
^{241}Am	High	1.9×10^{-6}	1.4×10^{-7}	1.4×10^{-7}	5.2×10^{-6}	2.9×10^{-6}	8.0×10^{-5}	1.6×10^{-6}	1.4×10^{-7}
	Low	1.2×10^{-8}	2.6×10^{-9}	3.6×10^{-9}	6.3×10^{-8}	4.0×10^{-8}	8.4×10^{-7}	1.8×10^{-8}	2.8×10^{-9}
^{244}Cm	High	2.0×10^{-6}	6.4×10^{-8}	6.5×10^{-8}	3.8×10^{-6}	1.9×10^{-6}	4.6×10^{-5}	9.1×10^{-7}	6.4×10^{-8}

Default assumptions for members of the public: 1-μm activity mean aerodynamic diameter aerosol; type S for Th; type M for all others

[a] Doses from high-LET α- and low-LET β- and γ-radiation given separately; low LET not shown when < 1%

Table 113. Committed doses from ingestion of α-emitting actinide radionuclides by adults (Gy/Bq)

Nuclide	LET[a]	Lung	Stomach	Colon	Liver	Red bone marrow	Bone surface	Testis	Thyroid
^{232}Th	High	1.8×10^{-9}	1.8×10^{-9}	3.1×10^{-9}	9.2×10^{-9}	2.3×10^{-8}	5.9×10^{-7}	5.4×10^{-9}	1.8×10^{-9}
	Low	1.8×10^{-10}	1.7×10^{-10}	7.8×10^{-10}	2.5×10^{-10}	1.2×10^{-9}	4.6×10^{-9}	1.3×10^{-10}	1.9×10^{-10}
^{234}U	High	1.4×10^{-9}	1.4×10^{-9}	2.9×10^{-9}	5.4×10^{-9}	4.0×10^{-9}	3.9×10^{-8}	1.4×10^{-9}	1.4×10^{-9}
^{235}U	High	1.3×10^{-9}	1.3×10^{-9}	2.6×10^{-9}	5.0×10^{-9}	3.7×10^{-9}	3.7×10^{-8}	1.3×10^{-9}	1.3×10^{-9}
	Low	1.3×10^{-10}	2.2×10^{-10}	4.8×10^{-9}	3.4×10^{-10}	9.6×10^{-10}	2.6×10^{-9}	1.2×10^{-10}	1.2×10^{-10}
^{238}U	High	1.2×10^{-9}	1.3×10^{-9}	2.5×10^{-9}	4.7×10^{-9}	3.6×10^{-9}	3.5×10^{-8}	1.2×10^{-9}	1.2×10^{-9}
	Low	2.6×10^{-10}	2.8×10^{-10}	1.7×10^{-9}	1.0×10^{-9}	3.2×10^{-9}	8.3×10^{-9}	2.6×10^{-10}	2.6×10^{-10}
^{237}Np	High	3.5×10^{-10}	4.1×10^{-10}	1.9×10^{-9}	4.3×10^{-9}	1.0×10^{-8}	2.7×10^{-7}	3.7×10^{-9}	3.5×10^{-10}
	Low	6.1×10^{-11}	1.4×10^{-10}	2.7×10^{-9}	3.1×10^{-10}	8.4×10^{-10}	1.4×10^{-8}	2.4×10^{-10}	5.7×10^{-11}
^{238}Pu	High	6.4×10^{-10}	7.1×10^{-10}	2.4×10^{-9}	7.8×10^{-8}	1.8×10^{-8}	3.7×10^{-7}	4.9×10^{-9}	6.4×10^{-10}
^{239}Pu	High	7.2×10^{-10}	7.8×10^{-10}	2.4×10^{-9}	8.6×10^{-8}	2.0×10^{-8}	4.1×10^{-7}	5.5×10^{-9}	7.2×10^{-10}
^{241}Am	High	7.6×10^{-10}	8.3×10^{-10}	2.5×10^{-9}	2.8×10^{-8}	1.5×10^{-8}	4.5×10^{-7}	8.7×10^{-9}	7.6×10^{-10}
	Low	1.9×10^{-11}	8.8×10^{-11}	1.9×10^{-9}	3.4×10^{-10}	2.2×10^{-10}	4.4×10^{-9}	9.9×10^{-11}	1.5×10^{-11}
^{244}Cm	High	3.4×10^{-10}	4.1×10^{-10}	2.2×10^{-9}	2.0×10^{-8}	1.0×10^{-8}	2.5×10^{-7}	4.8×10^{-9}	3.4×10^{-10}

[a] Doses from high-LET α- and low-LET β- and γ-radiation given separately; low LET not shown when < 1%

The values given for ^{3}H, inhaled as either $^{3}H_2O$ or organically bound, are for intakes as a vapour, with complete absorption to blood. The doses are therefore the same. For very soluble radionuclides such as ^{137}Cs and ^{131}I, the doses from ingestion are higher than those from inhalation, because the ingested radionuclide is assumed to be completely absorbed to the blood while a proportion of the inhaled material is immediately exhaled (see Table 102). For the least soluble radionuclides, including those of thorium, plutonium and other actinides, the doses are greater after inhalation because of their low absorption to blood from the intestine (see Table 103) and their longer retention in the lungs.

While a number of radionuclides, including ^{3}H, ^{14}C and ^{137}Cs, deliver similar doses to all tissues, many others deliver most of their dose to a small number of tissues. The radioiodines ^{125}I and ^{131}I deliver more than 99% of their dose to the thyroid gland. Ingested ^{90}Sr delivers most of its dose (> 90%) to bone surfaces and red bone marrow. Inhaled ^{90}Sr delivers about half of its dose to the lungs and half to bone surfaces and red bone marrow. For inhaled ^{89}Sr, because of its shorter half-life (50 days as compared with 29 years for ^{90}Sr), a greater proportion of the dose is delivered to the lungs. Similar considerations apply to doses from isotopes of radium and differences between ^{224}Ra (half-life, 3.6 days) and ^{226}Ra (half-life, 1600 years).

The presentation of committed doses conceals differences between the radionuclides in the temporal pattern of dose delivery, resulting from differences in their physical half-lives and their retention times in tissues. For example, doses from ^{3}H as $^{3}H_2O$ or organic matter are delivered in weeks or months because of their short retention times; doses from ^{89}Sr are negligible the first year after intake because of the short half-life of this radionuclide, while doses from ^{90}Sr and particularly from actinide isotopes such as ^{239}Pu and ^{241}Am are delivered over the entire 50-year integration period specified for calculation of the committed dose.

ICRP do not give dose coefficients for ^{222}Rn and its progeny; however, various estimates have been made of the doses delivered under various conditions of exposure. The doses to the lungs from exposure to radon and its progeny are usually expressed in terms of working-level months (WLM). The values given by Birchall and James (1994) and Marsh and Birchall (2000) allow calculation of the doses to the lung after inhalation of radon progeny as about 3×10^{-9} Gy/Bq ^{222}Rn after exposures in mines and about 5×10^{-9} Gy/Bq ^{222}Rn after domestic exposures. The higher value after domestic exposure is attributable to the larger contribution to the dose from unattached decay products. An analysis of the doses from ingested and inhaled radon gas suggests that the dose to the stomach dominates after exposure by ingestion, with a value of about 4×10^{-9} Gy/Bq, and that the red bone marrow may receive doses of approximately 6×10^{-11} Gy/Bq after ingestion and 2×10^{-11} Gy/Bq after inhalation (Khursheed, 2000).

4.1.24 *Models for the embryo and fetus*

ICRP task groups are developing models for embryos and fetuses; the approaches being used are described briefly below.

(*a*) *Dosimetry*

Implantation in the uterus occurs 2–3 days after the fertilized egg enters the uterus or about six days after fertilization. The implanted embryo is imbedded in the epithelial lining of the uterus and becomes closely surrounded by maternal tissue, the progressive erosion of this tissue constituting a source of nourishment for the embryo before development of the placenta. The period of organ formation in the embryo may be considered to last up to about the end of the second month, at which time the developing embryo still weighs less than 10 g. Because of the close apposition between the embryo and the uterine wall, it may be assumed, in the absence of more specific information, that the dose to the embryo up to the end of the second month of gestation can be approximated by the dose to the uterus.

The mass of the fetus increases rapidly from eight weeks to term at 38 weeks (birth). Calculation of doses to the fetus requires consideration of contributions from 'cross-fire' from penetrating radiation emitted in maternal organs and tissues and contributions from penetrating and non-penetrating radiations from radionuclides incorporated into the fetus. The absorbed fractions resulting from cross-fire from maternal tissues have been calculated for the fetus (average for all tissues) by the use of geometric 'phantoms' for the first, second and third trimesters of pregnancy. The doses to fetal organs and tissues from radionuclides retained in the fetus depend on the masses of the organs and tissues during the period of interest and the geometric relationships between them. Postnatal doses from activity retained in the fetus at term are also calculated.

Dose coefficients will be provided for maternal intakes by ingestion or inhalation, for long-term and single intake, before and during pregnancy for radioisotopes of the 31 elements considered in ICRP publications (ICRP, 1989, 1993c, 1995a,b).

(*b*) *Biokinetics*

For a number of radioactive elements, sufficient data for humans and animals exist to allow the development of specific models of transfer to the fetus. This applies principally to iodine and the alkaline earth elements. These models account for placental transfer throughout the fetal period and distribution within the fetus. A general approach has been applied to all other elements on the basis of limited data. The concentrations of the element in maternal and fetal tissues are compared, and doses are calculated from the ratio of the whole-body concentrations in the fetus and the mother (C_F/C_M).

For plutonium and related elements, C_F:C_M ratios are specified for each trimester. The distribution of activity between fetal organs and tissues is taken to be the same as that specified for three-month-old infants (ICRP 1989, 1993c, 1995a,b). The concentrations in the placenta may contribute doses to the fetus in some cases; the placental concentrations are specified as C_{Pl}:C_M ratios (Table 114).

Table 114. Ratios of concentrations of elements in the fetus and the mother (C_F:C_M) for intake before or during pregnancy

Element	Intake	
	Before pregnancy	During pregnancy
^{3}H	1.4	1.4
Cs	1	1
Pu	0.03	0.1/0.3/1[a]
Am	0.01	0.1

Modified from Stather & Phipps (1998)
[a] 1st, 2nd and 3rd trimesters

A model for the transfer of alkaline earth elements is being developed (Fell *et al.*, 1998), which takes into account information on: (i) calcium deposition in the human fetus, i.e. skeletal development; (ii) bidirectional flow of calcium and strontium across the placenta (in animals); (iii) the placental content of calcium and strontium (low); (iv) placental discrimination in transfer to the fetus (calcium > strontium > barium > radium); and (v) the following maternal changes during pregnancy: increased gut transfer of radionuclides, increased urinary excretion and increased bone turnover.

Table 115 gives examples of model predictions for strontium reaching maternal blood after ingestion or inhalation. The highest transfer to the fetus, in which 20% of total strontium reaches the maternal blood, occurs after intake late in pregnancy (36

Table 115. Intakes of Sr before conception or during pregnancy

Time of intake	Retention at term (%) in maternal blood		
	Fetus	Mother	C_F:C_M[a]
1 year before conception	0.1	11	0.1
8 weeks	0.3	9	0.5
12 weeks	0.7	9	1
24 weeks	3	16	3
30 weeks	7	18	6
36 weeks	14	26	9
8–38 weeks[b]	5	16	5

From Fell *et al.* (1998)
[a] Fetus:mother whole-body concentration ratio at term
[b] Long-term intake

weeks) and corresponds to a C_F:C_M ratio of 9. For calcium, the corresponding C_F:C_M ratio at 36 weeks is 19. Preliminary estimates of the doses after intake of ^{90}Sr at this late stage of pregnancy gave committed doses, to e.g. the red bone marrow, in offspring that are twice those for adults.

4.1.25 *Studies of decorporation (chelation)*

Several physical methods and a number of pharmacological approaches have been developed for the removal of radioactive materials from the body. The physical approaches include, for example, the use of lung lavage to remove recently inhaled material (Muggenburg *et al.*, 1977). The pharmacological approaches include the use of diuretics to promote urinary excretion and administration of chelating compounds. Various chelating compounds have been described, and their use has been extensively reviewed (Levine, 1979; Bulman, 1990). The presumption in most of these studies is that the likelihood of adverse effects will decrease by accelerating the removal of the radioactive material from the body. Thus, the 'efficacy' of a particular treatment or therapeutic strategy is based on its ability to reduce the body burden of the radioactive material in comparison with that in untreated controls. Table 116 lists some of the compounds used in 'decorporation' studies, the animal model used and the radionuclide targeted.

4.2 Toxic effects

4.2.1 *Deterministic effects*

Incorporated radionuclides may induce a wide variety of deterministic effects in irradiated tissues and organs. These effects, which may be related to carcinogenesis in some tissues (see, e.g., Lord *et al.*, 1991) and may precede neoplasia (Ober *et al.*, 1994), were formerly referred to as non-stochastic effects. Deterministic effects may arise in any tissue or cell system, provided that the radiation dose is sufficient to initiate the necessary cellular and matrix changes. The effects produced by incorporated radionuclides are, in most respects, qualitatively similar to those produced by external irradiation from X-rays, γ-rays and neutrons, although the latter effects are better understood, partly because of the vast experience accumulated from the medical uses of radiation for radiotherapy (see, e.g., Withers, 1986, 1989; IARC, 2000).

Deterministic effects result from cell damage or cell death and give rise to impairment of tissue function. Within any tissue or organ, the effect may be either the direct result of irradiation of component cells or an indirect effect of damage to vascular components. With respect to the latter, damage to blood vessels may be particularly important (Field & Upton, 1985). The extent of tissue damage is a function of the number of damaged cells, and a large number is frequently required to produce the effect (UNSCEAR, 1993). The minimum radiation dose needed to cause enough cellular damage to have an effect is usually more or less well defined and is referred to

Table 116. Studies of 'decorporation' (chelation)

Chelating compound tested	Species	Route of administration	Nuclide(s)	Reference
N-(2,3-Dimercaptopropyl)phthalamidic acid; meso-dimercapto-succinic acid	Rat	Injection	^{210}Po	Bogdan & Aposhian (1990)
2,3-Dimercaptopropanol; *N*,*N*′-diethylamine-*N*-carbodithioate (diethyldithiocarbamate); *N*-(2,3-dimercaptopropyl)phthalamidic acid	Rat	Injection	^{210}Po	Rencova *et al*. (1993)
N,*N*′-Diethylamine-*N*-carbodithioate (diethyldithiocarbamate); *N*,*N*′-di(2-hydroxyethyl)ethylenediamine-*N*,*N*′-biscarbodithioate	Rat	Injection	^{210}Po	Rencova *et al*. (1995)
Alginate	Mouse	Oral	^{226}Ra	Schoeters et al. (1983)
Zn-Diethylenetriaminepentaacetic acid; Ca-diethylenetriamine-pentaacetic acid	Rat	Injection	^{239}Pu, ^{241}Am, ^{242}Cm	Seidel & Volf (1972)
Zn-Diethylenetriaminepentaacetic acid	Mouse	Injection	^{239}Pu	Jones *et al*. (1986)
N^1,N^5,N^{10},N^{15}-Tetrakis(2,3-dihydroxy-4-carboxybenzoyl)tetra-azatetradecane; diethylenetriaminepentaacetic acid (Ca or Zn salt); desferrioxamine	Mouse, rat, hamster	Injection, oral	^{238}Pu, ^{241}Am	Volf (1986)
N^1,N^5,N^{10},N^{15}-Tetrakis(2,3-dihydroxy-4-carboxybenzoyl)tetra-azatetradecane	Mouse, rat, baboon	Injection	^{238}Pu, ^{239}Pu	Gerasimo *et al*. (1986)
Diethylenetriaminepentaacetic acid (Ca or Zn salt)	Rats	Injection, oral	^{238}Pu	Sullivan & Ruemmler (1986)
N^1,N^5,N^{10},N^{15}-Tetrakis(2,3-dihydroxy-4-carboxybenzoyl)tetra-azatetradecane; *N*,*N*′,*N*″,*N*‴-tetra(2,3-dihydroxybenzoyl)-spermine	Mouse	Injection	^{239}Pu	Szot *et al*. (1986)
N^1,N^5,N^{10},N^{15}-Tetrakis(2,3-dihydroxy-4-carboxybenzoyl)tetra-azatetradecane	Mouse	Injection	^{239}Pu	Durbin *et al*. (1989)

Table 116 (contd)

Chelating compound tested	Species	Route of administration	Nuclide(s)	Reference
Zn-Diethylenetriaminepentaacetic acid; Ca-diethylenetriamine-pentaacetic acid	Dog	Injection	^{239}Pu	Bruenger *et al*. (1991b)
Hydroxypyridinone desferrioxamine; dihydroxamic diethylene-triaminepentaacetic acid; diethylenetriaminepentaacetic acid (Ca or Zn salt)	Rat	Injection	^{238}Pu, ^{241}Am	Stradling *et al*. (1991)
Linear hydroxypyridinone derivative, $C_{34}H_{34}O_{12}N_8Na_4$; di-ethylenetriaminepentaacetic acid (Ca or Zn salt)	Rat	Injection	^{238}Pu, ^{241}Am	Stradling *et al*. (1992)
Diethylenetriaminepentaacetic acid (Ca or Zn salt)	Dog	Injection, infusion	$^{238}Pu(NO_3)_4$	Guilmette & Muggenburg (1993)
Docosyltriethylenetetraminepentaacetic acid	Rat	Oral	^{239}Pu	Miller *et al*. (1992)
Docosyltriethylenetetraminepentaacetic acid	Rat	Oral	^{239}Pu, ^{241}Am	Miller *et al*. (1993)
Zn-Diethylenetriaminepentaacetic acid	Rat	Oral	^{238}Pu, ^{241}Am	Gray *et al*. (1995)

as the 'threshold dose' (Field & Upton, 1985). With increasing radiation dose, both the fraction of damaged cells and the severity of the effect increase (UNSCEAR, 1982). This contrasts to radiation-induced cancer, in which the frequency of the effect is a function of radiation dose but the severity is independent of dose. The effects of radiation may be greater in children, whose tissues and organs are rapidly growing, than in adults. In contrast, the severity of the effect decreases with dose protraction; this is attributed to the increased time available for either repair of cell damage or tissue repopulation by undamaged cells (ICRP, 1984). Dose–response curves established for deterministic effects are sigmoidal (UNSCEAR, 1993). The deterministic effects of radiation may be enhanced by administered drugs, e.g. in radiotherapy patients who receive chemotherapy (Withers, 1986). Other factors that affect the expression of deterministic effects include oxygen tension, hormone status, temperature, sex, stress and trauma (Hall, 1978; Elkind, 1980; UNSCEAR, 1982, 1993).

Deterministic effects are commonly initiated by radiation damage to the stem cells within a tissue. As a result, the number of maturing cells and mature, functional cells recruited by the irradiated tissue is reduced, leading to impaired tissue function. The time of onset of overt tissue damage is commonly a function of the cell kinetics, the adverse effects being produced faster in tissues with a rapid turnover of cells, such as the bone marrow, than in tissues and organs comprising cells with long cycle times, such as the liver (Coggle, 1983).

Deterministic effects of radiation are found at the sub-cellular, cellular, tissue and whole-body levels. The most serious short-term effect of radiation is acute radiation syndrome, which leads to death. Smith and Stather (1976) estimated that the radiation dose that leads to a 50% rate of death within 60 days ($LD_{50/60}$) in humans is 3.5 Gy, with a threshold dose of 2 Gy; in the absence of substantial medical intervention, the mortality rate after whole-body exposure to 4 Gy or more is nearly 100%. External, whole-body doses exceeding 100 Gy result in cerebral and cardiovascular death — usually within two days. The effects include nausea, vomiting, diarrhoea, headache, erythema, disorientation, agitation, ataxia, weakness, somnolence, coma, convulsions, shock and finally death. At doses exceeding 20 Gy, death normally results from gastrointestinal complications within two weeks. At doses of 3–4 Gy, death is delayed, perhaps for a month, and is caused by bone-marrow ablation. The effects include nausea, vomiting, diarrhoea, weakness, fatigue, anorexia, fever, haemorrhage, epilation and, without medical intervention, death (Young, 1987).

Acute radiation syndrome is unlikely to result from internal deposition of radionuclides. In contrast, specific tissues and organs may be damaged after exceptional intake of some radionuclides, in particular when they are deposited in a single organ or a small volume of tissue. Experience with the use of radiotherapy (Rubin & Casarett, 1972) suggests a wide range of threshold doses for effects in different tissues and organs, depending on the radiosensitivity of the tissue and the age of the subject. The thresholds for effects in different tissues are given by ICRP (1984). At tissue doses of 5–10 Gy, bone-marrow hypoplasia and permanent sterility of the ovary may occur

in 25–50% of irradiated patients. At tissue doses of 10–30 Gy, the threshold doses for cataract of the lens, permanent sterility of the testis and siderosis of the kidney may be exceeded. The threshold doses for liver failure, pneumonitis and fibrosis of the lung, nervous tissue necrosis and skin ulceration lie in the region 40–70 Gy. Effects such as bone necrosis and fracture, hypothyroidism and hypopituitarism occur when the local tissue dose exceeds 100–300 Gy. In children, the threshold doses for deterministic effects are generally lower: 15 Gy for failure of breast development, 30 Gy for arrested growth and 40–50 Gy for muscle hypoplasia (ICRP, 1984).

In other mammalian species, the $LD_{50/30}$ varies from about 2 Gy in pigs to 7 Gy in rats, 8 Gy in rabbits and 10 Gy in gerbils.

The radiation-induced effects at the cellular level include DNA strand breakage and micronucleus formation, which are reviewed in section 4.4.

4.2.2 *Effects on specific tissues and organs*

Of the wide range of deterministic effects described above, relatively few are important under most conceivable conditions of human exposure to internally deposited α- and β-emitting radionuclides, since most radionuclides tend to be deposited in a limited range of tissues within the body. Only the Group I metals, such as sodium and caesium, and some non-metals, such as the noble gases and hydrogen, are relatively uniformly distributed in the body and capable of delivering a similar radiation dose to a wide range of tissues, approximating whole-body irradiation. For this reason, the deterministic effects of, e.g., ^{137}Cs, an important component of fall-out resulting from nuclear fission, have generally been assumed to be deducible from observations made following exposure to similar doses of whole-body external X-radiation (McClellan *et al.*, 1962; McClellan & Bustad, 1964).

Studies with $^{3}H_2O$ in mice suggest that β-radiation from tritium is more damaging to spermatogonia than γ-rays from ^{137}Cs. Similarly, enhanced effects were seen in assays of post-implantation death of mouse embryos and injury to haematopoietic tissue, suggesting that the biological effectiveness of ^{3}H relative to that of external γ-rays is 2–6 (Balonov *et al.*, 1993). Care must therefore be exercised in extrapolating data collected after external exposures to electromagnetic radiations to exposures from more or less uniformly distributed internally deposited radionuclides.

After their intake, metals other than those in Group I and some non-metals become located in a limited number of tissues as they follow the metabolic pathways for use of essential elements (Priest, 1990). For example, radium and strontium, which are alkaline earth elements, follow the metabolic pathways of calcium and are deposited almost exclusively in the skeleton. Therefore, the radionuclides of these elements are likely to substantially irradiate only skeletal tissues. The lanthanide isotopes and most actinide isotopes are deposited in the skeleton, but these nuclides also attach to iron transport proteins and are deposited to variable extents in the liver, spleen and bone marrow. As a consequence, internal exposure to such radionuclides could result in

deterministic effects within a wider range of organs (ICRP, 1984; UNSCEAR, 1993). The inhalation of radioisotopes — either as radioactive gases or as particles — results in irradiation of tissues within the respiratory tract. In a review (Park *et al*., 1997) of the outcome of life-span studies in beagle dogs that inhaled ^{238}Pu (an α-particle emitter), a wide range of deterministic effects was seen in many tissues of animals that had received an initial lung deposit in excess of 3 kBq. The effects included radiation pneumonitis, osteodystrophy, hepatic nodular hyperplasia, lymphopenia, neutropenia, sclerosing tracheobronchial lymphadenitis, hypoadrenocorticism and increased activity of serum alanine aminotransferase, which is indicative of liver damage.

Another important difference between exposure to external electromagnetic radiation and to internal radiation from deposited radionuclides is the temporal distribution of radiation dose. Under most circumstances, the deterministic effects after X- and γ-irradiation were quantified after exposure to high doses, either at a single exposure or to relatively few fractionated doses (Withers, 1986). In contrast, incorporated radionuclides may deliver their radiation dose over periods of days or years. Under such circumstances, the tissue repair processes may be more effective, and the doses required to produce the same effect under various exposure conditions may be quite different. The many models used to quantify the relationship between the extent of tissue damage, total dose, dose protraction and overall duration of exposure have been described by ICRP (1984).

The deterministic effects that have been observed in a limited but important range of tissues are described below. The most important target tissues are often the lungs, lymph nodes and liver for inhaled radioactive particles, and the liver, bone and bone marrow and thyroid for dissolved deposits. All ingested radionuclides may irradiate the gastrointestinal tract.

(*a*) *Bone*

Deterministic effects of radionuclides incorporated within the human skeleton were first described in the late 1920s after observation of osteonecrosis and fractures in the bones of dial painters who had ingested the α-particle-emitting radionuclide ^{226}Ra (Martland *et al.*, 1925; see also section 1.2.2(*k*)). The first author also described changes in blood and radiation-induced bone cancers (Martland, 1926, 1931). Since then, studies of the skeletons of dial painters and of experimental animals exposed to α-particle-emitting, bone-seeking radionuclides have demonstrated many radiation-induced changes, including areas of bone sclerosis, abnormally large resorption cavities and blocked haversian canals within individual osteons (Rowland & Marshall, 1959; Taylor *et al*., 1965). Other disparate effects observed after intakes of both ^{226}Ra and ^{239}Pu include significant peritrabecular fibrosis (Jee *et al*., 1969) and the formation of a fibrotic layer between the mineralized endosteal bone surface and marrow cells (Lloyd & Henning, 1983), as seen in a female radium-dial painter with osteosarcoma 60 years after intake of ^{226}Ra. The layer was up to 50 μm in depth and occurred at a cumulative skeletal dose of 66 Gy. The filling of osteocyte lacunae, the presence of

hypermineralized osteons and new regenerative bone production have also been described after contamination with radium (Pool *et al.*, 1983).

Radiation 'osteitis', 'osteodystrophy' and 'osteodysplasia' are terms used to describe the entire spectrum of radiation-induced disturbances to the remodelling mechanism of bone tissue. The process is characterized by areas of bone infarction, i.e. bone necrosis, vascular damage, peritrabecular fibrosis and new bone formation (Stover & Jee, 1963). A proliferative fibro-osseous response is frequently seen in the marrow. This response resembles those seen in the active phases of Paget disease and fibrous dysplasia. The pathology of these deterministic bone lesions has been described in detail in persons exposed to ^{226}Ra. Lisco (1956) published a detailed analysis of the bone lesions in a dial painter with a fibrosarcoma of the ischium. Osteodysplasia was distributed widely in many of the non-tumorous bones available for study. It was postulated that the tumour developed at a site of radiation-induced skeletal damage, presumably originating from the cells of the peritrabecular fibrous tissue.

Radiation osteodysplasia has been studied extensively in mice treated with the short-lived radionuclides ^{224}Ra and ^{227}Th (Gössner *et al.* 1976; Gössner, 1986). A comprehensive survey of the literature on the pathology of deterministic radiation effects on bone has been published (Luz *et al.*, 1991).

Simmons and Holzmann (1983) and Morgan *et al*. (1983) showed that the severity of deterministic effects, within a dose range to 200 Gy, is a function of average skeletal α-particle dose, with a threshold of approximately 1 Gy. It has been claimed that in ultrastructural studies of ^{238}Pu-injected mice the changes were somewhat analogous to those that occur in ageing (Mohelská *et al*., 1988), the primary effect on bone cells being cell hypertrophy and destruction of endosteal cell organelles, followed by deformation and hypertrophy of osteocytes and then abnormalities in osteocyte self-burial and abnormal formation of bone tissue structure.

