

# Molecular, cellular, and tissue impact of depleted uranium on xenobiotic-metabolizing enzymes

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**Abstract** Enzymes that metabolize xenobiotics (XME) are well recognized in experimental models as representative indicators of organ detoxification functions and of exposure to toxicants. As several *in vivo* studies have shown, uranium can alter XME in the rat liver or kidneys after either acute or chronic exposure. To determine how length or level of exposure affects these changes in XME, we continued our investigation of chronic rat exposure to depleted uranium (DU, uranyl nitrate). The first study examined the effect of duration (1–18 months) of chronic exposure to DU, the second evaluated dose dependence, from a level close to that found in the environment near mining sites (0.2 mg/L) to a supra-environmental dose (120 mg/L, 10 times the highest level naturally found in the environment), and the third was an *in vitro* assessment of whether DU exposure directly affects XME and, in particular, CYP3A. The experimental *in vivo* models used here demonstrated that CYP3A is the enzyme modified to the greatest extent: high gene expression changed after 6 and 9 months. The most substantial effects were observed in the liver of rats after 9 months of exposure to 120 mg/L of DU: CYP3A gene and protein expression and enzyme activity all decreased by more than 40 %. Nonetheless, no direct

effect of DU by itself was observed after *in vitro* exposure of rat microsomal preparations, HepG2 cells, or human primary hepatocytes. Overall, these results probably indicate the occurrence of regulatory or adaptive mechanisms that could explain the indirect effect observed *in vivo* after chronic exposure.

**Keywords** Uranium · Xenobiotic · Cytochrome P450 · Liver · Kidney · Hepatocyte

## Introduction

Uranium in the environment results from leaching from natural deposits, release in mill tailings, nuclear industry emissions, combustion of coal and other fuels, and the use of phosphate fertilizers containing uranium (Bleise et al. 2003). It can thus be found in quantities that vary between areas by a factor of more than a thousand (Wrenn et al. 1985). As a consequence, populations (civil and military) living in contaminated areas can be exposed to various amounts of uranium.

Animal studies as well as the studies of occupationally exposed people have shown that the major health effects of both natural and depleted uranium are linked to chemical toxicity rather than to radiation (Craft et al. 2004; Leggett 1989). Following ingestion, uranium rapidly appears in the bloodstream; it accumulates particularly in the kidneys and the skeleton, whereas little is found in the liver (Igarashi et al. 1987; La Touche et al. 1987). Nonetheless, the liver, through the portal vein, is the first organ exposed to compounds (nutrients and xenobiotics) absorbed through the digestive tract, and it is the most important organ involved in the detoxification process. It is thus subject to the action of numerous potentially toxic compounds, even if they do

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not accumulate in large quantities. The kidney, because of its role in urine formation, is the second key organ of the detoxification process. The many hepatotoxic and nephrotoxic substances include numerous drugs and environmental toxins but also some heavy metals, such as lead, cadmium, mercury, arsenic, and uranium.

To minimize the insults caused to the body by these xenobiotics, the liver is equipped with diverse xenobiotic-metabolizing enzymes (XME). The kidney, a target tissue of uranium (Gueguen and Rouas 2012; Vicente–Vicente et al. 2010), can also actively metabolize many drugs, hormones, and xenobiotics and plays a major role in their excretion (Lohr et al. 1998). The detoxification pathway for a vast variety of exogenous and endogenous molecules is composed of three distinct phases. The phase-I reaction is essentially catalyzed by the members of the CYP superfamily of enzymes (Gueguen et al. 2006a; Honkakoski and Negishi 2000). The phase-II metabolizing enzymes consist of numerous proteins belonging to the UDP-glucuronosyltransferases (UGT), glutathione-S-transferases (GST) in particular (Rushmore and Kong 2002). ATP-binding cassette (ABC) transporter proteins belonging to the third phase are important determinants of drug metabolism and clearance by the liver and kidneys (Faber et al. 2003). CAR (constitutive androstane receptor) and PXR (Pregnane X receptor), both nuclear receptors, are also known to play central roles as sensors and transcriptional mediators in highly integrated drug/xenobiotic signaling networks (Kliwer and Willson 2002).

As several studies have shown, uranium can alter XME in the rat liver or kidneys after either acute exposure (Gueguen et al. 2006b; Moon et al. 2003) or chronic exposure to low levels (Gueguen et al. 2005; Souidi et al. 2005). These *in vivo* studies have demonstrated changes in CYP3A1 and CYP3A2 gene expression after a 9-month exposure to uranium through drinking water (40 mg/L, i.e., 2.7 mg/kg BW).

To determine whether length or level of exposure affected these changes in XME, we continued our investigation of chronic rat exposure to DU through drinking water. The first study examined the effect of duration (1–18 months) of chronic exposure to DU through drinking water (40 mg/L). This concentration was chosen as a non-nephrotoxic level close to the maximum environmental level found in well-water and generally used in our model of long-term DU exposure (Dublineau et al. 2007; Gueguen et al. 2007; Lestaevel et al. 2005; Rouas et al. 2011; Tissandie et al. 2007). Secondly, a study evaluated dose dependence, that is, exposure to DU concentrations starting from a level close to that found in the environment near mining sites (0.2 mg/L) to a supra-environmental dose (120 mg/L, 10 times the highest level naturally found in the environment, i.e., 12 mg/L) (Juntunen 1991). Thirdly,

because only one publication has reported *in vitro* effects of DU on XME in a hepatoma cell line (Miller et al. 2004), we conducted an *in vitro* study to assess whether DU exposure affects XME and, in particular, CYP3A, directly. This study used different *in vitro* models from the liver: the HepG2 hepatoma cell line, primary human hepatic cells, and a microsomal liver fraction.

## Materials and methods

### In vivo studies

#### Animals

Experiments were performed with 3-month-old male Sprague–Dawley rats (250 g) obtained from Charles River Laboratories (L'Arbresle, France). Animals were housed at constant room temperature ( $21\text{ }^{\circ}\text{C} \pm 1^{\circ}$ ) with a 12-h light/dark cycle. Experiments were approved by the Animal Care Committee of the Institute (IRSN) and conducted in accordance with the French regulations for animal experimentation (Ministry of Agriculture Act No. 2011-110, June 2011) and with ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

#### Animal exposure to depleted uranium (DU)

The rats in the contaminated group ( $n = 12$  for each group) were exposed to uranyl nitrate ( $\text{U}^{238}$ : 99.74 %,  $\text{U}^{235}$ : 0.255 %,  $\text{U}^{234}$ : 0.0055 %) (AREVA-NC, France) via their drinking water for 1–18 months at a concentration of 40 mg/L (study 1) or for 9 months at concentrations from 0.2 to 120 mg/L (study 2). During the DU-exposure period and through the end of the experiments, animals were carefully monitored once a week (body weight and food and water intake). This monitoring confirmed that DU exposure had no influence on the rats' food consumption, body weight, or general health status (data not shown). Control animals ( $n = 12$ ) had mineral drinking water (vehicle water of the DU preparation). Uranium levels in livers and kidneys were measured by ICP-MS as previously described (Paquet et al. 2006).

#### Tissues collection and histopathology

At the end of the contamination, animals were anesthetized by inhalation of 5 % isoflurane (Abbot France, Rungis, France) and euthanized by exsanguination by intracardiac puncture. After weighting, organ samples (liver and kidneys) were frozen immediately in liquid nitrogen and stored at  $-80\text{ }^{\circ}\text{C}$ . For histological examination, liver and kidney samples were fixed in 4 % formaldehyde solution

(Carlo Erba, Rueil Malmaison, France) at room temperature during 48 h. After fixation, tissues were dehydrated, embedded in paraffin, cut in 5- $\mu$ m-thick sections, and stained with hematoxylin–eosin–safran. Histopathologic analysis was assessed by an independent laboratory (Bio-doxis, Romainville, France).

#### Biochemical characteristics of plasma

Blood was centrifuged at 4,000 *g* for 10 min (4 °C), and plasma was then stored at –80 °C. Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total bilirubin, creatinine, alkaline phosphatase (AP), and urea were measured by an automated Konelab 20 (biological chemistry reagents, Thermo Electron Corporation, Villebon-sur-Yvette, France).

