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## Brain accumulation of inhaled uranium in the rat depends on aerosol concentration, exposure repetitions, particle size and solubility

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1 **Brain accumulation of inhaled uranium in the rat depends on aerosol concentration, exposure repetitions,**  
2 **particle size and solubility.**

3

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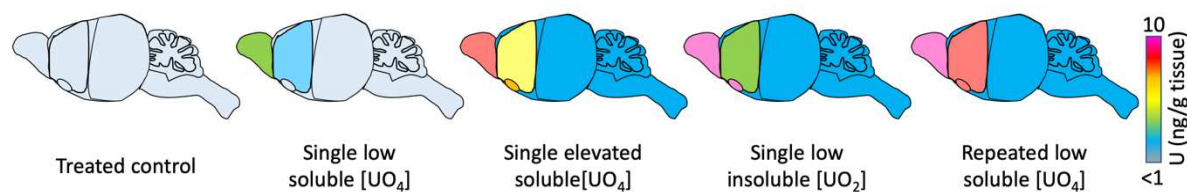
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26 **Graphical abstract.**

27



31 **Abstract.**

32 A rostro-caudal gradient of uranium (U) in the brain has been suggested after its inhalation. To study the factors  
33 influencing this mapping, we first used 30-min acute inhalation at 56 mg/m<sup>3</sup> of the relatively soluble form UO<sub>4</sub>  
34 in the rat. These exposure parameters were then used as a reference in comparison with the other experimental  
35 conditions. Other groups received acute inhalation at different concentrations, repeated low dose inhalation of  
36 UO<sub>4</sub> (10 exposures) or acute low dose inhalation of the insoluble form UO<sub>2</sub>. At 24 hours after the last exposure,  
37 all rats showed a brain U accumulation with a rostro-caudal gradient as compared to controls. However, the total  
38 concentration to the brain was greater after repeated exposure than acute exposure, demonstrating an  
39 accumulative effect. In comparison with the low dose soluble U exposure, a higher accumulation in the front of  
40 the brain was observed after exposure to higher dose, to insoluble particles and following repetition of  
41 exposures, thus demonstrating a dose effect and influences of solubility and repetition of exposures. In the last  
42 part, exposure to ultrafine U particles made it possible to show 24 hours after exposure the presence of U in the  
43 brain according to a rostro-caudal gradient. Finally, the time-course after exposure to micronic or nanometric U  
44 particles has revealed greater residence times for nanoparticles.

45

46

47 **Keywords.**

48 Uranium, inhalation, nanoparticles, solubility, rat

49

50 **Abbreviations.**

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

53 **1. Introduction.**

54 Uranium (U) is a natural radioactive transuranic element with a wide range of industrial uses, but its main  
55 application concerns energy production and military use (Craft et al., 2004). The natural fissile isotope uranium-  
56 <sup>235</sup>U is used primarily in most nuclear power plants. The preparation of uranium to be used as fuel in nuclear  
57 power plants includes different successive steps (mining, milling, conversion, enrichment, fabrication of nuclear  
58 fuel and reprocessing in some cases). Importantly, depending on the steps in the uranium fuel cycle, the chemical  
59 nature of U compounds, notably solubility, varies. Ammonium diuranate (NH<sub>4</sub>)<sub>2</sub>U<sub>2</sub>O<sub>7</sub>, a component of  
60 yellowcake, is converted to uranium hexafluoride (UF<sub>6</sub>) prior to <sup>235</sup>U enrichment. The enriched UF<sub>6</sub> is then  
61 converted into UO<sub>2</sub>, the final product in the manufacture of nuclear fuel pellets used in most reactors  
62 (UNSCEAR, 2016). In nuclear fuel cycle facilities, workers involved in the concentration or enrichment process  
63 and in fuel fabrication could face repeated inhalations of airborne U compounds (Fulco et al., 2000). Indeed, dust  
64 inhalation is one of the major risk of contamination for nuclear workers (Blanchin et al., 2004; Durakovic et al.,  
65 2003; Fulco et al., 2000; Salbu et al., 2005). Furthermore, in addition to micrometric U dusts, metal ultrafine or  
66 nanometric (<100 nm) U particles can be also generated as byproducts in several industrial steps of the nuclear  
67 fuel cycle or during the decommissioning of nuclear facilities (Dewalle et al., 2010; Onodera et al., 1991;  
68 Trelenberg et al., 2006).

