RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

IRSN
INSTITUT DE RADIOPROTECTION ET DE SÛRETÉ NUCLÉAIRE

MULTIGENERATIONAL EPIGENETIC AND METABOLOMIC EFFECTS OF INTERNAL EXPOSURE TO NON-TOXIC DOSES OF URANIUM IN RATS

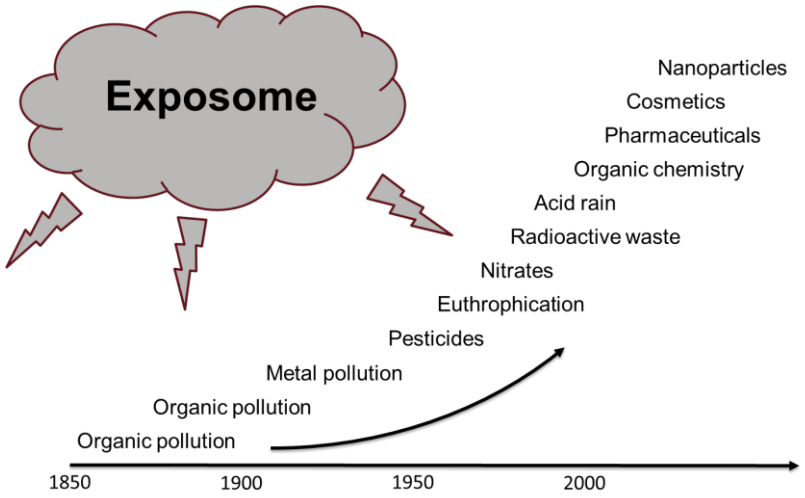
Stéphane Grison[1], Audrey Legendre[1], Céline Gloaguen[1], Dimitri Kereselidze[1], Christelle Elie[1], Benadjaoud Mohamed Amine[1], Philippe Lestaevel[1], Jean-Charles Martin[2], Maâmar Souidi[1]

1 Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE/SESANE/LRTOX, Fontenay-aux-Roses, F-92260 France
2 C2VN, BIOMET, Aix Marseille Université, France

stephane.grison@irsn.fr

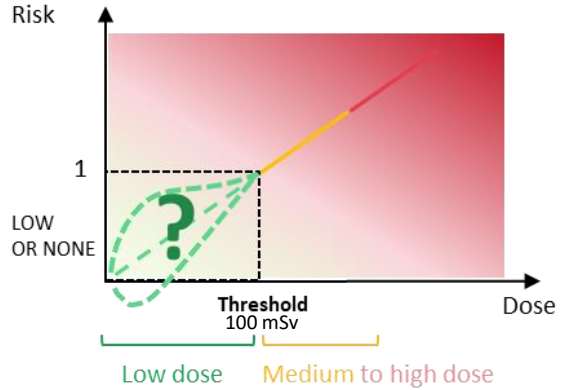
MEMBRE DE ETSON

Environmental, health and social context :



Anthropogenic source of ionizing radiation

Nuclear fuel cycle (uranium mining, refining)
Nuclear weapons (1945 Hiroshima & Nagasaki bombing, 1945-1980 : 520 Atmospheric tests)
Nuclear accidents (1957: Mayak, 1986: Tchernobyl, 2011: Fukushima Daiichi)
Medical procedures (CT Imaging, Nuclear medicine)
CBRN terrorism (internationnal political context)

