Environmental aspects of the ENVIRHOM program : first results

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Abstract: In the field of environmental radioprotection, the knowledge gaps concern situations leading to chronic exposure at the lower doses typical of the living conditions of organisms influenced by radioactive releases. For any radionuclide and ecosystem, the specificities of these situations are as followed : (i) various chemical forms occur in the environment as a function of the physico-chemical conditions of the medium; (ii) each transfer from one component to another can lead to a modification of these forms with a "chemical form-specific" mobility and bioavailability; (iii) different categories of non-radioactive toxicants are simultaneously present. In this multipollution context, the biological effects of ionising radiation may be exacerbated or reduced with the potential for action or interaction of all the pollutants present simultaneously. These situations of chronic exposure at low levels are likely to cause toxic responses distinct from those observed after acute exposure at high doses since long-term accumulation mechanisms in cells and tissues may lead to microlocalised accumulation in some target cells or subcellular components. The assessment of these mechanisms is primordial with regard to internal exposure to radionuclides since they increase locally both the radionuclide concentration and the delivered dose, coupling radiological and chemical toxicity. This is the main purpose of the ENVIRHOM research programme, recently launched at IRSN. This paper provides a global overview of the experimental strategy and of the first results obtained for phytoplankton and uranium.

1. INTRODUCTION

The international community is showing a priority need for the development of design, knowledge and methods for risk assessment in connection with ionising radiation on biocenosis [1, 2, 3] in the field of radioprotection. One of the main challenges is to improve knowledge of situations that lead to the environment's and man's components being subject to chronic exposure from radionuclides present at a trace[1] level. In these situations and throughout their lifetime, the biocenosis and the general public are subject to external radiation that is linked to evolution in contaminated surroundings and to internal radiation alongside chemo-toxicity that is linked to the integration processes of radionuclides in living organisms (direct transfer due to abiotic compartments and trophic transfers). For both of the following cases, the risk is, in particular, a function of the type of radiation:

(1) in the case of internal contamination, the risk linked to the biological incorporation of α?or β emitting radionuclides will be more important than that linked to the incorporation of radionuclides that mainly emit radiation which is not directly ionising i.e. neutrons and photons (low LET, high penetration);

[1] The word «traces» in geochemistry is the opposite of «major». The latter type of element enters into the composition of the earth's crust (O, Si, Al, Fe, Ca, Na, K, Mg, Ti, K, H, P, Mn) and may be found in medium sized quantities greater than or equal to 1 g/kg (or 1 $^{o}/_{o}$) whereas the «elements in a state of trace» are characterised by quantities lower than 1g/kg. The boundary between major elements and elements in a state of trace is conventional (a major element in the lithosphere may be in traces in the hydrosphere or in the atmosphere). Virtually all the elements including oligo-elements are found in a state of trace in the living world– in the geochemical sense – [4].

(2) in the case of external radiation, this hierarchy works in the opposite direction. The risk that is therefore associated with γ emitters is more important in front of that associated with α or β emitters. Furthermore, the potentially associated chemo-toxic risk thus becomes insignificant.

Within the framework of point (1), the ENVIRHOM programme, launched last year at the IRSN suggests data acquisition to understand and quantify biological effects involved by the accumulation of radionuclides by living organisms (biological components of ecosystems and the general public for human populations) in chronic exposure situations [5]. The accumulation mechanisms in cells and tissues may lead to microlocations on some target cells or subcells according to the biochemical behaviour of the studied radionuclide, bringing chemo-toxic phenomena into play and energy deposits of a very different size and flow rate, characterized by heterogeneity at the cell scale. Biological effects may result from these phenomena. These effects on living organisms, man included, are not precisely known to date as the vast majority of available data corresponds to studies performed on high doses of radionuclides, short term exposure and not within a multipollution context (i.e. without taking into account the simultaneous presence of other categories of pollutants: metals, organic micropollutants, …).