69 After inhalation, the first organs impacted by the uranium deposition are the lungs as described in the Human  
70 Respiratory Tract Model (HRTM biokinetic model) (ICRP, 1994). Accordingly to their pulmonary transfer rate,  
71 uranium compounds can be classified according to their solubility as fast (F), moderate (M) and slow (S) (ICRP,  
72 2017). As an example, the solubility of UO<sub>4</sub> is 50% type F and 50% type M. The ICRP model aims at predicting  
73 translocation and deposition of uranium according to particle size parameters and then subsequent retention  
74 following its deposition depending on the different anatomical pulmonary regions (bronchial and alveolar  
75 compartments). U distribution is then predicted by the systemic biokinetic model to the whole organism *via* the  
76 blood pathway. After its absorption to blood, uranium is present mainly as uranyl ions complexed to proteins  
77 (e.g. transferrin, albumin) or bicarbonate anions (Ansoborlo et al., 2006). The main sites of deposition of  
78 uranium from the circulation are the skeleton and the kidneys. In addition, experimental data predict that part of  
79 the metal enters the brain (Ballou et al., 1986; Diamond et al., 1989; La Touche et al., 1987; Lemerrier et al.,  
80 2003). Most recently, several publications suggested that uranium deposition in brain is higher after inhalation  
81 than after ingestion or injection, suggesting that the cerebral accumulation of U depends on the mode of  
82 contamination (for review, see (Dinocourt et al., 2015)). For instance, 24 hours after the end of repeated

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

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

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

108 **2. Material and methods.**

109

110 *2.1. Animals*

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

120

<b>Group name</b>	<b>Low</b>	<b>High</b>	<b>Ins.</b>	<b>Rep.</b>	<b>UFP</b>
	<b>Micrometric aerosol</b>				<b>Nanometric aerosol</b>
Chemical form	UO <sub>4</sub>	UO <sub>4</sub>	UO <sub>2</sub>	UO <sub>4</sub>	UO <sub>2</sub>
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 <sup>3</sup> )	56 ±28	<u>412 ±82</u>	52 ±14	61 ±17	<u>1 ±0</u>
Duration of the exposure (min)	30	30	30	30	<u>60</u>
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	<sup>238</sup> U = 99.55% <sup>236</sup> U = 0.051% <sup>235</sup> U = 0.39% <sup>234</sup> U = 0.0047%	<sup>238</sup> U = 99.55% <sup>236</sup> U = 0.051% <sup>235</sup> U = 0.39% <sup>234</sup> U = 0.0047%	<sup>238</sup> U = 99.75% <sup>235</sup> U = 0.24% <sup>234</sup> U = 0.001%	<sup>238</sup> U = 99.55% <sup>236</sup> U = 0.051% <sup>235</sup> U = 0.39% <sup>234</sup> U = 0.0047%	<sup>238</sup> U = 99.799% <sup>235</sup> U = 0.200% <sup>234</sup> U = 0.001%

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

125

126

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<sub>2</sub>) and U peroxide (UO<sub>4</sub>) powders found at workplaces in U fuel  
130   cycle facilities were supplied by ORANO-AREVA NC (Pierrelatte, France). Briefly, micronic UO<sub>4</sub> and UO<sub>2</sub>  
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<sub>4</sub> powder was <sup>238</sup>U=  
133   99.55%; <sup>236</sup>U= 0.051%; <sup>235</sup>U= 0.39%; <sup>234</sup>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

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

160

#### 161 2.4. *Sample collection*

162 Deeply isoflurane anaesthetized (inhalation of isoflurane 5% / air 95%) rats were euthanized and their brain  
163 quickly removed. The olfactory bulbs, olfactory tubercles, frontal cortex and the rest of the brain (indicated in  
164 the rest of the document as “brain residue”) were dissected out on ice, weighed and stored at  $-80^{\circ}\text{C}$  until  
165 required. Peripheral organs (nasal turbinates and kidneys) were also dissected on ice, weighed and stored at  
166  $-80^{\circ}\text{C}$ .