In all species, α-particle emitters have been shown to produce different effects in cortical and trabecular bone — a function of the different structures and turnover of these tissues. A landmark study on the effects of ^{239}Pu on dog bone was published by Jee (1972). A wide variety of histopathological lesions was induced by a single intravenous injection of ^{239}Pu (IV) citrate in beagles. The changes noted in trabecular bone were cell death, including the death of osteoblasts, osteocytes and osteoclasts; a reduction in cell division and DNA synthesis in bone cells; endothelial cell damage and reduced marrow-space vascularity; suppressed bone resorption in regions of high plutonium concentration; atypical bone formation, with mosaic trabeculae of woven bone, growth arrest lines and peritrabecular fibrosis; bone necrosis with spontaneous microfractures; altered bone remodelling rates; and pathological bone resorption and osteosarcoma. In cortical bone from the same dogs, haversian canal plugging and inhibited canalicular transport were observed, as well as excessive bone resorption, decreased vascularity, osteocyte death, atypical bone formation, endothelial cell depletion and, in rare cases, osteosarcoma. In an earlier paper (Jee *et al*., 1962), it was suggested that these effects are attributable not only to the direct interaction of α-

particles with bone cells but also to indirect effects caused by radionuclide- and dose-dependent radiation damage to both small and larger blood vessels within the bone marrow. The attribution of effects to these different causes is problematic, but both are likely to be important. The lowest average skeletal doses that produced significant vascular depletion were reported to be 23 Gy for ^{226}Ra, 3.5 Gy for ^{239}Pu, 5 Gy for ^{228}Ra and 2.5 Gy for ^{238}Th. In contrast, vascular effects were not seen up to a dose of 80 Gy in dogs injected with the β-particle emitter ^{90}Sr. The differences in the toxicity of these radionuclides is a consequence of their different deposition patterns or those of their progeny and, hence, dose distribution within the skeleton (Priest, 1990), and of the lower toxicity of β-particle-emitting isotopes. Momeni *et al.* (1976) used X-ray radiography to visualize endosteal and periosteal cortical sclerosis and thickening, osteolytic lesions, fractures and trabecular coarsening in beagles that were fed diets containing ^{90}Sr chloride in equilibrium with ^{90}Y or were given ^{226}Ra chloride by repeated injections. The α-particle-emitting alkaline earth isotope ^{226}Ra was estimated to be five to six times more toxic than the β-particle-emitting alkaline earth isotope ^{90}Sr and its co-located decay product ^{90}Y. Effects were seen with the β-particle emitters only when the skeletal dose rate exceeded 0.025 Gy per day.

Changes such as those described above, other than the occlusion of vessels, have also been seen in mice after administration of the short-lived, α-particle-emitting isotopes ^{227}Th and ^{244}Ra (Müller *et al*., 1978) and after injection of ^{241}Am (Nilsson & Broomé-Karlsson, 1976). In the latter study, the effects that were not seen in dogs included a reduction in the numbers of nucleated cells in the epiphyseal growth plate cartilages, loss of a substantial fraction of the long-bone metaphyseal trabeculation and a reduction in longitudinal bone growth. Growth retardation has also been demonstrated in rabbits injected with ^{224}Ra and in boys and girls injected with this short-lived, α-particle-emitting isotope for the treatment of tuberculosis during the period 1946–50. Significant growth inhibition was observed among these children at a mean skeletal dose of 3–25 Gy (Spiess *et al*., 1986). Hitchman and colleagues (1978) failed to reproduce this effect in three-month-old dogs that had been given 2.86 μCi (106 kBq)/kg bw ^{239}Pu, perhaps because plutonium, unlike radium, does not concentrate in the mineralizing zones of the epiphyseal growth plate cartilages.

Given the relatively low doses at which skeletal deterministic effects were observed in animals injected with the bone surface-seeking radionuclides ^{239}Pu and ^{241}Am, efforts were made to see if similar effects could be demonstrated in humans. In a recent publication (Stebbings, 1999), radiographs prepared from the bones of five persons injected with 11–15 kBq ^{239}Pu in the years 1945–47 were described. Two of these subjects died within two years of injection, but the others survived for 29–47 years. The cumulative skeletal doses calculated up to 1977 were 0.04–0.05 Gy and 0.81–1.4 Gy, respectively. Although the underlying disease complicated the analysis, bony changes were seen in all cases, and changes attributable to the plutonium were suspected for three subjects, comprising two cases of osteoporosis and non-specific degenerative changes associated with hip and vertebral fractures and areas of increased bone density. At a

higher dose of internal α-radiation, pathological changes resembling those described in dogs were found in the bones of a worker at the Hanford nuclear weapons plant who had been extensively contaminated with ^{241}Am (Priest *et al*., 1995b). When he died 11 years after his accident from a pre-existing heart condition, his skeleton contained an estimated 500 kBq of radionuclide — an amount that had already been much reduced by decorporation therapy from an initial estimated intake of 185 MBq. The bones examined were the patella, clavicle, sternum, rib, vertebral body and ossified thyroid cartilage; all showed evidence of radiation damage. The cellularity of most bones was reduced, and little evidence of recent active bone remodelling was seen in any bone other than the vertebra, as concluded from the redistribution of the americium in the vertebral body. In several bones, the architecture was disrupted, with woven bone, abnormal appositional bone deposits, bizarre trabecular structures and marked peri-trabecular fibrosis. Growth arrest lines were common. When compared with trabecular bone modelling, that of cortical bone in the rib appeared less disrupted. Overall, the results obtained are consistent with those observed in dogs (Jee, 1972) at a similar level of actinide intake.

Sharpe (1983) discussed the possibility that the bone infarcts and osteonecrosis seen in radium-dial painters might be linked to the production of bone cancers. While such links have not been confirmed, it is clear from the above, given the low dose thresholds for some effects and the apparent 10-Gy threshold for osteosarcoma induction by ^{226}Ra in humans (Rowland, 1997), that osteosarcoma is unlikely to develop in bone that is free of radiation-induced damage.

Benign radiation-induced tumours such as osteochondromas (cartilagenous exostosis) usually arise in growing bone and cartilage. They are recorded in children who have received external irradition and have also been observed in children and adolescents treated with ^{224}Ra for osteoarticular tuberculosis. Spiess and Mays (1979a) reported 55 exostoses in 28 of 218 children and adolescents treated with this radio-nuclide. These lesions developed in the long bones, usually at sites where the growing metaphysis had been irradiated. This suggests that disturbance of normal skeletal growth was the mechanism of induction. Patients who were younger at the time of injection of ^{224}Ra had a higher incidence of exostosis. None of these radiation-induced exostoses has been reported to have become malignant, although 36 of the 218 children developed bone sarcomas elsewhere in the skeleton.

(*b*) *Teeth*

Several publications have described the effect of ^{226}Ra intake on teeth. The term ‘radium jaw’ was coined by Blum (1924) to describe the tooth loss that was common in the radium-dial painters. Radiation damage to either dental tissues or to their blood supply initiates excessive resorption of dentine, particularly around the gum line, causing teeth to break with a minimum of trauma. In the dial painters, one tooth after another broke until all were lost. A similar loss was seen in young persons injected with the short-lived α-particle emitter ^{224}Ra (Sonnabend *et al*., 1986). This effect was

greatest (20–30%) in patients injected at the age of 16–21 years, with much lower frequencies at earlier and later ages of administration. Radiation-induced tooth loss has also been described in beagles injected with either ^{226}Ra or ^{239}Pu (Jee & Arnold, 1960) and in mice injected with ^{224}Ra (Humphreys *et al*., 1985). Robins (1990) described osteopenia equivalent to radium jaw in mice after injection of > 16 kBq of ^{224}Ra.

(*c*) *Eye*

Cataracts were described in 119 of 813 women who were radium-dial painters before 1930 (Adams *et al*., 1983) and in 58 of 831 patients injected with high doses of ^{224}Ra (Chmelevsky *et al*., 1988). In the dial painters, latency was negatively correlated with accumulated radiation dose; in the ^{224}Ra-injected patients, the incidence was dose-dependent, with a 14% incidence in those patients receiving the highest doses of radium and an overall mean of 5–6% for patients injected either as children or as adults (Stefani *et al*., 1986). In this population, about 55–60% of the cataracts were thought to be associated with irradiation; the remainder were accounted for by the normal age-related incidence. By plotting incidence against dose, Chmelevsky *et al*. (1988) concluded that the incidence increased either as a function of the square of the dose or linearly with dose, with an intake threshold of 0.5 MBq/kg. Griffith *et al*. (1985) described the case of a worker who had been potentially exposed to external β- and γ-radiation and had possibly ingested or inhaled plutonium and other radionuclides. In three known incidents, his face had been contaminated with plutonium, some of which must have reached the bloodstream. After 24 years of work, the man had developed impaired vision due to cataract. The estimated radiation dose to the eye — measured by external dosimeters — was ~ 0.8 Sv, which is below the threshold for this effect derived for γ-radiation in the atomic bomb survivors, 1.1–1.5 Gy (Griffith *et al.*, 1985; see also Otake & Schull, 1990). The authors noted that ^{239}Pu concentrates in the iris of dogs and suggested that the cataract seen in this worker may have arisen because of the additional dose of α-particles from his internal body-burden of ^{239}Pu, which was 2 nCi (74 Bq).

(*d*) *Skin*

Unlike in most other tissues, damage to the skin from α- and β-particle-emitting radionuclides generally results from energy released by externally deposited radionuclides, rather than internally deposited isotopes; these external sources may be in the form of diffuse deposits or hot particles. Experimental studies on pig and mouse skin show that the effects produced are dependent on the range of the radiation emission, the area of skin irradiated, the thickness of skin and the degree of skin penetration achieved by the radionuclide (Hopewell *et al*., 1986, 1993). For example, application of sources of ^{90}Sr, ^{170}Tm and ^{147}Pm (high-, medium- and low-energy β-particle emitters, respectively) to the skin of pigs (thick) or mice (thin) *in vivo* resulted in a range of deterministic effects, varying from slight breakdown of the most superficial

layers of the epidermis, produced by high doses from ^{147}Pm, to extensive local damage and moist desquamation, produced at ~ 28 Gy from large-area ^{90}Sr sources (22.5 mm in diameter). To produce the same effect with thulium, 80 Gy of β-radiation from this radionuclide were required. The thresholds for acute tissue breakdown due to the larger-diameter sources of β-radiation were 17 Gy for ^{90}Sr/^{90}Y and 30 Gy for ^{170}Tm. In contrast, the threshold for less serious epidermal necrosis after irradiation by ^{147}Pm was 150 Gy. This is important, since exposure of 50% or more of the total body surface led to death of Chernobyl liquidators due to skin desquamation and subsequent infection when the doses to the skin exceeded 30 Gy for penetrating β-radiation and 200–300 Gy for less energetic emitters (Barabanova & Osanov, 1990).

Similar dermal effects of radionuclides deposited externally were seen in Marshall Islanders exposed to fall-out rich in β-particle emitters from a thermonuclear explosion at Bikini atoll in March 1954 (Cronkite *et al*., 1995; see section 1.1.1(*c*)(ii)). On Rongelap atoll, where the exposure was greatest (~ 1.9 Gy total dose of γ-radiation in air), hair loss and hyperpigmentation of the skin were seen. Some of the skin lesions were painful. The lesions were initially small but gradually coalesced. Later, scaly, dry desquamation proceeded from the centre of each lesion, and the skin colour was lost. Repigmentation began in the central region of each lesion and spread, so that after a few weeks the skin appeared normal. In some more severe cases, lesions, mostly on the head and neck, became necrotic with moist desquamation, followed by ulceration. By three months after the explosion, the hair had begun to grow, and within 6–12 months the skin had returned to normal.

(*e*) *Liver*

The deterministic effects of radionuclides deposited in the human liver have been studied extensively in patients who received the radiographic contrast agent Thorotrast and in animals given a variety of α- and β-particle-emitting isotopes. Thorotrast is a colloidal preparation of $^{232}ThO_2$, which, after its injection into the bloodstream, is deposited within reticuloendothelial organs, principally the spleen, liver and bone marrow. Thorotrast is essentially insoluble and continues to irradiate tissues at all times after its entry (radioactive half-life, 1.1×10^{10} years), with a mixture of α- and β-emissions from ^{232}Th and its progeny (principally ^{228}Ra, ^{228}Th and ^{230}Th).

A special study was made of one Thorotrast patient who died from myelodysplasia in June 1989, 36 years after receiving an injection of Thorotrast, and who donated her body to the Transuranium and Uranium registries in the USA. At autopsy, the patient's liver was found to contain 44.3% of the whole-body ^{232}Th content, and the estimated accumulated radiation dose received by this organ was 15 Gy (Kathren & Hill, 1992). The liver was of normal weight, but its exterior appearance was characterized by widespread, reticulated capsular fibrosis (Graham *et al*., 1992). Histological examination showed generalized, moderate, subcapsular and portal fibrosis. The hepatocytes were iron-loaded — a consequence of multiple blood transfusions. Foci of mild hepatocellular dysplasia, occasionally associated with intranuclear inclusions, were identified.

Focal extramedullary haematopoiesis, consistent with myelodysplasia, was present. No neoplasm was identified.

Liver cirrhosis was seen in groups of Japanese patients 20 years after receiving Thorotrast, and the incidence rose to 10% after 36 years (Mori *et al*., 1983). Cirrhosis occurred at organ dose rates of 0.15–0.6 Gy/year, with a mean cumulative dose of 9.5 Gy (Kato *et al*., 1983). In an equivalent group of 2326 German patients with a longer latency since injection (approaching 50 years), the incidence of cirrhosis was somewhat higher at 12.6% (van Kaick *et al*., 1989). However, no cirrhosis was present in the liver of the whole-body donor.

Moroz and Parfenov (1972) summarized changes in the livers of 10 children and four adolescents exposed accidentally to polonium from polonium–beryllium sources. The amounts of polonium deposited in these two groups ranged from 18.5 kBq to over 370 kBq. Transitory changes were observed in liver function, and decreased numbers of leukocytes and platelets was seen during the first few months after exposure.

In dogs injected with the α-particle emitter ^{239}Pu at doses ranging from 0.0168 to 2.9 μCi (620 Bq–107 kBq)/kg bw, hepatic-cell necrosis was a consistent finding. However, regeneration was sufficient to maintain the normal liver mass in all dogs except those that received the highest dose. Changes in the distribution of plutonium produced by regeneration were seen at lower doses and at average cumulative doses to the liver ≤ 0.8 Gy. The dogs given the highest dose of plutonium showed severe centrilobular degeneration and fibrosis (Taylor *et al*., 1972b).

In dogs that had inhaled ^{238}Pu, the initial lung was 3–200 kBq, which, after translocation of the radionuclide, gave rise to doses of α-radiation ≤ 3.4 Gy to the liver. Effects were seen at doses below those at which cancer was induced. At necropsy, the most consistent change was an increased incidence of adenomatous hyperplasia as the dogs aged. These regions were typically 20–30 mm in diameter, well-circumscribed and pale grey, with cells that were enlarged and often distended with glycogen or cytoplasmic vacuolation. Excess activity of liver enzymes, including alanine aminotransferase and alkaline phosphatase, in serum indicated liver damage. The increased activity was positively correlated with the dose rate and the cumulative dose of α-radiation (Weller *et al*., 1995c).

Similar changes were described in the livers of mice given 8 or 16 μCi (300 or 600 kBq)/kg bw, i.e. high doses, of ^{241}Am (Nilsson & Broomé-Karlsson, 1976) and in dogs given 6 mg/kg bw (corresponding to 150 kBq) of ^{237}Np citrate (Mahlum & Clarke, 1966). In the mice, slight periportal fibrosis was a common finding. The parenchymal changes varied from changes in cell nuclei to extensive fatty degeneration, multiple focal necroses and microabscesses. Binucleate cells were increased in number. In a significant number of mouse livers, the epithelial cells of the bile canaliculae were swollen, and multi-focal proliferations of these structures were seen. In the dogs, the liver damage comprised cloudy swelling of tissues, fatty degeneration, lobular necrosis, biochemical changes and a marked increase in the number and size of the littoral cells. Given the low specific activity of ^{237}Np, the effects seen might have been

due to the chemical toxicity of the compound rather than the emitted radiations (Boulahdour & Galle, 1998).

Hepatic changes have also been described after intake of β-particle-emitting radionuclides. Injections of $^{144}Ce/^{144}Pr$ at 0.33–0.42 mCi (12.2–15.5 MBq)/kg bw to sheep produced effects in the liver similar to those described above, including cell swelling and death. The hepatocyte nuclei were clearly damaged by nine days after injection, when the effects were most frequent near the central veins of the lobules; later, all parts of the lobule were affected. By 18–24 days after injection, foci of necrotic fibril material containing gram-positive pleomorphic cocci were seen, which resulted in terminal bacteraemia (Sullivan *et al.*, 1969).

Hepatic effects have also been seen in dogs that inhaled ^{144}Ce at doses resulting in long-term body burdens of 0.096–13.3 MBq/kg bw. In the dogs that lived longer than five years after treatment, the radionuclide produced an average cumulated dose of β-radiation to the liver of 60 Gy per MBq ^{144}Ce/kg bw. The animals survived 1–10 years after exposure. Hepatocellular degeneration or hepatic atrophy with fibrosis and hepatic failure was either the primary cause of death or contributed to death in 18 animals that received cumulative doses of 6.4–210 Gy. It may be recalled that 10 animals died from liver tumours after exposure to doses over the same range (10–240 Gy) (Hahn *et al.*, 1995).

(*f*) *Haematopoietic bone marrow*

The effects of α-radiation on the bone marrow of humans and of α- and β-radiation on the bone marrow and stem cells of animals have been studied extensively. In the patient who died in 1989 and donated her body to the Transuranium and Uranium registries in the USA (see section *e*, above), the estimated accumulated dose of radiation to the skeleton and bone marrow from a Thorotrast injection given 36 years previously was 3.9 Gy (Kathren & Hill, 1992). A bone-marrow biopsy from the iliac crest *post mortem* showed failure of cell maturation beyond the myelocyte stage of the granulocytic series, with virtually no polymorphonuclear cells. Blasts and pro-myelocytes comprised about 15% of all the elements. Erythropoiesis was virtually absent. Megakaryocytes were present in adequate numbers but were often mononuclear or binuclear. No significant fibrosis was seen. The authors concluded that the findings in bone marrow were compatible with refractory anaemia with an excess of blast cells. Consistent with this diagnosis was the presence of extramedullary haematopoiesis in the liver.

Similar changes were described in the bone marrow of the worker in Hanford who had been contaminated with ^{241}Am (Priest *et al.*, 1995b). The bone marrow of this patient had been substantially damaged by α-irradiation from americium, principally on the bone surfaces. A common finding was a marked decrease in bone marrow cellularity associated with dilatation of blood sinusoids. The severity of these effects varied according to site and was greatest in the vertebral body, where the marrow was almost acellular, and least in the clavicle. In addition, extensive peritrabecular marrow

fibrosis was present in some bones, including the rib and clavicle. As noted above, fibrosis is a common observation in bones irradiated by bone-seeking radionuclides and has been linked to bone sarcoma induction (Rowland, 1994).

Aplastic anaemia resulting from α-irradiation by Thorotrast was seen at high frequency in all groups of Thorotrast-treated patients studied (Mole, 1986), although at a slightly lower frequency than that of leukaemia. However, the bone marrow of all the patients was damaged. Similarly, slight or severe bone marrow damage was recorded in 38% of patients given 10 MBq or more of the short-lived α-particle emitter ^{224}Ra more than 5 years previously (Arnold & Weber, 1989).

Male Wistar rats were given a single intravenous injection of $^{239}PuO_2$ (particle size, 1–2 μm) at a dose of 23.2, 46.3 or 92.5 kBq/kg bw, and the numbers of cells in the bone marrow and peripheral blood were analysed for 365 days at different times after injection. Dose-dependent impairment of haematopoiesis was observed, with an increase in the number of lymphocytes and a reduction in the pool of maturating granulocytes in the bone marrow. The number of circulating lymphocytes was decreased for a long time after injection (Murzina *et al.*, 1988).

In dogs, ^{239}Pu-labelled macrophages accumulated in the bone marrow at lower injected doses of plutonium (0.5 kBq/kg bw), but at higher doses (up to 10 kBq/kg bw) the influx of macrophages was depressed because of inhibition of bone resorption by radiation. At 0.5 kBq, the number of macrophages reached a maximum 100 days after injection. After four years, ^{239}Pu-labelled macrophages were no longer detectable. Other responses in the bone marrow included fibrosis, hypoplasia and hyperplasia (Jee, 1972). In mice irradiated to a level of about 2 Gy from ^{239}Pu injected one year previously at a dose of 13.3 kBq/kg bw, the bone-marrow tissues were about 10 times more sensitive to subsequent irradiation with X-rays (Svoboda *et al.*, 1987).

^{241}Am administered to mice at 8 or 16 μCi (300 or 600 kBq)/kg bw also had profound effects on the marrow, the effects ranging from slight hypoplasia to complete aplasia. All cell types were usually depleted, but the erythroid series was most sensitive. Most of the marrows were reported to have become loaded with fat, and the blood sinusoids were heavily congested. In some mice, the sinusoids were destroyed and replaced by blood lakes. The effects were most severe in the vertebral column, where americium is concentrated, and least severe in the long bones (Nilsson & Broomé-Karlsson, 1976).

In mice, both ^{239}Pu and ^{226}Ra have been shown to damage haematopoietic stem cells and their regulatory stromal microenvironment (Lord *et al*., 1991). After a single injection of 960 Bq of ^{239}Pu (35 Bq/g bw) per mouse (strain $B_6D_2F_1$), the number of viable stem cells in the femur fell to about 50% of the normal number eight days after injection, with a gradual recovery to 90% of control values 120 days after injection. In contrast, the total cell numbers in the marrow remained at control levels for 300 days then decreased to 60% after 1.5–2 years. After a single injection of ^{224}Ra to CBA/H mice (555 Bq/g bw), the total cell number in the bone marrow of the femur fell to 60% of the control value by 4 h after injection, before reaching a minimum of 25% at four

days then recovering to 75% at about 90 days. The number of viable stem cells was initially unaffected but then decreased rapidly, reaching < 5% of control values eight days after treatment; the number then recovered slowly to a level of 50% of the control value by 90 days. The failure to recover fully was attributed to microenvironmental damage. Similar effects on the numbers of stem cells have been described in adult and neonatal mice after administration of ^{241}Am. Even at a dose of 33 Bq/g bw, the stromal cells in the bone marrow showed a reduced capacity to support viable stem-cell proliferation (Van den Heuvel *et al*., 1987). The inability of a damaged microenvironment to host a complement of normal stem cells can induce extraproliferative activity and stress on the pluripotent progenitor cells outside that microenvironment, which is required to maintain normal cellular output. It has been suggested that such perturbation, while not leading to the development of leukaemia *per se*, could be a prerequisite for its induction by other, non-radiological hazards. Damage to stroma may, however, directly precede the development of osteosarcoma (Lord *et al*., 1991).

Studies with β-particle emitters have also revealed effects on the haematopoietic system. Mice were maintained on $^{3}H_2O$ at 11×10^7 Bq/L starting at four weeks of age, and the bone marrow of the femur and tibia from these animals and from controls given tap water was analysed at regular intervals. No significant difference between treated and control mice was found in the total cellularity of the bone marrow over the entire 80-week observation period. A decrease was seen in the relative number of colony-forming cells as early as 12 weeks in the males and 20 weeks in the females. After a brief recovery, the decrease continued throughout the experiment, indicating maintenance of normal cell numbers in the bone marrow with fewer than normal stem cells (Carsten *et al*., 1977).

In mice given $^{3}H_2O$ at a concentration of 1.9×10^{10} Bq/L, haematopoietic failure resulted in death after 45 days. The marrow became severely hypoplastic, and replacement by fatty tissue was observed. At concentrations > 1.5×10^{11} Bq/L, all bone-marrow cells were destroyed, the marrow space became filled with diffuse haemorrhage, and death occurred within 15 days. At a dose of 9.25×10^9 Bq/L, the mice survived, but the marrow showed decreased cellularity (Yamamoto *et al*., 1990).

The β-particle emitters ^{90}Sr/^{90}Y, ^{32}P-phosphate and ^{144}Ce/^{144}Pr are bone-seeking radionuclides that attach to bone surfaces, from which they irradiate the marrow, and the depth of penetration of the radiation often exceeds that of similarly located α-particle emitters. Inhalation of aerosols containing ^{90}Sr chloride produced dose-related pancytopenia in dogs with a retained burden of > 0.37 MBq/kg bw. Thrombocytopenia and neutropenia were persistent until the death of the animals. The effects were reported to be similar to those seen after external irradiation (Gillett *et al*., 1987b). In rodents given 1 μCi (37 kBq)/g ^{32}P-phosphate per day for 14 days per month for three months, bone-marrow damage was inferred from decreases in the numbers of erythrocytes and lymphocytes and an increase in the fraction of circulating neutrophils and immature cells (Malhotra & Srivastava, 1978).

After administration of 0.33–0.42 mCi (12.2–15.5 MBq)/kg ^{144}Ce, in equilibrium with its decay product ^{144}Pr, to sheep, the production of granulocytes within the bone marrow decreased rapidly, and it ceased by three days after injection. The replacement cells were judged to be lymphoid in appearance, and haemorrhage was common. Megakaryocytes were also damaged, showing nuclear fragmentation and pyknosis. Regeneration of the bone marrow was seen 24 days after injection, but the delivery of granulocytes to the peripheral circulation was impaired (Sullivan *et al.*, 1969).

Given the importance of iron metabolism within the bone marrow, the effects of administration of the Auger electron-emitter ^{55}Fe were studied in mice. Doses in excess of 1.3 mCi (48 MBq) per mouse resulted in a dose-dependent depletion in the population of nucleated red blood cell precursors in the bone marrow that was similar to that seen after external whole-body irradiation. However, at very high doses, the effect was less severe than expected, probably because of diversion of excess iron to the liver. At an administered dose of 15 mCi (550 MBq) per mouse, the number of bone-marrow erythrocyte precursors decreased to 10% of the control value, a 60% reduction in the number of granulocyte precursors was seen, and total bone-marrow cellularity was decreased by 50%; the number of viable stem cells was also decreased. The absence of marked changes in the granulocyte-cell population probably reflects both the deposition of iron in the erythroblasts and on bone surfaces and the short range of Auger electrons (track length in tissue, < 1 μm) (Reincke *et al.*, 1975).

(*g*) *Gonadal tissues*

The effects of radiation on gonadal tissues are also discussed in section 4.3. Less is known about the deterministic effects of internally deposited radionuclides in the human testis and ovary than about the effects of external exposure to ionizing electromagnetic radiation (UNSCEAR, 1982, 1993); however, studies have been conducted with both α- and β-particle-emitting radionuclides in experimental animals.

Single intraperitoneal injections of $^{3}H_2O$ at a dose of 92.5 or 185 kBq/g bw to female Swiss albino mice caused a reduction in ovarian volume and an almost total depletion of follicles. Oocytes showed atretic changes, including fragmentation of nuclear materials, pseudo-maturation, spindle formation and shrinkage of the oocyte membrane. Cells in the granulosa layer showed pyknosis and cell lysis (Kapoor *et al.*, 1985). The survival of oocytes was studied in juvenile ICR mice given single injections of $^{3}H_2O$ at doses of 170–1020 kBq/g bw, corresponding to cumulative doses of 0.039–0.3 Gy. Other groups of mice were exposed to comparable doses of either external γ-rays from ^{60}Co or external neutrons from ^{252}Cf. An exponential decrease with increasing dose in the number of surviving oocytes was found. The effectiveness of the different radiation types decreased in the order: external ^{252}Cf neutrons > $^{3}H_2O$ β-radiation > ^{60}Co γ-radiation.

The effects of $^{3}H_2O$ and [^{3}H]thymidine (which is incorporated into DNA) on the mass of the testis of CBA mice was measured. A 30% reduction in testicular mass was observed five weeks after injection of $^{3}H_2O$ at 40 μCi (1.5 Mbq)/g bw, whereas a dose of 10 μCi (0.37 MBq)/g bw [^{3}H]thymidine resulted in a 20% reduction. The mass of

the testes had recovered by 16 weeks. The biological effectiveness of the β-radiation from $^{3}H_2O$ relative to that from external γ-radiation, calculated on the basis of the average absorbed dose to the testis, was 1.43. The equivalent ratio for [^{3}H]thymidine was 2.07, illustrating the stronger effect of the short-range β-emitter when incorporated directly into DNA (Carr & Nolan, 1979).

Injection of ^{241}Am into mice at a dose of 0.04, 8 or 16 μCi (1.5, 300 or 590 kBq)/kg bw also resulted in a reduction in testicular mass. Histological examination of the treated mice showed either marked hypospermia or aspermia and tubular degeneration. In older mice, slight interstitial fibrosis was seen, with a reduction in the number of Leydig cells. Necrosis of superficial testicular vessels with fibrosis and calcification were observed. In the smaller arteries, proliferation of the endothelium, generally in association with proliferation of adventitial tissue, was seen. Effects were found even at 0.04 μCi/kg bw (Nilsson & Broomé-Karlsson, 1976).

(*h*) *Lung*

In patients treated with Thorotrast, the lungs were irradiated by both thorium and its decay products, including ^{220}Rn, deposited within the lung and by ^{220}Rn emanating from thorium deposits throughout the body and transported to the lungs by the blood. Calculation of an average α-radiation dose to the lung is therefore complicated. For a Thorotrast intake of 18.5 kBq, the cumulative dose to basal cells in the region of the terminal bronchi (assuming 40 years of exposure) has been estimated to be about 2.5 Gy (Hornik & Kaul, 1995). At this level of irradiation, increased incidences of pulmonary cancer are not seen (Hoffmann & Daschil, 1986), but deterministic effects have been described in the woman who donated her body to the Uranium and Trans-uranium registries in the USA (Graham *et al*., 1992). In this case, the lungs showed focal emphysematous changes and atelectasia (areas of deflated lung associated with failure of surfactant production).

