Uranium behaviour in the baboon after the deposition of a ceramic form of uranium dioxide and uranium octoxide in the lungs : implications for human exposure

H. METIVIER*, J.L. PONCY**, G. RATEAU**, G.N. STRADLING***,
J.C. MOODY***, S.A. GRAY***

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RÉSUMÉ

Des quantités allant de 1 à 20 fois les LAI pour l'uranium (sous les formes chimiques UO_2 et U_3O_8) ont été inhalées par des babouins suivis pendant 1 an par anthroporadiométrie puis sacrifiés pour analyse des différents organes où l'uranium peut migrer en quantité significative. Les résultats ont montré qu'un an après : 1) la rétention d'uranium dans le poumon est plus forte lorsqu'il est inhalé sous forme d' UO_2 que sous forme d' U_3O_8 ; 2) le rapport "concentration dans les ganglions/concentration dans les poumons" est plus élevé lorsque l'uranium est inhalé sous forme d' UO_2 ; 3) pour les deux formes, le transfert vers les ganglions n'est pas influencé par la massé inhalée ; 4) les concentrations relatives d'uranium, quelles que soient les formes inhalées évoluent dans l'ordre : ganglions thoraciques > poumons >> crâne > fémur-clavicule > vertèbres lombaires – cage thoracique > sternum > reins > foie ; 5) la mesure de certains enzymes présents dans les urines peut être utile pour surveiller les surexpositions d' U_3O_8 , mais, à ce jour et dans la gamme de doses étudiée, l'anthroporadiométrie et l'excrétion urinaire restent des procédés de choix.

ABSTRACT

UO_2 and U_3O_8 were inhaled by baboons in amounts such that the mass concentrations in the lungs were between about 3 and 20, and 1 and 7.4 times respectively those calculated in human lungs after exposure to 1 ALI of the compounds. The results show that 1 year later : 1) the initial lung deposits of uranium retained in the chest are greater for UO_2 than for U_3O_8 ; 2) the ratios of the amounts of uranium retained in the lymph nodes vs those in the lungs, expressed both in terms of concentration and mass, are greater for UO_2 than for U_3O_8 ; 3) for both UO_2 and U_3O_8 , the amounts of uranium retained in the lymph nodes, expressed as a percentage of that in the chest, do not appear to be affected to a great extent by the initial lung deposits ; 4) after inhalation of UO_2 and U_3O_8 in different amounts, the trend in the mass concentrations of uranium in the various tissues assayed were lymph nodes > lungs >> skull > femora-clavicles > lumbar vertebra – thoracic cage > sternum > kidneys > liver ; 5) the measurement of urine concentrations of some enzymes may be a useful index for assessing substantial overexposures to UO_2 and U_3O_8 , however, so far, external chest monitoring and urinary assays of uranium should remain the procedures of choice.

* Commissariat à l'énergie atomique, Institut de protection et de sûreté nucléaire, BP 6, 92265 Fontenay-aux-Roses Cedex, France.

** Commissariat à l'énergie atomique, Direction des sciences du vivant, BP 12, 91680 Bruyères-le-Chatel, France.

*** National radiological protection board, Chilton, Didcot, Oxon, OX11 0RQ, UK.

1. Introduction

The most likely route of intake for workers exposed to UO_2 and U_3O_8 , which are important intermediates in the fabrication of nuclear fuels, is by inhalation. One method for assessing intakes of these compounds is by external monitoring of the chest. The interpretation of such measurements will be affected by the intake pattern, the amounts inhaled and individual biological variations. However, other factors such as the amounts of uranium assumed to have accumulated in the tracheobronchial lymph nodes and the possible influence of uranium deposited in the rib cage and skull after its translocation to the blood, will also be important.

At human autopsy, it is a common practice to estimate the skeletal content of uranium by extrapolation from that present in selected bones such as the femur. However, since the concentration of uranium in the skeleton is unlikely to be uniform, information on the variations that can occur between the bones is of practical value when using this method of assessment.

The evidence available suggests that the overall biokinetic behaviour of uranium in humans can be predicted reasonably accurately by combining data obtained on the translocation of uranium to blood in the rat with mechanical clearance data obtained from humans exposed to clay particles labelled with ^{85}Sr or ^{88}Y (Stradling *et al.*) [23-25]. However, in order to assess uranium distribution between the lung and thoracic lymph nodes, and to investigate uranium concentration in various bones of the skeleton, a large primate such as the baboon is the most appropriate animal species. In our experiments, the factors have been investigated after the administration of UO_2 and U_3O_8 to male and female baboons in amounts corresponding to acute human exposure of between about 3 and 20 times the annual limit on intake (ALI) for class Y uranium compounds as recommended by the International commission on radiological protection (ICRP) [15]. In addition, the study has included an investigation of urinary metabolites which could be used as biochemical indicators of uranium exposure.

