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Article

Pulmonary retention of actinides after dissolution of PuO_2 aerosols: interest in modelling DTPA decorporation

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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 PuO_2 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 (f_r) 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: PuO_2 aerosols / dissolution parameters / bound fraction / DTPA / modelling

RÉSUMÉ **Rétention pulmonaire des actinides après dissolution d'aérosols de PuO_2 : 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 PuO_2 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 (f_r) avant la phagocytose des particules de PuO_2, principalement par les macrophages alvéolaires. L'administration précoce (+2 heures) de poudres sèches d'acide diéthylène triamine penta-acetique (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 ($f_r = 1$, $s_r = 100\ d^{-1}$, $s_s = 0\ d^{-1}$), type M, as moderate absorption ($f_r = 0.1$, $s_r = 100\ d^{-1}$, $s_s = 5 \times 10^{-3}\ d^{-1}$) and type S, as slow absorption ($f_r = 1 \times 10^{-3}$, $s_r = 100\ d^{-1}$, $s_s = 1 \times 10^{-4}\ d^{-1}$). All americium chemical forms are considered as type M compounds, whereas, for plutonium, soluble forms (nitrate, oxalate…) are

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 $^{241}Am_2O_3$ 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 ($s_b = 2 \times 10^{-4}$ 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 $^{238}PuO_2$ 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 *in vitro*, in presence of DTPA (Sato *et al*., 1994; Cooper *et al*., 1979). In the case of PuO_2 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 PuO_2 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, *in vitro* and *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, σ_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 administrated 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_s) 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 × 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 PuO_2, 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 1×10^3 Bq so that a few PuO_2 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 3.2× ~ 0.7 mm^2). 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 PuO_2" aerosol (^{241}Am: 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 (^{241}Am: ~15% of the total alpha activity) and biokinetic data have been reported after inhalation exposure (Lataillade *et al.,* 1995). In addition, a second PuO_2, 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 PuO_2". Table I compares the dissolution parameters of Pu/Am after inhalation exposure to the different aerosols.

After exposure to the "young PuO_2" aerosols, similar dissolution parameters are measured for Pu. The large value of f_r is due to the fact that the calculation

TABLE I

Influence of the delay between fabrication of PuO_2 and aerosol generation on the Pu/Am dissolution after inhalation exposure of rats. Two industrial oxides were studied, referred to as a and b. Dissolution parameters (f_r and s_s) were calculated by a linear correlation method (Ramounet *et al.,* 2000) by using experimental data reported by * Lataillade *et al.* (1995), ** recent unpublished data, and * Ramounet *et al.* (2000). NM : not measured.**

Influence du délai entre la fabrication du PuO_2 et la génération aérosols sur la dissolution du Pu/Am après inhalation chez le rat. Deux oxydes industriels ont été étudiés référencés comme a et b. Les paramètres de dissolution (f_r et s_s) ont été calculés à l'aide d'une méthode basée sur une corrélation linéaire (Ramounet *et al*., 2000) en utilisant les données expérimentales issues de * Lataillade *et al*. (1995), ** travaux récents non publiés, et * Ramounet *et al*. (2000). NM : non mesuré.**

PuO_2	Delay of the study	Pu		Am	
		f_r	s_s	f_r	s_s
"young" a	< 3 years*	1.4×10^{-2}	3.5×10^{-5} d^{-1}	NM	NM
"old" a	> 15 years**	9.5×10^{-3}	2.0×10^{-4} d^{-1}	1.1×10^{-1}	4.2×10^{-3} d^{-1}
"young" b	< 3 years***	2.2×10^{-2}	1.5×10^{-5} d^{-1}	3.0×10^{-2}	3.6×10^{-4} d^{-1}

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 (1×10^{-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.

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_s 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_s 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

