ICRP TG121 : Effects Of Radiation Exposure On Offspring And Next Generations: Heritable Effects In Non-Human Species

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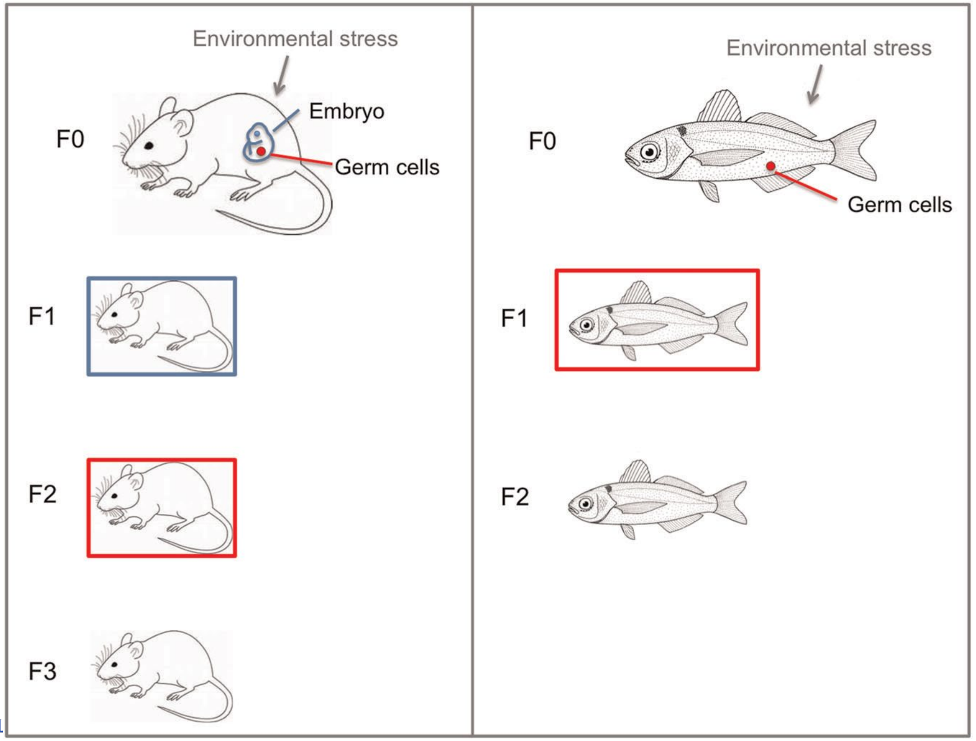
Background and scope of TG121 for biota

- Scientific reviews for radiation effects on biota (UNSCEAR 2008; ICRP108 and 124) provide perspectives to the radiation effects on animal and plant species. Effect data have been reviewed to propose new Derived Consideration Reference levels for environmental radiological protection (TG99) but **they do not include data on heritable effects**.

- **Due to varied generation times in animal and plant species** (life cycle of ~20 days for worms vs > 1000 years for some trees) and long exposure time, **this topic is of major importance for biota**.

Some definitions

- **Multigenerational experiments** : same irradiation pattern (transient, seasonal or chronic) for each generation
- **Transgenerational experiments** : aim at measuring an effect in an organism where no cell has been exposed to irradiation after parental exposure.



Head J. 2019

Are there any evidence of multi- or transgenerational effects in biota?

Study of descendants of *Rana japonica* obtained 1, 2, 3 and 4 generations after male and female gamete irradiation with X rays (1.5, 2.5 and 3.5 Gy). **In F4, fewer laid eggs and higher male frequency were observed** (Nishioka, 1978)

Continuous exposure of microcrustaceans (*Daphnia magna*) to ^{137}Cs (from 7 µGy/h to 35 mGy/h) **over 3 generations (78 days). Threshold** of effects on reproduction decreased from 35 mGy/h in F0 to 70 µGy/h in F2 (Parisot et al., Aquat. Toxicol., 2015). Genotoxicity increased also in offsprings over generations. Role of genetic factors?

Is there a role of genetic factors in these heritable effects?