167

#### 168 2.5. *Uranium measurements using an inductively coupled plasma mass spectrometer*

169 Uranium concentration analyses were performed as previously reported (Ibanez et al., 2019). Selected brain  
170 areas and peripheral organs were digested using 8 mL nitric acid  $\text{HNO}_3$  (Ultrapur, JT Becker) and 2 mL of 30%  
171 hydrogen peroxide (Normapur, VWR) in a microwave oven (Milestone StartD microwave digestion system 1000  
172 W, Ethos TC; Milestone, Italy) as follows: 20 min to reach  $200^{\circ}\text{C}$  and then held at  $200^{\circ}\text{C}$  for 10min in the  
173 microwave oven at 1000 W using the digestion rotor system. Samples were then evaporated and dissolved in 5  
174 ml of 10%  $\text{HNO}_3$ . Samples were diluted and elemental uranium concentration was measured using an  
175 inductively coupled plasma mass spectrometer (ICP-MS, PQ EXCELL, ThermoElectron with S-Option) using  
176 bismuth as the internal standard (Claritas PPT Grade). The detection limit of uranium measured was: 1 ng/L for  
177  $^{238}\text{U}$  and  $^{235}\text{U}$ .

178

#### 179 2.6. *Statistics*

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



184 **3. Results**

185

186 **3.1. Unexposed animals**

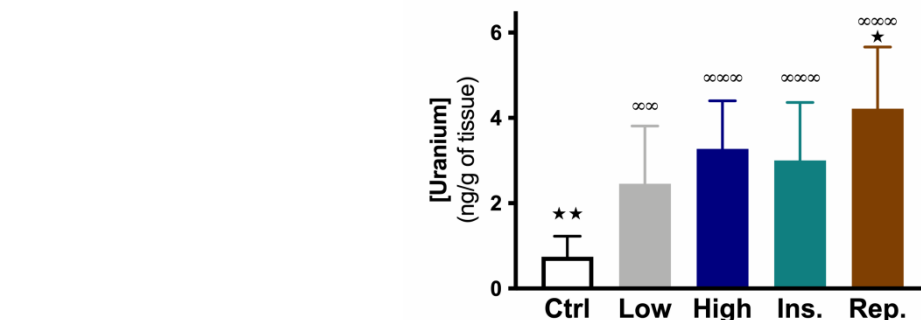
187

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

194

195 **3.2. Uranium concentration in the whole brain**

196 The experimental design showing aerosols characteristics and exposure modes are indicated in the Table 1:  
197 animals received acute exposure to a low concentration of  $UO_4$  (Low group), acute exposure to an elevated  
198 concentration of  $UO_4$  (High group), acute exposure to a low concentration of the insoluble  $UO_2$  (Ins. group) or  
199 repeated exposure to a low concentration of  $UO_4$  (Rep. group). As compared to control animals, all groups  
200 exposed to microparticles of uranium showed increased brain U concentrations (Low: +230%,  $p < 0.01$ ; High:  
201 +340%,  $p < 0.001$ ; Ins.: +300%,  $p < 0.001$ , Rep.: +465%,  $p < 0.001$ ) (Figure 1). Concerning differences between  
202 uranium treated groups, the group exposed repeatedly to  $UO_4$  showed higher U levels in comparison with the  
203 acute exposure to a low concentration of  $UO_4$  (+72%,  $p < 0.05$ ). Raw data are given in the Supplemental Table 2.



204

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

210 28 animals). Significant differences from the Low group are indicated at \* $p < 0.05$  and \*\* $p < 0.01$  and from the  
211 control group at  $^{\infty}p < 0.01$  and  $^{\infty\infty}p < 0.001$ , using one-way ANOVA (treatment as factor) with the LSD *post hoc*  
212 test.

