The treatments currently available for the decontamination of persons who have incorporated one or more radionuclides are few in number and often inefficacious. To develop new treatments, scientists are now taking two approaches, one chemical, the other pharmacokinetic.

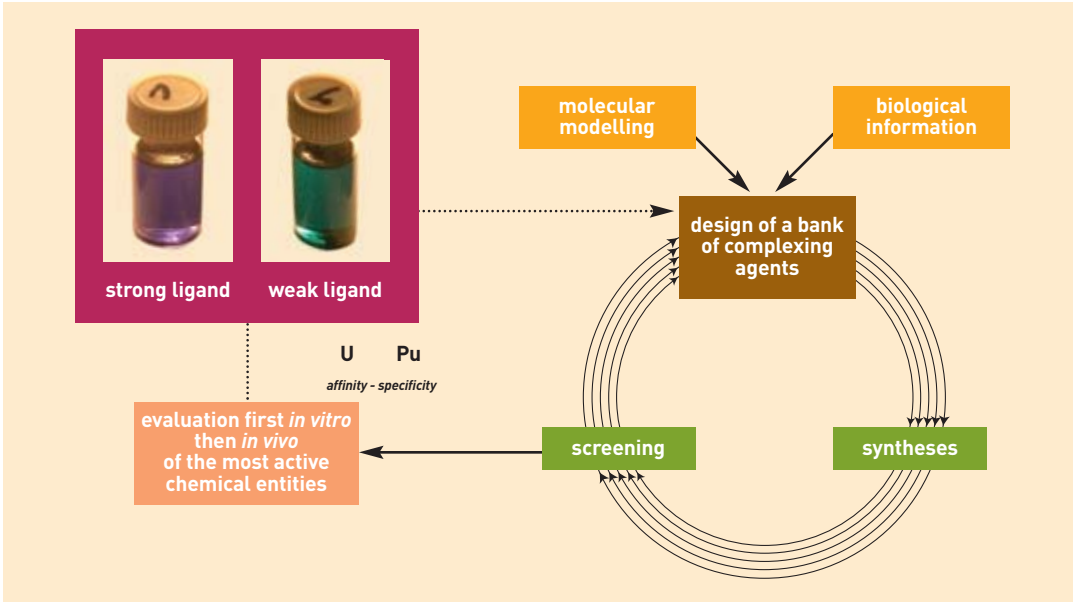
CEA on the trail of **new treatments**

L. Médard/CEA

Preparation at CEA Saclay of liposomes, lipid spheres mixed with solvents in which decorporating agents can be encapsulated to direct them towards contaminant transfer and deposition sites *in vivo*.

The development and exploitation of **radionuclides** in the second part of the twentieth century, mainly in the nuclear industry, drew attention to the hazards faced by persons handling these toxic materials. The effects on health of **internal contamination** depend on the **activity** incorporated, its distribution in the different tissues and the duration of action of the contaminant, which in turn depends on its **effective half life**.

Two extreme situations are observed: the massive **intake** of cæsium-137 causes the **irradiation** of all the tissues, resulting within days in the destruction of the bone marrow and an acute irradiation syndrome; incorporated radium-236 is retained in the skeleton leading decades later to a bone cancer (**osteosarcoma**). The usual therapeutic solution consists essentially in administering **decorporation** agents, which will accelerate the excretion of the radionuclide or radionuclides harboured in the body. These substances are **chelating agents**, which are designed to form **complexes** with radionuclides that are both stable and easy for the body to excrete. Unfortunately, the human treatments currently available are very few in number and often inefficacious. For example, diethylene triamine penta-acetic acid (DTPA) is the only agent used in human treatment after contamination by **transuranics**, and its efficacy varies according to the radionuclide and its physicochemical form: it efficiently traps plutonium and americium in the blood, but not uranium or neptunium.



CEA

Figure.
General strategy for the design of ligands potentially able to complex uranium and plutonium *in vivo*. Stringent screening ensures that these synthesised entities meet certain criteria. Illustrated: example of a strong uranium ligand (left), and a weak one (right).

