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Journal of Trace Elements in Medicine and Biology

Renal adaptive response to exposure to low doses of uranyl nitrate and sodium fluoride in mice --Manuscript Draft--

Manuscript Number:	JTEMB-D-20-00473R1
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Abstract:	<p>BACKGROUND: Despite their differences in physicochemical properties, both uranium (U) and fluoride (F) are nephrotoxicants at high doses but their adverse effects at low doses are still the subject of debate. METHODS: This study aims to improve the knowledge of the biological mechanisms involved through an adaptive response model of C57BL/6J mice chronically exposed to low priming doses of U (0, 10, 20 and 40 mg/L) or F (0, 15, 30 and 50 mg/L) and then challenged with acute exposure of 5 mg/kg U or 7.5 mg/kg NaF. RESULTS: We showed that an adaptive response occurred with priming exposures to 20 mg/L U and 50 mg/L F, with decreased levels of the biomarkers KIM-1 and CLU compared to those in animals that received the challenge dose only (positive control). The adaptive mechanisms involved a decrease in caspase 3/7 activities in animals exposed to 20 mg/L U and a decrease in in situ VCAM expression in mice exposed to 50 mg/L F. However, autophagy and the UPR were induced independently of priming exposure to U or F and could not be identified as adaptive mechanisms to U or F. CONCLUSION: Taken together, these results allow us to identify renal adaptive responses to U and F at doses of 20 and 50 mg/L, probably through decrease apoptosis and inflammatory cell recruitment.</p>
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	Edward Calabrese, PhD University of Massachusetts Amherst edwardc@schoolph.umass.edu Expertise in toxicology, risk assessment, environmental health, dose response, hormesis
Response to Reviewers:	

Highlights :

- Uranium and fluoride induce an adaptive response in mice exposed chronically
- Apoptosis regulation is involved in uranium-induced adaptive response
- Inflammatory control is involved in fluoride-induced adaptive response
- Uranium induces UPR and autophagy in the kidney
- Fluoride induces UPR in the kidney

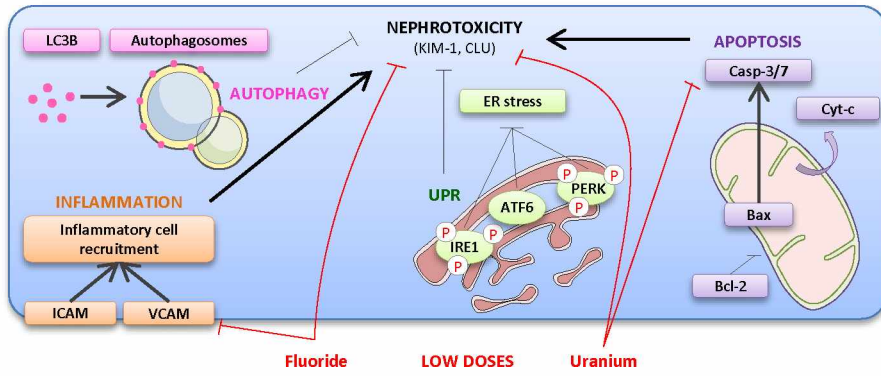


Figure 1

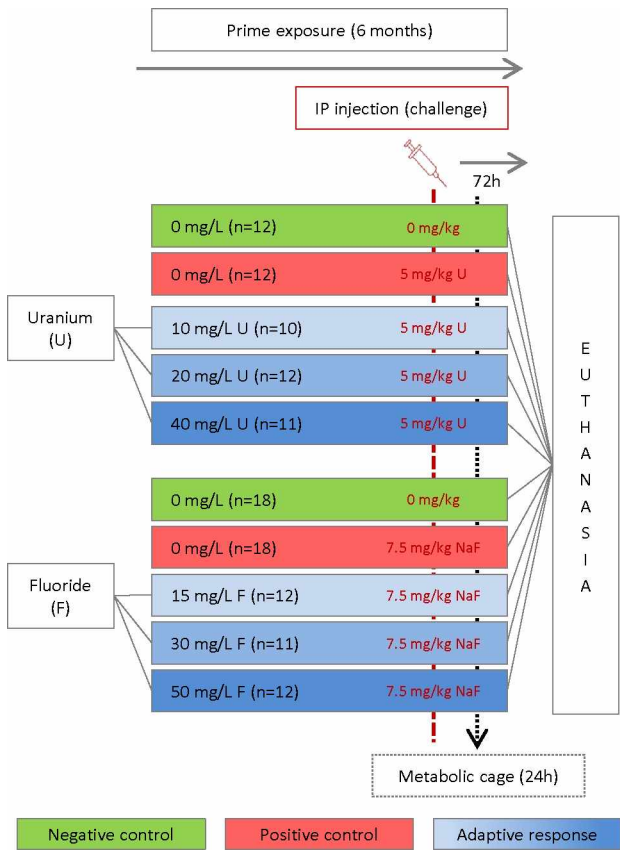


Figure 2

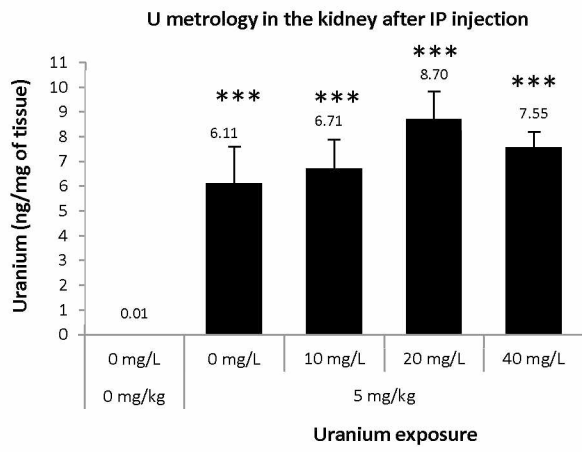


Figure 3

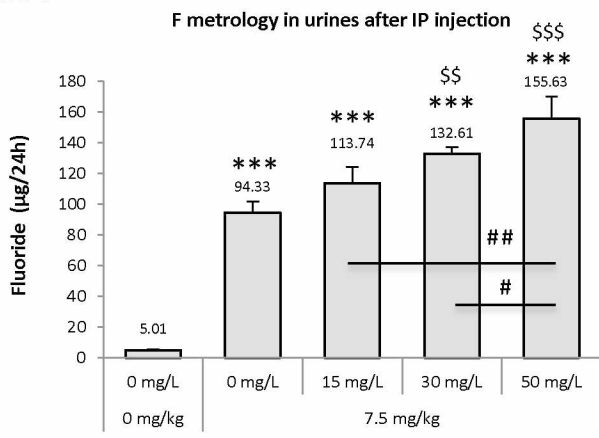


Figure 4

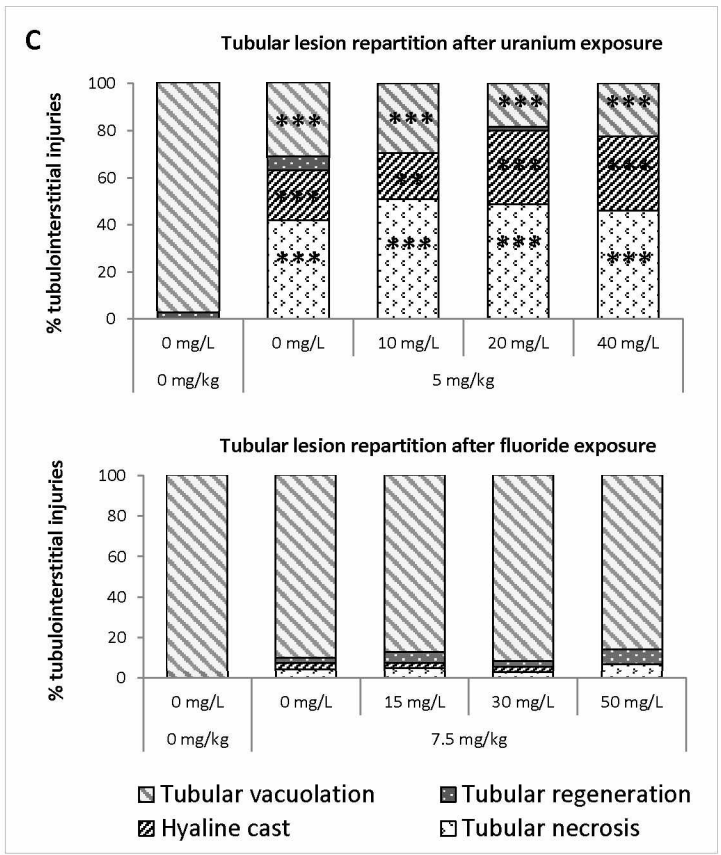
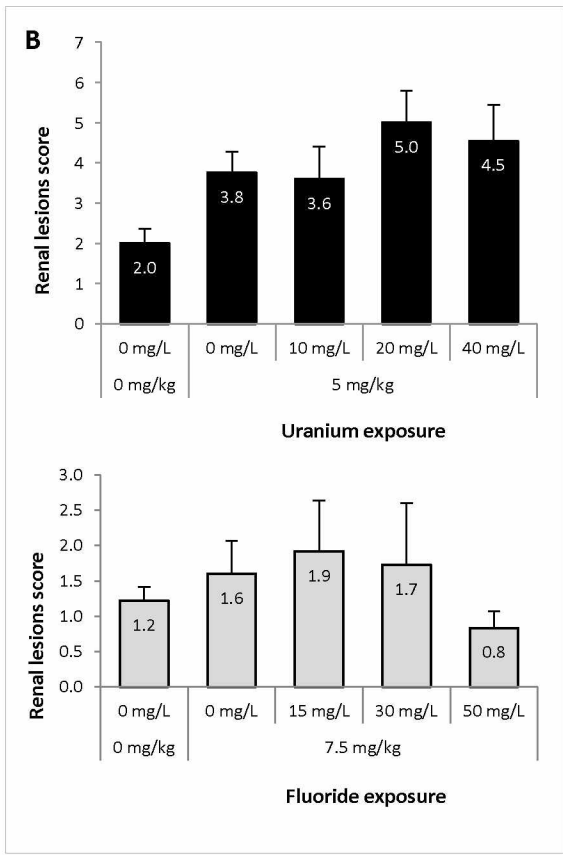
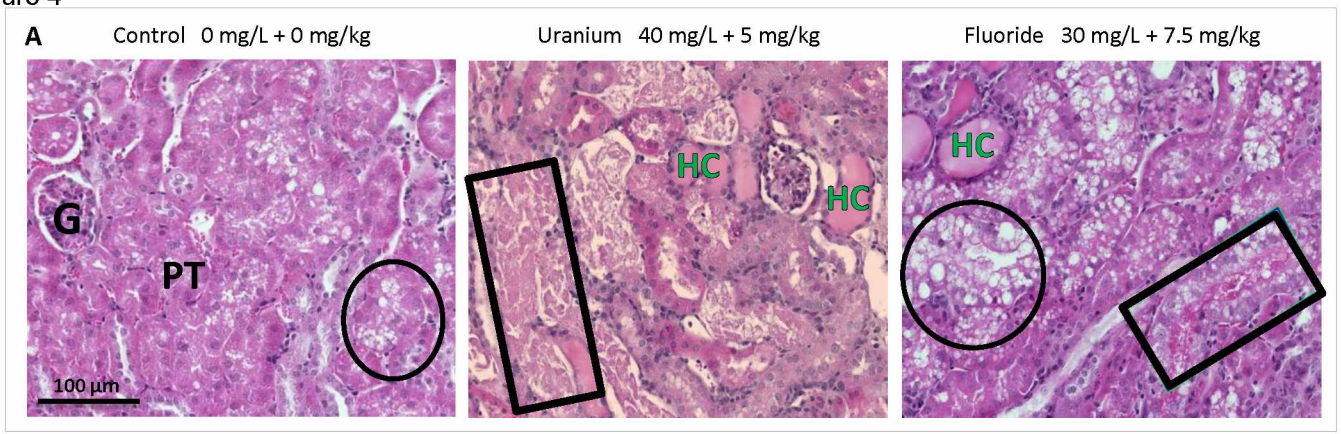
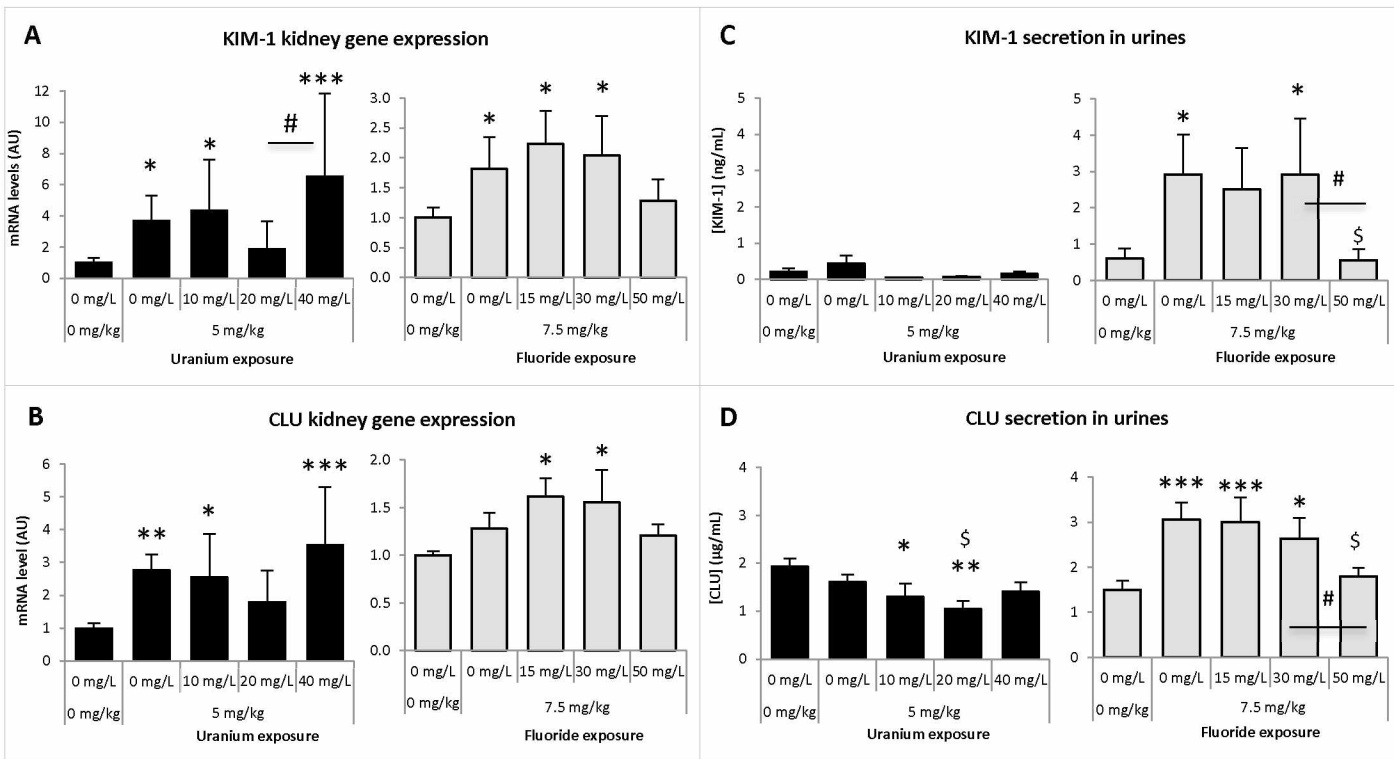