2. Materials and methods

2.1 Physico-chemical characteristics of uranium oxides

Enriched ceramic grade UO_2 (2.2 % ^{235}U), and U_3O_8 containing natural uranium (0.71 % ^{235}U), were obtained from nuclear fuel process lines. The oxide samples were first ground as a slurry in ethanol using a micronising mill with agate elements (McCrone research associates, London) to provide particles less than about $20\mu\text{m}$ in diameter. For the animal experiments, the respirable fractions of the dusts were separated from the milled samples by sedimentation in absolute ethanol. The particle size of

the respirable fraction was determined at NRPB using an aerodynamic particle sizer (Model APS 33, TSI, Inc., St. Paul, MN, USA). The count median diameter (CMD) and mass median diameter (MMD) for UO_2 particles were respectively $1.48 \mu\text{m}$ (geometric standard deviation, σ_g 1.37) and $2.19 \mu\text{m}$ (σ_g 1.32); the CMD and MMD for U_3O_8 particles were $0.82 \mu\text{m}$ (σ_g 1.24) and $1.29 \mu\text{m}$ (σ_g 1.46).

2.2 Animals

Adult male and female baboons (*Papio papio*), weighing between 7.5 and 12 kg at the time of exposure were used. The health of the animals was confirmed by X-ray examinations of the lungs and biochemical analysis of the blood and urine. Dried food (U.A.R., Villemoisson, France) supplemented with fresh fruits and water were freely available throughout the experiment.

2.3 Exposure of animals to uranium oxides

Eight baboons were used for the study. Groups of two animal (one male and one female) were exposed by inhalation to either UO_2 or U_3O_8 in amounts that differed by about 1 order of magnitude.

Prior to inhalation of the oxides, the baboons were tranquilized by intramuscular injection of Valium (Roche, France; 0.5 mg kg^{-1} body wt) and anaesthetised by an intramuscular injection of Imalgene (Rhône Mérieux, France; 10 mg kg^{-1} body wt). To facilitate administration of the aerosol, and maximise deposition in the pulmonary region of the lungs, a polythene tube (inner diameter 7.5 mm) was first inserted into the trachea to the first bifurcation. To avoid external contamination during exposure, the anaesthetised animal was encapsulated in a polythene bag whilst breathing continued spontaneously via the tracheal tube.

The aerosol was generated from suspension of the oxides in isotonic saline (2 g in 25 ml or 0.3 g in 15 ml at the higher and lower dose levels respectively) using a compressed air device (Acorn nebulizer, Medic-Aid, Pagham, UK). The animals were exposed for 2 h or 1.5 h respectively in a facility described in details by André *et al.* [4].

2.4 Uranium assessment in the chest

Uranium amounts in the chest were assessed from gamma spectrometric measurements of the 17, 62, 92 and 185 keV emissions from uranium. The measurements were made immediately after exposure to assess the initial lung content, and at 2, 7, 14, 30, 62, 84, 140, 173, 210, 281 and 365 d to evaluate the retention kinetics in the chest. The detection system included two sodium iodide crystals (type 121 SM 51, Scintiflex, Q and S, Ltd) each protected by a $93 \mu\text{m}$ thick beryllium window. The detectors, 130 mm apart, were located each side of the chest wall of the baboon whilst it was restrained in the sitting position. The

counter was calibrated using a phantom in which known amounts of the oxides were embedded in tissue equivalent plastic. The data obtained for the chest uranium content was corrected by subtracting the amounts measured before exposure. The limits of detection of the counting system were 280 $\mu\text{g UO}_2$ and 650 $\mu\text{g U}_3\text{O}_8$.

2.5 Analysis of body tissues

After killing the anaesthetised animals by exsanguination, the following tissues and bones of the skeleton were removed for assay of their uranium content : lungs, trachea *minus* connective tissue, thoracic lymph nodes, liver, kidneys, skull cap, sternum, clavicle, right and left sides of the thoracic cage, lumbar vertebra (5th), right and left femurs. After dissection, the samples were reduced in bulk by successive heat treatments at 500°C in a muffle furnace and digestion of the residue with 8M HNO_3 . The residues were subsequently dissolved in 4M HNO_3 and analysed for their uranium content by delayed neutron counting. The procedure used was similar to that described in detail by Amiel, Donoghue *et al.*, Ide *et al.* [1, 9, 13]. The limit of detection (95 % confidence limits) using a statistical method [26] and based on a background count of 25 cpm and a calibration figure of 550 cpm μg^{-1} natural uranium, was 0.04 $\mu\text{g U-nat}$ or 0.013 μg for U containing 2.2 % ^{235}U .

2.6 Enzymatic analysis of urine

The concentrations of some enzymes in urine can be used as indicators of the nephrotoxicity of uranium (Diamond, Leggett) [8, 19]. In these experiments, the urine was tested for the presence of lactate dehydrogenase (LDH) with the reagent Biotrol SFBC (Biotrol Pharma, France) and N-acetyl- β -glucosaminidase (NAG) colorimetrically with Böehringer solution 875406 (Böehringer Mannheim, France). In the same urine samples, we also measured glutamic-oxaloacetic transaminase (SGOT) with Biotrol A03027, glutamic-pyruvic transaminase (SGPT) with Biotrol A03020, glutamyl gamma transferase (CGT) with Biotrol A03010 for the purpose of a complete health check of the baboons.