213

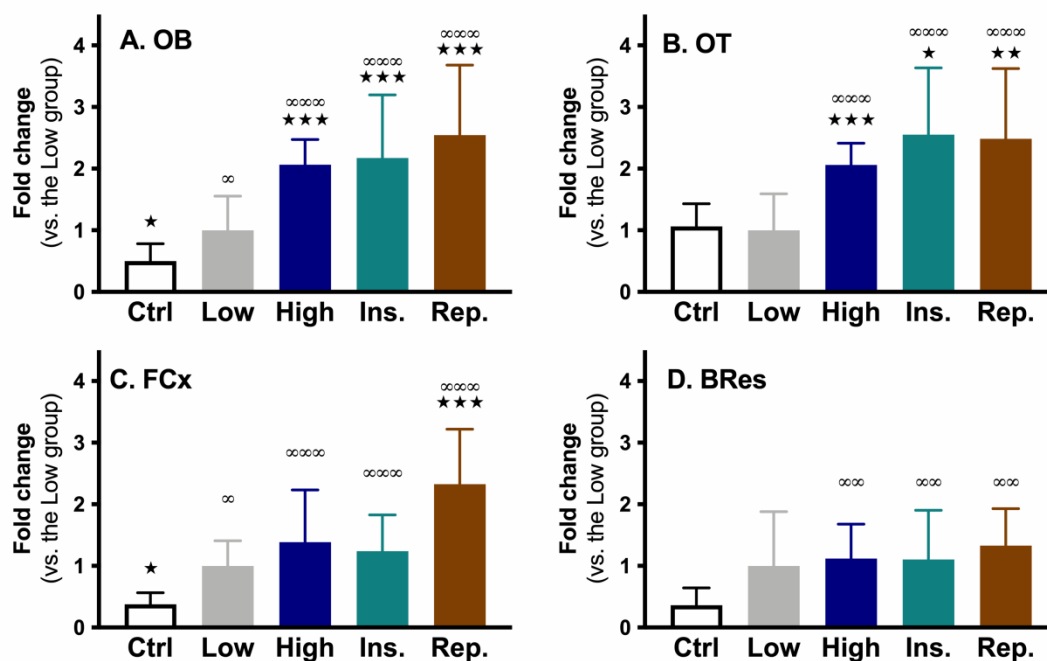
### 214 **3.3. Uranium concentration depends on the exposure mode.**

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

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

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

230



231

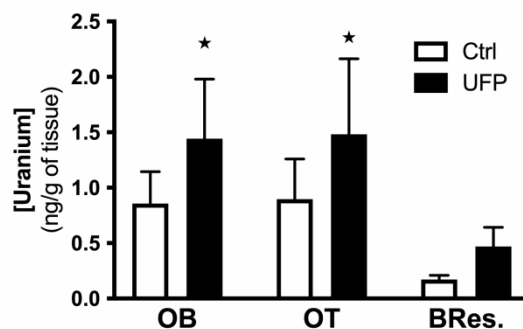
232 **Figure 2. Uranium concentrations in rat cerebral areas at 24h after inhalation.** Uranium concentrations  
 233 were measured in olfactory bulbs (OB, **A**), olfactory tubercles (OT, **B**), frontal lobe of the cortex (FCx, **C**) and in  
 234 brain residue (BRes, **D**). Animals were exposed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

242

#### 243 3.4. Cerebral distribution of the inhaled ultrafine particles of uranium at 24h after exposure.

244 A group was exposed to nanometric uranium UO<sub>2</sub> aerosol. The characteristics of this aerosol and of its exposure  
 245 mode are given in the Table 1. The Figure 3 shows the significant higher accumulation of U in OB (+68%,  
 246  $p<0.05$ ) and OT (+65%,  $p<0.05$ ) which is absent in the brain residue 24 hours after inhalation of UFP as  
 247 compared with the 1-hour ambient air group.

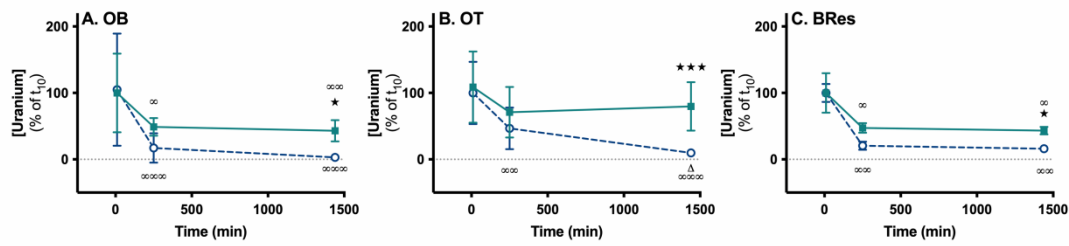
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249  
 250 **Figure 3. Uranium concentrations at 24h after inhalation of Uranium nanoparticles.** Animals were exposed  
 251 to ambient air (open bars) or to ultra-fine particles of uranium (UFP, black bars). Data are given as mean  $\pm$  SD of  
 252 8 animals. Significant differences from control are indicated at \* $p < 0.05$ . *Abbreviations:* BRes, brain residue;  
 253 OB, olfactory bulbs; OT, olfactory tubercles.