#### Liver microsomal and cytosol preparation

Liver samples were crushed in an aqueous buffer (pH 7.40) containing  $\text{KH}_2\text{PO}_4$  (50 mM), sucrose (300 mM), DTT (0.5 mM), EDTA (10 mM), and NaCl (50 mM) and centrifuged for 20 min at 20,000 *g*. The supernatant was then centrifuged for 1 h at 100,000 *g* at 4 °C. The ensuing microsomal pellet was homogenized in buffer. The supernatant obtained after the second centrifugation corresponded to the cytosol fraction.

Both microsomal and cytosolic fractions were stored at –80 °C. Protein content was determined by the Bradford method, with bovine serum albumin used as the standard.

#### Real-time RT-PCR

Total RNA from the liver was extracted with the RNeasy Total RNA Isolation Kit (Qiagen, Courtaboeuf, France) and reverse-transcribed with random hexamers and the

Superscript II First Strand Synthesis System (Invitrogen, Cergy-Pontoise, France). Real-time PCR was used to analyze the mRNA level of the major cytochromes such as P450 3A1, 3A2, 2E, and 2C11 (CYP3A1: EC 1.14.14.1, CYP3A2: EC, CYP2E: EC 1.14.14.1, and CYP2C11: EC 1.14.14.1), glutathione-S-transferase A2 (GSTA2: EC), UDP-glucuronosyltransferase 1A1 and 2B1 (UGT1A1: EC 2.4.1.17 and UGT2B1: EC 2.4.1.17), sulfotransferase 1A1 (ST1A1: EC 2.8.2.1), multidrug resistance protein 1 (MDR1), and multi-resistance protein 2 (MRP2). An Abi-Prism 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France) was used with 10 ng of cDNA for each reaction. A mix of primers (Invitrogen, Cergy-Pontoise, France) (2.5 % v/v), SYBR (Applied Biosystems, Courtaboeuf, France) (83 % v/v), and sterile water (14.5 % v/v) was added to each well to give a final volume of 25  $\mu$ L. Samples were normalized to hypoxanthine–guanine phosphoribosyltransferase (HPRT), and fold induction calculated relative to the control (unexposed group). Sequences of forward and reverse primers are listed in Table 1 (Gueguen et al. 2007; Rekka et al. 2002; Ropenga et al. 2004; Rouas et al. 2009; t Hoen et al. 2002; Theron et al. 2003).

#### Western blot

Proteins from liver homogenate, or microsomal or cytosolic fractions, were separated by 10 % sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto nitrocellulose membrane. The membranes were blocked for 1 h in 5 % nonfat dry milk in TBS. The blots were incubated overnight with antibodies diluted in 2 % nonfat dry milk in TBS at 4 °C. Microsomal CYP3A1 and CYP3A2 were detected with rabbit polyclonal antibodies (Abcam, Paris, France), microsomal CYP2C11 with goat polyclonal antibodies (Daiichi Pure

**Table 1** Primer sequences for gene encoding for xenobiotic-metabolizing enzymes

Gene	Forward	Reverse	Accession no	Amplicon size (pb)	References
HPRT	GCTCGAGATGTCATGAAGGAGA	TCAGCGCTTTAATGTAATCCAGC	NM012583.2	108	Ropenga et al. (2004)
CYP3A1	GATGTTGAAATCAATGGTGTGT	TTCAGAGGTATCTGTGTTTCC	NM01310.2	290	Rekka et al. (2002)
CYP3A2	AGTAGTGACGATTCACATAT	TCAGAGGTATCTGTGTTTCTT	NM153312.2	250	Rekka et al. (2002)
CYP2E	GGAGGAGCTCAAAAACACCAAA	GGTGCGCAGCCAATCAG	NM031543.1	62	Gueguen et al. (2007)
CYP2C11	ATGGGATGCAATGGAAGGAG	TCTTGCCCATCCAAAAGTC	X79081	67	Gueguen et al. (2007)
GSTA2	TTGACATGTATTCAGAGGGT	TTGTTTTGCATCCATGGCTG	K00136	362	t Hoen et al. (2002)
UGT1A1	TGGCATCCCCAAAACGATCT	GGAACCGGAGTGTGTGATGAA	NM175846.2	65	Rouas et al. (2009)
UGT2B1	TGGAGAACATGGTGTAGTGGT	TTGGCTTTTTCTCAGTAGTCAGG	NM173295.1	68	Gueguen et al. (2007)
ST1A1	AGGGTGGCAAGCTAGAGAAGTG	GAGGGAACCCCTGGACATTT	NM031834	67	Rouas et al. (2009)
MDR1	ATCAACTCGAAAAGCATCC	AATCAACTTCAGGATCCGC	NM1334401.1	115	Theron et al. (2003)
MRP2	TTCGAAGCTGGATGATGTGTTT	GCCATGCAGATCATGACAAGAG	NM012833.1	60	Gueguen et al. (2007)

Chemicals, Tokyo, Japan), microsomal UGT1A1 with goat polyclonal antibodies, and microsomal UGT2B1 with rabbit polyclonal antibodies (both Santa Cruz Biotechnology, Heidelberg, Germany). Cytosolic GSTY $\alpha$  was detected with goat antibody (Oxford Biomedical Research, Oxford, USA). Immune complexes were revealed by rabbit anti-goat IgG and goat anti-rabbit IgG (Santa Cruz Biotechnology) coupled to horseradish peroxidase (HRP) and the luminol derivative of Immobilon Western (Millipore, Billerica, USA). Samples were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was detected with rabbit anti-rat antibody (Santa Cruz Biotechnology). Reaction intensity was determined by computer-assisted densitometry (Fuji LAS3000, Raytest, France).

#### *Testosterone hydroxylase assay*

Testosterone hydroxylase activity was determined as previously described (Rouas et al. 2009) with freshly prepared microsomal fractions. Testosterone (10  $\mu$ L) solubilized in potassium phosphate buffer containing 45 % (w/v) hydroxypropyl  $\beta$  cyclodextrin (HP $\beta$ CD) as well as buffer (390  $\mu$ L) containing KH<sub>2</sub>PO<sub>4</sub> (15 mM), K<sub>2</sub>HPO<sub>4</sub> (60 mM), EDTA (1 mM), DTT (0.5 mM), and MgCl<sub>2</sub>·6H<sub>2</sub>O (5 mM), was added to the microsomal solution (50  $\mu$ L). NADPH (1 mM) was used to begin the reaction, which was stopped after 20 min at 37 °C by the addition of 2 mL methanol/chloroform (2:1, v/v). Cortisolone (10 nmol) was used as an internal standard. Testosterone and its metabolites were extracted with 2 mL chloroform and 1.5 mL water. Tubes were vortexed for 1 min, and 2 mL of the organic phase was collected and evaporated (nitrogen gas). The dry residue was reconstituted with 250  $\mu$ L of acetonitrile by vortexing for 1 min. The solution was injected into the HPLC for the analysis (column Lichrospher 100 RP18, 250  $\times$  4 mm, 5  $\mu$ m), and components were quantified at 247 nm. The mobile phase was acetonitrile/water (26:74). Typical retention times were 8 min for 4-androsten-7 $\alpha$ , 17 $\beta$ -diol-3-one (characteristic of CYP2A activity), 10 min for 4-androsten-6 $\beta$ , 17 $\beta$ -diol-3-one (characteristic of CYP3A activity), 11 min for 4-androsten-16 $\alpha$ , 17 $\beta$ -diol-3-one (characteristic of CYP2B activity), and 4-androsten-2 $\alpha$ , 17 $\beta$ -diol-3-one (characteristic of CYP2C activity). Enzymatic activity was expressed as picomoles per minute per whole liver.

#### *In vitro studies*

HepG2 is the standard model for in vitro study of the effects of toxic compounds on liver cells; nevertheless, to assess XME and particularly cytochrome P450 in greater

detail, we also used primary liver cells due to their greater ability to respond to CYP inducers.

#### *Components of cell culture media*

Roswell Park Memorial Institute medium (RPMI 1640, ref 21875034), penicillin/streptomycin 10,000 U/mL (PS, ref 15140), fetal bovine serum (FBS, ref 10270-106), and L-glutamine (ref 25030-024) were purchased from Invitrogen (Cergy-Pontoise, France). Non-essential amino acids (NEAA, ref M7145), amphotericin B (ref A2942), and retinoic acid (ref R4643) were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France).

HepG2 cells were obtained from ATCC (Molsheim, France). Primary hepatocytes, ISOM's medium, ascorbic acid, Hepato-STIM medium, and collagen plates were obtained from BD Biosciences (le Pont de Claix, France). Plastics used for HepG2 cell culture were purchased from VWR (Fontenay-sous-Bois, France).