Figure 5



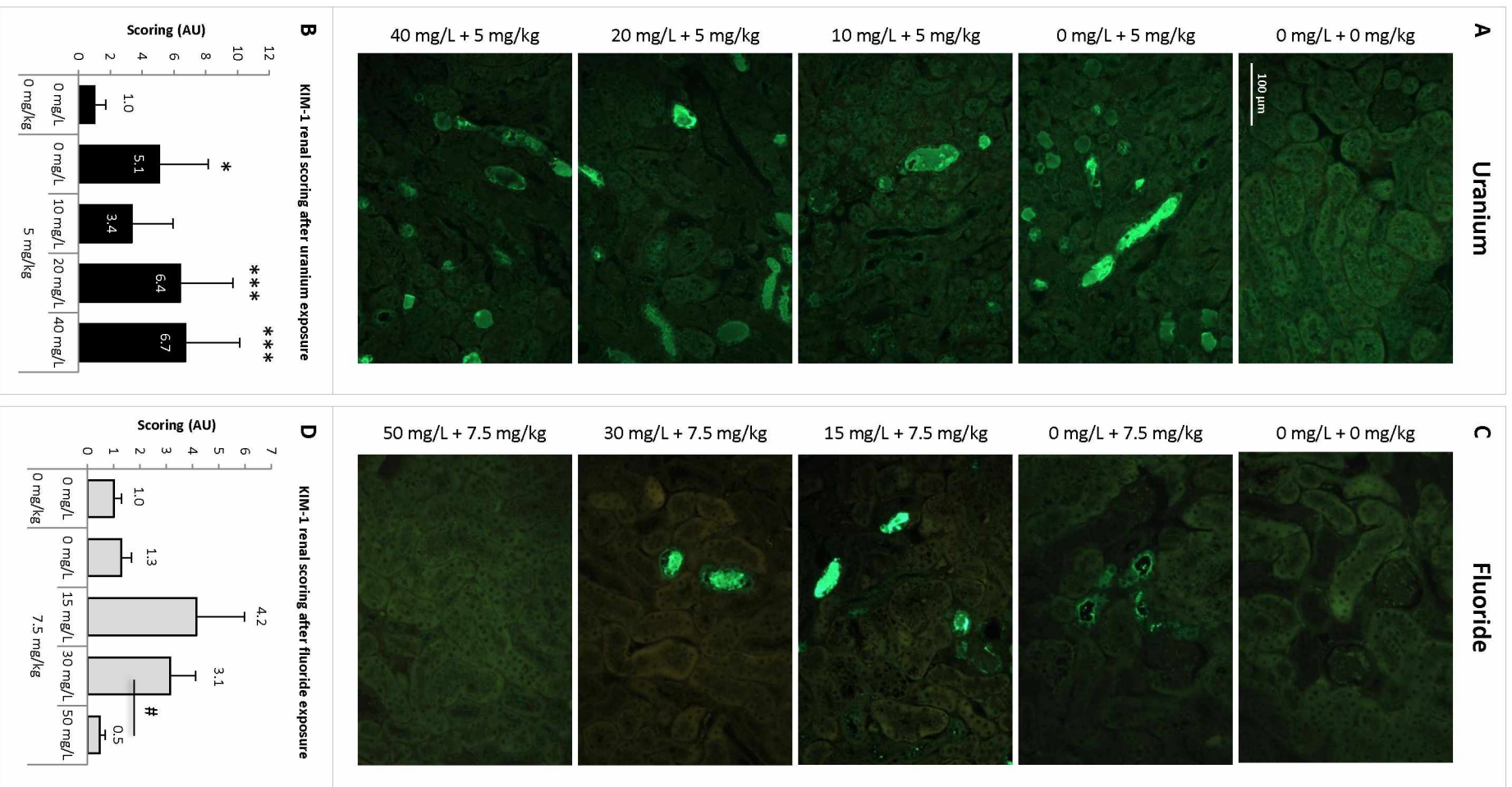


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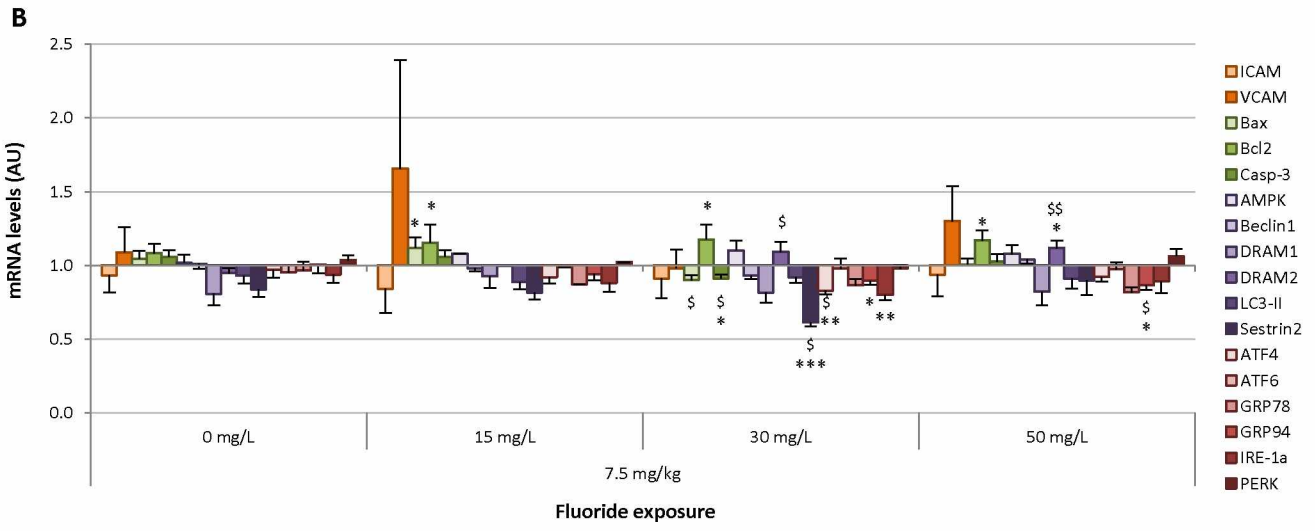
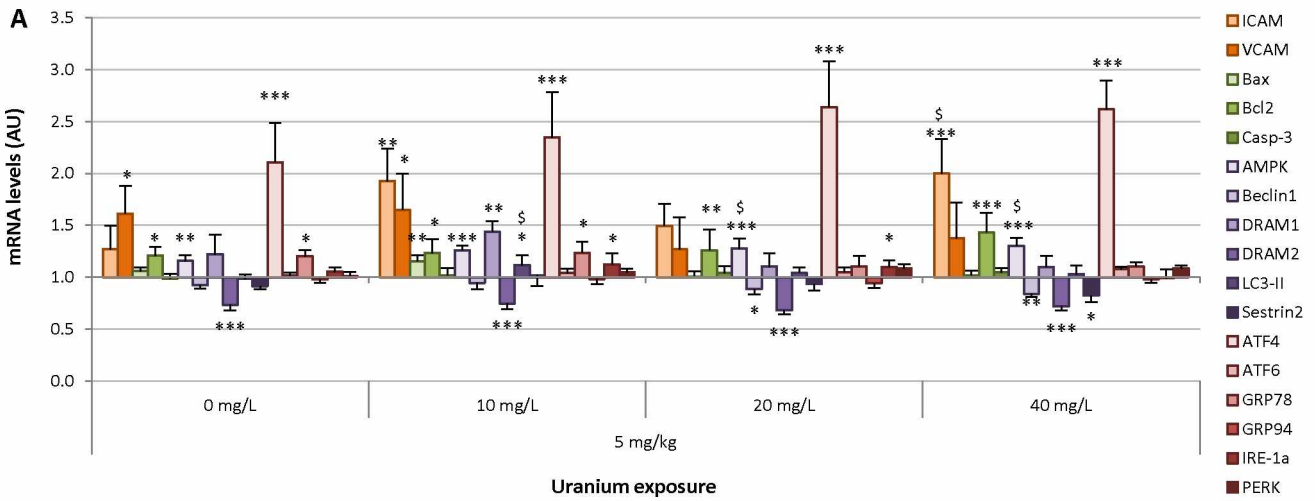


