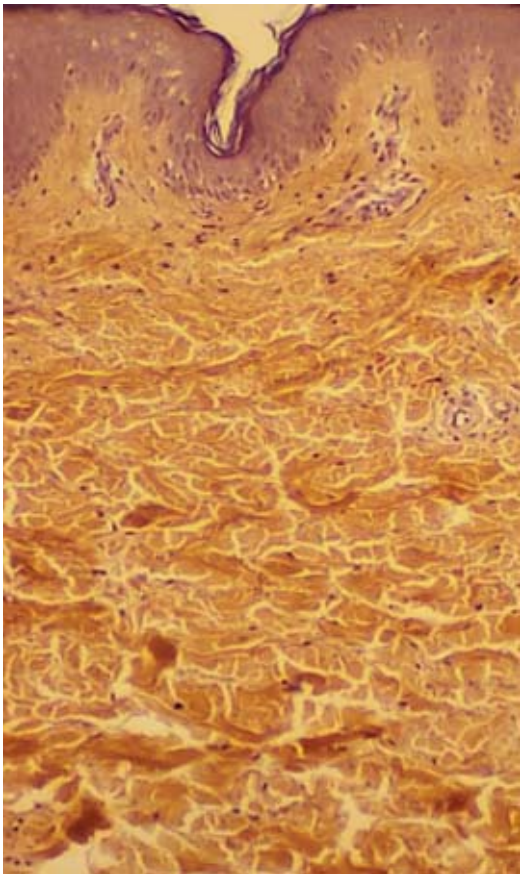
Effects of ionising radiation on human skin

The skin is the first tissue that is damaged by external exposure to ionising radiation. The early and late responses to irradiation in this complex organ are still poorly understood. Thanks to DNA microarrays, the response of skin cells to irradiation, and in particular that of epidermal stem cells, can now be studied globally.



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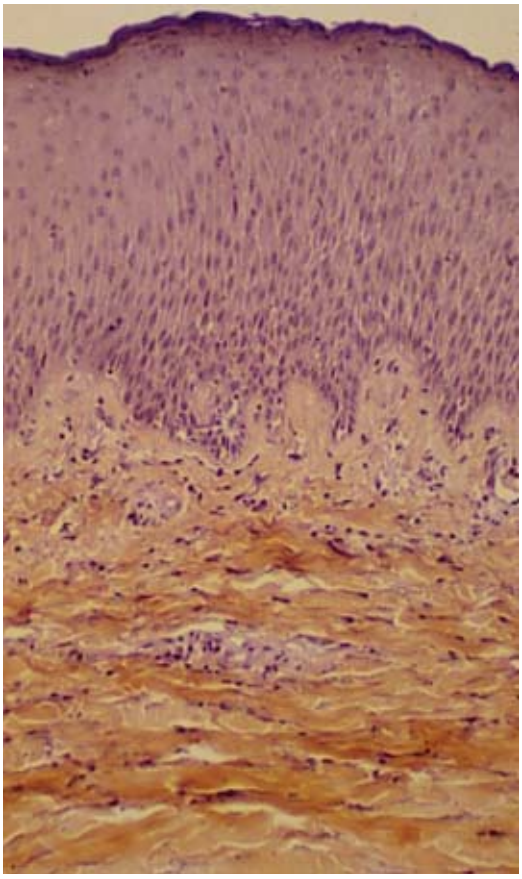

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Hyperplasia (increased tissue volume) of the skin, a complication of radiotherapy. Comparison of a normal human skin section (left) with an irradiated skin section (right) showing **fibrosis** of the dermis and acanthosis(1) of the epidermis (magnification x 10).

The skin is the first tissue that is damaged during **external exposure** to **ionising radiation**. Historically, burns to fingers and hands among radiologists and physicists using **X-rays**, and the description of **radiocarcinoma**, revealed the toxicity of this radiation. Today, exposure of the skin can occur in medical or industrial uses of radiation.

Non-cancerous skin damage after ionising radiation is called *radiodermatitis*. It results from lesions in several cell types possessing different radiosensitivities, e.g., the **keratinocytes** of the *epidermis*, the **fibroblasts** and the **endothelial** cells of the dermis (Figure in Box). In addition, the effects on the skin depend on many factors linked to the exposure, such as the energy of the radiation, the total **dose** and the **dose rate**, and to the patient, e.g., age, sex and anatomical location of the target. After a heavy dose (from 15 **grays** [Gy]), a rapid evolution similar to that of a burn can be observed. Late complications, which may appear several years after exposure, are also observed. They can take the form of skin atrophy, with thin fragile skin, which can lead to skin necrosis, or conversely hypertrophy, with thick inflamed skin. **Fibrosis** of the dermis and **keratosis** of the epidermis are frequent late complications of irradiation, and are examples of skin hypertrophy (illustrations above and on the following page).