The time course of pulmonary changes was studied in beagle dogs after inhalation of $^{239}PuO_2$ particles in an aerosol, to give lung burdens of 0.1–48 μCi (3.7 kBq and 1.8 MBq) and a cumulative dose of α-particles to the lung of 0.004–140 Gy. Most animals at the higher doses that were not killed died from lung disease within one year. Examination of autopsy specimens showed that few changes had occurred in the lungs during the first week after inhalation. Between one week and one month after inhalation, some small bronchioles were swollen, with desquamation of epithelial cells. Multiple foci of septal, peribronchiolar and perivascular fibrosis also appeared. Between 55 and 63 days, moderate septal thickening was seen, as was alveolar collapse, infiltration of fibrotic areas with neutrophils and the first signs of alveolar cell metaplasia. By 63–79 days after inhalation, the focal fibrosis had become increasingly severe and resulted in local obliteration of the normal pulmonary architecture. The alveolar-cell metaplasia was now focal and moderate, but it was overshadowed by the appearance of moderate-to-severe peribronchiolar squamous and bronchiolar-type metaplasia, the alveolar lining cells taking on the appearance of squamous or simple columnar epithelium. Alveolar

macrophages were common, and giant cells were present within fibrotic foci. Between 80–107 days, the septal fibrosis had become increasingly severe, and diffuse fibrosis associated with increased alveolar and squamous metaplasia was also described. At 120–124 days, the developing metaplasia was extensive, and thickening of the alveolar septa and foci of advanced dense fibrosis caused considerable distortion of the entire lung architecture. In some bronchi, the lining surface was either denuded of epithelium or covered with a layer of flat cells. Focal areas of alveolar 'fibrin balls', neutrophil clusters, giant cells and haemorrhage were present. After 168 days, the pathological changes to the lung were severe; the lungs were twice their normal mass and visibly damaged. Emphysematous spaces were associated with the most severely damaged areas (Clarke & Bair, 1964). Nevertheless, the immune responses in dogs that had inhaled $^{239}PuO_2$ (initial lung burden, 19–35 kBq) were not suppressed by large continuous doses of α-particles, indicating that pulmonary immune responses are preserved despite severe radiation-induced alteration of tissues (Galvin *et al*. 1989).

At lower doses, the effects of α-radiation are less severe. In the study of Thomas *et al*. (1972), the average cumulative radiation dose to the lungs of four dogs at various times after inhalation of ^{241}Am oxide as an aerosol was determined to be 30 Gy at 127 days, 32 Gy at 256 days, 38 Gy at 512 days and 53 Gy at 1022 days after exposure. Only minor pulmonary changes were found 127 days after inhalation, with a few isolated areas of inflammation and minor alterations in bronchiolar and alveolar epithelia. By 256 days, these changes were more extensive. Some local dense fibrosis was present, which was predominantly sub-pleural and associated with marked pleural thickening, but was also present as a diffuse deposit throughout the lungs. At times up to 1022 days, some focal mineralization was seen, with local proliferation of alveolar cells and minimal squamous metaplasia. The fibrosis was more severe, with obliterative fibrosis of small arteries and some dense parabronchial fibrosis.

The lesions induced by internal exposure to α-radiation have been described in mice (Talbot & Moores, 1985), rats (Sanders, 1972; Métivier *et al*., 1975, 1978), dogs (Galvin *et al*., 1989; Diel *et al*., 1992) and baboons (Métivier *et al*., 1975) after irradiation by $^{239}PuO_2$ and in hamsters after inhalation of $^{238}PuO_2$ (Pickrell *et al*., 1983).

The changes after administration of $^{239}PuO_2$ to rats were similar to those produced by external irradiation. At cumulative doses of α-particles ranging from 3 to 130 Gy, the severity of the effects produced was a function of dose. During the intermediate stages of developing pneumonitis, the number of type II alveolar epithelial cells was increased, and these cells contained an increased number of osmiophilic inclusions. Also found was accumulation of a membranous–proteinacious exudate in the air spaces, accumulation of lipid in the septal walls, severe disruption of endothelia and accumulation of collagen, elastin, fibroblasts, plasma cells and mast cells in greatly thickened septal walls. Type I cells were relatively unchanged, the changes being restricted to a few areas of focal cytoplasmic disruption; however, about one week before the death of the animals, generalized oedema was present, with swelling of type I cells. A decrease was also seen in the proliferation of macrophages, and these

cells were less able to phagocytose latex spheres. Fewer macrophages were recoverable by lavage, suggesting a long-term cytocidal effect of plutonium on these cells (Sanders, 1972). A similar plutonium-induced reduction in macrophage activity was found in dogs with an accumulated lung dose of 23 Gy from single or repeated exposure. At this dose, about 80% of the animals died from deterministic effects (radiation pneumonitis, pulmonary fibrosis) and most of the remainder from pulmonary cancer (Diel *et al*., 1992). Similarly, in mice that had inhaled an aerosol of $^{239}PuO_2$ providing an average lung dose of 32 Gy, marked changes were seen in the lungs, including increased lung mass, protein and total collagen, fibrotic patches with accumulation of foamy cells and decreased cellularity with areas of compensatory hypertrophy (Talbot & Moores, 1985).

The survival rate of baboons exposed to an aerosol of $^{239}PuO_2$ was three times shorter than that of dogs in comparable studies, and varied from about 15 days at a lung burden at death of 400 nCi/g (14.8 MBq/kg) to 900 days at 3 nCi/g (111 kBq/kg). Scar tissue and fibrosis associated with local accumulation of plutonium were found. Some early deaths resulted from cell necrosis and alveolar oedema; later deaths resulted from interstitial pneumonitis and respiratory insufficiency due to fibrosis and were preceded by high arterial pCO_2 and low arterial pO_2 (Métivier *et al.*, 1975). In rats, the lung fibrosis observed 200 days after administration of $^{239}PuO_2$ aerosol at lung doses of up to 3 Gy regressed as the dose rates gradually decreased due to clearance of plutonium particles, so that, at 400 days, the lung collagen content had returned to normal (Métivier *et al*., 1978). A similar pattern of collagen reabsorption was observed in hamsters that had received 50 nCi (1.85 kBq) of relatively soluble $^{238}PuO_2$ by inhalation. Diffuse interstitial fibrosis was evident 10 weeks after inhalation, which had peaked by 15–20 weeks and then reached a plateau before returning to control values at 50 weeks. In this group of animals, dense fibrotic scars were rare; however, in animals that received twice as much ^{238}Pu, areas of heavy fibrosis was more common and, in these, the collagen concentration remained high. The authors concluded that diffuse fibrosis resolves spontaneously under conditions of limited exposure to α-radiation (Pickrell *et al*., 1983).

Pneumosclerosis and malignant lung tumours were also described in rats after intratracheal administration of ^{237}Np as the nitrate or oxalate. These changes occurred at cumulative lung doses of 0.05–32.2 Gy. The oxalate resulted in more severe fibrosis than the more soluble neptunium nitrate. As in other tissues, ^{237}Np may induce effects as a consequence of both its radiotoxicity and its chemical toxicity (Levdik *et al*., 1972).

The responses of pulmonary tissues to irradiation have also been studied in dogs after administration of β-particle-emitting radionuclides (McClellan *et al*., 1970; Hobbs *et al*., 1972; Slauson *et al*., 1976, 1977). Pneumonitis and fibrosis (as well as carcinogenesis) developed after inhalation of insoluble aerosols containing either ^{90}Sr, $^{90/91}$Y or ^{144}Ce, and the sequential alterations in the function of the lungs leading to death from pulmonary failure were described. In a later study (Mauderly *et al*., 1980),

the time course of histological and functional changes in the lungs of dogs exposed by inhalation to 230–630 μCi (8.5–23.3 MBq) of ^{144}Ce incorporated in aluminosilicate particles were evaluated comprehensively. The total score for histological changes in serially sacrificed animals was found to rise exponentially with cumulative radiation dose within the dose range 0–500 Gy; once disease was clinically evident, it progressed little with further increases in dose. The effects produced were changes to the vasculature (inflammation and thrombosis of arteries and veins, dilatation of lymphatic vessels and perivascular fibrosis), changes in bronchioles (inflammation and epithelial degeneration, metaplasia), changes in alveolar structures (interstitial-cell proliferation, alveolar lining-cell proliferation, fibrosis, collagenous scars, changes in macrophages and leukocytes, fibrin, oedema, emphysema) and changes to the pleura (fibrosis). Changes in pulmonary function were also observed. In dogs with pulmonary failure, a fourfold increase in respiratory frequency, a 50% reduction in tidal volume, a fourfold increase in alveolar dead space and many changes in gas exchange were noted. Cardiac output and blood pressure were also increased. Overall, it was concluded that the dogs developed progressive radiation pneumonitis and pulmonary fibrosis similar to that produced by external irradiation of the lungs. Moderate functional impairment was associated with more severe inflammatory and proliferative changes in the airways and alveoli. The severe impairment was found to have resulted from progressive fibrosis and scarring.

(*i*) *Thyroid*

The thyroid gland in adults is considered to be radioresistant in terms of cell death and failure of function. It has the capacity to actively concentrate iodine [and presumably the chemically related element astatine, although no experimental evidence exists to support this suggestion]. Radioiodine can, therefore, deliver considerable doses to the gland. Both medical diagnostic and therapeutic procedures are based on this effect: irradiation of the thyroid is a common treatment for the purposes of reducing its metabolic rate and controlling symptoms of angina in patients with cardiac insufficiency. A dose of at least 300 Gy is required to cause total ablation of the thyroid within a period of two weeks. This can be achieved with a single oral dose of 1850–3700 MBq of ^{131}I, resulting in an uptake of about 37 MBq by the thyroid (Goolden & Davey, 1963).

Reduced thyroid function caused by internal irradiation with ^{131}I or ^{125}I has been reported frequently. Orally administered ^{131}I is widely used for treatment of a hyperactive thyroid, which is more radioresponsive. After fractionated doses of 1.5–3.7 MBq, giving estimated total doses of 2–8 Gy, a return to normal activity or even hypothyroidism was observed (Werner *et al.*, 1952; UNSCEAR, 1982). When it occurs, hypothyroidism after treatment with radioiodine develops slowly: in 7.5% of the cases it appeared within the first year after treatment, and this percentage increased by approximately 3% each subsequent year, until approximately 26% of the patients had symptoms of hypothyroidism at seven years (Beling & Einhorn, 1961). It is not clear whether the rate of delayed hypothyroidism caused by ^{125}I is different from that after

treatment with ^{131}I (Bremner *et al.*, 1973). The loss of thyroid function in hypothyroid patients can be compensated by administration of synthetic thyroid hormone.

Little is known about the incidence of hypothyroidism after exposure to low doses of ^{131}I, e.g. during diagnostic tests for uptake. Hypothyroidism occurred in three of 146 patients who had received doses in the range of 0.31–0.80 Gy and in five of 151 patients at 0.81–19 Gy (UNSCEAR, 1982). However, the incidence of overt hypothyroidism in children exposed to ^{131}I in fall-out was not significantly different from that in unexposed controls (Rallison *et al*., 1974).

Radioactive fall-out from a thermonuclear explosion at Bikini in the Pacific Ocean was deposited on the Marshall Islands in 1954 (see section 1.1.1(*c*)(ii)). Inhalation and ingestion of radioactive iodine (mainly ^{131}I, ^{132}I, ^{133}I, ^{134}I) by the population resulted in significant internal exposure. Twenty-five years later, the population on the nearby atoll of Rongelap still showed a significantly impaired thyroid reserve, while at least four of 43 persons suffered from thyroid malfunction. Three of these were estimated to have received doses < 3.5 Gy (Larsen *et al.*, 1978).

Radiation from ingested ^{131}I and iodine deficiency have a combined effect on the development of thyroid abnormalities. The frequencies of diffuse euthyroid goitre and nodular abnormalities were significantly increased among children who received doses to the thyroid > 1 Gy and lived in areas with iodine deficiency (renal excretion, 7.5–5.0 μg/mL) than in children who received similar doses but lived in regions with adequate iodine provision (> 10 μg/mL) (Tsyb *et al*., 1999).

When children are exposed to radiation during the first two decades of life, the thyroid gland is susceptible to radiation-induced effects. Few data are available on the effects of absorbed doses of ^{131}I in the thyroid of children. In a study cited by the National Council on Radiation Protection and Measurements (1977; UNSCEAR, 1993), eight of 443 children < 16 years of age showed hypothyroidism after diagnostic tests with ^{131}I. The incidence of hypothyroidism increased with dose from 0% at < 0.3 Gy to 0.23% per year after > 0.8 Gy. Hypothyroidism was observed in eight of 30 young patients (8–18 years) receiving ^{131}I therapy for hyperthyroidism after administration of a mean amount of 244 MBq, during a nine-year follow-up (Hayek *et al*., 1970). A 92% prevalence of hypothyroidism was found among 51 patients aged 6–18 years after ^{131}I therapy (mean activity, 240 MBq) for hyperthyroidism (Freitas *et al*., 1979).

Within nine years after the thermonuclear explosion at Bikini in 1954, thyroid nodules were noted in children on Rongelap atoll, who had received the highest dose (Larsen *et al.*, 1978). Of those aged < 10 years, 67% developed nodules. The doses to the thyroid were estimated to be 10–43 Gy. Five children who were exposed when below the age of five years showed growth retardation, which was most prominent among children aged 1–1.5 at the time of exposure (Sutow *et al.*, 1965). The incidence of subclinical hypothyroidism was 31% among children who were < 10 years old at the time of exposure to estimated doses of > 2 Gy from ^{131}I (Conard, 1984).

A 23-year-old woman with Graves disease was given symptomatic treatment with propanolol and then received ^{131}I at 370 MBq as a definitive treatment. Three weeks

later, she was hospitalized with acute radiation thyroiditis. The clinical symptoms and signs persisted for over 30 days, and one month later she developed hypothyroidism. Of the three therapeutic options for the treatment of Graves disease — antithyroid drugs, radioiodine and surgery — treatment with radioactive iodine, without pretreatment with antithyroid drugs, can lead to acute thyroiditis or 'thyroid storm' (Zúñiga-González, 2000).

Detrimental effects on the thyroid of the developing human fetus may occur as a result of ^{131}I treatment for thyrotoxicosis of the mother during the first trimester of pregnancy. Estimates of the dose received under typical clinical circumstances indicate that the effective dose to the fetus is 100–450 Sv. This dose may be considerably higher if the blood concentration of ^{131}I in the mother remains high. Under such circumstances there may be fetal thyroid dysfunction, which can lead to severe abnormalities (Pauwels *et al.*, 1999).

The effects of a low dose of ^{131}I and ^{131}I-induced maternal hypothyroidism on the development of the thyroid gland and brain were studied in rat embryos. The dose given (150 μCi [5.6 MBq]) produced an estimated absorbed thyroid dose of 0.5 Gy, a dose similar to that received by the populations of the regions polluted by radioactive isotopes of iodine as a result of the Chernobyl accident in 1986. Thirty-five female Wistar rats and their 168 newborn pups were divided into a control group and four experimental groups distinguished by the time of ^{131}I injection: group I, no less than 12 days before mating; groups II, III and IV, days 5, 10 and 16 of gestation, respectively. In all tested females, the incorporated dose of ^{131}I led to hypothyroidism, accompanied by a 43% reduction in the thyroxin level and a nearly eightfold increase in the amount of thyroid-stimulating hormone. It was found, however, that the effect of maternal hypothyroidism on the development of the thyroid gland and brain of the embryo depends on the time at which ^{131}I took effect. The weight of the newborn brain and thyroid gland and total body mass were reduced. The hormonal status of the newborns' thyroid gland was also changed (Usenko *et al.*, 1999).

The radiotoxicity of ^{123}I, ^{125}I and ^{131}I to the thyroid gland was compared in groups of mice injected with the three isotopes at doses ranging from 100 kBq to 100 MBq. Thyroid function was determined 15 months later on the basis of the 24-h uptake of tracer activity of ^{131}I. A reduction in uptake to 20% of the control value for untreated mice was found for mice injected with 35 MBq of ^{123}I, 13 MBq of ^{125}I or 2.2 MBq of ^{131}I. The average absorbed dose in different parts of the thyroid was estimated by means of a refined method. The absorbed dose in the cell layers surrounding the follicles seemed to be most indicative of impairment of thyroid function (Van Best, 1982).

(*j*) *Gastrointestinal tract*

Animals receiving single doses of radiation (10–50 Gy) to the gastrointestinal tract died with signs of the gastrointestinal syndrome (Stather *et al.*, 1988; UNSCEAR, 1988). The symptoms in humans include anorexia, lethargy, diarrhoea, infection and loss of

fluids and electrolytes. Other signs include weight loss, reduced food and water intake, gastric retention and decreased intestinal absorption. Haemorrhage and bacteraemia may be present which aggravate injury and contribute to death. In various animal species, the mean time to death after doses of the order of 50 Gy is 4–10 days.

Given that the gastrointestinal tract is less radiosensitive than the bone marrow, death due to irradiation of the gut is likely to predominate over death due to irradiation of the bone marrow only when radioactivity delivering large internal doses has been ingested. Owing to the normal clearance times through the gastrointestinal tract, the dose would be delivered within a few days. Severe injury to the intestinal mucosa has not been reported after ingestion of radionuclides in humans, although many cases of accidental intakes have been published (Fry & Sipe, 1986).

Dose–effect relationships for intestinal damage have been calculated from data for animals, showing that different species respond in a similar way to irradiation of the gut (Bond *et al.*, 1965; Maisin *et al.*, 1971). After ingestion of radiotoxic doses of insoluble β-particle emitters, death was due to damage to the large intestine in both rats and dogs. Values for the LD_{50} of about 33 Gy for rats (25–41 Gy) and 40 Gy for dogs (20–52 Gy) were obtained in experiments in which rats ingested either $^{106}Ru/^{106}Rh$ (average, 1.4 MeV β) or ^{147}Pm (average, 0.06 MeV β) and dogs were given $^{106}Ru/^{106}Rh$ (Cross *et al.*, 1978; Sullivan *et al.*, 1978). The estimated dose to crypt cells in rats was the same with both $^{106}Ru/^{106}Rh$ and ^{147}Pm (about 35 Gy), although the dose to the mucosal surface was 30–35 times greater with ^{147}Pm than with $^{106}Ru/^{106}Rh$. On the basis of these data, an LD_{50} of 35 Gy has been suggested, with a simple linear function (LD_0, 20 Gy; LD_{100}, 50 Gy) (Pochin, 1983).

Comparison of the toxicity — on the basis of dose per body weight — of $^{106}Ru/^{106}Rh$ in rats of different ages showed decreasing sensitivity in the order: newborn > adults > weanlings (Sullivan *et al.*, 1987). The greater sensitivity of adults than of weanlings probably reflects the longer residence time of the gastrointestinal contents in the small bowel and caecum in adult animals. The newborn is more sensitive because of the uptake and retention of these radionuclides in the mucosal cells of the intestine, particularly in the proximal small intestine. Other radionuclides, including the actinides, are also retained in the mucosa of the immature gut of neonatal rats (see, e.g., Sullivan & Gorham, 1982). It would appear that at the high levels of retention observed in young rats, doses of up to 100 Gy/day may be received by cells located towards the tips of the intestinal villi, without evidence of mucosal injury. Indeed, a much lower concentration of radionuclide was seen in the region of the more sensitive crypt cells.

4.2.3 *Association between deterministic effects and cancer*

It has been suggested (Van den Hooff, 1984; Islam, 1985; Mole, 1986; Lord *et al.*, 1991; Gössner *et al.*, 2000) that cancer induction in humans and animals is linked to the occurrence of deterministic effects in the tissues in which the cancer arises. This suggestion has been made for several tissues, but the arguments have been most fully

explored in the case of human bone tumours resulting from skeletal irradiation by α-particle-emitting radium isotopes, and this is considered in detail below.

The first cases of malignant bone tumours occurring after therapeutic X-irradiation were reported by Beck (1922) in patients treated for tuberculous arthritis. Since then, detailed reports of post-irradiation neoplasia in bone after therapeutic external irradiation have been published (reviewed by Huvos, 1991; Schajowicz, 1993; Unni, 1996). The latent period, the time between exposure to external irradiation and the appearance of sarcoma, varies between three and 55 years with an average of about 15 years. It has been suggested that a dose of at least 30 Gy and a 3–4-year latent period is sufficient to establish a universally acceptable cause-and-effect relationship between exposure to radiation and tumour (Huvos & Woodard, 1988; Huvos, 1991).

Bone sarcomas associated with internal irradiation from ^{226}Ra and ^{228}Ra were first reported as an industrial hazard in radium-dial workers by Martland *et al.* (1925; see section 2.2.1). The long-term analysis of bone sarcomas in radium dial-painters is a classic epidemiological study in occupational medicine. In studies in the USA covering about 2600 individuals, 64 cases of malignant bone tumours were observed among persons in whom measurements were made (Rowland, 1994; Fry, 1998). A detailed study of the histopathology of ^{226}Ra- and ^{228}Ra-induced bone sarcomas in humans has been published (Schlenker *et al.*, 1989). There appears to be a practical threshold dose of 10 Gy for bone sarcomas in radium-dial painters (Rowland, 1997; Thomas, 1999).

Bone sarcomas have been induced in humans after incorporation of the short-lived α-particle-emitting ^{224}Ra. In the most recent reports on a group of 899 patients treated with ^{224}Ra in Germany (1945–55) (see section 2.2.2), 56 malignant bone tumours were described in 55 patients in this cohort (one person developed a second bone sarcoma two years later). Most of the cases occurred within the first 25 years after exposure; only four bone sarcomas have been diagnosed since 1980. According to data extracted from the cancer registries of the Saarland and of the former German Democratic Republic, the expected number of bone sarcomas in a group of this size would have been < 1 (about 0.3) over the entire observation period. The age at first injection of the patients who developed bone sarcomas ranged between 2 and 55 years. The time to tumour appearance peaked eight years after exposure. Thirty-seven bone sarcomas were reported in the 217 patients under the age of 21, whereas only 19 bone sarcomas occurred in 18 patients among the 682 adults. In the group of 393 patients with ankylosing spondylitis, only six bone sarcomas were seen. The lowest dose to the bone surface associated with a bone sarcoma in the total study cohort of patients exposed to ^{224}Ra was 9 Gy (Nekolla *et al.*, 1999, 2000).

More recent treatment of 1577 patients with ankylosing spondylitis with ^{224}Ra led to a mean bone surface dose of about 5 Gy. Among 626 deceased patients, only four cases of malignant primary bone tumour (compared with 1.3 cases expected in the general population) were observed, comprising one fibrosarcoma of the bone, one malignant fibrous histiocytoma, one reticulum-cell sarcoma (malignant lymphoma) of

the bone and one medullary plasmacytoma (myeloma), originally observed in the bone marrow of the sternum and pelvis. There was no osteosarcoma. In the control group, only one case, a medullary plasmacytoma, was observed (Wick *et al*., 1999).

Several studies have been devoted to the anatomical distribution of radiation-induced bone sarcomas in humans and beagles and their correlation to the distribution of radiation dose and bone mass or bone surface area (Spiers *et al.,* 1977*;* Spiers & Beddoe, 1983; Lloyd *et al.*, 1991). Spiers *et al.* (1977) observed a strong correlation between tumour frequency and the extent of the trabecular areas (endosteal surface areas). ^{226}Ra- and ^{224}Ra-induced bone sarcomas predominantly involved the appendicular skeleton; however, comparison of the sites of bone sarcomas induced by ^{226}Ra and and ^{224}Ra showed an axial:appendicular ratio of 14:85 with ^{226}Ra and 24:75 with ^{224}Ra. In contrast to spontaneously occurring tumours or those induced by external radiation, relatively few radium-induced tumours are located in the knee joint (Gössner, 1986).

In a retrospective study of patients who developed histopathologically confirmed bone tumours after receiving ^{224}Ra, the two commonest histological types were bone-producing osteosarcomas and non-bone-producing sarcomas of the fibrocytic and fibrohistiocytic type (Gössner *et al*., 1995). The first case of malignant fibrous histiocytoma of the bone after internal irradiation was described in this study. The unusually high incidence of this tumour type in ^{224}Ra patients, with a ratio of osteosarcoma:fibrosarcoma or malignant fibrous histiocytoma of 1.9:1 differs significantly from that among spontaneously occurring skeletal tumours (ratio, 5.9:1) (Gössner, 1999).

The types of bone tumour in patients exposed to ^{224}Ra have been compared with those in persons exposed to ^{226}Ra and ^{228}Ra (Schlenker *et al*., 1989), patients exposed to external irradiation (Huvos, 1991; Unni, 1996) and unirradiated patients who developed bone tumours at the sites of pre-existing bone lesions, such as Paget disease (Schajowicz, 1993) and bone infarct (Desai *et al*., 1996). The types of bone sarcomas among both radiation-induced and ‘secondary’ bone tumours are different from those among spontaneously occurring bone tumours (Table 117), with a higher incidence of bone tumours of the fibrosarcoma–malignant fibrous histiocytoma type and a lower incidence of chondrosarcomas in the first two groups than among the spontaneous tumours. These observations strongly suggest a close histogenic relationship between changes in the microenvironment (deterministic effects on bone remodelling and the fibro-osseous response, i.e. ‘radiation osteitis’) and the production of bone-producing and non-bone-producing tumour types (Gössner, 1999).

4.3 Reproductive and developmental effects

In the broad sense, the effects of perinatal exposure to radionuclides include a wide array of stage-dependent reproductive and developmental alterations. The adverse effects seen after prenatal exposure fit the classic model of developmental toxicity: prenatal and neonatal deaths, reduced growth and malformations. The effects of neonatal exposure, especially in rodents, follow a parallel pattern.

Table 117. Differential distribution of osteosarcomas, fibrosarcomas–malignant fibrous histiocytomas and chondrosarcomas among radiogenic, non-radiogenic 'secondary' bone tumours and spontaneous bone tumours

Source of bone tumours	No. of tumours (%)		
	Osteosarcomas	Fibrosarcomas/malignant fibrous histiocytomas	Chondrosarcomas
Radium-224	22 (53)	14 (33)	6 (14)
Radium-226 and -228	32 (70)	14 (30)	0 (–)
External irradiation	155 (63)	82 (33)	9 (4)
Paget disease	39 (64)	17 (28)	5 (8)
Spontaneous	3148 (55)	538 (10)	1990 (35)

From Gössner *et al*. (2000)

Dosimetric considerations are important in determining the qualitative and quantitative nature of the responses. When the overall content of the radionuclide remains constant, absolute and differential growth lead to continuing decreases in the concentration of the radionuclide, so that the distribution of radiation dose over time is different after perinatal deposition of most nuclides than after administration to adults. Differences in sensitivity cannot be dissociated completely from dose rate and dose rate reduction. Although precise timing can be achieved with external irradiation, the persistence of a radionuclide after perinatal deposition inevitably results in exposure of successive developmental stages. With many radionuclides, fetal deposition, retention and radiation dose are greater when they are administered at late stages of gestation.

In order to establish dose–effect relationships by stage of development for perinatal exposure to radionuclides, placental transfer and deposition and retention in the embryo, fetus or neonate must be quantified. The conceptus may receive radiation from radionuclides external to or within the mother's body. The radionuclide must be absorbed by the mother, enter her blood circulation and be transferred through the placenta, and these processes are influenced by the route of entry, the physicochemical form and the chemical characteristics of the radionuclide. Depending on the element and its biological behaviour in the mother and conceptus, the placenta may act as a 'transparent' intermediate compartment between the circulation of the mother and the conceptus; its structures may serve as barriers or may facilitate transfer (Sikov & Kelman, 1989; von Zallinger & Tempel, 1998). Passive diffusional transfer from the uterine mucosa may occur very early during gestation, but thereafter transfer is governed by ordinary transfer kinetics involving concentration gradients in the maternal and placental blood circulation. Selective deposition of a radionuclide in a fetal organ or tissue reduces its concentration in fetal blood and enhances transfer rates, whereas deposition of the nuclide in placental structures restricts transfer but

could lead to selective irradiation of the placenta *per se* or of primitive stem cells that originate in the blood island of the yolk sac placenta.

Most of the information about stage-dependent effects of radiation on prenatal development derives from studies of mice and rats exposed to X-rays or γ-rays at relatively high dose rates (UNSCEAR, 1986; Sikov & Hui, 1996; National Council for Radiation Protection and Measurements, 1998; IARC, 2000), and the discussion below is based on those studies.

The developmental processes constituting embryonic induction, differentiation and histogenesis are unique events. Thus, the effect of radiation on cancer induction and subsequent promotion in embryos or fetuses may differ from that in adults. Although the actual carcinogenic responses should be similar, the responses and consequences may change with the stage of neonatal development.

Effects are often categorized as early, delayed and late on the basis of their nature and the time at which they arise. The responses of embryos and early fetuses to irradiation are similar in human, non-human primate and lower mammalian species, reflecting the comparability of the developmental patterns. Differences between species in the relative and absolute duration of individual developmental stages lead to species-specific response characteristics. These differences become more prominent later in gestation, and differences in maturity at birth are associated with differences in response and apparent sensitivity. The distinctions become less clear with protracted exposure, and the spectrum often shifts with different radionuclides.

