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Anne van Der Meeren, Jaime F. Angulo, Sandra Bohand, Nina M. Griffiths. A quick and simple in vitro assay to predict bioavailability of actinides following accidental exposure. *Toxicology in Vitro*, 2019, 58, pp.142 - 149. 10.1016/j.tiv.2019.03.027 . hal-03485696

HAL Id: hal-03485696

<https://hal.science/hal-03485696>

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A quick and simple *in vitro* assay to predict bioavailability of actinides following accidental exposure

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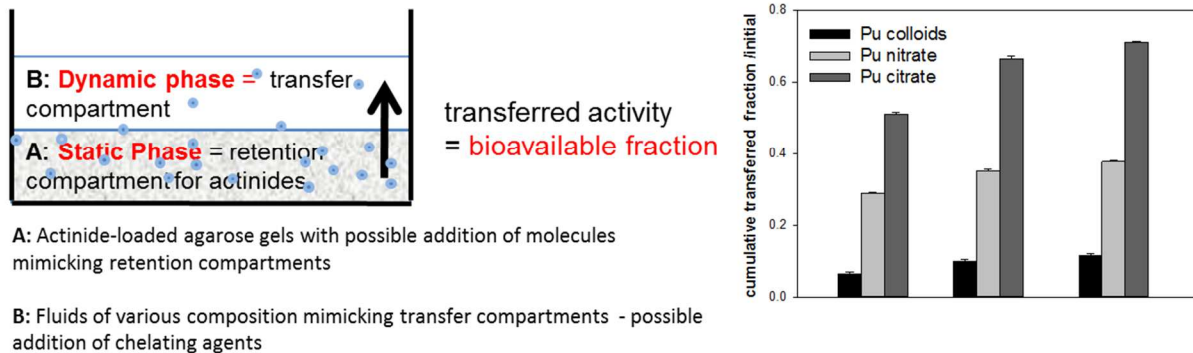
Key words: Bioavailability, Retention/Dissolution, Actinides, *in vitro* model development, chelating agent

Highlights:

- Actinide transfer from the static retention phase to the dynamic phase varies with nature and intrinsic properties of the contaminants in agreement with their *in vivo* behavior
- The presence of bioligands in the static retention phase modifies the transfer of actinides in the dynamic phase
- Chelating agents added to the dynamic phase enhance transfer of the contaminant
- The assay can be used as a first approach to predict *in vivo* behavior of radiocontaminants

Graphical abstract:

Demonstration of differential actinide transfer *in vitro* using a simple novel assay



Abstract (200 words)

Physicochemical properties of actinides highly influence internal intake and biodistribution. An *a priori* knowledge of the dissolution properties of compounds involved in accidental exposure would be of great help in early dose assessment. However, this information is rarely available, leading to difficulties in interpreting excretion data from contaminated victims.

We developed an *in vitro* acellular assay to predict *in vivo* bioavailability of actinides and improve medical handling of the victims. Various actinides of different physicochemical properties were used to validate the reliability of the assay to mimic *in vivo* behavior of the contaminants. Our assay was designed as a dynamic multicompartmental system in which an agarose gel represents the retention compartment of actinides and a dynamic phase the transfer compartment. Relevant physiological conditions were obtained by introducing various components both in the static and dynamic phases.

The proposed model may provide a good prediction of *in vivo* behavior and could be used as a first assessment to predict the fraction of actinides that could be potentially transferred from retention compartments, as well as the fraction available to chelating drugs.

Introduction:

Internal contamination of workers with radioactive elements such as actinides can arise from accidents involving nuclear reactors, industrial or medical sources. The understanding of the biodistribution of actinides following internal contamination is an important step in the process of dose estimation and risk assessment, as well as in determining an appropriate decorporation treatment.

Workers can be exposed to various actinides, such as Plutonium (Pu), Americium (Am) and Uranium (U), and to various physicochemical forms of these elements during the life-cycle of nuclear fuel production, use and recycling or disposal. Physicochemical properties determine biodistribution of actinides following intake. Committees for radiation protection such as International Commission on Radiological Protection (ICRP) or National Council on Radiation Protection (NCRP) propose biokinetic models where dissolution properties are recognized among the most critical factors governing the biodistribution of radionuclides, and thus influencing dose calculation. Default dissolution parameters are proposed in the ICRP Human Tract Respiratory Model (HTRM) (ICRP 1994), and in the NCRP wound model (NCRP 2007). However, for an accurate effective dose calculation, ICRP recommends the use of values specific to the contaminant whenever possible. In addition to values depending on the intrinsic properties of the elements and to their *a priori* physicochemical form when they penetrate into the body, transformed state is considered to reflect interactions with biological ligands and thus must also be taken into account. Indeed, initial status of a radiocontaminant can be modified according to the environment encountered at the site of contamination or during circulation and retention in the body. Among factors influencing actinide biodistribution are the presence of proteins with affinity for the element thus forming complexes (metal-protein interaction), as well as factors modifying redox status or pH (acidic conditions can favor dissolution). These factors are particularly important for Plutonium (Pu) which displays a very complex chemistry. Biodistribution reflects a multi-stage process, integrating the dissolution of the

contaminant, the interactions with biological ligands and the absorption into fluids. The default parameters proposed by ICRP illustrate different types of absorption: fast (type F), moderate (type M) and slow (type S), described by the default values f_r corresponding to the rapid absorption rate, at a half-life s_r . The remaining fraction $(1 - f_r)$ is absorbed at a slower rate s_s .

It is generally assumed that ionic forms are more prompt to form complexes with biological ligands or chemical chelatants than polymeric or particulate forms. However, it is unlikely that actinides remain as simple ions at physiological pH and in the presence of biological ligands (ICRP 1986). Therefore, formation of complexes of various natures must be expected. Consequently, changes in the bioavailability of the contaminant after penetration in the body will occur. Furthermore, the presence of chelating drugs used as decorporating agents, modifies the biodistribution of the actinide by limiting interactions with biological ligands and thus increasing urinary excretion of the complex.