After a low dose of radiation there are no clinical manifestations, but effects are detectable at the cellular level. A dose of only 0.5 Gy is sufficient to induce cell death in the basal layer of the epidermis. Using **DNA microarrays**, CEA Évry researchers recently observed that a dose of 10 mGy can modify the activity of the skin cells, for example by altering the activities of **transcription factors**.

Ionising radiation can also induce both benign and malignant skin tumours. In the *epidermis basocellular* and *spinocellular* carcinomas are observed, with an average latency of 25 years. Cancers of the fibroblast, or **sarcomas**, can appear in the dermis, along with tumours of the endothelial cells, **angiomas** or angiosarcomas, but these are less frequent than carcinomas. Although there seems to be no safe threshold, skin cancers are mostly observed after exposure to high doses of radiation.

(1) Acanthosis: benign thickening of the stratum spinosum (prickle cell layer) of the epidermis.

The skin, a complex organ

The skin is a complex organ composed of two superimposed layers, the *epidermis* and the *dermis* (Figure). The epidermis is an **epithelium** formed of several layers and composed chiefly of ***keratinocytes***, which are renewed monthly. The deepest stratum, called the basal layer of the epidermis, contains the non-**differentiated** stem cells that ensure this renewal. These stem cells seem to be the main victims of **ionising radiation** in the epidermis. When they migrate into the outer strata, the keratinocytes no longer divide, but they undergo differentiation ultimately to form the outermost horny layer (*stratum corneum*). The dermis feeds the epidermis, and supports blood vessels and nerves.

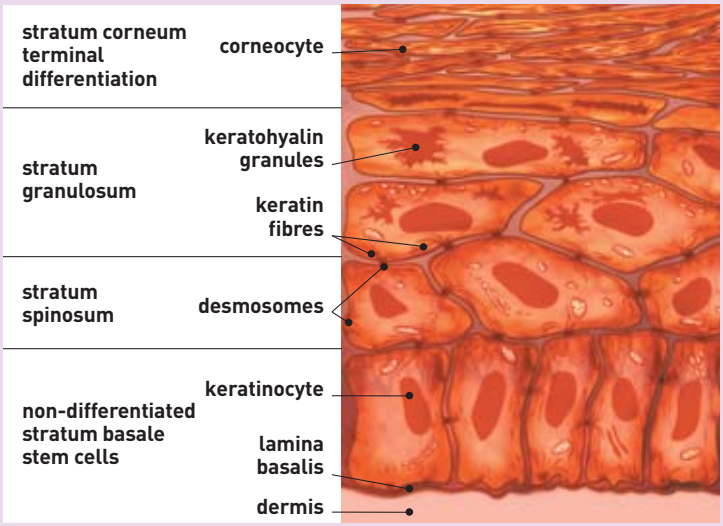
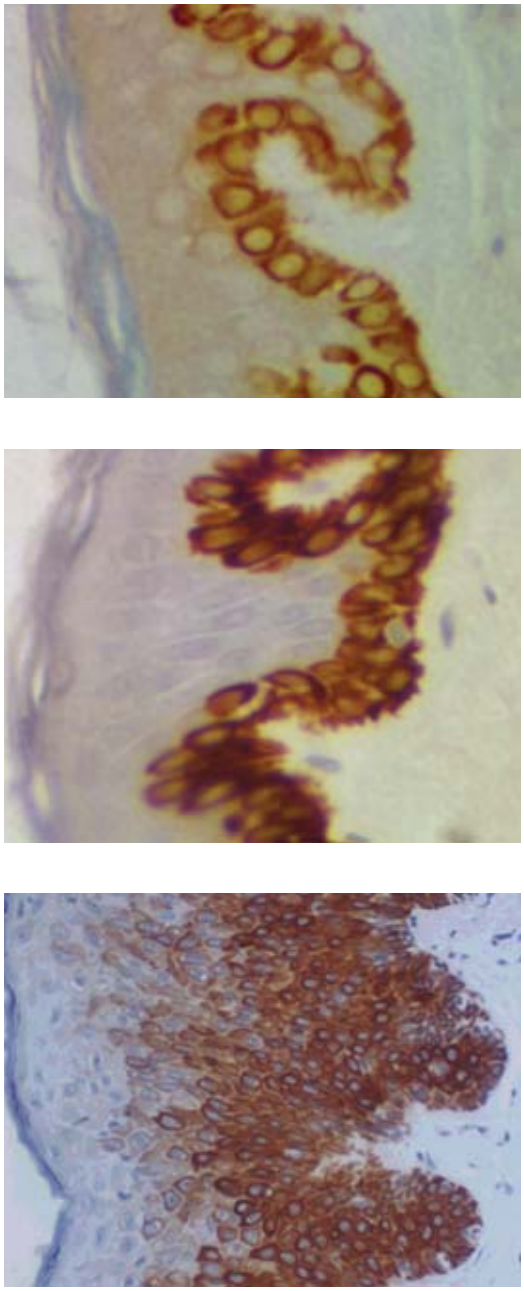


