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Inhibition of DNA double strand break repair by uranium and links with effects on reproduction of the zebrafish

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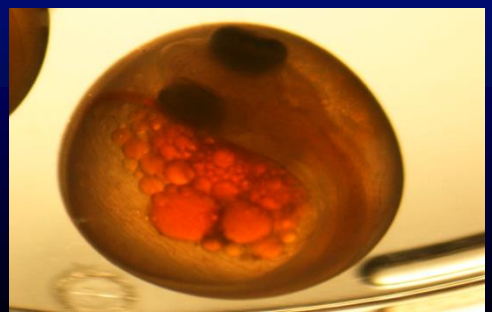

Uranium

nuclear fuel cycle, military, industrial & agricultural applications

92 238.03
U
Uranium

Contamination of aquatic ecosystems

Background [U] surface water : ~ ng/L to 6 µg/L

Vicinity of former uranium mines : ~ tens – 100 µg/L

U drinking water guideline : 15 µg/L WHO

Environmental Quality Standards range from 1 µg/L to 100 µg/L

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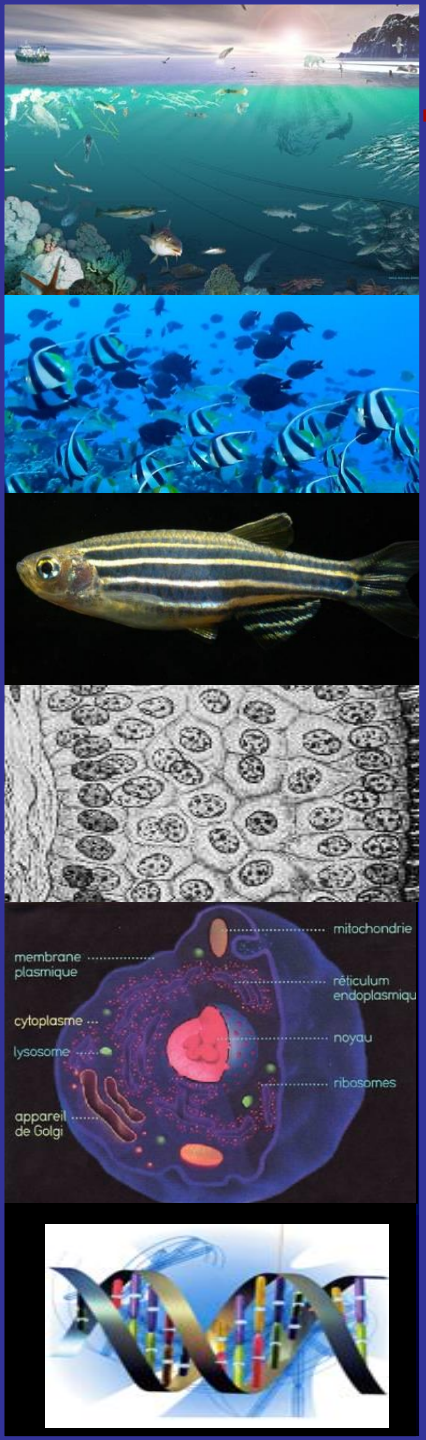
Biological endpoints



Ecological relevance

Mechanisms

Genotoxicity

⇒DNA is the 1st cellular target for radionuclides

·DNA damages in :

⇒ somatic cells : carcinogenesis

⇒ germ cells : teratogenesis and reprotoxicity

Objective

• Assess genotoxicity markers as predictive biomarkers for U effects on individuals or populations

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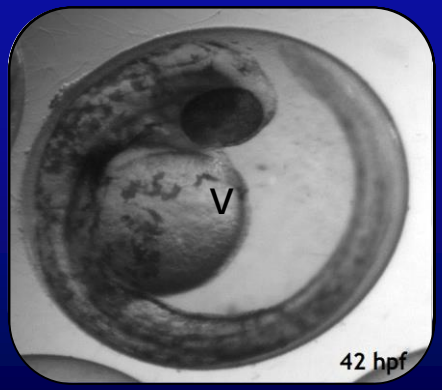
Zebrafish