3. Results

3.1 Initial lung deposits of uranium

The initial lung deposits of uranium, as UO_2 , estimated for animals 607, 608, 609 and 616, were respectively 17.5, 18.2, 2.66 and 3.28 mg. The weights of the lungs, calculated from the equation "lung weight (g) = 6.11 x animal weight (kg) + 6.73" [20] were respectively 62.7, 58.3, 53.0 and 66.0 g. Hence, the amounts of uranium deposited in the lungs corresponded to about 19, 21, 3.3 and 3.3 times the mass concentrations calculated to be in the pulmonary region of human lungs after acute exposure to the ALI for class Y compounds of natural uranium

(1.5×10^3 Bq, 60 mg U) [15]. This calculation assumes an AMAD of $1 \mu\text{m}$, a pulmonary deposit of 25 % of the amount inhaled [15] and a mass of the human lungs of 1000 g [14]. The initial lung deposits of uranium, as U_3O_8 , for animals 611 and 617 were estimated to be respectively 5.30 and 8.05 mg ; the values for animals 615 and 623 were estimated to be about 1 mg. The respective weights of the lungs were 79.4, 72.1, 69.6 and 58.6 g. Using the same method of calculation as above, the mass concentrations in the lungs were equivalent to respectively 4.5, 7.4, ~ 0.96 and ~ 1.1 times those predicted after the inhalation of 1 ALI of a class Y uranium compound. The relationships of the mass concentrations of uranium in the lungs vs the ALI after inhalation of an aerosol of AMAD $5 \mu\text{m}$ would be similar to the values above, since the lower fractional deposition in the pulmonary region, about 10 %, would be largely offset by the greater value of the ALI under these conditions [15, 23].

3.2 Uranium retention in the chest

The amounts of uranium retained in the chest, expressed as a percentage of the initial lung deposits are shown in table I, and figure 1 for UO_2 and figure 2 for U_3O_8 . Attempts to express the retention data in terms of a two component exponential function using a weighted least-squares method [12] proved unsuccessful. This was due to the variability of the results in the early lung clearance phase and the difficulty in measuring uranium in the chest at later times. The data suggest that after the inhalation of UO_2 at the high dose level, the long term retention half-times in the chest are about 350-400 d. At the high dose levels, the data show that U_3O_8 is more transportable than UO_2 . The plateaus observed in the chest retention data (tab. I, fig. 2) are most unlikely to reflect impairment of lung clearance. The data probably reflects errors in measurement of uranium in the chest, or movement of the uranium from the lungs to a transformed compartment or other body tissues. The results indicate that, for each exposure group, uranium is retained most avidly in the chest of the male baboons. However, since each group only contained one animal of either sex, it would be premature to draw any firm conclusions about the effect of sex on the lung retention kinetics of uranium. The difference observed here is probably due to hazard since this effect could not be confirmed when compiling all the results obtained with other particles such as beryllium metal, cobalt oxide or UF_4 [2-3, 5].

The apparent chest retention kinetics in baboons exposed to the lower amounts of UO_2 and UO_3 did not allow any conclusions to be drawn about the relative transportability of uranium. The values for UO_2 are given in table I, those for U_3O_8 are excluded since no significant decrease could be identified after the administration of about 1 mg.

3.3 Tissue distribution of uranium in body tissues

The amounts of uranium present in pre-selected body tissues and bones of the baboons after exposure to UO_2 and U_3O_8 are given

respectively in tables III and IV, and tables V and VI ; the values have been corrected for the uranium present in the tissues of an unexposed animal (tab. II). The results are expressed in terms of the total amount of uranium present in the tissue (μg), the relative concentration in that tissue ($\mu\text{g kg}^{-1}$ wet wt) and the percentage of the initial lung deposit in that tissue (% ILD). In each experimental group, similar amounts of uranium were administered to 1 female and 1 male baboon.

Fig. 1 – Retention of uranium in the chest after inhalation of UO_2 (10 mg).
Rétention thoracique après inhalation d' UO_2 (10 mg).

Fig. 2 – Retention of uranium in the chest after inhalation of U_3O_8 (10 mg).
Rétention thoracique après inhalation d' U_3O_8 (10 mg).

TABLE I
Retention of uranium in the chest.
Rétention thoracique.

Days after inhalation	Uranium dioxide (UO ₂)				Uranium octoxide (U ₃ O ₈)	
	607	608	609	616	611	617
2	88 ± 2	87 ± 2	84 ± 6	101 ± 5	94 ± 3	102 ± 2
7	95 ± 2	92 ± 2	95 ± 6	103 ± 4	75 ± 3	84 ± 3
14	82 ± 2	85 ± 2	94 ± 6	91 ± 5	91 ± 3	87 ± 3
28	76 ± 2	81 ± 2	98 ± 6	90 ± 5	92 ± 3	72 ± 3
56	62 ± 2	77 ± 2	102 ± 5	97 ± 5	45 ± 5	62 ± 4
88	50 ± 2	65 ± 2	91 ± 6	86 ± 5	25 ± 8	47 ± 5
140	43 ± 2	62 ± 2	—	—	13 ± 15	34 ± 6
173	—	—	74 ± 7	80 ± 6	—	—
210	36 ± 3	52 ± 2	61 ± 9	79 ± 6	13 ± 15	35 ± 6
290	30 ± 3	44 ± 3	44 ± 12	75 ± 6	14 ± 14	37 ± 6
365	29 ± 3	41 ± 3	29 ± 17	63 ± 7	8 ± 23	22 ± 9
Sex	f	m	f	m	f	m
ILD mg U	17.5	18.2	2.66	3.28	5.30	8.05

Results are expressed as % ILD (from $N(t)$) $\pm \frac{2\sqrt{2n(t)}}{N(t)}\%$

where $N(t)$ = nett chest count rate (cpm) at t days after subtraction of background count rate,
 $n(t)$ = gross chest count rate.