Figure.
Organisation of the differentiation of keratinocytes in the human epidermis.

Skin fibrosis: biology and therapy of a disease induced by ionising radiation

At the CEA, cases of late and chronic radiodermatitis are studied on samples from radiotherapy patients and accident victims at the Department of Functional Genomics, Évry (Essonne), and on a pig model at the Department of Radiobiology and **Genome Studies** at Jouy-en-Josas (Yvelines). The lesions observed are fibrosis and fibro**necrosis** of the skin and subcutaneous tissues after high-dose exposure.
Research has shown that radio-induced fibrosis is an endless scarring process. In this dynamic self-sustaining process, the myofibroblast, a particular type of fibroblast, plays an essential role, together with a **cytokine**, TGF-β1, which orchestrates chronic cell activation. These lesions were considered irreversible and not amenable to any specific treatment. However, some important results concerning their treatment were obtained by a group of radiotherapists at the St Louis Hospital (Dr S. Delanian), at the Curie Institute and by researchers at the CEA. One major result was that the irreversibility of radio-induced fibrosis could be challenged, and antioxidant compounds were found to be effective in the treatment of fibrosis. In a more recent approach the response to irradiation of sensitive patients are being characterised using DNA microarrays.



Comparison of a normal human epidermis (top), with an epidermis 6 hours after gamma irradiation (dose 10 Grays, centre), and with the epidermis of a patient with fibrosis 12 years after radiotherapy (bottom). Keratin 14, a marker of non-differentiated keratinocytes of the basal layer, is over-expressed in both cases.

The response of human keratinocytes to ionising radiation: study of the transcriptome using DNA microarrays

The Department of Functional Genomics of the Life Sciences Division of the CEA at the Évry Genopole, produces DNA microarrays(2), glass slides on which 7,600 **sequences** of human **genes** are deposited. Using this tool, the response to radiation of human skin cells can be studied globally instead of gene by gene. The scientists in this department are particularly interested in the relation between radiosensitivity and cell **differentiation** (Box).

(2) See on this subject *Clefs CEA* 47, p. 75.

A first study developed by the CEA Évry group of researchers focused on cultures of differentiated keratinocytes. As early as three hours after exposure, 5 to 10% of the genes, depending on the dose delivered, have a modified expression, and many functional pathways are impaired. An **activation** of the genes implicated in the synthesis of **ATP** and glycolysis(3) is observed, leading to the production of energy in the irradiated cell (Figures 1 and 2). Conversely, many of the genes involved in the differentiation of the keratinocytes are repressed by **gamma irradiation**, with, in parallel, an activation of specific markers of non-differentiated keratinocytes of the basal layer of the epidermis. The results obtained show that the irradiation produces a very rapid energy activation reaction and loss of differentiation in human keratinocytes. This approach also makes it possible to demonstrate new markers of skin irradiation. The CEA research group sought the **expression** of some of these new markers of cell activation in irradiated human epidermis. Markers such as the gene of keratin 14, a marker of the non-differentiated keratinocyte, are over-expressed in irradiated human skin six hours after exposure (illustration p. 54, centre). The effect is conserved for a long time, as keratin 14 is still abnormal in the epidermis of patients with skin complications 12 years after radiotherapy (illustration p. 54, bottom). Researchers at the CEA have suggested that the irradiation may immediately induce cell activation, which is conserved in the descendants of the irradiated cells, and plays a role in the appearance of late effects in radiosensitive patients.