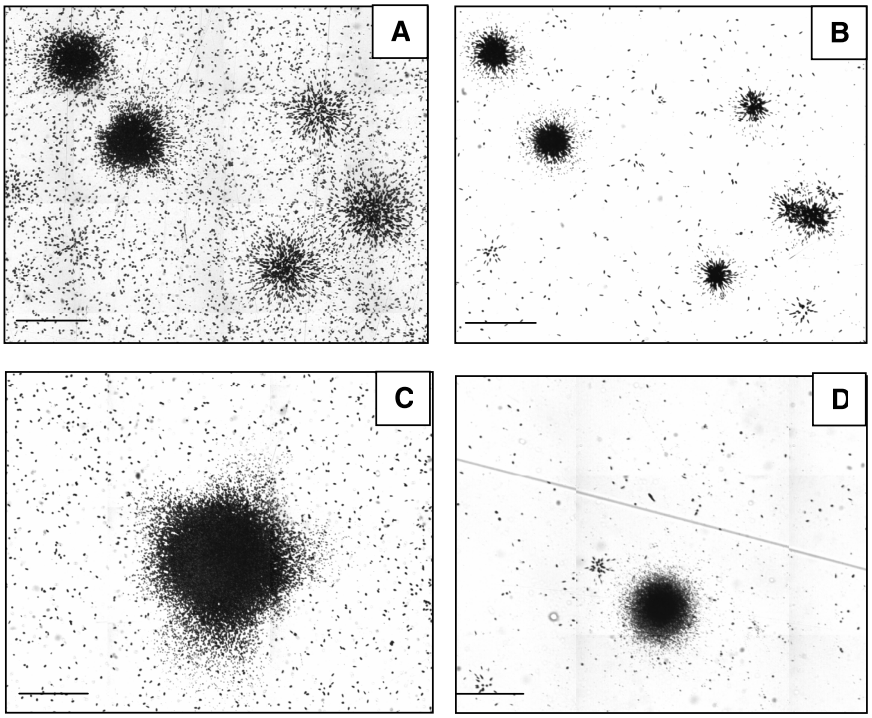

Figure 1 – ***Comparison of contact autoradiographs from the same area of lung sections before and after its incubation in a DTPA solution. Animals were euthanized 2 hours (A and B) or 1 week (C and D) after PuO_2 inhalation. Lung sections were incubated in a DTPA solution (B and D) or not (A and C). Lung retention at death: 327 Bq (A and B) and 245 Bq (C and D). Bar: 500 µm. The different diameter of spots visualizing PuO_2 particles is explained by slight variations of the distance between particles and solid track detector which occur for each contact autoradiography.***

Comparaison d'autoradiographies par contact obtenues à partir de mêmes zones de coupes de poumon avant et après leur incubation dans une solution de DTPA. Les animaux ont été euthanasiés 2 heures (A et B) ou 1 semaine (C et D) après l'exposition au PuO_2. Les coupes ont été incubées (B et D) ou non (A et C) dans une solution de DTPA. Rétention pulmonaire à la mort : 327 Bq (A et B) et 245 Bq (C et D). Barre : 500 µm. Les différents diamètres des spots visualisant les particules de PuO_2 sont expliqués par de faibles variations de la distance séparant la coupe du détecteur qui est spécifique à chaque autoradiographie par contact.

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

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 PuO_2 contamination. The mean number of isolated alpha tracks per unit of autoradiography area was determined in embedded lung sections and after their rehydratation 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.

Analyses quantitatives d'autoradiographies par contact de zones alvéolaires sous pleurales avant et après l'incubation de coupes de poumon dans une solution de DTPA. Les animaux ont été euthanasiés 2 heures ou 1 semaine après l'exposition au PuO_2. Le nombre moyen de traces alpha isolées par unité de surface de l'autoradiographie a été déterminé sur des coupes non déparaffinées et après réhydratation et incubation dans une solution de DTPA. L'activité correspondant aux actinides dissous retenus dans les poumons est calculée comme indiqué dans matériel et méthodes et exprimée en Bq et % de l'activité totale pulmonaire à la mort des animaux.

Time post-contamination	Lung sections	Mean number of tracks/unit area (± SD)	Bq/lung	Lung activity at death (Bq)	% of total lung activity
2 hours	embedded	412 ± 34	72 ± 6	327	22 ± 2
	+ DTPA *in vitro*	62 ± 13	11 ± 2		3.4 ± 0.6
1 week	embedded	244 ± 51	43 ± 8	245	17.5 ± 3
	+ DTPA *in vitro*	20 ± 5	3.5 ± 1		1.4 ± 0.4

(data not shown). Estimation of the fraction of lung activity associated with dissolved actinides performed after 2 hours is in agreement with the large f_r value measured for Am. Altogether, these data suggest that most of the dissolved actinides retained in the lungs could be decorporated *in vivo* by DTPA and might partly correspond to the bound fraction f_b as defined in Publication 66 of ICRP.

3.3. Decorporation of dissolved actinides in vivo

After inhalation of the "old PuO_2", 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 $\pm$ 5% (n = 5) of the controls, whereas, after a delayed treatment performed after 1 week, this reduction is limited to 47 $\pm$ 11% (n = 4). Assuming that decorporation mainly involves Pu/Am retained in the lungs, these results suggest that (1) most of f_r has dissolved during the first 2 hours after inhalation and can be decorporated by DTPA and (2) the dissolved material from f_r and/or $(1 - f_r)$ is slowly transferred to blood.