The dissolution process results from dynamic interactions between a number of different variables, including the above described components and microenvironmental conditions. Furthermore, it is also necessary to take into account the presence of contaminants of other origins, or the specific microenvironment composition at the site of entry (other agents in lungs for example). Acellular *in vitro* tests are often used to screen for dissolution as they provide information on how compounds may behave in chemically well-defined conditions. However, despite the advances of *in vitro* dissolution tests in particle qualification, the *in vivo* bioavailability of radiocontaminants remains difficult to evaluate using *in vitro* assays (Ansoborlo et al. 1999, Kastury et al. 2017). Many attempts have been made to develop *in vitro* methods to assess dissolution properties of radiocontaminants, mainly those recovered in the environment or at the work place. Concerning actinides, efforts have mainly concerned the assessment of particle solubility in lung simulated fluids (Eidson and Mewhinney 1983, Khokhryakov et al. 1998, Aladova et al. 2007). However, although none of these tests have been found satisfying different authors have provided dissolution data using simple dissolution assays in good accordance to the expected physicochemical properties of various

compounds ((Ansoborlo et al. 1998), Miglio et al. 1977) (Eidson and Mewhinney 1983) (Aladova et al. 2007),

Based on the multi-compartmental approach used in ICRP models, we developed a biphasic *in vitro* acellular test combining a static (retention sites) and a dynamic (transfer compartments) phase. Both the chemical nature of these phases and the biologically-relevant temperature of 37°C, mimic *in vivo* conditions. This makes possible the use of multiple combinations to provide relevant physiological conditions of contamination such as various contaminants and routes of contamination. In addition, it is also possible to predict the decorporation efficacy of various chelating drugs.

In a previous paper (Griffiths et al. 2016), principle and proof of concept of the assay were described. Notably, correlation of the *in vitro* data to the ones obtained from animal experiments was carried out to evaluate the capacity of our test to mimic *in vivo* behavior of actinides following intake. Here, we provide a full description of the test, its validation and applicability.

We focus on how this acellular test can help to predict biological behavior, i.e retention/dissolution balance and availability to chelating drugs according to physicochemical forms of Pu, or the nature of various elements. A three-tier approach with the following endpoints was followed to:

- 1- Determine the bioavailability of actinides according to the three solubility classes described by ICRP, based upon their transfer from lungs to blood (slow, medium and fast)
- 2- Mimic the behavior of actinides in specific biological compartments representing retention sites or transfer compartments
- 3- Evaluate efficacy of chelating agents according to complex the radionuclide of interest

Three different physicochemical forms of Pu (colloid, nitrate and citrate) and three actinides (Pu, Am, U) were considered to represent various scenarios of internal contamination at the workplace in the nuclear industry. Furthermore, the data available from *in vivo* studies conducted with these contaminants were used to validate of our assay.

Materials and methods

Actinide compounds

Three different physicochemical forms of plutonium (Pu) were used and will be referred as citrate, nitrate or colloids, respectively representing fast, medium and slow transferabilities. These were prepared from a stock solution of Pu (12.5 % ^{239}Pu and 86% ^{238}Pu in activity, CEA, France) in 2N HNO_3 as previously described (Van der Meeren et al. 2012). Briefly, following evaporation of stock solutions, the “citrate” and “nitrate” compounds, were made so final concentrations were 20 mM citrate and 25 mM nitrate respectively. For the “colloid” compound, colloids were formed *in vitro* as follows: after evaporation of the stock solution, Pu was diluted so Pu concentration was 25 μM and nitrate concentration was 25 mM then the pH was elevated at 11 with NaOH.

After evaporation of a stock solution in 2N HNO_3 (^{241}Am and 0.2% ^{238}Pu in activity), Am is resuspended in water, and thereafter referred as Am nitrate.

Uranium (^{233}U) was obtained from Eckert Ziegler (Les Ulis, France) as uranyl (uranium solution in HNO_3 1 N). After evaporation of the stock solution, U is resuspended in water, and thereafter referred as U nitrate.

Preparation of agarose gels and incubation media

Agarose gels were prepared by dissolution of agarose powder type IIA (Sigma) in physiological saline (140 mM NaCl, 5 mM KCl, pH 5.6) as a 2.5% solution. Gels were also prepared in order to simulate the biological composition of the sites where the contaminants entered in the body or the systemic retention organs, respectively lungs and bone.

Since the first identification of calcium phosphate biomineral in bones of vertebrates, Hydroxyapatite (HAPA) has been commonly used as a model of bone mineral matrix (Borchers and Pieler 2010). This compound has also been used to determine *in vitro* Pu binding (Guilmette et al. 1998). HAPA was

included in gels to mimic bone as the main retention organ for Pu to give a final amount in gels around 2 mg/gel.

Three different dynamic phases were used: NaCl/KCl (140/5 mM), Gamble's solution to represent extracellular lung fluid and Artificial Lysosomal Fluid (ALF) to represent intracellular lysosomal fluid (Midander et al. 2007)). pH of the solutions was adjusted to 5.6 for NaCl/KCl, 7.4 for Gamble's and 4.5 for ALF. pH was measured at each collection time and found to be stable over the incubation period.

In some cases the dynamic phase contained chelators: (a) Diethylene Triamine Pentaacetic Acid (DTPA, 10 or 500 μ M; Pharmacie Centrale des Armées, Fleury les Aubrais, France) or (b) the octadentate hydroxypyridone compound 3,4,3-Li(1,2-HOPO (HOPO, 10 or 500 μ M obtained from Novalix, Illkirch, France). Alternatively, the natural ligand apotransferrin (iron-free transferrin, 30 μ M) or holotransferrin (diferric transferrin, 30 μ M) was included in the dynamic phase at around circulating plasma levels. Unless otherwise stated, all reagents were obtained from Sigma-Aldrich, St Quentin Fallavier, France.