The role of human keratinocyte stem cells

It has often been suggested that radiation-induced lesions are mainly caused by stem cell damage. Evidence for this is still not fully conclusive, in particular for the skin. CEA scientists are currently studying the stem cells of the human epidermis, which are non differentiated cells that ensure the continuous renewal of this **epithelium**. By adapting a procedure designed for isolating stem cells from bone marrow, a method to isolate a population enriched in stem cells from human epidermis has been developed. This advance will enable the CEA team to characterise these cells by means of DNA microarrays and to study their radiosensitivity. In the frame of a new European contract, this team will test the use of these stem cells to reconstitute tissues damaged by ionising radiation.

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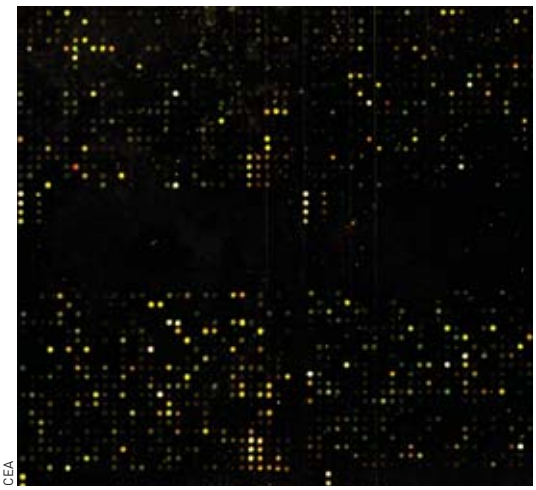
(3) Glycolysis: **enzymatic** breakdown of glucose.



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Hybridisation of a DNA microarray with **RNA** isolated from irradiated keratinocytes labelled with Cy3 (green fluorochrome) and RNA isolated from control keratinocytes labelled with Cy5 (red fluorochrome).

gene	induction factor	
	0.5 Gy	2 Gy
ATPase 1B3	3.5	3
ATPase ATP1A1	2	1.9
ATPase 5G3	2	2.2
ATPase 5C1	2.2	2.1
ATPase 6E	1.9	2

Figure 1.
Activated genes (irradiated against control) three hours after gamma irradiation (two doses) of cultured human keratinocytes. The use of DNA microarrays has revealed new cell activation markers, e.g., the 5 ATPases indicated.

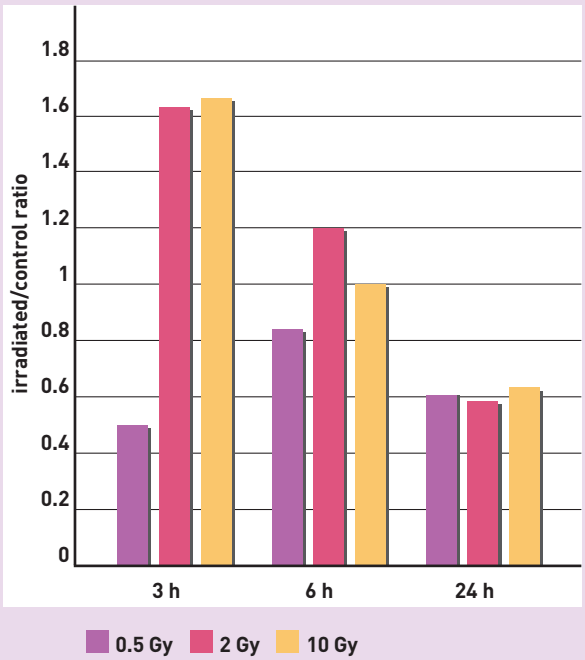


Figure 2.
Demonstration of an increase in intracellular ATP content in differentiated human keratinocytes for three doses of gamma irradiation.

A Natural and artificial radioactivity

Everything on the earth's surface has always been exposed to the action of ionising radiation from natural sources. Natural radiation, which accounts for 85.5% of total radioactivity (natural plus artificial), is made up of 71% telluric radiation and about 14.5% cosmic radiation. The radionuclides formed by the interaction of **cosmic rays** arriving from stars, and especially the Sun, with the nuclei of elements present in the atmosphere (oxygen and nitrogen) are, in decreasing order of dose (Box F, *From rays to dose*) received by the population, carbon-14, beryllium-7, sodium-22 and tritium (hydrogen-3). The last two are responsible for only very low doses.

