Pulmonary retention of actinides after dissolution of PuO₂ aerosols: interest in modelling DTPA decorporation

A.-L. SÉRANDOUR¹, P. FRITSCH¹

ABSTRACT This study estimates in terms of amount and localisation the pulmonary retention of dissociated Pu/Am in the rat for the first week following an inhalation exposure to industrial PuO₂ aerosols by combining standard biokinetic methods, quantitative analysis of contact autoradiograph obtained from lung section, and treatments by DTPA performed either in vivo or in vitro. The dissociated actinides mainly involved dissolved forms which are homogeneously distributed within lung parenchyma. Most of these chemical forms appears to come from the fraction (fₚ) of radioelements which seems to dissolve before particles phagocytosis mainly by alveolar macrophages. Early pulmonary administration of dry diethylenetriaminepentaacetic acid (DTPA) powder (+2 hours) decorporates ~90% of these actinide forms, whereas, a delayed treatment (+1 week) is far less efficient. By contrast, a similar extraction (~90%) of the dissolved actinides from lung sections of rat untreated by the chelating agent is measured after their incubation in a DTPA solution for both 2 hours and 7 days post-exposure times. These results can be explained by a gradual internalisation of a fraction of the early dissolved actinides (mainly Am) in alveolar cells, but not preferentially in alveolar macrophages, whereas the remaining fraction of dissolved actinides are transferred to blood. From these observations, a new model is proposed to help for interpretation of human bioassay data obtained after internal contamination and DTPA treatments.

Keywords: PuO₂ aerosols / dissolution parameters / bound fraction / DTPA / modelling

RÉSUMÉ Retention pulmonaire des actinides après dissolution d’aérosols de PuO₂ : intérêt dans la modélisation de la décorporation par le DTPA. Le but de cette étude est d’estimer, en termes de quantité et de localisation, la rétention pulmonaire de formes dissociées de Pu/Am chez le rat durant la première semaine suivant une exposition à des aérosols de PuO₂ industriel en combinant des études biocinétiques conventionnelles, l’analyse quantitative d’autoradiographies par contact obtenues à partir de coupes de poumons, et des traitements par du DTPA effectués in vivo et in vitro. Ces formes, essentiellement générées après dissolution des particules, sont distribuées de manière homogène au sein du parenchyme pulmonaire et apparaissent issues de la fraction des éléments transuraniens qui se dissout rapidement (fₚ) avant la phagocytose des particules de PuO₂, principalement par les macrophages alvéolaires. L’administration précoce (+2 heures) de poudres sèches d’acide diéthylène triamine penta-acétique (DTPA), par insufflation, permet de décorporer la plupart de ces formes d’actinides (~90%), alors qu’un traitement différé (+1 semaine) apparaît moins efficace (~50%). Il faut noter que les formes dissoutes ne représentent alors que 10 à 20 % de l’activité alpha totale retenue dans

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les poumons. En revanche, pour ces 2 temps post-inhalation, l’incubation de coupes de poumons de rats non traités in vivo par du DTPA, dans une solution de l’agent chélateur permet une extraction similaire des formes dissoutes (~90 %). Ces résultats peuvent être expliqués par une internalisation progressive d’une fraction des actinides solubilisés (principalement de l’Am) au sein des cellules du parenchyme alvéolaire qui n’intéresse pas préférentiellement les macrophages alvéolaires et par le transfert vers le sang de la fraction restante. Ces différentes observations permettent de proposer la structure d’un nouveau modèle qui pourrait être utile pour aider l’interprétation des données biologiques humaines recueillies après contamination interne accidentelle traitée par le DTPA.

1. Introduction

After inhalation of radioactive aerosols, doses are usually calculated after application of the Human Respiratory Tract Model (ICRP, 1994) combined with the Gastrointestinal Tract Model (ICRP, 1979) and systemic models which are specific for the different elements involved in the contamination. The dissolution of aerosols in the respiratory tract is described by a simple mathematical model in which a fraction $f_r$ of the total amount of retained radionuclides dissolves rapidly at a daily rate $s_r$, the remaining fraction $(1 - f_r)$, dissolves slowly at a daily rate $s_s$ (ICRP, 1994).