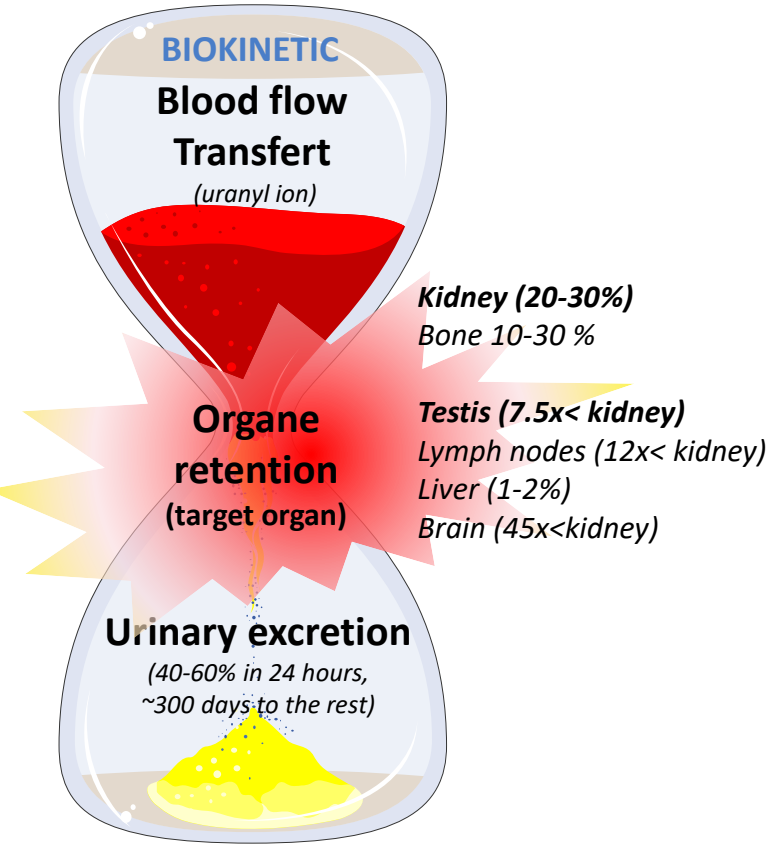


RADIATION PROTECTION STANDARDS ARE NECESSARY, BUT THEY NEED IDENTIFIED RISKS.

- **Low-dose effect relationship?**
- **Late effect?**
- **Impact on the next generations?**

ICRP Health risks to offspring and subsequent generations (TG 121)
ATSDR
UNSCEAR
MELODI

Uranium toxicity



Kidney (20-30%)
Bone 10-30 %

Testis (7.5x< kidney)
Lymph nodes (12x< kidney)
Liver (1-2%)
Brain (45x<kidney)

Dose

EXPOSURE TIME, ISOTOPE RATIO, SOLUBILITY, SPECIES, SEX, AGE, ORGAN, BIOLOGICAL SYSTEM AND METABOLIC PATHWAYS

(INTESTINAL ABSORPTION RAT = 0.2%; MAN = 2%, urinary excretion in males > in females), newborns absorb 20 to 100 times more than adults),

Radiological/**Chemical**

Toxicity

*Carcinogenicity, **nephrotoxicity,** urotoxicity, **reprotoxicity***

* ICRP (1959) maximum admissible uranium concentration in human kidneys : 3 µg.g-1

Low-dose effects of uranium

No preclinical effect

Physiological systems

- **Central Nervous System:** EU 4% 40 mg/L (Houpert 2005, 2007)
- **Behavior:** NU 40 mg/L males versus females (Lestaevel 2016)
- **Reproductive system:** DU 40-120 mg/L (Legendre 2016)
- **Bone remodeling:** NU 40 mg/L (Wade-Gueye 2012)

Metabolism

- **Cholesterol:** DU 40 mg/L, transcriptional (brain, liver), post-translational and metabolic (liver) effects (Racine 2009, 2010)
- **Steroid hormones**: DU-$EU_{4\%}$ 40mg/L, transcriptional effects in testis (Grignard 2008)
- **Vitamin D**: DU-$EU_{4\%}$ 40mg/L, transcriptional and metabolic effects (liver, brain, kidney) (Tissandie 2006, 2008)
- **Xenobiotics**: DU 40 mg/L, transcriptional effects (phase I for liver, brain, kidney, lung) (Souidi 2005, Gueguen 2007)
- **Iron**: DU 40mg/L, transcriptional effects (kidney) (Berradi 2008)
- **Acetylcholine**: DU 40 mg/L, metabolic effect (cortex and cerebellum) (Bensoussan 2009)
- **Bone** metabolism: DU 40 mg/L, transcriptional biological effects in juveniles (Wade-Gueye 2012)

Epigenetic mechanisms

- **DNA methylation**: DU 40 mg/L, reversibility of methyltransferase expression (liver, kidney) and global kidney methylation (Souidi 2016)