Carbon-14, with a half life of **5,730 years**, is found in the human body. Its activity per unit mass of carbon has varied over time: it has diminished as carbon dioxide emissions from the combustion of fossil fuels have risen, then was increased by atmospheric nuclear weapon tests.

Beryllium-7, with a half life of **53.6 days**, falls onto the leaf surfaces of plants and enters the body by ingestion (Box B, *Human exposure routes*). About 50 Bq (becquerels) per person per year of beryllium-7 are ingested.

The main or **"primordial" radionuclides** are potassium-40, uranium-238 and thorium-232. Along with their radioactive decay products, these elements are present in rocks and soil and are therefore found in many building materials. Their concentrations are generally very low, but vary according to the nature of the mineral. The gamma radiation emitted by these radionuclides forms the **telluric radiation**, which is responsible for the external exposure of the body. The primordial radionuclides and many of their long-lived descendants are also found in trace amounts in drinking water and plants: this results in an internal exposure by ingestion, plus an additional low exposure by inhalation of airborne suspended dust particles.

Potassium-40 is a beta and gamma emitter with a half life of **1.2 thousand million years**, and has no radioactive descendants. This radioactive isotope makes up 0.0118% of all natural potassium, and enters the body by ingestion. The mass of natural potassium in the human body is independent of the quantity ingested.

Uranium-238 is an alpha emitter with a half life of **4.47 thousand million years**. It has thirteen main alpha-, beta- and gamma-emitting radioactive descendants, including **radon-222** (**3.82 days**) and **uranium-234** (**0.246 million years**). Uranium-238 and its two descendants **thorium-234** (**24.1 days**) and **protactinium-234m**(1) (**1.18 min**), and **uranium-234** are essentially incorporated by ingestion and are mainly concentrated in the bones and kidneys.

Thorium-230, derived from uranium-234, is an alpha emitter with a period of **80,000 years**. It is an osteotrope, but enters the body mainly by the pulmonary route (inhalation). **Radium-226**, a descendant of thorium-230, is an alpha emitter with a half life of **1,600 years**. It is also an osteotrope and enters the body mainly *via* food. Another osteotrope, **lead-210** (**22.3 years**), is incorporated by inhalation though mostly by ingestion.

Thorium-232 is an alpha emitter with a half life of **14.1 thousand million years**. It possesses ten main alpha-, beta- and gamma-emitting radioactive descendants including **radon-220** (**55 s**). Thorium-232 enters the body mainly by inhalation. **Radium-228**, a direct descendant of thorium-232, is a beta-emitter with a half life of **5.75 years**. It enters the body mainly in food.

Radon, a gaseous radioactive descendant of uranium-238 and thorium-232, emanates from the soil and building materials, and along with its short-lived alpha-emitting descendants constitutes a source of internal exposure through inhalation. Radon is the most abundant source of natural radiation (about 40% of total radioactivity).

The human body contains nearly 4,500 Bq of potassium-40, 3,700 Bq of carbon-14 and 13 Bq of radium-226 essentially imported in food.

Natural radiation is supplemented by an anthropic component, resulting from the medical applications of ionising radiation and to a lesser extent from the nuclear industry. It accounts for about 14.5% of the total radioactivity worldwide, but much more in the developed countries. In the medical field (more than 1 mSv/year on average in France), irradiation by external sources predominates: radiodiagnosis (X-rays) and radiotherapy, long based on cæsium-137 and cobalt-60 sources, but now more and more often using linear accelerators. Irradiation by internal routes (curietherapy with iridium-192) has more specialised indications (cervical cancer, for example). The metabolic and physicochemical properties of some twenty radionuclides are put to use for **medical activities** and in **biological research**. The medical applications comprise radiodiagnostics (scintigraphy and radio-

(1) m for metastable. A nuclide is said metastable when a transition delay exists between the excited state of the atom and the stable one.

immunology), and treatment, including thyroid disorders using iodine-131, radioimmunotherapy in certain blood diseases (phosphorus-32) and the treatment of bone metastasis with strontium-89 or radiolabelled phosphonates alongside other uses of radiopharmaceuticals. Among the most widely used radionuclides are: **technetium-99m** (half life **6.02 hours**) and **thallium-201** (half life **3.04 days**) (scintigraphy), **iodine-131** (half life **8.04 days**) (treatment of hyperthyroidism), **iodine-125** (half life **60.14 days**) (radioimmunology), **cobalt-60** (half life **5.27 years**) (radiotherapy), and **iridium-192** (half life **73.82 days**) (curietherapy). The average contribution of radiological examinations to total radioactivity amounts to 14.2%.