ENVIRHOM suggests the assessment of the integration potentialities of radionuclides into the trophic networks from the soil and sediment considered as reservoir compartments of the biosphere likely to be gradually and significantly enriched in radionuclides released into the environment, and acting as secondary source-terms for the other components of the ecosystems. These transfers within ecosystems are characterised by a wide variety, concerning both the biogeochemical behaviour of radionuclides and the feeding strategies of plants and animals. The studies of long term induced biological disturbance as a consequence of bioaccumulation processes will be systematically focused on behaviour, growth and ability to reproduce, without excluding other more subtile effects (such as cytogenetic effects for example). The aim is to go on establishing relationships between the concentration of bioavailable chemical species in the various exposure sources for organisms, and the ecological repercussions, as a result of individual disturbances, in particular in terms of population dynamics and community structure (figure 1).

One of the programme's original features is displayed by the similarity between the approach strategy employed for a limited number of biological models, thoroughly incorporating the variety of biological interfaces met with in the plant and animal Kingdoms, including the human organism.

In order to limit the framework of this very broad research programme, a list of a limited number of radionuclides has been decided on the basis of a multi-criteria approach. The choice has been made within the list of radionuclides with a significant occurrence in the different source-terms from nuclear installations under normal operating conditions (nuclear power plant, fuel reprocessing plant), storage sites for radioactive wastes, uranium-bearing ore mining sites in operation or after closure, and more generally industries or particular geochemical situations generating a significant increase in naturally occurring radionuclide concentrations in the environment, post-accident situations such as Chernobyl. The first criteria is the type of radiation with selection of α and β emitters that develop the highest risk associated with internal contamination. The second criteria is the physical period, which should be significant in terms of chronic contamination at human lifespan scale, or 70 years. Thereafter, the remaining nuclides were ranked according to their propensity to react with biomolecules directly dependent on the affinity of radionuclides for hydroxyl groups, thiols and / or phosphates and therefore to bioaccumulate (In general, the tendency to form organic complexes is proportional to the tendency to hydrolyse and the electric charge, and inversely proportional to the ionic radius). The main differences within these biochemical properties help to distinguish two categories of elements : radioactive isotopes of an element (stable isotope or chemical analogous) involved in the constitution of living matter as macro-nutrients or oligo-elements or radioactive isotopes without any known biological function. Applying this selection method, the priority for radionuclides is as followed : α emitters with three actinids : natural

uranium, americium-241 and neptunium-237, long-lived β emitters with technicium-99, iodine-129, selenium-79 and Cs-135. The first experimental development was launched with uranium.