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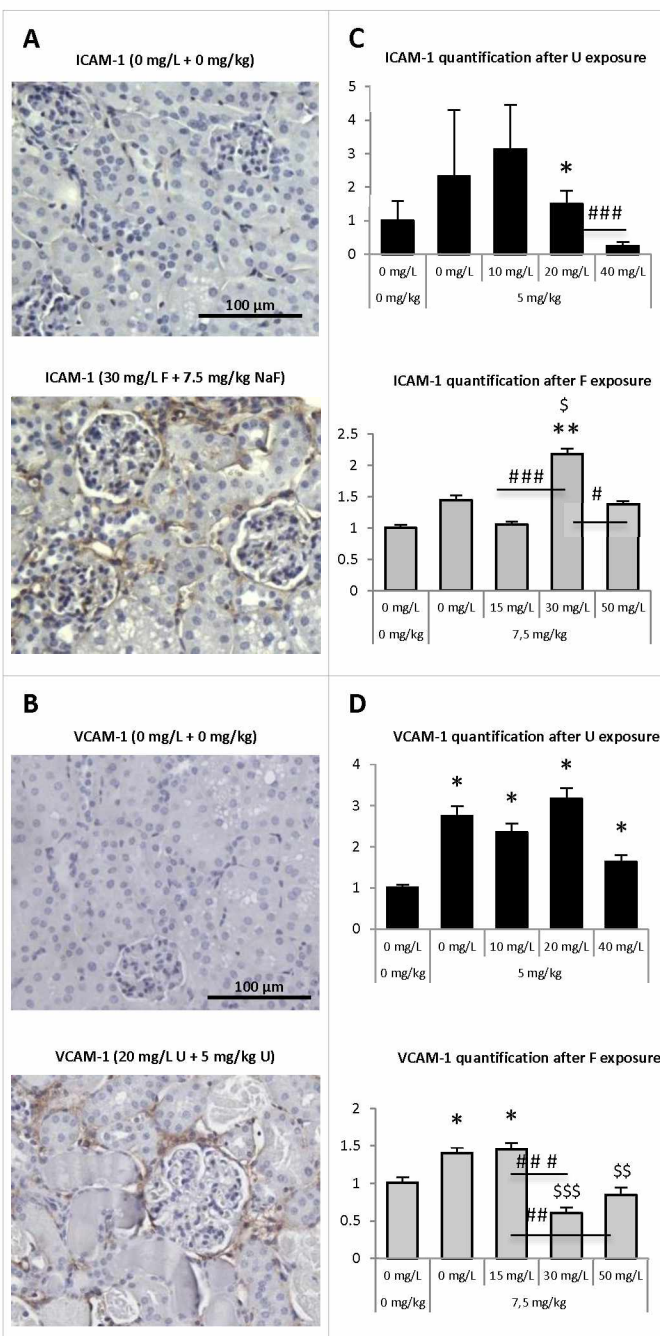
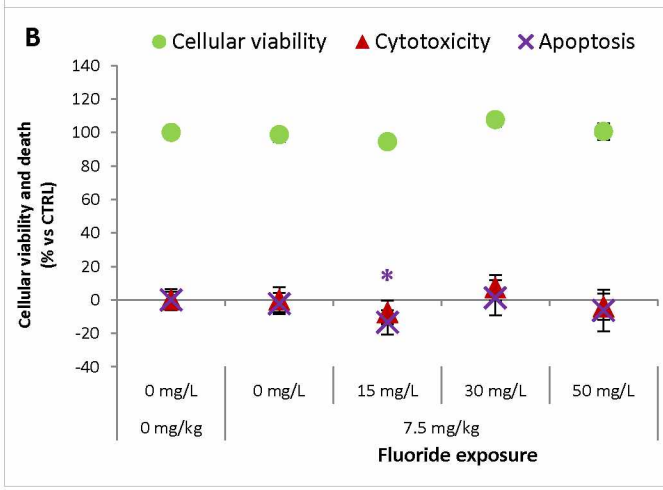
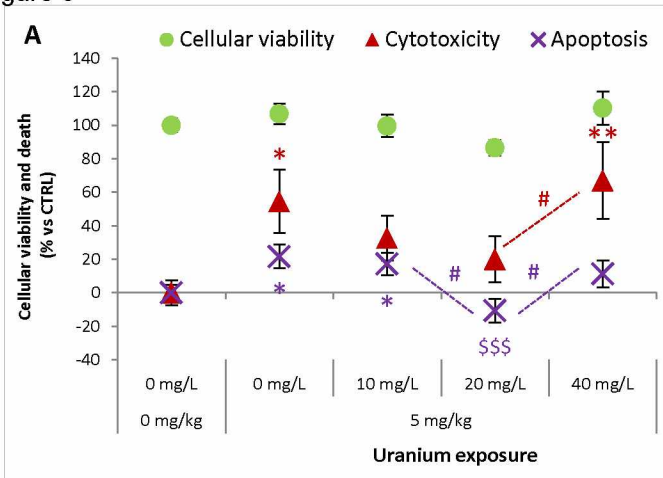
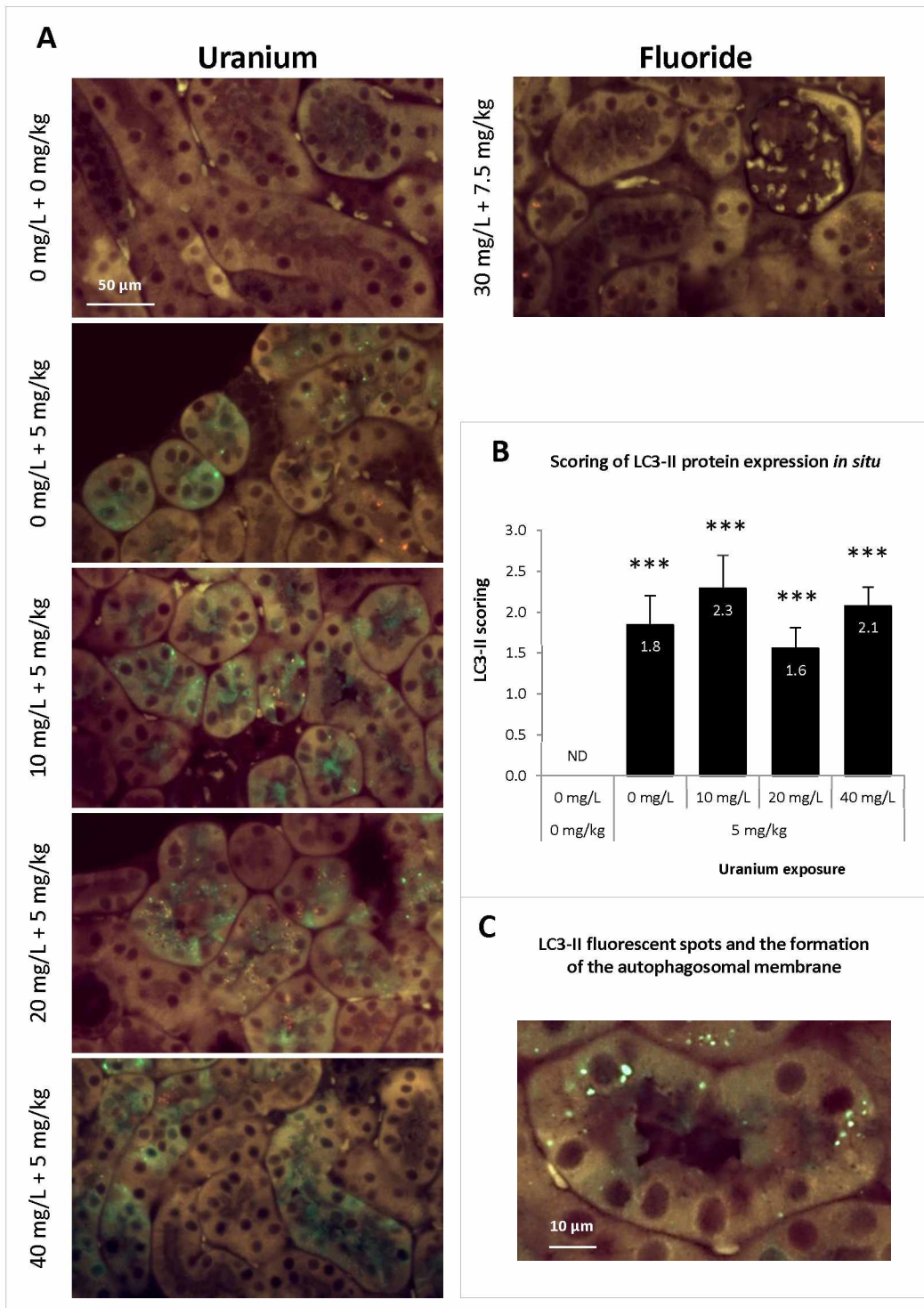
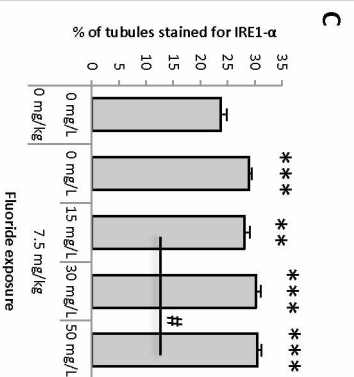
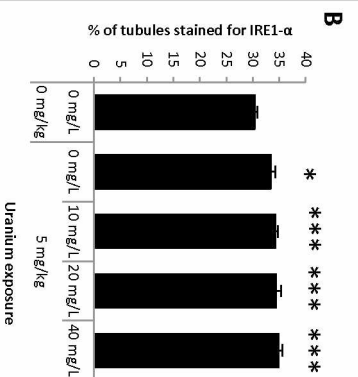
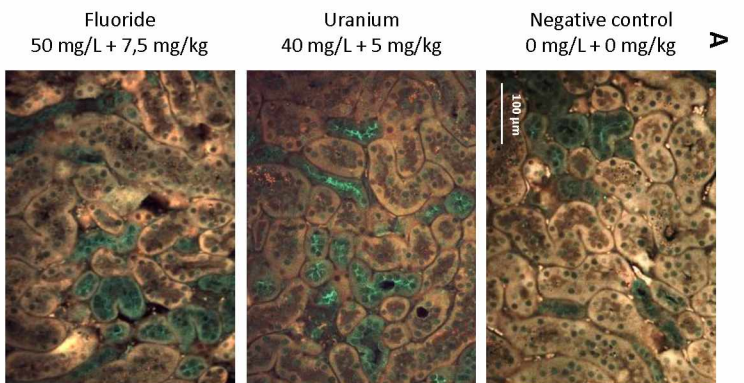


Figure 9







Author contributions

A.B. and Y.G. proposed the concept of the manuscript and designed the experimental study. A.B., Y.G. applied for research ethics approval. A.B., L.C., C.E., V.M., C.G., D.K. and Y.G. performed the experiments and collected data. All authors contributed to data analyses and interpretation. A.B. and Y.G. wrote the manuscript with intellectual input from L.R. and O.B. All authors read and provided input in finalizing the manuscript. Supervision and project administration was done by Y.G.

1 **Renal adaptive response to exposure to low doses of uranyl nitrate**
2 **and sodium fluoride in mice**

3

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18

19

20 Abbreviations:

21 CLU, clusterin; F, fluoride; KIM-1, kidney injury molecule-1; U, uranium; UN, uranyl nitrate;
22 UPR, unfolded protein response; NMDR, non-monotonic dose-response

23 **Abstract:**

24 **BACKGROUND:** Despite their differences in physicochemical properties, both uranium (U)
25 and fluoride (F) are nephrotoxics at high doses but their adverse effects at low doses are still
26 the subject of debate. **METHODS:** This study aims to improve the knowledge of the biological
27 mechanisms involved through an adaptive response model of C57BL/6J mice chronically
28 exposed to low priming doses of U (0, 10, 20 and 40 mg/L) or F (0, 15, 30 and 50 mg/L) and
29 then challenged with acute exposure of 5 mg/kg U or 7.5 mg/kg NaF. **RESULTS:** We showed
30 that an adaptive response occurred with priming exposures to 20 mg/L U and 50 mg/L F, with
31 decreased levels of the biomarkers KIM-1 and CLU compared to those in animals that received
32 the challenge dose only (positive control). The adaptive mechanisms involved a decrease in
33 caspase 3/7 activities in animals exposed to 20 mg/L U and a decrease in *in situ* VCAM
34 expression in mice exposed to 50 mg/L F. However, autophagy and the UPR were induced
35 independently of priming exposure to U or F and could not be identified as adaptive
36 mechanisms to U or F. **CONCLUSION:** Taken together, these results allow us to identify renal
37 adaptive responses to U and F at doses of 20 and 50 mg/L, probably through decrease apoptosis
38 and inflammatory cell recruitment.

39 **Keywords:** uranyl; fluoride; kidney; adaptation; apoptosis; inflammation

40

41 **Introduction**

42 Both uranium (U) and fluoride (F) are environmental and anthropogenic contaminants due
43 to mining, or the use of pesticides in agriculture fields, for example. U and F concentrations can
44 vary greatly from one place to another throughout the world. As an example, high water
45 concentrations of fluoride are found in the northeastern region of Niger, where the concentration
46 reaches 2.07 mg/L in surface waters and 2.42 mg/L in groundwaters [1]. The highest
47 concentrations found in drinking water are 20 mg/L for U in well water of the south of Finland
48 [2, 3] and 30 to 50 mg/L for F in water contaminated by geological sources [4], whereas the
49 world health organization (WHO) recommends maximal concentrations of 30 µg/L U, and
50 1.5 mg/L F [5, 6].

51 Once ingested, U and F mainly accumulate in the kidney [7-12], which is the most sensitive
52 organ to U and F because of the nephron's functions of filtration, transport and reabsorption [4,
53 8, 13, 14]. After high-dose exposure in animals, U- and F-induced kidney lesions are mainly
54 localized in the cortical and juxtaglomerular regions of the kidney, where the proximal
55 convoluted tubules are located [15-19] but the effects of low doses are controversial in
56 epidemiological [10, 20-27] or experimental studies [28-36]. A previous study by our
57 laboratory, showed that chronic exposure to different doses of U, from 1 to 600 mg/L U in
58 drinking water, did not induce any nephrotoxicity in rats despite renal accumulation at a level
59 known to be nephrotoxic when induced acutely ($> 3\mu\text{g/g}$ of kidney tissue) [18]. In this study,
60 Poisson et al. (2014) showed that U even induced antioxidative mechanisms, such as
61 glutathione overexpression, which suggests that renal adaptation occurs in response to chronic
62 exposure to low doses, as also hypothesized in other *in vivo* and *in vitro* studies [37-39]. Chronic
63 exposure to F, from 15 ppm to 100 ppm in rats, induces specific biomarkers of nephrotoxicity
64 [40-43] but it can reduce the nephrotoxicity induced by a second exposure to high dose of

65 gentamicin in rats [44]. The authors demonstrated that F induces cytoprotective factors such as
66 heat shock protein 72, also suggesting a potential adaptive response.

67 The non-linear relation or the nonmonotonic dose-response (NMDR) model, stipulates that
68 below a certain level, low doses of radiations or chemicals could be consider beneficial for the
69 organism [45-48]. The adaptive response that can explain this NMDR and the ability of a
70 biological system to exhibit fewer detrimental effects in response to exposure to high dose of a
71 toxicant when it has been pre-exposed to a small priming stress [49]. To study this potential
72 renal adaptation to U and F and improve our understanding of health risks at low levels, an
73 animal model of exposure was used. Chronic priming exposures to low doses of U or F were
74 followed by a challenge treatment, delivered acutely and at moderate nephrotoxic doses. This
75 strategy allowed us to detect the effects triggered by priming exposures to low doses of U and
76 F and to identify some underlying mechanisms.

77

78 **Materials and Methods**

79 *2.1. Animals*

80 The study was performed in 8-week-old male C57BL/6J mice weighing 25 ± 5 g at the
81 beginning of the study provided by Charles River (Saint Germain Nuelles, France). Animals
82 were housed in groups of three under controlled conditions. Three weeks of acclimatization was
83 provided before the beginning of the study. Water and food were supplied *ad libitum*. Body
84 weight was monitored once a week and did not show differences between groups (data not
85 shown). The animals were housed for a 24-h period in metabolic cages every month. The study
86 was approved by the IRSN Animal Care Committee #81 and conducted in accordance with
87 French legislation on the protection of animals used for experimental purposes (EC Directive
88 2010/63/EU and French Decret 2013–118). All experiments were approved by the Ethics

89 Committee #81 and authorized by the French Ministry of Research under the reference
90 APAFIS#9324-2017031613376362 v1 (internal project number P16-05), delivered on 4 July
91 2017.

92 *2.2. Chronic exposure to and acute exposure with U or F and urine collection*

93 U was obtained as uranyl nitrate (UN) (Merck-Prolabo, Fontenay-sous-bois, France),
94 which had a specific activity of 14×10^3 Bq/g and was composed of 99.74% ^{238}U , 0.26% ^{235}U ,
95 and 0.001% ^{234}U . F was obtained as sodium fluoride from Sigma (Saint-Quentin Fallavier,
96 France). Ketamine (Imalgène 1000) and Xylazine (5% Rompun) solutions were obtained from
97 Centravet (Dinan, France). Volvic® mineral water (Volvic) was used for exposure of animals
98 through drinking water due to its low minerals concentration to avoid precipitation. Fluoride
99 level (0.2 mg/L) and uranium level (0.28 $\mu\text{g/L}$) were measured in Volvic mineral water.