In the dissolution model of Publication 66 of ICRP (1994), a fraction $f_b$ of the dissolved activity can be bound to tissues of the respiratory tract before to be transferred to blood at a daily rate $s_b$, but there are often insufficient data to take account of this bound fraction, so $f_b$ is usually set as 0. In order to estimate $f_b$ and $s_b$ values for plutonium, Birchall et al. (1995) analysed the results of experiments previously reported in which the behaviour of $^{239}$Pu was followed in rats for 180 days after intratracheal injection of plutonium nitrate. The lung clearance observed over period 1–30 days, indicated values for $f_b$ of about 0.5, and for $s_b$ of about 0.2 d$^{-1}$ (ICRP, 2002). This evaluation did not take into account colloid formation which might occur for the masses of Pu administered. Nevertheless, because the mean dose delivered to the lungs from the bound materials is negligible in front of that delivered from the other actinide forms retained in the respiratory tract, $f_b$ and $s_b$ are not taken into account for dose calculation according to ICRP recommendations when insufficient data are available to determine their values.

Three different types of absorption to blood are proposed by the ICRP, to be used as default when no specific parameter value can be determined: type F, as fast absorption ($f_r = 1$, $s_r = 100$ d$^{-1}$, $s_b = 0$ d$^{-1}$), type M, as moderate absorption ($f_r = 0.1$, $s_r = 100$ d$^{-1}$, $s_b = 5 \times 10^{-3}$ d$^{-1}$) and type S, as slow absorption ($f_r = 1 \times 10^{-3}$, $s_r = 100$ d$^{-1}$, $s_b = 1 \times 10^{-4}$ d$^{-1}$). All americium chemical forms are considered as type M compounds, whereas, for plutonium, soluble forms (nitrate, oxalate…) are
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considered as type M and oxides as type S (ICRP, 1994). In fact, the term of absorption to blood of dissociated forms is used in Publication 66 of ICRP (1994) in order to take into account transfer of both radionuclides dissolved within the lungs and particulate material which appears to be important only for particles smaller than a few nanometres, here referred to as nanometre particles. The systemic behaviour of these 2 chemical forms appears quite different, the urinary excretion of nanometre particles being much larger than that of dissolved actinides (Stadling et al., 1978a, 1978b; Métivier et al., 1980).

Experimental studies in dogs have shown that a fraction of inhaled ²⁴¹Am₂O₃ dissolves very slowly and might come from largest particles (Guilmette and Muggenburg, 1988). Such a slow dissolution has been also recently reported in human after inhalation of Pu-nitrate aerosols (James et al., 2007). The authors assumed that the fraction which dissolved very slowly corresponded to bound materials and the value of \( f_b \) has been estimated to 0.08 (\( s_b = 2 \times 10^{-4} \text{ d}^{-1} \)). In this case, \( f_b \) and \( s_b \) might not actually correspond to the bound fraction as defined in Publication 66 of ICRP (1994).

After inhalation exposure to ²³⁸PuO₂ or Pu-nitrate, early studies have shown that a significant decorporation of lungs can be obtained after pulmonary administration of DTPA, even several weeks after the contamination (Stather et al., 1982; Stradling et al., 1984). The lung plutonium chelated by DTPA might correspond to dissolved forms associated with biological ligands, i.e. bound material as defined in Publication 66 of ICRP, but some of these forms might not be directly transferred to blood. Such dissolved forms of actinides are not the only one’s which can be potentially decorporated by DTPA. Small particles (oxides or colloids) containing a few actinide atoms can be also dissolved \textit{in vitro}, in presence of DTPA (Sato et al., 1994; Cooper et al., 1979). In the case of PuO₂ inhalation exposure, nanometre particles could be obtained by actinide oxide fragmentation and absorbed directly into blood (Fleischer and Raabe, 1977; Diel and Mewhinney, 1983; Guilmette et al., 1994; Métivier, 1997).