The results for UO₂ administered at the higher dose levels (ILD 17.5 and 18.2 mg uranium) given in table III have several noteworthy features. As expected, the largest amounts of uranium are present in the lungs and thoracic lymph nodes, although the amounts in the two animals varied appreciably. The largest amounts of uranium, other than in the lungs and lymph nodes, are present in the femora and skull cap. The concentrations of uranium in the various tissues and bones differ markedly and are in the order lymph nodes > lungs >> skull cap > femora > clavicles > lumbar vertebra ~thoracic cage > sternum > kidneys > liver. The concentration in the skull cap is about twice that in the femora and the difference between the skull cap and sternum about fivefold. The concentrations in the right and left thoracic cage are similar as are those for the right and left femora. The total amounts of uranium present in male tissues and bones are, in

general, greater than in females, whereas the uranium concentrations in these tissues tend to be higher in females. This latter observation may reflect differences in mass between the sexes.

TABLE II
Uranium content of tissues in unexposed baboon^a.
Teneur en uranium dans les tissus de babouin non exposé.

Tissue	Wt ^b g	Uranium content $\mu\text{g kg}^{-1}$	
Lungs + trachea	78.1	0.50 ± 0.03^c	6.4 ± 0.4
Thoracic lymph nodes	1.8	0.20 ± 0.02	110 ± 10
Liver	255.0	0.16 ± 0.02	0.63 ± 0.08
Kidneys	33.7	0.05 ± 0.01	1.5 ± 0.3
Skull cap	57.9	0.80 ± 0.04	14 ± 0.7
Sternum	21.9	0.10 ± 0.01	4.6 ± 0.5
Clavicle	10.1	0.11 ± 0.01	11 ± 1.0
Thoracic cage (r)	67.3	0.35 ± 0.03	5.2 ± 0.4
Thoracic cage (l)	66.4	0.38 ± 0.03	5.7 ± 0.4
Lumbar vertebra (5th)	24.0	0.21 ± 0.02	8.8 ± 0.8
Femur (r)	76.9	0.62 ± 0.04	8.1 ± 0.5
Femur (l)	77.9	0.56 ± 0.04	7.2 ± 0.5

a) animal number 422 ; female ; wt at death 11.1 g

b) wet weight

c) uranium content of trachea $0.08 \pm 0.01 \mu\text{g}$

The results for UO_2 administered at lower doses (ILD 2.66 and 3.28 mg uranium) are given in table IV. In general, the amounts of uranium measured in the various body tissues and bones are about 1/6 of those in the previous experiment reflecting the difference in the initial lung deposits. However, when expressed as the percentage of the initial lung deposits, the amounts of uranium in the various tissues are similar to those found after the higher intake (tab. III). As in the previous experiment, the amounts of uranium in the lungs and lymph nodes differ markedly between the animals. Uranium concentrations in the various bones exhibit the same trend as at the higher dose level and, as before, the greatest amounts of translocated uranium are present in the skull caps and femora. In this case, both the concentrations and absolute amounts of uranium tend to be higher in males than in females.

TABLE III
 Uranium content of tissues in baboons exposed to UO_2 at high dose level.
 Teneur en uranium des tissus de babouins exposés à de fortes doses d' UO_2 .

Tissue	Animal 607 ^a				Animal 608 ^b			
	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD
Lungs + trachea	63.3	3.14×10^3 $\pm 20^d$	4.96×10^4 ± 320	17.9	92.7	2.82×10^3 $\pm 20^e$	3.04×10^4 ± 110	15.5
Thoracic lymph nodes	3.3	1.86×10^3 ± 20	5.64×10^5 $\pm 6 \times 10^3$	10.6	2.9	4.46×10^3 ± 70	1.54×10^6 $\pm 7 \times 10^3$	24.5
Liver	169	10.3 ± 0.2	61 ± 1	0.06	261	11.1 ± 0.2	43 ± 1	0.06
Kidneys	32.5	4.63 ± 0.14	143 ± 4	0.03	43.1	2.41 ± 0.08	56 ± 2	0.01
Skull cap	32.7	23.9 ± 0.4	730 ± 12	0.14	49.1	23.1 ± 0.4	536 ± 9	0.13
Sternum	13.1	1.89 ± 0.08	144 ± 6	0.01	20.2	2.06 ± 0.08	102 ± 4	0.01
Clavicles	8.4	2.64 ± 0.08	315 ± 10	0.02	13.3	3.57 ± 0.14	268 ± 11	0.02
Thoracic cage (r)	59.2	14.1 ± 0.2	239 ± 3	0.08	74.1	17.2 ± 0.2	233 ± 3	0.09
Thoracic cage (l)	39.9	10.4 ± 0.2	262 ± 5	0.06	79.2	15.9 ± 0.2	201 ± 3	0.09
Lumbar vertebra (5th)	22.3	5.40 ± 0.14	242 ± 6	0.03	24.7	5.70 ± 0.14	231 ± 6	0.03
Femur (r)	74.6	24.0 ± 0.4	321 ± 5	0.14	109	35.0 ± 0.4	321 ± 4	0.19
Femur (l)	69.8	23.9 ± 0.4	343 ± 6	0.14	108	32.9 ± 0.4	305 ± 4	0.19

