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Benjamin B. Tournier[1,2], Chrystelle Ibanez[1], Elie Tourlonias[1,3], Fabrice Petitot[1,4], François Paquet[1,5], Isabelle Dublineau[1,6], Philippe Lestaevel[1,7*]

[1] Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE, SESANE, Laboratoire de Radiotoxicologie et Radiobiologie Expérimentale, 92262 Fontenay-aux-Roses, France.

[2] Division of Adult Psychiatry, Department of Psychiatry, University Hospitals of Geneva, Switzerland.

[3] Nucléagis SAS, 63480 Vertolaye, France.

[4] CEA, DEN, DUSP, Service de Protection contre les Rayonnements, 30207 Bagnols sur Cèze Cedex, France.

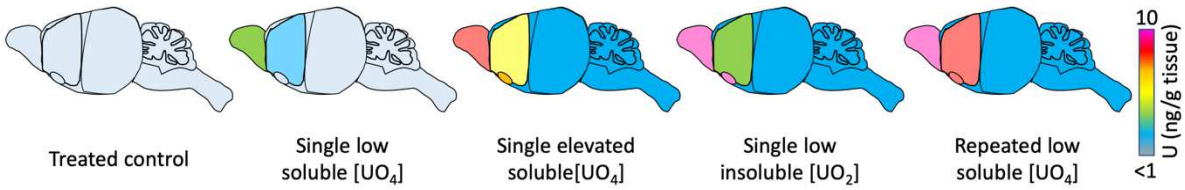
[5] Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-ENV, SRTE, 13115 Saint Paul-lez-Durance, Cedex, France.

[6] Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-ENV, SEDRE, Unité d'expertise des sites et des déchets radioactifs, 92262 Fontenay-aux-Roses, France.

[7] Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE, SER, Bureau d'Analyse et de Suivi des Expositions Professionnelles, 92262 Fontenay-aux-Roses, France.

*Corresponding author:
Philippe Lestaevel
Institut de Radioprotection et de Sûreté Nucléaire (IRSN)
PSE-SANTE, SESANE, Laboratoire de Radiotoxicologie et Radiobiologie Expérimentale, Fontenay-aux-Roses, 92262, France
Tel : +33 1 58 35 80 42
philippe.lestaevel@irsn.fr

Graphical abstract.



Abstract.

A rostro-caudal gradient of uranium (U) in the brain has been suggested after its inhalation. To study the factors influencing this mapping, we first used 30-min acute inhalation at 56 mg/m^3 of the relatively soluble form UO_4 in the rat. These exposure parameters were then used as a reference in comparison with the other experimental conditions. Other groups received acute inhalation at different concentrations, repeated low dose inhalation of UO_4 (10 exposures) or acute low dose inhalation of the insoluble form UO_2. At 24 hours after the last exposure, all rats showed a brain U accumulation with a rostro-caudal gradient as compared to controls. However, the total concentration to the brain was greater after repeated exposure than acute exposure, demonstrating an accumulative effect. In comparison with the low dose soluble U exposure, a higher accumulation in the front of the brain was observed after exposure to higher dose, to insoluble particles and following repetition of exposures, thus demonstrating a dose effect and influences of solubility and repetition of exposures. In the last part, exposure to ultrafine U particles made it possible to show 24 hours after exposure the presence of U in the brain according to a rostro-caudal gradient. Finally, the time-course after exposure to micronic or nanometric U particles has revealed greater residence times for nanoparticles.

Keywords.

Uranium, inhalation, nanoparticles, solubility, rat

Abbreviations.

BRes, brain residue; FCx, frontal cortex; Ins., insoluble; NT, nasal turbinates; OB, olfactory bulbs; OT, olfactory tubercles; Rep., repeated exposures; UFP, ultrafine particles; WB, whole brain.

1. Introduction.

Uranium (U) is a natural radioactive transuranic element with a wide range of industrial uses, but its main application concerns energy production and military use (Craft et al., 2004). The natural fissile isotope uranium-235 is used primarily in most nuclear power plants. The preparation of uranium to be used as fuel in nuclear power plants includes different successive steps (mining, milling, conversion, enrichment, fabrication of nuclear fuel and reprocessing in some cases). Importantly, depending on the steps in the uranium fuel cycle, the chemical nature of U compounds, notably solubility, varies. Ammonium diuranate $(NH_4)_2U_2O_7$, a component of yellowcake, is converted to uranium hexafluoride (UF_6) prior to ^{235}U enrichment. The enriched UF_6 is then converted into UO_2, the final product in the manufacture of nuclear fuel pellets used in most reactors (UNSCEAR, 2016). In nuclear fuel cycle facilities, workers involved in the concentration or enrichment process and in fuel fabrication could face repeated inhalations of airborne U compounds (Fulco et al., 2000). Indeed, dust inhalation is one of the major risk of contamination for nuclear workers (Blanchin et al., 2004; Durakovic et al., 2003; Fulco et al., 2000; Salbu et al., 2005). Furthermore, in addition to micrometric U dusts, metal ultrafine or nanometric (<100 nm) U particles can be also generated as byproducts in several industrial steps of the nuclear fuel cycle or during the decommissioning of nuclear facilities (Dewalle et al., 2010; Onodera et al., 1991; Trelenberg et al., 2006).