The social context and radiation protection issue

- **Can uranium exposure affect offspring?**
  - *Metabolism, gene expression, DNA methylation profile*
  - *Kidney and reproductive system*

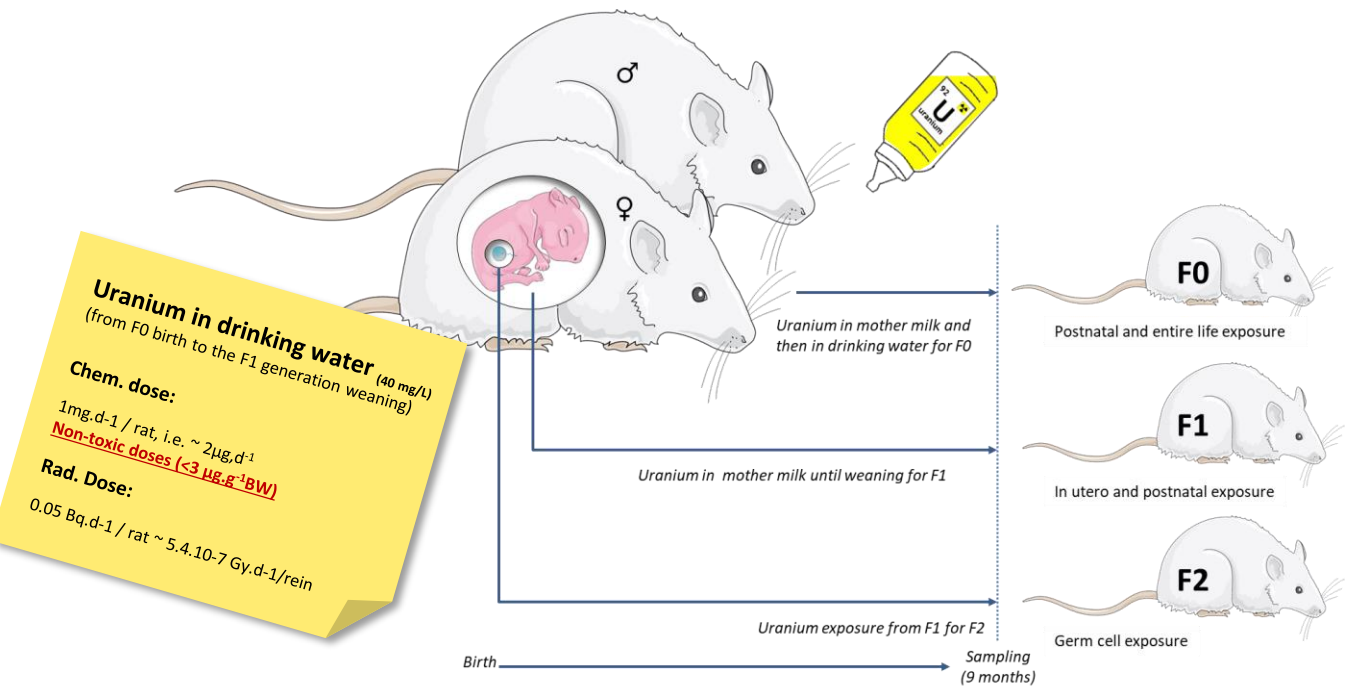
Scientific context : the reproductive system

- Infertility affects 8-12% of human couples and half of all men,
- Experimental chronic exposure to a supra environmental concentration of depleted uranium (DU) does not impair testicular steroidogenesis in adult rats[1] but, lifelong exposure to uranium from embryo* to adult age induces subtle testicular and hormonal defects in rats[2]

*Fetal development is a critical and vulnerable period of life and gametes are directly involved in transgenerational effects.