Two approaches are currently being considered by scientists for the development of new treatments. The first is a chemical approach to the problem with the search for new entities with higher and more selective chelating power (see below *Synthesis and screening of new actinide ligands*). The second approach is pharmaceutical and **pharmacokinetic**, and seeks to superimpose the distribution of the decorporation agent on that of the contaminant to improve efficiency (see *Pharmacokinetic and pharmaceutical approach*). Neither approach would be feasible without the development of high performance methods (see *Methods of pharmacological evaluation*).

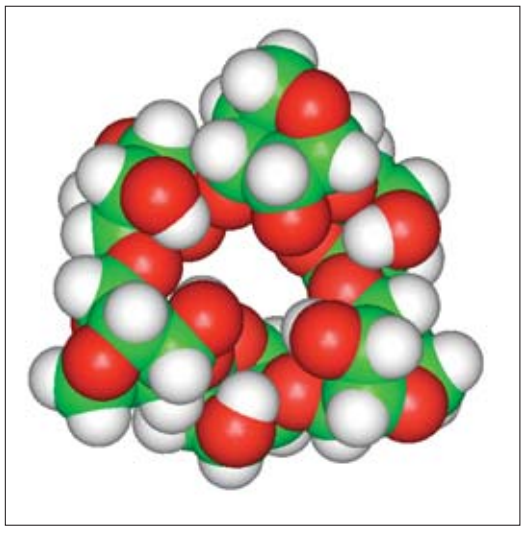
Synthesis and screening of new actinide ligands

The CEA's objective is to design and synthesise **ligands** able to complex the **major actinides** (uranium and plutonium) ***in vivo***. This aim is difficult to achieve because the chemical entities synthesised have to meet multiple criteria: strong association with the toxic metal, selectivity with respect to biological **cations**, non-toxicity and high **bioavailability**. To endeavour to solve this difficult problem, these researchers have opted for an innovative approach, now used in the pharmaceutical industry in the search for new drugs. It is based on the association between organic synthesis and rapid screening of chemical banks. To design the bank of potential ligands rationally, it is necessary first to develop **molecular modelling** methods to fully understand and ultimately predict how a metal ion such as uranium is complexed in an aqueous phase. This theoretical framework, coupled to the information already obtained on the mechanisms of intoxication by **actinides**, is used to guide chemical synthesis (Figure), which will involve both classical synthesis of highly structured ligands such as cyclodextrin(1) derivatives, and high throughput chemistry combining different complexing sub-units on structural supports. The chemical bank will then be rapidly multi-screened

(1) Cyclodextrin: a compound made up of several different or identical basic parts, (usually six or eight), associated in a chain, with a repeat motif, the sugar D-glucose (or dextrose).

CEA/DAM/df

In vivo experimentation in the rat to analyse the risk of radio-induced cancer. After inhalation of radioactive substances in aerosols, an autopsy of the animals and analysis of their sensitive organs (liver, kidney, lung, etc.) are carried out to determine the types of tumour they have developed.



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Molecular model of modified cyclodextrin studied for the decorporation of heavy metals.

Cyrille Dupont/CEA

Vials of calcium DTPA for the decorporation of transuranics or lanthanides. The efficacy of a new potential decorporating agent is systematically compared with that of this reference agent.

to select ligands that display both high affinity and high selectivity ***in vitro***. After complementary work (**speciation**, toxicity, etc.), the efficacy of the selected compounds will be assessed *in vivo*.