Recently, observation of contact autoradiographs obtained from lung sections of rats which were exposed to PuO₂ aerosols and treated or not by an intratracheal insufflation of dry DTPA powders suggested the existence of dissociated forms of actinides which might be decorporated by the chelating agent (Sérandour et al., 2007). The aim of this study was to characterize these dissociated forms by combining autoradiographic analysis after their extraction, \textit{in vitro} and \textit{in vivo} by DTPA with standard biokinetic studies. The obtained results allowed us to propose a new model which could be useful to help the interpretation of biological data collected after human contamination associated to chelating agent treatments.
2. Material and methods

2.1. Biokinetic studies after PuO$_2$ inhalation and DTPA treatment

Four groups of 30 male Sprague-Dawley rats (~250 g, Charles River Laboratories, France) were exposed nose-only to a mixture of industrial plutonium and americium oxide aerosol (André et al., 1989). Activity median aerodynamic diameter (AMAD) of the aerosols was measured using cascade impactor (AMAD 4.8 µm, $\sigma_g$ 2.2). The isotopic composition of aerosol in terms of percentage of total alpha activity was: 12% $^{238}$Pu, 18% $^{239}$Pu, 23% $^{240}$Pu and 47% $^{241}$Am. This compound, referred to as "old PuO$_2$", was received in 1987 and at this time it contained 3000% of $^{241}$Pu (beta) in terms of total alpha activity but only 13% $^{241}$Am.

These rats were treated or not by insufflation of a DTPA dry powder (25 µmol kg$^{-1}$) 2 hours (early treatment) or 1 week (delayed treatment) after the contamination. The DTPA (~4 mg of powder; mass median aerodynamic diameter: 0.95 µm) was administered intratrachealy to anaesthetised rats using a dry powder insufflator (model DP-4, Penn-Century, Philadelphia, USA) according to the manufacturer instructions (Gervelas et al., 2007; Sérandour et al., 2007).

Standard biokinetic methods have been detailed previously (Ramounet et al., 2000; Rateau-Matton et al., 2004). Briefly, radioactive measurements included chest X-ray spectrometry, liquid scintillation counting (total alpha activity measurement), and alpha spectrometry after radiochemical separation (evaluation of Pu and Am content). Biological samples included excreta, lungs, liver, femurs and kidneys.

Dissolution parameters of Pu/Am ($f_r$ and $s_r$) were calculated using a linear correlation method assuming $s_r = 100$ d$^{-1}$ and $f_b = 0$. This approach takes into account lung clearance by particle transport in terms of cumulated lung retentions and assumes that 50 and 33% of the dissolved Pu and Am are retained in the skeleton, respectively (Ramounet et al., 2000).

For these standard biokinetic studies, the initial deep lung deposit (IDLD) of rats measured on day 3 or 7 after inhalation exposure by X-ray spectrometry was larger than 1 x 10$^3$ Bq.

2.2. Contact autoradiograph of lung sections

For some control and DTPA treated animals, the left lobe of the lungs was fixed in formalin and embedded in paraffin, whereas the azygos was used for Pu/Am
measurements to estimate the total lung retention. Contact autoradiographs with a solid track detector (CR39) were performed on 10 µm thick-sections. At the end of the exposure (14 days), two kinds of alpha tracks were visualized after etching of the detector at 80 °C for 2 hours in 6 M KOH: isolated tracks, which presumably correspond to dissociated PuO₂, and spots containing tracks with a symmetrical point, corresponding to actinide oxide particles. For these studies, the IDLD of rats have to be less than 1x10³ Bq so that a few PuO₂ particles are encountered in the lung sections to allow a clear visualisation of isolated alpha tracks.

Quantitative analysis was performed after counting isolated alpha tracks on at least 10 different microscopic fields (objective 3.2x ~ 0.7 mm²). These images were obtained with a light microscope coupled to a CCD camera and a motorized stage controlled by specific softwares developed in the laboratory. Each image analysed corresponded to sub-pleural areas so that similar alveolar regions could be compared. In control rats, contact autoradiographs were successively performed on the same lung sections, crude, rehydrated and incubated in a DTPA solution (250 mg/ml) for 2 hours at 37 °C, whereas, in DTPA treated animals, only crude sections were analysed. An estimation of the total activity corresponding to isolated alpha tracks was performed taking into account the total area and thickness of the lung section, and assuming a detection yield of the alpha tracks of 0.3, a tissue compression of 50% due to microtome sectioning, and a total lung volume of 10 ml.