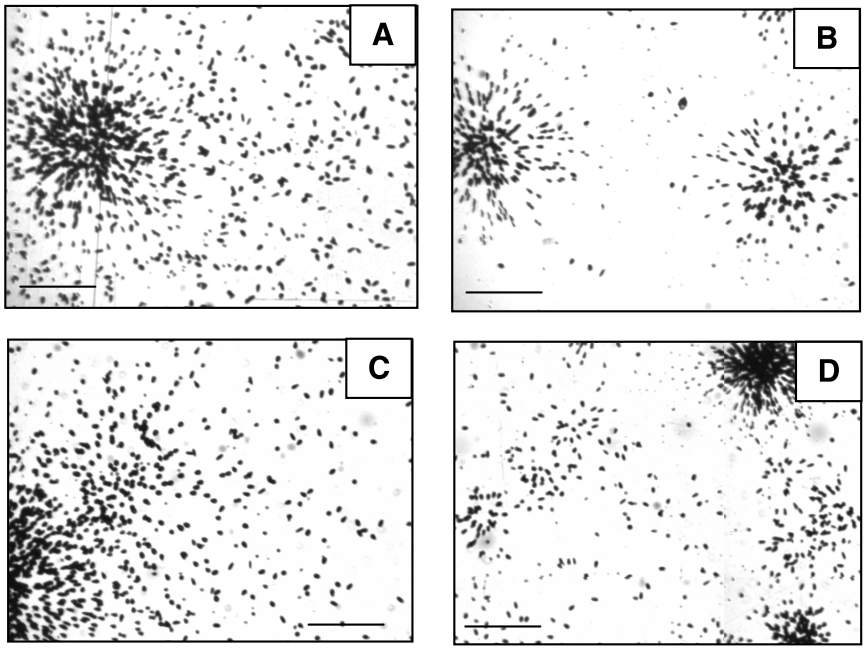

Figure 2 – ***Effect of DTPA dry powder insufflation on the pulmonary retention of dissolved actinides. Contact autoradiographs of lung sections were obtained from control or DTPA-treated animals. Control animals were euthanized 2 hours (A) or 1 week (C) after PuO_2 exposure. Treated animals were insufflated by a DTPA dry powder 2 hours (B) or 1 week (D) after PuO_2 exposure and euthanized 2 hours after the treatment. Lung retentions at death ranging from 200 to 400 Bq for all studied animals. Bars: 200 µm.***

Effet de l'insufflation de poudres sèches de DTPA sur la rétention pulmonaire des actinides dissous. Des autoradiographies par contact avec des coupes de poumon ont été réalisées chez des animaux traités ou non par le DTPA. Les témoins ont été euthanasiés 2 heures (A) ou 1 semaine (C) après exposition au PuO_2. Les animaux traités par une insufflation de poudres sèches de DTPA effectuée 2 heures (B) ou 1 semaine (D) après l'exposition au PuO_2 ont été euthanasiés 2 heures après le traitement. Pour tous les animaux étudiés, la rétention pulmonaire à la mort est comprise entre 200 to 400 Bq. Barre : 200 µm.

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.

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 or 1 week after PuO_2 contamination, and treated 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.

Analyses quantitatives d'autoradiographies par contact obtenues à partir de coupes de poumons d'animaux traités ou non par du DTPA. Le nombre moyen de traces alpha isolées par unité de surface de l'autoradiographie a été déterminé sur des coupes non déparaffinées d'animaux témoins, euthanasiés 2 heures ou 1 semaine après l'exposition au PuO_2, et d'animaux traités, euthanasiés 2 heures après un traitement DTPA précoce ou différé. L'activité correspondant aux actinides dissous retenus dans les poumons est calculée comme indiqué dans matériel et méthodes et exprimée en Bq et % de l'activité totale pulmonaire à la mort des animaux.

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

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 *in vivo* decorporation observed after the early treatment appears similar to the actinide extraction obtained after *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 *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, *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.

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 *in vitro* from lung sections of untreated animals. The efficacy of the *in vivo* DTPA treatment, as concerns lung decorporation, decreases as a function of the delay between therapy and contamination, whereas, *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_b, 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

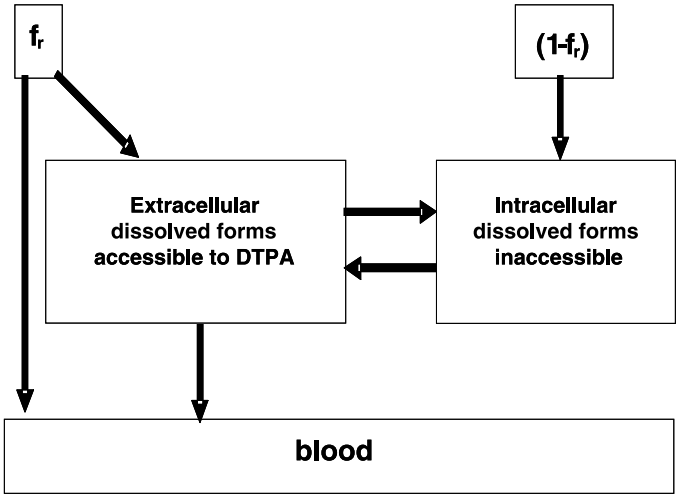

Figure 3 – ***Structure of a new model proposed to describe decorporation of dissolved actinides by DTPA based on the experimental data here reported.***

Structure d'un nouveau modèle proposé pour décrire la décorporation des actinides dissous par le DTPA basée sur les données expérimentales rapportées dans cette étude.

(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 PuO_2 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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