Early effects arise from alterations of cells present at the time of irradiation or of the first several daughter-cell generations, and may comprise mitotic inhibition, cell death and interruption of pregnancy. Depending on the species and the time of exposure, early prenatal deaths may manifest as reduced fertility, miscarriage or abortion, or as decreased litter size or increased resorptions in rodents. Defects in the developmental process that lead to malformations of surviving embryos may also manifest as alterations of metabolism or physiology.

Delayed effects produced by prenatal exposure to radiation are considered to be overt or latent defects that arise later or biologically modified expressions of previously induced defects. Prenatal exposure may result in decreased body or organ weights, with minor stage differences among species. The more severe effects occur after exposure during organogenesis and at the beginning of fetal development, but the period of sensitivity to postnatal growth retardation extends throughout the perinatal period, at least in rats and mice. Particularly in the case of exposure during the fetal period, effects on the weights of certain organs, e.g. the brain, may be disproportionate to the effect on body weight, resulting in substantially larger decreases in absolute and relative brain weights.

Differences in the nature of the response to radiation are also related to compensatory cell proliferation, which may occur later in gestation and during the postnatal period. Histopathological and cytological examinations of the human central nervous system after prenatal exposure to radiation consistently indicate that the effects of high

radiation doses on neurogenic processes are similar in humans and in experimental animals; this is true particularly of those effects that involve changes in the matrix and cell migration in specialized neural epithelia (ICRP, 1986). There is a programmed sequence of events that leads to qualitatively different cell populations at successive stages, which is probably also related to the lower capacity of neural tissue to repair lesions. Neuronal cell formation ceases early in the postnatal period, so that subsequent compensation of tissue damage is accomplished through gliosis, which can lead to an imbalance between neural and glial cells. In humans and in experimental animals, microcephaly or cerebral dysmorphology and underdevelopment are the most distinctive and frequent retardation effects.

After early cell inactivation, gametogenesis appears to be one of the most radiosensitive developmental processes. Nevertheless, germ cells have a great capacity for regeneration in the early stages of gametogenesis and also high rates of redundancy and natural elimination in adults. After acute and chronic irradiation, postnatal fertility is one of the most sensitive indicators of prenatal damage.

Late effects include the various degenerative diseases, solid tumours and leukaemia, or lesions of later life. A number of factors make it difficult to extrapolate the results of animal experiments to risk estimates for radiation-induced late effects in humans. In many experiments, the animals were selectively bred to have a high spontaneous incidence of neoplasms or degenerative diseases, so that the predisposition to development of various lesions in later life may be specific to the stock of animals studied. Factors such as interactions between differences in life span, altered endocrine status, competing risk factors in mortality, tumour latency and destruction of cells of target tissues are known to play a role in the development of late effects after prenatal irradiation of animals. Extrapolations should therefore be restricted to general features rather than to quantitative estimates of risk.

4.3.1 *Sensitivity at different stages of gestation*

The effects of irradiation depend on the period of gestation. Moreover, transfer of radionuclides to the conceptus, their subsequent biological disposition and the doses of radiation are also related to the stage of gestation.

(*a*) *Preimplantation period*

The earliest phases of development involve pluripotent cells with high mitotic activity, which develop into the blastocyst that is implanted into the uterine mucosa. Individual cells are radiosensitive during this period, when there is a high capacity for regeneration and reorganization.

The effects of exposure to radiation during the preimplantation period are characterized by a moderately high threshold, and most studies of rodents exposed during this period have not shown persistent effects. There is an all-or-none response over a wide range of doses, i.e. preimplantation or early postimplantation death, or complete

restitution, so that the survivors develop into fetuses with normal morphology. No other clear-cut types of effects have been found after exposure to radiation during this period.

(*b*) *Embryonic stage*

The period of organ formation in developing embryos is referred to as organogenesis. Cell death, mitotic delay or genomic alterations during organogenesis can lead to defects in the developmental process and have been considered the major cause of morphological lesions or malformations. Cell proliferation during this phase is accompanied by remodelling: the neural plate forms into the neural tube, outpouchings develop into the primitive brain, the complex structure of the heart is attained and the external body form develops. Extensive intrauterine selection, especially in humans, often avoids further prenatal development of embryos with major defects. Malformations are the characteristic effect of exposure to radiation during the period of major organ formation. Changes in radiosensitivity have been noted in various stages of organ differentiation.

(*c*) *Fetal period*

Gestation from organogenesis through term is referred to as the fetal period. It is characterized by growth and histogenic development, through which organs and tissues progress from primordial structures into more differentiated histological entities that are present at birth. This phase of development is relatively brief in many rodent species, but it comprises more than two-thirds of the prenatal period in primates. Acute exposure of rats or mice to radiation during later stages of this period has less effect on intrauterine mortality than exposure during organogenesis. Irradiation tends to result in developmental retardation but has little effect on the basic shape or structure of most organs. The sensitivity at the cellular level remains essentially the same, however, which is important in the formation of specific populations such as germ cells and structures in the central nervous system.

Irradiation often yields a mosaic of surviving and reproductively inactive cells in embryonic or fetal tissues. Damage to the cell nucleus or chromosomes may lead to acute cell death, chromosomal aberrations or aneuploidy and to inactivation or delayed death of daughter cells. Depending on the stage of gestation, there is a selection against aneuploid cells and those with chromosomal aberrations or micronuclei. The cell losses that result from cytogenetic alterations, rather than the defects *per se*, seem to be the determining factor in many developmental effects; however, genetic alterations may be involved in carcinogenesis and other late effects.

Progenitor cells of the gametic and haematopoietic lines are formed in blood islands of the yolk sac during early organogenesis and migrate into the embryo. These early cells are susceptible to apoptotic or reproductive death and to induction of latent effects expressed in subsequent cell lineages. Inactivation has been detected after acute irradiation during developmental phases ranging from early primordial cell formation through the fetal period of spermatogonial and oogonial precursors. This

effect is of particular relevance with regard to exposure to radionuclides that localize in these cells.

The central nervous system is formed through complex interactions between cell proliferation and migration, so that interference with either process affects its development. Defects induced by low doses of radiation are believed to involve the migratory processes and/or subsequent differentiation of the neural cells. The capacity for compensation decreases progressively during histogenesis. More damage will manifest because it is more likely that affected fetuses will survive than after exposure at earlier stages. Thus, deficits tend to progress and become apparent as delayed or late effects during the postnatal period.

Mammalian species generally have similar teratological characteristics in response to exposure to radiation, including short, stage-specific sensitive periods during early development and a relatively high capacity for restoration. The effects of radiation on the central nervous system may be exacerbated by progressive loss of neuronal reproductive capacity, which can lead to functional deficits in the absence of gross anatomical brain malformations. As has been noted, scaling is required when susceptibility to specific effects is related to developmental stage at exposure. Phylogenetic differences must also be considered when comparing interactions in humans with those that are important in rodents.

4.3.2 *Malformations in human populations after the Chernobyl accident*

Several research groups have examined the incidence of mutations, chromosomal abnormalities and congenital anomalies in the areas surrounding the 1986 reactor accident in Chernobyl (see section 2.7.2(*d*)). No clear consensus has been reached with regard to the incidence of malformations, and its attribution to exposure to radiation, either directly or indirectly, poses numerous difficulties.

A well-documented health consequence of the Chernobyl disaster was a dramatic increase in the number of pregnancy terminations in regions nearby and far from the accident site, as anxiety about possible prenatal exposure led to an increased demand for abortions and delays in planned conception. Reductions in birth rates over the 5-year period after the accident were seen not only in the Russian Federation but also in Denmark, Hungary, Italy and Norway (Castronovo, 1999).

Congenital malformations were studied in 16 590 5–12-week-old embryos and fetuses obtained from pregnant women during legal medical abortions in Minsk (control area) and from 2578 women in the Gomel and Mogilev regions where the soil was contaminated with ^{137}Cs at a rate of > 0.6 MBq/m^2. The malformation frequency during the period 1986–94 was 7.41 per 100 abortuses in the contaminated areas and 4.66 per 100 in the control regions. No information was given on malformations in abortuses obtained before 1986 (Lazjuk *et al.*, 1997).

In the same study, congenital malformations among neonates were also analysed. The frequency was 7.00 per 1000 live births in the contaminated areas in the period

1987–94 and 3.87 in 1982–85 ($p < 0.05$). In the control regions, the frequencies per 1000 neonates were 5.58 and 3.90 during those periods, respectively ($p < 0.05$). It was not possible to correlate the individual doses of pregnant women with the incidence of congenital malformations. No convincing data were obtained that structural changes in chromosomes are associated with the increase in malformation frequency in the contaminated area (Lazjuk *et al.*, 1997).

A retrospective analysis of the birth archives in two large hospitals in Kiev over the period 1969–90 showed no change in the rates of miscarriage, congenital anomalies or perinatal mortality between the periods before and after 1986 (Buzhievskaya *et al.*, 1995).

Castronovo (1999) concluded that there is no evidence that exposure of pregnant women to radiation from ^{137}Cs released during the Chernobyl accident has had any harmful effects.

4.3.3 *Developmental responses to radionuclides*

(*a*) *Radon and progeny*

A group of 43 pregnant Sprague-Dawley rats was exposed to ^{222}Rn in air at a dose of 1.3×10^7 Bq/m^3 radon and its progeny adsorbed onto ore dust for 18 h/day on days 6–19 of gestation. Another 26 rats were exposed to a filtered-air atmosphere. No developmental toxicity or teratological changes were detected. The calculated dose rate on the last day was 1.5 mGy, which was estimated to have resulted in 20 mGy during the post-implantation period, with a protracted dose equivalent of 0.4 Sv (Sikov *et al.*, 1992).

(*b*) *Radium*

Seventeen children of 10 mothers who had been employed as radium-dial painters were evaluated. One child was born while the mother was still employed, and the others were born 2–15 years after the mother had stopped dial painting. At the time of the study, seven of the 10 mothers had died from radium poisoning, but no health problems were detected in any of the children. Whole-body measurements of γ-radiation (detection limit, 0.2 μg radium) showed none in 14 of the children, with inconclusive results for the three others. No radon was found in the breath of these three children (detection limit, 0.1 pCi/L [0.0037 Bq/L]) (Martland & Martland, 1950).

The fertility of women who had been employed as radium-dial painters was investigated in epidemiological studies. The study population consisted of 199 women who had been employed in the dial-painting industry in Illinois, USA, between 1916 and 1929. The doses of α- and γ-radiation were calculated on the basis of the physical characteristics of the luminous paint used in Illinois during that period (essentially all ^{226}Ra) and the number of days that the women worked as dial painters. In most of the analyses, the estimates of total dose to the ovaries from internal radiation (α-particles from ingested radium) and of external radiation (γ-rays emitted by the luminous paint)

were combined, with a quality factor of 20 for α-particles. Internal comparisons by dose to the ovary were conducted because suitable cohorts could not be established before the analysis. There was no significant difference in the proportion of childless women in the different groups, so that differences in live-birth rates (the number of reported live births divided by the number of years of marriage until age 45) were examined only in women who had had at least one live infant. The mean of the natural logarithm of the live-birth rate decreased with increasing ovarian dose. Multiple linear regression analyses showed that the body burden, but not duration of employment, was a significant predictor of the live-birth rate (Polednak, 1980).

The study was expanded to include women who had been employed as dial painters in Connecticut and New Jersey, USA. Their exposure involved not only ^{226}Ra but also ^{228}Ra and larger doses to the ovary; however, the general approach was similar to that of the previous study. The mean numbers of pregnancies (1.2) and live-births (1.1) were significantly reduced in women in categories of internal dose to the ovary ≥ 5 Sv. Measures of health status and confounding factors that could have affected fertility, at least indirectly, were evaluated but did not appear to be important. There was no indication of an increased fetal death rate, suggesting that the findings for the live-birth rate did not involve post-implantation dominant lethal mutations, although losses before implantation could not be evaluated (Schieve *et al.*, 1997).

Most of the data on prenatal effects of exposure of animals to radium are derived from a study reported by Bagg (l922), who administered an isotonic saline solution containing dissolved radium by subcutaneous or intravenous injection to pregnant rats [strain not stated] at various stages of gestation or at times before mating. Controls were injected with the same solution after time had been allowed for decay. For comparison, other rats were exposed to external γ-rays from the same preparation of radium. The rats were killed at weekly intervals after treatment for detailed evaluation of embryos and offspring or allowed to bear their litters. Three experimental groups were considered. Sixty-five rats were mated and injected subcutaneously on days 7, 10–14, 15–17 or 18–21 of gestation. Preimplantation loss and early resorption were common, and embryonic death with continued placental growth was seen. The effects included fetal death with macroscopic haemorrhages of the placentas or fetuses, but the within-litter responses were variable so that newborn litters contained both normal and affected offspring. Another 77 rats were injected 5–7, 10–14 or 20 days before mating. Eleven died before mating, and some of the others produced either fetuses that were normal at evaluation or normal full-term young. Some females killed after mating had haemorrhagic or cystic ovaries, with either fertilization failure or early embryonic death. In order to verify the early effects seen after subcutaneous injection, a third group of pregnant rats was injected intravenously with the same preparation. Deaths with extensive haemorrhage occurred within 24 h after intravenous injection of higher doses during late gestation. The litters from rats that were exposed to γ-rays during late pregnancy showed effects that are now considered characteristic of the stage and dose.

(*c*) *Uranium*

The effects of prenatal and neonatal exposures to uranium on the development of Sprague-Dawley rats was studied in a series of experiments. Pregnant rats were injected intravenously with 0, 1.8, 3.33, 5.75 or 10 μCi/kg bw of ^{233}U [0, 66.6, 123, 213 or 370 kBq/kg bw] in citrate on day 9 or 15 of gestation and were killed at 20 days. The sizes of the groups were about 20 and 13 at the two times. The highest dose was toxic to the adults, and a statistically significant trend towards increased prenatal mortality was seen with dose. Exposure reduced the fetal and placental weights. Cleft palate was detected in nine fetuses from three litters after the highest dose at nine days (but not 15 days), and exposure at this time led to a dose-related increase in the numbers of litters and fetuses with rib anomalies. Several fetuses at the two highest doses were oedematous at 15 days (Sikov, 1987a).

Calculations of the radiation doses from parallel radioanalyses indicated that these effects were attributable to chemical toxicity rather than to radiation. Other results suggested that some of the fetal effects were mediated through alterations of maternal fluid balance, which is consistent with the known nephrotoxicity of uranium. In order to examine postnatal toxicity, groups of 10–14 newborn, 12-day-old and weanling rats of each sex from each age and dose group received an intraperitoneal injection of 0, 2, 5 or 10 μCi/kg bw of ^{233}U [0, 74, 185 or 370 kBq/kg bw]. The lowest dose did not affect the growth of weanlings, whereas 185 kBq/kg tended to decrease growth and the highest dose had a statistically significant growth-reducing effect. Only the highest dose significantly decreased the growth of the female juveniles, but not the male juveniles, and there was no effect on the growth of the neonatal rats (Sikov, 1987a).

(*d*) *Neptunium, plutonium and americium*

Numerous investigators have examined the placental transfer, fetoplacental distribution and neonatal absorption and metabolism of plutonium and americium (National Council on Radiation Protection and Measurements, 1998; see section 4.1). There are quantitative differences in the effects of these nuclides at various stages of gestation. They can be transferred to offspring via lactation, and their gastrointestinal absorption by neonatal animals is greater than that by adults.

Autoradiographic studies in several species during various periods of gestation showed that the highest fetoplacental concentrations of plutonium and americium are found in fetal membranes, especially in the developing yolk sac; the placenta contains lower concentrations and the embryo or fetus even less. The villous yolk sac is a functional nutritive structure present in early development, which contains blood islands from which stem cells of the haematopoietic system and gametes originate. Autoradiographic studies in rodents and other mammalian species, including non-human primates, have shown consistently that most of the radioactivity in embryonic membranes is contained within this structure.

Studies have shown stage-dependent changes in the deposition and localization of radionuclides in developing liver and skeleton during both the prenatal and postnatal periods. Plutonium is deposited primarily on the bone surfaces of adult animals, but there is progressive, relatively rapid burial in the bone matrix in the fetus and during the perinatal period as a result of bone remodelling.

The doses of α-radiation to embryos tend to be relatively low and homogeneous; they represent a small fraction of the average whole-body dose received by the pregnant woman and an even smaller fraction of the dose to the tissues in which the radionuclide is deposited. The localized concentrations in placental structures may, however, result in radiation doses that are as large as or larger than the doses to any maternal tissue. Because of their short path length, α-particles from radioactive decay in these extraembryonic volumes would not reach the embryo, but β-particles could irradiate embryonic tissue.

(i) *Neptunium*

Intravenous injection of rats with 0.3–5 μCi/kg [11.1–185 kBq/kg] ^{237}Np as the oxalate increased the incidence of preimplantation mortality (Ovcharenko & Fomina, 1982).

(ii) *Plutonium*

Wistar rats at day 9 of gestation were injected with graded doses of ^{239}Pu prepared with sufficient excess citrate so that it was predominantly in the monomeric form. The rats were killed for radioanalysis and for evaluation of toxicity in their litters at various times between days 10 and 15 of gestation. In the rats injected with doses < 1.5 μCi [55.5 kBq], there was no measurable increase in the mortality rate of fetuses; at doses of 3–12 μCi [111–444 kBq], there was 60% mortality, and at 25 or 50 μCi [925 or 1850 kBq] all fetuses died. None of the surviving fetuses showed gross morphological defects. When the highest dose was given by injection to pregnant rats on day 15 or 19 of gestation, no prenatal mortality was seen during the remainder of the prenatal period, although there was some microscopic damage to the fetal liver (Sikov & Mahlum, 1972).

A series of studies was conducted to examine age-related differences in the biological behaviour and effect of ^{239}Pu in relation to the physicochemical form in which it was injected, either monomeric or predominantly polymeric. In most cases, intravascular injection was used in weanling and adult rats, whereas newborn animals received injections directly into the heart. Prenatal exposure was ensured by intravenous injection of their dams.

A dose of 30, 60 or 90 μCi/kg [1.1, 2.2 or 3.3 MBq/kg] of each of the two ^{239}Pu preparations was injected intravenously into groups of 12 newborn, weanling and young adult Sprague-Dawley-derived rats. The LD_{50} and relative sensitivities were calculated on the basis of deaths that occurred over the subsequent 60 days. The polymer was about twice as toxic as the monomer to weanlings and adults, but there

was little difference between the two forms in the newborns soon after administration. Especially in weanlings and adults, a much higher percentage of the injected activity of the polymeric form than of the monomeric form was present in the liver. The difference between the physicochemical forms with respect to initial tissue distribution was less prominent in the newborns but developed as they matured (Mahlum & Sikov, 1974).

Another experiment was performed to examine differences in the effects of the monomeric and polymeric forms of plutonium on liver function in male and female Sprague-Dawley rats. Newborn and seven-day-old rats received injections into the ventricle of the heart, and 21- and 110-day-old animals received injections into the tail vein. The doses were chosen to produce minimal mortality over a 21-day period, and were 60 μCi/kg bw [2.2 MBq/kg bw] of the monomer and 30 μCi/kg bw [1.1 MBq/kg bw] of the polymer. Some of the animals were killed at sequential times for macroautoradiographs. At 21 days after injection, six rats from each age group were injected intravenously with ^{198}Au-labelled colloid and killed 10 min later to determine the reticuloendothelial function of the liver from hepatic incorporation of the radiolabelled colloid. Phagocyte function was unaffected by either form in the adults and weanlings, but in the seven-day-old animals the monomer produced a marked decrease and the polymer had only a slight effect. The polymer reduced the colloid uptake to less than half of the control value in the animals exposed at birth, and the monomer inhibited the uptake almost completely. Another six rats from each age group were injected intravenously with [^{131}I]sodium tetraiodotetrachlorofluorescein (rose bengal) to evaluate the function of the liver parenchyma, i.e. the blood clearance of radioiodine. This function was decreased in the livers of animals of the two younger age groups, the monomer being more effective than the polymer. Little effect was detected in the older animals (Kashima *et al.*, 1972).

In a series of studies to evaluate the effects of plutonium on bone strength, weanling and adult Sprague-Dawley rats were injected intravenously and newborn animals were injected intracardially with ^{239}Pu citrate at doses ranging from 0.006 to 0.09 μCi/g bw [222–3330 Bq/g bw] [group sizes not reported]. The animals were radiographed at intervals, and some from each group were killed at intervals for radioanalysis and histological examination. Subjective observations at one month after exposure indicated that the long bones of rats injected as weanlings with 0.06 or 0.09 μCi/g bw were extremely fragile, and the radiographic examinations revealed many spontaneous fractures in these animals. The frequency of fractures, which were accompanied by abnormal healing, was even greater three months after injection; histological examination showed abnormal callus at the break points in the bones, consisting of dense connective tissue. Rats in the three groups were killed and necropsied nine months after exposure for various analyses, including measurement of the mechanical breaking strength of the femur. The highest doses resulted in high doses of radiation to the femurs of adults but had little effect on their strength; there was also no effect on the strength of this bone in the newborn animals, which received only small doses to the femur. In contrast, there was a marked, dose-dependent decrease in the breaking strength of femurs from the animals injected as

weanlings, although the radiation doses were similar to those of the adults. The calcium and phosphorus contents of these femurs were not appreciably altered, but the water content was greatly increased (Mahlum & Sikov, 1969).

In experiments to investigate the late effects of plutonium, three-month-old adult and 21-day-old weanling Wistar rats were injected intravenously with 0.3, 1 or 3 μCi/kg bw [11.1, 37 or 111 kBq/kg bw]. Pregnant rats were injected intravenously on day 19 of gestation with 6, 20 or 60 μCi/kg bw [222, 740 or 2220 kBq/kg bw] of the same solutions for exposure of the fetuses, and newborns were injected intracardially with 3, 10 or 30 μCi/kg bw [111, 370, 1110 kBq/kg bw]. Other rats were injected with citrate solutions at the same concentration to serve as controls. Groups of about 25 rats of each sex from each group were maintained. Longevity decreased with increasing dose, and the effect was statistically significant in the three groups exposed postnatally. Although no significant effect on longevity was found in the group exposed prenatally in this experiment, subsequent experiments found significantly decreased survival of similarly exposed rats (see below). The incidence of bone tumours is described in section 3.3 (Sikov *et al*., 1978).

In experiments to examine the effect of gestational stage on the delayed effects of plutonium, pregnant rats were injected intravenously with ^{239}Pu citrate preparations at representative stages (day 9, 15 or 19 of gestation) at a dose of 0.011, 0.11 or 1.1 kBq/g bw. The cumulative radiation dose rates and doses to the embryo or fetus and offspring increased with prenatal age at injection. Prenatal exposure resulted in a dose-related decrease in postnatal growth that was more severe among offspring from litters injected on day 19 of gestation than in those treated on day 9; animals exposed on day 15 of gestation showed damage of intermediate severity. The exposure also shortened the lifespan (Sikov, 1989).

In studies on the influence of foster–rearing of rats on the postnatal effects of prenatal exposure to plutonium, pregnant rats were injected intravenously with 2.2 kBq/g bw ^{239}Pu citrate or with a citrate (control) solution on day 19 of gestation. At one day of age, the offspring of some control and exposed litters were kept with their own dams, while others were fostered to lactating females that had received the same (or the opposite) exposure as had their dams. 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 growth curves of control offspring that were nursed by exposed dams were depressed. Offspring exposed prenatally lived significantly less long than control groups, but fostering had no consistent effect on longevity. The incidences of several histopathological lesions of soft tissues, including tumours of the liver and adrenal gland, were elevated in the three groups exposed to plutonium prenatally, but the incidences were not affected by fostering or by the exposure history of the dams that reared the offspring. These observations 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, 1989).

Pregnant C57BL6 mice were injected intravenously on day 13 of gestation with 30 kBq/kg bw ^{239}Pu nitrate. The fetuses were evaluated on day 17 of gestation and the offspring at two and seven days and four, eight, 22 and 55 weeks of age. The numbers of colony-forming units in spleen and bone marrow increased rapidly after birth but at lower rates in exposed than in control mice. The quality of the haematopoietic microenvironment in femoral marrow was adversely affected in the exposed offspring (Mason *et al.*, 1992).

In experiments to investigate the possibility that transgenerational effects from preconceptional paternal irradiation might render offspring more vulnerable to secondary exposure to an unrelated carcinogen, ^{239}Pu citrate (at 0, 128 or 256 Bq/g) was administered by intravenous injection to male mice 12 weeks before mating with normal females. Two strains of mouse were used — CBA/H and BDF1. Haematopoietic spleen colony-forming units and fibroblastoid colony-forming units, a component of the regulatory microenvironment, were assayed independently in individual offspring at six, 12 and 19 weeks of age. Female offspring of BDF1 mice were injected with *N*-methyl-*N*-nitrosourea (MNU) as a secondary carcinogen at 10 weeks of age and monitored for the onset of leukaemia or lymphoma. The mean values of colony-forming units were unaffected by preconceptional paternal injection with ^{239}Pu, although there was an apparent increase in variation in fibroblastoid colony-forming units between individual animals. By 250 days, 68% of MNU-treated control animals (no preconceptional paternal injection) had developed thymic lymphoma (62%) or leukaemia (38%). The first case arose 89 days after MNU administration. In the groups in which the male parent had been treated preconceptionally, leukaemia or lymphoma developed 28 days earlier, the incidence rising to 90% by 250 days, so that leukaemia (65%) predominated over lymphoma (35%). This second-generation excess of leukaemia appears to be the result of preconceptional paternal injection with ^{239}Pu and may be related to inherited changes that affect the development of haematopoietic stem cells (Lord *et al.*, 1998c).

The transmission of chromosomal instability to the haematopoietic stem cells of offspring after exposure *in utero* to X- or ^{239}Pu radiation was investigated after pregnant CBA/Ca mice were injected with 80 kBq/kg bw ^{239}Pu nitrate in sodium-citrate solution or X-irradiated with 1 Gy on day 13 or 14 of gestation. Colony-forming units were grown from haematopoietic stem cells from fetal liver and from bone marrow from the offspring and from the dam. Non-clonal, unstable chromosomal aberrations were scored in metaphases from individual stem-cell colonies. It was concluded that irradiation *in utero* was not more efficient in inducing chromosomal instability in the offspring than in the fetus or the dam. All three cell populations showed a similar degree of unstable aberrations, in terms of both absolute numbers of non-clonal aberrations and relative excess (Rosemann *et al.*, 1999).

The effect of combined exposure to external γ-radiation and α-radiation from intratracheal injection of ^{239}Pu on the reproductive function of female rats and their progeny was studied in 609 female Wistar rats, 998 young rats of the first generation

and 80 young rats of the second generation. Female rats were exposed to γ-radiation at a dose of 12.9, 25.8, 51.6 or 103.2 mC/kg. Half of the irradiated rats received an intratracheal injection of ^{239}Pu nitrate (37 kBq/kg) immediately after γ-irradiation. The absorbed dose of α-radiation from ^{239}Pu to the ovary was 0.026 Gy 30 days after the beginning of the experiment, 0.23 Gy at the time of mating (90 days after injection of ^{239}Pu) and 0.70 Gy at the end of the experiment. No radioactivity was detected after radiometry of the progeny. No disorders of the oestrus cycle or fertility were detected in the treated rats, and no difference between the treated and control groups was observed with regard to the number of young rats or their survival, body mass or physical development. The number of erythrocytes in young rats born to females exposed to both γ-radiation (12.9 and 25.8 mC/kg) and α-radiation was higher than in young rats born to intact controls or to rats exposed to γ-radiation only or ^{239}Pu only. The ratio of brain volume to body mass in young rats of the second generation did not differ from that in the control group after combined exposure to γ- and α-radiation or exposure to γ-radiation or α-radiation only (Ovcharenko & Fomina, 1983).

In studies on the effects on the germ cells of male mice of exposure to ^{238}Pu, groups of 20–60 male hybrid mice (CBA × C57BL)F_1 aged 2.5 months received a single intraperitoneal injection of ^{238}Pu nitrate (pH 2; dose range, 7–1850 Bq/g bw). The plutonium content in the testis represented 0.02–0.04% of the amount injected. The average absorbed doses of α-radiation in the testis were 0.02–0.96 Gy, and the dose rates from α-radiation were 0.004–1 cGy/day. In order to evaluate the relative biological effectiveness of α-radiation from ^{238}Pu, the mice were exposed continuously to γ-radiation from ^{137}Cs at doses of 0.92–4.5 Gy. Exposure to α-radiation from ^{238}Pu was shown to induce dominant lethal mutations, reciprocal translocations, fragmentation of chromosomes and abnormal sperm-head morphology. No association with the average dose of α-radiation to the testis was observed for any of the end-points. The biological effectiveness of α-radiation relative to continuous exposure to γ-rays for the end-points studied was reported to be 10–20 (Pomerantseva *et al.*, 1987a,b, 1988).