3. Results and discussion

3.1. Standard biokinetic studies

After inhalation of the “old PuO₂” aerosol (²⁴¹Am: 47% of the total alpha activity), biokinetic data have been previously reported for the total alpha emitters (Pu + Am) (Gervelas et al., 2007; Sérandour et al., 2007). Data are now available regarding the specific biological behaviour of each element. This compound was also studied few years after its fabrication (²⁴¹Am: ~15% of the total alpha activity) and biokinetic data have been reported after inhalation exposure (Lataillade et al., 1995). In addition, a second PuO₂, generated by the same industrial process, was also studied for the first years following fabrication (Ramounet et al., 2000). These 2 last compounds are referred to as “young PuO₂”.

Table I compares the dissolution parameters of Pu/Am after inhalation exposure to the different aerosols.

After exposure to the “young PuO₂” aerosols, similar dissolution parameters are measured for Pu. The large value of fₚ is due to the fact that the calculation...
takes into account the IDLD. Thus, because the deposition in the upper respiratory tract has been estimated to 10–20 times the IDLD from the faecal excretion, the dissolution parameter $f_r$ of Pu appears similar to that proposed for a type S compound ($f_r = 1 \times 10^{-3}$), whereas $s_s$ is 3–6 times less than the default value ($1 \times 10^{-4}$ d$^{-1}$). A larger dissolution parameter $s_s$ than that of Pu is calculated for Am.

An increased dissolution of both Pu and Am is associated with PuO$_2$ ageing. This increase involves mainly $s_s$ for Pu (factor at about 10) and both $f_r$ and $s_s$ for Am (factors at about 4 and 10, respectively). This ageing related phenomenon might be due to the alteration of the crystalline structure in relation with both, the increase of $^{241}$Am amount with time, and alpha irradiation which can produce particle fragmentation (Fleischer and Raabe, 1977; Diel and Mewhinney, 1983; Guilmette et al., 1994; Métivier, 1997). However, because the Am/Pu ratio of actinides deposited in the skeleton (ratio of 4–5) appears quite different than that expected if a similar dissolution of Am and Pu occurs from particles containing few actinide atoms (ratio of 0.7, Ramounet et al., 2000), it appears that these nanometre particles might have a negligible contribution in both the early dissolution process and the blood absorption of actinides. Because the very low mass of PuO$_2$ deposited within the lung, formation of colloids from dissolved actinides is unexpected. Moreover, the relative distributions of Pu/Am either as concerns retention in skeleton and liver, or excretion in urines are similar to those measured after intravenous contamination with Pu/Am citrate. Thus, we can conclude that the transfer of actinides to the systemic compartments involves mostly dissolved forms within the lungs.

### TABLE I

<table>
<thead>
<tr>
<th>PuO$_2$</th>
<th>Delay of the study</th>
<th>$f_r$</th>
<th>$s_s$</th>
<th>$f_r$</th>
<th>$s_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&quot;young&quot; a &lt; 3 years*</td>
<td>$1.4 \times 10^{-2}$</td>
<td>$3.5 \times 10^{-5}$ d$^{-1}$</td>
<td>NM</td>
<td>NM</td>
</tr>
<tr>
<td></td>
<td>&quot;old&quot; a &gt; 15 years**</td>
<td>$9.5 \times 10^{-3}$</td>
<td>$2.0 \times 10^{-5}$ d$^{-1}$</td>
<td>$1.1 \times 10^{-4}$ d$^{-1}$</td>
<td>$4.2 \times 10^{-3}$ d$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>&quot;young&quot; b &lt; 3 years***</td>
<td>$2.2 \times 10^{-2}$</td>
<td>$1.5 \times 10^{-5}$ d$^{-1}$</td>
<td>$3.0 \times 10^{-2}$</td>
<td>$3.6 \times 10^{-4}$ d$^{-1}$</td>
</tr>
</tbody>
</table>
Recently, new experimental data have been obtained which allow us to characterize the early biokinetics of Pu/Am after inhalation exposure to the “old PuO$_2$” aerosols. The early bone retentions of the actinides measured during the first days after inhalation of the “old” PuO$_2$ do not fit to the linear correlation and appear far lower than those expected. Moreover, daily urine excretion of Am followed for 2 weeks in 5 animals shows a maximal activity recovered on the first day (20% of the cumulated excretion) with a nearly linear decrease by a factor of 4 up to day 7, and then a plateau value. If the specific $f_r$ and $s_r$ of Am are used with a $s_r$ value of 100 d$^{-1}$, the urinary excretion collected on day 1 might be about 60% of the cumulated excretion, whereas, the plateau value observed during the second week appears about 2 times larger than the excretion calculated from $s_r$ and $(1-f_r)$. Two hypothesis can explain these discrepancies: (1) the $s_r$ value of 100 d$^{-1}$ is too large to describe the early PuO$_2$ dissolution, (2) the bound fraction $f_b$ as defined by the Publication 66 of ICRP is not negligible, and need to be estimated using a specific approach. For this purpose, autoradiographic analysis of lung sections has been performed on some of the PuO$_2$ exposed animals.