100 The mice were divided into different groups (n=10 to 18 per group), as shown in Figure 1,
101 and were chronically exposed with either U (10, 20, or 40 mg/L) or F (15, 30, or 50 mg/L) in
102 Volvic drinking water for 6 months and then injected acutely with 5 mg/kg UN ($\text{UO}_2(\text{NO}_3)_2$)
103 or 7.5 mg/kg F (corresponding to 16.6 mg/kg NaF). There was a negative control group, which
104 drank Volvic water during the chronic contamination period and received an ip injection of
105 NaCl, and a positive control group, which drank Volvic water during the chronic contamination
106 period and received an injection of either 5 mg/kg UN or 7.5 mg/kg NaF. For chronic
107 contamination, UN and NaF were diluted in Volvic water, and the drinking solutions were
108 mixed with a magnetic agitator. During the contamination period, water consumption was
109 monitored weekly and do not show differences between groups (data not shown). For the acute
110 injections, UN and NaF were diluted in a NaCl solution and filtered the day of the injection.
111 The animals were then euthanized 72 hours later by exsanguination under anesthesia. The
112 challenge doses and the delay before euthanasia were based on our previous results [28] and on
113 the literature. Previous experiments showed a peak of renal effects 72 hours post-injection of U

114 and toxicity at the dose chosen [15, 30, 31, 34, 50, 51]. The experimental design and the animal
115 grouping are presented in Figure 1.

116 Every month during the chronic contamination period and immediately after the acute
117 injection, the animals were housed individually in metabolic cages (Tecniplast, Decines
118 Charpieu, France) for a 24-hour period to collect urine samples at room temperature without
119 food or water contamination. After collection, the urine samples were subsequently centrifuged
120 at 3000 x g for 2 min at 4°C, and the supernatants were isolated and stored at -80°C.

121 *2.3. Euthanasia and kidney collection*

122 Seventy-two hours after injection, the animals were anaesthetized by an ip injection of
123 100 mg/kg ketamine and 10 mg/kg xylazine and euthanized by exsanguination. Both kidneys
124 were collected, weighed wet and sagittally cut. No differences in kidney weight were found
125 between groups (data not shown). Half of the right kidney was placed in 4% formaldehyde for
126 histopathological analysis, and the other half was used for U or F quantification. The entire left
127 kidney was designated for biomolecular analysis after being flash-frozen in liquid nitrogen and
128 stored at -80°C.

129 *2.4. U and F measurements in the urine and kidney tissues*

130 U was quantified in the kidney by inductively-coupled plasma mass spectrometry
131 (ICP-MS) (ICP-MS, PQ, Excell, Thermo Electron, Villebon-sur-Yvette, France), as previously
132 described [18, 28]. The half-kidney was digested in nitric acid and hydrogen peroxide, and the
133 samples were then evaporated and dissolved in nitric acid. After the appropriate dilution, the U
134 content was measured using bismuth as an internal control and a U calibration curve. The
135 detection limit of U was determined by ICP-MS: 0.5 ng/L for ²³⁸U and 0.01 ng/L for ²³⁵U. After
136 dilution, F was quantified in the urine by a potentiometric method using an ion selective
137 electrode (Thermo Scientific Orion, Villebon-sur-Yvette, France).

138 *2.5. Histopathology and immunostaining*

139 After 24 hours in 4% formaldehyde, the preserved half-kidney was dehydrated before
140 being embedded in paraffin and cut into 5 μm slices with a microtome. Hematoxylin, eosin,
141 and saffron (HES) staining was conducted, and the slides were sent blinded to an outside
142 laboratory of pathologists for anatomopathological analyses (Biodoxis Laboratories,
143 Romainville, France). Glomerular damage was defined by the presence of glomerulosclerosis
144 and glomerular cystic dilatation, whereas tubular lesions were defined by the following: (1)
145 necrosis; (2) regeneration and dilatation of the tubules; (3) interstitial inflammation; and (4)
146 fibrosis. The different types of lesions were scored from 0 to 4 for each animal. The total sum
147 of the scores of all lesions corresponded to the global score, and the percentage of each lesion
148 among the tubulointerstitial lesions was then calculated as previously described [28, 52].
149 Paraffin-embedded slices were deparaffinized and hydrated in descending gradations of ethanol
150 and in 3% H_2O_2 diluted in PBS to block endogenous peroxidase activity. For kidney injury
151 molecule-1 (KIM-1) staining, antigen retrieval was achieved with citrate buffer at $\text{pH}=6$.
152 Sections were then incubated overnight with anti-KIM-1 (Abcam, Paris, France, ab47635) at a
153 200-fold dilution at 4°C in a moist chamber. After washing, the slices were incubated with an
154 Alexa Fluor-conjugated goat anti-rabbit secondary antibody (1/1 000, Abcam, ab150061).
155 Finally, the nuclei were stained with Vectashield antifade mounting medium containing DAPI
156 for fluorescence visualization. Ten photomicrographs per animal were collected with a LEICA
157 DM 4000B fluorescence microscope, and semiquantification of fluorescent labeling was
158 performed with Histolab Software (version 8.12, Microvision Instruments, Lisses, France). For
159 intercellular adhesion molecule and vascular cell adhesion molecule (VCAM) staining, antigen
160 retrieval was achieved with Tris/EDTA buffer at $\text{pH}=9$. Sections were then incubated for 1 hour
161 with anti-ICAM-1 (Abcam, ab119871) diluted 250-fold or with anti-VCAM-1 (Abcam,
162 ab134047) diluted 1000-fold at room temperature in a moist chamber. After washing, the slices

163 were incubated with anti-rat and anti-rabbit secondary antibodies and finally developed using a
164 DAB revelation kit (MMFrance, Brignais, France). Counterstaining with hematoxylin was
165 performed to identify renal structures. For Microtubule-associated protein 1A/1B-light chain
166 3B (LC3-II) and Endoribonuclease/protein kinase IRE1-like protein (IRE1- α), antigen retrieval
167 was achieved respectively with Tris/EDTA buffer at pH=9 or citrate buffer at pH=6. Sections
168 were then incubated respectively for 1 or 2 hours with anti-LC3-II (Abcam, ab192890) diluted
169 1500-fold or with anti-IRE-1 α (Novus Biologicals, NB100-2323) diluted 250-fold at room
170 temperature in a moist chamber. After washing, the slices were incubated with an Alexa Fluor-
171 conjugated goat anti-rabbit secondary antibody (1/1 000, Abcam, ab150061). Finally, the nuclei
172 were stained with Vectashield antifade mounting medium containing DAPI for fluorescence
173 visualization. Ten photomicrographs per animal were collected with a LEICA DM 4000B
174 fluorescence microscope.

175 *2.6. KIM-1 and CLU detection in the urine*

176 KIM-1 and clusterin (CLU) were measured in urine samples using ELISA kits according
177 to the manufacturer's instructions (R&D Systems, Lille, France). To comply with the
178 concentration intervals of the assay, the urine samples were diluted 1:100 for the CLU assay
179 and 1:10 to 1:100 for the KIM-1 assay.

180 *2.7. Real-time RT-PCR*

181 Total RNA was extracted from the renal cortex, dissected under binocular microscope, by
182 following the manufacturer's instructions (Total RNA Isolation Kit, Qiagen, Les Ulis, France)
183 and was reverse-transcribed into cDNA with a High-capacity cDNA Reverse Transcription Kit
184 (Applied Biosystems Life Technologies, Courtabœuf, France). Real-time polymerase chain
185 reactions (RT-PCR) was performed using two methods, namely, SYBR Green and Taqman,
186 with 10 μ L of 1 ng/ μ L cDNA per well and 2.5% v/v primers (Fisher Scientific, Illkirch, France),
187 83% v/v SYBR Green (Fisher Scientific, Illkirch, France), and 14.5% v/v sterile water. Table

188 1 shows the accession number and primer sequences of each gene used in this study for the
 189 SYBR Green method, whereas Table 2 shows the accession number of the Taqman genes used
 190 to analyze the mRNA levels of proteins involved in nephrotoxicity, inflammation, apoptosis,
 191 autophagy and the unfolded protein response (UPR). QuantStudio 12K Flex (Applied
 192 Biosystems Life Technologies, Courtabœuf, France) was used to detect the real-time RT-PCR
 193 products. The comparative $\Delta\Delta C_t$ method was used to determine the relative quantification of
 194 each gene expression in comparison with the geometric average of the C_t values of the
 195 housekeeping genes β -actin (ACTB), hypoxanthine-guanine phosphoribosyltransferase
 196 (HPRT) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Finally, the fold induction
 197 for each treated group was calculated relative to the control group (NaCl).

198 **Table 1.**Primer sequences of the genes analyzed by real-time RT-PCR with SYBR Green.

Gene	Accession Number	Forward	Reverse
BAX	NM_007527	CCCCCGAGAGGTCTTCTT	GCGGCCCCAGTTGAAGT
BCL2	NM_009741	TGGGATGCCTTTGTGGAAGT	CAGAGACAGCCAGGAGAAATCAA
CASP3	NM_001284409	CTGGACTGTGGCATTGAGACA	GCCTCCACCGGTATCTTCTG
CLU	AF182509	TCGGGCATCTGGCATCA	AAGCTCACGGGCGAAGAAC
GAPDH	GU214026	TCCACTCACGGCAAATCAACG	TAGACTCCACGACATACTCAGC
HPRT	NM_013556	TGCTGCGTCCCCAGACTTTTG	AGATAAGCGACAATCTACCAGAGG
ICAM-1	NM_010493	CACCCCAAGGACCCCAAGGAGAT	CGACGCCGCTCAGAAGAACCAC
KIM-1	BC053400	TTTCAGGCCTCATACTGCTTCTC	TGACCCACCACCCCTTT
VCAM-1	BC029823	TCGCGGTCTTGGGAGCCTCA	TGACCGTGACCGGCTTCCCA

199

200 **Table 2.**Accession numbers of the Taqman genes used to analyze autophagy and the UPR.

Gene	Accession Number	Taqman gene reference
ACTB	NM_007393.5	Mm02619580_g1
HPRT	NM_013556.2	Mm03024075_m1
GAPDH	NM_001289726.1	Mm99999915_g1

AMPK	NM_001013367.3	Mm01296700_m1
ATF4	NM_001287180.1	Mm00515325_g1
ATF6	NM_001081304.1	Mm01295319_m1
BECLIN1	NM_019584.3	Mm01265461_m1
DRAM1	NM_027878.2	Mm00503627_m1
DRAM2	NM_001025582.2	Mm00509019_m1
GRP78	NM_001163434.1	Mm00517691_m1
GRP94	NM_011631.1	Mm00441926_m1
IRE1-A	NM_023913.2	Mm00470233_m1
LC3-II	NM_026160.4	Mm00782868_sH
PERK	NM_010121.2	Mm00438700_m1
SESTRIN2	NM_144907.1	Mm00460679_m1

201

202 *2.8. Cellular viability and cellular death measurements*

203 Pieces of the renal cortex (25 mg) were placed into 1.5 mL Eppendorf tubes with 250 μ L
204 hypotonic buffer containing 25 mM HEPES, 5 mM MgCl₂, 1 mM EDTA, 1 mM Pefabloc,
205 1 μ g/mL pepstatin, 1 μ g/mL leupeptin, and 1 μ g/mL trypsin inhibitor. Then, the pieces were
206 ground with micropestles and centrifuged at 13,000 rpm for 15 min at 4°C. The supernatants
207 were collected and diluted 50-fold, and the protein concentrations of the supernatants were
208 determined by the Bradford test. Then, the concentrations of the samples were adjusted to
209 1 mg/mL before the determination of cellular viability, cytotoxicity and caspase activity with a
210 detection kit (Promega “ApoTox-Glo™ Triplex Assay”, Charbonnières-les-Bains, France,
211 G6320). The cleavage of the peptide GF-AFC substrate generated a fluorescent signal, which
212 was used to measure live-cell protease activity in the kidneys. Another fluorogenic peptide
213 substrate, called bis-AAF-R110 substrate, was used to measure dead-cell protease activity
214 released during the loss of the cellular membrane integrity. Caspase 3/7 activity was determined

215 by measuring the release of luciferin after the cleavage of the luminogenic DEVD-peptide
216 substrate by caspases 3 and 7.

217 *2.9. Statistical analyses*

218 Statistical analyses were performed using SigmaPlot 11.0 software (Systat Software, Inc.,
219 Erkrath, Germany), except for the semiquantification of the immunostainings, for which
220 Histolab Software (Microvision Instruments) was used as described previously [28]. One-way
221 analysis of variance (ANOVA) was used to compare each group, and the Holm-Sidak method
222 was applied for multiple comparisons. For the semiquantification of immunostainings, a general
223 estimating equation (GEE) was calculated using R 3.4.4 (version 3.4.4, R Core Team, Boston,
224 MA, USA) and RStudio software packages (version 1.1.423, RStudio Inc., Boston, MA, USA).
225 The level of significance was set at $p < 0.05$.

226

227 **3. Results**

228 *3.1. U and F measurements*

229 U content was measured in the mouse kidneys, 72 hours after ip treatment (Figure 2),
230 whereas F content was measured in the urine 24 hours after ip treatment (Figure 3). The basal
231 content of U in the kidneys of negative controls was very low (9.3 ng/g of tissue \pm 0.3),
232 corresponding to previously described natural U concentrations [18, 28]. For all treated groups
233 a significantly increased U content was observed ($p < 0.001$), reaching a maximum of
234 8.70 ng/mg of tissue (Figure 2). The level of U in the kidneys did not differ between groups
235 regardless of the dose of U in the drinking water (10 to 40 mg/L) and it was similar to the level
236 measured in the positive control group.