- ✓ Easy breeding (mature in 3 month)
- ✓ Fully sequenced genome

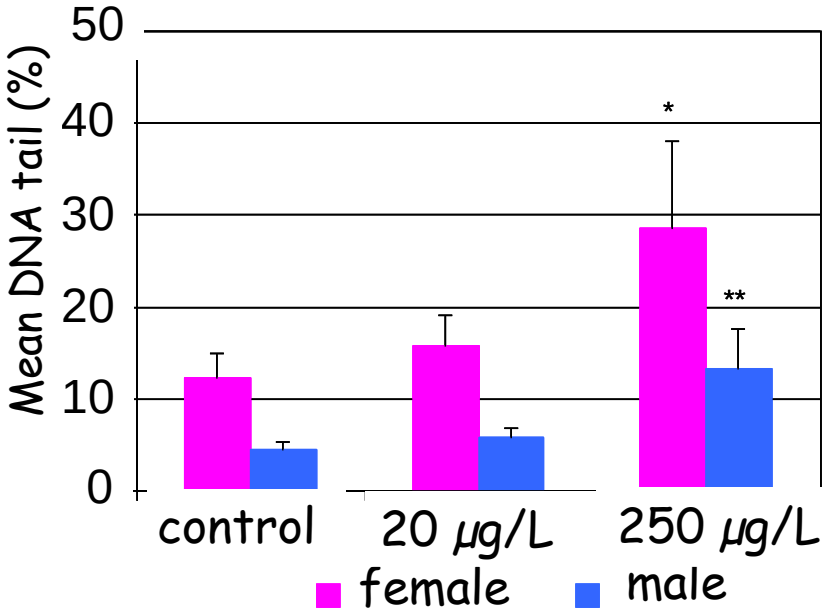


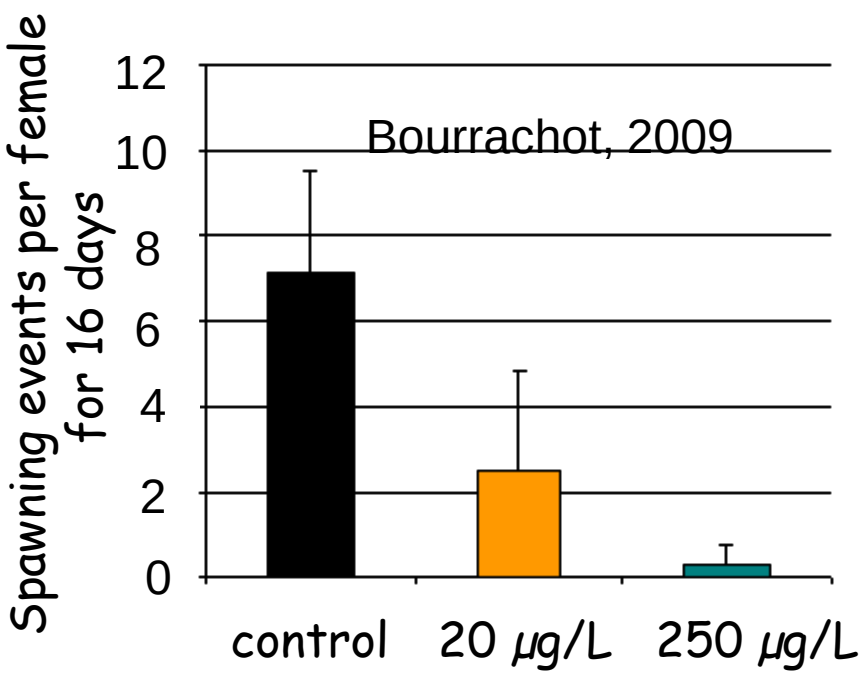
- ✓ Model for developmental studies

DNA damages and reproduction

Zebrafish exposure to uranium (20 days) and reproduction

Comet assay (gonads after 20 d exposure)