The **early atmospheric nuclear weapon tests** scattered fallout over the whole of the earth's surface and caused the exposure of populations and the contamination of the food chain by a certain number of radionuclides, most of which, given their short radioactive half lives, have now vanished. There remain **cæsium-137** (**30 years**), **strontium-90** (**29.12 years**), some **krypton-85** (**10.4 years**) and **tritium** (**12.35 years**), and the isotopes of **plutonium** (half lives **87.7 years** to **24,100 years**). Currently, the doses corresponding to the fallout from these tests are essentially attributable to fission products (cæsium-137) and to carbon-14, rather than activation products and plutonium.

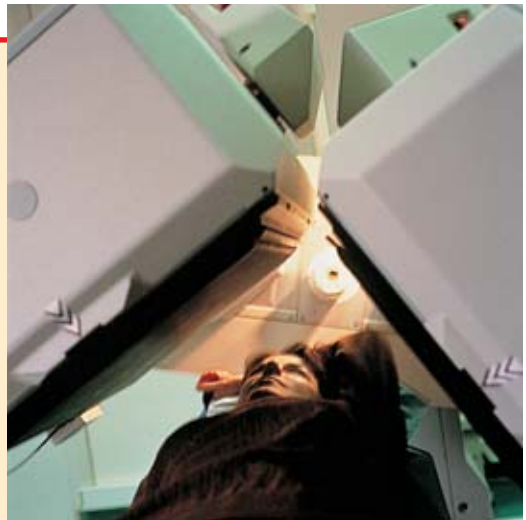
In the **Chernobyl accident** (Ukraine), which occurred in 1986, the total radioactivity dispersed into the atmosphere was of the order of 12 milliard milliard (10^{18}) becquerels over a period of 10 days. Three categories of radionuclides were disseminated. The first consisted of volatile fission products such as **iodine-131**, **iodine-133** (**20.8 hours**), **cæsium-134** (**2.06 years**), **cæsium-137**, **tellurium-132** (**3.26 days**). The second was composed of solid fission products and actinides released in much smaller amounts, in particular the strontium isotopes ^{89}**Sr** (half life **50.5 days**) and ^{90}**Sr**, the ruthenium isotopes ^{103}**Ru** (half life **39.3 days**) and ^{106}**Ru** (half life **368.2 days**), and **plutonium-239** (**24,100 years**). The third category was rare gases which although they represented most of the activity released, were rapidly diluted in the atmosphere. They were mainly **xenon-133** (**5.24 days**) and **krypton-85**.

The contributions of the early atmospheric nuclear weapon tests and the Chernobyl accident to the total radioactivity are roughly 0.2% (0.005 mSv) and 0.07% (0.002 mSv) respectively.

The whole of the **nuclear-powered electricity production** cycle represents only about 0.007% of total radioactivity. Almost all the radionuclides remain confined inside the nuclear reactors and the fuel cycle plants. In a nuclear reactor, the reactions that take place inside the fuel yield transuranics. **Uranium-238**, which is non-fissile, can capture neutrons to give in particular plutonium isotopes ^{239}**Pu**, ^{240}**Pu** (half life **6,560 years**) and ^{241}**Pu** (half life **14.4 years**), and **americium-241** (**432.7 years**). The main fission products generated by the fission of **uranium-235** (**704 million years**) and **plutonium-239** are **iodine-131**, **cæsium-134**, **cæsium-137**, **strontium-90** and **selenium-79** (**1.1 million years**).

The main radionuclides present in releases, which are performed in a very strict regulatory framework are, in liquid release, **tritium**, **cobalt-58** (**70.8 days**), **cobalt-60**, **iodine-131**, **cæsium-134**, **cæsium-137** and **silver-110m** (**249.9 days**). In gaseous releases **carbon-14** is the most abundant radionuclide, emitted most often as carbon dioxide. In all the reactors in the world, the total production of radiocarbon dioxide amounts to one tenth of the annual production formed naturally by cosmic radiation.

In addition, certain radionuclides related to the nuclear industry exhibit chemical toxicity (Box D, *Radiological and chemical toxicity*).