After inhalation, the first organs impacted by the uranium deposition are the lungs as described in the Human Respiratory Tract Model (HRTM biokinetic model) (ICRP, 1994). Accordingly to their pulmonary transfer rate, uranium compounds can be classified according to their solubility as fast (F), moderate (M) and slow (S) (ICRP, 2017). As an example, the solubility of UO_4 is 50% type F and 50% type M. The ICRP model aims at predicting translocation and deposition of uranium according to particle size parameters and then subsequent retention following its deposition depending on the different anatomical pulmonary regions (bronchial and alveolar compartments). U distribution is then predicted by the systemic biokinetic model to the whole organism *via* the blood pathway. After its absorption to blood, uranium is present mainly as uranyl ions complexed to proteins (e.g. transferrin, albumin) or bicarbonate anions (Ansoborlo et al., 2006). The main sites of deposition of uranium from the circulation are the skeleton and the kidneys. In addition, experimental data predict that part of the metal enters the brain (Ballou et al., 1986; Diamond et al., 1989; La Touche et al., 1987; Lemercier et al., 2003). Most recently, several publications suggested that uranium deposition in brain is higher after inhalation than after ingestion or injection, suggesting that the cerebral accumulation of U depends on the mode of contamination (for review, see (Dinocourt et al., 2015)). For instance, 24 hours after the end of repeated

inhalations in rat, the total brain U amounts were about 0.47% of the total U retained in the body but they were only about 0.007 and 0.24% after respectively an acute injection or chronic ingestion (Houpert et al., 2007).

In addition, compared to other modes of exposure, inhalation of U resulted in the presence of an important rostro-caudal gradient with the olfactory bulbs containing the highest concentration (Barber et al., 2005; Houpert et al., 2007; Houpert et al., 2005; Monleau et al., 2005; Paquet et al., 2006; Tournier et al., 2009). A direct transfer from the nasal cavity to the brain have been proposed to explain such a heterogeneous brain distribution (Ibanez et al., 2019; Ibanez et al., 2014; Tournier et al., 2009). This additional route of entry to the brain without transit through the lungs and bloodstream in rats appears to modify the U brain mapping which, if it exists in humans, could also represent a new mode of contamination for U particles that is not taken in account in the HRTM ICRP model. Therefore, the knowledge of the brain distribution of inhaled U, which may depend on its solubility, granulometry and concentration, is of importance in the case of U intoxication via inhalation.

The objective of this study was to highlight the link between physical-chemical characteristics of uranium and the uranium distribution into the brain after inhalation. Specifically, the aims of our study were to compare different U aerosol inhalation protocols on resulting concentrations in some brain parts, using soluble (F/M) and insoluble (M/S) forms of uranium, acute or repeated exposure mode, micronic or nanometric U particles. To this end, a first group was exposed acutely to a “low” concentration of soluble U aerosol (UO_4) The term “low” is then used as a reference term and is indicated as “low” in terms of aerosol volumic concentration and in comparison with the other experimental conditions exposed to a higher concentration. The frontal cortex, olfactory bulbs and tubercles and the rest of the brain were used as a reference brain map. The distribution of the inhaled U was then compared with other groups having inhaled acutely the same aerosol (UO_4) but at higher dose, or having inhaled acutely an aerosol formed a less soluble U physico-chemical form (UO_2) at low dose. Another group was then exposed repeatedly at the same low dose of the same soluble aerosol (UO_4, 10 exposures). In a second part, the entry to the brain of U nanoparticles (UO_2) was sought. Finally, the kinetics of retention of U in the brain was compared between two U aerosols formed of whether nanoparticles or microparticles.

2. Material and methods.

2.1. Animals

Adult male Sprague–Dawley rats (Charles River Laboratories, France) were housed in pairs under standard conditions (light on: 8.00 am/8.00 pm; temperature: 22 ± 1 ◦ C) with food (105, CERJ, France) and water provided *ad libitum* and were randomly assigned to one of the experimental groups (n=8/group). Group details are given in the table 1. To avoid stress, rats were progressively acclimatized to the nose-only exposure tubes over a three-week period. Treated control rats have also performed the habituation to the nose-only exposure tubes and were only exposed to ambient air. All animal procedures were approved by the Animal Care Committee of the IRSN and complied with French regulations for animal experimentation (Ministry of Agriculture Act N°87-848, October 19th 1987, modified 29th of May 2001. They were approved by the IRSN Ethics Committee and authorized by the French Ministry of Research.

Group name	**Low**	**High**	**Ins.**	**Rep.**	**UFP**
		Micrometric aerosol			**Nanometric aerosol**
Chemical form	UO_4	UO_4	UO_2	UO_4	UO_2
ICRP reference absorption type	Intermediate type F/M	Intermediate type F/M	Intermediate type M/S	Intermediate type F/M	Intermediate type M/S
Aerosol concentration (mg/m^3)	56 ±28	412 ±82	52 ±14	61 ±17	1 ±0
Duration of the exposure (min)	30	30	30	30	60
Number of exposures	1	1	1	10 (3x/wk)	1
Mass median aerodynamic diameter (µm) (range)	1.99 ±0.13 (1.72-2.2)	1.79 ±0.07 (1.71-2.1)	1.81 ±0.02 (1.74-1.83)	1.64 ±0.17 (1.47-2-2)	0.15 ±0.09 (0.08-0.3)
Radiological nature	Reprocessed	Reprocessed	Depleted	Reprocessed	Depleted
Isotopic ratio	^{238}U = 99.55% ^{236}U= 0.051% ^{235}U = 0.39% ^{234}U = 0.0047%	^{238}U = 99.55% ^{236}U= 0.051% ^{235}U = 0.39% ^{234}U = 0.0047%	^{238}U = 99.75% ^{235}U = 0.24% ^{234}U = 0.001%	^{238}U = 99.55% ^{236}U= 0.051% ^{235}U = 0.39% ^{234}U = 0.0047%	^{238}U = 99.799% ^{235}U = 0.200% ^{234}U = 0.001%

Table 1. Characteristics of uranium aerosols. Characteristics of uranium used in the present study for aerosol generation as well as some parameters of animal exposure design are indicated in this table. Data are indicated as mean ±SD. Abbreviations: Ins., insoluble; Rep., repeated exposures; UFP, ultrafine particles. ICRP reference absorption types: F: fast; M: moderate; S: slow (from ICRP 137, 2017).