#### *Cell culture*

All cells were grown in incubators with humidified atmosphere (i.e., 37 °C, 5 % CO<sub>2</sub>) to a confluence of 80 %. HepG2 in a monolayer culture in RPMI supplemented with 10 % FBS, 1 % PS, and primary hepatocytes with ISOM's medium supplemented with ascorbic acid 25 mM, FBS 1 %, and EGF 0.25 %. Primary liver cells come from a non-smoking healthy adult aged 25 years who died accidentally.

#### *Preparation of DU solution*

A solution of uranyl nitrate hexahydrate (UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O) (10 mM) was prepared from DU powder dissolved in 100 mM bicarbonate (HCO<sub>3</sub><sup>-</sup>) solution. The radioactive-specific activity of DU is 1.4  $\times$  10<sup>-4</sup> Bq/g and its isotopic composition is <sup>238</sup>U = 99.74 %, <sup>235</sup>U = 0.255 %, and <sup>234</sup>U = 0.0055 % (AREVA-NC, France).

Depleted uranium (DU) solutions used for experiments were prepared by diluting stock solution in cell culture media. Cells were incubated with increasing DU concentrations (10, 100, and 500  $\mu$ M) for 24 h.

#### *CYP inducer and inhibitor exposure*

Pharmaceutical substances frequently used in CYP3A induction studies were used to specifically enhance or repress CYP3A isoforms for in vitro models.

Dexamethasone was chosen as the CYP3A inducer in HepG2 cells. After testing different concentrations, we chose 50  $\mu$ M, because it moderately induced CYP3A for DU co-treatment.

Rifampicin was used as the CYP3A inducer in human primary hepatocytes. After testing different concentrations, we chose 20  $\mu\text{M}$  because it moderately induced CYP3A for DU co-treatment.

Isoniazid was used as a CYP inhibitor in rat liver microsomes. Different concentrations were tested (from 1 to 1,000  $\mu\text{M}$ ) and compared with DU treatment.

#### *CYP3A activity in vitro*

Cytochrome P450 (CYP450) activity was measured by a luminescent method with the P450-Glo assay (Promega, Charbonnières, France), performed by incubating the samples (source of CYP450 isoenzymes) and a lumino-genic cytochrome P450 substrate. The substrates are converted to luciferin by CYP450, which in turn reacts with luciferase to produce a luminescent signal. This signal is measured by Luminometer Mithras LB940 (Berthold, Thoiry, France). The amount of light produced is directly proportional to the CYP3A activity. These measurements were taken in primary hepatocyte cells and are expressed as luminescence arbitrary units/mg protein. Mean values  $\pm$  standard deviations were obtained in three independent experiments.

#### Statistical analysis

To compare the time-course or dose–response effects of DU exposure in vivo, statistical analyses were performed by two-way analysis of variance with DU contamination and time after DU exposure as the two factors. For the in vitro studies of mRNA level or enzymatic activities, statistical analysis used Student's *t* test (*p* values are reported between brackets). All results are expressed as their mean  $\pm$  SEM. The acceptable level of significance was established as *p* < 0.05.

## Results

### Time-course study

#### *General health characteristics*

Food and water intake was monitored weekly, starting from the beginning of the contamination; at no point, did it differ significantly between DU-exposed and control animals (data not shown). Accordingly, the final body weight was similar between DU and control groups as was the kidney and liver weights (Online Resource 1).

Plasma levels of various biochemical indicators related to kidney (creatinine, urea) or liver function or integrity (transaminases, alkaline phosphatase) were

measured for each exposure period and no abnormal level was observed (Online Resource 1). Accordingly, the histological examination of livers and kidneys of rats exposed to DU for 9 months showed no deleterious effects in this study, as reported earlier at this level (40 mg/L).

#### *Assessment of xenobiotic-metabolizing enzymes*

Figure 1 reports the effect of chronic uranium exposure (40 mg/L) over time on the gene expression of the main XMEs in rat livers (Fig. 1a) and kidneys (Fig. 1b). Expression of these various genes changed differently over time. The mRNA levels of liver CYP2C11 (–49 %) and kidney CYP3A1 (–73 %) and 3A2 (–97 %), all phase I enzymes, had decreased after 6 months, whereas gene expression of the latter two (CYP3As) increased in the livers and kidneys of rats exposed to uranium for 9 months. No significant change was noticed in shorter (1 and 3 months) or longer (18 months) DU-exposure groups. The lack of modification after an 18-month exposure might be due to the age of the animals, as variability increases with age. CYP2A, 2B, 2C, and 3A activities were measured in the microsomal liver fraction and did not differ from those of control rats regardless of the duration of DU exposure (data not shown).

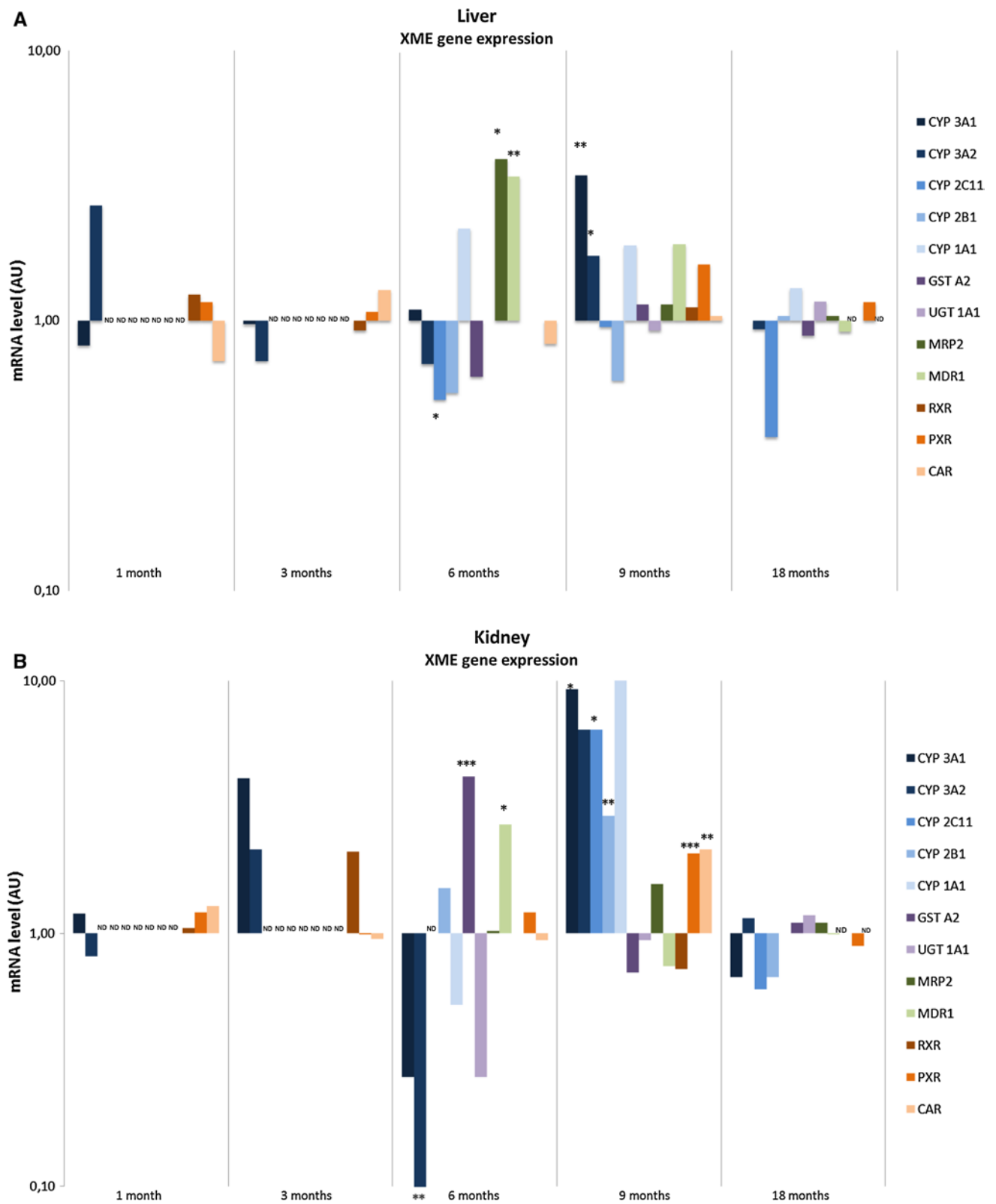
At 6 months, unlike the phase-I (cytochrome P450) enzymes that were repressed, gene expression of one phase-II enzyme (GSTA2) in the kidney and two phase-III transporters, MDR1 and MRP2, in the liver tripled compared with controls (*p* < 0.01 and *p* < 0.05, respectively). No other significant change was observed at any other duration of DU exposure.