The agarose solution was melted by heating in a microwave. After 5 min cooling at room temperature, the actinide to be tested was added under gentle stirring. The agarose solution was then distributed in 24 well-culture dish (300 μ l/well). When gel had hardened, then representing the static phase, the dynamic phase was added in the wells (700 μ l/well). For all experimental conditions, the plates were then incubated at 37°C in a 5% CO₂ atmosphere on an orbital shaker to prevent radiocontaminant settling. All conditions were done in triplicate.

In each individual experiment, controls were represented by the contaminant added to gels prepared in saline and incubated in dynamic phase made of saline.

Collection of fluids and activity measurement

After two hours of incubation, the dynamic phase was collected, placed in a scintillation counting glass vial. Seven hundred μ l of fresh dynamic phase of the same composition were added to replace

the collected samples. The plates were further incubated for 22 hours, after which similar collection process occurred. Forty-eight hours after the beginning of the incubation, the dynamic phase was collected as described above. The gels were removed from the wells and placed in glass vials. Gels were dried and wet-ashed with nitric acid. Activity (in Bq) was then measured by liquid scintillation counting (TriCarb 2500, Perkin Elmer, Villebon-sur-Yvette, France) in the presence of scintillant (Ultima Gold^{TMAB}; Perkin Elmer, Villebon-sur-Yvette, France)

Data processing

Recovered activity was calculated as the addition of the activity measured in each well (sum of activity from the dynamic phase fluid at the 3 time points and the activity found in the gel at the end of experiment). Each data point represents cumulative fraction of transferred activity measured in the dynamic phase at each sampling time/recovered activity deposited in each well.

Box-and-whisker plot representation was used to illustrate the distribution of the dataset (Tukey 1977). Box plots display the full range of variation (from minimum to maximum), the likely range of variation (the IQR), and a typical value (the median). The upper limit of the box represents the upper quartile, and the lower limit, the lower quartile. The error bars represent the upper and lower extremes. The dots represent outliers, either 3×IQR or more above the third quartile or 3×IQR or more below the first quartile. This graph representation was chosen as it allows a clear summary of large amounts of data, as it provides a graphical display of the distribution of results as well as indications of symmetry within the data.

For data sets of smaller size, results were expressed as mean \pm sd.

Results and discussion

1-Evaluation of the bioavailable fraction of different forms of radionuclides

The variable of this first assessment was the physicochemical form and the nature of the contaminant. Both gels and incubation fluid of the dynamic phase consisted of physiological saline (NaCl/KCl).

1.1 Various physicochemical properties of a single element: the example of Pu.

Pu solutions were prepared to obtain different physicochemical properties, representing the three categories slow, moderate and fast described by ICRP (ICRP 1994). We asked whether the assay was able to reflect their solubility properties and the transferability observed *in vivo*. The results presented in figure 1 show the progressive transfer of Pu in the dynamic phase, with various proportions according to the solubility properties, citrate being the fastest and colloid the slowest, as expected from the initial physicochemical form of the Pu compound. In addition to the differences in transfer kinetics, the final percentage of the recovered activity transferred after 48 h varied accordingly with mean values of 15.7%, 29.2% and 76.1% for the colloid, nitrate and citrate respectively. This is to be compared to the highly insoluble properties of actinide oxides, such as mixed oxides (MOX), for which transferred fraction in our assay was shown to be less than 0.1% of the recovered activity (Griffiths et al. 2016).

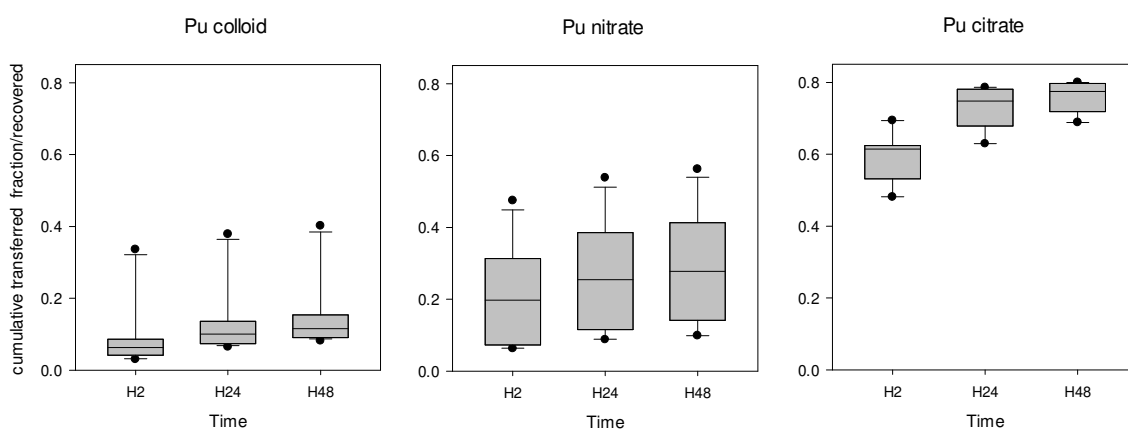


Figure 1: Pu under different physicochemical forms i.e colloid, nitrate and citrate, was included in the static retention phase (agarose gels) prepared in NaCl/KCl. After 2, 24, and 48 h incubation in NaCl/KCl, the transferred activity was measured in the dynamic phase using liquid scintillation counting. Results are expressed as cumulative activity/recovered activity in the gel. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 6 times for colloids and nitrate and three times for citrate.