Significant increase of DNA damage in males and females gonads

Decrease of fecundity & of egg and larvae viability

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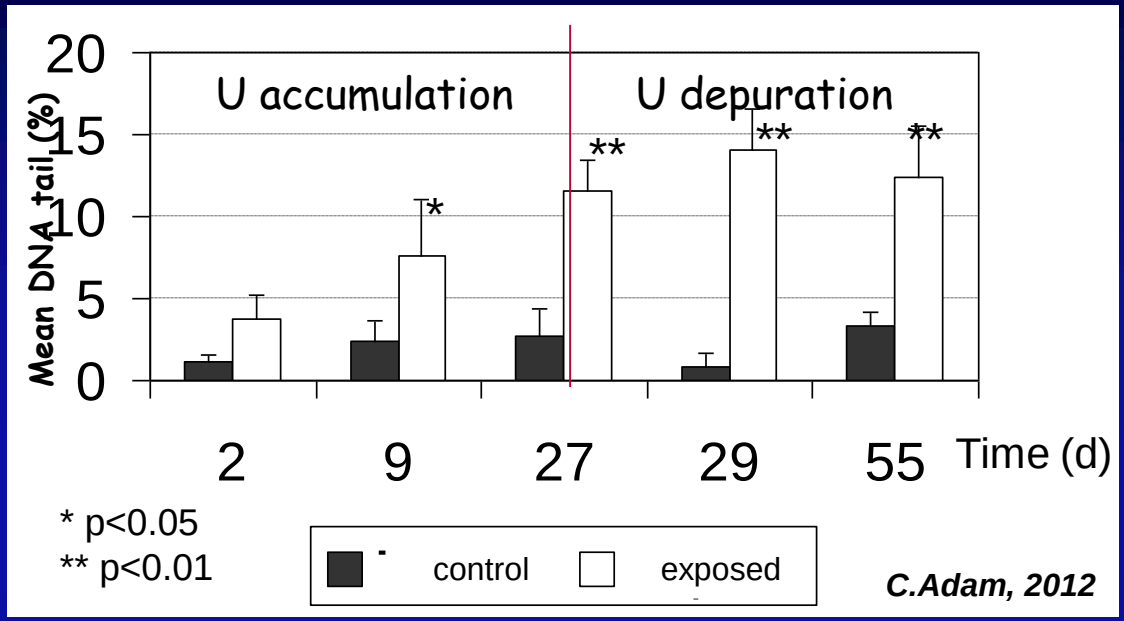
Reversible DNA damages ?

Exposure of zebrafish to 20 µg/L of uranium during 27 days
U depuration during 28 days

Comet assay in male gonad cells



- DNA damages are still observed after 28 days of depuration → occurence of DNA Double Strand breaks (DSBs) ? correlation with the decrease of fertility ?

Does U induce DNA DSBs in zebrafish?