237 F could only be measured in the urine, as the tissue content of F in the control and treated
238 animals was too low to show any difference by potentiometry (data not shown) [28]. Urine was

239 collected during two 24-hour periods, one before ip injection and another immediately after.
240 Before acute ip treatment, only the F level of the 50 mg/L F group, with a concentration of
241 36.2 $\mu\text{g F}/24\text{h}$, was significantly increased compared to that of the control group ($p<0.05$) (data
242 not shown), whereas after ip treatment a significant dose-dependent increase of F in the urine
243 was observed (Figure 3). The negative control mice showed a basal level of 5.0 $\mu\text{g F}/24$ hours,
244 whereas the positive controls excreted 94.3 μg of F in the urine 24 hours after ip treatment. The
245 highest priming dose of 50 mg/L F induced an increase in F excretion (155.6 $\mu\text{g}/24$ hours \pm
246 14.4) that was significantly different from that in the 15- and 30-mg/L F-exposed groups
247 ($p<0.001$ and $p<0.05$, respectively), confirming that the increases in these measurements were
248 dependent on the dose of F chronic exposure.

249 *3.2. Histopathological scoring in the kidney after U and F exposure*

250 Seventy-two hours after challenge, HES staining (Figure 4A) allowed us to determine
251 global scoring of damage induced in the kidney by U and F (Figure 4B). In addition, the extent
252 of these damages (hyaline cast appearance, tubular vacuolization, necrosis and regeneration)
253 was quantified for each experimental group (Figure 4C) as previously done in our studies [28,
254 52].

255 The extent of renal lesion was detected in a limited number of animals, and there was no
256 significant increase between the scores of the exposed and control groups (Figure 4B). All
257 animals in the negative control group showed tubular vacuolization in their kidneys, but with
258 low severity ($p>0.05$). Global scores were increased after U exposure, but not significantly,
259 probably because of the basal damage in the negative control animals. However, U significantly
260 induced the formation of hyaline casts, tubular vacuolization and necrosis in all treated animals
261 compared to the negative control animals ($p<0.005$ for hyaline casts in 10 mg/L U-treated
262 animals, and $p<0.001$ for all other lesions) (Figure 4C). Non-exposed animals presented tubular
263 vacuolization, whereas hyaline cast formation and tubular necrosis were specifically induced

264 after U exposure. However, no significant differences between pre-exposed animals and
265 positive control animals were observed under our experimental conditions, and no significant
266 differences were observed between the groups of animals exposed to U or F through drinking
267 water. The HES scores of the F groups were similar but lower. Nevertheless, hyaline cast
268 formation, tubular regeneration and necrosis appeared in some animals exposed to F.

269 The histopathologic analysis revealed that neither U nor F induced glomerular damage in
270 the kidneys.

271 *3.3. Biomarkers of U- and F-induced nephrotoxicity: KIM-1 and CLU mRNA, urinary secretion* 272 *and in situ detection*

273 CLU and KIM-1 are proteins considered early biomarkers for a wide variety of acute and
274 chronic renal impairments [53-56].

275 As shown in Figure 5A, the gene expression of KIM-1 was 3.7- and 1.8-fold increased by
276 U and F challenge treatment, respectively ($p<0.05$). Similarly, the gene expression of CLU was
277 induced in the kidneys by acute treatment with U, increasing 2.8-fold ($p<0.005$) (Figure 5B),
278 but not after F acute exposure. Interestingly, for both U and F, all groups exposed to the priming
279 doses through drinking water, except for the 20 mg/L U and the 50 mg/L F prime-exposed
280 groups, had significantly induced KIM-1 and CLU gene expression. Indeed, the KIM-1 and
281 CLU expression levels were not significantly different from those of the negative control group
282 at these concentrations, indicating a possible decrease in nephrotoxicity and a potential adaptive
283 response to these doses.

284 When looking at the urinary secretion of these proteins in Figures 5C and 5D, we can see
285 that the levels of both proteins were significantly augmented in the urine of animals from the
286 positive F control group, showing 4.8- and 2.1-fold increases in KIM-1 and CLU, respectively.
287 Interestingly, the groups previously exposed to 15 and 30 mg/L F and then challenged with 7.5
288 mg/kg F injection, but not the group exposed to the priming dose of 50 mg/L, exhibited

289 increased urinary secretion of KIM-1 and CLU. Indeed, under these conditions (50 mg/L + 7.5
290 mg/kg), the KIM-1 and CLU urinary levels were not significantly different than those of the
291 negative control group. Their urinary levels were also significantly lower than those of the
292 positive control group (0 mg/L + 7.5 mg/kg) ($p < 0.05$), confirming the hypothesis of reduced
293 nephrotoxicity and a potential adaptive response after pre-exposure to 50 mg/L F. In the case
294 of U, treatment with the challenge dose alone did not induce KIM-1 or CLU 24 hours after ip
295 injection. On the other hand, a priming exposure to 10 or 20 mg/L U induced 1.5- and 1.8-fold
296 decreases, respectively, in the basal level of CLU secretion in the urine ($p < 0.05$ and $p < 0.005$).
297 These differences between urinary and mRNA levels could be explained by the difference in
298 the timing of the analysis (24 and 72 hours after ip injection) and suggested that U-induced
299 nephrotoxicity cannot be detected 24 hours after ip treatment but can only be detected after a
300 longer period (48 or 72 hours).

301 The *in situ* detection of KIM-1 is shown in Figure 6. This protein was detected in the
302 proximal tubules of some control animals, revealing a basal level of renal impairment, and in
303 all U- and F-exposed mice (Figures 6A and 6C). In the group exposed to 50 mg/L F, only a few
304 animals showed *in situ* KIM-1 expression in the kidneys. *In situ* KIM-1 expression was
305 analyzed relative to that in the control group (fixed at 1.0), and the analysis revealed that acute
306 treatment with U alone significantly induced a 5.1-fold increase in protein expression in the
307 kidneys ($p < 0.05$), whereas F did not, as shown in Figures 6B and 6D. A significant increase in
308 KIM-1 was detected in all groups that underwent priming exposure to U ($p < 0.001$), except those
309 that were exposed to a dose of 10 mg/L. For F, *in situ* KIM-1 expression in the kidneys was not
310 significantly induced in any group, even though increasing tendencies, specifically 4.2- and 3.1-
311 fold increases were observed in mice contaminated with 15 and 30 mg/L F, respectively before
312 challenge. Interestingly, chronic priming exposure to 50 mg/L F led to a level of KIM-1 that
313 appeared to be lower than the level in negative controls (50% of the basal expression).

314 Nevertheless, these results are in accordance with the gene expression of KIM-1 and its urinary
315 secretion and consolidate the hypothesis of an adaptive response for the group exposed to a
316 priming concentration of 50 mg/L F.

317 *3.4. Gene expression of factors involved in the potential adaptive mechanisms of U and F*

318 The mRNA expression of the genes shown in Figure 7 allows us to provide general
319 guidance on the mechanisms potentially implicated in the renal adaptive response to U and F.
320 The fold induction of each group was calculated relative to the control group which was fixed
321 at an mRNA level of 1.0 (group not shown in the figure).

322 ICAM and VCAM are both proteins involved in the recruitment of inflammatory cells and
323 therefore are good markers of the potential inflammation induced by U and F. The mRNA level
324 of ICAM was not induced in positive controls, whereas it was induced 1.9- and 2.0-fold in the
325 kidney of mice that received the priming doses of 10 and 40 mg/L U ($p < 0.005$ and $p < 0.001$,
326 respectively). The overexpression of VCAM was slightly induced in animals from the positive
327 control group and the group pre-exposed to 10 mg/L, ($p < 0.05$). No induction of either ICAM
328 or VCAM was observed at the priming concentration of 20 mg/L, suggesting that no
329 inflammation was induced at this concentration (Figure 7A). In contrast, no induction of either
330 ICAM or VCAM mRNA expression was observed after F exposure under our conditions
331 (Figure 7B).

332 Bax and Bcl2 are known to be pro- and anti-apoptotic proteins, whereas the gene Casp-3
333 encodes the effector apoptotic protein caspase 3. Under our conditions, all groups of mice that
334 received the challenge dose of U showed a slightly increased expression of the anti-apoptotic
335 protein Bcl2, not exceeding a 1.4-fold increase ($p < 0.05$ to $p < 0.001$) (Figure 7A). In contrast,
336 the gene expression of the pro-apoptotic protein Bax was increased only after a priming
337 exposure to 10 mg/L U ($p < 0.005$), but showed a low 1.2-fold increase. No induction of Casp-3
338 was observed after U exposure under our conditions. In contrast with the induction observed

339 with U, no induction of BCl2, Bax or Casp-2 gene expression was induced with the challenge
340 dose of F (Figure 7B). However, a slight 1.2-fold induction of BCl2 mRNA was observed in
341 all groups of mice exposed to the priming concentrations of F ($p<0.05$), whereas the gene
342 expression of Bax was increased only after a priming exposure to 15 mg/L F ($p<0.05$). Bax and
343 Casp-3 gene expression was significantly reduced compared to that in the positive control group
344 after a priming exposure to 30 mg/L F.

345 Beclin1 and LC3-II are both genes coding for proteins involved in autophagy, whereas
346 Sestrin2, AMPK and DRAMs are genes known to be induced by autophagy. In the case of U,
347 as shown in Figure 7A, the gene expression of AMPK was slightly increased (1.2-fold increase)
348 in positive controls ($p<0.005$), whereas the same treatment significantly reduced the gene
349 expression of DRAM2 ($p<0.001$). Mice previously contaminated with the priming doses of U
350 also showed increased mRNA levels of AMPK but showed a low 1.3-fold induction ($p<0.001$).
351 Interestingly, the groups that received the priming doses of 20 and 40 mg/L presented
352 significantly higher AMPK gene expression than that presented by the positive control group
353 ($p<0.05$). In contrast, all groups exposed to the priming doses of U showed significantly reduced
354 gene expression of DRAM2 ($p<0.001$), similar to the positive control group, whereas the
355 priming dose of 10 mg/L U increased the mRNA expression of DRAM1 ($p<0.005$). At the same
356 priming dose of 10 mg/L U, mice presented a small increase in the mRNA expression of LC3-
357 II ($p<0.05$), a protein known to be involved in autophagosome formation and therefore in the
358 last step of autophagy. As shown in Figure 7B, the F challenge dose did not induce any of the
359 genes implicated in autophagy we investigated. However, F induced DRAM2 gene expression
360 in the group exposed to 50 mg/L and then treated with the challenge dose of 7.5 mg/kg NaF
361 when compared to the negative and positive controls ($p<0.05$ and $p<0.005$, respectively).
362 Moreover, at the priming dose of 30 mg/L F the mRNA expression of SESTRIN2 was

363 significantly decreased compared to that in the negative and positive control groups ($p < 0.001$
364 and $p < 0.05$, respectively).

365 PERK, ATF6, IRE1- α , and ATF4 are involved in the three pathways of the UPR and
366 GRP78 and GRP94 are both genes induced by this biological response. Interestingly, ATF4
367 gene expression was induced in all groups treated with U (Figure 7A) regardless of whether
368 they experienced a priming exposure before being challenged ($p < 0.001$). Nevertheless, only the
369 animals pre-exposed to 10 and 20 mg/L U showed an increased IRE1- α mRNA level compared
370 to that in the negative control group ($p < 0.05$), and GRP78 gene expression was also increased
371 after the priming exposure of 10 mg/L followed by the challenge dose ($p < 0.05$). In contrast,
372 there was no induction of the genes involved in the UPR after F treatment, as shown in Figure
373 7B.

374 In summary, Figures 7A and 7B show that U challenge treatment induced the expression
375 of genes involved in inflammatory cell recruitment, apoptosis, autophagy and the UPR, whereas
376 none of these genes were induced after F challenge in mice. It seems that U priming exposure
377 slightly induced genes involved in all these mechanisms, whereas only genes implicated in
378 apoptosis and autophagy were slightly modulated by F priming exposure. To further explore
379 the implication of these mechanisms, we observed specific markers, through enzymatic activity
380 measurements and *in situ* immunochemistry.

381 3.5. *In situ ICAM-1 and VCAM-1 expression in the kidney*

382 To determine whether either U or F can induce the recruitment of inflammatory cells in the
383 kidney, the quantification of *in situ* ICAM-1 and VCAM-1 expression was performed. A basal
384 level of CAM proteins was detected (Figure 8A and 8B), and *in situ* ICAM and VCAM
385 expression was quantified relative to that in the negative control group (fixed at 1.0). Mice did
386 not show any significant induction of ICAM-1 in the kidney after U challenge treatment (Figure
387 8C), but U did induce VCAM-1 expression in the kidney of mice challenged with 5 mg/kg,

388 regardless of whether they received priming exposure ($p < 0.05$) (Figure 8D). It seems that a
389 priming exposure to 20 mg/L U induced the level of ICAM-1 significantly, eliciting a 1.5-fold
390 induction compared to that in the control group ($p < 0.05$). When challenged with F, mice did
391 not show any significant induction of ICAM-1 in the kidney (Figure 8C) but showed a 1.4-fold
392 induction of *in situ* VCAM-1 expression ($p < 0.05$) (Figure 8D). A priming exposure to 30 mg/L
393 F followed by a challenge injection of F, induced the *in situ* level of ICAM-1 significantly,
394 eliciting a 2.2-fold induction compared to that in the negative control group ($p < 0.005$). On the
395 other hand, the groups exposed to the priming doses of 30 and 50 mg/L F showed significantly
396 lower expression levels of VCAM-1 compared to those in the positive control group ($p < 0.001$
397 and $p < 0.005$, respectively) but did not show any significant difference compared to the
398 nonexposed animals.