After an extensive review of the literature, Davesne et al. (Davesne et al. 2010) derived values of absorption parameters for Pu in various physicochemical forms. The analysis was performed on experimental data from both *in vitro* and *in vivo* studies. From their work, type S was assigned to

MOX and Pu oxide, type M to Pu nitrate and type F to Pu citrate. The mean f_r values calculated for the three categories were respectively 3.2×10^{-2} and 6.4×10^{-2} for type S, 2.6×10^{-1} for type M and 4.5×10^{-1} for type S. f_r values cannot be derived from our study, as dissolution was studied at too short periods of time. However, the mean of the transferred ratio in 2 hours was of 10.1×10^{-2} , 2.1×10^{-1} and 5.9×10^{-1} for colloid, nitrate and citrate respectively, indicating consistent values with the f_r values calculated by Davesne et al. These data indicate the possible use of our assay in a first approach to provide pertinent information on the solubility characteristics of a Pu compound.

Electrophoresis on agarose gels is widely used to separate nucleic acids or proteins by their molecular size. The three-dimensional matrix formed by agarose allows the traffic of fluids and biomolecules. The percentage of agarose in the gel is an important parameter in controlling pore size. Smaller molecules diffuse faster than larger molecules in a given gel. We can hypothesize that retention results from interactions between the actinide and the polysaccharide although we do not have any clear explanation for the retention of Pu in the agarose gels. Non-specific retention, based on size-dependent effects (bigger polymers such as colloids being retained to an higher extent in the 3D structure than ions) could be involved as well.

1.2 Various elements in a single physicochemical form: Pu, Am, U nitrate

Pu, Am and U were prepared as a nitrate form, and incubated in NaCl/KCl. The three elements showed different transfer rates, Pu being the less transferred, in accordance to the intrinsic solubility properties of the various elements observed in *in vivo* studies. The mean percentage of the recovered activity transferred after 48 h, was of 17.9 %, 77.5 % and 69.1 % for Pu, Am and U respectively. These data are consistent with higher rate of elimination of U as compared to Pu observed *in vitro* (Eidson and Mewhinney 1983) and *in vivo* (Ellender et al. 1995). However, our results do not reflect the higher bioavailability of U as compared to Am observed in the same studies. This illustrates the extents to which the presence of bioligands can influence the *in vivo* behavior, thus highlighting the necessity of complexification of *in vitro* testing to better mimic the *in vivo*

situation. In addition to bioligands, the presence of ions, such as carbonate, can markedly influence the *in vivo* behavior particularly of U.

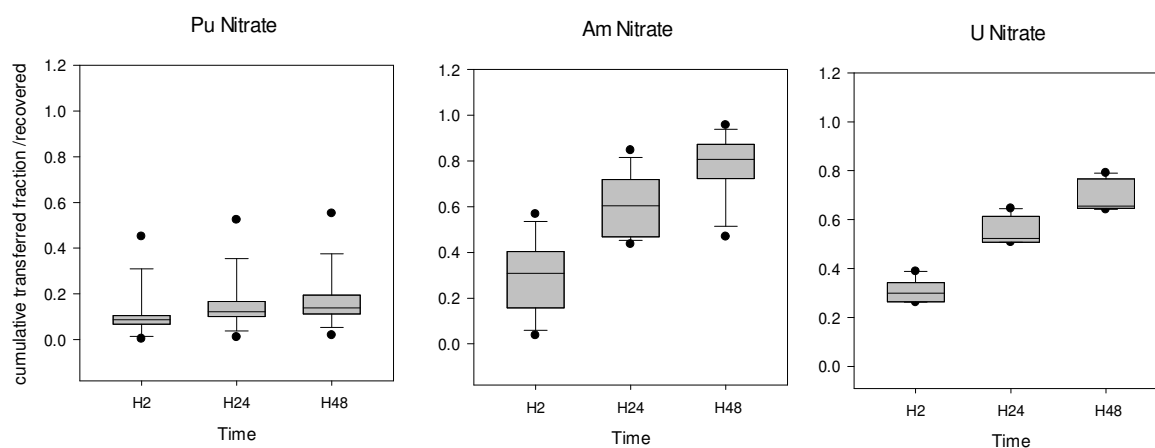


Figure 2: Pu, Am and U nitrate were included in the static retention phase prepared in NaCl/KCl. After 2, 24, and 48 h incubation in NaCl/KCl, the transferred activity was measured in the dynamic phase using liquid scintillation counting. Results are expressed as cumulative activity/recovered activity in the gel. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 29 times for Pu, 10 times for Am and twice for U.

2- Presence of biological ligands to mimic retention/mobilization

To better understand the biochemical factors affecting the initial dissolution properties of the contaminant as well as retention in a specific organ, agarose gels were prepared in various media mimicking different retention compartments of the body, and then incubated in physiological saline. Agarose gels prepared in physiological saline served as controls. Pu and Am were prepared as nitrate.

2.1 Pu retention in lung compartments

The commonly used surrogate fluids representing lung environment are Gamble's solution and artificial lysosomal fluid (ALF). Gamble's solution mimics the interstitial and airway lining fluids whereas ALF solution simulates intercellular conditions in phagocytosing cells, namely macrophages (Marques et al. 2011). Similarly to what is classically found for particulate matter (Kastury et al. 2017, Pelfrene et al. 2017), dissolution/bioavailability was found to be higher in ALF than in Gamble's solution. These may result either from differences in pH (4.5 vs 7.4) or from the presence of

phosphate in Gamble's solution and citrate in ALF than can respectively increase retention or facilitate transferability, as it has been observed for U (Guilmette et al. 2000).