[1]Grignard et coll., Contamination with depleted or enriched uranium differently affects steroidogenesis metabolism in rat, 2008)
[2]Legendre, A et al. Endocrine effects of lifelong exposure to low-dose depleted uranium on testicular functions in adult rat, 2016

Experimental strategy for the multigenerational effects of prenatal uranium exposure: from phenotype to epigenome (kidney and reproductive system)

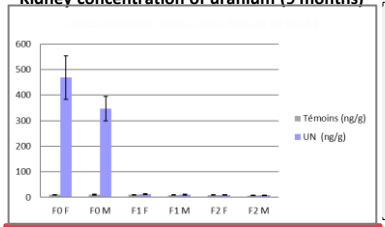


PHENOTYPES

MOLECULAR ANALYSIS

Phenotype and function: body weight, kidney, testis and sperm

Kidney concentration of uranium (9 months)



Only F0 contaminated

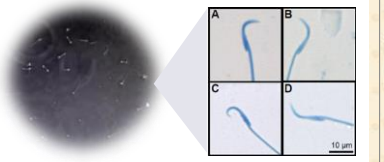
Epididymides concentration of uranium

Unpublished data
All rights reserved

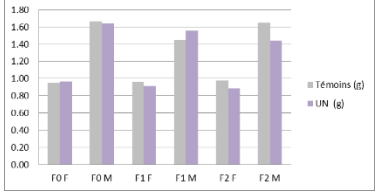
Testis concentration of uranium (ng/g)

Unpublished data
All rights reserved

Sperm morphology
(10 parameters)



Relative kidney weight (9 months)



Relative epididimal weight (g/100 BW)

Unpublished data
All rights reserved

Relative testis weight (g/100 BW)

Unpublished data
All rights reserved

No difference in concentration
Number of abnormal spermatozoa higher in F1
(Head and the middle-piece always modified)

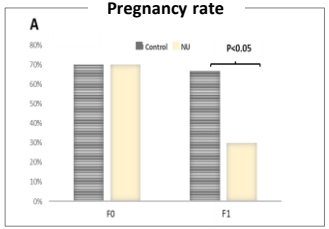
Amylase, uric acid, urea, glucose, calcium, chlorine, potassium, sodium, phosphorus, creatinine, urine volume

Urinary clinical effect

Decreased of epididimal relative weight for the generation F1

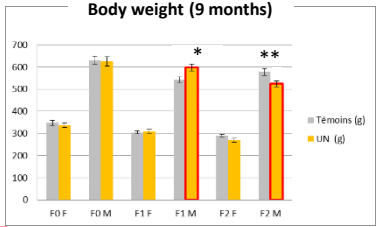
Increased of gonads relative weights for the generation F2

Pregnancy rate



Pregnancy rate reduced to 30 %
Number of pups per litter and male/female ratio unchanged

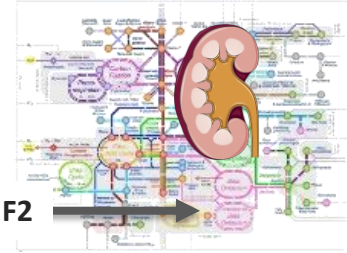
Body weight (9 months)



Decrease of body weight for the generation F2

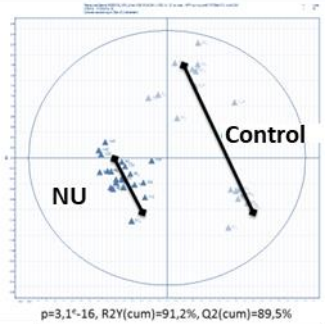
Metabolomics profiles in Blood, kidney, urine

Simultaneous analysis of all metabolites present in a tissue or biological fluid at a given time

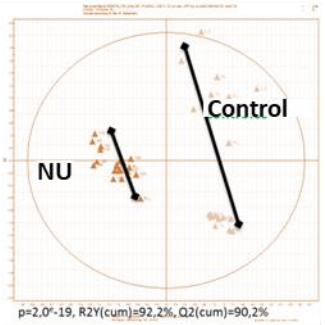


Kidney sample (F0 generation PLS-DA)

Male

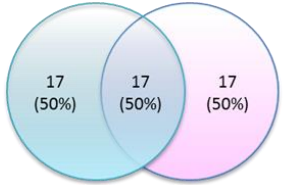


Female



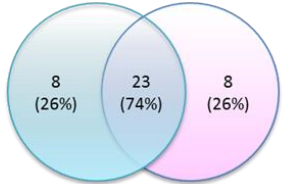
F0 — **F1** — **F2**

Plasma:

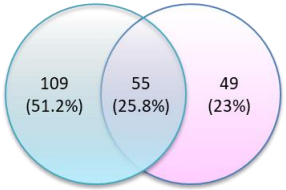


Males: 34 variables (blue); females: 34 variables (pink).
17 common variables in the plasma (F0).