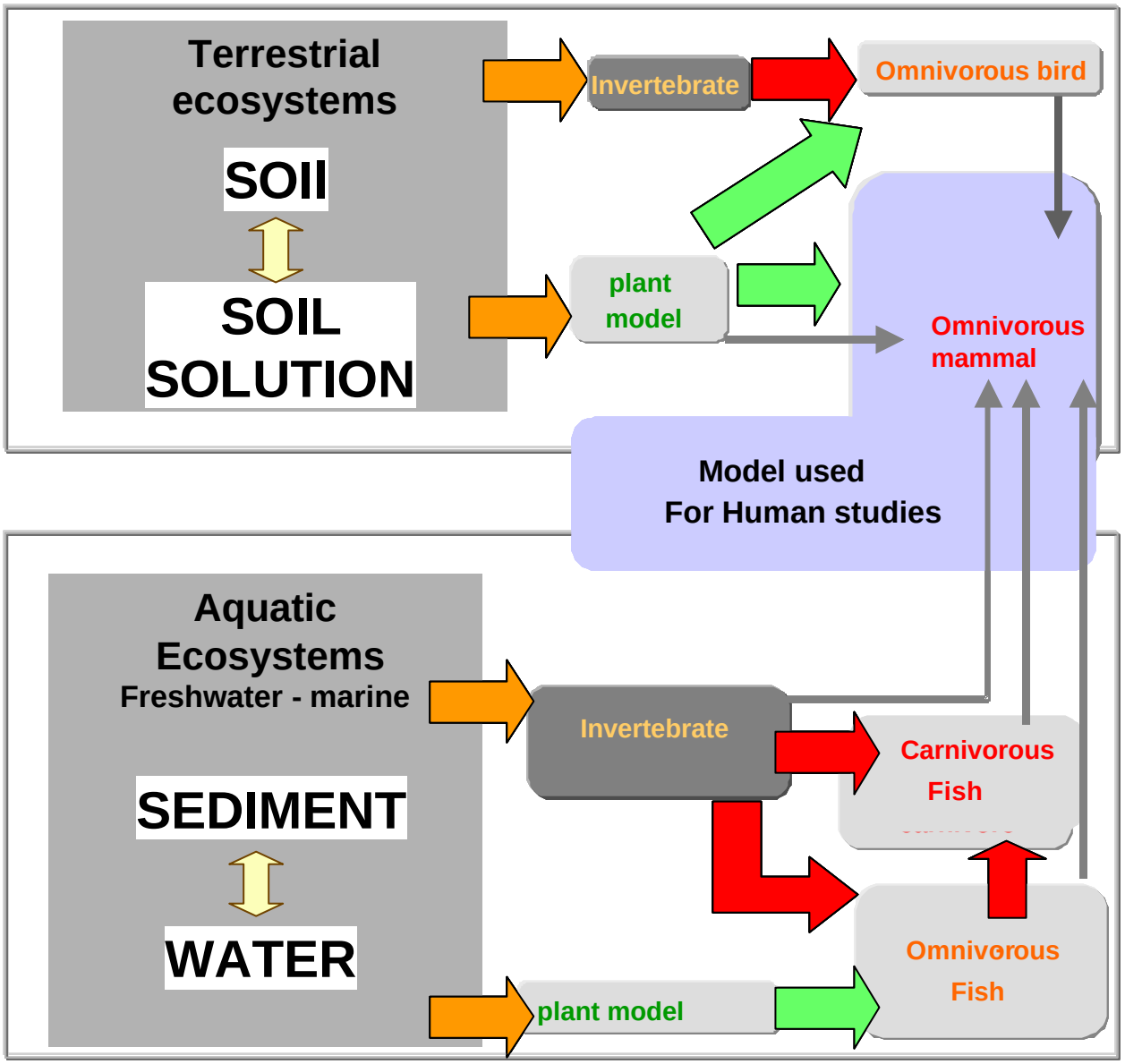

Figure 1 – Articulation of initiated experimental developments on uranium. Each of the studied transfers portrayed by an arrow from the exposure source component to the target organism will be investigated for the four research areas that define the ENVIRHOM programme: (1) characterisation of the bioaccumulation processes, (2) induced biological effects, (3) dosimetric consequences, (4) understanding of the mechanisms employed.

2. WHY URANIUM?

Uranium concentrations in terrestrial and aquatic surroundings may be increased in connection with various anthropogenic contributions, originating from uses throughout the different stages of the nuclear fuel cycle (mines and waste storage sites in particular), and up to agricultural use (phosphate based fertilizers), the medical surroundings, research laboratories and military use of depleted uranium. Several phenomena linked to the biogeochemical behaviour of uranium, in connection with the implementing of physical processes of solid form transport (fluid erosion, sedimentation …) and water transport (colloid and dissolved forms), may lead to the existence of accumulation zones in soils and sediments that are the biosphere's physical compartments: horizons that are rich in organic matter and/or iron

oxyhydroxides in an oxidising condition, flooded soil or sediments in a reducing condition (uranium is therefore at the (+IV) valency and tends to enter into zones that are rich in organic matter, in sulphur and/or minerals rich in Fe(II)). Uranium's environmental geochemistry quite schematically enables U transport into high Eh zones [U(+VI)] and a deposit by reduction and precipitation in low Eh zones [U(+IV)] to be predicted [6]. The existence of these accumulation zones may aggravate reactions that are likely to occur at the biological interface level and consequently, the mechanisms leading to an implementation of the bioaccumulation processes on various intercellular biological targets in plants and animals.

To summarise, according to the geochemical contexts, uranium may be transported and/or accumulated in some ecosystem zones in connection with its physico-chemical properties. These properties, in association with the bioaccumulation potential of animals and plants, are such that its presence in the environment may create a potentially chronic risk for the biocenosis both on the chemical and radiological scale (effect, in particular, of α particle ionising radiation).

The operational codes (ABRICOT 2.2., AQUAREJ 2.3 or FOCON 96 1.0 for example) currently in use at the IRSN for chronic exposure situations, consider the environment compartments to be highly simplified, to equilibrium, with a homogenous concentration, performing exchanges on the basis of transfer coefficients that characterise the element without distinguishing chemical species or even mobile and bioavailable fractions. The accumulation processes in some areas of the biosphere are not taken into account, in the same way as the bioaccumulation processes in connection with the internal contamination of organisms. Effects on the biocenosis are not investigated.