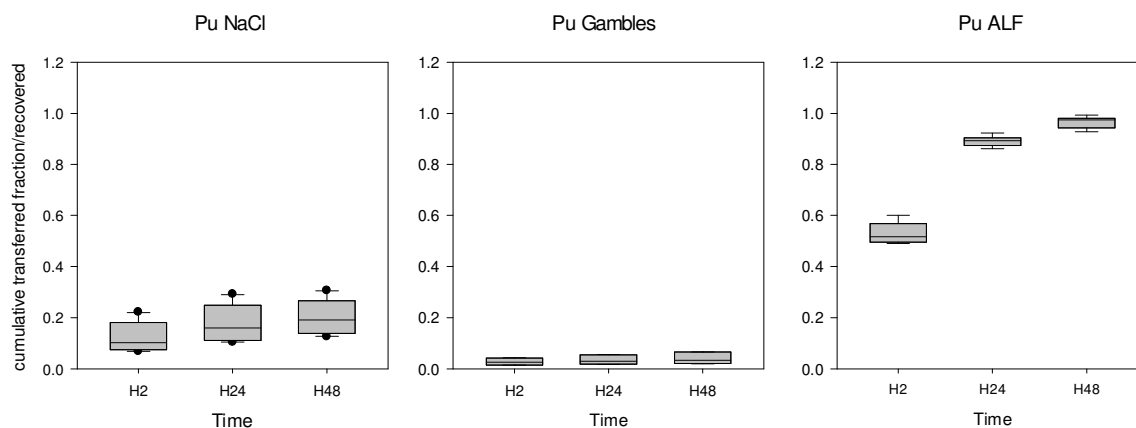


Figure 3: Pu nitrate was included in the static retention phase prepared in NaCl/KCl, Gamble's or ALF solutions. After 2, 24, and 48 h incubation in the corresponding dynamic phase of similar composition, the transferred activity was measured using liquid scintillation counting. Results are expressed as cumulative activity/recovered activity in the static phase. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 4 times for NaCl, twice for Gambles and twice for ALF.

2.2 Pu and Am in bone as systemic retention tissue.

We evaluated the ability of static phases containing Hydroxyapatite (HAPA), a major mineral fraction in bone, to retain Pu and Am. Bone is, together with liver, the main retention organ of Pu and Am following transfer to the blood. Results shown in figure 4 indicate that both Pu and Am presented an increased retention in the presence of HAPA in the static phase compared with that containing physiological saline. The increased retention of Pu and Am in the presence of HAPA is in accordance with the bone being a systemic retention organ for both elements.

In the presence of HAPA in the static phase, 91 times less Am and 3.4 times less Pu were transferred to the dynamic phase in 48 hours, as compared to physiological saline-containing gels. These results confirm the higher retention potential of Hydroxyapatite towards Am as compared to Pu (Guilmette et al. 1998).

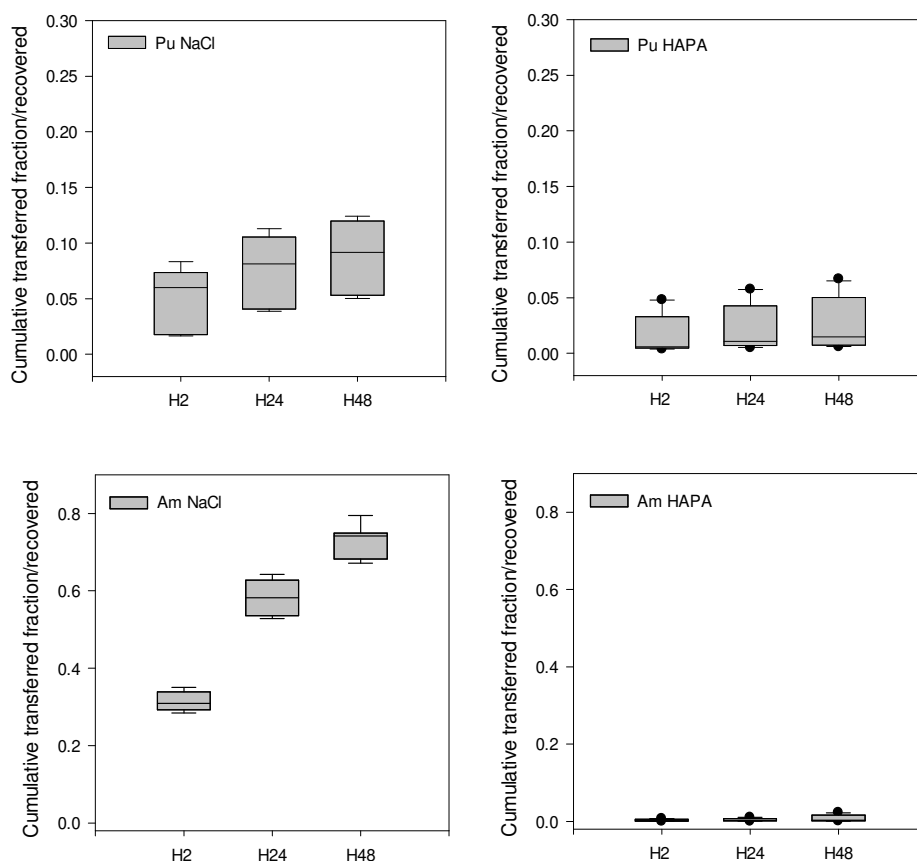


Figure 4: Pu and Am nitrate were included in the static retention phase prepared in NaCl/KCl, with or without Hydroxyapatite (HAPA). After 2, 24, and 48 h incubation in NaCl/KCl, the transferred activity was measured using liquid scintillation counting. Results are expressed as cumulative activity/recovered activity in the gel. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 4 times for both elements.

3-Transferability of actinides in the presence of chelating agents

We seek to evaluate the efficacy of chelating molecules to enhance the transferability of radionuclides from the static phase into the dynamic phase. For this purpose, the actinide-loaded agarose gels were incubated in NaCl/KCl supplemented with various chelating agents.

3.1 Pu in the presence of the biological ligand transferrin

Transferrin (Tf) is the predominant Pu-transporter protein in the plasma. To evaluate the possible mobilization of Pu from the static phase, the iron-free form of transferrin, apotransferrin or the diferric transferrin (holotransferrin) were included in the dynamic phase at around plasma levels (30

μM). The Pu-loaded gels were prepared in physiological saline. The results show that apotransferrin markedly increases Pu in the medium with almost the entire activity being transferred after 48 h, likely as Pu-Tf complexes (figure 5). Similarly dynamic phases containing holotransferrin also increased Pu transfer from the gels with around $64.2 \pm 1.1 \%$ at 48 h although less than apotransferrin ($93.1 \pm 0.5 \%$). Presumably the Pu binding site is more accessible on the apoprotein as compared with iron-containing holotransferrin.