### 3.2. In situ visualisation of different physico-chemical forms of actinides

The observation of contact autoradiographs shows that the alpha activity retained in the lungs corresponded either to spots with a symmetrical point, *i.e.* PuO$_2$ particles, or isolated alpha tracks, which could correspond to actinides associated to different lung ligands after particle dissolution (Figs. 1A and 1C). At the post-exposure times studied (2 hours and 1 week), these isolated tracks are homogeneously distributed within the deep lung. This indicates the lack of a preferential localisation of dissolved actinides in alveolar macrophages, suggesting a fast dissolution of PuO$_2$ particles ($f_r$) before their phagocytosis. The largest number of isolated tracks per unit of section area is observed 2 hours after inhalation exposure and a gradual but slight decrease of this number appears to occur thereafter (Fig. 1C) which might visualize a slow transfer of the dissolved actinides to blood.

In our experimental conditions, incubation of lung sections in a DTPA solution allows the chelating agent to access potentially to all the retained actinides, whatever their localisation (extracellular and intracellular). Comparison of contact autoradiographs obtained from the same area of a lung section, before and after incubation in a DTPA solution, shows that most of the isolated tracks disappears, suggesting that actinides retained in the lung parenchyma after particle dissolution can be chelated by DTPA (Figs. 1B and 1D). By contrast, the mean number of tracks in spots corresponding to particles appears not to be influenced by the incubation in the DTPA solution, even for the smallest particles which can be
visualized by a few alpha tracks. This indicates the absence of a significant dissolution of particles in presence of the chelating agent.

Table II shows the results of a quantitative analysis of the autoradiographs. As control, the incubation of rehydrated lung sections in a solution which does not contain DTPA leads only to a loss of about 20% of isolated tracks and particles.
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3.3. Decorporation of dissolved actinides in vivo

After inhalation of the “old PuO₂”, a local treatment by insufflation of DTPA dry powder induces a large increase of the urinary excretion of actinides, and a significant reduction of their retention in liver and skeleton but not in the lungs (Sérandour et al., 2007). The systemic retention of Pu and Am depends on the delay between the contamination and the DTPA treatment. Thus, for example, a treatment performed 2 hours after inhalation exposure, here referred to as early treatment, reduces the skeletal burden measured at 10 days to 15 ± 5% (n = 5) of the controls, whereas, after a delayed treatment performed after 1 week, this reduction is limited to 47 ± 11% (n = 4). Assuming that decorporation mainly involves Pu/Am retained in the lungs, these results suggest that (1) most of $f_d$ has dissolved during the first 2 hours after inhalation and can be decorporated by DTPA and (2) the dissolved material from $f_d$ and/or $(1 - f_d)$ is slowly transferred to blood.

### Table II

Quantitative analysis of contact autoradiographs from subpleural parenchyma of lung sections before and after incubation in a DTPA solution. Animals were euthanized 2 hours or 1 week after PuO₂ contamination. The mean number of isolated alpha tracks per unit of autoradiography area was determined in embedded lung sections and after their rehydration and incubation in a DTPA solution. The activity corresponding to the dissolved actinides retained in the lungs was calculated as reported in the material and methods section and expressed as Bq and % of the total lung activity at death.