With regards to the global dosimetric assessment models that exist for man and are to be implemented for non-human living organisms, the concepts in use are not adapted to (1) the existence of a radioactive nuclide concentration heterogeneity at the scale of the cell/organ/full organism; (2) integration of the time factor in exposure and the appearance of the biological effect; (3) use of biokinetic parameters that characterise chronic exposure at the scale of the organism's life expectancy; (4) low exposure levels; (5) the potential existence of a relationship between radioactive and chemical poisoning.

The selection of uranium for the first experimental studies should ultimately enable results to be gathered on a two to four year scale that provides evidence as to the relevance in obtaining knowledge specific to the low exposure context (*v high dose*), in the long term (*v short term*) with the aim of a dynamic model (*v to equilibrium*) taking into account the heterogeneous spreading process (*v homogenous*) on the most relevant spatial scale with respect to accumulation processes in the biotopes and bioaccumulation in living organisms.

3. THE FIRST EXPERIMENTAL DEVELOPMENTS ON URANIUM.

3.1 Chosen biological models and transfers studied

The aim of the first experimental developments is to assess the uranium accumulation and elimination kinetics and to highlight the bioaccumulation mechanisms that are likely to be employed by organisms that are representative of the continental ecosystems and by the human organism. The main aim first of all breaks down into establishing a link between the uranium physico-chemical species formation in the various exposure sources for living organisms (uranium in thermodynamically plausible forms in the water column – dissolved and solid phases – sediments, soils in the case of direct exposure pathway) and the bioavailability for various biological models (algae, higher plants, invertebrates). These biological models are chosen as first trophic levels that enable the integration of the radionuclide in the trophic nets: transfer from the water column (dissolved phase) to a green unicellular algae, from water filtration and ingestion of mineral particles for two freshwater lamellibranches molluscs, via root transfer from the soil solution for a higher plant model. Two trophic relationships will be studies in order to screen in a

simplified manner, the diet panel that may exist in the animal world: transfer of a plant model towards a filtering animal model (phytoplankton ->bivalves); transfer from one animal model (invertebrate acting as prey) towards a carnivorous animal model (prawn acting as predator) and towards a fish model. Secondly, the link with the biological effects will be investigated by taking up positions in organism chronic exposure situations.

3.2 Conceptual basis: link between chemical speciation of the radionuclide in the exposure source, bioaccumulation and toxic effects

The suggested research comprises the understanding of the phenomenology linking the pollutant chemical species formation in the exposure source, passing through the biological barriers, bioaccumulation and induced biological effects. These research topics represent the principles of all studies that endeavour to understand interactions between a metal and a living organism [7]. An application in ecotoxicology in a more or less in-depth manner, for a few cationic transition metals (Ag, Cd, Cu, Ni, Pb and Zn in particular) has already taken place. Various theorems and concepts have been developed in the aim of rationalising the substantiation and expression of accumulation and/or toxicity observations of cationic metals in aquatic organisms.

The free ion model (or FIAM for Free Ion Activity Model) was initially developed by Morel [8]. It transforms the interaction of an M^{n+} metal in an aquatic organism in three successive stages: (i) advection/diffusion of the metal to equilibrium in the “bathing” solution, the interface making up the biological membrane and the exposure surroundings, (ii) complexing reaction with a surface site, (iii) transport through the membrane (Figure 2). These three stages are a pre-requisite to all biological responses (level of input in the cell and/or targeted biological effects called “endpoint”). The taking into account of additional theorems (models that are derived from the free ion model, such as the biological ligand model for example – BLM -, or the theory of the biological receiver – BRT-) also enables substantiation of the fact that metal bioavailabililty decreases according to two processes: (1) through a decrease in the free ion activity in the solution leading to a reduction in the metal fixation on the biological receiver sites, (2) through an increase in the competitor ion concentration and therefore a reduction in the metal quality linked to receiver sites. It is therefore possible to take the physico-chemical quality of the water into account (pH, hardness, dissolved organic matter) in the assessment of a metal’s toxicity implementing two sub-models: a «conventional » sub-model concerning chemical species formation of the metal in the surroundings (MINEQL+ or JCHESS) enhanced by a complexing reaction reduction on the receiver site being taken into account (for example, fish gills) where the equilibrium constant would be experimentally characterised and a toxicity sub-model linking the metal load that has thus been accumulated on the action sites and the toxic effect. The application examples for this type of model, for the moment, only deal with metal fixation on the receiver sites at the gill level, the direct exposure channel from water and short term toxicity phenomena [9, 10]. Within the scope of the ENVIRHOM problem, it will also be necessary to take the biological effects in the assessment into account:

- The exposure chronicity as such examples given in publications show that this changes biokinetics with respect to short term exposure,

- The exposure channel, directly from physical surroundings, but also trophic as the latter leads to an accumulation and a clean property tissue/cell distribution.

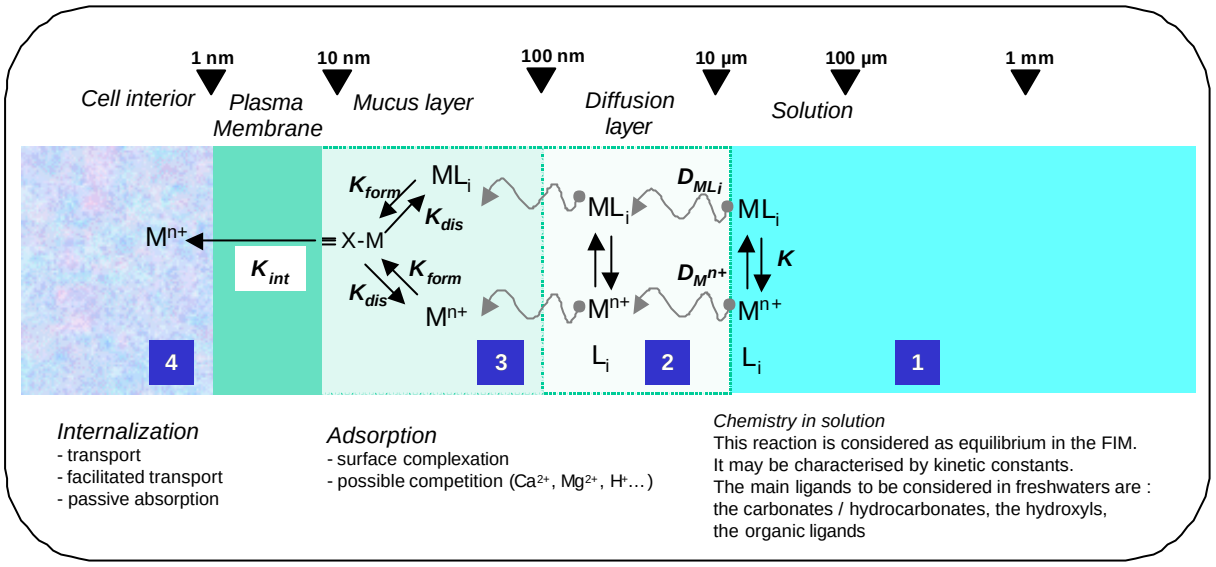

Figure 2 – The main metal-cell interaction stages and the model theorems that substantiate them (Free Ion Model, Biological Ligand Model and biological Receptor Theory) [modified from Campbell (1995) [11]].

3.3 Experimental procedure

3.3.1 Chemical speciation/bioaccumulation link: short term exposure experiments

Bioaccumulation of a pollutant is a function of the species formation of the latter in an exposure source (physical compartments, food). Where uranium is concerned, a geochemical model may fairly reliably support the experimental approach as long as enough thermodynamic data is available and its consistency has been verified, at least in matters of reactions with mineral ligands. The exposure sources are in the first stage of simplified physico-chemical composition (artificial water of mineral composition that is gradually complexed as knowledge and understanding deepens, up to the taking into account of the presence of dissolved organic matter) in such a manner as to predict in the most reliable way possible, the forms of U that are present and likely to cross over biological barriers. The geochemical speciation code JChess [12] using a database compiled from the OECD/NEA thermochemical data base project was used to perform the solution speciation calculations. Three main variables are then with a complexification of the water column chemical composition : (1) pCO_2 and pH (from atmospheric pression to 10 atm and from acid to basic conditions respectively); (2) competitive cations such as Ca, Mg; (3) presence of ligands such as phosphates, or dissolved organic matter. For these complementary short-duration well-defined laboratory experiments in simplified conditions; biokinetics for short exposure times (in hours for algae, in days for animal models) and characterisation of the input mechanism(s) are investigated.