Errors are 95 % confidence limits based on counting statistics
 a) female ; weight at death 9.8 kg ; initial lung deposit 17.5 mg U
 b) male ; weight at death 11.5 kg ; initial lung deposit 18.2 mg U
 c) wet weight
 d, e) uranium contents of trachea respectively $1.17 \pm 0.05 \mu\text{g}$ and $1.32 \pm 0.06 \mu\text{g}$
 (r) and (l) : right and left

TABLE IV
Uranium content of tissues in baboons exposed to UO_2 at lower dose level.
Teneur en uranium dans les tissus du groupe le moins exposé à UO_2 .

Tissue	Animal 609 ^a				Animal 616 ^b			
	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD
Lungs + trachea	67.6	0.45×10^3 $\pm 10^d$	6.66×10^3 ± 150	17.3	84.0	1.18×10^3 $\pm 10^e$	1.40×10^4 ± 120	35.7
Thoracic lymph nodes	3.1	0.24×10^3 ± 10	8.06×10^4 $\pm 3.2 \times 10^3$	9.2	2.3	0.76×10^3 ± 10	3.30×10^5 $\pm 4.3 \times 10^3$	23.0
Liver	241	1.17 ± 0.04	4.9 ± 0.2	0.05	267	1.58 ± 0.06	5.9 ± 0.06	0.05
Kidneys	40.0	0.54 ± 0.04	13 ± 1	0.02	39.9	0.59 ± 0.04	15 ± 1	0.02
Skull cap	66.5	4.67 ± 0.08	70 ± 1	0.18	61.4	5.50 ± 0.14	90 ± 2	0.17
Sternum	20.0	0.39 ± 0.04	20 ± 2	0.02	23.5	0.50 ± 0.04	21 ± 2	0.02
Clavicles	8.4	0.44 ± 0.04	52 ± 5	0.02	14.0	0.80 ± 0.04	57 ± 3	0.02
Thoracic cage (r)	61.2	1.74 ± 0.06	28 ± 1	0.07	93.6	3.13 ± 0.08	33 ± 1	0.10
Thoracic cage (l)	59.9	2.02 ± 0.06	34 ± 1	0.08	68.8	2.86 ± 0.08	42 ± 1	0.09
Lumbar vertebra (5th)	29.0	1.01 ± 0.04	35 ± 1	0.04	29.3	1.31 ± 0.06	45 ± 2	0.04
Femur (r)	84.4	3.84 ± 0.08	45 ± 1	0.15	103	6.37 ± 0.14	62 ± 1	0.19
Femur (l)	78.2	4.24 ± 0.08	54 ± 1	0.16	108	6.63 ± 0.14	61 ± 1	0.20

Errors are 95 % confidence limits based on counting statistics
a) female ; weight at death 11.0 kg ; initial lung deposit 2.66 mg
b) male ; weight at death 12.3 kg ; initial lung deposit 3.28 mg U
c) wet weight
d, e) uranium contents of trachea respectively $1.28 \pm 0.05 \mu\text{g}$ and $0.78 \pm 0.05 \mu\text{g}$
(r) and (l) : right and left

The data for uranium tissue distribution after U_3O_8 administration at the higher dose level (5.3 and 8.05 mg) are given in table V. The trend in uranium concentration in the various tissues and bones, namely lymph nodes > lungs >> skull cap > clavicles > femora > lumbar vertebra ~thoracic cage > sternum > kidneys > liver is similar to that obtained after UO_2 administration. The concentration in the skull cap is about 1.5 times that in the femora and clavicles, and about 5 times that in the sternum. The concentrations in the left and right femora, and in the left and right thoracic cage are similar, as before. However, uranium amounts in the various tissues, expressed as a percentage of the initial lung deposits, are about 3-6 times greater than after UO_2 administration. This might be due to the greater transportability of uranium after U_3O_8 deposition in the lungs. In this case, the higher concentrations in the bones occur in the female baboons.

The results obtained after U_3O_8 administration at the lower dose level (ILD about 1 mg) are given in table VI. The amounts of uranium present in the various body tissues and bones are generally about 10-20 times lower than at the higher dose level. The fact that the amounts in the tissues are somewhat lower than expected on the basis of the differences in the initial lung deposits reflects uncertainty in the lower dose group measurements. However, the uranium concentrations in body tissues follow the same trend as in the higher dose group.

3.4 Distribution of uranium between lungs and lymph nodes

The ratio of the amounts of uranium present in the thoracic lymph nodes and lungs of each animal, expressed in terms of both concentration and mass, are given in table VII, together with the percentage of the total lung contents retained in the lymph nodes. All the data were calculated from those given in tables II-VI. The results have five noteworthy features : 1) taking into account the different amounts administered, the ratios of uranium concentrations in the lymph nodes vs lungs are greater after UO_2 than after U_3O_8 administration ; 2) after UO_2 administration, the concentration ratio appears to be higher in males than in females but this trend was not observed for U_3O_8 ; however, since each exposure group consisted of two animals only, no conclusions are drawn as to the influence of sex ; 3) after the intake of each compound by each sex, the concentration ratios are reasonably independent of the amounts administered ; 4) taking into account the different amounts administered, the ratios, by mass, of uranium in the lungs vs that in the lymph nodes are greater for UO_2 than U_3O_8 ; 5) for both UO_2 and U_3O_8 , the percentage of the lung deposits present in the lymph nodes at death does not appear to be substantially affected by the initial lung deposits, although the values for U_3O_8 are about 4-6 times lower than those for UO_2 .