399 3.6. Viability and cell death measurements in the kidneys

400 Cell viability and death were studied by measuring enzymatic activities in tissue
401 homogenates prepared from the renal cortex (Figure 9). A challenge treatment of 5 mg/kg U
402 induced cytotoxicity and apoptosis to 155% and 122% of the control basal level, respectively
403 ($p < 0.05$). Under our conditions, priming exposure to 10 or 20 mg/L U abolished the cytotoxicity
404 induced by the U challenge dose alone. Moreover, contamination with 20 mg/L U through
405 drinking water also abolished the apoptosis induced challenge with ip U injection. Indeed, this
406 group did not present significant differences compared with the negative control group, and its
407 caspase 3/7 activity was significantly lower than that of the positive control group ($p < 0.001$).
408 All F-treated groups, except for the 15 mg/L F pre-exposed mice, which presented a small
409 decrease in caspase 3/7 activity to 87% of the basal level, presented similar rates of cell viability
410 and death compared to those in the negative control group ($p < 0.05$). In conclusion, F did not
411 modulate cell viability or death at our working concentrations, but a renal adaptive response to
412 cytotoxicity and apoptosis induced by chronic exposure to 20 mg/L U in mice was suggested.

413 3.6. *In situ LC3-II measurement in the kidneys and the induction of autophagy*

414 LC3-II is the form of microtubule-associated protein 1A/1B-light chain 3 (LC3), that is
415 conjugated to phosphatidylethanolamine and recruited to the autophagosomal membranes [57].
416 Therefore, the induction of autophagy in kidney cells has been studied through the
417 immunodetection of the LC3-II protein *in situ* (Figure 10).

418 In negative control animals, no LC3-II was detected, as shown in Figure 10A. U induced
419 LC3-II in the kidneys of challenged mice regardless of whether the animals were pre-exposed
420 to priming doses of U, with scores ranging from 1.6 to 2.3 ($p < 0.001$) (Figures 10A and 10B).
421 Indeed, the scattered staining observed in the positive control and 10 mg/L U and 20 mg/L
422 U-pre-exposed groups, indicated the formation of LC3-II from the cytosolic protein LC3-I,
423 whereas LC3-II puncta, particularly noticeable in the magnified image of a 40 mg/L
424 U-pre-exposed mouse, indicated the recruitment of the protein to the autophagosomal
425 membranes. In addition, regardless of the type of exposure (challenged animals with or without
426 priming exposures), F did not induce *in situ* LC3-II expression.

427 3.7. *In situ IRE1- α measurement in the kidneys and the induction of the UPR*

428 The IRE1- α protein is mainly involved in one of the three known pathways of UPR
429 induction. Therefore, its overexpression in the cell cytosol was a good marker for the study of
430 UPR induction after contamination.

431 In the negative groups, there was a basal level of tubule staining (approximately 24-30 %),
432 as observed in Figure 11A. Animals challenged with U showed a significant increase in *in situ*
433 IRE1- α expression compared to that in the negative control group ($p < 0.05$), as the animals
434 previously exposed to priming doses of U showed a staining rate of tubules up to 35% ($p < 0.001$)
435 (Figure 11B). The F positive control group showed the induction of IRE1- α *in situ* compared
436 to that in the negative control group ($p < 0.001$) (Figure 11C), similar to the priming exposed
437 groups to 15, 30 and 50 mg/L ($p < 0.005$, $p < 0.001$ and $p < 0.001$, respectively). Interestingly, the

438 animals exposed to 50 mg/L F through drinking water presented overexpression of the *in situ*
439 protein compared to that in the group pre-exposed to 15 mg/L ($p<0.05$). Moreover, this group
440 presented a higher expression of IRE1- α than the positive control group, but this difference was
441 not statistically significant ($p=0.068$).

442 **4. Discussion**

443 The adaptive response is a process that can occur at exposures to low doses of chemicals
444 or radiation stress [47, 49, 58]. The most frequently described protocol for inducing the adaptive
445 response is challenge following a previous priming exposure to a low dose [38, 48, 59]. It is
446 known that U and F are both nephrotoxic after acute exposures [15, 28-32, 50, 60, 61], but
447 previous studies including ours have shown that U and F may trigger a renal adaptive response
448 after chronic low-dose exposures [18, 37, 38, 44, 62]. This work allowed us to identify the
449 mechanisms of this adaptive response to exposures to chronic low doses of U and F through a
450 chronic priming chronic exposure to low doses of U or F followed by challenge treatment
451 delivered acutely and at nephrotoxic doses to mice. The challenge dose was previously selected
452 from our recent study of U and F acute treatments [28].

453 In this study, we have shown that the adaptive response is induced after priming exposure
454 to 20 mg/L for U and 50 mg/L for F. A pre-exposure to 20 mg/L U decreased the overexpression
455 of CLU and KIM-1 mRNA triggered by the challenge dose of 5 mg/kg U without inducing any
456 difference relative to the negative control (Figures 5A and 5B). On the other hand, U induced
457 *in situ* KIM-1 expression in all groups (challenge or prime + challenge) except in the 10 mg/L
458 U-pre-exposed group. Thus, the renal adaptive response to U observed under our conditions
459 mainly occurred at a dose of 20 mg/L. Our previous studies performed in rodents showed that
460 a 9-month exposure to 40 mg/L U does not induce renal histopathological lesions, nephrotoxic
461 biomarkers [39, 52], or glomerular filtration rate changes [18, 63]. Moreover, renoprotective
462 mechanisms such as the induction of glutathione are induced dose-dependently [18]. Previous

463 studies have shown that UN is not nephrotoxic when delivered chronically to rodents at
464 concentrations ranging from 1 to 600 mg/L UN [18, 39, 52, 63], despite one study showing the
465 appearance of tubular lesions without dose-dependent increases in severity, after a 28- or 91-
466 day exposure to doses ranging from 0.96 to 600 mg/L [36]. The renal adaptive response
467 triggered by U in drinking water has not yet been identified, but it was previously described
468 that U is able to induce a renoprotective response *in vivo* after a single acute subtoxic dose of
469 UN [38, 62]. Our study is the first to clearly demonstrate that U induces a renal adaptive
470 response *in vivo* after chronic exposure to low doses of U in drinking water. Analyses of both
471 the mRNA levels and the urinary protein secretion of CLU and KIM-1 revealed an adaptive
472 response to F at 50 mg/L (Figure 5). Indeed, the KIM-1 and CLU levels in this group were not
473 significantly different from those in the negative control group, whereas the mRNA expression
474 and urinary secretion of these proteins were significantly decreased compared to those in the
475 positive control group. Interestingly, chronic exposure to 50 mg/L F for 40 days induces
476 nephrotoxic biomarkers such as the KIM-1, CLU or OPN genes and urinary protein levels in
477 rats [40], but a 60-day period of exposure does not induce any histological damage [64].
478 However, rats treated with a priming exposure of the same dose before nephrotoxic treatment
479 with gentamicin, compared to animals treated with gentamicin only, exhibit a decrease in F-
480 induced nephrotoxicity, gene expression and protein secretion of KIM-1 and CLU [44]. Our
481 findings are in accordance with this study and confirm the adaptive response observed at 50
482 mg/L F.

483 The mechanisms involved in this adaptive response have been studied to attain a better
484 comprehension of the toxicological effects of low-dose exposure to U or F. Several mechanisms
485 have been described in the literature as being involved in the adaptive response to chemicals or
486 irradiation [49]. Among them, apoptosis, autophagy, the inflammatory response and the UPR
487 have been reported to be induced by U and F [40, 44, 65-68] and could be involved in the U

488 and F adaptive response. It was previously shown that autophagy is able to reduce the toxic
489 effects induced by U in organs other than the kidneys [66, 69] and those induced by other
490 chemicals in the kidneys [70]. However, to our knowledge, we are the first to demonstrate that
491 autophagy is induced by U in the kidneys, *in vivo*. Indeed, through gene expression analysis
492 (Figure 7), we observed that the 20 mg/L U and 40 mg/L U-treated groups showed an adaptive
493 increase in AMPK gene expression and that the priming dose of 10 mg/L induced LC3-II and
494 DRAM1, genes known to be induced by autophagy. Moreover, animals pre-exposed to 10 and
495 20 mg/L U had an increased IRE1- α mRNA level, whereas the challenge dose alone did not
496 induce IRE1- α gene expression. Interestingly, our results showed the induction of autophagy
497 and the UPR after U challenge, regardless of whether there was a priming exposure (Figures 10
498 and 11). The UPR was also induced by F in the positive control group, as in the animals that
499 received the priming doses. This was the first study to show that U or F induces the UPR in the
500 kidneys *in vivo*. Nevertheless, our results do not identify autophagy and the UPR as mechanisms
501 involved in the adaptive response observed under our conditions. However, we cannot exclude
502 these mechanisms, as it is possible that autophagy and/or the UPR are induced during priming
503 exposure or after a recovery period between the priming and challenge doses. Further studies
504 will be necessary to investigate the induction of these mechanisms at other periods of exposure
505 at similar doses.

506 The gene expression analysis shown in Figure 7 indicates that inflammation and the
507 modulation of CAM mRNA expression could be mechanisms involved in the adaptive response
508 to U and F. Interestingly, in the case of F, *in situ* VCAM-1 levels were induced in the kidneys
509 by the challenge dose alone but not when mice were exposed to the priming doses of 30 and 50
510 mg/L F. This decrease in *in situ* VCAM-1 expression could partly explain the adaptive response
511 to F observed at 50 mg/L, under our conditions. As ICAM-1 is overexpressed in the kidneys of
512 animals pre-exposed to 30 mg/L F, the potential recruitment of inflammatory cells in the group

513 of mice that received this priming exposure needs further investigation (Figure 8A). It has been
514 shown that inflammation is modulated in the intestine after the chronic ingestion of U in rodents
515 [63, 71], but our results showed that U induced VCAM-1 in the kidneys of challenged mice
516 (Figure 8B) regardless of whether the mice received priming exposures. Therefore, similar to
517 autophagy and the UPR, the modulation of inflammatory cell recruitment cannot be identified
518 as a mechanism of the adaptive response to U.

519 Apoptosis, another mechanism studied, was identified as a mechanism involved in adaptive
520 response to U under our conditions, whereas no changes were observed after F exposures
521 (Figure 9). Indeed, both groups of animals with priming exposures to 20 and 40 mg/L U had
522 similar caspase 3/7 levels as those of the negative control group. Moreover, a priming exposure
523 to 20 mg/L U significantly reduced the caspase 3/7 activity induced by the challenge dose alone,
524 showing that apoptosis is at least partly involved in the adaptive response to U. In comparison,
525 other metals such as cadmium have been shown to reduce oxidative stress and induce less
526 apoptosis after chronic exposure compared to acute exposure [72, 73]. In contrast, apoptosis
527 and autophagy are induced in mouse hepatocytes after chronic exposure to 50 mg/L NaF for
528 150 days [74]; this difference can be explained by a tissue-specific response or the duration of
529 the exposure.

530 Our data demonstrate the existence of a renal adaptive response to chronic exposure to low
531 doses of U and F involving the induction of adaptive mechanisms including apoptosis and
532 inflammatory regulation in mice. Our results allowed us to identify molecular targets partly
533 responsible for these adaptive mechanisms: downregulation of the activities of the effector
534 caspases 3/7 and a decrease in *in situ* VCAM expression. Moreover, KIM-1 protein is
535 cytoprotective because of its role in the phagocytosis of cellular debris produced by the
536 apoptosis of tubular cells [75, 76] and its overexpression in our study may have been
537 cytoprotective and played a role in the adaptive response observed in the group pre-exposed to

538 20 mg/L U. Overall, these results allow a better understanding of the effects of chronic exposure
539 to low doses of U and F on an organism and provide new knowledge for the (radio)protection
540 of individuals. They raise the question of a higher or lower risk window - in certain ranges of
541 doses where the adaptive response is induced - and of their applications in humans. Indeed, the
542 use of fine and specific biomarkers of renal impairment in humans could allow better
543 assessment of renal function during exposures to low doses of U or F.

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553 **Conflict of interest statement**

554 The authors report no conflict of interest.

555

556 **Figures captions**

557

558 **Figure 1.** General scheme of the distribution of mice into uranium- and fluoride-treated groups
559 (n=10-18 per group) and the doses administered.

560 **Figure 2.** Measurement of U in the kidneys of mice that drank contaminated water (0, 10, 20, 40
561 mg/L U) for 6 months and were acutely injected with 5 mg/kg U or NaCl (n=10-12 per group). The
562 results are presented as the mean \pm standard error of the mean. The asterisk represents a significant
563 difference compared with the negative control group (** $p < 0.001$).

564 **Figure 3.** Measurement of F in the urine of mice that drank contaminated water (0, 15, 30, 50 mg/L
565 F) for 6 months and were acutely injected with 7.5 mg/kg NaF or NaCl (n=11-18 per group). The results
566 are presented as the mean \pm standard error of the mean. The asterisk represents a significant difference
567 compared with the negative control group (0 mg/L + 0 mg/kg), and the dollar symbol represents a
568 significant difference compared with the positive control group (0 mg/L + 7.5 mg/kg). The number of
569 symbols (1, 2, or 3) corresponds to the level of significance ($p < 0.05$, $p < 0.005$, and $p < 0.001$).

570 **Figure 4.** Representative photomicrographs (A) (200 \times) of noticeable renal lesions after HES
571 staining in control mice, U-contaminated (40 mg/L and 5 mg/kg U) mice and F-contaminated (30 mg/L
572 F and 7.5 mg/kg NaF) mice. The letters G, PT, and HC indicate glomeruli, proximal tubules, and hyaline
573 casts, respectively. Tubular vacuolization is indicated by black circles, whereas tubular necrosis is
574 indicated by black rectangles. The renal lesion scores (B) are presented as the mean \pm standard error of
575 the mean (n=10-18 per group) were done blinded. The percentage of tubular and interstitial injuries
576 (basophilic tubules, tubular necrosis, vacuolization and hyaline casts) in kidneys 72 hours after ip
577 injection (C) is presented as the mean. The asterisk represents a significant difference compared with
578 the negative control group (0 mg/L + 0 mg/kg) (two-way ANOVA with Holm-Sidak correction,
579 * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$).