127 *2.2. Aerosol generation*

128 The aerosol generation and the measure of their characteristics were previously described (Petitot et al., 2013;
129 Tournier et al., 2009). Industrial U dioxide (UO_2) and U peroxide (UO_4) powders found at workplaces in U fuel
130 cycle facilities were supplied by ORANO-AREVA NC (Pierrelatte, France). Briefly, micronic UO_4 and UO_2
131 aerosols were generated from uranium powder with the RBG1000 rotating brush generator (PALAS®, France)
132 used for aerosol generation from dry powder. The isotopic composition by mass of the UO_4 powder was ^{238}U=
133 99.55%; ^{236}U= 0.051%; ^{235}U= 0.39%; ^{234}U= 0.0047%. Microparticle aerosol concentrations, particle size
134 distribution and their mass median aerodynamic diameter were monitored using an aerodynamic particle sizer
135 (APS 3321, Trust Science Innovation, TSI®, France) combined with a diluter, as previously described (Monleau
136 et al., 2006; Tournier et al., 2009).
137 Nanoparticles aerosol was generated using a uranium metal bar and the spark-discharge aerosol generator
138 (PALAS GFG 1000). Nanoparticle aerosol concentrations and their mass median aerodynamic diameter were
139 determined using a filter sampling unit and a differential mobility analyzer (DMA, TSI®) combined with a
140 condensation particle counter (CNC, TSI®). To avoid saturation of the measuring devices, a dilution system of
141 the aerosol (VKL100, 2 bar) was installed upstream of the Scanning Mobility Particle Sizer (i.e. DMA + CNC)
142 (Petitot et al., 2013). The characteristics of the aerosols are given in Table 1. The uranium concentrations used
143 here in microparticle aerosols are lower than those previously used showing none effects on general health and
144 blood-plasma biochemical parameters (Monleau et al., 2006; Monleau et al., 2005; Tournier et al., 2009). More
145 details on the characterization of nanoparticles such as the ultrastructure of the uranium particles contained in the
146 air stream from the inhalation chamber and the elemental composition of these particles were published
147 previously (Petitot et al., 2013).
148
149 *2.3. Inhalation exposures*

150 Aerosol exposures were performed using the nose-only inhalation system contained within a glove box, a
151 procedure previously described (Monleau et al., 2005). The nose-only inhalation system is composed of three
152 parts: the aerosol generation part with the generators (RBG or PALAS), the metrology part containing the
153 devices used to perform real time monitoring (APS or SMPS), and the inhalation chamber itself on which the
154 nose-only tubes can be connected. Before uranium inhalation exposure, all animals are progressively
155 acclimatized to the tubes as mentioned previously. The noses of the rats contained within the tubes are then in
156 direct contact with the aerosols generated inside this inhalation chamber. At the end of each exposure, the nose

of each animal was washed to remove residues of U particles, in order to eliminate any risk of ingestion. The characteristics of aerosols and exposures are indicated in the Table 1. All exposed groups were associated with a respective treated control group (i.e. exposed to ambient air).

2.4. Sample collection

Deeply isoflurane anaesthetized (inhalation of isoflurane 5% / air 95%) rats were euthanized and their brain quickly removed. The olfactory bulbs, olfactory tubercles, frontal cortex and the rest of the brain (indicated in the rest of the document as "brain residue") were dissected out on ice, weighed and stored at −80°C until required. Peripheral organs (nasal turbinates and kidneys) were also dissected on ice, weighed and stored at −80°C.

2.5. Uranium measurements using an inductively coupled plasma mass spectrometer

Uranium concentration analyses were performed as previously reported (Ibanez et al., 2019). Selected brain areas and peripheral organs were digested using 8 mL nitric acid HNO_3 (Ultrapur, JT Becker) and 2 mL of 30% hydrogen peroxide (Normapur, VWR) in a microwave oven (Milestone StartD microwave digestion system 1000 W, Ethos TC; Milestone, Italy) as follows: 20 min to reach 200°C and then held at 200°C for 10min in the microwave oven at 1000 W using the digestion rotor system. Samples were then evaporated and dissolved in 5 ml of 10% HNO_3. Samples were diluted and elemental uranium concentration was measured using an inductively coupled plasma mass spectrometer (ICP-MS, PQ EXCELL, ThermoElectron with S-Option) using bismuth as the internal standard (Claritas PPT Grade). The detection limit of uranium measured was: 1 ng/L for ^{238}U and ^{235}U.

2.6. Statistics

The whole brain U concentration was compared between groups using a one-way ANOVA with the LSD *post hoc* test. Two-way ANOVA with the LSD *post hoc* test were used to estimate differences in uranium concentrations in rat cerebral areas (treatment and cerebral areas as factors) and to analyze the kinetics of U in the brain (treatment and time as factors).