The concentration range used in total uranium in the exposure sources goes up to a maximum of 1 $mg \cdot l^{-1}$, a value that may be encountered in an aquatic ecosystem that is influenced by mining discharge.

Where bivalve is concerned, the uranium transfer associated with model mineral particles will be assessed, in the same way as the trophic transfer due to ingestion of phytoplankton [9]. In order to quantify the bioaccumulation processes that may be employed during transfer through ingestion when in a predator-prey relationship, experimental studies on the bivalve (asiatic clam as prey) or the fish (rainbow trout as predator) will begin with a pharmacokinetic approach in order to model the fate of an

alimentary bolus where the main chemical "pools" of uranium in the prey (subcellular fractionation) have been explored.

3.3.2 Bioaccumulation/biological effect link: chronic exposure (long term) experiments

Based on knowledge gained from previously described experiments, the exposure scenario that defines the most important bioavailability will be chosen to be transposed into experiments that enable simulation of chronic exposure under controlled conditions (significant duration in front of the organisms' lifespan). During these experiments, the bioaccumulation processes will be investigated in parallel with the involved biological effects. The primary aim of the studies of microlocalisation will be to determine, for a few target organs, whether uranium is evenly distributed in the tissues and cells or whether, on the contrary, it is localised in particular structures. In the latter case, the position of the radionuclides in relation to or within target cells will be established. The chosen observation technique will be electron microscopy in transmission associated with a spectral analysis of X energy dispersion.

Certain biochemical responses will be measured to assess the early effects of stress at cellular or subcellular level, involving dosage and validation techniques borrowed from ecotoxicology. Several (sub)cellular endpoints will be investigated : (1) The responses to oxidative stress (catalase, superoxide dismutase, glutathione transferase, forms of glutathione); (2) The exploration of energy expenditure (adenylate load, glycogen reserves, protein, lipid and glucid content); (3) Other biomarkers of more general effects (induction of metallothioneins (or phytochelatines for plants), of stress proteins (hsp). At the individual scale, the investigation of biological disturbances following bioaccumulation will be undertaken mainly on three essential functions of great importance for the functionning and structure of any ecosystem : the growth, the behavior and the reproduction, this latter including cytogenetic effects on germinal cells. For organisms with a sufficient organisation level (fish), this will be mainly viewed as investigations on the immune system, the central nervous system and the reproductive system.

The suggested work on phytoplankton corresponds to the assessment of the bioaccumulation and the microlocalisation of uranium (extracellular, intracellular including the cytosolic fraction) and the effects that are induced more particularly on the photosynthetic activity and/or the growth rate. The experiments will be performed in turbidostat, which enables the algae to be continuously harvested at a constant cellular density, by limiting the species formation modifications of uranium in the surroundings.

4 FIRST RESULTS OBTAINED FOR THE UNICELLULAR ALGAE MODEL

Phytoplankton represents the basic part of the productivity chain, at the lowest trophic level in the freshwater trophic networks. It is therefore a key player in the elements cycle in the ecosytems, especially with regards to their integration into the food chain from the water column.

Two distinct phenonema may be identified for algae : adsorption (metal fixation at the algae surface without penetration of the cellular membrane) and absorption (metal internalisation). Particular attention is given to the difference between these two phenomena. The first is of a chemical nature whereas the second is of a biological nature. By using short exposure time (< 1 h) and low cellular density for the experimental population, it is possible to keep under control the uranium solution chemistry and to achieve the identification of one or more chemical species that govern the uranium/algae interactions [13].