The most important effects of DU exposure were observed after 9 months of chronic intake, when gene expression of CYP3A enzymes increased along with gene expression of their associated nuclear receptors, in both the liver and the kidneys, although there was no difference in enzymatic activity (data not shown). In view of these results, the study of increasing DU concentrations that followed was conducted for a 9-month contamination period.

#### Dose–response study

Increasing concentrations of DU (from 0.2 to 120 mg/L) were administered to rats for 9 months. Uranium levels in livers and kidneys were measured by ICP-MS, as previously described (Paquet et al. 2006). Dose-dependent accumulation of uranium was measured in the kidneys (from 4.7 to 352.8 ng/g of tissue) and in the liver (from 0.2 to 4.2 ng/g of tissue) of rats exposed to increasing concentrations of DU.





**Fig. 1** Xenobiotic-metabolizing enzymes and nuclear receptor mRNA level variation in the liver (a) and the kidney (b) of rats exposed to DU through drinking water (40 mg/L) during 1, 3, 6, 9, or 18 months. *ND* not determined

### General health characteristics

Weekly monitoring of the animals' body weight and food and water intake showed no differences between the groups (data not shown). Kidneys and livers were weighed (and related to BW). Surprisingly, liver weight increased by 13 % for DU concentrations above 40 mg/L ( $p < 0.05$ ) but remained nonetheless within the normal range (Online Resource 2).

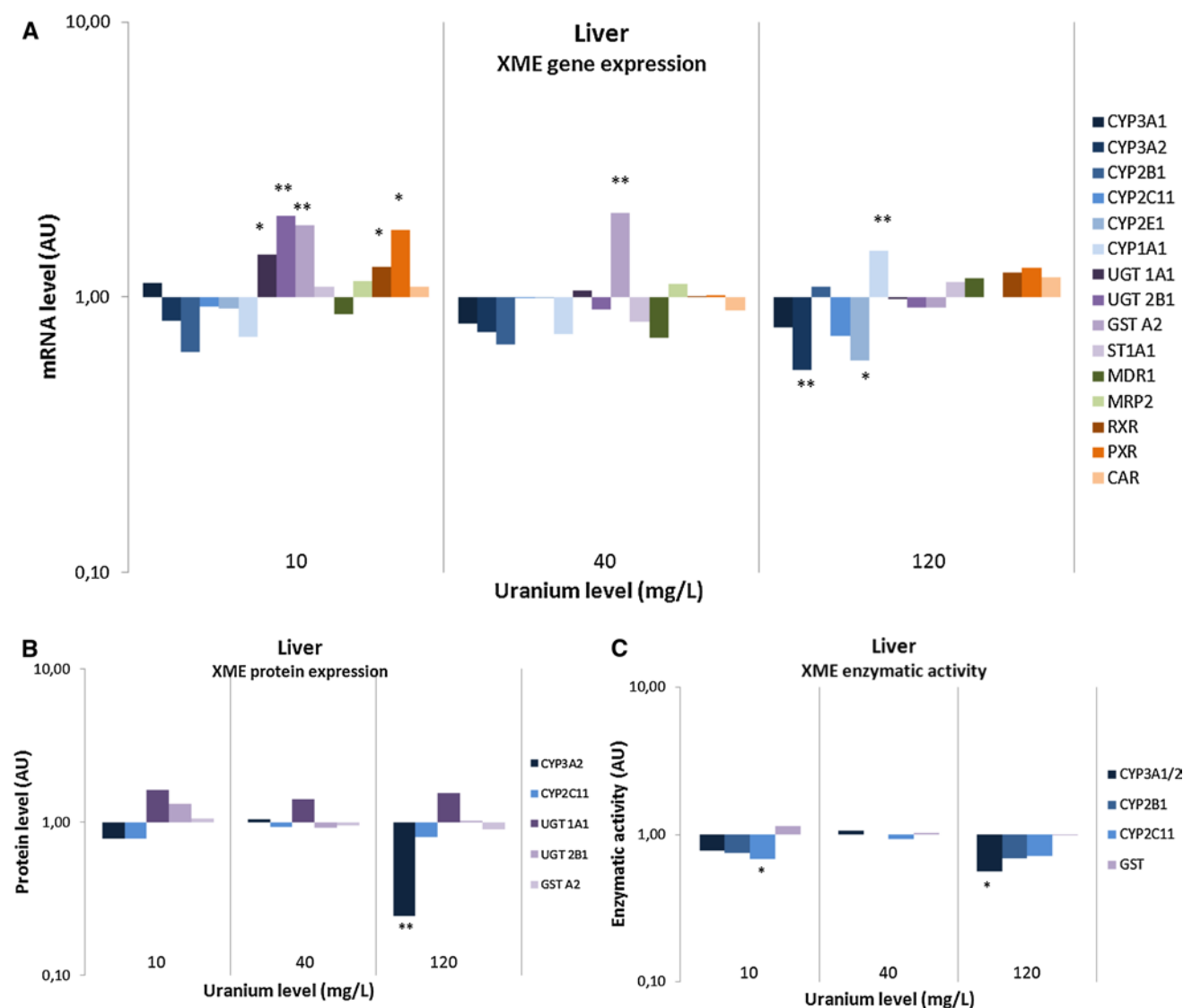
The plasma marker levels related to kidney functions and liver integrity or functions did not change significantly, regardless of the DU concentration in drinking water and despite the higher transaminase level in the 120 mg/L group.

Complementary histological analyses of livers and kidneys confirmed the absence of tissue alteration due to

chronic DU exposure (data not shown). This finding indicates that uranium did not produce major toxicity when administered through drinking water at concentrations between 0.2 and 120 mg/L.

### Analysis of xenobiotic-metabolizing enzymes

Our previous results obtained with animals exposed to DU at 40 mg/L for 9 months (Gueguen et al. 2007; Rouas et al. 2009; Souidi et al. 2005) showed that chronic exposure modified liver expression of CYP3A, a major phase-I enzyme. As shown in Figs. 2 and 3, the present dose-response study confirms this change, showing the greatest modification in CYP3A enzyme activity and gene and protein expressions in the liver of animals exposed to the



**Fig. 2** Xenobiotic-metabolizing enzymes and nuclear receptor mRNA level, protein level, and enzymatic activity in the liver of rats exposed to increasing concentration of DU through drinking water (10, 40, and 120 mg/L) during 9 months

highest DU concentrations (120 mg/L)(decreases of 44 % ( $p < 0.05$ ), 50 % ( $p < 0.01$ ), and 75 % ( $p < 0.05$ ), respectively) (Fig. 2).

Of the four key phase-II enzymes we studied, only one (ST1A1) changed with the level of exposure (Fig. 3a). The mRNA level of this enzyme increased in the kidneys as a function of uranium concentration in the drinking water (compared with control animals, increase of 96 % at 10 mg/L through 242 % at 120 mg/L). Future studies of the protein level and/or enzymatic activity of ST1A1 at the same or lower levels of contamination should determine whether this enzyme can be used as a kidney biomarker of chronic DU exposure.

Overall, these results confirm that some XME, notably CYP3A in the liver and ST1A1 in the kidneys, might be usable as markers of chronic DU contamination, because the effect observed increases with dose.

#### In vitro studies

The experimental in vivo models used here showed that CYP3A is the enzyme modified to the greatest extent, and the most substantial effects were observed in the liver—the key organ of detoxification process—of rats exposed to uranium. Then, we next studied the direct effect of uranium on XME and more specifically on CYP3A in different in vitro liver cell models.