Laurence Médard/CEA

Classical scintigraphy performed at the Frédéric-Joliot Hospital Service (SHFJ). The gamma-ray camera is used for functional imaging of an organ after administration, usually by the intravenous route, of a radioactive drug (radiopharmaceutical) to the patient. The radionuclides used are specific to the organ being studied: for example, technetium-99m for the kidneys and bones, thallium-201 for the myocardium. The injected radiopharmaceutical emits gamma photons, which are captured by two planar detectors placed at 180° or 45° according to the examination.

B Human exposure routes

Human **exposure**, i.e., the effect on the body of a chemical, physical or radiological agent (irrespective of whether there is actual contact), can be external or internal. In the case of **ionising radiation**, exposure results in an energy input to all or part of the body. There can be direct **external irradiation** when the subject is in the path of radiation emitted by a radioactive source located outside the body. The person can be irradiated directly or after reflection off nearby surfaces.

The irradiation can be **acute** or **chronic**.

The term **contamination** is used to designate the deposition of matter (here **radioactive**) on structures, surfaces, objects or, as here, a living organism. Radiological contamination, attributable to the presence of **radionuclides**, can occur by the **external** route from the receptor medium (air, water) and vector media (soils, sediments, plant cover, materials) by contact with skin and hair (cutaneous contamination), or by the **internal** route when the radionuclides are **intaken**, by **inhalation** (gas, particles) from the atmosphere, by **ingestion**, mainly from foods and beverages (water, milk), or by penetration (injury, burns or diffusion through the skin). The term **intoxication** is used when the toxicity in question is essentially chemical.

In the case of **internal contamination**, the dose delivered to the body over time (called the **committed dose**) is calculated for 50 years in adults, and until age 70 years in children. The parameters taken into account for the calculation are: the nature and the intaken quantity of the radionuclide (RN), its chemical form, its **effective half life**[1] in the body (combination of **physical** and **biological half lives**), the type of **radiation**, the mode of exposure (inhalation, ingestion, injury, transcutaneous), the distribution in the body (deposition in target organs or even distribution), the radiosensitivity of the tissues and the age of the contaminated subject.

Lastly, the **radiotoxicity** is the toxicity due to the ionising radiation emitted by the inhaled or ingested radionuclide. The misleading variable called **potential radiotoxicity** is a *radiotoxic inventory* that is difficult to evaluate and made imprecise by many uncertainties.

(1) The effective half life (Te) is calculated from the physical half life (Tp) and the biological half life (Tb) by $1 / Te = 1 / Tp + 1 / Tb$.

F From rays to dose

Radioactivity is a process by which certain naturally-occurring or artificial **nuclides** (in particular those created by **fission**, the splitting of a heavy nucleus into two smaller ones) undergo spontaneous **decay**, with a release of energy, generally resulting in the formation of new nuclides. Termed **radionuclides** for this reason, they are unstable owing to the number of nucleons they contain (protons and neutrons) or their energy state. This decay process is accompanied by the emission of one or more types of **radiation**, ionising or non-ionising, and (or) particles. **Ionising radiation** is electromagnetic or corpuscular radiation that has sufficient energy to ionise certain atoms of the matter in its path by stripping electrons from them. This process can be *direct* (the case with alpha particles) or *indirect* (gamma rays and neutrons).

Alpha radiation, consisting of helium-4 nuclei (two protons and two neutrons), has low penetrating power and is stopped by a sheet of paper or the outermost layers of the skin. Its path in biological tissues is no longer than a few tens of micrometres. This radiation is therefore strongly ionising, i.e., it easily strips electrons from the atoms in the matter it travels through, because the particles shed all their energy over a short distance. For this reason, the hazard due to radionuclides that are **alpha emitters** is **internal exposure**.

Beta radiation, made up of electrons (beta minus radioactivity) or positrons (beta plus radioactivity), has moderate penetrating power. The particles emitted by **beta emitters** are stopped by a few metres of air, aluminium foil, or a few millimetres of biological tissue. They can therefore penetrate the outer layers of the skin.

Gamma radiation composed of high energy photons, which are weakly ionising but have high penetrating power (more than the **X-ray** photons used in radiodiagnosis), can travel through hundreds of meters of air. Thick shielding of concrete or lead is necessary to protect persons.

The interaction of **neutron radiation** is random, and so it is stopped only by a considerable thickness of concrete, water or paraffin wax. As it is electrically neutral, a neutron is stopped in air by the nuclei of light elements, the mass of which is close to that of the neutron.