Cells in G0/G1

DNA Double Strand Breaks

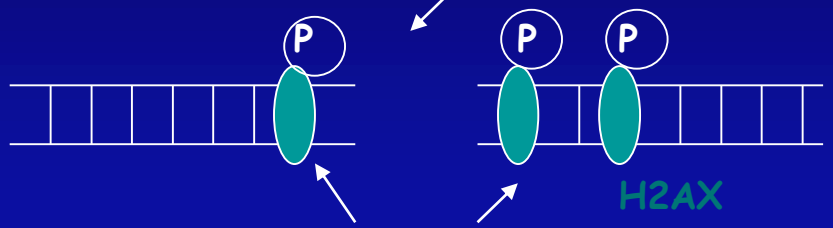
DSB repair

Time-response Experiments

DNA repair kinetics
Number of foci at 10min, 1h,2h, 4h, 24h

DSB detection

Dose-response experiments



ATM P

Ku70 Ku80 DNApK

NHEJ repair Pathway

P MRE11

MRE11 dependant recombination Pathway

Cytogenetic abnormalities

Micronuclei number

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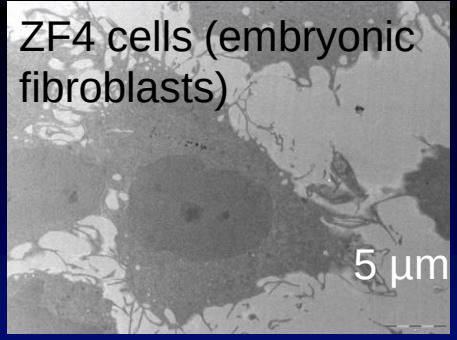
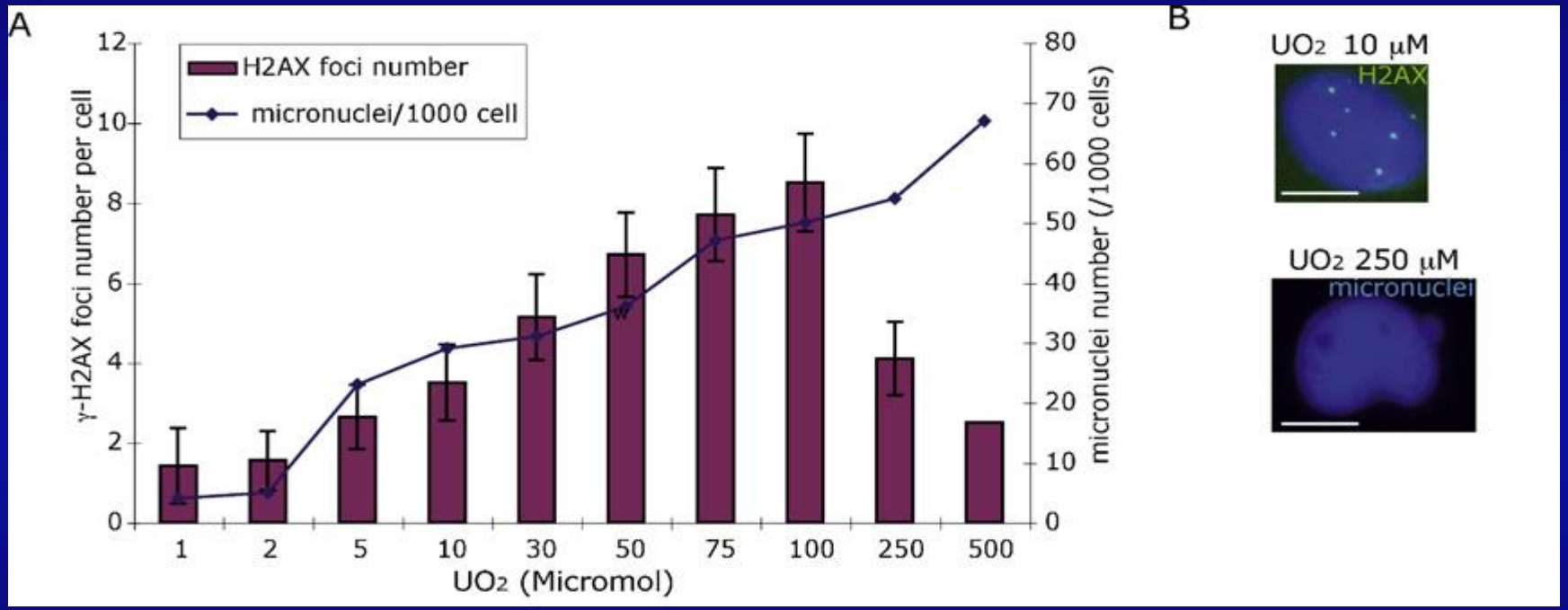