3. Results

3.1. Unexposed animals

Control groups were used at each aerosol exposure experiment type (low, high, insoluble and repeated). No difference was measured between them in terms of uranium concentration (see Supplemental table 1). Thus, data from these groups were consequently pooled as a single control group. However, the statistical analysis was doubly performed, considering the respective control group of each exposure experiment type and considering the overall control group. The data presented below relate to comparisons with the pooled control group unless otherwise indicated.

3.2. Uranium concentration in the whole brain

The experimental design showing aerosols characteristics and exposure modes are indicated in the Table 1: animals received acute exposure to a low concentration of UO_4 (Low group), acute exposure to an elevated concentration of UO_4 (High group), acute exposure to a low concentration of the insoluble UO_2 (Ins. group) or repeated exposure to a low concentration of UO_4 (Rep. group). As compared to control animals, all groups exposed to microparticles of uranium showed increased brain U concentrations (Low: +230%, $p<0.01$; High: +340%, $p<0.001$; Ins.: +300%, $p<0.001$, Rep.: +465%, $p<0.001$) (Figure 1). Concerning differences between uranium treated groups, the group exposed repeatedly to UO_4 showed higher U levels in comparison with the acute exposure to a low concentration of UO_4 (+72%, $p<0.05$). Raw data are given in the Supplemental Table 2.

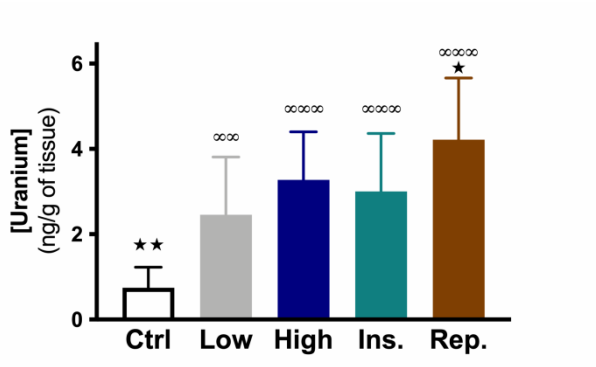


Figure 1. Uranium concentrations in the whole brain at 24h after inhalation. Uranium concentrations were measured in the whole brain and expressed as fold change in comparison with animals exposed to the low concentration of UO_4 (Low group). Animals were exposed to a low concentration of soluble uranium (Low), to a high concentration of soluble uranium (High), to a low concentration of insoluble uranium (Ins.) or to repeated exposures of soluble uranium at a low concentration (Rep.). Data are given as mean ± SD of 8 animals (control:

28 animals). Significant differences from the Low group are indicated at $^{\star}p<0.05$ and $^{\star\star}p<0.01$ and from the control group at $^{\circ\circ\circ\circ}p<0.01$ and $^{\circ\circ\circ\circ\circ\circ}p<0.001$, using one-way ANOVA (treatment as factor) with the LSD *post hoc* test.

3.3. Uranium concentration depends on the exposure mode.

In comparison with controls, increases in U concentrations in olfactory bulbs (OB), olfactory tubercles (OT), frontal cortex (FCx) and brain residue was observed in the High, the Insoluble and the Repeated groups (from $p<0.05$ to $p<0.001$, Fig. 2). In main contrast, increases in U concentrations in the Low group were restricted to OB ($p<0.05$) and FCx ($p<0.05$), suggesting a rostro-caudal gradient of U levels, as OB and FCx are more rostral than OT and the rest of the brain (brain residue, BRes). A significant difference in U levels between OB and BRes was observed in the High ($p<0.001$), the Insoluble ($p<0.05$) and the Repeated groups ($p<0.05$).

In addition, a minimum of 2-fold increase in U levels in the OB and the OT was observed in the High, the Insoluble and the Repeated groups, as compared to the Low group (from $p<0.05$ to $p<0.001$) (Fig. 2A-B). In the frontal cortex (FCx), an increase in U levels was observed in animals repeatedly exposed to UO_4 as compared to the Low group (2.3-fold change, $p<0.001$, Fig. 2C). In the brain residue, there was no difference between groups of various exposure modes (Fig. 2D). Raw data are given in the Supplemental Table 2.

In all exposed groups, the levels of U were increased in the nasal turbinates and the kidneys as compared to controls (see Supplemental Table 2). In addition, a significant increase in U concentration was observed in the nasal turbinates in the High group (+660%) and in the kidneys in the High (+2050%) and the Repeated (+680%) groups, as compared to the Low group (Supplemental Table 2).

