IV. HOW CAN THE EFFECTS OF TOXICS BE PREVENTED AND TREATED?

Deeper knowledge of the mechanisms involved in the action of nuclear toxics (Chapter II) and their effects on health (Chapter III) will ultimately make it possible to devise methods of prevention, efficient monitoring schemes and, when needed, satisfactory remedial solutions.

Methods of prevention might, for example, aim to identify genetic predisposition and other individual vulnerability factors. Biosensors are offering promising monitoring solutions.

Remedial treatment already includes many existing methods for the decontamination, depollution or decorporation of elements distributed in certain compartments of the geosphere, the trophic chain or the body. Alongside certain traditional processes, such as isotopic dilution, mechanical action or chemical neutralisation, in particular the use of derivatives of Prussian blue, methods linked to the metabolic competition of a non-toxic substance with the unwanted element to be eliminated are progressing. Besides achieving the further improvement of the only treatment – DTPA – which in its present form is efficacious against certain transuranics, researchers are working on new *chelating agents*, which are able to bind strongly to toxics to form a complex stable in biological media. These agents are able to trap radionuclides on their way through the body and speed up their successful elimination. At CEA, research on these "new extractive structures" now extends a programme launched as far back as 1998.

In the development of new chemical entities with decontaminating properties, an alternative exists between a strictly chemical approach, the aim of which is to design more efficient and more selective chelating agents, and a more "pharmaceutical" approach that aims to match the distribution in the body of the decorporating agent to that of the contaminant. For this purpose, scientists are using the most advanced methods of the pharmaceutical industry, namely the association of organic synthesis and rapid screening of banks of chemical entities, the properties of which may one day be predicted by molecular modelling.

To detect toxic metals directly *in situ*, and to identify and localise them *in vivo* in the body if necessary, scientists are developing, alongside chemical sensors, biological sensors that are specific to particular toxins.

Biosensors to track down toxic metals

S. Berger/CEA

Visualisation of the calmodulin structure in 3-D.

The decontamination of sites and persons **contaminated** (Box B, ***Human exposure routes***) by toxic metals derived from a nuclear-related activity requires, first, the means to neutralise the toxicity of these metals in the environment and in the bodies of contaminated persons, and second, efficient specific reagents to detect the metals in question. The detection and identification of such metals is normally carried out *ex situ* by inductively coupled plasma mass spectrometry (ICP-MS). This method is cumbersome and costly, but methods for the direct detection of toxic metals *in situ* and their localisation ***in vivo*** in different biological and cellular **compartments** are still lacking. Accordingly, in the framework of the CEA's Nuclear Toxicology Programme, teams of chemists and biochemists are working on the development of sensors and specific biosensors for these toxic metals.

From chemical sensors to biosensors

Different approaches have been chosen to detect metal ions, in particular in aqueous media or in biological samples: one uses chemical sensors, three others biochemical sensors.

Chemical sensors

Chemical sensors are made of a **complexing** entity coupled to a fluorescent molecule, the fluorescence of which is modified upon complexation of a metal ion. However, these sensors bind families of ions and are not very specific to any particular metal: they are therefore of limited utility. Importantly, no specific chemical sensor has yet been described for uranium, plutonium and the other ions of interest in nuclear toxicology.

Peptide biosensors

Among the biosensors, the fluorescent peptide sensors are made up of a 26 **amino acids peptide** derived from a zinc finger domain, chemically labelled with a fluorescent group. In the presence of zinc ions, they form a structure around the metal and expose the fluorescent group to changes in the environment,