(iii) *Americium*

In a study of fetal toxicity, rats were injected intravenously at various stages of gestation with high doses of ^{241}Am citrate (3–15 μCi/g bw; 111–555 kBq/g bw) and killed two days later. The amount of ^{241}Am retained in the placenta was 9–15 times higher than that in the fetuses at all times tested. The smaller the quantity of injected ^{241}Am, the larger the fraction of this radionuclide that was retained in the placenta. More ^{241}Am was transferred to the fetus towards the end of pregnancy. No malformations were noted, but injections at early stages of gestation led to lower fertility and higher intrauterine lethality (Moskalev *et al.*, 1969).

These and later studies have shown consistently that a smaller fraction of injected americium than plutonium enters the conceptus or fetoplacental unit. Proportionately less americium was selectively deposited in the placenta and membranes than that observed with plutonium. It is not clear if this difference in distribution is responsible

for the prenatal effects, but it is consistent with the higher incidence of embryolethality and malformation after administration of ^{239}Pu than of ^{241}Am at the same activities (Sikov *et al.*, 1986).

A group of 59 male BALB/c mice, 11 weeks old, were injected intraperitoneally with 103 Bq/g bw of ^{241}Am citrate, and groups of 15–62 14-week-old females received 45, 90 or 213 Bq/g bw. There were about 30 male and female controls. In a second experiment, pregnant mice were injected intravenously with 100, 500 or 1500 Bq/g bw of ^{241}Am citrate on day 14 of gestation, and the offspring were reared by untreated dams. The controls were sham-injected pregnant mice whose litters were raised by other dams. The longevity was slightly but significantly shortened by exposure of adult mice to all doses of americium; the response was non-linear. The longevity of the offspring exposed *in utero* was not reduced (Van den Heuvel *et al.*, 1995).

(*e*) *Hydrogen*

Experiments were performed to investigate the prenatal effects of ^{3}H in mice. Superovulated $BC3F_1$ (C57BL/C3H) female mice, 10–12 weeks old, were caged overnight with ICR males; the day after mating, as detected by a plug, was designated day 0. Two-cell embryos in the late G_2 phase and early S phase were isolated and exposed to $^{3}H_2O$ at 100–2000 μCi/mL [3.7–74 MBq/mL] or external γ-rays. In another experiment, the fallopian tubes of superovulated virgin $B6C3F_1$ mice were isolated and inseminated *in vitro* with sperm from ICR male mice, and the early pronuclear stage was exposed to $^{3}H_2O$ or γ-rays. More than 95% of the control embryos developed to the blastocyst stage. The immediate effect of irradiation was a delay in cleavage among embryos, which was severe in pronuclear embryos exposed to γ-radiation. Although a few embryos died during the cleavage period, most continued through several divisions before arrest at the morula stage, after which they degenerated. The LD_{50} values (in MBq/mL) were 15.8 for late G_2 phase two-cell embryos, 8.5 for early S phase two-cell stage embryos and 4.4 for the pronuclear stage; the differences were statistically significant. Similar LD_{50} values were obtained with γ-rays. The biological effectiveness of β-radiation from $^{3}H_2O$ relative to γ-radiation did not differ significantly from 1, and was < 2 (Yamada *et al.*, 1982).

In similar studies with mice (strain: Radiologisches Institut, Freiburg), an average of 92% of 178 control embryos was reported to develop to the blastocyst stage within 66 h. Development was not significantly inhibited by exposure to $^{3}H_2O$ at a concentration < 370 kBq/mL. The percentage that reached the blastocyst stage declined progressively, and the incidence of degeneration at the morula stage, or earlier, increased as the concentration of $^{3}H_2O$ increased. No blastocysts developed at the highest concentration (18.5 MBq/mL). In experiments with [^{3}H]thymidine, only 83% of the 21 embryos reached the blastocyst stage when exposed to 0.74 kBq/mL, and a concentration of 18.5 kBq/mL almost completely inhibited development to the blastocyst stage. [^{3}H]Thymidine was about 1000 times more effective than $^{3}H_2O$. Further analyses indicated that $^{3}H_2O$ inhibited the late stages of blastulation, while [^{3}H]thymidine delayed

the rapid cleavage stages. This conclusion is compatible with the intracellular distribution of energy (Streffer *et al.*, 1977).

The radiotoxicity of [^{3}H]thymidine and [^{3}H]arginine in early mammalian embryo development was studied in two-cell stage mouse embryos (strain, Radiologisches Institut, Freiburg) isolated 30 h after conception and incubated *in vitro* with 370 or 925 Bq/mL of each of the radiolabelled compounds up to the formation of the inner cell mass, 192 h after conception. No difference in the radiotoxicity of the two compounds was seen with respect to cell proliferation. In contrast, the formation of blastocysts, the outgrowth of trophoblasts and the formation of inner cell mass were impaired more strongly by [^{3}H]arginine than by radiolabelled thymidine, at similar external concentrations. Increased micronucleus formation was found 96 h after conception with the higher concentration, [^{3}H]arginine again being more effective than [^{3}H]thymidine. The authors noted that arginine is taken up more rapidly, so that the intracellular dose remained longer; furthermore, histone synthesis is not restricted to the S phase during early development, and thus arginine was incorporated while thymidine incorporation was delayed until the start of DNA synthesis (Müller *et al.*, 1987).

An assay in mouse embryo chimaeras was used to determine whether the radiosensitive target for the effects of ^{3}H on embyronic cell proliferation is nuclear or extranuclear. This in-vitro assay involves aggregating an irradiated cleavage-stage embryo with an untreated embryo, culturing them for two or three cell cycles and then dissociating them to determine the number of cells contributed by each of the two embryos. With eight-cell embryos from superovulated CD1 mice cultured in ^3H_2O or [^{3}H]thymidine, the concentrations were adjusted so that both radioactive compounds would deliver comparable calculated doses to the nucleus, which resulted in about 100-fold greater extranuclear doses delivered by ^3H_2O. In a control experiment, embryos were exposed to ^{137}Cs γ-rays. Fifteen to 20 embryos were incubated at a range of concentrations for 2 h during the S phase of the eight-cell stage, aggregated with unirradiated fluorescein isothiocyanate-labelled control embryos and incubated for about 20 h. Chimaeras of two untreated embryos (one labelled with fluorescein isothiocyanate) were used as controls. Each embryo was then partially dissociated and examined to obtain the total cell number and the number of unlabelled (non-fluorescent) cells, which were expressed as a ratio. The ratios were averaged for each dose group, and differences between the three modalities were compared. At the highest nuclear doses (up to 1 Gy), a small but statistically significant decrease in proliferation ratio was seen with all three treatments. [^{3}H]Thymidine consistently produced lower mean proliferation rates than ^3H_2O over the dose range 0.14–0.43 Gy. The authors concluded that the radiosensitive target for effects on embryonic cell proliferation is nuclear (Wiley *et al.*, 1994)

In studies on the dosimetry and effects of ^{3}H on rat embryos and fetuses, rats [strain and numbers not stated] were injected subcutaneously with ^3H_2O (0.3–11.1 MBq/g bw) at several times before mating or during gestation. Effects were found at all but the lowest dose. Injection before implantation led to failure of implantation or

defects that resulted in intrauterine death. The typical disorders in dams, fetuses and placentas were vascular. Both the maternal and fetal parts of the placenta showed oedema and congestion. Oedema and subdermal haematomas were found in 21% of 17–19-day fetuses given a dose of 3.0 MBq/g bw and in 41% at 11.1 MBq/g bw, but no other histological changes were detected in the fetus (Moskalev *et al.*, 1969).

Pregnant Sprague-Dawley rats were maintained throughout pregnancy at constant activities of ^3H_2O at 0.04–37 MBq/mL of body water via the drinking-water, providing doses of 3–300 mGy/day to the embryo and fetus. The biological effects included sterility, reduced growth, reduced litter size, increased resorptions and microencephaly. The ^3H incorporated into fetal organs represented 20–30% of the average maternal ^3H_2O activity during gestation (Cahill & Yuile, 1970).

In a similar study, groups of six Sprague-Dawley rats received ^3H-labelled drinking-water from conception of the F_1 generation through delivery of the F_2 generation. The equilibrium concentrations were 0.37, 3.7, 37 and 370 kBq/mL, which gave daily dose rates of 0.03–30 Gy to the dams in each group. The offspring were weaned at 21 days, and two males and two females from each litter continued to receive ^3H_2O. At about 110 days, the females were bred to males at the same dose but from different litters, and exposure was continued through the birth of the F_2 animals. Ovaries and term fetuses were removed for evaluation, while other fetuses and F_1 males were used for radioanalysis. No morphological abnormalities were observed in offspring of either generation; the litter sizes and postnatal growth of the F_1 generation were unaffected, although the highest dose resulted in a 30% reduction in testis weight at 125 days of age. Statistically significant effects were seen in the F_2 generation, including increased resorption and decreased litter size at the highest dose, although there appeared to be no difference in the preimplantation death rate. Birth weight was decreased at 37 and 370 kBq/mL, and the relative brain weights were reduced at all doses except 0.37 kBq/mL. The maximum tissue concentrations were reached during exposure *in utero*, with concentrations in the brain and testis that were greater than the average tissue concentration (Laskey *et al.*, 1973).

In a similar study, ^3H_2O produced a dose-related reduction in brain weight in F_2 neonates, and differences in eye opening and righting reflex, startle reflex and hypoactivity in a residential maze were reported (Cahill *et al.*, 1976).

Sprague-Dawley rats were injected intraperitoneally on day 1 of gestation with ^3H_2O and given ^3H-labelled drinking-water to maintain an equilibrium level of 0, 37, 370 or 3700 kBq/mL throughout gestation, which yielded a calculated cumulative dose of 0, 0.066, 0.66 or 6.6 Gy to the conceptus over the gestation period. Dose-related reductions in body weight and brain weight were seen after exposure to 0.66 or 6.6 Gy, but the relative brain weight was reduced only at 6.6 Gy. No changes were found in a variety of neurological indicators (Bursian *et al.*, 1975).

In another series of studies, large amounts of ^3H_2O were given by a single injection to evaluate the effects on prenatal and postnatal development. NMRI mice were injected intraperitoneally with ^3H_2O at doses of 2.5–50 MBq/g bw on day 7, 9 or

11 after conception. The doses of 40 and 50 MBq/g bw were lethal to many of the dams within the 11-day period after injection on day 7, and all embryos were resorbed in the litters that were available for sacrifice and evaluation. The rate of resorption was about 50% per dam at 30 MBq/g bw (14 litters, on day 9), with a significantly elevated frequency of dead fetuses and reduced fetal and placental weights. More than half of the live fetuses had cleft palate. Injection of 5 MBq/g bw on day 7 or 9 (13 litters each) significantly increased the frequency of resorption and decreased the fetal and placental weights. The dose of 20 MBq/g bw on day 9 or 11 (12 litters each) produced even greater reductions in weights, but the rate of resorption was increased only at the earlier time. A low incidence of skeletal anomalies was found in the fetuses of dams at 20 and 30 MBq/g bw. Histological examination of some of these fetuses showed that administration of the lower dose on day 9 or 11 of gestation retarded brain histogenesis and resulted in hypoplasia of the gonads (Török *et al.*, 1979).

In a study of postnatal development, groups of nine pregnant NMRI mice were injected with ^3H_2O at a dose of 2.5, 5 or 10 MBq/g bw on day 9 after conception. Litter size and perinatal mortality were not affected, although the number of deaths before weaning was increased at the highest dose, and birth weight and growth rate were reduced at 10 MBq/g bw. Mating tests at two months of age showed that the offspring of dams given 10 MBq/g bw were not fertile (both sexes), and female but not male offspring at 5 MBq/g bw had reduced fertility. There was no effect on the rate of mortality in the interval between weaning up to 4–5 months of age, when most offspring were killed. The weights of the gonads were reduced in males and females at all doses, and the brain weight was reduced at the higher doses. The ovaries were often cystic; the number of oocytes was drastically reduced at the lowest dose, and they were virtually absent at the highest dose. The seminiferous epithelium was in a state of disintegration in the four males at the lowest dose examined histologically and was absent in most of the tubules of five males at the high dose; spermatozoa were observed only occasionally (Török *et al.*, 1979).

In a multi-generation study, 35-day-old male inbred C57BL/6M mice were given ^3H_2O in drinking-water at a concentration of 370 kBq/mL for 35 days. The mice were then mated to unexposed females. The offspring were separated after weaning, and the new generation of males was given ^{3}H-labelled drinking-water according to the same dose regimen, followed by mating with unexposed siblings. This sequence was repeated until the 18th generation. The number of offspring and the sex ratio were recorded. Some mice of each generation were maintained for life, and others were killed. At the ninth generation, 50 pairs from the two lines were entered into a separate evaluation to obtain data on parameters such as fertility in subsequent generations. In general, there was a progressive reduction in the relative fertility of the successive generations of exposed males, which became apparent as reduced litter sizes, reduced birth weights and increased perinatal mortality, and was paralleled by increased intrauterine mortality (Méwissen *et al.*, 1984).

In a study of morphological changes in the cerebral cortex after exposure to ^3H during the neonatal period, one-day-old Swiss albino mice were injected [route unstated] with ^3H_2O at a dose of 120.62 kBq/mL of body water [assumed to represent 90% of the body weight] and then maintained with their dams on ^3H-labelled drinking-water at 185 kBq/mL throughout the experimental period. The young mice [numbers not stated] were killed at intervals up to six weeks of age. The total radiation dose delivered was calculated to be about 64 mGy/week. In sections of the brain, the overall thickness and the thickness of the visual region of the cerebral cortex relative to that of controls was significantly decreased at five and six weeks *post partum*. Decreased relative thickness was also observed in other regions of the cortex. Changes were found in total cell packing density, which became consistently statistically significant at five weeks of age. Glial packing density increased during the first and second weeks (Bhatia & Sisodia, 1988).

In a study on the testicular effects in the F_1 generation, pregnant mice were given an initial intramuscular injection of ^3H_2O at a dose of 118 kBq/mL of body water (assumed to represent 62% of the body weight) on day 16 of gestation and were then maintained on ^3H-labelled drinking-water at 185 kBq/mL. F_1 mice were killed at three, four or five weeks of age, the testes were fixed and prepared as histological sections, and cells in various stages were counted. The calculated total doses delivered were 0.23, 0.29 and 0.35 Gy at the three times. At three weeks of age, pronounced vacuolization of the cytoplasm was found in the testis, with pyknotic nuclei in many tubules. Many of these showed giant cells, haemorrhage and oedema of interstitial tissues, which led to an increased tubular diameter at four weeks; a lesser degree of damage was seen at five weeks, when fibrosis was the primary feature. By three weeks of age, the numbers of type A, I and B spermatogonial cells were reduced to about 80, 70 and 60% of the control values, respectively; these levels remained approximately the same throughout the remainder of the experiment. Various stages of spermatocytes were maintained at about 70% of the level of controls, and spermatids at 45% of control levels. The usual appearance of sperm in the lumen by five weeks of age was not observed (Bhatia & Srivastava, 1982).

A similar design was used in a study of the effect of exposure to ^3H_2O at various gestational stages. Pregnant Swiss albino mice [number not stated] were divided into three groups: one group received an intramuscular injection of 74 kBq/mL of body water at day 0 (preimplantation), day 6 (organogenesis) or day 14 (fetal period) of gestation and was then maintained on a 34% higher concentration of ^3H in the drinking-water on days 0–5, 6–12 and 14–18, respectively. Further groups of animals were given H_2O by injection at a dose of 111 or 185 kBq/mL of body water and then maintained on ^3H-labelled drinking-water during similar periods of gestation. Control pregnant females were injected with distilled water. Animals in all groups were killed on day 18 of gestation for examination of the uterus, implantation sites and prenatal mortality. During the preimplantation period, but not during the other periods, the average number of implantation sites was significantly reduced and the percentage of

resorbed embryos increased at all doses. The percentage of resorbed embryos was also increased by exposure to the higher two doses during organogenesis. Only a few types of anomalies were seen, which included open eyelids in roughly one-third of the fetuses exposed during organogenesis (the actual percentage increased with dose) and a few instances of 'short tail' at the two higher doses (Sharma & Saini, 1993).

In studies on the effects of exposure to ^3H on brain development, 14 Sprague-Dawley-derived female rats were given ^3H_2O at a concentration of 111 kBq/mL, the average daily intake being 2.9 MBq. To study the effects in newborn offspring, ^3H_2O was supplied from 30 days before pregnancy until parturition. For the multigenerational study, ^3H_2O was also supplied after birth to the offspring and their lactating dam until they reached maturity. At this time, the female offspring were mated with control males, and the process was repeated for three additional generations. Animals were analysed as newborns or at 30 or 120 days of age. None of the treated animals showed indications of radiation illness. The blood cell volume and number, blood glucose and bone marrow were not significantly altered, although alkaline phosphatase activity in blood was significantly decreased in exposed when compared with control rats. The various measures of pregnancy outcome, including litter size, neonatal body weights and weights of the cerebral hemispheres, were essentially the same as in the controls. The total DNA content of the newborns' brains showed a slight decrease in all generations, but this varied in magnitude and was not statistically significant in all cases. The protein concentration was decreased in all generations except F_3, and the reductions were statistically significant; the protein:DNA ratio relative to the controls was variable across generations (Zamenhof & van Marthens, 1979, 1981).

As part of a longitudinal study to evaluate the behavioural effects of prenatal exposure to ^3H_2O, C57BL/6J pregnant mice received a single intraperitoneal injection at 12.5 days of gestation, to give a calculated total cumulative absorbed dose *in utero* of 50, 100 or 300 mGy. The pregnant control mice received saline. The litters were weaned at 21 days, and male offspring were used for behavioural tests; there was a total of 110 offspring from 59 mothers. The exposed animals, especially at the two higher doses, showed hyperactivity in the open field test at day 21, but they were hypoactive at 100 days of age. Other tests showed that the exposed animals had difficulties in both learning and memory retention for skilled performance (Wang & Zhou, 1995).

In a study on the effect of exposure to ^3H_2O in the squirrel monkey (*Saimiri sciureus*), groups of 28–43 females were assigned to one of seven dose groups on the day of insemination and given an intraperitoneal injection of ^3H_2O to produce the desired body concentration. They were then maintained on ^3H_2O for the rest of the study. The concentration of ^3H_2O was adjusted upwards twice during the experiment to achieve preselected body water concentrations. The mean radioactivity of the drinking-water during weeks 7–33 was 0, 3, 11, 27, 52, 107 and 218 kBq/mL in the seven dose groups, respectively. The animals were weighed at four-week intervals, and urine samples were collected. Blood samples were obtained from the progeny within 24 h of birth, and they were then killed and samples taken for radioanalysis and

histological evaluation. There were three to nine full-term deliveries in each group, and a total of four full-term deliveries were stillborn. The numbers varied but there were essentially no differences among the groups in terms of delivery rate, proportion of discernible abortuses or gestation time. With occasional exceptions that were considered not meaningful, the body dimensions, body weights and organ weights did not correlate with the concentration of ^3H in the drinking-water. Haematological parameters were also unaffected. All of the 46 full-term progeny and four of 10 abortuses were necropsied and examined. No correlation was found between the distribution of grossly observable lesions and ^3H_2O concentration. Except in the gonads, the few histological lesions observed did not indicate a dose–response relationship, and the lesions reported in neonatal rats (see Zamenhof & van Marthens, 1979) were not observed in these monkeys. The ovaries of the newborn animals were markedly affected by exposure to ^3H, but the testes were not. The control progeny had ovaries with many large oocytes, each surrounded by inconspicuous follicular cells and little connective tissue. Particularly at the highest dose, few oocytes were discernible in the exposed groups (Jones *et al.*, 1980).

Male and female Hale-Stoner-Brookhaven random-bred mice [numbers not stated], four weeks of age, were exposed continuously to ^3H-labelled drinking-water at 111 kBq/mL. Control groups received tap water. When the animals reached eight weeks of age, they were bred randomly within their treatment group to produce offspring, which were maintained on ^3H_2O or tap water. When the offspring reached eight weeks of age, there were divided into four groups with exposure to ^3H_2O continued for females only, for males only, for males and females or terminated. Significantly more early embryonic deaths and fewer viable embryos were found when both parents or only the female received ^3H_2O. The authors concluded that the lower sensitivity of males was the result of elimination of radiation injury during the several cell divisions that occur in spermatogenesis (Carsten & Commerford, 1976).

Pregnant rats of the Donryu strain [number not stated] received a single dose of 50, 75, 100, 125 or 150 mCi of ^3H_2O [1850, 2775, 3700, 4625 or 5550 MBq] by intraperitoneal injection on day 8 or 9 of gestation. The rats were killed on day 18, and their fetuses were examined for external and internal abnormalities. Thirteen anomalous fetuses were found among the 327 control fetuses, which included nine with defects of the ventricular septa, three with defects of the vascular ring and two cases of umbilical hernia. All the implants of dams injected with 125 mCi or 150 mCi on day 8 of gestation (and with 150 mCi on day 9) were dead. At 100 mCi, 6/14 and 13/16 fetuses of dams injected on days 8 and 9, respectively, survived; all had anomalies, particularly in the cardiovascular system. There were fewer deaths and malformations at the lower doses at either time (Satow *et al.*, 1989).

As indicated in section 3.3, [^3H]thymidine has been of interest from a radiobiological standpoint, especially relative to tumorigenesis, because part of it is incorporated into the DNA of proliferating cells while the remainder is rapidly catabolized and excreted. The incorporated [^3H]thymidine remains in the DNA until the cell divides, at

which time the radioactivity is partitioned among the daughter cells, or until the cell dies, after which it can be reused. Because of their short range, the β-particles emitted by [^{3}H]thymidine in DNA selectively irradiate the nucleus.

A system of continuous intravenous infusion by means of a small pump delivering 1 mL/day to pregnant rats was used to compare the effects of [^{3}H]thymidine and ^3H_2O. Autoradiographic evaluation showed that all cell nuclei in newborn rats are labelled with [^{3}H]thymidine after continuous exposure of the dam from day 9 of gestation through term, and that there is a linear relationship between the dose administered to the dam and the incorporation into DNA and non-DNA fractions in various organs of the developing rat. Four-month-old Wistar rats were infused continuously with [^{3}H]thymidine or ^3H_2O on days 9–21 of gestation. The concentrations of each compound (0.8 and 1.6 μCi/g bw [29.6 and 59.2 kBq/g bw] per day of [^{3}H]thymidine and 8 and 16 μCi/g bw [296 and 592 kBq/g bw] per day of ^3H_2O) had been shown previously to reduce the numbers of oocytes by approximately 50 and 95%. On the basis of the dose to the nuclei of oocytes, the biological effectiveness of [^{3}H]thymidine relative to that of ^3H_2O was calculated to be 3.7. The oocyte depletion test was used as the biological end-point, and ^{3}H incorporation at birth as the basis for the dose calculation (Schreml & Fliedner, 1977).

Delayed and late effects of exposure to ^3H_2O and [^{3}H]thymidine *in utero* were investigated in pregnant SAS/4 mice that received ^{3}H-labelled drinking-water from day 1 of gestation at a concentration of 1.8, 11, 16 or 30 μCi/mL [66.6, 407, 592 or 1110 kBq/mL]. Another group of mice received in-dwelling subcutaneous catheters to provide continuous infusion of [^{3}H]thymidine during the 12 days between day 7 of gestation through term, which resulted in total amounts of ^{3}H infused of about 10, 27, 50 and 130 kBq/g bw. The surviving fraction of oocytes of all types decreased progressively as a function of total body ^{3}H at birth after administration of ^3H_2O or [^{3}H]thymidine, the latter being two to three times more effective than ^3H_2O. Growth and survival were adversely affected at the highest doses (Lambert & Phipps, 1977, 1983).

A solution of [^{3}H]thymidine was injected intraperitoneally at a dose of 7.4 MBq/mL into two-month-old male mice [strain not stated]. The total dose per mouse (11.1 MBq) was given in six fractions over two days, which produced uniform labelling of a wide band of maturing sperm. Since the early spermatocytes are the latest cells to pick up thymidine during spermatogenesis, the first labelled sperm would appear in the fifth week after injection. From the fourth week onwards, injected males were mated with untreated females. After mating, the uterine contents of some of these females were analysed by autoradiography. The first labelled sperm appeared on day 30 after the first injection, when 34% of all sperm were labelled. By day 32, all sperm cells were labelled. The proportion of labelled sperm decreased to 40% on day 40 after the first injection. The other females were dissected on day 13 of pregnancy, and the frequency of dominant lethal mutations was scored. This frequency paralleled the degree of labelling of the sperm (Bateman & Chandley, 1962).

(*f*) *Carbon*

The genetic effects associated with exposure to [^{14}C]glucose were investigated in male (CBA × C57Bl)F_1 hybrid mice. In one experiment, the compound was administered orally as an aqueous solution to three groups of 10 mice, each at graded doses that resulted in calculated radiation doses to the gonads after three months of 0.22, 0.5 and 1 Gy, respectively. In a second experiment, the animals received curds with labelled glucose for 33 days, resulting in estimated gonadal doses of 0.74 and 1.47 Gy at the end of this period. In the third experiment, the glucose was given in drinking-water for six or 12 months. The doses to the gonads were estimated to be 0.006 and 0.031 Gy at six months and 0.013 and 0.066 Gy at 12 months. Males were mated to females at intervals after exposure, and the frequencies of dominant lethal mutations, reciprocal translocations and sperm-head abnormalities were evaluated. Pre- and post-implantation losses occurred during the first mating intervals after single exposures, and pre-implantation losses occurred during the second interval at the two higher doses, indicating dominant lethal mutation of pre-meiotic cells and also post-meiotic cells. There was a transient decrease in male fertility at the high dose, apparently due to spermatogonial death, with subsequent recovery. After the 33-day exposure, only post-meiotic exposure increased post-implantation loss at both doses. In both of these experiments, the frequency of abnormal sperm heads was not clearly different from that in controls, but translocations were more frequent than in controls although not dose-related. Exposure in the third experiment did not affect the frequency of reciprocal translocations, but it increased the frequency of sperm-head abnormalities at 12 months, although this frequency was not altered at six months. The higher dose reduced fertility at both six and 12 months, but this was apparently not related to genetic change (Pomerantseva *et al.*, 1983).

(*g*) *Phosphorus*

Pregnant Sprague-Dawley rats received ^{32}P as sodium phosphate by intraperitoneal injection at a dose of 37 or 111 kBq/g bw on day 16, 18 or 20 of gestation. Measurements of radioactivity in femur and liver indicated a total dose per fetus of 0.1 Gy, with an inhomogeneous distribution, as judged from autoradiography, that resulted in the highest doses to the skeleton. The results suggested a substantial reduction in the lifespan of offspring after exposure of the dam to 111 kBq/g bw but not to 37 kBq/g bw. The testes of male offspring of dams at the high dose were about 25% of the normal size and showed histological changes, including an absence of spermatogenesis. Castration cells were seen in the pituitary. Nine adult animals of each sex were injected with 37 kBq/g bw and mated with fertile unexposed rats to assess effects on fertility. Only three of the treated females and two of the treated males were fertile (Berry *et al.*, 1983).

Pregnant Wistar rats were injected intraperitoneally with 18.5, 37, 55.5 or 74 MBq of ^{32}P phosphate in saline solution on day 6, 8, 9 or 10 of gestation and killed and

dissected at intervals thereafter for determination of radioactivity. A second series of animals received graded doses of 11.1–74 MBq of ^{32}P at the same stages of pregnancy as the first group. These animals were killed on day 14 of gestation. A dose-related decrease in fetal weight was found in all treated animals. At the dose that killed about 50% of fetuses, their weight was about 60% of that of controls. The LD_{50} values for each day of injection differed significantly from that on all other days. Gross and detailed histological examinations of the fetuses showed consistent patterns of growth retardation and anomalous morphology. In general, the incidence and severity increased with increasing dose, and the type of malformation was somewhat dependent on the gestational day at injection. After exposure on day 6 of gestation, some of the 14-day old fetuses had the appearance normally seen at 13 days. The malformations included limb reduction, decreased size of the mandible and occasional instances of incomplete closure of the facial grooves. Histologically, some fetuses at the highest doses showed a distinct decrease in the density of the liver cords, and the sinusoids were almost completely devoid of fetal erythrocytes. The malformations after injection at day 8 or 9 were similar but more severe and frequent. They also included ocular and facial defects, with cases of anophthalmia at the latter time. Defects of the eye were most common after injection on day 10, as were facial defects and a marked reduction of limb size. Several of the fetuses in this group showed decreased density of liver cords that were similar to those described above (Sikov & Noonan, 1957, 1958).

The effects of exposure to ^{32}P later in gestation were evaluated in subsequent studies with pregnant Wistar rats receiving a dose of 22.2, 37 or 74 MBq on day 14 or 17 of gestation. Animals were killed at intervals after injection, starting after 8 h and then daily afterwards. Other animals received an injection of 7.4 or 14.8 MBq and were kept until the birth of their litters. The number of live births per litter was reduced by injection of 74 MBq at either time but was not affected at lower doses. Injection of 74 MBq at 14 days produced fetal deaths during the last two days of gestation, while neonatal deaths attributable to the trauma of parturition were also noted. No prenatal deaths were seen with injection on day 17; a few deaths occurred during the birth process. A total of 33 litters were born, and there was no delay of parturition. Birth weight was reduced at a dose of 14.8 MBq at 14 days, and injection of the higher doses at 17 days produced statistically significant decreases in birth weight (Sikov & Lofstrom, 1957).