Kidney:

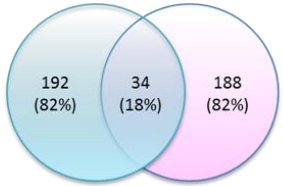


Males: 31 variables (blue); females: 31 variables (pink)
23 common variables in the kidney (F0).

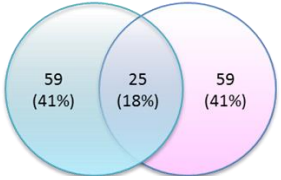


Males: 164 variables (blue); females: 104 variables (pink).
55 common variables in the kidney (F1).

Urine:

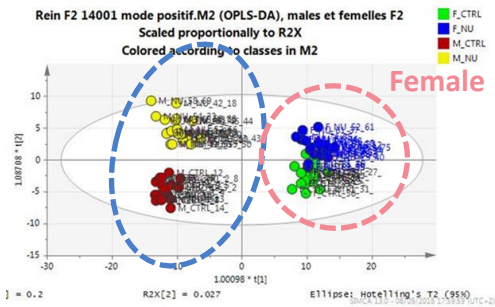


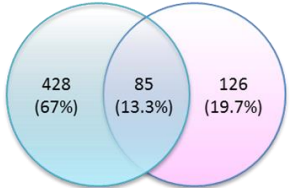
Males: 226 variables (blue); females: 222 variables (pink)
34 common variables in the urine (F0).



Males: 84 variables (blue); females: 84 variables (pink).
25 common variables in the urine (F1).

Male

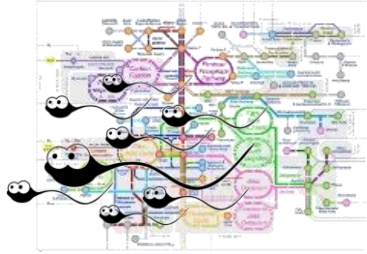


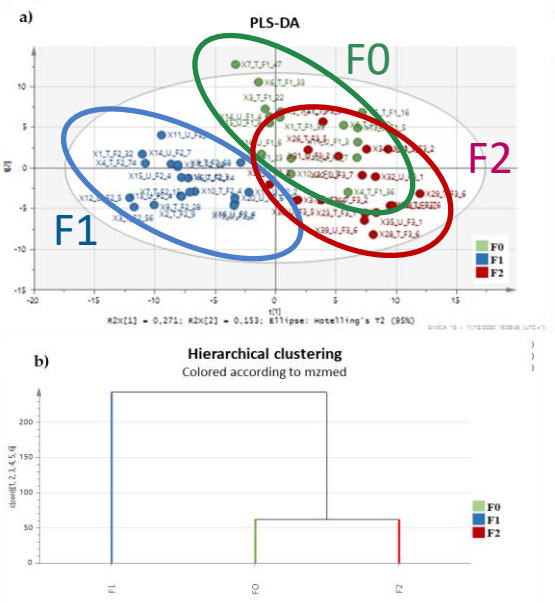


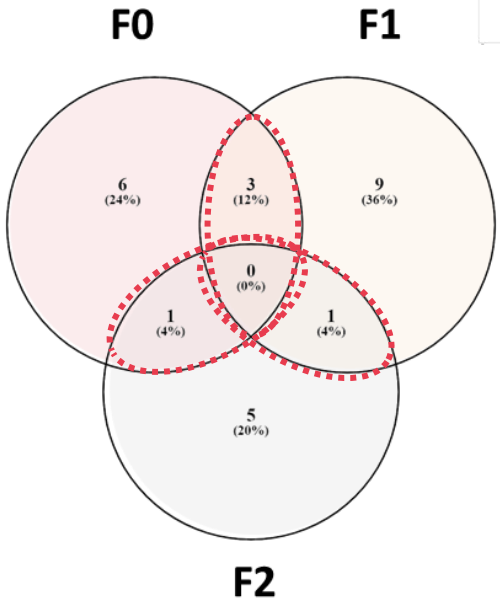
Males: 513 variables (blue); females: 211 variables (pink). 85 common variables in the kidney (F1).