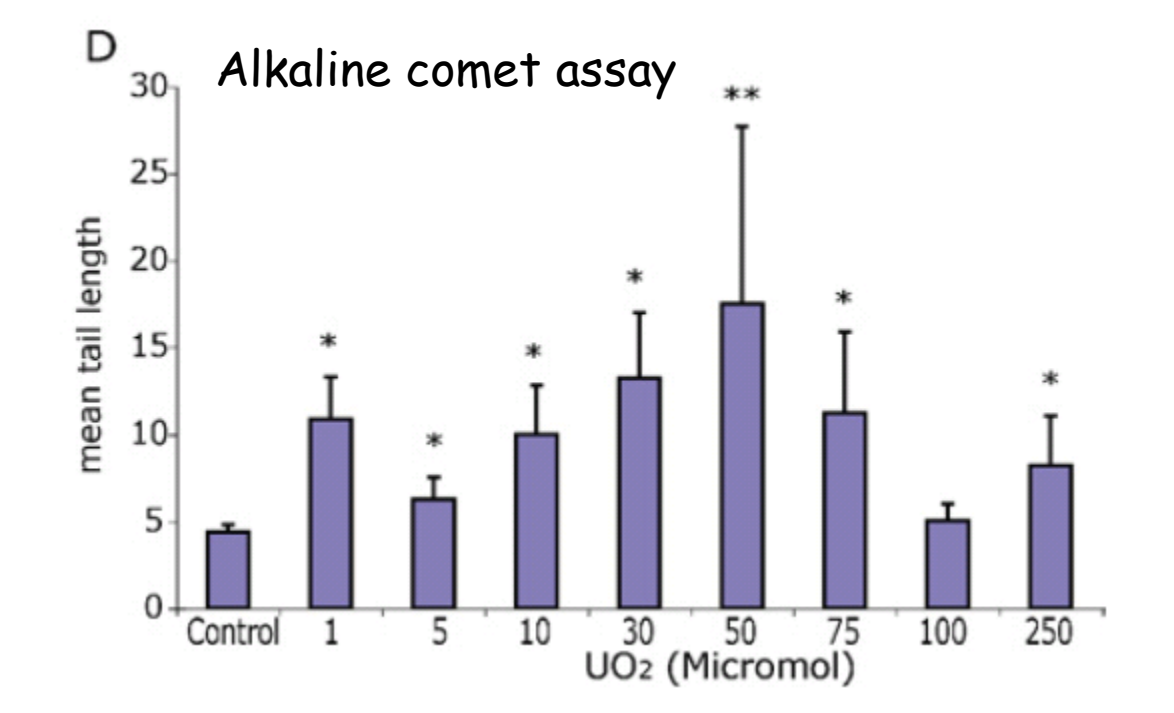
Uranium induces DSB in ZF4 cells



Pereira et al, Aquatic Toxicology, 2011

- ✓ No cytotoxicity up to 500 µM