<table>
<thead>
<tr>
<th>Time post-contamination</th>
<th>Lung sections</th>
<th>Mean number of tracks/unit area (± SD)</th>
<th>Bq/lung</th>
<th>Lung activity at death (Bq)</th>
<th>% of total lung activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>embedded</td>
<td>412 ± 34</td>
<td>72 ± 6</td>
<td>327</td>
<td>22 ± 2</td>
</tr>
<tr>
<td></td>
<td>+ DTPA in vitro</td>
<td>62 ± 13</td>
<td>11 ± 2</td>
<td>3.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>1 week</td>
<td>embedded</td>
<td>244 ± 51</td>
<td>43 ± 8</td>
<td>245</td>
<td>17.5 ± 3</td>
</tr>
<tr>
<td></td>
<td>+ DTPA in vitro</td>
<td>20 ± 5</td>
<td>3.5 ± 1</td>
<td>1.4 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>
The diminution of lung retention due to early DTPA treatment which corresponds to about 10–20% of IDLD cannot be measured in vivo with standard X-ray spectrometry, because of the presence of large amounts of actinides in the gastrointestinal tract, especially during the first days post inhalation. This leads us to analyze contact autoradiographs obtained from lung sections in order to point out a local effect of DTPA insufflation. This approach needs the comparison of autoradiographs obtained from control and DTPA treated rats, which is shown in Figure 2.
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After DTPA treatments, most of the isolated alpha tracks disappears (Figs. 2B and 2D) as compared with control (Figs. 2A and 2C), indicating that DTPA, administered at the site of contamination, can chelate most of the dissolved actinides retained in the lungs. Moreover, the amount of Pu/Am decorporated from the lung by DTPA is similar to that recovered in the urines. This indicates that, quantitatively, decorporation mainly involves the lungs rather than the systemic compartments. The \textit{in vivo} decorporation observed after the early treatment appears similar to the actinide extraction obtained after \textit{in vitro} incubation of lung section of an untreated rat in presence of DTPA. By contrast, the efficacy of the delayed treatment seems to be less than that observed \textit{in vitro} for a post-inhalation time of 1 week.

These observations are confirmed after a quantitative analysis of the autoradiographs the results of which are shown in Table III. From these results it appears that, \textit{in vivo}, the accessibility of dissolved actinides decreases depending on time after the aerosol exposure. This phenomenon might be associated with the internalisation of the dissolved actinides within cells of the lung parenchyma which are not preferentially alveolar macrophages.

### TABLE III

Quantitative analysis of contact autoradiographs from lung sections of control and DTPA treated animals. The mean number of isolated alpha tracks per unit of autoradiography area was determined in embedded lung sections of control animals which were euthanized 2 hours after an early or a delayed DTPA insufflation. The activity corresponding to the dissolved actinides retained in the lungs was calculated and expressed as Bq and % of the total lung activity at death.

<table>
<thead>
<tr>
<th>DTPA dry powder insufflation</th>
<th>Mean number of tracks/unit area (± SD)</th>
<th>Bq/lung</th>
<th>Lung activity at death (Bq)</th>
<th>% of total lung activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 hours)</td>
<td>412 ± 34</td>
<td>72 ± 6</td>
<td>327</td>
<td>22</td>
</tr>
<tr>
<td>Control (1 week)</td>
<td>244 ± 51</td>
<td>43 ± 8</td>
<td>245</td>
<td>17.5</td>
</tr>
<tr>
<td>Early DTPA treatment</td>
<td>61 ± 23</td>
<td>11 ± 4</td>
<td>393</td>
<td>2.8</td>
</tr>
<tr>
<td>Delayed DTPA treatment</td>
<td>75 ± 19</td>
<td>13 ± 3</td>
<td>203</td>
<td>4.3</td>
</tr>
</tbody>
</table>

After DTPA treatments, most of the isolated alpha tracks disappears (Figs. 2B and 2D) as compared with control (Figs. 2A and 2C), indicating that DTPA, administered at the site of contamination, can chelate most of the dissolved actinides retained in the lungs. Moreover, the amount of Pu/Am decorporated from the lung by DTPA is similar to that recovered in the urines. This indicates that, quantitatively, decorporation mainly involves the lungs rather than the systemic compartments. The \textit{in vivo} decorporation observed after the early treatment appears similar to the actinide extraction obtained after \textit{in vitro} incubation of lung section of an untreated rat in presence of DTPA. By contrast, the efficacy of the delayed treatment seems to be less than that observed \textit{in vitro} for a post-inhalation time of 1 week.