Deregulation up to F2 generation / High sexual differences

Metabolomics profiles in sperm







F1 clearly separated by PLS-DA.
F0 and F2 more similar
(CV-ANOVA; p = 2.19E-27; R2Y(cum) = 96.3%; Q2(cum) = 88.5%)

High generational dimorphism
Only 12% similarity between F0 and F1 (4% for F1-F2)

Grison, S., Legendre A., et al., Multigenerational Exposure to Uranium Changes Sperm Metabolome in Rats. Int J Mol Sci, 2022. 23(15).

Testis overview

F1 and F2 generation seem deregulated

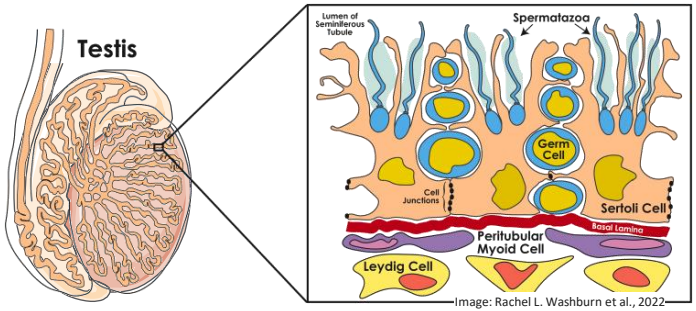


Image: Rachel L. Washburn et al., 2022

	F0	F1	F2
Zoometrie			
Relative weights			
Histology			
Seminiferous tubules diameter			
Seminiferous tubules lumen diameter			
% of seminiferous tubules in stage I-VI			
Leydig cells volume			
Gene expression (mRNA)			
Certoli cells			
Germ cells			
Sex hormones and steroidogenesis			
Sexual hormone levels			
Gonadotropin			
Testosterone			
Estradiol			
Gene expression (mRNA)			
Gonadotropin			
Steroidogenesis			

	F0	F1	F2
Cells mortality			
Apoptotic cells			
Gene expression (mRNA)			
Apoptotic pathway			
Oxydative stress			
Inflammation			
Blood-testis barrier (BTB) integrity			
Vitamin D			
Gene expression (mRNA)			
VDR			
Cyp27A1			
CYP27B1			
CYP24A1			
Protein levels			
Cyp27A1			
CYP27B1			
CYP24A1			
Metabolite levels			
Cholecalciferol			
25(OH)D3			
24,25(OH)2 D3			