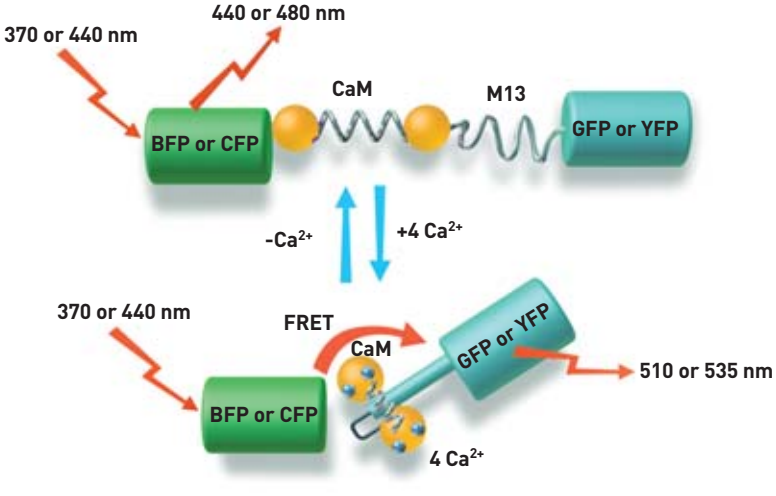

Figure.
Schematic structure of a "cameleon" biosensor for calcium. The scheme shows how the transfer of fluorescence (FRET) in a pair of fluorescent proteins GFP (BFP and GFP, or CFP and YFP) can measure calcium. The GFP proteins are depicted as cylinders, calmodulin (CaM) as two spheres connected by a spiral, and the substrate peptide M13 is disordered. In the absence of calcium (top), proteins BFP and CFP (fluorescence acceptors), after excitation at wavelengths 370 or 440 nm, emit a fluorescence at 440 or 480 nm, respectively. After addition of calcium (bottom), this metal binds to the four sites of calmodulin, which then binds peptide M13 in an ordered conformation, bringing the biosensor into a favourable position for the fluorescence transfer (FRET) between the acceptor protein (BFP or CFP) and the donor protein (GFP or YPF) which emits a fluorescence at wavelength 510 or 535 nm. The calcium is then detected by the intensity of the emitted fluorescence and the colour change of the biosensor, hence the nickname "cameleon" (adapted from *Nature*, vol. 338, 28/08/97 © Macmillan Publishers Ltd.).

resulting in a variation in fluorescence emission that depends on the metal concentration. Alternatively, when the peptides have been conjugated with two appropriate fluorescent groups, zinc binding by the peptide causes a conformational modification that favours an efficient transfer of energy between the two fluorophores (*Fluorescence Resonance Energy Transfer* or FRET), resulting in the emission of a fluorescence signal that is proportional to the metal concentration.

"Cameleon" biosensors

Called "cameleons", these fluorescent **protein** sensors (Figure) consist of a fusion protein comprising in succession, from its amino-end to its carboxy-end: a blue or cyan mutant (BFP or CFP, fluorescence donor) of the fluorescent protein GFP derived form the jellyfish *Aequorea victoria*, calmodulin (CaM), which can bind up to four calcium ions, a peptide-substrate (M13) binding to calmodulin and another green or yellow mutant of the same fluorescent protein (GFP or YFP, fluorescence acceptor). Calcium binding by calmodulin causes a conformational change in the fusion protein, which forms a new site to which the peptide-substrate binds, and produces a new conformation in the fusion protein, resulting in a more efficient energy transfer from the fluorescence donor (BFP or CFP) to the fluorescence acceptor (GFP or YFP). The result is an increase in the fluorescence emitted by the fluorescence acceptor (GFP or YFP). This fluorescent indicator system, which relies on the variations in the conformation induced by calcium binding by the calmodulin-peptide complex, is calcium-specific. A similar system has been described that uses the zinc-specific protein metallothioneine as complexing agent. The development of "cameleons" specific to toxic metals therefore depends on the engineering of new calmodulin and (or) metallothioneine proteins that are specific to these metals. These indicators offer the advantage that they can be genetically coded and addressed to precise intracellular compartments *in vivo*.

Bacteria

Bacteria containing a promoter that can translate the presence of a toxic metal by a luminescent signal form the third approach. In this system, the metal acts as a cellular stress factor and induces an altered expression of a bioluminescent protein, which thus provides the detection signal. These systems are not therefore specific to any one metal, and cannot be used as metal-specific sensors until specific promoters have been developed. Various CEA research teams are working on these various approaches to the development of specific sensors and biosensors.

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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.

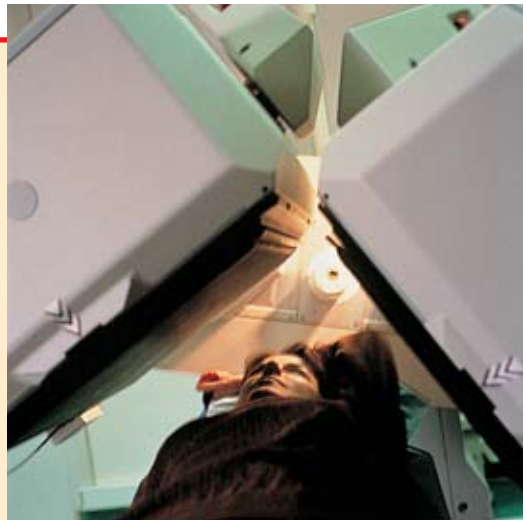
In the **Chernobyl accident** (Ukraine), which occurred in 1986, the total radioactivity dispersed into the atmosphere was of the order of 12 milliard milliard (10^{18}) becquerels over a period of 10 days. Three categories of radionuclides were disseminated. The first consisted of volatile fission products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