Injection of a low dose (7.4 MBq) of ^{32}P on day 14 or 17 of gestation led to subdural haemorrhage at birth and the preceding days, with bleeding into the abdominal viscera and brain at higher doses. There was a general decrease in the size of the skeleton in late fetuses and newborns after injection on day 14 of gestation, with alterations in the relative dimensions of several bones at high doses; injection on day 17 produced less pronounced changes. Administration of the highest dose (74 MBq) on day 14 decreased the density of lymphocytes in the cortical region of the thymus; the effect was even greater with injection on day 17. The effects on the gonads were variable; the effects of 74 MBq given on day 14 of gestation ranged from relatively normal testes to large degenerated areas, depressed mitotic rate and a pronounced decrease in the number of

primordial spermatagonia. Only a moderate decrease in mitotic rate was found after injection on day 17 (Sikov *et al.*, 1958).

The effect of radiophosphorus on the development of the anterior pituitary was studied in Swiss albino mice. A dose of 37 kBq/g bw injected intramuscularly into pregnant females seven days after fertilization did not affect the pituitary of the fetuses or newborn mice. The same dose given intraperitoneally to one-day-old mice caused hypertrophy of acidophilic cells in the pituitary. After injection of this dose into seven-day-old mice, cell death and an increased number of acidophils were seen, but a decreased number was observed in 14- and 21-day-old animals. Males and females differed markedly with respect to effects on the pituitary when injected at the age of 21 and 28 days, the females showing more severe damage (Dev & Srivastava, 1981).

(*h*) *Strontium*

The effects of ^{90}Sr on the development of the ovaries in fetal mice were studied in CBA mice injected intravenously on day 11 of gestation with 185, 370 or 740 kBq of ^{90}Sr nitrate per animal. Five randomly selected female offspring from each dose group were killed at 56 days of age, and the histological structure of one ovary was examined. Starting at 70 days of age, the remaining females (19–26 per group) were tested for fertility by mating with an untreated male for 100 days, at which time they were killed and the ovaries prepared for microscopic evaluation. There were marked quantitative changes in the histological composition of oocyte and follicle types in the controls between 56 and 170 days of age, including a substantial reduction in the total number of cells per ovary. The numbers of cells at both times were reduced to about 50% of control values by the dose of 185 kBq. A progressively greater reduction was seen at the higher doses, which was more marked at 170 than at 56 days. The earliest ovarian developmental stages (oocytes I–III and primordial follicles) were most sensitive. The mating tests did not indicate effects on fertility, as judged by the fraction of females that were fertile, the mean litter size and the total number of young weaned. Thus, despite the considerable reduction in the number of oocytes, the pool of mature follicles was adequate for production of litters at normal frequency and of normal size (Rönnbäck *et al.*, 1971).

In a subsequent study of similar design, 370 kBq ^{90}Sr nitrate were injected to female rats on day 8, 11, 13, 16 or 19 of gestation. When the offspring reached the ages of 28, 56 and 84 days, five randomly selected females in each group were killed and the ovaries prepared for histological examination. The control ovaries showed the expected changes with age. Exposure to ^{90}Sr decreased the total number of cells per ovary relative to the control, and the effect was progressively more marked with exposure later in gestation. As in other studies, the oocytes and primordial follicles were the most sensitive to radiation (Rönnbäck, 1979).

In a further study of similar design, pregnant CBA mice were injected with 185 kBq of ^{90}Sr on day 19 of gestation, and some of the litters were cross-fostered with controls at birth. This provided four groups of five or six litters that received either no

^{90}Sr, prenatal exposure to ^{90}Sr but control milk, no prenatal exposure but milk with ^{90}Sr or ^{90}Sr *in utero* and in milk. The rate of postnatal mortality was increased among mice exposed to ^{90}Sr, especially those that received exposure only in milk. No malformations were observed. As in other studies, the ovaries of mice that received prenatal and postnatal exposure contained significantly fewer cells and showed retarded cell differentiation. A smaller but significant reduction in the number of ovary cells was found in the group that received only prenatal exposure to ^{90}Sr, while the group that received only ^{90}Sr in milk showed an even smaller effect, which was not statistically significant (Rönnbäck, 1981a).

In a further study, the effects of radiostrontium on fertility after exposure in late gestation were investigated, and the reproductive capacity of individual CBA female mice was correlated with changes in the histology of their ovaries after mating. Pregnant CBA mice were injected intravenously on day 19 of gestation with ^{90}Sr nitrate at a dose of 46, 92, 185, 370 or 740 kBq, and the female offspring (15–28 per group) were bred from 12 weeks of age with control CBA males throughout a seven-month period of continuous breeding. The number of litters born, the time between the litters and the numbers of live offspring were recorded; the litters were weaned at 20 days, just before the birth of the next litter. The mating period was considered to have been completed when more than 40 days had passed after the last birth for more than 50% of the females. This occurred at about 10 months of age, at which time they were killed and their ovaries prepared for microscopic examination. One control female, one at 370 kBq and 19 of the 24 at 740 kBq were infertile. The other five mice in the latter group became pregnant and delivered one litter each, with an average interval of 21.6 days between mating and delivery. All the other females produced one or more litter. The interval between the start of mating and the birth of the first litter increased with increasing dose (except for the highest dose), but the time to birth of the last litter was not correlated. Measures of the total reproductive capacity of the females were not markedly affected, except at 370 kBq and 740 kBq, where there was a significant decrease. A progressive decrease in litter size was seen with dose (other than the lowest) during most intervals; however, fertility was not affected by doses below 370 kBq per dam. Microscopic examination of the ovaries showed that even the 46-kBq dose produced a 37% reduction in the total number of oocytes and follicles. The ovaries were severely depleted of follicles and oocytes at higher doses, but there were multiple corpora lutea at lower doses (Rönnbäck, 1981b).

In an experiment performed to obtain information on gonadal effects in male offspring after prenatal exposure of the female parent to radiostrontium, pregnant CBA mice were injected intravenously on day 19 of gestation with a dose of 92.5, 185 or 370 kBq of ^{90}Sr nitrate. The male offspring of these females and of controls were killed in groups of four to six at 14, 28 or 56 days of age, and the testes were weighed and prepared for histological examination and quantification. There was no effect on relative or absolute testis weight, measured at 28 days. The two lower doses had no microscopic effect. At 28 days, the dose of 370 kBq resulted in an almost complete absence of elongating spermatids, a number of cross-sections showing spermatocytes

as the most advanced cell type. The number of round spermatids at 28 days was decreased, but only at the highest dose. The weight of the testes, the number of spermatids per cross-section and the relative proportion of the various cell types were unaffected in animals evaluated at 56 days. A second experiment was performed to determine the time course of changes in spermatogenesis at a dose of 740 kBq of ^{90}Sr. In the control animals, almost all of the tubular cross-sections showed spermatocytes at 14 days, but spermatagonia were the most advanced cell type found in 30% of the tubules of the exposed animals. The spermatocytes that were present were generally less advanced than in the controls. At 28 days, all tubular cross-sections showed spermatocytes in controls and exposed animals, although they were somewhat more advanced in the controls; no differences were observed at 56 days. The fertility of males from litters that had been exposed *in utero* to 740 kBq of ^{90}Sr was evaluated by mating each of 20 males to a control female for about 140 days. Eight untreated couples served as controls. Reproductive capacity was measured in terms of the number of litters, mean litter size and time interval between litters for each couple. None of these parameters was affected by exposure. Counts of Leydig cells relative to Sertoli cells at 28 days showed no difference between the exposed and the control animals. The authors concluded that the fetal testis is less sensitive to the effects of radiation from radiostrontium than is the ovary. Although germ cells may be killed, the results suggest that the surviving stem cells restore their numbers, even though this might result in a delay of differentiation (De Rooij & Rönnbäck, 1989).

(*i*) *Iodine*

The uptake of iodide by the fetal thyroid has been measured in several studies, but the kinetics of iodine in the fetus is not well understood. Zanzonico and Becker (1992) modified previous metabolic models and calculated fetal absorbed doses in clinically relevant situations such as in hyperthyroid pregnant women receiving radioiodine therapy. Analysis of data from 17 published and unpublished reports of cases in which pregnant women had received therapeutic radioiodine indicated that thyroid function at birth was not affected when the mother had been treated before the 10th week of pregnancy. When radioiodine was administered after that time, even at doses < 555 MBq, fetal thyroid function was at great risk.

A case report was published of radiation-induced sterility after administration of massive doses of ^{131}I to a 24-year-old man as therapy for metastatic thyroid cancer. The patient was pretreated with thiouracil. A dose of 246 mCi [9.1 GBq] of ^{131}I was administered and another 317 mCi [11.7 GBq] after seven months. The disease was apparently arrested six years after treatment. The patient, who had previously been fertile, was evaluated for fertility three years after treatment: his ejaculate was aspermic, and biopsies of the testis showed a complete lack of germ cells in tubules, with no evidence of spermatogenesis. The Sertoli and Leydig cells appeared normal, but the amounts of pituitary gonadotrophins in the patient's urine were markedly increased. The authors concluded that injury due to treatment with ^{131}I was responsible for the infertility. They

noted that a previously reported case showed similar findings, and that comparable changes in women had been noted in the literature (Kammer & Goodman, 1959).

A six-month-old conceptus from a pregnancy that was terminated when it was detected during a protracted course of external radiotherapy and repeated doses of ^{131}I for papillary thyroid cancer was studied. The woman had received 3.7 GBq of radioiodine twice during the pregnancy, during the second and 22nd weeks. It was estimated that the radiation dose to the fetal thyroid was 370 Gy; actual measurements of radioactivity allowed extrapolation to a fetal thyroid dose of 260 Gy. The fetal thyroid was undersized for its age, atrophic and sclerotic, and fibrosis and calcifications were seen microscopically. Although post-mortem changes could not be excluded, complete 'necrobioses' of follicular epithelial cells were seen, and autoradiography showed ^{131}I deposition in these areas and none in adjacent fibrotic areas (Arndt *et al.*, 1994).

The effect of exposure of pre-implantation Swiss Webster mouse embryos to [^{125}I]iododeoxyuridine *in vitro* was compared with that of irradiation with ^{137}Cs γ-rays. ^{125}I decay is characterized by localized deposition of its energy through emission of numerous low-energy Auger electrons. The dose for survival of 37% of the mouse embryos was about 15 cGy for the ^{125}I Auger electrons and 175 cGy for the γ-rays (Narra *et al.*, 1991).

It has been established that iodine does not concentrate in the fetal thyroid gland until follicles are observable. The offspring of mice injected with high doses of iodine had necrosis, fibrosis and compensatory hyperplasia of the thyroid. These and other studies showed that injection of pregnant animals with iodine after onset of fetal thyroid function leads to retarded neonatal growth of the offspring, a phenomenon that is also observed after neonatal exposure. A similar physiological dependence has been demonstrated during development of the human thyroid (Speert *et al.*, 1951).

In a study of the early and delayed effects of ^{131}I relative to age at exposure, young adult male and female Sprague-Dawley-derived rats, pregnant rats at 19 days of gestation, weanlings (21 days) and newborns were injected intraperitoneally with carrier-free radioiodine in dilute sulfite solution at a dose of 18.5, 37 or 111 kBq/g bw or with the sulfite solution alone. The rats were fed a low iodine diet for one week before and one week after injection, when they were returned to the stock diet. Three randomly selected rats from each age and dose group were killed at one, three and seven days after injection. The fraction of the ^{131}I that was incorporated into the thyroid and its retention were different for the four age groups. Proportionately less ^{131}I was retained at higher doses as a consequence of radiation damage to the gland, especially in the older animals. Four months after injection, a dose-dependent retardation of overall growth was seen, with statistically significant differences in the body weights at the highest dose. The reduction in size of the thyroid at this time was statistically significant in all age and dose groups and was most pronounced in male animals exposed *in utero* on day 19 of gestation. Four months after the initial injection, some of the remaining rats were placed on a low-iodine diet for one week and then injected with a tracer dose of 1 μCi [37 kBq]

of carrier-free ^{131}I to test thyroid function. Thyroid radiosensitivity was quantified in terms of the initial doses required to reduce incorporation or iodine-trapping capacity to 50% of that in the controls four months after exposure. The resulting values were 9.7 Gy in the fetuses, 53 Gy in the newborns, 120 Gy in the weanlings and 180 Gy in the adults (Sikov, 1969).

Microscopic evaluations of thyroid specimens from rats in the older age groups in the preceding study (exposed as young adults or weanlings) showed dose-related degeneration of the thyroid, which was followed by fibrosis; this was presumed to be responsible for the reduced thyroid function. In the two groups exposed in the perinatal period (*in utero* or as newborns), however, inhibition of thyroid growth and a failure of differentiation into definitive follicles were the primary morphological changes. Constriction of the trachea underlying the thyroid was seen, which was most pronounced in the two youngest groups, and the severity was dose-dependent. This effect was similar to changes that had been reported in sheep and, on the basis of the histological appearance, seemed to result from failure of the segments to develop (Sikov *et al.*, 1972).

(*j*) *Cerium*

Weanling and adult Sprague-Dawley rats were injected intravenously with ^{144}Ce chloride at a dose of 9.3, 18.5 or 37 kBq/g bw, and newborn animals were injected intracardially. The animals were radiographed at intervals, and some from each group were killed for radioanalysis, histological examination and assessment of bone strength. The cerium-exposed weanlings showed only a slight decrease in femur strength, in contrast to the results of exposure to plutonium (see section 4.3.3(*d*)(ii)) (Mahlum & Sikov, 1969).

4.4 Genetic and related effects

Interactions of ionizing radiation with various components of living cells induce many different types of molecular damage, which lead to diverse cellular responses. It is well established that efficiency in producing biological damage varies with radiation type. For many biological responses, DNA is believed to be the critical target. Ionizing radiation causes various types of DNA damage, ranging from isolated base damage, single-strand breaks or simple double-strand breaks to more complex DNA alterations involving clustered damage sites with multiple breaks and/or base changes within a few base pairs. The tertiary structure may lead to damage over longer distances. The more complex forms of damage are potentially unique to ionizing radiation and are not seen spontaneously or with other DNA-damaging agents. Subsequent processing by enzymes may accurately repair radiation-induced damage, re-establishing the normal sequence and structure. Alternatively, the processing may fail and lead to alterations in DNA, which may be in the form of changes in DNA sequence, deletions or genetic rearrangements, with large alterations seen as

chromosomal aberrations. These alterations may lead to the death of the cell or to viable inherited mutations (Hutchinson, 1985; Goodhead, 1994; Ward, 1994).

4.4.1 *α-Particle emitters*

(*a*) *In-vitro studies*

(i) *DNA double-strand breaks*

Measurements of DNA double-strand break induction in mammalian cells induced by high-LET, slow α-particles (3.0–3.4 MeV) from an external source (^{238}Pu, ^{241}Am) have shown a biological effectiveness relative to that of X- or γ-radiation of < 1 (Coquerelle *et al*., 1987; Prise *et al*., 1987; Fox & McNally, 1990; Jenner *et al*., 1993), although earlier studies indicated a value of 1.6 (Blöcher, 1988) or 3.5 (Kampf & Eichhorn, 1983), whereas values for cell inactivation and mutation of up to > 6 and > 10 have been found (depending on dose), respectively, in the same cell line (Thacker *et al*., 1982). Experimental studies have shown a significantly reduced ability of cells to rejoin α-particle-induced double-strand breaks when compared with those produced by low-LET X-rays and γ-rays after incubation of cells at 37 °C (Coquerelle *et al*., 1987; Jenner *et al*., 1993), with < 50% rejoining after 3 h, while most low-LET radiation-induced breaks rejoined within 1 h. Evidence for increased clustering of damage on DNA after exposure to α-particles comes from experiments in which plasmid DNA was irradiated under conditions that mimic the cellular environment with respect to scavenger capacity. In this study, a cell-free system derived from human embryo kidney cells was used to determine the rejoining of single-strand breaks produced by α-particles, which was found to be significantly less than that of breaks induced by γ-radiation. In addition, 50% of the α-particle-induced single-strand breaks were converted to double-strand breaks, compared with only ~12% of those induced by γ-radiation (Hodgkins *et al.*, 1996).

(ii) *Chromosomal and chromatid aberrations*

α-Particles have been shown to induce chromosomal aberrations (Purrott *et al*., 1980; Welleweerd *et al*., 1984; Griffin *et al*., 1995; Simmons *et al*., 1996) and micronuclei (Bilbao *et al*., 1989; Mill *et al*., 1996) in many studies of transformed and primary mammalian cells irradiated *in vitro*. In primary human fibroblasts, a large proportion (38–47%) of the exchange aberrations observed were complex, resulting from three or more breaks in two or more chromosomes. The complex aberrations most frequently observed were insertions (Griffin *et al*., 1995). Sister chromatid exchange following irradiation with α-particles has also been shown in human lymphocytes in G_0 phase (Aghamohammadi *et al*., 1988) and in V79 hamster cells in G_2 and S phases (Griffin *et al*., 1994). Significantly increased frequencies of sister chromatid exchange have been observed in both human and rodent cells exposed to doses of α-particles (from a ^{238}Pu source) as low as 0.31 mGy (Nagasawa & Little, 1992; Deshpande *et al*., 1996): 30% of the cells showed an increased frequency of sister chromatid exchange at

this dose, although < 1% of the cell nuclei were traversed (Nagasawa & Little, 1992). This implies that the cell nucleus need not be hit directly in order to produce sister chromatid exchange (Nagasawa & Little, 1999; see also section vii below).

(iii) *Mutation*

The lethal effect and the induction of reverse gene mutations by α-radiation was studied in *Salmonella typhimurium* strain TA1538, which has a mutation that increases the permeability of the cell wall. ^{239}Pu citrate (pH 7.0–7.3) was used as a source of α-radiation, admixed with the culture medium, and was given at doses of 74–18 500 kBq/mL. The control series contained bacterial culture and sodium citrate in the same concentration. The results showed dose-dependent cell killing and induction of gene mutations. The dependence was exponential. The estimated LD_{37} and LD_{50} values were 34.8 Gy and 21.8 Gy, respectively, and the estimated mutation doubling dose was 19 Gy (Gafieva & Chudin, 1988).

The hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) mutation system is widely used for quantitative studies to detect mutations in mammalian cells, ranging from single-base changes to large intrachromosomal deletions (Albertini *et al.*, 1982). α-Particles typically induce a higher frequency of mutants per unit dose in rodent and human cell lines than low-LET radiation (Thacker *et al*., 1982; Chen *et al*., 1984; Metting *et al*., 1992; Griffiths *et al*., 1994; Bao *et al*., 1995), with relative biological effectiveness values as large as 7–10. Smaller values of 2–3 have been reported for *Hprt* mutation after α-particle irradiation of some hamster and mouse lines, and, after account was taken of survival, the effectiveness was similar or inferior to that of X-rays. This appears to be due to the highly effective cell killing and mutagenesis of low-LET radiation on these cells when compared with other rodent cell lines. The mutagenic effectiveness of low-LET radiation is more variable than that of high-LET radiation, presumably because cell lines have different abilities to repair damage due to low-LET radiation, while damage due to high-LET radiation is generally regarded as less repairable (Barnhart & Cox, 1979; Iliakis, 1984).

The cytotoxic and mutagenic effects of radon and its progeny in murine lymphoblast L5178Y-R16 cells were compared after exposure *in vitro* to a steady-state ratio of radon and its progeny (^{222}Rn:^{218}Po:^{214}Po = 1:3.5:4.5) under various experimental conditions. In one series of experiments, the cells were added to growth medium, through which a filtered mixture of radon and air had previously been passed for a ≥ 4-h equilibration period, giving a dose rate of 0.03–0.1 Gy/h. In a second series, a mixture of radon, CO_2 and air was passed over the medium overnight before the cells were added and continued throughout the 2–4-h incubation period, giving a dose rate of 0.1–0.6 Gy/h. In the third experiment, cells were added to growth medium containing ^{212}Bi — a decay product of ^{220}Rn — in the presence or absence of 0.1 mol/L diethylenetriaminepentaacetic acid, a nontoxic chelator for bismuth. In all cases, a dose-dependent increase in the induced frequency of mutation at the thymidine kinase locus was found. The frequency as a fraction of the surviving cell fraction was similar in the three experiments. The mutation

frequency was lower for a given dose of chelated compared with unchelated bismuth (Evans *et al.*, 1993).

In experiments with Chinese hamster ovary cells containing a single copy of human chromosome 11, direct evidence was obtained that passage of a single α-particle from a microbeam through the cell nucleus can induce mutation in surviving cells, measured as loss of all or part of the human chromosome (Hei *et al.*, 1997). In the same system, mutations were reported to be induced when α-particles traversed the cytoplasm, with little or no cell killing (Wu *et al.*, 1999).

Deletion-pattern analysis of α-particle-induced mutations at the *Hprt* locus of V79 Chinese hamster cells revealed a larger fraction of deletions than that caused by X-rays for the same level of survival. Furthermore, non-contiguous, partial deletions were present among the α-particle-induced mutants, which were not found after X-irradiation (Schmidt & Kiefer, 1998). In contrast, no difference was found in the ratio of large deletions:point mutations at doses of low- or high-LET radiation that resulted in about 20% survival, in mutations at the *HPRT* and *Hprt* loci classified by molecular analysis (Thacker, 1986; Aghamohammadi *et al.*, 1992). The average size of radon-induced deletions of the *HPRT* gene in human TK6 lymphoblasts was not as large as those produced by X-rays (Bao *et al.*, 1995; Chaundhry *et al.*, 1996).

(iv) *Mutations in tumour-related genes*

Activation of the Ki-*RAS* protooncogene and inactivation of the *TP53* tumour-suppressor gene are events common to many types of human cancers.

After exposure of rats by inhalation of a $^{239}PuO_2$ aerosol, resulting in an initial lung burden of about 100 nCi [3.7 kBq], specific Ki-*ras* point mutations were present in 46% of the radiation-induced malignant neoplasms of the lung. Spontaneous pulmonary neoplasms, which are rare in rats, contained similar activating mutations and frequencies (40%), and similar mutation frequencies were found in radiation-induced adenomas and foci of alveolar epithelial hyperplasia. No mutations were identified in normal lung tissue, and *ras* expression in hyperplastic lesions and neoplasms was similar to that observed in normal pulmonary epithelia (Stegelmeier *et al.*, 1991). Further studies indicated that *p53* gene mutations are relatively unimportant in the development of most lung tumours in rats exposed to ^{239}Pu by inhalation (Kelly *et al.*, 1995; Belinsky *et al.*, 1997).

Immunohistochemical studies of gene alterations in lung tumours from beagle dogs exposed to $^{239}PuO_2$ by inhalation and in lung tumours from unexposed dogs indicated that activation of the K-*ras* gene is not essential for the development of lung tumours in either exposed or unexposed dogs. The study also indicated that elevated expression of *p53* is infrequent in canine lung tumours (Tierney *et al.*, 1996). In a study of lung tumours from 25 beagle dogs exposed to $^{239}PuO_2$ by inhalation (Griffey *et al.*, 1998), the rate of K-*ras* mutations (8%) was higher than that described in canine plutonium-induced lung tumours (see above) but lower than that reported in spontaneous canine lung cancers (16%), spontaneous human non-small-cell lung cancer (13–36%) (Fong

et al., 1995) and spontaneous lung cancer (40%) or lung tumours in rats exposed by inhalation to $^{239}PuO_2$ (46%) (Stegelmeier *et al.*, 1991). These results suggest that there are species differences in the involvement of K-*ras* in the development of plutonium-induced lung tumours. [The Working Group noted that specific mutations in tumour tissue may not be directly attributable to radiation.]

(v) *Cell transformation*

The induction of oncogenic transformation by α-particles has been reported from a number of laboratories where different α-particle sources and different cell culture systems were used (Lloyd *et al.*, 1979; Robertson *et al.*, 1983). Although no significant increase in the induction of preneoplastic transformation of primary rat tracheal epithelial cells was observed when the cells were exposed directly on a planar ^{210}Po α-source (distance to source, 0–9 μm) (Ford & Terzaghi-Howe, 1993), up to 10-fold increases were seen when the cells were exposed to similar fluences of α-particles from remote ^{238}Pu and ^{241}Am sources (distance, 18 μm) (Terzaghi-Howe *et al.*, 1996). The results suggest that the geometry of the tracks of α-particles through the cell and the range of LETs to which the cell is exposed are important in determining the probability of cell survival and transformation.

Malignant transformation was induced in immortalized human bronchial epithelial cells in culture by a single 300-mGy dose of α-particles. The transformed cells were able to produce progressively growing subcutaneous tumours after inoculation into athymic nude mice (Hei *et al.*, 1994). Exposure of SV40-immortalized human thyroid epithelial cells *in vitro* to single doses (0.14–1.57 Gy) of 3.26-MeV α-particles also induced malignant transformation. Tumours were detected 50–160 days after subcutaneous transplantation of the irradiated cells into athymic mice. The first estimate of the relative biological effectiveness at peak tumour induction was 3.8 (Riches *et al.*, 1997). The oncogenic transformation potential of precisely known numbers of α-particles from a microbeam traversing mammalian cell nuclei has been measured. Traversal of the nucleus of a C3H10T½ cell by a single α-particle was found to be significantly less effective in inducing cell transformation than traversal by a mean (from a Poisson distribution) of one α-particle. Furthermore, the single particles were not significantly more effective than no irradiation (Miller *et al.*, 1999). α-Particle-induced C3H10T½ transformants were reported to be less tumorigenic after injection into mice than transformants induced by X-rays. This was ascribed to induction by the α-particles of genomic instability in the parent cells of the neoplastic foci. Although tumours produced by the X-ray-induced transformants appeared earlier, they grew at similar rates to those produced by α-particles (Lehane *et al.*, 1999).

(vi) *Genomic instability*

Radiation has been shown to induce genomic instability, a characteristic of which is a longer delay between exposure and the appearance of an effect, despite a number of mitotic divisions (Morgan *et al.*, 1996; Little, 2000).

Genomic instability has been observed in clones of cultured CBA/H mouse haematopoietic stem cells derived from marrow irradiated in vitro with 0.25–1 Gy of α-particles from an external ^{238}Pu source (Kadhim *et al.*, 1992). These doses correspond to the passage of an average of 0.5–2 α-particles through each cell. Aberrations were observed in approximately 50% of the clones that survived irradiation and were mostly chromatid-type aberrations, suggesting they had arisen after many generations, whereas aberrations occurred in only 2% of survivors of X-irradiation. Similarly delayed chromosomal effects were observed in α-particle-irradiated bone-marrow samples obtained from two of four normal human subjects (Kadhim *et al.*, 1994). When bone-marrow cells obtained from a male mouse were irradiated with α-particles *in vitro* and subsequently transplanted into female recipients, the repopulated haematopoietic system showed chromosomal instability that persisted for up to at least one year (Watson *et al.*, 1996).

The frequency of mutation at the *Hprt* locus was measured in clonal populations of Chinese hamster ovary cells derived from single cells that survived exposure to doses of 2–12 Gy of X-rays or 2 Gy of α-particles from an external ^{238}Pu source. Approximately 8–9% of the clonal populations showed high frequencies of late-arising mutations when examined 23 population doublings after irradiation, as indicated by mutation fractions 10^2–10^4-fold greater than the background. These results confirm that radiation can induce a type of transmissible genetic instability in some surviving cells that can lead to persistently increased frequencies of new mutations in their progeny for up to 23 population doublings after exposure (Little *et al.*, 1997). Studies of the clonal descendants of murine haematopoietic stem cells in vitro revealed a 5–10-fold increase in the frequency of non-clonal *Hprt* mutations after high- and low-LET irradiation at similarly effective killing doses (Harper *et al.*, 1997).

(vii) *'Bystander' effects*

Cellular effects, including mutations and chromosomal aberrations, can result not only from radiation tracks directly through the nucleus but also from tracks through the cytoplasm (Wu *et al.*, 1999), and some responses can be induced in nearby 'bystander' cells (Little, 2000). As mentioned above, Nagasawa and Little (1992) observed sister chromatid exchange in 30% of a population of Chinese hamster ovary cells after exposure to low doses of α-particles, in which < 1% of the cell nuclei were actually traversed. Subsequent studies of primary human fibroblasts confirmed this finding, with a threefold higher frequency of cells with an increased number of sister chromatid exchanges (Deshpande *et al.*, 1996) or a five times higher *HPRT* gene mutation frequency (Nagasawa & Little, 1999) than predicted from the actual number of nuclei traversed. In an experiment with mouse bone-marrow cells, the use of a shielding grid between the α-particle source and these cells gave the expected reduction in cell killing of approximately 50%, but no reduction in chromosomal instability was observed from that seen after irradiation without the grid. These results show that α-particles induce chromosomal instability in the progeny of unirradiated

cells due to unexpected interactions between irradiated and unirradiated cells (Lorimore *et al.*, 1998).