The first results suggest that uranium adsorption at the surface happens very quickly and reaches a stationary state (equilibrium) in just a few minutes (Figure 3). The absorption, however, increases with exposure time. In addition to this, saturation phenomena are noted when the uranium concentration reached a certain level (some µM *i.e.* around 0.1 mg/L), that is to say that the metal internalisation capacity of the algae reaches a maximum level. One other stage was overcome when observing that phosphates (which are found in the environment due to human activities and which are responsible for the eutrophication of waterways), by forming chemical complexes with uranium, unaffect absorption of this metal by the algae. The uranium accumulation is significantly lower in acidic (pH 5) than it is in neutral media (pH 7). This remark leads to important questions as uranium chemistry is greatly altered within this range. Michaelis-Menten kinetic parameters K_m and V_{max} were determined using the Marquardt-Levenberg algorithm to obtain the best fit to the observed uptake levels (see equation and curves in Fig. 3b). The half-saturation constant is nearly doubled at pH 7 when the data is analysed based on the total uranium concentrations. Growth toxicity tests, representative of long term exposure (the lifespan for an algae within our experimental conditions is in the order of 10 h), are in progress, underlying the importance of the water quality variables (such as pH). Microlocation data are also expected.

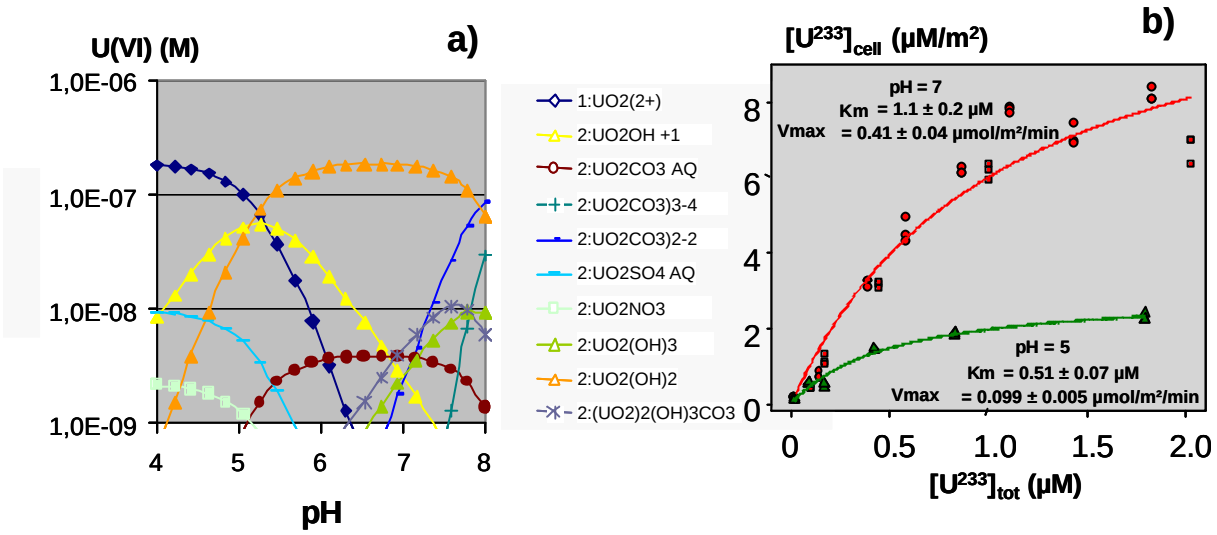

Figure 3 – a) Uranium speciation diagramme in a very simple artificial water in equilibrium with atmospheric CO_2 b) U intracellular uptake for phytoplankton after 30 minutes of exposure at various total uranium concentrations (? = pH 5; ? = pH 7). Error bars represent the standard deviation from the average of three measurements.

5 CONCLUSIONS - PERSPECTIVES

The knowledge gained about uranium, in association with a State of the Art calculation of biocenosis doses almost entirely comprised of base data intended to establish a dose-effect relationship for individuals or the population in cases of severe external γ? radiation, will be substantiated as an illustration in order to put forward concepts linked to the assessment of doses that are delivered consecutively to

internal contamination and to effects on the individuals. The original feature is the integration of the element's geochemical speciation to define potential accumulation zones in biotopes, to take into account the bioavailable form(s) in order to understand the bioaccumulation processes and the resulting effects. The work carried out in a similar manner on the mammal model that was used for studies that may be extrapolated to man, ensures the consistency of the integration of all the results in a complete operational environmental and general public radioprotection system for chronic exposure situations.

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