TABLE V
Uranium content of tissues in baboons exposed to U_3O_8 at higher dose level.
Teneur en uranium dans les tissus du groupe le plus exposé à U_3O_8 .

Tissue	Animal 611 ^a				Animal 617 ^b			
	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD	wt ^c g	Uranium content μg	Uranium content $\mu\text{g kg}^{-1}$	% ILD
Lungs + trachea	76	382 $\pm$ 1	5.03 $\times 10^3$ $\pm 10^d$	7.20	96.5	1 490 $\pm 3^e$	1.54 $\times 10^4$ ± 30	18.5
Thoracic lymph nodes	2.76	57 $\pm$ 0.6	2.07 $\times 10^4$ ± 220	1.08	2.15	202 $\pm$ 1	9.40 $\times 10^4$ ± 470	2.51
Liver	203	8.10 $\pm$ 0.24	40 $\pm$ 1	0.15	214	7.52 $\pm$ 0.24	37 $\pm$ 1	0.09
Kidneys	37.4	5.46 $\pm$ 0.20	146 $\pm$ 5	0.10	44.6	4.14 $\pm$ 0.18	93 $\pm$ 4	0.05
Skull cap	75.0	68.2 $\pm$ 0.8	909 $\pm$ 11	1.29	74.3	53.2 $\pm$ 0.6	716 $\pm$ 8	0.66
Sternum	21.4	3.68 $\pm$ 0.16	172 $\pm$ 7	0.07	24.1	3.91 $\pm$ 0.16	162 $\pm$ 7	0.05
Clavicles	9.7	5.97 $\pm$ 0.20	615 $\pm$ 21	0.11	14.2	6.90 $\pm$ 0.22	486 $\pm$ 15	0.09
Thoracic cage (r)	54.6	28.0 $\pm$ 0.4	513 $\pm$ 7	0.53	77.8	23.3 $\pm$ 0.4	299 $\pm$ 5	0.29
Thoracic cage (l)	52.1	23.4 $\pm$ 0.4	449 $\pm$ 8	0.44	80.0	18.2 $\pm$ 0.4	228 $\pm$ 5	0.23
Lumbar vertebra (5th)	21.9	10.3 $\pm$ 0.4	470 $\pm$ 18	0.19	28.4	8.72 $\pm$ 0.26	307 $\pm$ 9	0.11
Femur (r)	78.4	44.8 $\pm$ 0.6	571 $\pm$ 8	0.85	105	51.8 $\pm$ 0.6	493 $\pm$ 6	0.64
Femur (l)	79.6	42.7 $\pm$ 0.6	545 $\pm$ 8	0.81	106	46.9 $\pm$ 0.6	442 $\pm$ 6	0.58

Errors are 95 % confidence limits based on counting statistics
a) female ; weight at death 10.8 kg ; initial lung deposit 5.30 mg
b) male ; weight at death 12.9 kg ; initial lung deposit 8.05 mg
c) wet weight
d, e) uranium contents of trachea respectively 0.16 $\pm$ 0.03 μg and 0.10 $\pm$ 0.03 μg
(r) and (l) : right and left

TABLE VI
 Uranium content of tissues in baboons exposed to U_3O_8 at lower dose level.
 Teneur en uranium dans les tissus du groupe le moins exposé à U_3O_8 .

Tissue	Animal 615 ^a				Animal 623 ^b			
	wt ^c g	Uranium content μg	Uranium content $\mu g\ kg^{-1}$	% ILD	wt ^c g	Uranium content μg	Uranium content $\mu g\ kg^{-1}$	% ILD
Lungs	84.4	207 $\pm$ 1	2.60 $\times 10^3$ $\pm 10^d$	20.7	79.2	172 $\pm$ 1	2.17 $\times 10^3$ $\pm 10^e$	17.2
Thoracic lymph nodes	1.86	25 $\pm$ 0.4	1.34 $\times 10^4$ ± 220	2.5	2.40	18.6 $\pm$ 0.4	7.75 $\times 10^3$ ± 170	1.86
Liver	230	0.46 $\pm$ 0.08	2.0 $\pm$ 0.3	0.05	228	0.68 $\pm$ 0.10	3.0 $\pm$ 0.4	0.07
Kidneys	43.8	0.29 $\pm$ 0.06	6.6 $\pm$ 1.4	0.03	46.3	0.24 $\pm$ 0.06	5.2 $\pm$ 1.3	0.02
Skull cap	59.0	2.24 $\pm$ 0.20	38 $\pm$ 3	0.22	76.7	3.14 $\pm$ 0.24	41 $\pm$ 3	0.31
Sternum	20.3	0.33 $\pm$ 0.06	16 $\pm$ 3	0.03	26.2	0.28 $\pm$ 0.06	11 $\pm$ 2	0.03
Clavicle	10.1	0.28 $\pm$ 0.06	28 $\pm$ 6	0.03	13.0	0.46 $\pm$ 0.08	35 $\pm$ 6	0.05
Thoracic cage (r)	54.4	1.02 $\pm$ 0.14	19 $\pm$ 3	0.10	77.7	1.83 $\pm$ 0.16	24 $\pm$ 2	0.18
Thoracic cage (l)	66.6	1.01 $\pm$ 0.14	15 $\pm$ 2	0.10	80.2	1.59 $\pm$ 0.14	20 $\pm$ 2	0.16
Lumbar vertebra (5th)	24.8	0.54 $\pm$ 0.08	22 $\pm$ 3	0.05	36.7	0.83 $\pm$ 0.10	23 $\pm$ 3	0.08
Femur (r)	84.4	2.03 $\pm$ 0.20	24 $\pm$ 2	0.20	113	3.41 $\pm$ 0.18	30 $\pm$ 2	0.34
Femur (l)	88.9	1.99 $\pm$ 0.16	22 $\pm$ 2	0.20	121	4.14 $\pm$ 0.20	34 $\pm$ 2	0.41