(*b*) *In-vivo studies*

In the study of Guilmette *et al.* (1989) in Chinese hamsters, described in section 3.1.2(*c*), the frequency of chromosomal aberrations in animals exposed to $^{232}ThO_2$ was 0.47/cell per Gy and was similar to that in animals exposed to ^{239}Pu citrate.

The effects of α-emitting radionuclides were studied *in vivo* in Chinese hamsters injected intravenously with ^{239}Pu citrate (0.6 μCi or 22.2 kBq/kg bw) or $^{239}PuO_2$ particles (diameter, 0.17, 0.30, 0.44 and 0.84 μm) with activities up to 6 μCi/kg bw [222 kBq/kg bw]. The distribution of the particles was traced by labelling them with ^{51}Cr. They were found to concentrate in the reticuloendothelial system, such that 90% of the injected activity was in the liver several days after injection, 3% in the spleen and the remainder in the bone and bone marrow. ^{239}Pu citrate produced a linear increase in chromosomal aberration frequency with increasing dose. After injection of $^{239}PuO_2$, the aberration frequency in the liver again increased with increasing average organ dose, the response plateauing at high doses, and seemed to be independent of particle size (Brooks *et al.*, 1974).

The effects of internally deposited radionuclides in the testis of male mice and their offspring have been studied after intravenous injection of α-particle-emitting radionuclides. No significant difference from age-matched controls was found in the frequency of reciprocal translocations in primary spermatocytes of (C57BL/Cne × C3H/Cne)F_1 mice 724 days after a single intravenous injection of 185 Bq (7.5 kBq/kg bw) of monomeric ^{239}Pu citrate (Pacchierotti *et al.*, 1983). With a higher level of injected activity, there was again no significant increase over that in controls in the translocation frequency in spermatocytes of (C3H/HeH × 101/H)F_1 mice (at 21, 28 and 34 weeks) after intravenous injections of 4 μCi/kg bw [148 kBq/kg bw] ^{239}Pu citrate (Searle *et al.*, 1976).

Although initial experiments indicated no significant excess in the rate of intra-uterine death among offspring of male CBA mice injected intravenously with up to 0.5 μCi [18.5 kBq] per animal of ^{239}Pu nitrate solution, significant differences were found in subsequent experiments in males after intravenous injections of 0.05–0.5 μCi [1.85–18.5 kBq] ^{239}Pu citrate. The animals at the higher doses became sterile between 12 and 20 weeks. At the lower doses, there was a significant excess of intra-uterine deaths in matings from week 4 onwards and an increasing proportion of late deaths in offspring. The lower doses seemed to have as severe a genetic effect as the higher doses. Among the offspring of F_1 males, a general increase was seen in the rate of intra-uterine death and an excess proportion of late deaths as compared with the controls (Lüning *et al.*, 1976).

In a study of the frequency and spectrum of chromosomal aberrations in somatic cells after exposure to ^{239}Pu, male Wistar rats were given a single intravenous injection of 23.2, 46.3 or 92.5 kBq/kg bw of $^{239}PuO_2$ (particle size, 1–2 μm). Metaphase spreads

were prepared from a bone-marrow cell suspension at 8, 32, 128, 256 and 412 days after the injection. The frequency of chromosomal aberrations in myelokaryocytes during the period of observation was increased by a factor of 1.7, 2.3 or 3.7, corresponding to the three doses of $^{239}PuO_2$ injected (Nikolaevskaya *et al.*, 1988).

The dynamics of the frequency and spectrum of chromosomal aberrations was studied in hepatocytes of rats after exposure to polymeric ^{239}Pu nitrate. Male Wistar rats were given a single intravenous injection of 18.5, 55.5 or 166.5 kBq/kg bw of ^{239}Pu polymer in nitric acid (pH 1.5). In previous experiments, it had been shown that these doses of ^{239}Pu resulted in liver cirrhosis. Metaphase spreads were prepared from homogenates of the regenerating liver (after partial hepatectomy) and analysed 16–365 days after the injection of the radionuclide. Two-phase changes in the frequency of chromosomal aberrations were observed. The frequency of aberrations increased significantly 16 days after exposure, decreased considerably during the following 1.5 months and increased again 256–365 days after exposure. The increase in the frequency of structural damage to chromosomes 16 days after exposure was due mainly to aberrations of the chromatid type, while the contribution of chromosome-type aberrations increased for longer after exposure, and the frequency was dose-dependent. No dose-dependence was observed for the early increase (Zakharova *et al.*, 1988).

Increased frequencies of chromosomal aberrations were detected in cells removed from the lungs of Syrian hamsters 24 h after exposure to $^{238}PuO_2/ZrO_2$ microspheres, giving an initial lung burden of approximately 140 nCi [5.18 kBq] (Stroud, 1977), and the induction of micronuclei in a range of cell types (deep lung fibroblasts and epithelial cells, tracheal and nasal epithelial cells) was increased in male Wistar rats exposed to radon and its progeny (up to 564 working-level months) (Brooks *et al.*, 1997). A similar effect was reported in mouse lung macrophages after exposure of female CBA/Ca mice to $^{238}PuO_2$ by inhalation (initial alveolar deposit, 67 and 424 Bq) (Kellington *et al.*, 1992).

Rhesus monkeys were exposed by inhalation to a $^{239}PuO_2$ aerosol, to achieve initial lung burdens of 2–1800 nCi [0.07–66.6 kBq]. The inhaled $^{239}PuO_2$ was retained in the body with an effective half-life of 1000 days, with some translocation from the lungs to pulmonary lymph nodes. Cytogenetic damage to blood lymphocytes was assayed at various times during the 43-month period of the study. Only animals with a cumulative lung dose > 10 Gy showed a significant increase in the frequency of rings and dicentrics when compared with controls (La Bauve *et al.*, 1980). Studies on specific-locus mutations in $(101 \times C3H)F_1$ mouse spermatogonial stem cells after injection with 0.37 MBq/kg bw of ^{239}Pu suggest that plutonium is two to three times more effective in producing mutations than protracted γ-radiation but much less effective than neutrons. Furthermore, the genetic damage induced by ^{239}Pu appeared to be more severe than that induced by low-LET radiation (National Council for Radiation Protection and Measurements, 1987b).

(*c*) *Human studies*

(i) *Workers exposed to radionuclides and residents of neighbouring areas*

Plutonium: Under industrial operating conditions, the most likely route of intake of plutonium is by inhalation of contaminated dust or droplets, although occasionally other exposure routes may be important (see section 1). If the material is insoluble, the principal region of deposition is the lungs and their associated lymph nodes. Soluble material is quickly transferred to the blood and then deposited preferentially in the bones and the liver, some being excreted.

Chromosomal aberrations in human peripheral blood lymphocytes are a recognized indicator of exposure to ionizing radiation *in vivo*. An increase in the frequency of chromosomal aberrations above the background level reflects direct exposure of circulating lymphocytes and also exposure of haematopoietic precursor cells in the bone marrow — either stem cells or cells during proliferation and maturation. Irradiated mature lymphocytes show a variety of symmetrical exchanges (translocations, inversions and insertions) and asymmetrical exchanges (dicentrics, centric rings and interstitial deletions); however, lymphocytes derived from irradiated haemopoietic precursor cells generally contain mainly symmetrical aberrations owing to selection during repeated divisions.

A banding technique that allows recognition of many symmetrical aberrations which would be missed with conventional staining was used to analyse peripheral blood lymphocytes from 54 plutonium workers from the British Nuclear Fuels facility at Sellafield, United Kingdom. These workers had body burdens in excess of 296 Bq and were divided into three groups by urine analysis of plutonium; all had been exposed at least 10 years before the analysis. These workers had also been exposed to significant levels of external γ-radiation. The controls were 39 newly hired workers with no known exposure to radiation or known clastogenic chemicals. The control group included more non-smokers and consisted of younger (average age, 33.7 years) persons than the exposed groups (average ages, 51–52 years). All groups of plutonium workers showed increased frequencies of both symmetrical and asymmetrical chromosomal aberrations over those in controls. The frequency of symmetrical exchanges exceeded that of asymmetrical exchanges in all groups, including the controls. The formation and survival of radiation-induced aberrations was randomly distributed among the chromosomes according to length. The distribution of the break-points within the cells showed an excess in the centromeres and telomeres (Tawn *et al.*, 1985).

Twenty-four of the workers in the above study were still employed at Sellafield and therefore available for resampling 10 years later. Analysis of chromosomes in G-banded peripheral blood lymphocytes was performed on two groups of workers who had 20–50% and > 50% of the maximum permissible body burden of plutonium. A significant increase was found in the frequencies of symmetrical aberrations in both

groups when compared with workers with similar histories of exposure to mainly external γ-radiation but with little or no intake of plutonium and with controls with negligible exposure, estimated to be < 50 mSv. In contrast, no significant differences in asymmetrical aberrations were found. As the latter are short-lived, this suggests that the recent exposure of mature lymphocytes was minimal. The frequencies of symmetrical aberrations had increased significantly since the earlier sampling time. Additional external exposure was negligible over this period. The results indicate that haematopoietic precursor cells are irradiated by internally deposited plutonium, and that subsequent selection results in only cells with symmetrical aberrations reaching the peripheral lymphocyte pool (Whitehouse *et al.*, 1998).

Peripheral blood from 22 workers at the Rocky Flats plutonium facility, Colorado, USA, was analysed for the presence of sister chromatid exchange and chromosomal aberrations. These workers were exposed to radiation from internal deposits of plutonium, continuous external irradiation and single or multiple chemicals. Sister chromatid exchange is sensitive to some chemical mutagens, while chromosomal aberrations are induced by moderate to high doses of ionizing radiation. The workers were grouped according to their internal burdens of plutonium (< 148, 148–740 and > 740 Bq). A significant increase in the frequency of chromosomal aberrations when compared with the control frequency was observed only in the cells of workers with > 740 Bq of internalized plutonium. There was no significant increase in the mean frequencies of sister chromatid exchange when analysed by estimated internal plutonium burden (Brandom *et al.*, 1990).

The frequency of symmetrical chromosomal translocations was measured in peripheral lymphocytes from 75 workers (40 men, 35 women, aged 53–80 years; mean, 66 ± 4) at the Mayak nuclear industrial complex (southern Urals, Russian Federation; see section 2.4.3). The workers received their main exposure between 1948 and 1963, approximately 35–40 years before blood sampling. Cumulative external γ-ray doses of 0.02–9.91 Gy and plutonium burdens of 0.26–18.5 kBq were reported. The controls consisted of 33 unexposed persons from uncontaminated areas of the southern Urals, aged 45–74 years (mean, 59 ± 8). Exchange aberrations (translocations) were scored by fluorescence in-situ hybridization with probes for chromosomes 1, 4 and 12, simultaneously with a pancentromeric probe. When compared with the control group, a significantly elevated translocation frequency was found for the total study group and for 48 subjects with and 27 without plutonium incorporation. The frequency of dicentrics did not significantly differ from that in the controls. The translocation frequency showed a significant dependence on external γ-ray dose, plutonium uptake having no substantial effect (Salassidis *et al.*, 1998).

Human T lymphocytes were used to determine the frequency and molecular spectrum of somatic gene mutations induced by ionizing radiation *in vivo* and *in vitro*. Blood lymphocytes from 17 former plutonium workers (mean age, 71.2 years) with a history of protracted exposure showed a 2.5-fold increase in *HPRT* mutation frequency when compared with unexposed adults of similar age (66–80 years). No increase in the

frequency of total gene deletions was found, which was consistent with the results for lymphocytes exposed to ^{222}Rn *in vitro*, but in contrast to the data obtained for humans exposed to ^{131}I (Albertini *et al*., 1997).

Uranium: Blood samples from 115 smokers (23–52 years of age) working in a nuclear fuel manufacturing facility who had been exposed to uranyl compounds over 1–25 years (mean lung dose, ~ 90 mSv) were analysed for various types of chromosomal aberrations. The control group comprised 94 smokers and 118 non-smokers who had not been exposed to uranyl compounds or any other known mutagens and belonged to the same age group. A significant increase in the frequency of chromosomal aberrations was found in the exposed smokers when compared with the control smokers. Smokers in the control group had a higher frequency of chromosomal aberrations than non-smokers, suggesting a clastogenic effect of smoking. The chromosomal aberrations observed in the exposed smokers were attributed to the cumulative effect of smoking and exposure to uranyl compounds (Prabhavathi *et al.*, 2000).

Cultured peripheral blood lymphocytes from 116 smokers and 80 non-smokers who were occupationally exposed to uranyl compounds were analysed for sister chromatid exchange. Blood samples were also collected from 59 control non-smokers and 47 control smokers who were not exposed to uranium. A significant increase in sister chromatid exchange frequency was observed among both smokers and non-smokers exposed to uranyl compounds when compared with their respective controls. In controls, a significant increase in the frequency of sister chromatid exchange was observed in smokers when compared with non-smokers (Prabhavathi *et al.*, 1995).

A cohort study was conducted with a group of miners from the Radium Hill uranium mine in South Australia, which was in operation from 1952 to 1961. Exposure to radiation underground in the mine was estimated from past measurements of radon gas. Persons who worked exclusively above ground according to the mine records were selected as controls. In 1991–92, the miners were interviewed, and blood was taken for measurement of somatic mutations. The mutation rates in *HPRT* and glycophorin A (*GPA)* were estimated with standard assay techniques. The frequency of homozygous mutations (NN) of *GPA* was increased in underground miners when compared with controls, and the mutation rate tended to rise with increasing exposure, except at the highest exposure (> 10 working-level months). However, there was no association between place of work and hemizygous (N0) mutations of *GPA* or the *HPRT* mutation. The authors concluded that there may be an association between *GPA* mutations and previous occupational exposure to ionizing radiation (Shanahan *et al.*, 1996).

A cross–sectional exploratory analysis of a possible relationship between environmental exposure to uranium and genetic effects included 56 volunteer residents from within a five-mile [9 km] radius around an uranium processing plant and 56 ‘control’ subjects from a geographically separate area who were not known to be exposed to uranium emissions. The groups were matched for age, sex and smoking habits. Three assays for human somatic gene mutations were carried out: the HPRT T-lymphocyte cloning assay to measure 6-thioguanine-resistant lymphocytes; the glycophorin A assay

to detect loss of expression of the M or N allele; and the micronucleus assay as a marker of chromosomal damage. The results showed no statistically significant difference between the groups. In both groups, age was significantly related to the *HPRT* mutant frequency (Wones *et al.*, 1995).

These studies of populations exposed to radiation from internally deposited radionuclides provide little consistent evidence for the induction of chromosomal or other cellular defects. The inconsistency may be related to the presence of other factors that affect aberration rates, such as external γ-rays, smoking, age and other chemical exposures, which were not adequately controlled for. Furthermore, several of the studies involved small numbers of subjects, and the findings are difficult to interpret. Because most radionuclides of plutonium or uranium are unlikely to be deposited in body sites from which there would be meaningful exposure of bone-marrow stem cells, it is not entirely clear that aberration frequencies should in fact be expected to be increased in the small numbers evaluated. The assessment of exposure was weak in many of the studies. As ^{238}U has a very low specific activity, it is unlikely that inhalation of even high concentrations would result in appreciable exposure of the bone marrow. Thus, these human studies provide little information on cellular damage induced by exposure to radiation from internally deposited radionuclides.

Radium: Chromosomes were studied in blood cultures from 62 women who had been radium-dial painters mainly in 1936–45 but also up to the middle of the 1950s. A whole-body counter was used to estimate the body burdens, which ranged from undetectable to as much as 0.56 μCi [20.7 kBq]; the women were allocated to one of three groups: 0–0.04 μCi [0–1.5 kBq] (nine women), 0.05–0.09 μCi [1.9–3.3 kBq] (20 women) and 0.10–0.56 μCi [3.7–20.7 kBq] (33 women). A control group of 57 women was chosen randomly from a general, representative population aged 35–64 years, a range similar to that of the luminizers. The proportion of cells with structurally abnormal chromosomes was higher in the luminizer population than among the sample of women without luminizing experience. The study also showed a consistent gradient of increasing structural abnormality with increasing body burden of radium (Boyd *et al.*, 1966). [The Working Group noted that the exposure during the painting of dials with radium also involved direct exposure to γ-radiation from the pots containing the paint and that the body burdens of radium would also be directly correlated to γ-ray exposure. Therefore, the association might be partly or entirely explained by occupational exposure to γ-rays.]

(ii) *Patients exposed to Thorotrast*

As Thorotrast is a colloidal solution, bone-marrow cells are exposed directly to α-radiation, and chromosomal aberrations can be detected in peripheral lymphocytes.

A 72-year-old man who had been given a 32-mL bolus dose of Thorotrast [not specified] during cerebral angiography performed in 1950 underwent whole-body radioactivity counts in 1993, which showed an estimated body burden of 4.65 g of thorium. This estimate may be in error by up to 50% owing to variation in counting

efficiency resulting from the distribution of thorium in various organs and in the weights of individuals. Peripheral T lymphocytes were cultured to quantify the frequencies and cellular distributions of asymmetrical and symmetrical types of chromosome aberrations in first-division metaphases and of micronuclei. Aberrations were scored by classic chromosome group analysis and chromosome painting techniques. *GPA* mutations in red blood cells were also analysed to obtain a relative measure of the damage sustained by the erythroid stem-cell population. About 30% of the lymphocytes in this patient contained one or more chromosomal aberrations, most of which were 'stable'. In addition, the frequency of unstable aberrations was significantly increased. Since any lymphocyte progenitor that sustained an asymmetrical aberration would not be expected to survive clonal expansion, mitogen-responsive T lymphocytes that bear dicentrics, rings or acentric fragments must be mature cells that were probably exposed to radiation in the recent past. Thus, radionuclides with long half-lives to which bone-marrow stem cells may be exposed would develop symmetrical and asymmetric aberrations. If the initial exposure occurred many years previously, the aberrations would be mainly symmetrical. Increased frequencies of *GPA* mutations were also observed, showing that genomic damage is induced in erythroid progenitors. The numbers of micronuclei in lymphocytes were only moderately increased when compared with the expected values for persons of comparable age (Littlefield *et al.*, 1997).

For a study of somatic mutation frequencies at the *GPA* and lymphocytic T-cell receptor (*TCR*) loci in erythrocytes from Thorotrast patients, peripheral blood samples were obtained from 18 Japanese men aged 67–83 (mean, 74 ± 4) years who had been treated with an unknown dose of Thorotrast 40–50 years previously. The control group consisted of male atomic bomb survivors aged 67–83 years, whose estimated dose of radiation had been < 0.005 Gy. Samples from 23 men were used for the assay of erythrocyte GPA and from 19 men for the assay of lymphocytic TCR. The men treated with Thorotrast had a significantly higher frequency of mutations at the lymphocytic *TCR* loci but not at the erythrocyte *GPA* loci (Umeki *et al.*, 1991).

Chromosomes in haematopoietic stem cells from the bone marrow of 50 Japanese veterans who had been injected with Thorotrast to evaluate injuries from war wounds and from two women who had been admitted to military hospitals (combined mean age, 65 years) were analysed. The frequency of cells with stable chromosomal abnormalities (4.35%) was significantly higher than that in the control group (0.48%), which consisted of 21 war-wounded veterans who had no record of Thorotrast administration (mean age, 66 years). Fourteen cases of clonal expansion of cells were found, with chromosomal aberrations in 11 patients. Clones observed in the cells of two of these patients had high frequencies of abnormalities (Tanosaki *et al.*, 1999).

The frequencies of chromosomal aberrations were measured 30–40 years after injection of Thorotrast in peripheral blood lymphocytes from 63 patients aged 49–77 years (average, 64.5 years). Of the 63 patients, 58 showed high frequencies of chromosomal aberrations (Sasaki *et al*., 1987).

(iii) *Residential exposure to radon*

DNA damage was measured in the alkaline single-cell gel electrophoresis ('Comet') assay in circulating lymphocytes in coded blood samples from 125 residents in 45 households in Sweden with various levels of ^{222}Rn in the drinking-water (10–2410 Bq/L) and indoor air (35–1025 Bq/m^3). Levels of radon in indoor air > 200 Bq/m^3 were found to be significantly associated with increased DNA damage in peripheral lymphocytes. No such correlation was detected for radon concentrations in the drinking-water, and there was no obvious relationship between the radon levels in drinking-water and in indoor air (Hellman *et al.*, 1999).

Chromosome analysis was performed on blood lymphocytes from 25 persons (14 female, 11 male aged 6–75 years (mean, 42 ± 21)) who had lived continuously in nine houses with indoor radon concentrations of 210–3000 Bq/m^3 (4–60 times the German average of 50 Bq/m^3). The mean frequency of cells containing dicentrics plus ring chromosomes and the incidence per cell of dicentrics plus ring chromosomes were significantly increased when compared with control levels. The mean frequency of symmetrical translocations, detected by means of fluorescence in-situ hybridization (target chromosomes 1, 4 and 12), in the group exposed to radon was slightly but not significantly increased. A similar tendency became apparent upon comparison of two groups of subjects exposed to above and below 2800 Bq/m^3-years (Bauchinger *et al.*, 1994, 1996).

The relationship between domestic exposure to radon and the occurrence of chromosomal aberrations, especially stable translocations, in peripheral blood lymphocytes was investigated by use of fluorescence in-situ hybridization with probes for chromosomes 1, 2 and 4. The study comprised a total of 84 non-smoking persons, divided into three groups according to indoor radon concentration: low (< 100 Bq/m^3; mean, 67 Bq/m^3), medium (200–400 Bq/m^3; mean, 293 Bq/m^3) and high (> 800 Bq/m^3; mean, 1737 Bq/m^3). The participants had lived for a minimum of 10 years in their present home. The groups were matched with regard to age, sex and medical exposure to radiation. Equal frequencies of translocations and other aberrations, e.g. dicentrics and complex rearrangements, were obtained in each group. As a significant correlation was found between translocations and age and the mean age was high (50 years), the genome-corrected frequency of translocations was high (about one in 100 metaphases). The study showed that continuous domestic exposure to high concentrations of radon did not increase the frequency of stable or unstable chromosomal aberrations (Lindholm *et al.*, 1999).

An apparent association ($p = 0.01$) was found between the log frequency of mutants at the *HPRT* locus in T lymphocytes from 19 non-smokers and indoor radon concentrations of 40–660 Bq/m^3 in their homes in England (Bridges *et al*., 1991). However, in a follow-up study by the same authors, no significant correlation was found between the *HPRT* mutant frequency or *BCL-2* translocation frequency and radon levels among 65 people in 41 houses in the same town (Cole *et al*., 1996).

(iv) *Mutations in tumour-related genes*

Two of five (40%) Thorotrast-induced hepatic angiosarcomas and five of 19 (26%) sporadic induced hepatic angiosarcomas contained K-*RAS*-2 gene mutations (Przygodzki *et al.*, 1997).

In nine cholangiocarcinomas and nine hepatic angiosarcomas from Thorotrast-exposed patients, only one *TP53* point mutation was found. This appeared to be a lower incidence than that in hepatocellular carcinomas in Europe as a whole (Andersson *et al.*, 1995c). In four hepatic angiosarcomas from Thorotrast patients, no *TP53* mutations (exons 5–8) were found (Soini *et al.*, 1995).

The *TP53* genes in lung tumours from 50 uranium miners in Germany and those in 13 liver cancers from Thorotrast-exposed patients were analysed. There was no evidence of a mutation hotspot at codon 249 of the *TP53* gene in either group, and no additional mutations were found in exons 5–8 in the Thorotrast-exposed patients (Hollstein *et al.*, 1997).

The association between residential exposure to radon and *TP53* mutations was investigated in samples of lung tumours from 83 non-smokers and 250 smokers obtained in a nationwide investigation in Sweden. The *TP53* status (exons 5–8) of a total of 243 tumours was determined. An increased prevalence of mutation was suggested among persons with heavy residential exposure to radon, but it was not significant. No specific mutational pattern was observed (Yngveson *et al.*, 1999).

In a study of *TP53* mutations (exons 5–7) in lung cancers from 16 former German uranium miners and 13 lung cancer patients without a mining history, no evidence was found for a mutational hot spot. Four of the tumours from miners contained mutations, two of which were double mutations. One G → T transversion was found in the only non-smoker (Popp *et al.*, 1999).

Mutations of the *TP53* gene were analysed in tumour and non-tumour tissues from 20 Thorotrast recipients who developed cancer, mainly of the hepatic bile duct and blood vessels. Of these patients, 19 were found to harbour *TP53* point mutations in their tumour tissue. Interestingly, *TP53* mutations were found even in non-tumorous tissues of the liver and small intestines, but at a lower frequency. The distribution pattern of the point mutations was significantly different in non-tumour and tumour tissues, most of the mutations in malignant tissues being located in the highly conserved domains of the *TP53* gene. It was noted that the predominant DNA damage expected as a result of exposure to α-radiation is deletion. The results support the idea that *TP53* alterations are important in the genesis of Thorotrast-induced tumours, but the point mutations may be the consequence of genomic instability induced by α-irradiation (Iwamoto *et al.*, 1999).

Genetic changes in the *TP53* gene were investigated in malignant liver tumours obtained at autopsy from Japanese patients with a history of Thorotrast treatment. These archival tissues were analysed for loss of heterozygosity at the 17p13 locus, followed by single-strand conformation polymorphism analysis and sequencing to detect mutations in exons 5–8 of the *TP53* gene. Fifteen cases were considered to be informative in terms

of polymorphism, and four cases showed loss of heterozygosity. Eight cases showed nine mutations in exons and two in introns, comprising seven transitions, two transversions and two deletions. It was suggested that the relatively large deletions, such as those indicated by loss of heterozygosity, could be attributed to the direct action of α-particles (Wada *et al.*, 1999).

Mutations of the K-*RAS* and the *TP53* genes were analysed in archival sections of intrahepatic cholangiocarcinomas from 22 Japanese patients, who had been injected with Thorotrast 33–49 years previously; 21 men had been injected between the ages of 20 and 30 years, and one woman had received treatment at the age of 14. The estimated total dose to the liver ranged from 2.7 to 22.1 Gy. For the analysis of *K-RAS* mutations, tumour tissues from four other Thorotrast-treated patients were included. The mutations in these two genes were compared with the spectrum in intrahepatic cholangiocarcinomas not associated with Thorotrast. The frequency of mutation of the K-*RAS* gene was lower (only one mutation found in 22 cases) while that of the *TP53* gene was more than two times higher (12 mutations in six of 22 samples) than in the non-Thorotrast-treated cases. The commonest mutation of the *TP53* gene was A → G transitions. *TP53* mutations were also found in non-cancerous areas of the livers in which Thorotrast had been deposited (Kamikawa *et al.*, 1999). [The Working Group noted that specific mutations in tumour tissue may not be directly attributable to radiation.]

4.4.2 *β-Particle emitters*

(*a*) *In-vitro studies*

(i) *Low-energy electrons*

Ultrasoft characteristic X-rays (0.1–5 keV) interact within cells, producing low-energy electrons similar not only to low-energy β-particles but also to the abundant low-energy secondary electrons produced by virtually all ionizing radiations (α-, β-, γ-, X-radiation and Auger emissions). Generally, the biological effectiveness relative to that of γ-rays and high-energy X-rays was seen to increase substantially with decreasing photon energy (and therefore electron energy) in a variety of cell lines for biological end-points such as DNA double-strand break induction (Prise *et al*., 1989; Botchway *et al*., 1997), cell inactivation (e.g. Goodhead & Thacker, 1977; Raju *et al*., 1987), chromosomal aberrations (Virsik *et al*., 1980; Griffin *et al*., 1998), mutations (Cox *et al*., 1977; Goodhead *et al*., 1979) and cell transformation (Frankenberg *et al*., 1995). In recent experiments, fluorescence in-situ hybridization was used with probes for chromosomes 1 and 2 to analyse chromosome exchanges in untransformed human fibroblasts exposed to 0.28-keV carbon K ultrasoft X-rays, which produce a single electron with a track length of < 7 nm. Despite the low energy and short range of these electrons, exchanges were produced with high efficiency. For simple exchanges between the target chromosomes, a linear dose–response relationship was observed, providing further support for the hypothesis that a single DNA lesion may form an exchange with undamaged DNA.

This suggests that the passage of a single electron may lead to genetic rearrangement (Griffin *et al.*, 1998).

(ii) *DNA strand breaks*

Methyl-labelled [^{3}H]- and [^{14}C]thymidine incorporated into DNA result in DNA single- and double-strand breaks, which are repaired rapidly (Cleaver *et al*., 1972; Cleaver & Burki, 1974; Burki *et al*., 1975; Sundell-Bergman & Johanson, 1980; Jorgensen *et al*., 1987). Incorporation of [5-^{3}H]uridine into RNA was found to be 80% less effective in causing single-strand breaks in DNA than incorporation of [^{3}H]thymidine in DNA, an intermediate result being obtained with cells irradiated by ^{3}H decays from $^{3}H_2O$ added to the medium before freezing (Burki *et al*., 1975). The Auger electron-emitter ^{125}I incorporated into DNA as iodo-2′-deoxyuridine was found to be significantly more efficient than [^{3}H]thymidine in introducing unrepairable DNA strand breaks in mammalian cells. The observed lack of repair may be due in part to the large number of ^{125}I decays per cell, which may interfere with enzymatic repair processes (Feinendegen *et al*., 1977; Sundell-Bergman & Johanson, 1980).