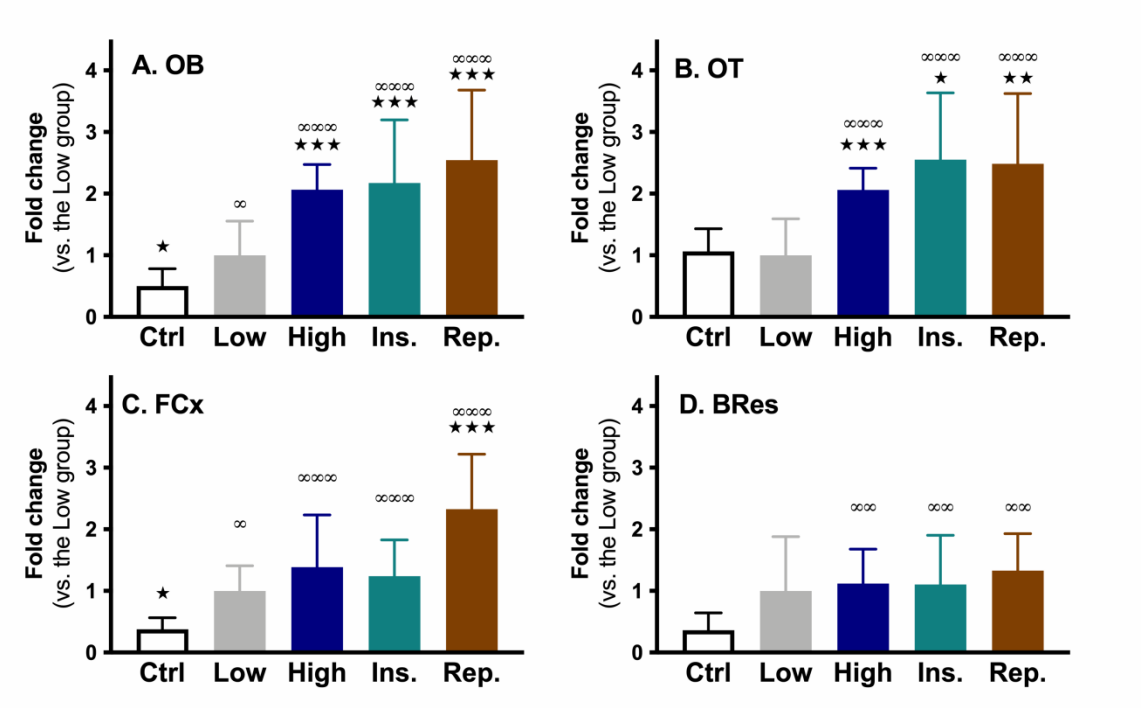


Figure 2. Uranium concentrations in rat cerebral areas at 24h after inhalation. Uranium concentrations were measured in olfactory bulbs (OB, **A**), olfactory tubercles (OT, **B**), frontal lobe of the cortex (FCx, **C**) and in brain residue (BRes, **D**). Animals were exposed to a low concentration of soluble uranium (Low), to a high concentration of soluble uranium (High), to repeated exposures of soluble uranium at a low concentration (Rep.) or to a low concentration of insoluble uranium (Ins.). Data are given as mean ± SD of 8 animals. The groups of control animals (exposed to ambient air) corresponding to each experimental group are represented together due to the absence of difference between them (Ctrl, mean ± SEM of 28 animals). Significant differences from the control group are indicated at $^{\circ}p<0.05$, $^{\circ\circ\circ\circ}p<0.01$ and $^{\circ\circ\circ\circ\circ}p<0.001$ and significant differences from the group exposed to a low concentration of soluble uranium (Low) are indicated at $^{\star}p<0.05$, $^{\star\star}p<0.01$ and $^{\star\star\star}p<0.001$ using two-way ANOVA (treatment x brain region) with the LSD *post hoc* test.

3.4. Cerebral distribution of the inhaled ultrafine particles of uranium at 24h after exposure.

A group was exposed to nanometric uranium UO_2 aerosol. The characteristics of this aerosol and of its exposure mode are given in the Table 1. The Figure 3 shows the significant higher accumulation of U in OB (+68%, $p<0.05$) and OT (+65%, $p<0.05$) which is absent in the brain residue 24 hours after inhalation of UFP as compared with the 1-hour ambient air group.

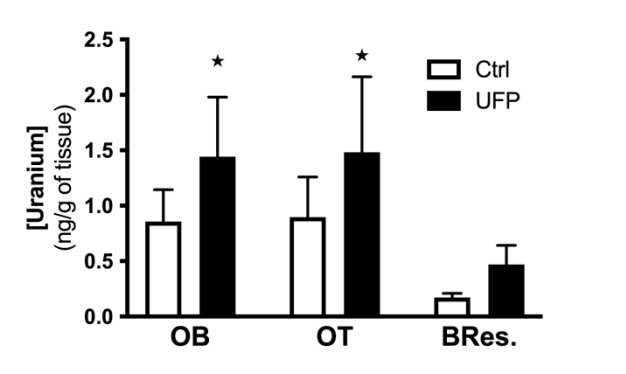


Figure 3. Uranium concentrations at 24h after inhalation of Uranium nanoparticles. Animals were exposed to ambient air (open bars) or to ultra-fine particles of uranium (UFP, black bars). Data are given as mean ± SD of 8 animals. Significant differences from control are indicated at ⋆p<0.05. *Abbreviations*: BRes, brain residue; OB, olfactory bulbs; OT, olfactory tubercles.

3.5. Kinetics of U concentrations in the brain, following acute exposure to micronic or nanometric particles of uranium.

Data showing uranium concentrations in OB, OT, and BRes at 10 min, 250 min, and 24 h after the end of the exposure to microparticles (UO_4) or to nanoparticles (U metal) are shown in Figure 4. In OB, as compared to the respective timepoint t_{10min}, U levels are significantly lower at t_{250min} and t_{24h} for both micro- and nanoparticles group ($p<0.05$, $p<0.01$ and $p<0.001$). However, the U concentration decrease in OB for micronic particles is greater compared to nanoparticles, resulting in a significant difference between the two types of particles at t_{24h} ($p<0.05$). At 24h post-exposure, U nanoparticle concentration is higher compared to microparticle one. In OT, levels of U microparticles decreased between t_{10min} and t_{250min} ($p<0.01$) and between t_{250min} and t_{24h} ($p<0.05$). In marked contrast, the concentration of U nanoparticles in OT remains stable throughout the measurement period, and result in a significant difference ($p<0.001$) between the two types of particles at t_{24h} with significant higher U concentration for the nanoparticle group. In the BRes, kinetics were identical to those observed in the OB: a significant decrease between t_{10min} and t_{250min} and t_{10min} and t_{24h} ($p<0.05$, $p<0.01$) for both types of particles. A significant difference between the two types of particles is also observed at t_{24h} with higher U nanoparticles concentration as compared to microparticles ($p <0.05$).