- ✓ Increase of the nb of γ-H2AX foci per cell up to 100 µM

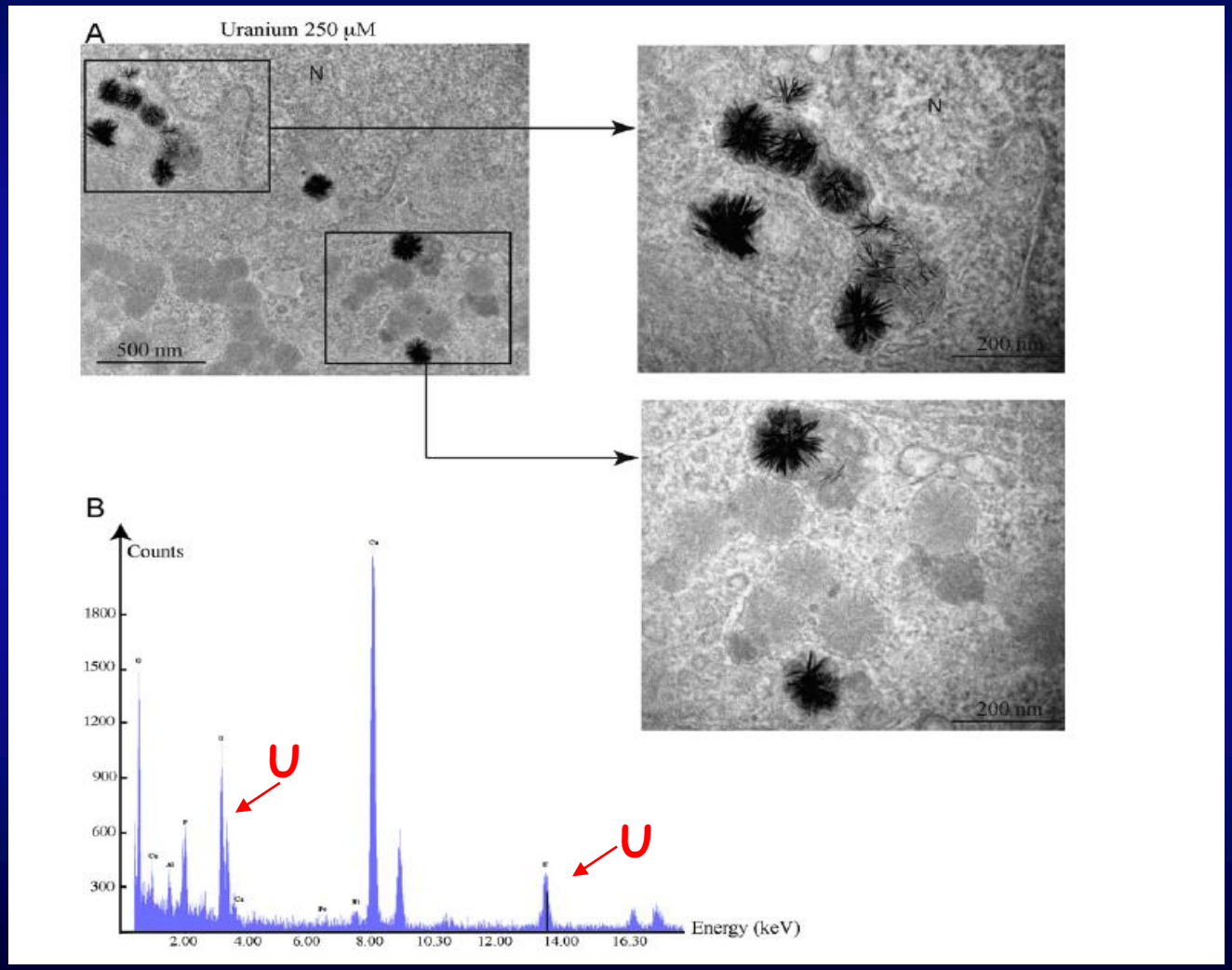
Occurrence of SSBs concomitant to DBSs formation