(iii) *Chromosomal aberrations*

Exposure of human lymphocytes *in vitro* to β-radiation from a ^{90}Sr/^{90}Y source (maximum β energy, 2.27 MeV) resulted in a biological effectiveness for micronucleus induction of 0.5 relative to 250-kVp X-rays (Mill *et al*., 1996). A dramatic increase in the frequency of chromosomal aberrations was observed after use of an Auger electron-emitting indium-111-labelled bleomycin complex, which binds to DNA in mouse glioma and human small-cell lung cancer cells, when compared with control incubations with bleomycin or $^{111}InCl_3$ (Hou *et al*., 1992). A number of studies in various test systems have shown an increased yield of chromosomal aberrations in cells exposed to β-radiation from $^{3}H_2O$, with a reported biological effectiveness relative to γ- and X-rays of 1.6–2.6 (Vulpis, 1984; Matsuda *et al*., 1986). The Auger electron-emitter ^{125}I incorporated into DNA in the form of [5-^{125}I]iodo-2′-deoxyuridine was considerably more effective than incorporated ^{131}I or [^{3}H]thymidine in cell killing and induction of chromosomal aberrations (Chan *et al*., 1976). Studies of chromosomal damage induced by the decay of [^{3}H]thymidine and [^{125}I]iodo-2′-deoxyuridine incorporated into the DNA of Chinese hamster cells indicated significantly greater effectiveness of ^{125}I than ^{3}H for induction of chromatid breaks (RBE, 17 ± 6), the sum of isochromatid breaks and chromatid exchanges (RBE, 21 ± 9) and the total number of chromatid aberrations (RBE, 18 ± 5) (Sundell-Bergman *et al*., 1985). An adaptive response *in vitro* in human lymphocytes after prior exposure to low-level irradiation from radioisotopes ([^{3}H]thymidine, [^{14}C]thymidine, $^{3}H_2O$ and [^{32}P]orthophosphate) was reported (Sankaranarayanan *et al*., 1989). The chromosomal aberration frequency observed after a challenge dose of 0.5 Gy of X-rays was lower than that expected on the basis of additivity of the effects of the individual treatments, although the response varied between samples from different donors.

(iv) *Mutation*

Cell killing and mutation to 6-thioguanine resistance were studied in growing mouse leukaemia cells in culture after exposure to ^{3}H-labelled amino acids and [^{3}H]thymidine. The greatest effect was seen with [^{3}H]thymidine, followed by [^{3}H]arginine and [^{3}H]lysine for a given concentration of ^{3}H (in kBq per mL medium). The differences between the ^{3}H-labelled amino acids disappeared almost completely when the effects were compared on the basis of the absorbed dose to the cells. The effects of [^{3}H]thymidine, however, remained more than twofold greater than those of the other ^{3}H-labelled compounds (Furuno-Fukushi *et al.*, 1987).

Incorporation of [^{125}I]iodo-2′-deoxyuridine into DNA was found to be more effective than that of the ^{131}I-labelled compound at inducing cell killing and mutations in human cells, both being more effective than unincorporated radioiodines or X-rays (Whaley & Little, 1990). Incorporated [^{125}I]iodo-2′-deoxyuridine was also more effective at inducing cell killing and mutations than the DNA-intercalating agent [^{125}I]acetylproflavine, probably because of the reduced energy deposition by the latter (Whaley *et al.*, 1990). Although [^{125}I]iodo-2′-deoxyuridine incorporated into cellular DNA was effective at producing both toxic and mutagenic effects in cells proficient in incorporating a thymidine analogue into DNA, virtually no effect was seen in cells that were deficient in this respect (Whaley & Little, 1990).

(v) *Cell transformation*

The biological effectiveness for the induction of malignant transformation in cultured mouse (C3H10T½) cells after exposure to 1–6 Gy of $^{3}H_2O$ at calculated dose rates of 51–307 mGy/h relative to γ-rays at 4 °C and 37 °C, respectively, was 1.6 and 1.7 (Yamaguchi *et al.*, 1989). A high efficiency for neoplastic transformation of BALB/3T3 mouse embryo cells was observed for ^{125}I incorporated into DNA: per radioactive decay, ^{125}I was about 25 times as effective as ^{3}H from incorporated [^{3}H]thymidine (LeMotte *et al.*, 1982).

(*b*) *In-vivo studies*

Chinese hamsters were injected with ^{144}Ce citrate (0.85 kBq/g bw), resulting in a low dose rate, estimated to be 7.3 mGy/day, of low-LET radiation to bone marrow. Although no significant increase in the total aberration frequency was observed, the treatment increased by more than twofold the number of chromatid exchanges in bone-marrow cells after a subsequent external exposure to ^{60}Co γ-rays when compared with controls exposed to γ-rays only (Brooks *et al.*, 1993).

No increase in lymphocyte aberration yields was found in hamsters injected with ^{137}Cs chloride to deliver a low committed effective dose of about 0.4 mGy (Lloyd *et al.*, 1997c).

The potential of vitamin C, an antioxidant, to protect spermatogonial cells in mouse testis against the effects of chronic irradiation from internally deposited radionuclides was investigated. A small, non-toxic amount of vitamin C (1.5 μg in 3 μL saline) injected

intratesticularly protected the spermatogonia against damage caused by Auger electrons from similarly administered [5-^{125}I]iodo-2′-deoxyuridine, as judged 29 days later by longer survival of treated spermatogonial cells than control cells without vitamin C. A dose modification factor of 2.3 was obtained. In contrast, no protection was observed when ^{210}Po citrate, an α-particle emitter, was administered (Narra *et al.*, 1994)

Ultrastructural modifications of pulmonary cells were investigated in rats after intravenous injection of ^{99m}Tc-labelled microspheres (2×10^5; radioactivity, 20 MBq), and nuclear expression of p53 protein was assessed by immunohistochemistry. Despite very high previously calculated doses [not specified] delivered to pulmonary cells, no morphological cell damage and no significant increase in nuclear expression of p53 were noted (Jacquet *et al.*, 1999).

(*c*) *Human studies*

(i) *Radioiodine therapy*

Chromosomal abnormalities in peripheral leukocytes were studied in groups of patients treated with ^{131}I for hyperthyroidism or thyroid cancer. The groups consisted of 48 patients (24–79 years of age) studied 3–14 years after treatment for hyperthyroidism with a total dose 8–54 mCi [0.3–2.0 GBq] of ^{131}I; 11 hyperthyroid patients (27–61 years of age) studied before and 0.5 h after administration of 8.3–12.7 mCi [0.31–0.47 GBq] of ^{131}I; and 11 thyroid cancer patients (16–49 years of age) studied before and 0.5, 2, 24 and 48 h after treatment with 150–200 mCi [5.6–7.4 GBq] of ^{131}I. The 21 controls (18–78 years of age) were matched for age and sex with the first group of 48 patients and had no history of irradiation or thyroid disease. Statistically significant increases in the frequency and severity of chromosomal abnormalities were observed after radioiodine therapy for hyperthyroidism. These abnormalities were detected as early as 0.5 h after administration and persisted for at least 14 years after treatment. The incidence and severity of abnormalities were greater after the larger doses of ^{131}I for thyroid carcinoma and in the period shortly after treatment (Nofal & Beierwaltes, 1964).

The presence of micronuclei was studied in binucleated peripheral blood lymphocytes from 22 women (aged 20–53 years; mean, 36.2 ± 2.1 years) with thyroid cancer who had received [^{131}I]sodium iodide orally as an adjuvant after total thyroidectomy (total dose, 3.4–37.5 GBq) 1–5 years before the study. The results showed no significant difference in the frequency of micronuclei between the patients and the control group, the latter being composed of 19 unexposed women (Gutiérrez *et al.*, 1995).

The micronucleus assay was used to investigate effects on chromosomes in peripheral blood lymphocytes from 47 patients with hyperthyroidism and 39 patients with thyroid cancer who were treated with ^{131}I. In the patients treated for hyperthyroidism, the micronucleus frequency was determined before ^{131}I therapy and one week, one month and three months afterwards; an additional sample was taken from a

subgroup of 17 patients six months after treatment. In the patients treated for thyroid cancer, samples were taken before treatment and one week, six months and one year later. At the same time, a cross–sectional study was performed with 70 control subjects and 54 thyroid cancer patients who had received the last therapeutic dose 1–6 years before the study. The patients treated for hyperthyroidism had a significantly increased average number of micronuclei over time. In the sample obtained six months after therapy, the mean micronucleus frequency was virtually the same as that in the sample taken three months earlier. In the patients treated for thyroid cancer, a twofold increase in the frequency of micronuclei was seen one week after therapy. Although this value decreased with time, the frequency of micronuclei one year after ^{131}I therapy remained higher than the value before therapy. In the cross-sectional study, a significant increase in the frequency of micronuclei was detected in the subgroup of thyroid cancer patients treated 1–3 years before the study. These results indicate that exposure to ^{131}I therapy induces chromosomal damage in peripheral lymphocytes (Gutiérrez *et al.*, 1999a).

No significant increase in the frequency of sister chromatid exchange or in the number of cells with unusually high sister chromatid exchange counts was found after ^{131}I therapy for hyperthyroidism or thyroid cancer. The study population was 46 patients treated for hyperthroidism (38 women, eight men) and 39 for thyroid cancer (27 women, 12 men), who received doses of 0.15–1.3 GBq and 3.7–5.6 GBq, respectively. In the follow-up analysis, four blood samples were drawn from each patient: the first before the radioiodine treatment and the remaining three taken sequentially over the year after therapy. In addition, a cross-sectional study was carried out with 78 control persons and 51 thyroid cancer patients who had completed radioiodine therapy (mean dose, 7.8 GBq) 1–6 years before the investigation. No statistically significant increase in the frequency of sister chromatid exchange or in the number of high-frequency cells was observed in the hyperthyroid patients or the thyroid cancer group when compared with controls (Gutiérrez *et al.*, 1999b).

Ten patients with thyroid cancer were treated with two doses of 1.85 GBq of ^{131}I given 24 h apart. Blood samples were taken from these patients before and at various times after exposure and analysed for the presence of chromosomal aberrations (dicentrics). The increase in the yield of aberrations after exposure to radioiodine was small but statistically significant. When compared with published values for whole-body doses after such treatment, the increase appeared to be somewhat smaller than expected after acute exposure of lymphocytes *in vitro* or *in vivo*. It was suggested that this was due to the low dose rate of ^{131}I (Baugnet-Mahieu *et al.*, 1994).

A significant increase in the mean number of micronuclei was measured in binucleated peripheral blood lymphocytes from patients with thyroid cancer after radio-iodine therapy, when compared with control subjects. Twenty-five patients (19 women, six men; age range, 36–72 years; mean age, 58.4 years) with differentiated thyroid carcinoma were treated with 3.7 GBq of ^{131}I after total thyroidectomy. Lymphocytes were collected from the patients before therapy and one week afterwards. The patients were classified into three groups: no prior ^{131}I treatment before the current therapy, one

prior administration of 3.7 GBq of ^{131}I and two prior administrations of 3.7 GBq of ^{131}I with at least six months between each. The mean number of micronuclei after treatment was significantly higher than that before treatment, but there was no effect on micronucleus frequency with cumulative exposure to radiation (Watanabe *et al.*, 1998).

Cytogenetic responses were investigated in 19 patients with differentiated thyroid cancer (11 papillary carcinomas and eight follicular carcinomas). After total or near-total thyroidectomy, seven patients were irradiated externally with daily fractions of 2 Gy of γ- and X-radiation (total, 50 Gy), and 12 patients underwent thyroid ablation and then received oral doses of 1734–2600 MBq of [^{131}I]sodium iodide. A further eight patients with intact thyroid glands were treated with ^{131}I at activities of 185–595 MBq for thyrotoxic diseases. For the determination of control aberration levels, 14 patients with thyroid cancer treated by surgery only and 14 healthy, age- and sex-matched controls were included in the study. Blood samples were taken 24 h after treatment in each case of external irradiation and five days after oral intake of radioiodine. The frequency of aberrant lymphocytes in the surgically treated cancer controls was significantly higher than that in matched healthy controls, and the radiation-treated patients had distinctly more chromosomal aberrations than either of the controls. External irradiation caused 10 times more aberrant cells than ^{131}I therapy. The doses of radioiodine given for ablation to the cancer patients were almost seven times higher than the doses given to the patients with thyrotoxic disease (185–595 MBq), who had intact glands. Nevertheless, the frequency of chromosomal aberrations was significantly lower among the cancer patients (Gundy *et al.*, 1996; Katz *et al.*, 1998).

Chromosomal aberrations were scored in the peripheral blood of 18 patients (14 women, four men) aged 25–79 years (mean, 48 years) with differentiated thyroid carcinoma who had received therapy with radioiodine. All the patients had undergone total thyroidectomy before the first ^{131}I treatment. Blood samples were obtained before and four days after each administration of 3.7 GBq of ^{131}I and were analysed according to conventional cytogenetics or by fluorescence in-situ hybridization with probes for chromosome 4. Repeated administration resulted in a cumulative dose of 1–3.5 Gy (two to seven treatments). An increase in the frequency of symmetrical and asymmetrical aberrations was observed with each treatment, but the number of chromosomal aberrations from the third treatment onwards was considerably lower than that expected from the calculated dose, perhaps due to killing of lymphocytes with multiple chromosomal anomalies (M'Kacher *et al.*, 1998).

Chromosomal aberrations and micronuclei were also analysed in peripheral blood lymphocytes from 19 patients with thyroid cancer who received therapeutic doses of 2.6 GBq (70 mCi) of ^{131}I. Oxidative stress was assessed by determining thiobarbituric acid-reactive substances in blood, total plasma antioxidant status and serum uric acid concentrations. All these parameters were assessed before treatment and one and six months afterwards. A significant increase in the frequency of micronucleated cells was observed at both one and six months after treatment when compared with controls before treatment. The frequency of cells with chromosomal aberrations, excluding gaps,

was also significantly higher one and six months after treatment than before treatment. Parameters of oxidative stress were slightly modified over the period studied, but the differences were not significant, except for a decrease in thiobarbituric acid-reactive products six months after therapy and in serum uric acid concentration one and six months after therapy. The overall results showed a slight but significant induction in persistent cytogenetic damage after ^{131}I therapy but no clear correlation between the cytogenetic findings and oxidative stress parameters (Monteiro Gil *et al.*, 2000).

Human T lymphocytes from 13 patients were used to determine the frequency and molecular spectrum of somatic gene mutations induced by radioimmunoglobulin therapy with ^{131}I-labelled antibody. The exposure induced *HPRT* mutations and a predominant molecular spectrum of partial deletions, rearrangements and total gene deletions. The mutation frequencies decreased with time after exposure (Albertini *et al.*, 1997).

A significant increase in *GPA* and lymphocytic *TCR* gene mutation frequencies was found in the peripheral blood of patients after ^{131}I therapy (Akiyama *et al.*, 1995).

(ii) *Technetium-99m*

Cytogenetic effects were assessed in blood samples from five patients with various arthrosic and periarthrosic diseases, obtained after bone scintigraphy with 925 MBq of [^{99m}Tc]hydroxymethylene diphosphonate. No cytogenetic effects were detected 3.6 and 24 h after administration of the radionuclide (Jacquet *et al.*, 1999).

Mutant frequencies were measured in T lymphocytes of patients undergoing radionuclide angiography with erythrocytes labelled *in vivo* with ^{99m}Tc. Blood from 13 patients was sampled before and 8–120 days after an injection of 750 MBq of ^{99m}Tc. The frequencies of *HPRT* mutants were measured by the T-cell cloning method. The mean frequency of mutants after treatment was significantly lower than that measured before exposure. Further analysis indicated that the decrease in mutant frequency after exposure could be accounted for by an effect on cloning efficiency (Van Dam *et al.*, 1991).

(iii) *Other medical treatment*

The induction of chromosomal aberrations after radiophosphorus treatment was studied in 48 patients with various forms of polycythaemia, 11 of whom received ^{32}P for polycythaemia vera. Patients without polycythaemia vera and seven regular blood donors served as controls. Peripheral blood was analysed for chromosomal aberrations on first referral to a hospital ward or clinic and was repeated once a year. A variety of non-specific chromosomal aberrations were found in polycythaemia vera patients who had had no previous treatment with radiation. Among radiophosphorus-treated patients, the frequency of these aberrations was moderately higher. Dicentric chromosomes were the aberration typical of ^{32}P-treated patients. Only recent injections of ^{32}P had an effect on the number of dicentric chromosomal aberrations detected (Modan *et al.*, 1970).

Forty-eight patients with rheumatoid arthritis who had received intra-articular injections of ^{198}Au and 22 who had been given intra-articular injections of ^{90}Y were investigated for the presence of chromosomal damage. Blood samples from some patients were not taken until several months or years after the injections. In nine cases, the blood samples were scanned before and at intervals after treatment to determine the distribution of the isotopes. ^{198}Au was administered in the form of a colloidal suspension of metallic gold stabilized with gelatine at particle sizes up to 20 μm, most of the activity being in the 10–13-μm particles. The ^{90}Y preparation was ionic yttrium bound to a colloidal ion-exchange resin with a particle size of 20–50 μm. The distribution of the radionuclides was scanned in 20 patients, and some leakage from the joint to the regional lymph nodes was detected, occasionally constituting up to 20% of the administered activity, which may account for the lack of correlation between the amount of either radionuclide and the percentage of cells with chromosomal damage. Damage was detected in many patients after 24 h; on average, there was an increase in detectable damage up to the last sampling time, 28 days after injection (Stevenson *et al.*, 1973).

The frequency of chromosomal aberrations was measured in 30 patients after ^{90}Y synovectomy. The patients (all > 45 years old) had chronic synovitis of the knee and received a dose of 5 mCi [185 MBq] of ^{90}Y silicate. Chromosomes from cultured peripheral blood lymphocytes were studied before and approximately three months after therapy. The frequency of cells with chromosomal aberrations increased significantly, from 0.33% before treatment to 0.87% after treatment (Doyle *et al.*, 1977).

^{165}Dy (dysprosium) hydroxide macroaggregates were developed for the treatment of arthritis in clinical trials, as dysprosium provides a better spectrum of decay energies and a shorter half-life than the conventionally used yttrium, permitting quicker and more efficient treatment. The micronucleus frequencies in peripheral blood lymphocytes were examined in 42 patients before and two weeks after radiation synovectomy, in which 21 patients received ^{165}Dy hydroxide macroaggregates (10 GBq) and the others received ^{90}Y silicate (185 MBq). In most patients in each group, no significant change in micronucleus frequency was observed, but ^{165}Dy and ^{90}Y treatment caused significant increases in four and six patients, respectively, and a significant decrease in two and one patient, respectively. The results indicate that, in most patients, the materials and methods of administration used currently do not result in leakage of radioactive material from the injection site (Prosser *et al.*, 1993).

(iv) *Hydrogen-3*

Chromosomal translocations were analysed by fluorescence in-situ hybridization in the blood lymphocytes of a person who, 11 years previously, had accidentally inhaled a substantial amount of $^{3}H_2O$. A comparison was made between previous estimates of radiation dose and contemporary dosimetry by urine analysis and scoring of dicentric chromosomes. The blood lymphocytes were analysed by two laboratories with different chromosome probe combinations, and good agreement in translocation

yields was found. Comparison of these values with the dicentric frequency obtained shortly after the accident and with a translocation frequency measured six years after exposure showed good agreement between all measurements. Thus, the translocations were completely stable for 11 years (Lloyd *et al.*, 1986, 1998).

(v) *Chernobyl accident*

A cytogenetic study was carried out with lymphocytes from children in Belarus, the Russian Federation and the Ukraine who had been exposed to fall-out consisting mainly of ^{137}Cs from the Chernobyl reactor accident in 1986. A total of 41 children, all aged 8–10 years, were selected for the study. The first group, five girls and four boys, was from the countryside of Navrovl'a (Belarus), 70 km from Chernobyl, where the ^{137}Cs contamination was 15–50 Ci/km^2 [555–1850 GBq/km^2]. The second group, eight girls and 16 boys, came from the area of Belarus surrounding Chernobyl; they had been evacuated soon after the accident and transferred to Gomel or Drujri 200–300 km from Chernobyl, where the ^{137}Cs ground contamination was 1–10 Ci/km^2 [37–370 GBq/km^2]. The third group, three girls and five boys, was from Stolin (Belarus), 250 km from Chernobyl, with ^{137}Cs contamination of 1–5 Ci/km^2 [37–185 GBq/km^2] and 5–15 Ci/km^2 [185–555 GBq/km^2] in part of the surrounding area. Blood samples were collected from all the children during 1991–92, and internal contamination was evaluated by whole-body counter analysis of ^{137}Cs, which gave values for the three groups of 460–2795 Bq, 44–397 Bq and 7714–32 343 Bq, respectively. Remarkably, the third group had the highest values in spite of the fact that the first group was from an area with higher ground contamination. The control group consisted of 10 healthy Italian children (five girls and five boys) selected on the basis of age. The overall frequency of acentric fragments, dicentrics and translocations in the three exposed groups and of acentric fragments in the first two groups were significantly higher than those in the controls (Padovani *et al.*, 1993).

DNA from 129 paired thyroid tumours and non-tumorous tissue samples from 102 Belarussian children (age at surgery, ≤ 18 years) and 27 adults (age at surgery, 19–35 years), who had been exposed to radioactive fall-out from the Chernobyl reactor accident, was examined for microsatellite instability and loss of heterozygosity. Twenty-eight microsatellite markers were chosen because of their vicinity to DNA repair genes or genes involved in tumorigenesis and to regions of chromosomal breakpoints in thyroid tumours. In 40 patients (31% of 129), a total of 73 alterations were observed, 80% of which were classified as loss of heterozygosity and only 20% as microsatellite instability. A subgroup of 11 patients was identified, mainly girls (8.5% of 129), who had alterations in at least two microsatellite markers. Comparisons were made with samples of spontaneous thyroid carcinomas without exposure to radiation from 20 adult patients in Munich, Germany (mean age at surgery, 56 ± 13 years). None of the tumour samples from this group showed alterations in the 28 microsatellite markers tested. The results indicate greater instability of microsatellite markers in thyroid cancers from Belarussian patients. It remains uncertain whether the increased genomic instability is

the result of exposure to radioactive iodine from the Chernobyl reactor accident or to the young age of the patients (Richter *et al.*, 1999).

(vi) *Techa River, southern Urals*

About 28 000 inhabitants of settlements on the banks of the Techa River were exposed internally (predominantly to ^{90}Sr), mainly due to the use of the River as a source of drinking-water, and externally due to ^{137}Cs γ-rays from contaminated sediments in the River (see section 2.9.2). Forty-three years after the beginning of the exposure, stable chromosomal aberrations were analysed in peripheral blood lymphocytes, and somatic mutations were measured in erythrocytes (*GPA*) and lymphocytes (*TCR*). Stable chromosomal aberrations including translocations, inversions and deletions were analysed by fluorescence in-situ hybridization. Mutant lymphocytes and erythrocytes were registered according to *TCR* and *GPA* mutation frequencies by flow cytometry. The study was carried out on 80 subjects whose individual accumulated doses and dose-rate dynamics, from ^{90}Sr and ^{137}Cs, in the red bone marrow were reconstructed on the basis of the findings of measurements with a whole-body counter *in vivo*. There was no significant difference in the incidence of chromosomal translocations between people exposed at various levels and the controls. The frequency of mutant lymphocytes defective in *TCR* gene expression increased with cumulative doses to the red bone marrow, and, at doses > 2 Gy, the difference from the control group was statistically significant. The frequency of mutations within the *GPA* system in individuals with long-term exposure did not differ from that in the comparison group, and the frequency of mutant erythrocytes of different types did not depend on accumulated dose to the red bone marrow (Akleyev *et al.*, 1995).

Symmetrical translocations were measured by fluorescence in-situ hybridization with probes for chromosomes 1, 4 and 12 in peripheral lymphocytes from residents of settlements along the Techa River. The study group consisted of 73 individuals born between 1911 and 1953 and residing in 13 villages along the River, 7–148 km downstream of the site of release of radioactive waste. Blood was sampled between 1994 and 1996. Data for dose reconstruction based on physical measurements and calculations for the population in settlements along the River were obtained from Degteva *et al*. (1994). External doses were calculated on the basis of long-term area measurements of γ-radiation at relevant sites in each village. The external exposure ranged from < 0.01 Gy/year in the lower regions of the River (> 150 km downstream) to an overall average dose for the year 1951 of 0.5–1.0 Gy, as was estimated for the inhabitants of Metlino, a village located only 7 km downstream from the site of release. Internal dosimetry was achieved by large-scale measurements of ^{90}Sr and β-irradiation on the surface of teeth in 1960 and by whole-body measurements started in 1974. The control group consisted of 39 healthy unexposed persons of a comparable age range and living in uncontaminated areas of the southern Urals. A significantly elevated mean translocation frequency was found when compared with controls for the total study group and for both groups of inhabitants of the villages in the upper reaches of the Techa

River (7–60 km) during 1950–51 (the time of maximum release of radioactive waste) and in villages in the lower reaches (78–148 km) until the time of blood sampling. The latter group was further divided into a subgroup comprising 14 individuals who had left the riverside settlements before 1965 and a subgroup consisting of 19 people who continued to live in the settlements until the time of blood sampling. While the translocation frequency of the first subgroup was not significantly elevated above background, a threefold higher value was found for the second. In the lower reaches of the Techa River, the influence of external exposure can be excluded. The reported difference in the response of these two subgroups may be attributable to internal exposure, since the members of the second subgroup had a further 30 years in which to incorporate the long-lived radionuclides, particularly bone-seeking ^{90}Sr (Bauchinger *et al.*, 1998).

(vii) *Mutations in tumour-related genes*

The identification of a genomic fingerprint that would provide proof of the interaction between a specific exposure and a target cell would be highly desirable for molecular epidemiology. However, a specific molecular lesion is almost always missing, probably because of the large number of factors that act on tumour induction and progression. Signalling via protein tyrosine kinases has been identified as one of the most important events in cellular regulation, and rearrangements of the tyrosine kinase domain of the *RET* proto-oncogene have been found in thyroid cancers thought to be associated with ionizing radiation (Ito *et al.*, 1993; Fugazzola *et al.*, 1995; Klugbauer *et al.*, 1995). However, the biological and clinical significance of *RET* activation remains controversial, and further studies of the molecular biology of radiation-induced thyroid cancers are needed before the carcinogenic pathway can be fully understood.

RET oncogene rearrangements were studied in papillary thyroid carcinomas of children exposed to radioactive fall-out in Belarus after the Chernobyl accident. Small tissue samples from thyroidectomy specimens were analysed, comprising 12 papillary thyroid carcinomas from children, two papillary thyroid carcinomas and one follicular carcinoma from adults and non-tumourous thyroid tissue from four children and four adults as controls. Two-thirds of the papillary thyroid carcinomas in children had a *RET* rearrangement, and all the tumours with *RET* rearrangements had lymph-node metastases. Intra-chromosomal rearrangement involving *RET* and the adjacent *H4* or *ELE* gene on chromosome 10 was a frequent event in the thyroid cancers of children in the zone of Belarus contaminated by the accident (Klugbauer *et al.*, 1995).

Mutations in the *TP53* tumour-suppressor gene (exons 5–8) were investigated in 31 thyroid tumours from children in Belarus (24 cases of papillary thyroid carcinoma, three benign tumours and two cases each of thyroiditis and goitre) and 33 thyroid tumours from adolescents and adults with no exposure to radiation (25 carcinomas of various histological types, including 11 papillary carcinomas and eight adenomas); six tumours from adults (four papillary carcinomas, one adenoma and one goitre) served as controls. The mutational spectrum of *TP53* in the thyroid carcinomas from Belarussian children differed greatly from those in the other groups. In the 29 malignant tumours in the

control groups, seven different mutations were detected on exons 5–8, none of which occurred among the 15 papillary carcinomas in this group. Five mutations were found in tissue samples from the 24 childhood papillary carcinomas, and they were all the same *TP53* point mutation (CGA → CGG) on codon 213 of exon 6 (Smida *et al.*, 1997).

Molecular biological studies showed that the proportion of cases of papillary carcinoma of the thyroid that expressed the *RET* gene was not significantly different in tumours from exposed and unexposed persons (mainly in other countries, such as France, Italy and the United Kingdom). Studies of the type of translocation leading to *RET* gene expression are inconclusive (UNSCEAR, 2000). *RAS* gene mutations were found (as expected) in follicular carcinomas but were absent from papillary carcinomas from either exposed or unexposed areas. Thyroid-stimulating hormone (*TSH*) receptor mutations, which are normally found in follicular tumours, were not found in any papillary carcinoma, nor were any *TP53* mutations identified. These results are consistent with the hypothesis that papillary carcinomas are associated only with *RET* translocation, and that *RAS* and *TSH* receptor mutations occur in follicular tumours and *TP53* mutations in undifferentiated carcinomas.

[The Working Group noted that specific mutations in tumour tissue may not be directly attributable to radiation.]