a) female ; weight at death 11.5 kg ; estimated initial lung deposit 1 mg
 b) male ; weight at death 15.2 kg ; estimated initial lung deposit 1 mg
 c) wet weight
 d, e) uranium contents of trachea respectively 0.06 $\pm$ 0.02 μg and 0.04 $\pm$ 0.02 μg
 (r) and (l) : right and left

TABLE VII

Distribution of uranium between the lungs (L) and thoracic lymph nodes (TLN).
 Distribution de l'uranium entre poumon (L)
 et ganglions lymphatiques thoraciques (TLN).

Compound	Animal No. Sex	ILD mg	Uranium ratio by conc.	TLN : L by mass	% lung deposit in TLN
UO ₂	607, F	17.5	11.4	0.59	37
UO ₂	608, M	18.2	50.7	1.58	61
UO ₂	609, F	2.66	12.1	0.53	35
UO ₂	616, M	3.28	23.6	0.64	39
U ₃ O ₈	611, F	5.30	4.1	0.15	13
U ₃ O ₈	617, M	8.05	6.1	0.14	12
U ₃ O ₈	615, F	~1	5.2	0.12	11
U ₃ O ₈	623, M	~1	3.6	0.11	10

3.5 Analysis of enzymes in urine

Increases in the SGOT concentrations in urine (see 2.6) were observed after exposure of both males and females to the higher dose levels of UO₂ (tab. VIII). The GGT concentrations were also increased in the male baboons exposed to the higher doses of UO₂ and U₃O₈. The SGOT concentrations in the urine of unexposed animals (tab. VIII) are compatible with those reported by Gerasimo *et al.* [10]. No increases in the urinary concentrations of the other enzymes investigated could be identified.

TABLE VIII

Enzymatic analysis of urine.
 Analyse enzymatique de l'urine.

Animal	Compound	ILD (mg U)	Enzyme	Concentration in urine (IU l ⁻¹) ^a	
				Day 0	Day 365
607	UO ₂	17.5	SGOT ^b	24	93
608	UO ₂	18.2	SGOT ^b	13	76
608	UO ₂	18.2	GGT ^b	69	91
617	U ₃ O ₈	8.05	GGT ^b	50	137

a) International units per litre

b) SGOT and GGT in unexposed animals, respectively 16-24 and 22-48 IU l⁻¹

3. Discussion

In these experiments, UO_2 and U_3O_8 have been administered in varying amounts to baboons in order to elucidate the lung retention kinetics of uranium, its distribution between thoracic lymph nodes and lung tissue, its concentrations in various body tissues and bones, and its effect, if any, on metabolites present in urine. The results are now discussed in terms of their implications for human exposure.

4.1 Uranium retention in the chest

In-vivo monitoring data obtained for 9 individuals over a 12-year period after termination of their occupational exposure to ceramic grade UO_2 have shown that the mean retention half-time of uranium in the chest is 390 d (range 280-490 d) (Stradling *et al.*) [25]. The values predicted from experiments in which the same compound was administered to rats ranged from 400 to 520 d and hence are in reasonable agreement with these observations. A feature of the human data is that an appreciable proportion of the chest content measured immediately after the cessation of exposure appears to be retained indefinitely and varies by about fourfold between individuals (Price) [21]. The results of the baboon experiments (tab. III, IV, VII) suggest that this must be due to the uranium retained in the thoracic lymph nodes rather than the data being affected by the small amounts of uranium that are likely to accumulate in the skull or rib cage as a consequence of uranium translocation into the blood. A twofold difference in uranium distribution between the lungs and lymph nodes is in reasonable agreement with the fourfold variation in the apparent long-term component in humans. Whilst the magnitude of the lymph node content in baboons, about 35-60 % of the total lung content, is higher than the apparent value in humans, the discrepancy may be due to the difference in the exposure patterns, *i.e.* acute vs chronic, and the fact that the amounts of uranium deposited in baboon lungs were 1 order of magnitude or more greater than those measured in human lung.