### 255 3.5. Kinetics of U concentrations in the brain, following acute exposure to micronic or nanometric 256 particles of uranium.

257 Data showing uranium concentrations in OB, OT, and BRes at 10 min, 250 min, and 24 h after the end of the  
 258 exposure to microparticles ( $UO_4$ ) or to nanoparticles (U metal) are shown in Figure 4. In OB, as compared to the  
 259 respective timepoint  $t_{10min}$ , U levels are significantly lower at  $t_{250min}$  and  $t_{24h}$  for both micro- and nanoparticles  
 260 group ( $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ ). However, the U concentration decrease in OB for micronic particles is  
 261 greater compared to nanoparticles, resulting in a significant difference between the two types of particles at  $t_{24h}$   
 262 ( $p < 0.05$ ). At 24h post-exposure, U nanoparticle concentration is higher compared to microparticle one. In OT,  
 263 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  
 264 marked contrast, the concentration of U nanoparticles in OT remains stable throughout the measurement period,  
 265 and result in a significant difference ( $p < 0.001$ ) between the two types of particles at  $t_{24h}$  with significant higher U  
 266 concentration for the nanoparticle group. In the BRes, kinetics were identical to those observed in the OB: a  
 267 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  
 268 significant difference between the two types of particles is also observed at  $t_{24h}$  with higher U nanoparticles  
 269 concentration as compared to microparticles ( $p < 0.05$ ).



271

272 **Figure 4. Effect of particle size on brain uranium distribution as a function of time.** Animals were exposed  
 273 to  $\text{UO}_4$  microparticles (white circles, blue dashed line) or to U metal nanoparticles (green squares, solid line) of  
 274 uranium. Uranium concentrations were measured in olfactory bulbs (A), olfactory tubercles (B) and brain  
 275 residue (C). Data are given as mean  $\pm$  SD of 8 animals. Significant difference from the respective timepoint  
 276  $t=10\text{min}$  group is indicated as  $\infty p < 0.05$ ,  $\infty\infty p < 0.01$  and  $\infty\infty\infty p < 0.001$ ; significant difference from the respective  
 277 timepoint  $t=250\text{min}$  group is indicated as  $\Delta p < 0.05$ ; significant difference in between micro and nanoparticles  
 278 group is indicated as  $* p < 0.05$  and  $*** p < 0.001$  using two-way ANOVA (treatment x time) with the LSD  
 279 *post hoc* test.

280

#### 281 4. Discussion

282 Our study demonstrated that whatever the intrinsic characteristics of the U aerosol or its exposure, inhalation  
 283 resulted in the presence of an important rostro-caudal gradient in the brain with the olfactory bulbs and the rest  
 284 of the brain containing the highest and the lowest U concentration, respectively, confirming previous  
 285 experiments (Houpert et al., 2007; Monleau et al., 2005; Tournier et al., 2009). However, the present  
 286 investigation demonstrated that U distribution in the brain depends on several parameters: U aerosol  
 287 concentration, U physico-chemical form such as its solubility, U particle size and the exposure number. Indeed,  
 288 several factors affect the U concentrations as measured in tissues located in the frontal regions of the brain,  
 289 closest to the nasal cavity on a neuroanatomical point of view (olfactory bulbs and tubercles). Data clearly  
 290 demonstrated that the U concentration in the front of the brain is increased when animals are exposed to  $\text{UO}_2$ , to  
 291 a high concentration of  $\text{UO}_4$  or to multiple exposures to  $\text{UO}_4$  as compared to an acute  $\text{UO}_4$  exposure. In addition,  
 292 the kinetics of the U in the brain is modified by use of nanoparticles instead of microparticles. As a whole, our  
 293 study shows that the ability of uranium to enter the brain depends on the conditions of inhalation in terms of  
 294 aerosol concentration, the nature of the uranium and the frequency of exposure.