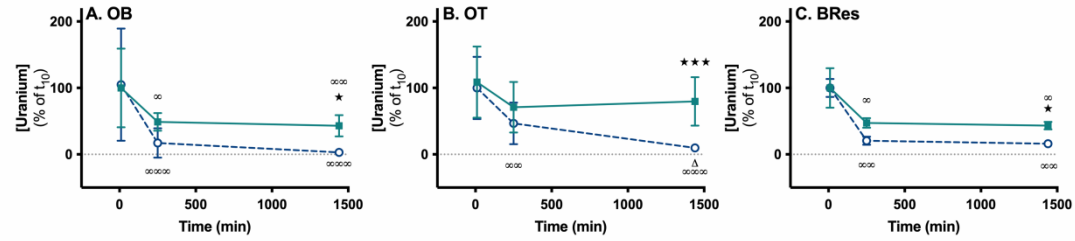


Figure 4. Effect of particle size on brain uranium distribution as a function of time. Animals were exposed to UO_4 microparticles (white circles, blue dashed line) or to U metal nanoparticles (green squares, solid line) of uranium. Uranium concentrations were measured in olfactory bulbs (**A**), olfactory tubercles (**B**) and brain residue (**C**). Data are given as mean ± SD of 8 animals. Significant difference from the respective timepoint t=10min group is indicated as $^{\circ\circ}p<0.05$, $^{\circ\circ\circ\circ}p<0.01$ and $^{\circ\circ\circ\circ\circ}p<0.001$; significant difference from the respective timepoint t=250min group is indicated as $^{\Delta}p<0.05$; significant difference in between micro and nanoparticles group is indicated as $^{\star}p<0.05$ and $^{\star\star\star}p<0.001$ using two-way ANOVA (treatment x time) with the LSD *post hoc* test.

4. Discussion

Our study demonstrated that whatever the intrinsic characteristics of the U aerosol or its exposure, inhalation resulted in the presence of an important rostro-caudal gradient in the brain with the olfactory bulbs and the rest of the brain containing the highest and the lowest U concentration, respectively, confirming previous experiments (Houpert et al., 2007; Monleau et al., 2005; Tournier et al., 2009). However, the present investigation demonstrated that U distribution in the brain depends on several parameters: U aerosol concentration, U physico-chemical form such as its solubility, U particle size and the exposure number. Indeed, several factors affect the U concentrations as measured in tissues located in the frontal regions of the brain, closest to the nasal cavity on a neuroanatomical point of view (olfactory bulbs and tubercles). Data clearly demonstrated that the U concentration in the front of the brain is increased when animals are exposed to UO_2, to a high concentration of UO_4 or to multiple exposures to UO_4 as compared to an acute UO_4 exposure. In addition, the kinetics of the U in the brain is modified by use of nanoparticles instead of microparticles. As a whole, our study shows that the ability of uranium to enter the brain depends on the conditions of inhalation in terms of aerosol concentration, the nature of the uranium and the frequency of exposure.

When U is inhaled, a fraction is found in the nasal cavity where a direct passage to the brain has been proposed, in addition to the transfer from the lungs via the bloodstream (Ibanez et al., 2014; Monleau et al., 2006; Tournier et al., 2009). The nasal cavity thus represents a potential route of entry of uranium to the brain.

We reported that only the High [UO_4] group shows an increase in the U concentration in the nasal turbinates compared to the Low [UO_4] group, thus showing a dose effect of the aerosol concentration. Conversely, the absence of a difference with the Low [UO_2] and Repeated low [UO_4] groups tends to show that the inhaled quantity per exposure are comparable, which is in line with the concentrations measured in the generated aerosols. It also demonstrates that there is no cumulative effect, from one day to the other, of the repeated exposure at least in the nasal turbinates suggesting that nasal cavity probably flush away the aerosol within the first 24 hours.

In the brain, we demonstrated that olfactory bulbs and tubercles are affected differently, unlike the rest of the brain, probably because a lower U quantity reaching these more posterior brain regions (frontal cortex and brain residue). These observations are in line with previous data (Ibanez et al., 2019; Monleau et al., 2005; Tournier et al., 2009). In addition, the rostro-caudal gradient of the brain U distribution is modified by the repetition of exposures. Indeed, in the Repeated low [UO_4] group, U levels were also found to be increased in the frontal cortex but not in the rest of the brain. This observation is of particular interest since no cumulative effect was observed in the nasal turbinates of the repeated group whereas U appears to cumulate in the olfactory bulbs and even finally reach the frontal cortex which is not the case for an acute exposure. However, although group differences are significant in brain areas, it is important to note that at the whole brain level, no group difference appeared. These observations are also important in the sense that dose calculations to the brain in response to exposure to a uranium aerosol do not consider the heterogeneity of the brain in terms of uranium accumulation. Thus, even if the doses received globally by the brain are identical, some structures, located at the front of the brain are more strongly exposed in case of inhalation. Therefore, brain toxicity calculations of U after inhalation considering the brain as a homogeneous tissue may underestimate the real impact at more targeted brain areas.