Pereira et al, Aquatic Toxicology, 2011

Genotoxicity of uranium on ZF4 cells

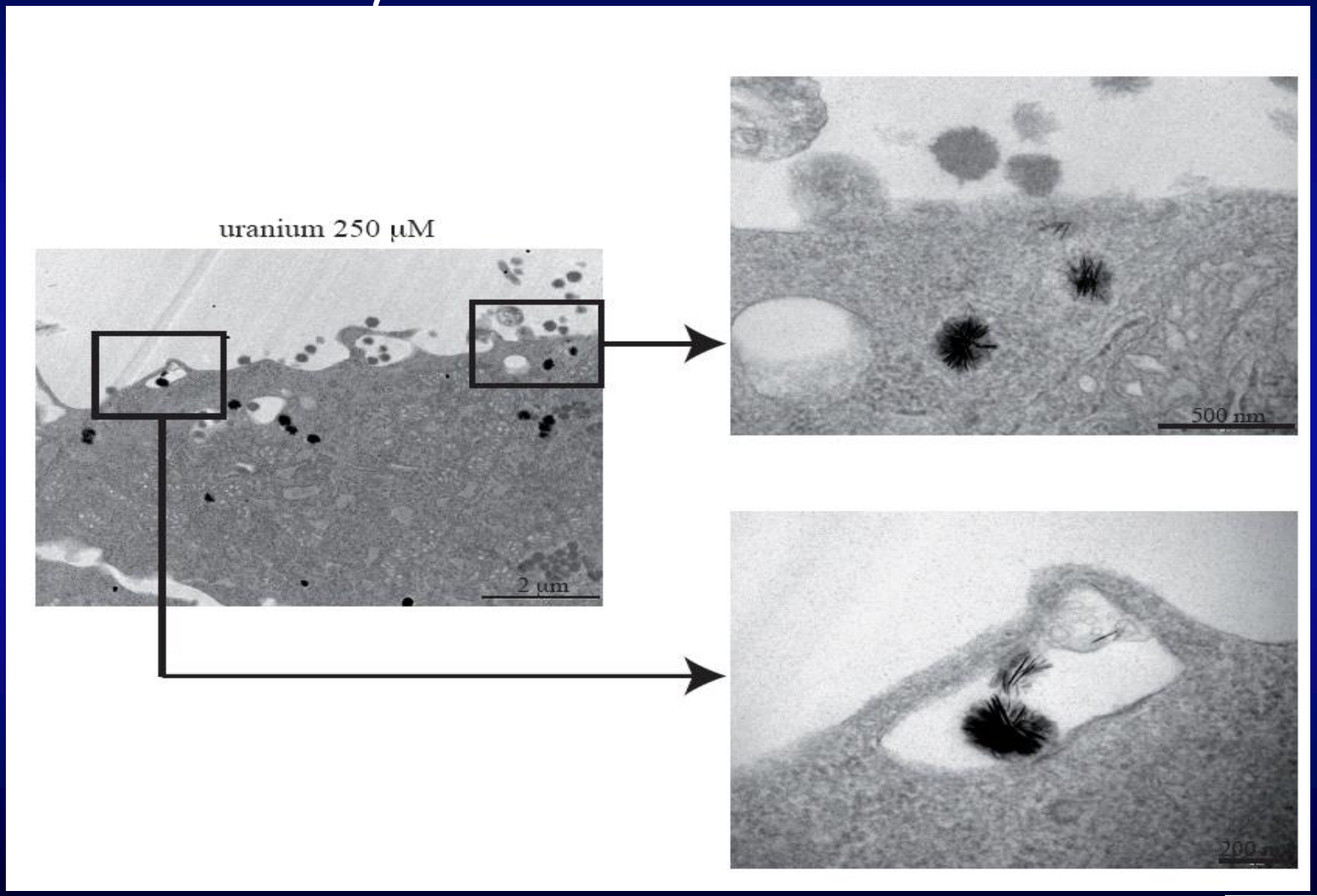
Increasing of U precipitates with the decrease of DSBs