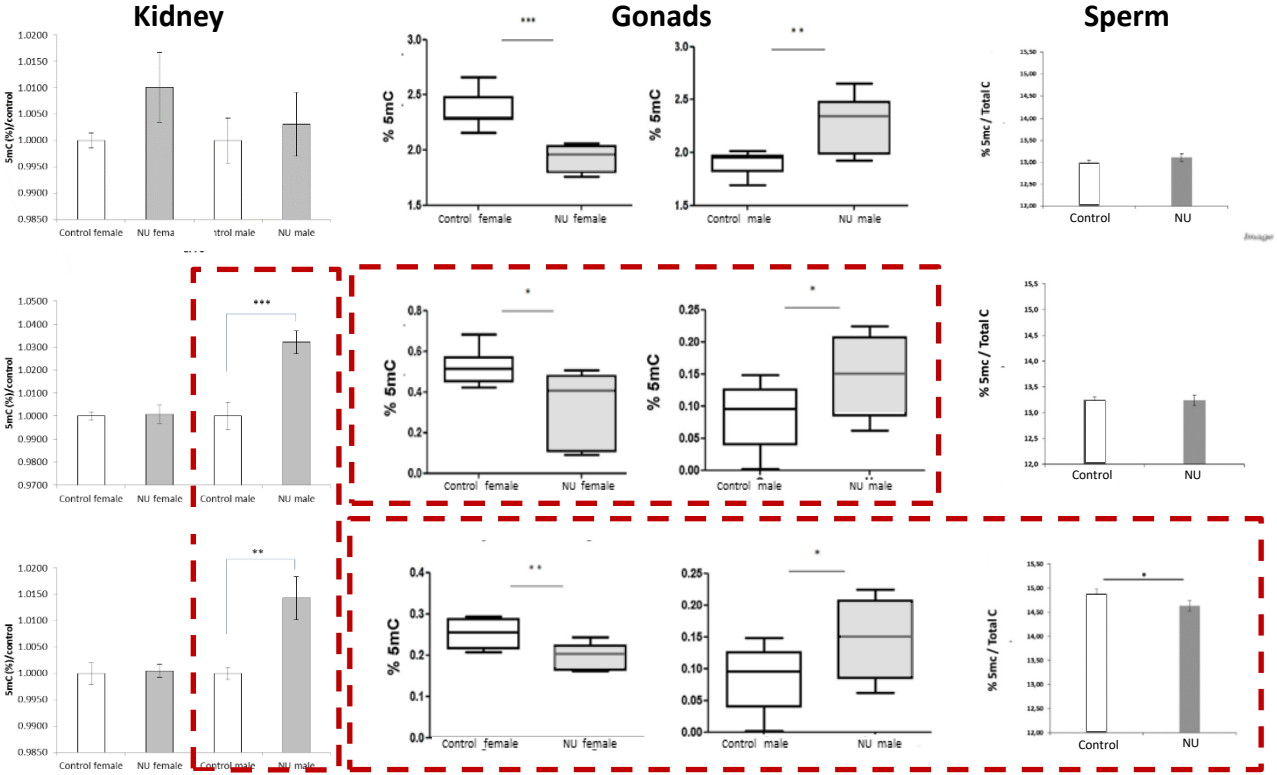
Legendre, A., et al. Multigenerational exposure to uranium induces testicular effects by hormonal disruptions. Article in preparation

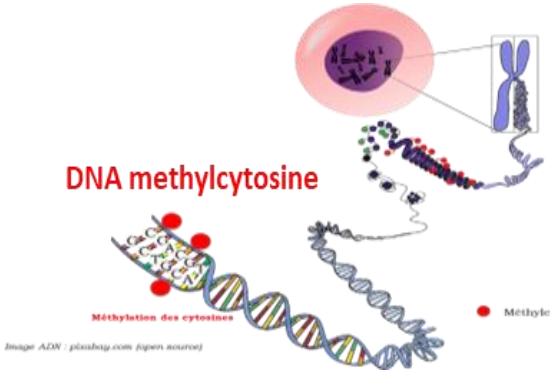
Epigenetic imprinting

F0

F1

F2





F1 and F2 seem deregulated

Grison, S., et al., Low dose of uranium induces multigenerational epigenetic effects in rat kidney. Int J Radiat Biol, 2018. 94(11): p. 975-984.

Elmhiri, G., et al., DNA methylation and potential multigenerational epigenetic effects linked to uranium chronic low-dose exposure in gonads of males and female rats. Toxicol Lett, 2018. 282: p. 64-70.

Legendre, A., et al., Multigenerational exposure to uranium changes morphometric parameters and global DNA methylation in rat sperm. C R Biol, 2019. 342(5-6): p. 175-185.