#### Microsomes study

The first set of experiments examined the potential inhibitory direct effect of DU on a purified fraction of cells containing CYP enzymes, specifically the microsomal liver fraction of control rats. Figure 4 shows the activity of CYP3A and CYP2C in liver microsomes exposed to the concentrations of uranium from 1 to 1,000  $\mu\text{M}$ . Enzyme activity was measured by HPLC of a testosterone hydroxylase assay. To confirm the inducibility/inhibition potential of cytochrome P450, microsomal fractions were exposed to various concentrations of isoniazid (1 to 1,000  $\mu\text{M}$ ), a known inhibitor of these cytochromes P450. A significant decrease in the activity of CYP2C (87 %,  $p < 0.01$ ) and CYP3A (35 %,  $p < 0.05$ ) was observed after treatment with 1 mM of isoniazid. This inhibition was dose-dependent and greater for CYP2C than CYP3A at concentrations from 10 to 1,000  $\mu\text{M}$ . By comparison, uranium had no effect on either CYP3A or CYP2C activity measured in rat liver microsomal fractions, regardless of the concentration used (from 1 to 1,000  $\mu\text{M}$ ). These results suggest that uranium had no direct effect on the activity of these CYPs measured in microsomal fractions.

In a next step, hepatocyte cultured cells were used to study the effect of uranium on XME at the cell level. With these more complex in vitro models, the hypothesis of an indirect effect occurring through cell regulation was examined.

#### HepG2 study

The human hepatoma cell line HepG2, a common in vitro model for toxicological and XME studies (Krusekopf et al. 2003; Maruyama et al. 2007), was used. A previously published study of cytotoxicity and localization of uranium inside the cells (Rouas et al. 2010) allowed us to define the concentrations used to study XME expression.

Dexamethasone was chosen as a standard CYP3A inducer in HepG2 cells. As expected and as Fig. 5 shows, dexamethasone administered at a concentration of 50  $\mu\text{M}$  induced expression of CYP3A7 (+150 %,  $p < 0.01$ ) and CYP3A4 (+200 %,  $p < 0.05$ ) genes. In comparison, DU treatment (10–500  $\mu\text{M}$ ) did not induce either an increase or a decrease in gene expression of these CYP3A isoforms. In co-exposure to uranium and dexamethasone (50  $\mu\text{M}$ ), however, uranium exposure inhibited CYP3A induction but only at the highest concentration used (500  $\mu\text{M}$ , but not at 10 and 100  $\mu\text{M}$ ). This inhibition might thus be related to the decreased cell viability at this DU level, as previously reported (Rouas et al. 2010).

#### Study of primary human hepatocytes

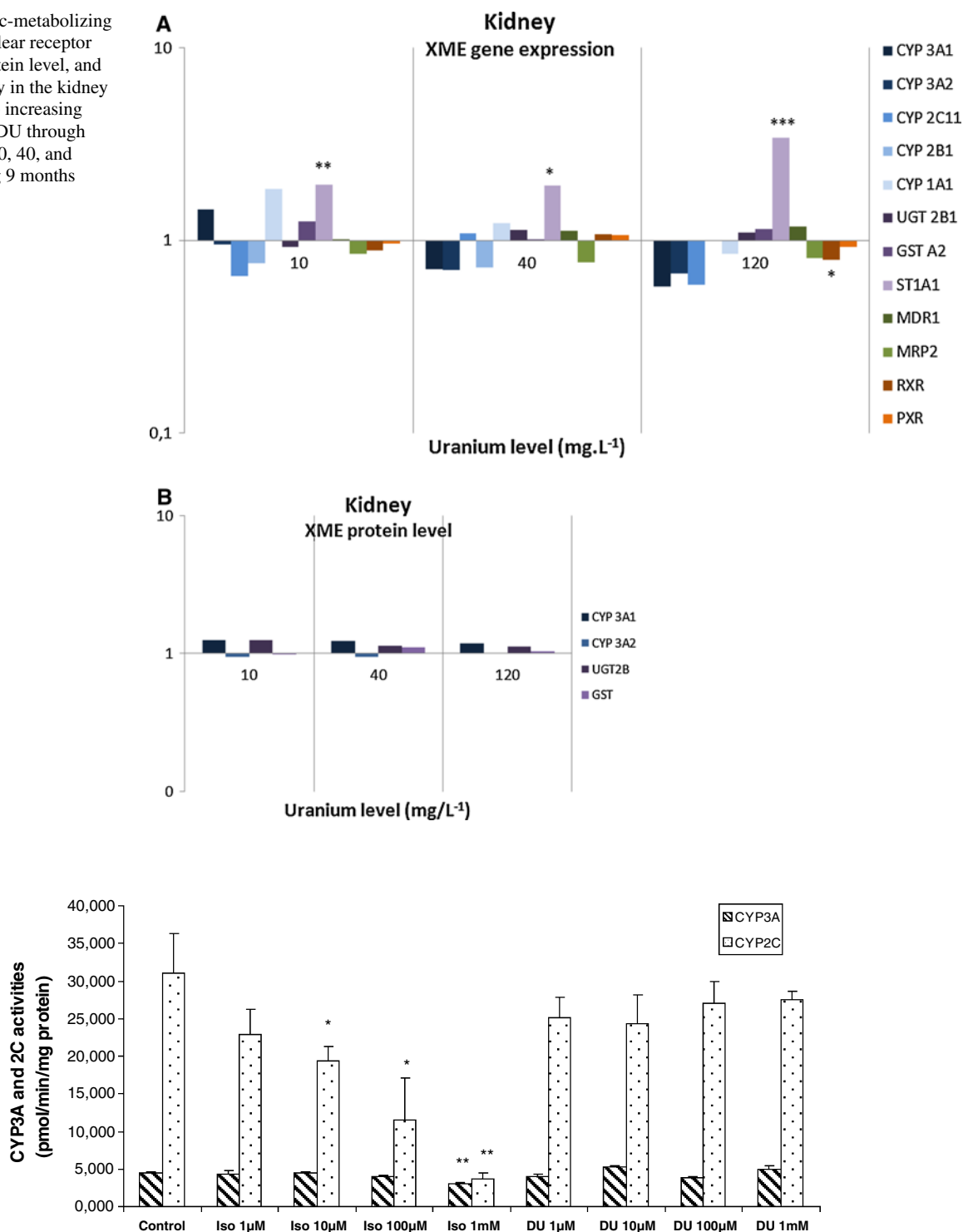
Figure 6 shows the effect of uranium on human primary hepatocyte cells. The increased gene expression of CYP3A4 induced by rifampicin (14-fold at 20  $\mu\text{M}$ ,  $p < 0.001$ ) confirms the responsiveness of these enzymes in the cells. Rifampicin also induced the other CYP3A isoforms (doubled level of CYP3A5,  $p < 0.01$ , and 11-fold increase of 3A7,  $p < 0.001$ ).

In the primary hepatocytes as in the HepG2 cells, uranium treatment did not alter the gene expression of CYP3A isoforms when it was administered alone (10, 100, or 500  $\mu\text{M}$ ). Nevertheless, when administered with rifampicin (20  $\mu\text{M}$ ), induction of CYP3A isoforms ( $p < 0.04$  for CYP3A7) was down-regulated at the highest uranium dose (500  $\mu\text{M}$ ). At the same time, gene expression of other CYPs (1A2, 2E1, 2C9), phase-II enzymes (GSTA1, GSTA4, GSTM1, ST1B1), and transporters (MDR1, MRP2) did not increase or decrease significantly in relation to exposure to uranium (data not shown).

These results were completed by measuring total CYP3A activity (Fig. 2b). No significant change in this activity was observed for uranium exposure alone, at any concentration used (10–500  $\mu\text{M}$ ). In case of co-exposure



**Fig. 3** Xenobiotic-metabolizing enzymes and nuclear receptor mRNA level, protein level, and enzymatic activity in the kidney of rats exposed to increasing concentration of DU through drinking water (10, 40, and 120 mg/L) during 9 months