580 **Figure 5.** Gene expression levels of the sensitive nephrotoxicity markers KIM-1 and CLU in the
581 renal cortex 72 hours after ip injection (A-B) and their urinary protein levels directly after IP injection

582 [77]. Mice were chronically exposed for 6 months by drinking contaminated water (0, 10, 20, 40 mg/L
583 U or 0, 15, 30, 50 mg/L F) and then acutely injected with either NaCl, 5 mg/kg U or 7.5 mg/kg NaF.
584 The results are presented as the mean \pm standard error of the mean (n=10-18 per group for mRNA and
585 n=10-12 for protein). The asterisk represents a significant difference compared with the negative control
586 group (0 mg/L + 0 mg/kg), and the dollar symbol represents a significant difference compared with the
587 positive control group (0 mg/L + 5 mg/kg for U and 0 mg/L + 7.5 mg/kg for F). The number of symbols
588 (1, 2, or 3) corresponds to the level of significance ($p<0.05$, $p<0.005$, and $p<0.001$).

589 **Figure 6.** Representative photomicrographs (200x) of the *in situ* renal expression of the protein
590 KIM-1 (in green) in mice chronically exposed for 6 months by drinking contaminated water (0, 10, 20,
591 40 mg/L U or 0, 15, 30, 50 mg/L F) and then acutely injected with either NaCl, 5 mg/kg U or 7.5 mg/kg
592 NaF (A; C) (n=10-18 per group). The results of the semiquantification of KIM-1 scoring (B; D) are
593 presented as the mean \pm standard error of the mean (n=10-18 per group). The asterisk represents a
594 significant difference compared with the negative control group (0 mg/L + 0 mg/kg). The number of
595 symbols (1, 2, or 3) corresponds to the level of significance ($p<0.05$, $p<0.005$, and $p<0.001$) (general
596 estimating equation for normal regression).

597 **Figure 7.** Gene expression of molecules involved in inflammation (in orange), apoptosis (in green),
598 autophagy (in purple), and the UPR (in red) in the renal cortex 72 hours after the ip injection of mice
599 chronically exposed to U (A) and F (B). Mice were exposed for 6 months by drinking contaminated
600 water (0, 10, 20, 40 mg/L U or 0, 15, 30, 50 mg/L F) and then acutely injected with either NaCl, 5 mg/kg
601 U or 7.5 mg/kg NaF. The results are presented as the mean \pm standard error of the mean relative to the
602 control group (n=10-18 per group). The asterisk represents a significant difference compared with the
603 negative control group (0 mg/L + 0 mg/kg), and the dollar symbol represents a significant difference
604 compared with the positive control group (0 mg/L + 5 mg/kg for U and 0 mg/L + 7.5 mg/kg for F). The
605 number of symbols (1, 2, or 3) corresponds to the level of significance ($p<0.05$, $p<0.005$, and $p<0.001$).

606 **Figure 8.** Representative photomicrographs (200x) of the *in situ* renal expression of the proteins
607 ICAM-1 (A) and VCAM-1 (B) in non-exposed and chronically exposed mice (0 and 30 mg/L F or 0 and

608 20 mg/L U) before challenge injection (0 and 7.5 mg/kg NaF or 0 and 5 mg/kg U). The results of the
609 semiautomatic quantification of ICAM-1 (C) and VCAM-1 (D) are presented as the mean \pm standard
610 error of the mean (n=10-18 per group). The asterisk represents a significant difference compared with
611 the negative control group (0 mg/L + 0 mg/kg), and the dollar symbol represents a significant difference
612 compared with the positive control group (0 mg/L + 5 mg/kg for U and 0 mg/L + 7.5 mg/kg for F). The
613 number of symbols (1, 2, or 3) corresponds to the level of significance ($p<0.05$, $p<0.005$, and $p<0.001$)
614 (general estimating equation for normal regression).

615 **Figure 9.** Cell viability, cytotoxicity and caspase 3/7 activity in the renal cortex of mice 72 hours
616 after ip U and F injections. Mice were chronically exposed for 6 months by drinking contaminated water
617 (0, 10, 20, 40 mg/L U or 0, 15, 30, 50 mg/L F) and then acutely injected with either NaCl, 5 mg/kg U or
618 7.5 mg/kg NaF. The results are presented as the mean percentage relative to the negative control group
619 \pm standard error of the mean (n=10-18 per group). The asterisk represents a significant difference
620 compared with the negative control group (0 mg/L + 0 mg/kg), and the dollar symbol represents a
621 significant difference compared with the positive control group (0 mg/L + 5 mg/kg for U and 0 mg/L +
622 7.5 mg/kg for F). The number of symbols (1, 2, or 3) corresponds to the level of significance ($p<0.05$,
623 $p<0.005$, and $p<0.001$).

624 **Figure 10.** Representative photomicrographs (400x) of the protein *in situ* renal expression of LC3-
625 II (in green) in mice chronically exposed for 6 months, by drinking contaminated water (0, 10, 20, 40
626 mg/L U or 30 mg/L F) and then acutely injected with either NaCl, 5 mg/kg U or 7.5 mg/kg NaF (A)
627 (n=10-12 per group). The results of the semiquantification of LC3B scoring after U exposure (B) are
628 presented as the mean \pm standard error of the mean. The asterisk represents a significant difference
629 compared with the negative control group (0 mg/L + 0 mg/kg) (general estimating equation for normal
630 regression, *** $p<0.001$). An enlarged image (C) after exposure to 40 mg/L and 5 mg/kg U to show the
631 green puncta of LC3-II, which represent protein recruitment to autophagosome membranes, is presented.

632 **Figure 11.** Representative photomicrographs (200x) of the *in situ* renal expression of the protein
633 IRE1- α (in green) in control mice and mice exposed over a 6-month period to either 50 mg/L F or 40

634 mg/L U in drinking water and then acutely injected with 7.5 mg/kg F or 5 mg/kg U, respectively (A)
635 (n=10-12 per group). The number of IRE1- α -positive tubules after U (B) or F exposure (C) is presented
636 as the mean \pm standard error of the mean. The asterisk represents a significant difference compared with
637 the negative control group (0 mg/L + 0 mg/kg) (* p <0.05, ** p <0.005, and *** p <0.001) (general
638 estimating equation for normal regression).

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Bibliography

- [1] M. Waziri, U. Musa, S.S. Hati, Assessment of fluoride concentrations in surface waters and groundwater sources in northeastern Nigeria, *Resour Environ* 2(2) (2012) 67-72.
- [2] R. Juntunen, Etelä-Suomen kallioporakaivojen uraani- ja radontutkimukset (Uranium and radon in wells drilled into bedrocks in southern Finland), Report of Investigation, Geological Survey of Finland, Espoo 98 (1991).
- [3] L. Salonen, 238U series radionuclides as a source of increased radioactivity in groundwater originating from Finnish bedrock, *Future Groundwater Resources at Risk (Proceedings of the Helsinki Conference, June 1994)* 222 (1994).
- [4] O. Barbier, L. Arreola-Mendoza, L.M. Del Razo, Molecular mechanisms of fluoride toxicity, *Chem Biol Interact* 188(2) (2010) 319-33.
- [5] WHO, *Fluoride in drinking-water*, Switzerland, 2006.
- [6] WHO, *Guidelines for drinking-water Quality*, World Health Organization, Geneva, Switzerland, 2011.
- [7] D. Browne, H. Whelton, D. O'Mullane, Fluoride metabolism and fluorosis, *Journal of dentistry* 33(3) (2005) 177-86.
- [8] Y. Guéguen, L. Roy, S. Hornhardt, C. Badie, J. Hall, S. Baatout, E. Pernot, L. Tomasek, O. Laurent, T. Ebrahimian, C. Ibanez, S. Grison, S. Kabacik, D. Laurier, M. Gomolka, Biomarkers for uranium risk assessment for the development of the CURE (Concerted Uranium Research in Europe) molecular epidemiological protocol, *Radiation Research* 187(1) (2017) 107-127.
- [9] UNSCEAR, *Sources, effects and risks of ionizing radiation*, United Nations Scientific Committee on the Effects of Atomic Radiation, U.S., 2016.
- [10] X. Xiong, J. Liu, W. He, T. Xia, P. He, X. Chen, K. Yang, A. Wang, Dose-effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children, *Environ Res* 103(1) (2007) 112-6.
- [11] Y.D. La Touche, D.L. Willis, O.I. Dawydiak, Absorption and biokinetics of U in rats following an oral administration of uranyl nitrate solution, *Health physics* 53(2) (1987) 147-62.
- [12] E. Craft, A. Abu-Qare, M. Flaherty, M. Garofolo, H. Rincavage, M. Abou-Donia, Depleted and natural uranium: chemistry and toxicological effects, *Journal of toxicology and environmental health. Part B, Critical reviews* 7(4) (2004) 297-317.
- [13] L. Vicente-Vicente, Y. Quiros, F. Perez-Barriocanal, J.M. Lopez-Novoa, F.J. Lopez-Hernandez, A.I. Morales, Nephrotoxicity of uranium: pathophysiological, diagnostic and therapeutic perspectives, *Toxicological sciences : an official journal of the Society of Toxicology* 118(2) (2010) 324-47.
- [14] S.A. Katz, *The Chemistry and Toxicology of Depleted Uranium* 2(1) (2014) 50-78.
- [15] T. Dote, K. Kono, K. Usuda, H. Nishiura, T. Tagawa, Acute renal damage dose response in rats to intravenous infusion of sodium fluoride, *Fluoride* 33(4) (2000) 210-217.
- [16] S.L. Manocha, H. Warner, Z.L. Olkowski, Cytochemical response of kidney, liver and nervous system of fluoride ions in drinking water, *The Histochemical journal* 7(4) (1975) 343-55.
- [17] F. Paquet, P. Houpert, E. Blanchardon, O. Delissen, C. Maubert, B. Dhieux, A.M. Moreels, S. Frelon, P. Gourmelon, Accumulation and distribution of uranium in rats after chronic exposure by ingestion, *Health physics* 90(2) (2006) 139-47.
- [18] C. Poisson, J. Stefani, L. Manens, O. Delissen, D. Suhard, C. Tessier, I. Dublineau, Y. Gueguen, Chronic uranium exposure dose-dependently induces glutathione in rats without any nephrotoxicity, *Free radical research* 48(10) (2014) 1218-31.
- [19] K. Usuda, K. Kono, T. Dote, K. Nishiura, K. Miyata, H. Nishiura, M. Shimahara, K. Sugimoto, Urinary biomarkers monitoring for experimental fluoride nephrotoxicity, *Arch Toxicol* 72(2) (1998) 104-9.
- [20] M.L. Zamora, J.M. Zielinski, G.B. Moodie, R.A. Falcomer, W.C. Hunt, K. Capello, Uranium in drinking water: renal effects of long-term ingestion by an aboriginal community, *Archives of environmental & occupational health* 64(4) (2009) 228-41.

690 [21] M.L. Zamora, B.L. Tracy, J.M. Zielinski, D.P. Meyerhof, M.A. Moss, Chronic ingestion of uranium in
691 drinking water: a study of kidney bioeffects in humans, *Toxicological sciences : an official journal of the*
692 *Society of Toxicology* 43(1) (1998) 68-77.

693 [22] H.M. Wasana, D. Aluthpatabendi, W.M. Kularatne, P. Wijekoon, R. Weerasooriya, J. Bandara,
694 Drinking water quality and chronic kidney disease of unknown etiology (CKDu): synergic effects of
695 fluoride, cadmium and hardness of water, *Environmental geochemistry and health* 38(1) (2016) 157-
696 68.

697 [23] O. Lantz, M.H. Jouvin, M.C. De Vernejoul, P. Druet, Fluoride-induced chronic renal failure,
698 *American journal of kidney diseases : the official journal of the National Kidney Foundation* 10(2)
699 (1987) 136-9.

700 [24] P. Kurttio, A. Harmoinen, H. Saha, L. Salonen, Z. Karpas, H. Komulainen, A. Auvinen, Kidney toxicity
701 of ingested uranium from drinking water, *American journal of kidney diseases : the official journal of*
702 *the National Kidney Foundation* 47(6) (2006) 972-82.

703 [25] P. Kurttio, A. Auvinen, L. Salonen, H. Saha, J. Pekkanen, I. Makelainen, S.B. Vaisanen, I.M. Penttila,
704 H. Komulainen, Renal effects of uranium in drinking water, *Environ Health Perspect* 110(4) (2002) 337-
705 42.

706 [26] M.I. Jimenez-Cordova, M. Cardenas-Gonzalez, G. Aguilar-Madrid, L.C. Sanchez-Pena, A. Barrera-
707 Hernandez, I.A. Dominguez-Guerrero, C. Gonzalez-Horta, O.C. Barbier, L.M. Del Razo, Evaluation of
708 kidney injury biomarkers in an adult Mexican population environmentally exposed to fluoride and low
709 arsenic levels, *Toxicology and applied pharmacology* 352 (2018) 97-106.

710 [27] K.B. Jayasekara, D.M. Dissanayake, R. Sivakanesan, A. Ranasinghe, R.H. Karunarathna, G.W.
711 Priyantha Kumara, Epidemiology of chronic kidney disease, with special emphasis on chronic kidney
712 disease of uncertain etiology, in the north central region of Sri Lanka, *Journal of epidemiology* 25(4)
713 (2015) 275-80.

714 [28] A. Bontemps, L. Conquet, C. Elie, V. Magneron, C. Gloaguen, D. Kereselidze, K. Tack, O.C. Barbier,
715 Y. Gueguen, In Vivo Comparison of the Phenotypic Aspects and Molecular Mechanisms of Two
716 Nephrotoxic Agents, Sodium Fluoride and Uranyl Nitrate, *International journal of environmental*
717 *research and public health* 16(7) (2019).