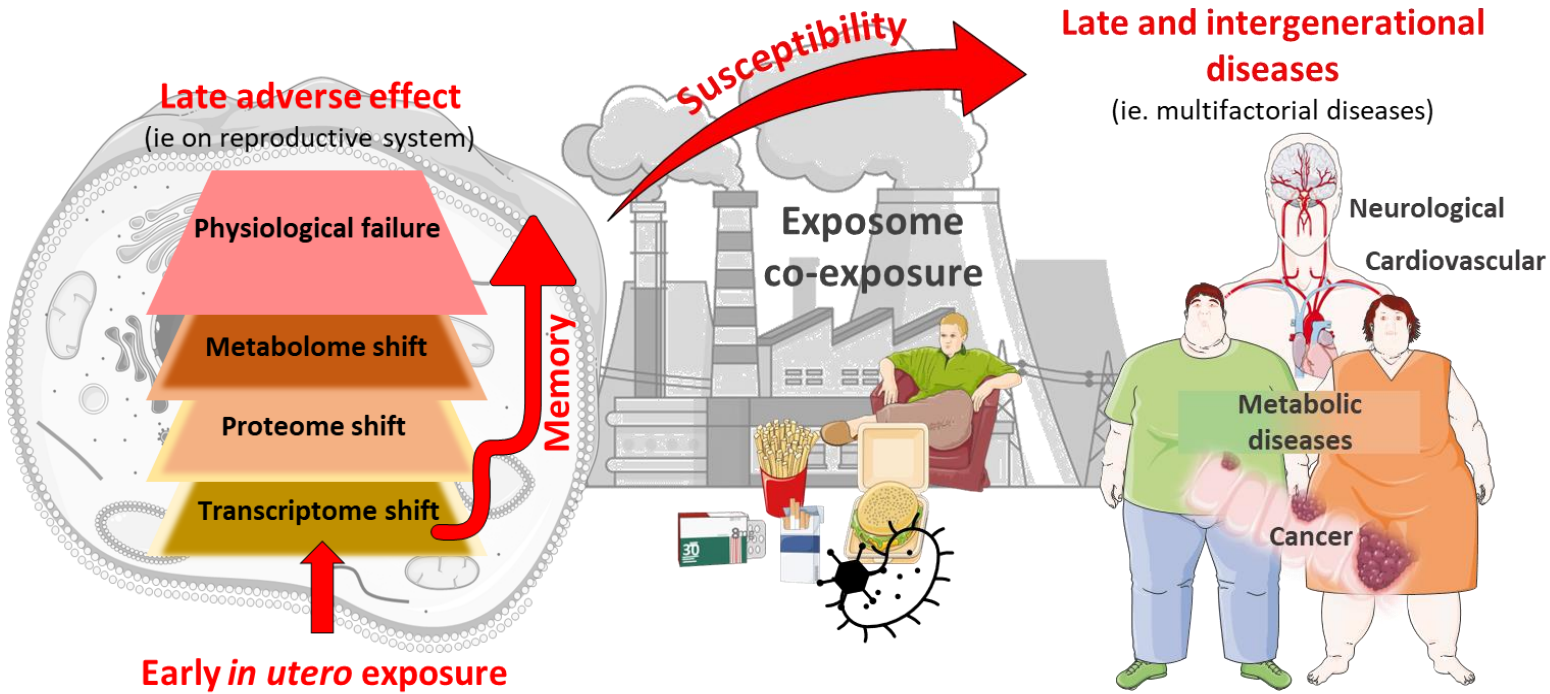
Grison, S. and M. Souidi, Use of omics analysis for low-dose radiotoxicology and health risk assessment: the case of uranium. Environ Epigenet, 2022. 8(1): p. dvac025.

Conclusion

- A non-toxic concentration of uranium induces epigenetic, metabolic and phenotypic changes over two generations.

- These effects can be adverse to reproductive function.

Risk at low dose : hypothesis



Reproductive system seems can be a target of uranium, especially for the fetus !

Grison, S., et al., In utero exposure to ionizing radiation and metabolic regulation: perspectives for future multi- and trans-generation effects studies. Int J Radiat Biol, 2024: p. 1-14.

RÉPUBLIQUE FRANÇAISE
Liberté
Égalité
Fraternité

IRSN
INSTITUT DE RADIOPROTECTION ET DE SÛRETÉ NUCLÉAIRE

THANKS FOR YOUR ATTENTION,

MANY THANKS TO MY COLLEAGUES

Stéphane Grison[1], Audrey Legendre[1], Céline Gloaguen[1], Dimitri Kereselidze[1], Christelle Elie[1], Benadjaoud Mohamed Amine[1], Philippe Lestaevel[1], Jean-Chales Martin[2], Maâmar Souidi[1]

1 Institut de Radioprotection et de Sûreté Nucléaire (IRSN), PSE-SANTE/SESANE/LRTOX, Fontenay-aux-Roses, F-92260 France
2 C2VN, BIOMET, Aix Marseille Université, France

stephane.grison@irsn.fr

MEMBRE DE ETSON