U_3O_8 intakes by baboons were too low for the lung retention kinetics of uranium to be assessed with confidence by chest monitoring. The long-term retention half-time predicted for workers on the basis of Stradling's experiments with rats exposed to a similar U_3O_8 preparation suggests it would be about 230 d [23, 25]. The baboon data quoted in tables V-VII suggest that the amounts of uranium likely to be retained indefinitely in the thorax as a consequence of accumulation in the lymph nodes will be appreciably lower after inhalation of U_3O_8 than after inhalation of UO_2 .

4.2 Concentration ratios of uranium in lungs and lymph nodes

The distribution of inhaled dusts between the thoracic lymph nodes and lungs is frequently expressed as a concentration ratio. The data in table VII show that the values for UO_2 vary from about 11 to 50 and those for U_3O_8 from about 3 to 6. It is noteworthy that ratios in excess of 100 have

been reported by Leach *et al.* in dogs and monkeys exposed chronically to $5 \text{ mg m}^{-3} \text{ UO}_2$ over an interval of 5 y [17-18]. The values reported for humans exposed to uranium are also extremely variable. Usually, however, they refer to uranium inhalation at low concentrations, but in many cases neither the chemical form nor the amounts inhaled have been specified. For example, concentration ratios of 1.5 have been reported after chronic inhalation to U_3O_8 (Donoghue *et al.* [9] ; 2.5 (σ g 5.5) for 6 individuals exposed to undefined forms of uranium (Campbell *et al.* [6]) and 10.4 and 21.2 for 2 uranium millers (Singh *et al.* [22]). The relatively high values in the study reported here, particularly for UO_2 , reflect the possible consequences of human exposure to acute intakes of uranium in excess of the ALI for class Y compounds. The data should not be extrapolated to predict the values that would occur after chronic inhalation of much lower amounts. The results summarised in table VII for UO_2 suggest that the concentration ratio may be higher in males than in females with an opposite trend for U_3O_8 . However, since only one animal of either sex was used at each dose group, the results should be interpreted with caution. Hence, no conclusions can be drawn from these experiments about the effect of sex on concentration ratios.

4.3 Deposition in tissues other than the lung

In general, the amounts of uranium present in the various bones of the baboon skeleton, expressed as percentages of the initial lung deposits, are about 5 times higher after U_3O_8 administration. This suggests that after intakes of U_3O_8 and UO_2 the transfer rates of uranium to blood also differ by about fivefold. A similar difference in transfer rates was observed by Stradling *et al.* when the same preparations of the compounds were inhaled by rats [24].

It is noteworthy that after the inhalation of UO_2 and U_3O_8 in different amounts by baboons of either sex, the trend in uranium concentration in the various bones and tissues is closely similar, namely skull > femora ~clavicles > lumbar vertebra ~thoracic cage > sternum > kidneys > liver. This appears to indicate that uranium is transported *via* the blood in the same chemical forms, *i.e.* complexed with transferrin, citrate and bicarbonate (Cooper *et al.*) [7]. In general, uranium concentrations in the skull cap are about 2 and 5 times that present respectively in the femora and sternum. The median concentration ratios are present in the clavicles, ribs and femora.

Other studies on the variability of uranium concentrations in human and animal tissues have also been published. The twofold difference in uranium concentrations in the ribs and sternum of a former uranium miner [22] were similar to those observed here for baboons. Welford and Baird [27] reported uranium concentrations in bone samples obtained from former residents of New York city ; the values varied from about $0.01 \mu\text{g}^{-1}$ ash in the long bone shaft to about $0.03 \mu\text{g U g}^{-1}$ ash in the skull, and hands and feet. Hamilton [11] determined uranium concentration in skull,

rib, femur (shaft), sternum, vertebra and cartilage samples from non-occupationally exposed persons in the UK. The ash concentrations were respectively 0.032, 0.020, 0.024, 0.018, 0.017 and 0.024 $\mu\text{g g}^{-1}$, and would appear to be compatible with the results obtained in this study. Animal data also show that skeletal distribution of uranium is non-uniform. For example, after intravenous injection of the citrate complex into 3 dogs, the ratios of maximum vs minimum concentrations in the various samples of bone ash ranged from 4.2 to 12 [22].

The results of this study, and others, illustrate the possible errors that can occur in the assessment of the skeletal content of uranium by extrapolation from that present in individual bones, and demonstrate that the analysis of several bones will be necessary in order to make the best estimates. The baboon experiments suggest that Donoghue's assessments based on the sternum [9] could underestimate the skeletal content by about 2. The low concentrations of uranium present in the kidneys and liver vs those in the bones in the baboon experiments are consistent with data obtained for former occupationally exposed individuals (Kathren *et al.*) [16].

4.4 Enzymatic analysis of urine

Increases in the concentrations of SGOT were observed in the urine of both baboons exposed to UO_2 in amounts that simulated human exposures to about 20 times the ALI for the compound. No increase could be found after acute exposures in amounts equivalent to 3 times the ALI. Enhanced levels of GGT were found in the urine of the male baboons exposed to the higher doses of UO_2 and U_3O_8 , the latter being equivalent to 7.4 times the ALI. We are unsure whether these increases in concentration are due to uranium toxicity. No increases were observed in the urinary concentrations of those enzymes that reflect nephrotoxicity.

As a conclusion, presently, external monitoring of the chest and urine analysis should remain the methods of choice for this purpose. ■

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