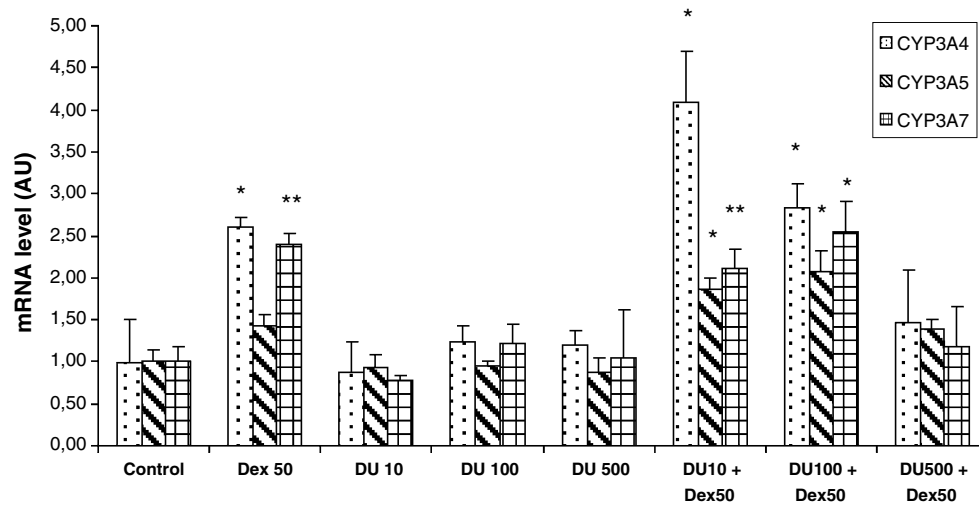


**Fig. 4** CYP2C and 3A activities in rat liver microsomal fraction treated by depleted uranium (DU) and Isoniazide (Iso) during 24 h ( $n = 6$  for each group) measured by testosterone hydroxylase assay. The results are expressed as picomoles/minutes/mg of micro-

somal proteins. Asterisk represents a significant difference between treated and control (C) groups (Student's  $t$  test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ )

(uranium with rifampicin), CYP3A4 induction decreased slightly, compared to rifampicin (20  $\mu$ M) exposure alone, at the highest uranium concentration (500  $\mu$ M,  $-20\%$ ,

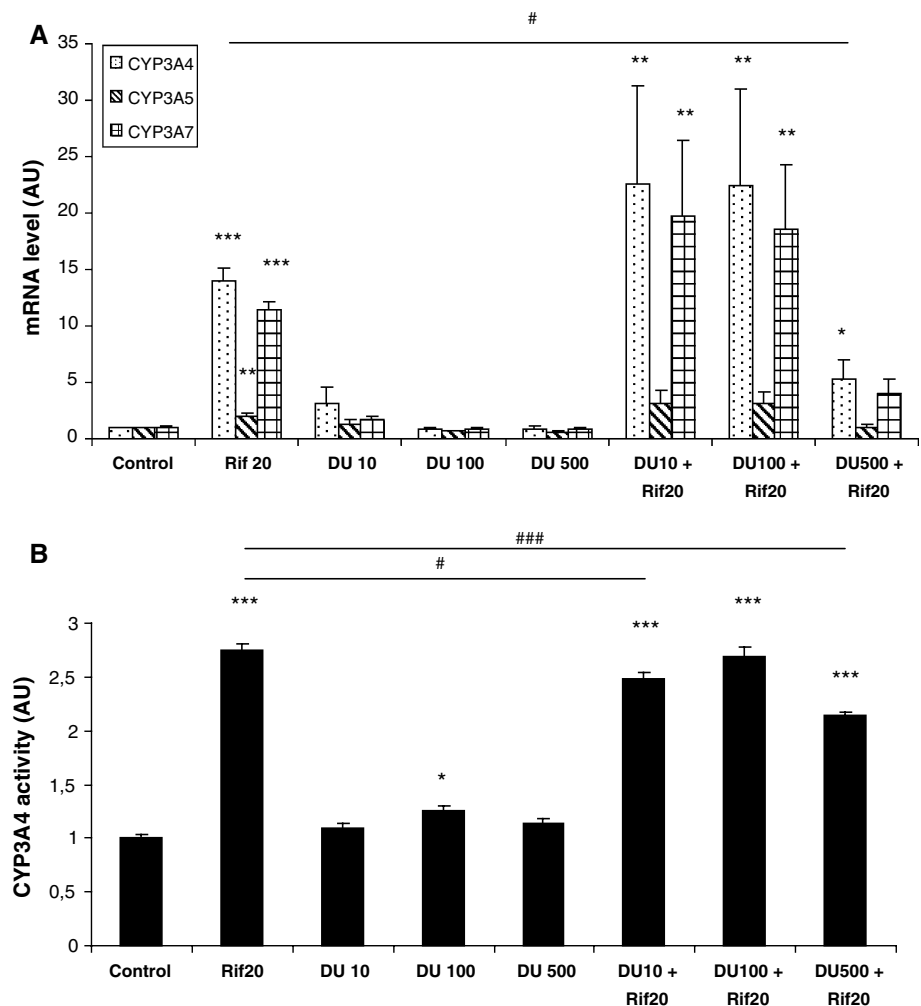
$p < 0.001$ ). This reduction was correlated with decreased gene expression, although the decrease observed with rifampicin at the lowest uranium dose, 10  $\mu$ M, was not.



**Fig. 5** Gene expression of CYP3A isoforms in HepG2 cells treated by depleted uranium (DU) and dexamethasone (Dex) during 24 h ( $n = 4$  for each group). The results are expressed as a ratio to the

housekeeping gene GAPDH mRNA levels, AU arbitrary units. Asterisk represents a significant difference between treated and control (C) groups (Student's *t* test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ )

**Fig. 6 a** Gene expression of CYP3A4, CYP3A5, and CYP3A7 isoforms or **b** CYP3A4 activity in primary human hepatic cells treated by depleted uranium (DU) and Rifampycin (Rif) during 24 h ( $n = 4$  for mRNA and  $n = 6$  for CYP3A4 activity). The results of gene expression are expressed as a ratio to the housekeeping gene GAPDH mRNA levels, AU arbitrary units. Asterisk represents a significant difference between treated or co-treated groups and control group (Student's *t* test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ). Sharp sign represents a difference between co-treated groups and Rif20 group (Student's *t* test, # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$ )



## Discussion

Enzymes that metabolize drugs or xenobiotics (DME or XME) play a central role in the biotransformation, metabolism, and detoxification of xenobiotics and foreign compounds that are introduced to the human body and can be used as sensitive indicators of liver or kidney damage. In particular, levels of cytochromes P450, for the analysis of liver detoxification functions or GST- $\alpha$  or - $\gamma$  to evaluate kidney damage, can be measured in serum, urines, or liver tissue (Fent 2003; Gueguen et al. 2006a, 2012; Waring and Moonie 2011).

It has been previously shown that XME expression can be altered in the liver and kidneys of rats exposed to uranium through drinking water for 9 months at a maximizing environmental level of 40 mg/L (i.e., 2.7 mg/kg BW) (Rouas et al. 2009; Souidi et al. 2005). In these previous studies, one XME in particular was altered after exposure to uranium—CYP3A and especially the CYP3A2 isoform—which seems to be a preferred liver target of uranium administered chronically at a low dose. Although these changes are rather slight, they have been correlated to the potentiation of production of a toxic metabolite of low-toxic doses of acetaminophen, a commonly used drug actively metabolized by cytochrome P450 (Gueguen et al. 2007).

In this work, we used different *in vivo* and *in vitro* models in an extensive study of the effects of uranium alone on the xenobiotic-metabolizing system. Overall, the experimental *in vivo* models used here demonstrated that CYP3A is the enzyme modified to the greatest extent. When the duration of uranium exposure varied from 1 to 18 months, CYP3A gene expression was substantially lower than in control rats at 6 months but higher at 9 months (Fig. 1). These time-dependent changes can be related to the lack of any gradual accumulation of DU in the rat organs, so it does not reach a steady state (Gueguen et al. 2007; Paquet et al. 2006). The biological modifications observed might depend on the tissue level of uranium. Accumulation of uranium in the kidneys and the liver as a function of time of DU exposure was measured and was similar to the previous results (Paquet et al. 2006). Adaptive mechanisms probably occur in the organs and lead to the changes in the level of XME expression. Greater effects were observed after 9 months of exposure, when the level of uranium in the organs was higher.

Nevertheless, these variations in terms of accumulation and biological effect on XME in the liver and kidneys did not modify either clinical plasma indicators or the histological appearance of these tissues (data not shown). Accordingly, in a previous study, sensitive and specific markers of tubular kidney injury revealed no significant structural change in the kidney in this protocol (9 months, 40 mg/L,

i.e., approximately 0.1  $\mu\text{g/g}$  kidney) (Rouas et al. 2011). The XME changes seen here probably did not involve deleterious effects to kidneys or livers but probably reflect functional changes. Globally, in our model of chronic exposure to uranium at a non-nephrotoxic level, the accumulation of uranium and its effect on XME in these organs depends on the time of exposure but it does not vary linearly, that is, from shorter to longer exposure. This lack of a linear time relation is probably due to a time-dependent adaptive mechanism to uranium presence in organs.