Pharmacokinetic and pharmaceutical approach

The objective of this research is dual. The first aim is to study the fate in the body (pharmacokinetics) of **decorporating** agents. It is most important to make sure that these agents are able to gain access to the **compartments** in which contaminants are carried and deposited *in vivo*. It is also important to know the distribution kinetics of the agents to set and optimise the decorporation protocols. The second aim is to propose original pharmaceutical formulations that will direct the decorporating agent more effectively towards the *in vivo* contaminant transfer and deposition sites. For example, liposomes are formulations that offer a broad range of useful carrier properties, in particular the concentration of a drug in the liver or an increase in its biological residence time in the bloodstream, and also improve drug efficacy. Liposomes are small hollow spheres, a few hundred nanometres in diameter, composed of a lipid double layer. The drug is inserted in the double layer where it is trapped in the central aqueous cavity as a function of its liposolubility.
This approach was recently applied with success for DTPA, the pharmacokinetics of which were studied in the rat and modified by means of a drug dosage form based on different liposomes(2). It was possible to study the distribution of DTPA in the plutonium target organs (liver and bone) and to modify this distribution in order to increase the contact time between the complexing agent and the radionuclide to favour decontamination. In addition, the pharmacokinetic study allowed the plutonium decorporation protocol to be optimised, thanks to the efficiency of the encapsulation, thereby obviating many long and costly animal experiments.

Pharmacological assessment methods

The objective of these studies was to measure the efficacy of newly synthesised decorporating agents in comparison with reference treatments. Two types of approach are currently being developed, an *ex vivo* approach and an *in vivo* approach concerning mainly uranium, plutonium (Pu) and americium. The evaluation of the chelating power of a compound towards a contaminant can be carried out *ex vivo*. First of all different biochemical forms of the radionuclide are isolated from isolates of the biological compartments targeted by the element, after experimental contamination of animals (mainly rats). After contamination by Pu, for example, in the rat, blood, liver and bones (femurs) are removed, to isolate Pu complexes with **transferrin**, the cytosol(3) compartment of liver cells (storing Pu) and the Pu-binding bone matrix of the femurs. The fractions thus obtained are then placed in the presence of different concentrations of the chelating agents to be tested. The affinity of the new decorporating agent is compared with that of DTPA (reference agent for the decorporation of Pu) by measuring the displacement of the radioelement from its biological target to the decorporating agent.
Another method for assessing the efficacy of a decorporating agent can be implemented *in vivo*. In these experiments, laboratory animals are contaminated with a radionuclide, and different treatments administered to them. The protocols of these treatments can vary according to the doses of chelating agents, their delivery forms, their administration routes, their injection time patterns, etc. After the contamination and the treatments the rats are kept in metabolism cages where their excreta can be collected regularly. At the end of the experiment, a material balance of Pu retention in the main organs and its excretion in urine and fæces is drawn up. The efficacy of the different treatments tested is compared with a reference treatment (e.g., DTPA, acute injection, one hour after contamination) in terms of variation of the retention of the radionuclide and its excretion. The tests carried out *ex vivo* make it possible to limit animal experiments to those on chemical entities that exhibit higher affinities for the contaminant than the reference agent. By means of these experiments *in vivo*, the efficacy of new treatment protocols can be evaluated in order to improve the decorporation of radionuclides.

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(2) As part of joint research by CEA/LRT, CEA/SPI and UMR 8612 of the University of Paris XI.

(3) Cytosol: the main constituent of the cytoplasm, other than membrane organelles such as the endoplasmic reticulum and the **mitochondria**.

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.

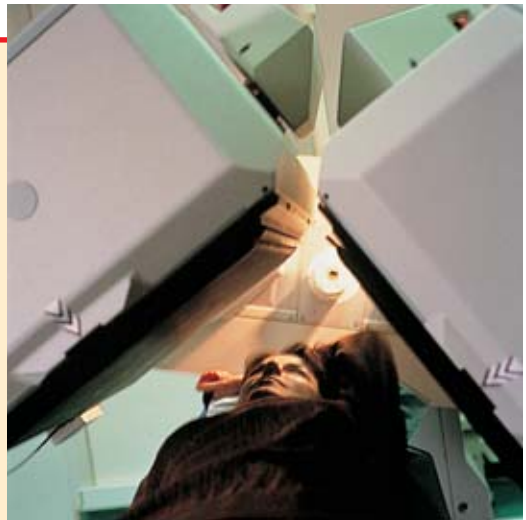
In the **Chernobyl accident** (Ukraine), which occurred in 1986, the total radioactivity dispersed into the atmosphere was of the order of 12 milliard milliard (10^{18}) becquerels over a period of 10 days. Three categories of radionuclides were disseminated. The first consisted of volatile fission products 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.