• The quantity of energy delivered by radiation is the **dose**, which is evaluated in different ways, according to whether it takes into account the quantity of energy absorbed, its rate of delivery, or its biological effects.

• The **absorbed dose** is the quantity of energy absorbed at a point per unit mass of matter (inert or living), according to the definition of the International Commission on Radiation Units and Measurements (**ICRU**). It is expressed in **grays** (Gy): 1 gray is equal to an absorbed energy of 1 joule per kilogramme of matter. The *organ absorbed dose* is obtained by averaging the doses absorbed at different points according to the definition of the International Commission on Radiological Protection (**ICRP**).

• The **dose rate**, dose divided by time, measures the intensity of the irradiation (energy absorbed by the matter per unit mass and per unit time). The legal unit is the gray per second (Gy/s), but the gray per minute (Gy/min) is commonly used. Also, radiation has a higher **relative biological effectiveness (RBE)** if the effects produced by the same dose are greater or when the dose necessary to produce a given effect is lower.

• The **dose equivalent** is equal to the dose absorbed in a tissue or organ multiplied by a **weighting factor**, which differs according to the nature of the radiation energy, and which ranges from 1 to 20. Alpha radiation is considered to be 20 times more harmful than gamma radiation in terms of its biological efficiency in producing random (or **stochastic**) effects. The equivalent dose is expressed in sieverts (Sv).

• The **effective dose** is a quantity introduced to try to evaluate harm

Foulon/CEA

Technicians operating remote handling equipment on a line at the Atalante facility at CEA Marcoule. The shielding of the lines stops radiation. The operators wear personal dosimeters to monitor the efficacy of the protection.

in terms of whole-body stochastic effects. It is the sum of *equivalent doses* received by the different organs and tissues of an individual, weighted by a factor specific to each of them (weighting factors) according to its specific sensitivity. It makes it possible to sum doses from different sources, and both external and internal radiation. For internal exposure situations (**inhalation**, **ingestion**), the effective dose is calculated on the basis of the number of **becquerels** incorporated of a given radionuclide (**DPUI**, **dose per unit intake**). It is expressed in sieverts (Sv).

• The **committed dose**, as a result of internal exposure, is the cumulated dose received in fifty years (for workers and adults) or until age 70 (for those aged below 20) after the year of **incorporation** of the radionuclide, unless it has disappeared by physical shedding or biological elimination.

• The **collective dose** is the dose received by a population, defined as the product of the number of individuals (e.g., those working in a nuclear plant, where it is a useful parameter in the optimisation and application of the ALARA system) and the average equivalent or effective dose received by that population, or as the sum of the individual effective doses received. It is expressed in man-sieverts (man.Sv). It should be used only for groups that are relatively homogeneous as regards the nature of their exposure.

D Radiological and chemical toxicity

The chemical toxics linked to the nuclear industry include **uranium** (U), **cobalt** (Co), **boron** (B), used for its neutron-absorbing properties in the heat-exchange fluids of nuclear power plants, **beryllium** (Be), used to slow neutrons, and **cadmium** (Cd), used to capture them. Boron is essential for the growth of plants. Cadmium, like lead (Pb), produces toxic effects on the central nervous system. When the toxicity of an element can be both radiological and chemical, for example that of plutonium (Pu), uranium, neptunium, technetium or cobalt, it is necessary whenever possible to determine what toxic effects are radiological, what are chemical, and what can be either radiological or chemical (see *Limits of the comparison between radiological and chemical hazards*).

For **radioactive** elements with long physical **half lives**, the chemical toxicity is a much greater hazard than the radiological toxicity, as exemplified by rubidium (Rb) and natural uranium. Thus the chemical toxicity of uranium, which is more important than its radiological toxicity, has led the French regulators to set the **ingested** and **inhaled** mass limits for uranium in chemical compounds at 150 mg and 2.5 mg per day respectively, regardless of the **isotopic** composition of the element.

Certain metals or **metalloids** that are non-toxic at low concentrations can become toxic at high concentrations or in their radioactive form. This is the case for cobalt, which can be **genotoxic**, selenium (Se) (naturally incorporated in **proteins** or **RNA**), technetium (Tc) and iodine (I).

Cyrille Dupont/CEA

Two-dimensional gel electrophoresis image analysis carried out in the course of nuclear toxicology work at CEA Marcoule Centre in the Rhone Valley.