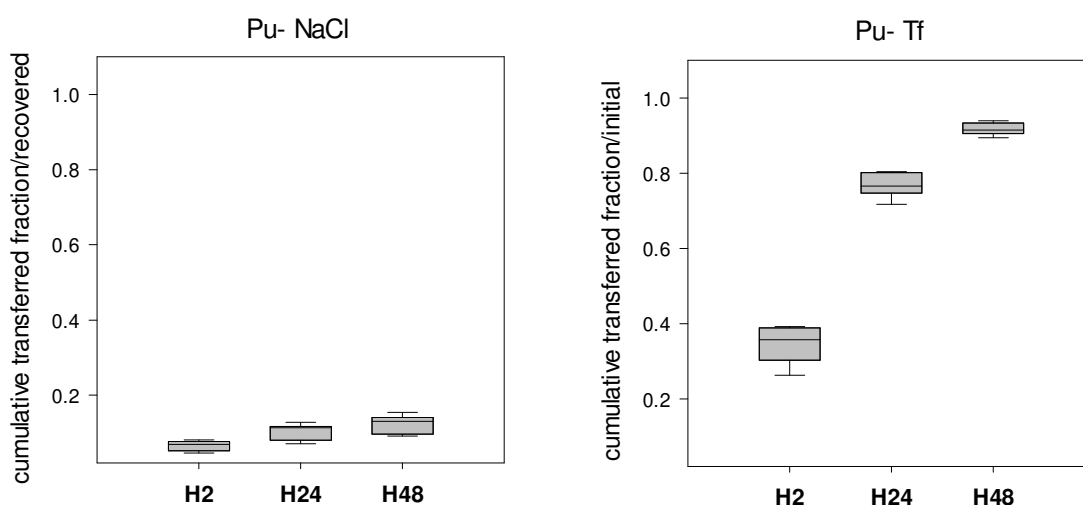


Figure 5: Pu nitrate was included in the static retention phase prepared in NaCl/KCl. After 2, 24, and 48 h incubation in NaCl/KCl containing (B) or not (A) apotransferrin ($30 \mu\text{M}$) the transferred activity was measured in the dynamic phase using liquid scintillation counting. Results are expressed as cumulative activity/recovered activity in the gel. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated twice.

These results suggest that Tf can penetrate the agarose gel where complexes between Tf and the available actinide compounds are formed. These complexes, more readily transferred, are then recovered in the dynamic phase due to the renewal of the medium.

3.2 Transfer of Pu, Am and U in the presence of synthetic chelating agents in the dynamic phase

We previously reported the efficacy of DTPA to enhance Pu transferability with a dose-response relationship using the biphasic assay (Griffiths et al. 2016). Here, we addressed additional objectives concerning DTPA efficacy.

a- Evaluation of DTPA efficacy on transferability using several physicochemical forms of Pu, with 500 μM DTPA in the dynamic phase.

Figure 6 illustrates the enhanced Pu transferability in the presence of DTPA whatever the initial physicochemical form of Pu, with all ratios (activity in the dynamic phase in the presence of DTPA as compared to activity in the dynamic phase without DTPA) being $\gg 1$. The efficacy of DTPA to enhance Pu transfer is inversely correlated to the solubility of Pu compound. A possible explanation for this observation is that DTPA chelates Pu on the surface of the colloid, which atoms would not have been easily transferred without a chelating agent. However, for the most soluble form, only a limited fraction of Pu remains trapped in the gel, and is thus available for DTPA chelation (see figure 1).

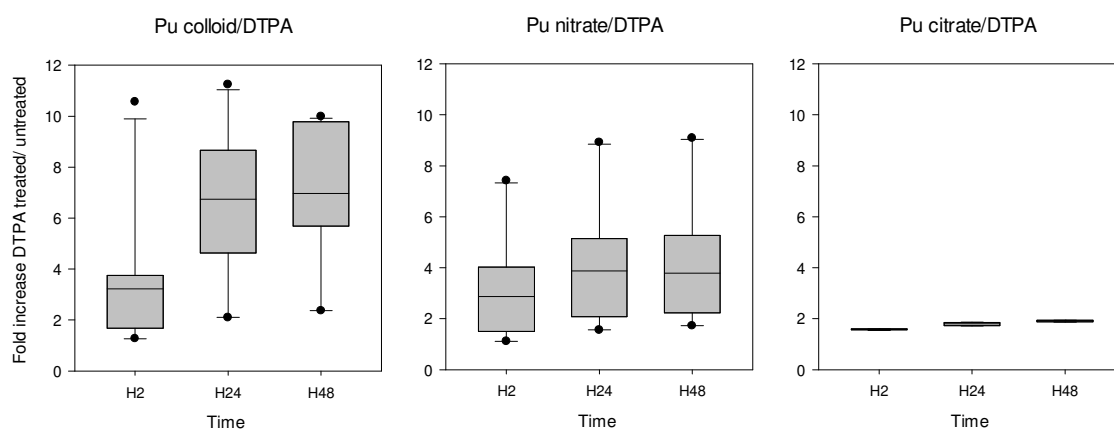


Figure 6: Pu under different physicochemical forms i.e colloid, nitrate and citrate, included in the static retention phase prepared in NaCl/KCl. After 2, 24, and 48 h incubation in NaCl/KCl containing or not DTPA (500 μM) the transferred activity was measured using liquid scintillation counting. Results are expressed as fold increase transferred activity in the dynamic phase in the presence of DTPA as compared to transferred activity in the absence of DTPA. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 4 times for both colloid and nitrate and twice for citrate.

b- Comparison of the effect of DTPA on transferability properties of Pu, Am and U as nitrate forms introduced in static phases prepared in saline.