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4. Conclusions

This study shows the presence of actinides which are partly retained in the lungs after dissolution of deposited PuO$_2$ aerosols. During the first week following inhalation exposure, autoradiographic analyses show that a fraction of these forms can be decorporated after a pulmonary administration of DTPA, or extracted \textit{in vitro} from lung sections of untreated animals. The efficacy of the \textit{in vivo} DTPA treatment, as concerns lung decorporation, decreases as a function of the delay between therapy and contamination, whereas, \textit{in vitro}, a similar fraction of dissolved actinides is extracted after DTPA incubation of lung sections of untreated rats killed 2 hours or 1 week after the inhalation exposure. This suggests that these chemical forms of actinides can be concomitantly internalised within cells of lung parenchyma without preferential accumulation in alveolar macrophages, and transferred to blood. In fact, most of the actinides transferred to the systemic compartments corresponds to early PuO$_2$ dissolution ($f_r$) and altogether, autoradiography and standard biokinetic data suggests that the dissolution rate $s_r$ is close to the default value (100 d$^{-1}$) but $f_{fr}$, as defined in Publication 66 of ICRP (1994), is not equal to 0. Because most of the actinides dissolved involves Am, these conclusions might be specific for this element.

From these results a new model can be proposed to describe pulmonary decorporation of transuranium elements by DTPA after its local administration (Fig. 3). In this model, the dissolution of $f_r$ occurs before particle internalisation in cells, mostly alveolar macrophages, which involves the fraction $(1 - f_r)$. The early dissolved actinides are promptly associated with different biological ligands located in extracellular compartments including cell surfaces and surfactant which are accessible to DTPA. Such chemical forms can be either internalized within different cell types to become inaccessible to the chelating agent, or transferred to blood. The internalized actinides can move again to the extracellular compartments. The dissolution of the remaining fraction $(1 - f_r)$ occurs within the cells and then dissolved forms behave as internalized actinides. Such a model might be applied to other kinds of contamination including wounds for which the delayed DTPA treatment can induced an increase urinary excretion of Pu much larger than the total amount of actinides circulating in blood and interstitial fluids (Fritsch et al., 2007).

The homogenous repartition of the dissolved actinides in the lung parenchyma contributes to a homogenous alpha irradiation. For the same dose delivered to the lungs, the risk to induce malignant lung tumours might be larger for a homogeneous than for a heterogeneous irradiation. The heterogeneous irradiation involves the presence of hot spots at the vicinity of actinide oxide particles having a high specific alpha activity which may kill most of the neighbouring target cells.
(Fritsch et al., 2003; Fritsch, 2007). Thus, decorporation of these dissolved actinides might lead to a significant decrease of the risk of cancer induction, even if no significant reduction of the equivalent dose delivered to the lungs can be observed. In fact, this calculated dose corresponds to a mean value which takes into account neither the heterogeneity of alpha irradiation, nor the dose rate. Thus, after human contamination with actinide oxides containing transuranium elements, repeated DTPA treatment might decrease significantly the risk for lung tumour induction but no effect is expected on the occurrence of deterministic lesions associated particle aggregation (Hahn et al., 2003).

Further experiments are in progress to warrant these modelling hypotheses by combining analyse of standard biokinetic data with quantitative analysis of dissolved actinides in tissue section visualised by autoradiography and chelating agent treatments. Contamination will be performed after inhalation exposure to PuO₂ and intratracheal injection of Pu and Am-nitrate, in order to characterize dissolved forms of each actinide within the lungs.

In conclusion, this study shows the need of new researches both in terms of mechanisms involved in the biological behaviour of actinides, including the identification and localisation of their ligands, and radioprotection, to develop new
models which can be applied to human to improve the efficacy of DTPA treatment, not only on the basis of a reduction of doses, but also on a reduction of risk for tumour induction.

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