To assess whether the effects described above might depend on dose and on the concentration at which the first effects on XME appear, we conducted a second study with different DU concentrations added to drinking water in a chronic exposure protocol. This appears to be only the second study of chronic exposure of rats to different doses of uranium through drinking water. The first study (Gilman et al. 1998) showed renal alterations in the control group (non-exposed to uranium) and no rise in histopathologic severity with increasing dose: histological lesions in the kidneys of rats exposed to the lowest concentration (0.96 mg/L) did not differ significantly from those observed at the highest concentration (600 mg/L). In the present study, rats were exposed to uranium in drinking water containing 0.2–120 mg/L of uranium for 9 months. No indicators of hepatotoxic or nephrotoxic effects were observed according to the plasma parameters or histological analysis.

The maximal kidney concentration of uranium that did not induce a deleterious effect in rats contaminated through their drinking water ranged from 0.4  $\mu\text{g/g}$  kidney to 1.9  $\mu\text{g/g}$  (Gilman et al. 1998; Linares et al. 2006; Ortega et al. 1989). To our knowledge, no study has shown any kidney effect of uranium at a tissue concentration below 0.4  $\mu\text{g/g}$  after oral exposure except in the previous studies from our group. We used the same contamination protocol and found some variation in the metabolism of vitamin D (Tissandie et al. 2007) and of xenobiotics (Rouas et al. 2009; Souidi et al. 2005). In these studies, the expression or activity of the cytochromes P450 involved in these metabolisms was altered in the liver or the kidneys of the uranium-exposed rats.

Heavy metals are known to be able to modify cytochrome P450 function and thus organ function. Lead, cadmium, and arsenic can affect the oxidative activity of cytochrome P450 through the inhibition of heme synthesis or the activation of its degradation (Moore 2004). Chronic uranium exposure can also modify some XME (Figs. 2, 3) as a function of dose. Again, the strongest effect is on CYP3A2; the change observed in its gene expression at 120 mg/L is correlated with reduced protein expression and decreased CYP3A activity (Fig. 2a–c). Both of these sets of *in vivo* results make it difficult to reach any definitive conclusion about the precise effect of chronic uranium

exposure on XME. We therefore conducted *in vitro* studies to examine whether uranium has a direct or indirect effect on XME.

CYP3A enzyme activity evaluated after *in vitro* exposure of a rat microsomal preparation showed that uranium by itself had no direct effect on an enriched preparation of CYP enzymes. Hepatocytes directly exposed to uranium were then used to analyze whether uranium could have a direct effect on cells but an indirect effect on CYP. Until now, most *in vitro* studies of the effects of uranium have analyzed cell death (Carriere et al. 2004; Milgram et al. 2008; Mirto et al. 1999; Thiebault et al. 2007). To our knowledge, only one study evaluated the effects of uranium on the detoxification system, such as the induction of XME, and only a few enzymes were studied (Miller et al. 2004). In comparison, many studies have analyzed the effects of heavy metal exposure on XME, such as CYPs. One hypothesis about uranium cytotoxicity is based on P450 reductase, which could play a role in inducing oxidative stress (Pourahmad et al. 2006). In our study, exposure of HepG2 cells to various concentrations (10–500  $\mu\text{M}$ ) of uranium did not induce expression of CYP1A1 or GST genes (data not shown), contrary to other heavy metals. These results are in line with a previous report that uranium exposure does not substantially modify the expression of these genes (Miller et al. 2004). Accordingly, in human hepatocytes, exposure to uranium did not affect the gene expression of CYP3A main isoforms (CYP3A4, CYP3A5, and CYP3A7) or of CYP3A4 enzyme activity. CYP3A gene expression and activity were altered only when cells were co-exposed to both an inducer (rifampicin or dexamethasone) and a cytotoxic concentration of uranium (500  $\mu\text{M}$ ). These results suggest that uranium might modify CYP3A expression when it is administered in specific conditions. Nevertheless, the finding that uranium enters cells and indeed cell nuclei (Rouas et al. 2010) provides a basis for supposing that it might act directly on a molecular target inside the cells and not only on cytoplasmic membrane receptors (Brady et al. 1989; Hori et al. 1985; Schwartz and Flamenbaum 1976).

Overall, the absence of any direct effect by uranium on CYP3A in *in vitro* models together with the augmentation or diminution of CYP3A *in vivo* probably means that the regulation or adaptive mechanisms occur. Indeed, the effects observed *in vivo* depend on the duration of exposure. We can suppose that the organism's homeostatic mechanisms induce some defenses—and signals of these defenses—in response to continuous uranium exposure. The modifications of XME in the liver, where uranium does not accumulate at a high level, could be related to other tissue signals in response to this exposure. This appears to be the case, for example, in a rat model of renal failure that induced a decrease in liver CYP levels (mainly CYP2C11,

CYP3A1, and CYP3A2) (Leblond et al. 2001), which might be due to the reduced levels of parathyroid hormone (PTH) (Michaud et al. 2010). Results from our previous experiments showed changes in CYP activity or gene expression related to endobiotic metabolism, especially vitamin D metabolism, but the plasma PTH level evaluated in animals exposed to uranium for 9 months did not change (Tissandie et al. 2007). Other adaptive mechanisms probably occurred in our model; they might involve oxidative stress or signaling disruption due to these changes, as observed in *in vitro* models (Periyakaruppan et al. 2007; Pourahmad et al. 2006; Prat et al. 2005). These hypothesized pathways should be investigated in future work to improve our understanding of the effect of uranium at the cellular level as a function of time of exposure.

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**Conflict of interest** The authors declare that there is no conflict of interest.

## References

- Bleise A, Danesi PR, Burkart W (2003) Properties, use and health effects of depleted uranium (DU): a general overview. *J Environ Radioact* 64(2–3):93–112
- Brady HR, Kone BC, Brenner RM, Gullans SR (1989) Early effects of uranyl nitrate on respiration and K<sup>+</sup> transport in rabbit proximal tubule. *Kidney Int* 36(1):27–34
- Carriere M, Avoscan L, Collins R et al (2004) Influence of uranium speciation on normal rat kidney (NRK-52E) proximal cell cytotoxicity. *Chem Res Toxicol* 17(3):446–452
- Craft E, Abu-Qare A, Flaherty M, Garofolo M, Rincavage H, Abou-Donia M (2004) Depleted and natural uranium: chemistry and toxicological effects. *J Toxicol Environ Health B Crit Rev* 7(4):297–317
- Dublineau I, Grandcolas L, Grison S et al (2007) Modifications of inflammatory pathways in rat intestine following chronic ingestion of depleted uranium. *Toxicol Sci* 98(2):458–468
- Faber KN, Muller M, Jansen PL (2003) Drug transport proteins in the liver. *Adv Drug Deliv Rev* 55(1):107–124
- Fent K (2003) Ecotoxicological problems associated with contaminated sites. *Toxicol Lett* 140–141:353–365
- Gilman AP, Villeneuve DC, Secours VE et al (1998) Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley rat. *Toxicol Sci* 41(1):117–128
- Gueguen Y, Rouas C (2012) New data on uranium nephrotoxicity. *Radioprotection* 47(3):345–359. doi:10.1051/radiopro/2012015
- Gueguen Y, Paquet F, Voisin P, Souidi M (2005) Effects of chronic contamination with depleted uranium on xenobiotic biotransformation enzymes in the rat. In: Medimond IP (ed) proceedings of the 14th international conference on cytochromes P450, pp 61–65. ISBN 88-7587-188-4
- Gueguen Y, Mouzat K, Ferrari L et al (2006a) Cytochromes P450: xenobiotic metabolism, regulation and clinical importance. *Ann Biol Clin (Paris)* 64(6):535–548