295 When U is inhaled, a fraction is found in the nasal cavity where a direct passage to the brain has been  
 296 proposed, in addition to the transfer from the lungs via the bloodstream (Ibanez et al., 2014; Monleau et al.,  
 297 2006; Tournier et al., 2009). The nasal cavity thus represents a potential route of entry of uranium to the brain.

298 We reported that only the High [UO<sub>4</sub>] group shows an increase in the U concentration in the nasal turbinates  
299 compared to the Low [UO<sub>4</sub>] group, thus showing a dose effect of the aerosol concentration. Conversely, the  
300 absence of a difference with the Low [UO<sub>2</sub>] and Repeated low [UO<sub>4</sub>] groups tends to show that the inhaled  
301 quantity per exposure are comparable, which is in line with the concentrations measured in the generated  
302 aerosols. It also demonstrates that there is no cumulative effect, from one day to the other, of the repeated  
303 exposure at least in the nasal turbinates suggesting that nasal cavity probably flush away the aerosol within the  
304 first 24 hours.

305

306 In the brain, we demonstrated that olfactory bulbs and tubercles are affected differently, unlike the rest of the  
307 brain, probably because a lower U quantity reaching these more posterior brain regions (frontal cortex and brain  
308 residue). These observations are in line with previous data (Ibanez et al., 2019; Monleau et al., 2005; Tournier et  
309 al., 2009). In addition, the rostro-caudal gradient of the brain U distribution is modified by the repetition of  
310 exposures. Indeed, in the Repeated low [UO<sub>4</sub>] group, U levels were also found to be increased in the frontal  
311 cortex but not in the rest of the brain. This observation is of particular interest since no cumulative effect was  
312 observed in the nasal turbinates of the repeated group whereas U appears to cumulate in the olfactory bulbs and  
313 even finally reach the frontal cortex which is not the case for an acute exposure. However, although group  
314 differences are significant in brain areas, it is important to note that at the whole brain level, no group difference  
315 appeared. These observations are also important in the sense that dose calculations to the brain in response to  
316 exposure to a uranium aerosol do not consider the heterogeneity of the brain in terms of uranium accumulation.  
317 Thus, even if the doses received globally by the brain are identical, some structures, located at the front of the  
318 brain are more strongly exposed in case of inhalation. Therefore, brain toxicity calculations of U after inhalation  
319 considering the brain as a homogeneous tissue may underestimate the real impact at more targeted brain areas.

320

321 The UO<sub>2</sub> (M/S) aerosol lead to a higher brain U accumulation in the olfactory bulbs and tubercles in  
322 comparison with the UO<sub>4</sub> (F/M) aerosol, even if, at the whole brain level, no significant difference was observed.  
323 These observations suggest that the olfactory pathway is taken by U particles, whether in soluble or insoluble  
324 form. Physicochemical U forms in the tissue could differ from the original aerosol form once inhaled (Ibanez et  
325 al., 2019). It is then possible that these chemical changes post-exposure could facilitate or prevent their  
326 translocation to the brain. An impact of the solubility on the brain translocation was also reported for other  
327 metals. As an example, solubility was shown to affect the manganese entry into the brain (Dorman et al., 2001;

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

339  
340 Repeated exposure to uranium particles leads to a higher concentration in the brain, as a whole and in frontal  
341 regions vs the brain residue. These data are consistent with the hypothesis that repeated exposure to uranium  
342 leads to larger amounts in the frontal brain structures compared to acute exposure (olfactory bulbs and frontal  
343 cortex) but also in cerebral structures spared by acute inhalation (olfactory tubercles). Our results show there  
344 might be a cumulative effect of repeated exposures. Indeed, the Low group shows a concentration ratio between  
345 OB and BRes of 1.44 on average vs 2.67 in the High group (see Supplemental Table 2). Thus, even if the  
346 transfer from the lungs to the brain via the bloodstream contributes in the uranium concentrations within the  
347 tissues, it is more likely that these gradients, and their amplitude, are due to the direct nose to brain passage.  
348 Supporting this idea, we have previously shown that a peripheral injection of U does not give rise to a  
349 preferential accumulation in any of the structures of the brain (Tournier et al., 2009).