Our data are in agreement with the known lower affinity of DTPA for Am than that of Pu, and that of U (Ansoborlo et al. 2007). Up to 5 times more Pu is transferred to the dynamic phase in the presence of DTPA, although less than a two fold increase is observed for Am (figure 7). Despite the lower

affinity of DTPA towards Am as compared to Pu, decorporation efficacy of DTPA *in vivo* is higher after Am intake than after Pu (Gremy et al. 2016). The efficacy of a decorporating drug *in vivo* to decrease retention and increase excretion, results from i) a good chelation efficacy of the drug towards the contaminant ii) the bioavailability of the contaminant to the drug thus allowing the formation of complexes and iii) the rate of elimination of the complexes by physiological paths (urine and faeces). Therefore, one explanation for *in vivo* higher decorporation potential of Am by DTPA as compared to Pu is the higher bioavailability of Am as a consequence of the lower affinity of Am towards biological ligands, such as Tf. In the present work, only the ability to form a complex was evaluated, since gels were prepared with saline. However, a more complex composition of agarose gels would be needed to be more relevant to *in vivo* conditions. Notably, biological ligands competitive to DTPA in the process of complex formation are to be considered.

In addition, the limited efficacy of DTPA to increase Am transfer in our assay might result from Interactions of the tested actinides with from a limitation of the method concerning compounds with a high intrinsic transferability; additional transfer being difficult to demonstrate when over 80% of activity rapidly diffuses in the dynamic phase (figure 2). Am and U in saline show equivalent transferability. However, whereas DTPA further increases Am transferability, no effect on U was obtained. The absence of an effect in U transfer illustrates the low affinity of DTPA for U, rather than a limit of the assay due to the high bioavailability of U.

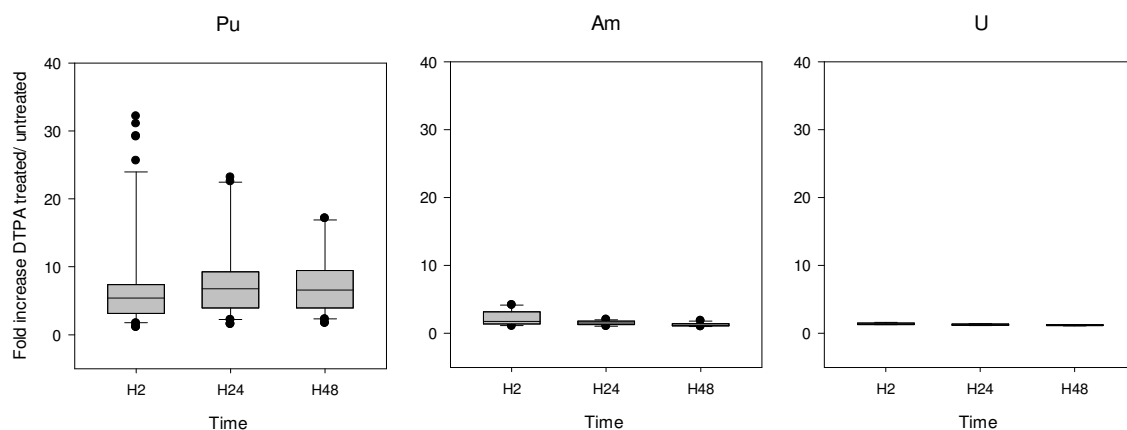


Figure 7: Pu, Am or U were included in the static retention phase prepared in NaCl/KCl. After 2, 24, and 48 h incubation in NaCl/KCl containing or not DTPA (500 μ M) the transferred activity was measured using liquid

scintillation counting. Results are expressed as fold increase transferred activity in the presence of DTPA as compared to transferred activity in the absence of DTPA. Boxes represent the full range of data, and the black line the median. Dots represent outliers. Each experimental condition was done in triplicate and repeated 19 times for Pu, 8 times for Am and twice for U.

c. Comparison of the efficacy of DTPA or HOPO (synthetic siderophore) on Pu nitrate transferability.

Pu was added to static phases prepared with saline, and 10 and 500 μM DTPA and HOPO were used in the dynamic phase.

Both DTPA and HOPO were able to mobilize Pu nitrate from the static phase (figure 8). We observed that at 10 μM HOPO is more efficient than DTPA. However, at 500 μM , both chelatants present similar efficacy at 24 and 48 hours. This could result from the chelation of the entire available fraction at the early time points leading to almost 100% of activity being transferred at 48 hours (figure 8). These results are in accordance with *in vivo* efficacy studies demonstrating the higher decorporating efficacy of HOPO compounds as compared to DTPA, with lower concentration of HOPO leading to higher efficacies (Volf et al. 1993, Fukuda et al. 2003).

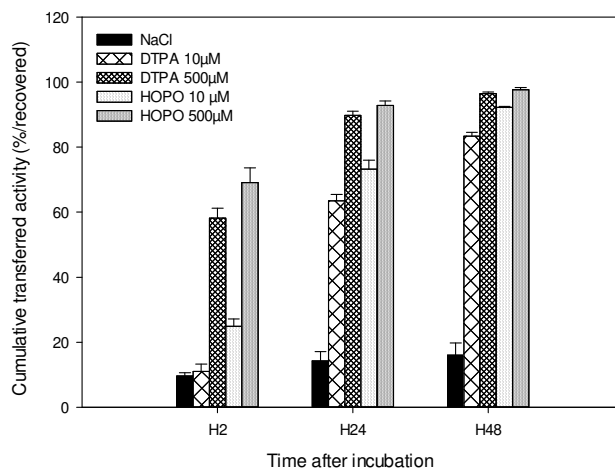


Figure 8: Pu nitrate was included in the static retention phase prepared in NaCl/KCl, as a nitrate form. After 2, 24, and 48 h incubation in NaCl/KCl containing or not DTPA (10 or 500 μM) or HOPO (10 or 500 μM), the transferred activity was measured using liquid scintillation counting. Results are expressed as cumulative transferred activity/recovered activity in the gel x100. Each experimental condition was done in triplicate. Bars represent mean \pm sd.