718 [29] T. Dote, K. Kono, K. Usuda, H. Nishiura, T. Tagawa, K. Miyata, M. Shimahara, N. Hashiguchi, J.
719 Senda, Y. Tanaka, Toxicokinetics of intravenous fluoride in rats with renal damage caused by high-dose
720 fluoride exposure, *International archives of occupational and environmental health* 73 Suppl (2000)
721 S90-2.

722 [30] P. Sangeetha Vijayan, P.D. Rekha, U. Dinesh, A.B. Arun, Biochemical and histopathological
723 responses of the Swiss albino mice treated with uranyl nitrate and its recovery, *Renal failure* 38(5)
724 (2016) 770-5.

725 [31] M.P. Santoyo-Sanchez, M. del Carmen Silva-Lucero, L. Arreola-Mendoza, O.C. Barbier, Effects of
726 acute sodium fluoride exposure on kidney function, water homeostasis, and renal handling of calcium
727 and inorganic phosphate, *Biological trace element research* 152(3) (2013) 367-72.

728 [32] J.M. Taylor, J.K. Scott, E.A. Maynard, F.A. Smith, H.C. Hodge, Toxic effects of fluoride on the rat
729 kidney. I. Acute injury from single large doses, *Toxicology and applied pharmacology* 3 (1961) 278-89.

730 [33] J.L. Domingo, J.M. Llobet, J.M. Tomas, J. Corbella, Acute toxicity of uranium in rats and mice, *Bull*
731 *Environ Contam Toxicol* 39(1) (1987) 168-74.

732 [34] Y. Gueguen, C. Rouas, Données nouvelles sur la néphrotoxicité de l'uranium, *Radioprotection*
733 47(3) (2012) 345-359.

734 [35] R.W. Leggett, The behavior and chemical toxicity of U in the kidney: a reassessment, *Health physics*
735 57(3) (1989) 365-383.

736 [36] A.P. Gilman, D.C. Villeneuve, V.E. Secours, A.P. Yagminas, B.L. Tracy, J.M. Quinn, V.E. Valli, R.J.
737 Willes, M.A. Moss, Uranyl nitrate: 28-day and 91-day toxicity studies in the Sprague-Dawley rat, *Toxicol*
738 *Sci* 41(1) (1998) 117-28.

739 [37] Y. Gueguen, D. Suhard, C. Poisson, L. Manens, C. Elie, G. Landon, C. Bouvier-Capely, C. Rouas, M.
740 Benderitter, C. Tessier, Low-concentration uranium enters the HepG2 cell nucleus rapidly and induces

741 cell stress response, *Toxicology in vitro : an international journal published in association with BIBRA*
742 30(1 Pt B) (2015) 552-60.

743 [38] T. Iwakura, Y. Fujigaki, T. Fujikura, N. Ohashi, A. Kato, H. Yasuda, Acquired resistance to rechallenge
744 injury after acute kidney injury in rats is associated with cell cycle arrest in proximal tubule cells,
745 *American journal of physiology. Renal physiology* 310(9) (2016) F872-84.

746 [39] Y. Gueguen, C. Rouas, A. Monin, L. Manens, J. Stefani, O. Delissen, S. Grison, I. Dublineau,
747 Molecular, cellular, and tissue impact of depleted uranium on xenobiotic-metabolizing enzymes,
748 *Archives of toxicology* 88(2) (2014) 227-39.

749 [40] M.C. Cardenas-Gonzalez, L.M. Del Razo, J. Barrera-Chimal, T. Jacobo-Estrada, E. Lopez-Bayghen,
750 N.A. Bobadilla, O. Barbier, Proximal renal tubular injury in rats sub-chronically exposed to low fluoride
751 concentrations, *Toxicology and applied pharmacology* 272(3) (2013) 888-94.

752 [41] Y.G. Long, Y.N. Wang, J. Chen, S.F. Jiang, A. Nordberg, Z.Z. Guan, Chronic fluoride toxicity decreases
753 the number of nicotinic acetylcholine receptors in rat brain, *Neurotoxicol Teratol* 24(6) (2002) 751-7.

754 [42] Y.M. Shivarajashankara, A.R. Shivashankara, P.G. Bhat, S.H. Rao, Lipid peroxidation and
755 antioxidant systems in the blood of young rats subjected to chronic fluoride toxicity, *Indian J Exp Biol*
756 41(8) (2003) 857-60.

757 [43] A.R. Shivashankara, Y.M. Shivarajashankara, P.G. Bhat, S.H. Rao, Lipid peroxidation and
758 antioxidant defense systems in liver of rats in chronic fluoride toxicity, *Bull Environ Contam Toxicol*
759 68(4) (2002) 612-6.

760 [44] M. Cardenas-Gonzalez, T. Jacobo Estrada, R. Rodriguez-Munoz, J. Barrera-Chimal, N.A. Bobadilla,
761 O.C. Barbier, L.M. Del Razo, Sub-chronic exposure to fluoride impacts the response to a subsequent
762 nephrotoxic treatment with gentamicin, *Journal of applied toxicology : JAT* 36(2) (2016) 309-19.

763 [45] F. Lagarde, C. Beausoleil, S.M. Belcher, L.P. Belzunces, C. Emond, M. Guerbet, C. Rousselle, Non-
764 monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment,
765 *Environmental health : a global access science source* 14 (2015) 13.

766 [46] L.E. Feinendegen, A.L. Brooks, W.F. Morgan, Biological consequences and health risks of low-level
767 exposure to ionizing radiation: commentary on the workshop, *Health physics* 100(3) (2011) 247-59.

768 [47] H. Matsumoto, N. Hamada, A. Takahashi, Y. Kobayashi, T. Ohnishi, Vanguard of paradigm shift in
769 radiation biology: radiation-induced adaptive and bystander responses, *Journal of radiation research*
770 48(2) (2007) 97-106.

771 [48] G. Olivieri, J. Bodycote, S. Wolff, Adaptive response of human lymphocytes to low concentrations
772 of radioactive thymidine, *Science* 223(4636) (1984) 594-7.

773 [49] Y. Gueguen, A. Bontemps, T.G. Ebrahimian, Adaptive responses to low doses of radiation or
774 chemicals: their cellular and molecular mechanisms, *Cellular and molecular life sciences : CMLS* 76(7)
775 (2019) 1255-1273.

776 [50] R.L. Kathren, R.K. Burklin, Acute chemical toxicity of uranium, *Health physics* 94(2) (2008) 170-9.

777 [51] K.L. Zimmerman, D.S. Barber, M.F. Ehrlich, L. Tobias, S. Hancock, J. Hinckley, E.M. Binder, B.S.
778 Jortner, Temporal clinical chemistry and microscopic renal effects following acute uranyl acetate
779 exposure, *Toxicologic pathology* 35(7) (2007) 1000-9.

780 [52] C. Rouas, J. Stefani, S. Grison, L. Grandcolas, C. Baudelin, I. Dublineau, M. Pallardy, Y. Gueguen,
781 Effect of nephrotoxic treatment with gentamicin on rats chronically exposed to uranium, *Toxicology*
782 279(1-3) (2011) 27-35.

783 [53] S.G. Emeigh Hart, Assessment of renal injury in vivo, *Journal of pharmacological and toxicological*
784 *methods* 52 (2005) 30-45.

785 [54] Y. Gueguen, C. Rouas, F.A. Leblond, Les biomarqueurs d'atteinte rénale, *Néphrologie et*
786 *Thérapeutique* 8 (2012) 146-155.

787 [55] S. Hidaka, B. Kranzlin, N. Gretz, R. Witzgall, Urinary clusterin levels in the rat correlate with the
788 severity of tubular damage and may help to differentiate between glomerular and tubular injuries, *Cell*
789 *and tissue research* 310(3) (2002) 289-96.

790 [56] T. Ichimura, C.C. Hung, S.A. Yang, J.L. Stevens, J.V. Bonventre, Kidney injury molecule-1: a tissue
791 and urinary biomarker for nephrotoxicant-induced renal injury, *American journal of physiology. Renal*
792 *physiology* 286(3) (2004) F552-63.

793 [57] I. Tanida, T. Ueno, E. Kominami, LC3 and Autophagy, *Methods Mol Biol* 445 (2008) 77-88.
794 [58] E.J. Calabrese, L.A. Baldwin, The frequency of U-shaped dose responses in the toxicological
795 literature, *Toxicological sciences : an official journal of the Society of Toxicology* 62(2) (2001) 330-8.
796 [59] S. Tapio, V. Jacob, Radioadaptive response revisited, *Radiation and environmental biophysics* 46(1)
797 (2007) 1-12.
798 [60] E. Birkner, E. Grucka-Mamczar, Z. Machoy, R. Tarnawski, R. Polaniak, Disturbance of protein
799 metabolism in rats after acute poisoning with sodium fluoride, *Fluoride* 33(4) (2000) 182-186.
800 [61] A. Shashi, J. Singh, S. Thapar, Toxic effects of fluoride on rabbit kidney, *Fluoride* 35 (2002) 38-50.
801 [62] Y. Sun, Y. Fujigaki, M. Sakakima, A. Hishida, Acquired resistance to rechallenge injury in rats
802 recovered from subclinical renal damage with uranyl acetate--Importance of proliferative activity of
803 tubular cells, *Toxicology and applied pharmacology* 243(1) (2010) 104-10.
804 [63] I. Dublineau, M. Souidi, Y. Gueguen, P. Lestaevel, J.M. Bertho, L. Manens, O. Delissen, S. Grison, A.
805 Paulard, A. Monin, Y. Kern, C. Rouas, J. Loyen, P. Gourmelon, J. Aigueperse, Unexpected lack of
806 deleterious effects of uranium on physiological systems following a chronic oral intake in adult rat,
807 *BioMed research international* 2014 (2014) 181989.
808 [64] C.A. Kobayashi, A.L. Leite, T.L. Silva, L.D. Santos, F.C. Nogueira, R.C. Oliveira, M.S. Palma, G.B.
809 Domont, M.A. Buzalaf, Proteomic analysis of kidney in rats chronically exposed to fluoride, *Chem Biol*
810 *Interact* 180(2) (2009) 305-11.
811 [65] G. Kroemer, G. Marino, B. Levine, Autophagy and the integrated stress response, *Molecular cell*
812 40(2) (2010) 280-93.
813 [66] V. Pierrefite-Carle, S. Santucci-Darmanin, V. Breuil, T. Gritsaenko, C. Vidaud, G. Creff, P.L. Solari, S.
814 Pagnotta, R. Al-Sahlane, C.D. Auwer, G.F. Carle, Effect of natural uranium on the UMR-106
815 osteoblastic cell line: impairment of the autophagic process as an underlying mechanism of uranium
816 toxicity, *Arch Toxicol* (2016).
817 [67] M. Schroder, The unfolded protein response, *Molecular biotechnology* 34(2) (2006) 279-90.
818 [68] I. Szumiel, Radiation hormesis: Autophagy and other cellular mechanisms, *International journal of*
819 *radiation biology* 88(9) (2012) 619-28.
820 [69] L. Hurault, G. Creff, A. Hagege, S. Santucci-Darmanin, S. Pagnotta, D. Farlay, C. Den Auwer, V.
821 Pierrefite-Carle, G.F. Carle, Uranium Effect on Osteocytic Cells In Vitro, *Toxicological sciences : an*
822 *official journal of the Society of Toxicology* 170(1) (2019) 199-209.
823 [70] W.J. Liu, M.N. Luo, J. Tan, W. Chen, L.Z. Huang, C. Yang, Q. Pan, B. Li, H.F. Liu, Autophagy activation
824 reduces renal tubular injury induced by urinary proteins, *Autophagy* 10(2) (2014) 243-56.
825 [71] I. Dublineau, L. Grandcolas, S. Grison, C. Baudelin, F. Paquet, P. Voisin, J. Aigueperse, P.
826 Gourmelon, Modifications of inflammatory pathways in rat intestine following chronic ingestion of
827 depleted uranium, *Toxicological sciences : an official journal of the Society of Toxicology* 98(2) (2007)
828 458-68.
829 [72] A.R. Nair, W.K. Lee, K. Smeets, Q. Swennen, A. Sanchez, F. Thevenod, A. Cuypers, Glutathione and
830 mitochondria determine acute defense responses and adaptive processes in cadmium-induced
831 oxidative stress and toxicity of the kidney, *Archives of toxicology* 89(12) (2015) 2273-89.
832 [73] J. Liu, W. Qu, M.B. Kadiiska, Role of oxidative stress in cadmium toxicity and carcinogenesis,
833 *Toxicology and applied pharmacology* 238(3) (2009) 209-14.
834 [74] Y. Zhao, Y. Li, J. Wang, R.K. Manthari, Fluoride induces apoptosis and autophagy through the IL-17
835 signaling pathway in mice hepatocytes, *Arch Toxicol* 92(11) (2018) 3277-3289.
836 [75] C.R. Brooks, M.Y. Yeung, Y.S. Brooks, H. Chen, T. Ichimura, J.M. Henderson, J.V. Bonventre, KIM-
837 1-/TIM-1-mediated phagocytosis links ATG5-/ULK1-dependent clearance of apoptotic cells to antigen
838 presentation, *The EMBO journal* 34(19) (2015) 2441-64.
839 [76] T. Ichimura, S. Mou, Kidney injury molecule-1 in acute kidney injury and renal repair: a review,
840 *Zhong xi yi jie he xue bao = Journal of Chinese integrative medicine* 6(5) (2008) 533-8.
841 [77] M. Sumarac-Dumanovic, D. Stevanovic, A. Ljubic, J. Jorga, M. Simic, D. Stamenkovic-Pejkovic, V.
842 Starcevic, V. Trajkovic, D. Micic, Increased activity of interleukin-23/interleukin-17 proinflammatory
843 axis in obese women, *Int J Obes (Lond)* 33(1) (2009) 151-6.
844