- Gueguen Y, Souidi M, Baudelin C et al (2006b) Short-term hepatic effects of depleted uranium on xenobiotic and bile acid metabolizing cytochrome P450 enzymes in the rat. *Arch Toxicol* 80(4):187–195. doi:10.1007/s00204-005-0027-3
- Gueguen Y, Grandcolas L, Baudelin C et al (2007) Effect of acetaminophen administration to rats chronically exposed to depleted uranium. *Toxicology* 229(1–2):62–72. doi:10.1016/j.tox.2006.10.006
- Gueguen Y, Rouas C, Leblond FA (2012) Kidney injury biomarkers. *Nephrol Ther* 8(3):146–155. doi:10.1016/j.nephro.2012.02.004
- Honkakoski P, Negishi M (2000) Regulation of cytochrome P450 (CYP) genes by nuclear receptors. *Biochem J* 347(Pt 2):321–337
- Hori R, Takano M, Okano T, Inui K (1985) Transport of p-aminohippurate, tetraethylammonium and D-glucose in renal brush border membranes from rats with acute renal failure. *J Pharmacol Exp Ther* 233(3):776–781
- Igarashi Y, Yamakawa A, Ikeda N (1987) Plutonium and uranium in Japanese human tissues. *Radioisotopes* 36(9):433–439
- Juntunen R (1991) Uranium and radon in wells drilled into bedrock in southern Finland. Report of investigation, geological survey of Finland 98
- Kliwer SA, Willson TM (2002) Regulation of xenobiotic and bile acid metabolism by the nuclear pregnane X receptor. *J Lipid Res* 43(3):359–364
- Krusekopf S, Roots I, Kleeberg U (2003) Differential drug-induced mRNA expression of human CYP3A4 compared to CYP3A5, CYP3A7 and CYP3A43. *Eur J Pharmacol* 466(1–2):7–12
- La Touche YD, Willis DL, Dawydiak OI (1987) Absorption and biokinetics of U in rats following an oral administration of uranyl nitrate solution. *Health Phys* 53(2):147–162
- Leblond F, Guevin C, Demers C, Pellerin I, Gascon-Barre M, Pichette V (2001) Downregulation of hepatic cytochrome P450 in chronic renal failure. *J Am Soc Nephrol* 12(2):326–332
- Leggett RW (1989) The behavior and chemical toxicity of U in the kidney: a reassessment. *Health Phys* 57(3):365–383
- Lestaevél P, Bussy C, Paquet F et al (2005) Changes in sleep-wake cycle after chronic exposure to uranium in rats. *Neurotoxicol Teratol* 27(6):835–840
- Linares V, Belles M, Albina ML, Sirvent JJ, Sanchez DJ, Domingo JL (2006) Assessment of the pro-oxidant activity of uranium in kidney and testis of rats. *Toxicol Lett* 167(2):152–161
- Lohr JW, Willsky GR, Acara MA (1998) Renal drug metabolism. *Pharmacol Rev* 50(1):107–141
- Maruyama M, Matsunaga T, Harada E, Ohmori S (2007) Comparison of basal gene expression and induction of CYP3As in HepG2 and human fetal liver cells. *Biol Pharm Bull* 30(11):2091–2097
- Michaud J, Naud J, Ouimet D, et al (2010) Reduced hepatic synthesis of calcidiol in Uremia. *J Am Soc Nephrol*:[Epub ahead of print]
- Milgram S, Carriere M, Thiebault C, Malaval L, Gouget B (2008) Cytotoxic and phenotypic effects of uranium and lead on osteoblastic cells are highly dependent on metal speciation. *Toxicology* 250(1):62–69. doi:10.1016/j.tox.2008.06.003
- Miller AC, Brooks K, Smith J, Page N (2004) Effect of the militarily-relevant heavy metals, depleted uranium and heavy metal tungsten-alloy on gene expression in human liver carcinoma cells (HepG2). *Mol Cell Biochem* 255(1–2):247–256
- Mirto H, Henge-Napoli MH, Gibert R, Ansoborio E, Fournier M, Cambar J (1999) Intracellular behaviour of uranium(VI) on renal epithelial cell in culture (LLC-PK1): influence of uranium speciation. *Toxicol Lett* 104(3):249–256
- Moon YJ, Lee AK, Chung HC et al (2003) Effects of acute renal failure on the pharmacokinetics of chlorzoxazone in rats. *Drug Metab Dispos* 31(6):776–784
- Moore MR (2004) A commentary on the impacts of metals and metalloids in the environment upon the metabolism of drugs and chemicals. *Toxicol Lett* 148(3):153–158
- Ortega A, Domingo JL, Llobet JM, Tomas JM, Paternain JL (1989) Evaluation of the oral toxicity of uranium in a 4-week drinking-water study in rats. *Bull Environ Contam Toxicol* 42(6):935–941
- Paquet F, Houpert P, Blanchardon E et al (2006) Accumulation and distribution of uranium in rats after chronic exposure by ingestion. *Health Phys* 90(2):139–147
- Periyakaruppan A, Kumar F, Sarkar S, Sharma CS, Ramesh GT (2007) Uranium induces oxidative stress in lung epithelial cells. *Arch Toxicol* 81(6):389–395
- Pourahmad J, Ghashang M, Ettehad HA, Ghalandari R (2006) A search for cellular and molecular mechanisms involved in depleted uranium (DU) toxicity. *Environ Toxicol* 21(4):349–354
- Prat O, Berenguer F, Malard V et al (2005) Transcriptomic and proteomic responses of human renal HEK293 cells to uranium toxicity. *Proteomics* 5(1):297–306
- Rekka E, Evdokimova E, Eeckhoudt S, Labar G, Calderon PB (2002) Role of temperature on protein and mRNA cytochrome P450 3A (CYP3A) isozymes expression and midazolam oxidation by cultured rat precision-cut liver slices. *Biochem Pharmacol* 64(4):633–643
- Ropenga A, Chapel A, Vandamme M, Griffiths NM (2004) Use of reference gene expression in rat distal colon after radiation exposure: a caveat. *Radiat Res* 161(5):597–602
- Rouas C, Souidi M, Grandcolas L et al (2009) Acetaminophen induces xenobiotic-metabolizing enzymes in rat: impact of a uranium chronic exposure. *Environ Toxicol Pharmacol* 28(3):363–369. doi:10.1016/j.etap.2009.06.004
- Rouas C, Bensoussan H, Suhard D et al (2010) Distribution of soluble uranium in the nuclear cell compartment at subtoxic concentrations. *Chem Res Toxicol* 23(12):1883–1889. doi:10.1021/tx100168c
- Rouas C, Stefani J, Grison S et al (2011) Effect of nephrotoxic treatment with gentamicin on rats chronically exposed to uranium. *Toxicology* 279(1–3):27–35. doi:10.1016/j.tox.2010.09.003
- Rushmore TH, Kong AN (2002) Pharmacogenomics, regulation and signaling pathways of phase I and II drug metabolizing enzymes. *Curr Drug Metab* 3(5):481–490
- Schwartz JH, Flamenbaum W (1976) Uranyl nitrate and HgCl<sub>2</sub>-induced alterations in ion transport. *Kidney Int Suppl* 6:S123–S127
- Souidi M, Gueguen Y, Linard C et al (2005) In vivo effects of chronic contamination with depleted uranium on CYP3A and associated nuclear receptors PXR and CAR in the rat. *Toxicology* 214(1–2):113–122
- t Hoen PA, Rooseboom M, Bijsterbosch MK, van Berkel TJ, Vermeulen NP, Commandeur JN (2002) Induction of glutathione-S-transferase mRNA levels by chemopreventive selenocysteine Se-conjugates. *Biochem Pharmacol* 63(10):1843–1849
- Theron D, Barraud de Lagerie S, Tardivel S et al (2003) Influence of tumor necrosis factor-alpha on the expression and function of P-glycoprotein in an immortalised rat brain capillary endothelial cell line GPNT. *Biochem Pharmacol* 66(4):579–587
- Thiebault C, Carriere M, Milgram S, Simon A, Avoscan L, Gouget B (2007) Uranium induces apoptosis and is genotoxic to normal rat kidney (NRK-52E) proximal cells. *Toxicol Sci* 98(2):479–487
- Tissandie E, Gueguen Y, Lobaccaro JM et al (2007) In vivo effects of chronic contamination with depleted uranium on vitamin D(3) metabolism in rat. *Biochim Biophys Acta* 1770(2):266–272
- Vicente-Vicente L, Quiros Y, Perez-Barriocanal F, Lopez-Novoa JM, Lopez-Hernandez FJ, Morales AI (2010) Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives. *Toxicol Sci* 118(2):324–347. doi:10.1093/toxsci/ktq178
- Waring WS, Moonie A (2011) Earlier recognition of nephrotoxicity using novel biomarkers of acute kidney injury. *Clin Toxicol (Phila)* 49(8):720–728. doi:10.3109/15563650.2011.615319
- Wrenn ME, Durbin PW, Howard B et al (1985) Metabolism of ingested U and Ra. *Health Phys* 48(5):601–633