Pereira et al, AquaticToxicology, 2011

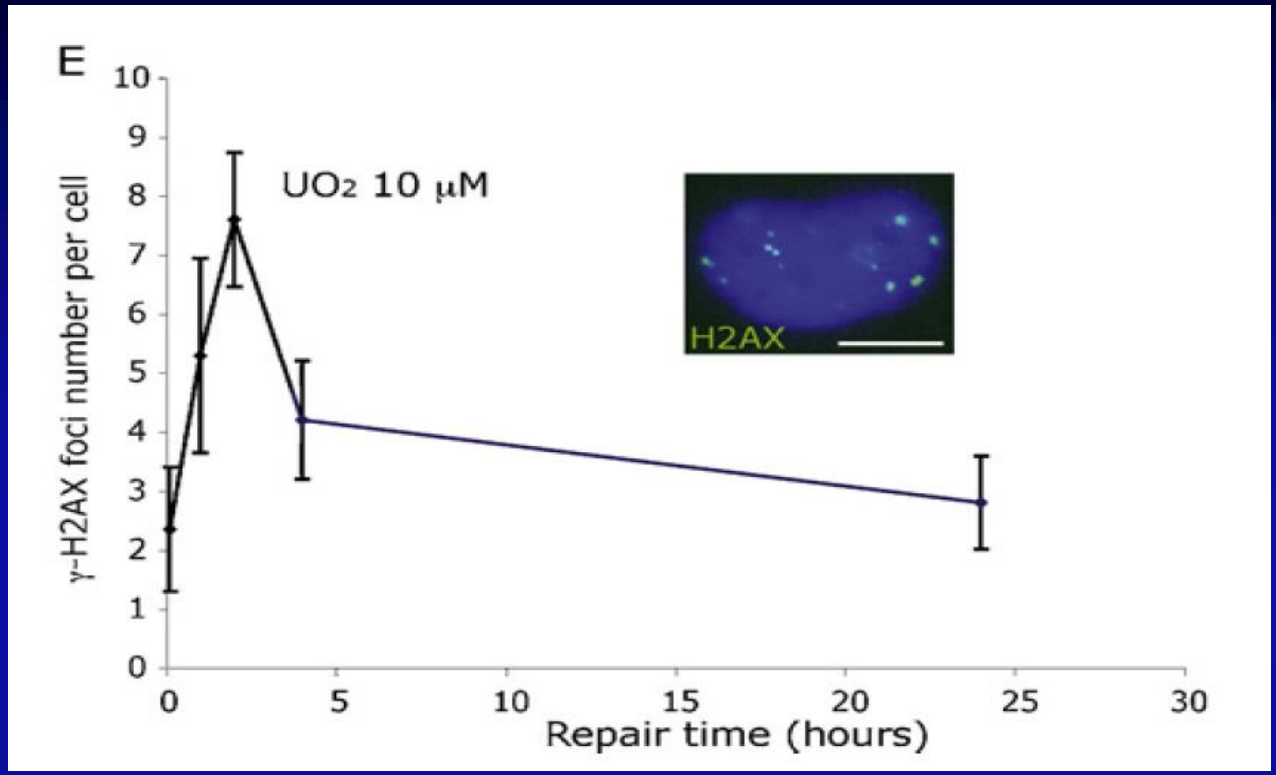
Genotoxicity of uranium on ZF4 cells

Urchins-like thin needle-shaped uranium structures, mainly concentrated in lyzosome-like vesicles



Pereira et al, Aquatic Toxicology, 2011

Unrepaired DBSs at low U concentrations



Pereira et al, Aquatic Toxicology, 2011

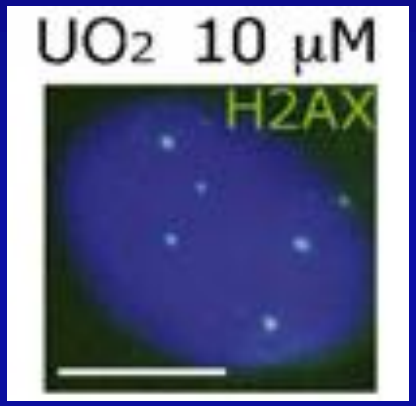
NHEJ repair was disrupted in uranium contaminated ZF4 cells: no DNApK foci was observed after 24 h of 10 µM uranium exposure

Conclusions

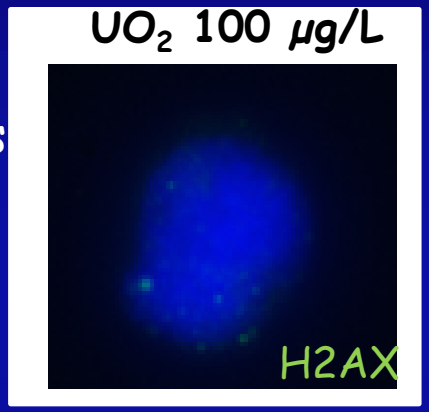
✓ Uranium induces DNA SSBs, DSBs and micronuclei

✓ These damages are probably linked to U reprotoxicity (similar internal [U] concentrations for in vivo & in vitro studies)

ZF4 cells



♂ gonad cells



✓ Uranium detoxication in lyzosome-like structures may explain the low cytotoxicity (< 10 %)

✓ Inhibition of NHEJ (DNA PK) complemented by another non specific DNA repair mechanism ?

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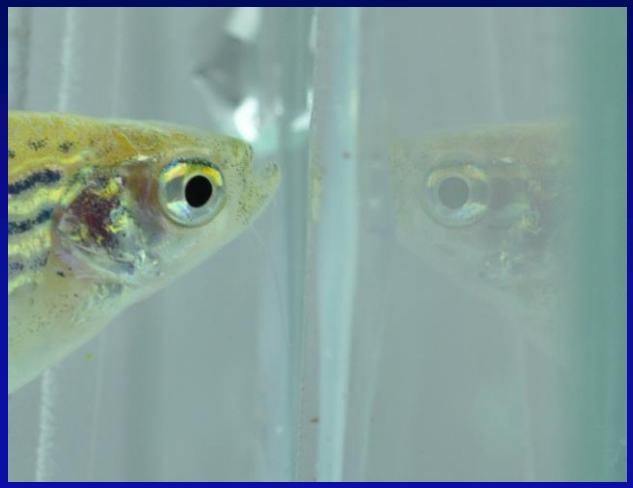
Thank you for your attention



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ANR HEMI-BREAKS
Envirhom project