The UO_2 (M/S) aerosol lead to a higher brain U accumulation in the olfactory bulbs and tubercles in comparison with the UO_4 (F/M) aerosol, even if, at the whole brain level, no significant difference was observed. These observations suggest that the olfactory pathway is taken by U particles, whether in soluble or insoluble form. Physicochemical U forms in the tissue could differ from the original aerosol form once inhaled (Ibanez et al., 2019). It is then possible that these chemical changes post-exposure could facilitate or prevent their translocation to the brain. An impact of the solubility on the brain translocation was also reported for other metals. As an example, solubility was shown to affect the manganese entry into the brain (Dorman et al., 2001;

Elder et al., 2006; Normandin et al., 2004). However, soluble forms of aluminium can better reach the brain than insoluble forms (Chalansonnet et al., 2018). Thus, the solubility parameter of the aerosol alone may not be sufficient to predict the ability of a particle to be transferred to the brain and that transfer assumptions should be formulated carefully if risk assessments are to be carried out. Differences found in the literature could come from a chemical property specific to each metal. In fact, several inhaled or instilled metals (eg aluminium, cadmium, cobalt, thallium, manganese, zinc) can gain olfactory bulbs while others remain trapped in the nasal cavity (eg iron and tungsten) (Henriksson et al., 1997; Kinoshita et al., 2008; Persson et al., 2003a; Persson et al., 2003b; Radcliffe et al., 2009; Rao et al., 2003; Sunderman, 2001; Tjalve et al., 1996; Vitarella et al., 2000). And, once in the brain some can migrate to more backward areas (Sunderman, 2001). Following a unilateral instillation the difference between the ipsilateral and contralateral side is 395% for manganese but only 53% for aluminum (Chalansonnet et al., 2018; Dorman et al., 2001).

Repeated exposure to uranium particles leads to a higher concentration in the brain, as a whole and in frontal regions *vs* the brain residue. These data are consistent with the hypothesis that repeated exposure to uranium leads to larger amounts in the frontal brain structures compared to acute exposure (olfactory bulbs and frontal cortex) but also in cerebral structures spared by acute inhalation (olfactory tubercles). Our results show there might be a cumulative effect of repeated exposures. Indeed, the Low group shows a concentration ratio between OB and BRes of 1.44 on average *vs* 2.67 in the High group (see Supplemental Table 2). Thus, even if the transfer from the lungs to the brain via the bloodstream contributes in the uranium concentrations within the tissues, it is more likely that these gradients, and their amplitude, are due to the direct nose to brain passage. Supporting this idea, we have previously shown that a peripheral injection of U does not give rise to a preferential accumulation in any of the structures of the brain (Tournier et al., 2009).

As with microparticles, exposure with nanometric particles induced a higher U concentration at the front of the brain. The data we observed agree with that obtained previously with the same exposure protocol (olfactory brain: 1.65 ng/g in the previous study vs 1.44 ng/g in the present study) (Petitot et al., 2013). As for the microparticles, metal-specific effects exist. For example in rodents, aluminum nanoparticles do not enter the brain (Chalansonnet et al., 2018) while iron, manganese, zinc, among others, pass through the olfactory pathway (Elder et al., 2006; Hopkins et al., 2018; Kao et al., 2012). Contradictory observations were also reported for

357 titanium dioxide nanoparticles capability to translocate to the brain after inhalation (Gate et al., 2017; Pujalte et
358 al., 2017).

359 For the reasons mentioned before, the microparticles- and nanoparticles-made aerosols differ widely and
360 cannot be directly compared. The data were therefore expressed in % of the measured concentration immediately
361 after the end of the inhalation period (t=10min). With this representation, we showed that the retention time in
362 the brain depends on the size of the particles: the nanoparticles are less easily cleared from the brain than the
363 microparticles in the three brain regions studied (OB, OT, BRes). In other words, the retention time and
364 therefore the duration of the contamination of U nanoparticles is longer than that of microparticles.

365 The kinetics of U microparticles in the brain appear to be closer to rubidium and thallium rather than that of
366 manganese, cadmium, cobalt, nickel and zinc. In fact, thallium has been shown to be rapidly transferred to the
367 OB, in less than 10 minutes (Kanayama et al., 2005). The kinetics of the rubidium concentration in OB was
368 evaluated at 3, 6, 12 and 24h in the brain after an intranasal administration and authors reported a gradually
369 decrease of the rubidium level throughout the 24h period (Kanayama et al., 2005). On the contrary, manganese,
370 cadmium and cobalt reach into the OB in around 24h (Brenneman et al., 2000; Persson et al., 2003b; Tjalve et
371 al., 1996). Regarding nickel or zinc, the metal peaks in OB were obtained only in 1 week after intranasal
372 administration (Henriksson et al., 1997; Persson et al., 2003a). Our observation of a longer retention time in the
373 case of nanoparticles is in line with previous observations. One study reported a greater retention of manganese
374 in the cortex and the same tendency in the olfactory bulb after inhalation of particles of 1 micron compared to 18
375 microns in mass median aerodynamic diameter (Fechter et al., 2002). A second study showed that inhaled
376 carbon nanoparticles remain present in the brain several days after the end of inhalation (Oberdorster et al.,
377 2004).
378