Conclusion:

We have developed a biphasic and dynamic *in vitro* acellular assay to assess the transferability of radiocontaminants in physiologically-relevant environments and their interactions with chelating drugs. The aim of this assay is to improve the characterization of bioligand-actinide interactions in biologically relevant environments. Predicting the behavior of radionuclides and decorporation process may help to enhance the reliability of internal dose assessment. The principle of the assay is based on the hypothesis that bioavailable ions would migrate into the dynamic fluid phase, although polymers of higher molecular weight or actinide-ligand complexes would remain trapped in the static phase. The transferability of the radionuclide corresponds to the activity contained in the dynamic phase, which is measured by scintillation counting. This assay is representative of the intrinsic dissolution properties of a radionuclide when both static and dynamic phases are made in saline. Alternatively, it may reflect interactions occurring in biological surroundings when static phases are made or incubated in the presence of potential ligands, either natural or synthetic.

Validation of the assay

To validate the assay we have followed three steps:

First, we verified that the transferability of the radionuclide in saline (intrinsic properties of the contaminant) was in accordance to what is expected from *in vivo* studies (urine excretion). Hence, our assay mimics the higher bioavailability (higher transfer from static to dynamic phase) of Am and U as compared to Pu. In addition, we could classify Pu of various chemical properties, as citrate being more soluble than nitrate than colloids, in accordance to previous data obtained *in vivo* (Van der Meeren et al. 2012). These results confirm the possible use of the assay to classify compounds in one

of the transportability classes described by ICRP. However, longer incubation time points are needed to be able to derive absorption values from our studies.

Second, we evaluated the possibility of modifying the transfer capacity of the contaminants by adapting the static phase composition to better mimic retention site composition. Hence, transferability of contaminant was evaluated in the presence of biological ligands that are representative components of organs of entry (lungs) or of systemic retention organs (bone). Differential bioavailability was evidenced in the different compartments of retention within the lungs (extracellular and intracellular), according to the previously published data on actinides or other metal oxides using *in vitro* dissolution tests (Wiseman 2015, Pelfrene et al. 2017). In addition, we confirmed the low transferability of Pu and Am when HAPA was included in the gel, thus illustrating the affinity of these actinides for bone. These results suggest the possible use of the assay to identify molecules showing affinity towards actinides which could influence the retention time in a given compartment, thus allowing a better prediction of *in vivo* biodistribution. This is of particular interest considering that the bound fraction (f_b) in lungs can highly influence committed effective dose calculation (Birchall et al. 2018).

Third, we assessed the possible mobilization of the radionuclide from the static phase by adding biological or synthetic ligands of actinides to the dynamic phase. Our results clearly illustrate that, when formed, actinide-ligand complexes are more easily transferred to the dynamic phase. Two applications could be thus envisioned for further development of the assay: the possibility of screening the efficacy of chelating drugs and the evaluation the bioaccessibility of a contaminant to chelating agents according to the presence of biological ligands representative of a retention site.

Potential applications and developments

The biphasic and dynamic properties of this assay provide a clear advantage as compared to the previously published *in vitro* models. In addition, of critical value is the multiplicity of combinations of molecules that can be used i) in the static phase to mimic retention sites from various organs and

tissues thus reflecting different contamination scenarios and route of entry and ii) in the dynamic phase to mimic composition of various transfer compartments, including those where a chelating drug can be added. For these reasons, our model can be described as a multicompartimental model, mimicking the multiple retention and transfer compartments.

Diverse uses and applications of the developed assay can be considered.

- In mechanistic studies to increase knowledge on biomolecule-radionuclide interactions, and identify biological ligands. Protein-actinide complex formation is often estimated in purely chemical non-physiological conditions. Our assay could provide experimental conditions more biologically relevant for such studies.

- For the screening of chelating drugs and the prediction of *in vivo* efficacy. It may also provide better conditions for efficacy evaluation (competition with bioligands). Dose-response studies in physiologically relevant situations can also be carried out.

- At the work place, to provide assistance on dose assessment and therapeutic decisions. After collection of the contaminants, the assay could allow transportability classification by comparison of transfer rates to a set of data obtained with compounds of known dissolution properties. Therefore more specific parameters could be used in dosimetric models instead of default parameters suspected from the event reconstitution, thus ensuring reliability of dose assessment.

- At the site of an accident or event involving a great number of internally contaminated victims, the characterization of the radio-contaminant encountered using our assay could bring rapid information regarding the properties of the contaminant involved. Triage of victims and therapeutic decision could then be facilitated.

- In risk assessment studies, our assay can provide information on retention sites of a given radio-contaminant, as well as dissolution properties. Thus, clues on toxicity study to perform can be

obtained; compounds with low retention or biological half-lives may only require short term toxicity tests while those with very long retention time may require long term chronic toxicity testing.

The assay is simple and easy to conduct. In addition it is flexible, relevant for various contaminants and representative of different conditions of exposure. This assay is easy to implement, rapid and cost-effective, small volumes of test fluids/suspension are needed and good reproducibility is obtained. In a first approach, this assay could help to better predict biodistribution after intake of contaminants encountered at the work place. This can be particularly appropriate in situations in which the dissolution properties of a contaminant are unknown, to the presence of cross-contamination, a mixture of compounds or aged actinides. In addition, we believe that the assay may be applicable to a wide class of contaminants, as long as a detection method exists to measure the transferred form in the dynamic phase.

Acknowledgements: Authors thank Agnès Moureau for technical assistance, Sylvie Coudert for advices in activity measurement and Stéphanie Lamart for useful discussions. This work has been funded within the frame of the CEA-Orano agreement.

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