350  
351 As with microparticles, exposure with nanometric particles induced a higher U concentration at the front of  
352 the brain. The data we observed agree with that obtained previously with the same exposure protocol (olfactory  
353 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  
354 microparticles, metal-specific effects exist. For example in rodents, aluminum nanoparticles do not enter the  
355 brain (Chalansonnet et al., 2018) while iron, manganese, zinc, among others, pass through the olfactory pathway  
356 (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  $\alpha$  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



387 form and particle size. In comparison, considering other metals, the association between particulate pollutants  
388 inhalation and increased risk of deleterious effects on brain is a rising concern. Indeed, evidences that the brain  
389 could be a direct target of inhalation exposures are piling up (Heusinkveld et al., 2016; Oberdorster et al., 2009),  
390 and also concern human as Maher et al. observed magnetite particles of exogenous origin in the human brain  
391 (Maher et al., 2016). More widely, deleterious effects of air pollution are thought to be mediated by the trigger of  
392 neuroinflammation processes (Jayaraj et al., 2017) causing subsequent neurodegenerative disorders (Costa et al.,  
393 2020).

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

400

## 401 **5. Conclusions**

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

413

## 414 **Conflict of interest**

415 Authors have no conflict of interest to declare.

416

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420 **Supplemental data.**

421

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

422

423 **Supplemental Table 1. Uranium concentrations in control groups.**

424 Quantification of uranium in the different control groups ([U] in ng/g of tissue). No difference was observed.

425 Data are indicated as mean  $\pm$  SD (6-8 animals per group). *Abbreviations:* BRes, brain residue; FCx, frontal  
426 cortex; OB, olfactory bulbs; OT, olfactory tubercles.

427

428

429

	<b>CTRL</b>		<b>Low [UO<sub>4</sub>]</b>		<b>High [UO<sub>4</sub>]</b>		<b>Insoluble [UO<sub>2</sub>]</b>		<b>Repeated low [UO<sub>4</sub>]</b>	
<b>WB</b>	<b>0.75</b>	$\pm 0.10^{**}$	<b>2.46</b>	$\pm 0.55$	<b>3.28</b>	$\pm 0.36$	<b>3.00</b>	$\pm 0.48$	<b>4.22</b>	$\pm 0.51^*$
<b>OB</b>	<b>1.31</b>	$\pm 0.74^*$	<b>2.61</b>	$\pm 1.45$	<b>5.40</b>	$\pm 1.06^{***}$	<b>5.67</b>	$\pm 2.68^{***}$	<b>6.65</b>	$\pm 2.97^{***}$
<b>OT</b>	<b>2.34</b>	$\pm 0.81$	<b>2.20</b>	$\pm 1.30$	<b>4.52</b>	$\pm 0.78^{***}$	<b>5.60</b>	$\pm 2.38^*$	<b>5.46</b>	$\pm 2.50^{**}$
<b>FCx</b>	<b>0.81</b>	$\pm 0.40^*$	<b>2.15</b>	$\pm 0.87$	<b>2.97</b>	$\pm 1.82$	<b>2.65</b>	$\pm 1.27$	<b>4.99</b>	$\pm 1.91^{***}$
<b>BRes</b>	<b>0.65</b>	$\pm 0.48$	<b>1.81</b>	$\pm 1.59$	<b>2.02</b>	$\pm 1.01$	<b>2.00</b>	$\pm 1.44$	<b>2.40</b>	$\pm 1.09$
<b>NT</b>	<b>2.15</b>	$\pm 5.00^*$	<b>191</b>	$\pm 186$	<b>1455</b>	$\pm 648^{***}$	<b>147</b>	$\pm 49.7$	<b>362</b>	$\pm 89.3$
<b>Kidneys</b>	<b>6.54</b>	$\pm 3.43^*$	<b>479</b>	$\pm 246$	<b>10283</b>	$\pm 1911^{***}$	<b>69.6</b>	$\pm 12.44$	<b>3730</b>	$\pm 766^{***}$

430

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

432 Quantification of uranium in the different groups at 24h after exposure to microparticles of uranium ([U] in ng/g  
433 of tissue). Data are indicated as mean  $\pm$  SD (8 animals per group). Difference from the Low [UO<sub>4</sub>] group are  
434 indicated at \*p<0.05, \*\*p<0.01, and \*\*\*p<0.001. *Abbreviations:* BRes, brain residue; FCx, frontal cortex; NT,  
435 nasal turbinates; OB, olfactory bulbs; OT, olfactory tubercles; WB, whole brain.

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