379 The concern of such direct translocation and retention of uranium particles into the brain is linked with its
380 dual chemical and radiotoxicity, as it is a transuranic element and an α emitter. In fact, cellular and cognitive
381 consequences may result from U exposure. In line with this idea, cognitive impairments were demonstrated in
382 rats following inhalation of aerosolized uranium (Monleau et al. 2005) and an inflammatory reaction was
383 observed in the spinal cord following chronic U ingestion (Saint-Marc et al., 2016). In addition, our data tend to
384 prove that physico-chemical changes of uranium particles (solubilized compounds, nanoparticles, ion forms) will
385 also need to be taken in account because they might influence subsequent neurotoxicity. Therefore, future
386 studies must be conducted to elucidate the physiological impact of inhaled U depending of its physico-chemical

form and particle size. In comparison, considering other metals, the association between particulate pollutants inhalation and increased risk of deleterious effects on brain is a rising concern. Indeed, evidences that the brain could be a direct target of inhalation exposures are piling up (Heusinkveld et al., 2016; Oberdorster et al., 2009), and also concern human as Maher et al. observed magnetite particles of exogenous origin in the human brain (Maher et al., 2016). More widely, deleterious effects of air pollution are thought to be mediated by the trigger of neuroinflammation processes (Jayaraj et al., 2017) causing subsequent neurodegenerative disorders (Costa et al., 2020).

Laboratory conditions with 56 mg/m^3 of uranium in aerosol and 30 minutes continuous exposure represent the "low" dose group compared to the other groups. This experimental dose was chosen according to the technical constraints of the vaporization of the uranium powder and the stability of the aerosol formed and is, therefore, not necessarily representative of the possible exposure values of populations with a risk of exposure. However, it remains relatively low compared to previous experimental studies (between 190 and 412 mg/m3 and between 30 and 120 min of exposition) (Monleau et al., 2006; Monleau et al., 2005; Tournier et al., 2009).

5. Conclusions

In conclusion, we have shown that the cerebral cartography of inhaled uranium has to be considered according to a panel of parameters: its solubility, the frequency of the exposures, the concentration of the aerosol and its granulometry. In all cases, a rostro-caudal gradient of uranium concentration from the olfactory bulbs to the rest of the brain is observed. In each scenario of exposure, we showed that all these parameters modulate either the concentrations, the brain regions involved or the retention time in the brain. These observations are of importance as they demonstrate that brain dose calculations for uranium inhalation cannot be done in terms of the whole brain effect but must consider the existence of a rostro-caudal gradient and the factors influencing it. This study in rats supports the hypothesis in rodents of a direct transfer of inhaled U from the nasal cavity to the brain, in addition to the supply through the bloodstream and bypassing the blood brain barrier. Further studies will be necessary to demonstrate such a pathway in humans to determine whether the human dosimetry models of ICRP for internal emitters (Leggett et al., 2018) should take this into account.

Conflict of interest

Authors have no conflict of interest to declare.

Acknowledgement

The authors wish to thank ORANO (AREVA NC) for partially supporting this work. They would also like to thank Dr. François Gensdarmes for his advice for the generation of uranium nanoparticles.

Supplemental data.

	CTRL (Low)		CTRL (High)		CTRL (Ins.)		CTRL (Rep.)	
OB	**1.06**	±0.45	**1.33**	±0.33	**1.56**	±0.47	**1.14**	±1.28
OT	**1.96**	±0.36	**2.78**	±0.76	**2.31**	±0.51	**2.21**	±1.11
FCx	**0.66**	±0.38	**0.85**	±0.37	**1.05**	±0.42	**0.65**	±0.36
BRes	**0.61**	±0.05	**0.69**	±0.53	**0.76**	±0.69	**0.71**	±0.35

Supplemental Table 1. Uranium concentrations in control groups.

Quantification of uranium in the different control groups ([U] in ng/g of tissue). No difference was observed. Data are indicated as mean ± SD (6-8 animals per group). *Abbreviations*: BRes, brain residue; FCx, frontal cortex; OB, olfactory bulbs; OT, olfactory tubercles.

	CTRL		Low [UO_4]		High [UO_4]		Insoluble [UO_2]		Repeated low [UO_4]	
WB	**0.75**	±0.10**	**2.46**	±0.55	**3.28**	±0.36	**3.00**	±0.48	**4.22**	±0.51*
OB	**1.31**	±0.74*	**2.61**	±1.45	**5.40**	±1.06***	**5.67**	±2.68***	**6.65**	±2.97***
OT	**2.34**	±0.81	**2.20**	±1.30	**4.52**	±0.78***	**5.60**	±2.38*	**5.46**	±2.50**
FCx	**0.81**	±0.40*	**2.15**	±0.87	**2.97**	±1.82	**2.65**	±1.27	**4.99**	±1.91***
BRes	**0.65**	±0.48	**1.81**	±1.59	**2.02**	±1.01	**2.00**	±1.44	**2.40**	±1.09
NT	**2.15**	±5.00*	**191**	±186	**1455**	±648***	**147**	±49.7	**362**	±89.3
Kidneys	**6.54**	±3.43*	**479**	±246	**10283**	±1911***	**69.6**	±12.44	**3730**	±766***

Supplemental Table 2. Uranium concentrations in the U microparticles treated rats.

Quantification of uranium in the different groups at 24h after exposure to microparticles of uranium ([U] in ng/g of tissue). Data are indicated as mean ± SD (8 animals per group). Difference from the Low [UO_4] group are indicated at $^{\star}p<0.05$, $^{\star\star}p<0.01$, and $^{\star\star\star}p<0.001$. *Abbreviations*: BRes, brain residue; FCx, frontal cortex; NT, nasal turbinates; OB, olfactory bulbs; OT, olfactory tubercles; WB, whole brain.

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