Natural populations of *Drosophila melanogaster* sampled in 2007 in Chernobyl sites of varying contamination levels (up to 49 µGy/h) were studied under laboratory conditions. **Survival rate of offspring decreased over 160 generations and significantly correlated with gross chromosomal rearrangements (dominant lethal mutation levels)** while no significant change of the frequency of point/gene mutations (recessive sex-linked lethal mutations) (Yushkova and Bashlykova, Env. Mol. Mutagen., 2020).

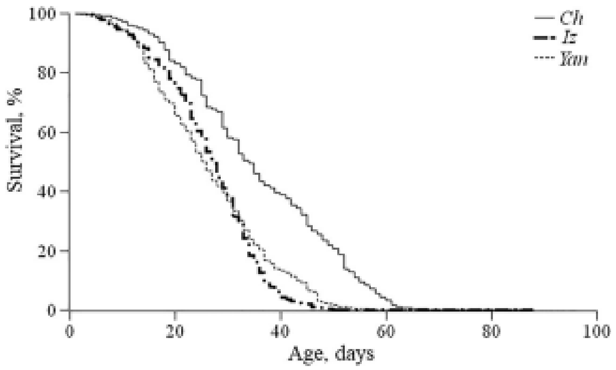


FIGURE 7 Mean survival rates of the F_{160} offspring from the study *Drosophila melanogaster*

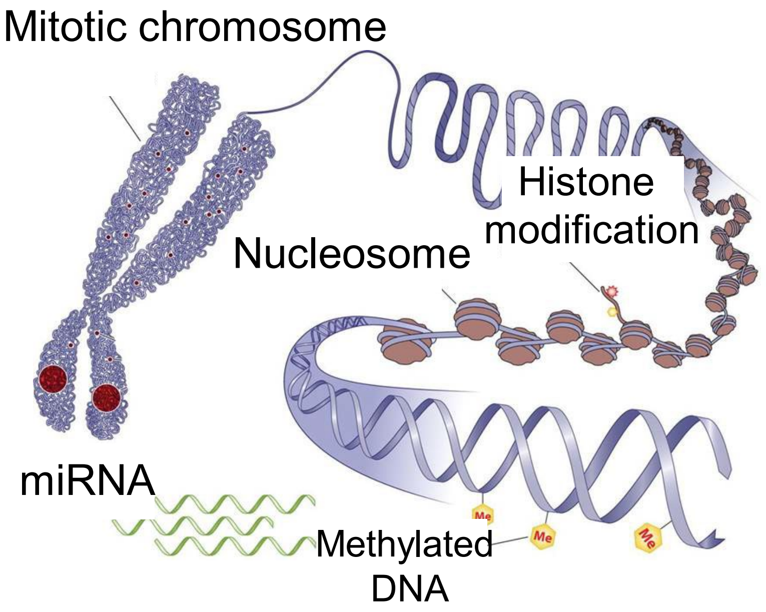
Is there a role of genetic factors in these heritable effects?

- In zebrafish exposed to 50 mGy/h of ^{137}Cs, **DNA alterations in gonads can be transmitted to offspring and be correlated to a decreased survival of F1s** (Guirandy et al., 2019). Decrease of fertility and increase of mutation rates, DNA damages or to chromosomes was also observed in birds (Ellegren et al., 1997 ; Bonisoli-Alquati et al., 2010) and mice in Chernobyl (Pomerantseva et al., 1997) or in the lab (>1 Gy) (Goodhead et al., 2013).

- Genetic markers have been examined in **19 frog populations in Chernobyl ~ 30 years after the accident**. **A higher mitochondrial diversity was observed for exposed populations**. Haplotype modeling indicates that Chernobyl frog population have a **high mitochondrial mutation rate and a small effective population size** (Car et al., 2021)

However, are there other mechanisms? Adaptation of pines to high doses in Chernobyl were observed despite deformities and DNA damages (Kovalchuck et al., 2003). These adaptive mechanism cannot be only genetic (10^{-5}-10^{-6} mutation per germ cell)…increase of DNA methylation : **epigenetic mechanism**

Epigenetic changes



Trygve and Tollefsbol (2012)
Burggren (2016)

Epigenetics : nuclear inheritance which is not based on changes in DNA sequence (Wu and Morris, 2001)

Results from interactions between **genome** and **environment leading to phenotypic changes (adaptation, adverse effects, evolution…)**

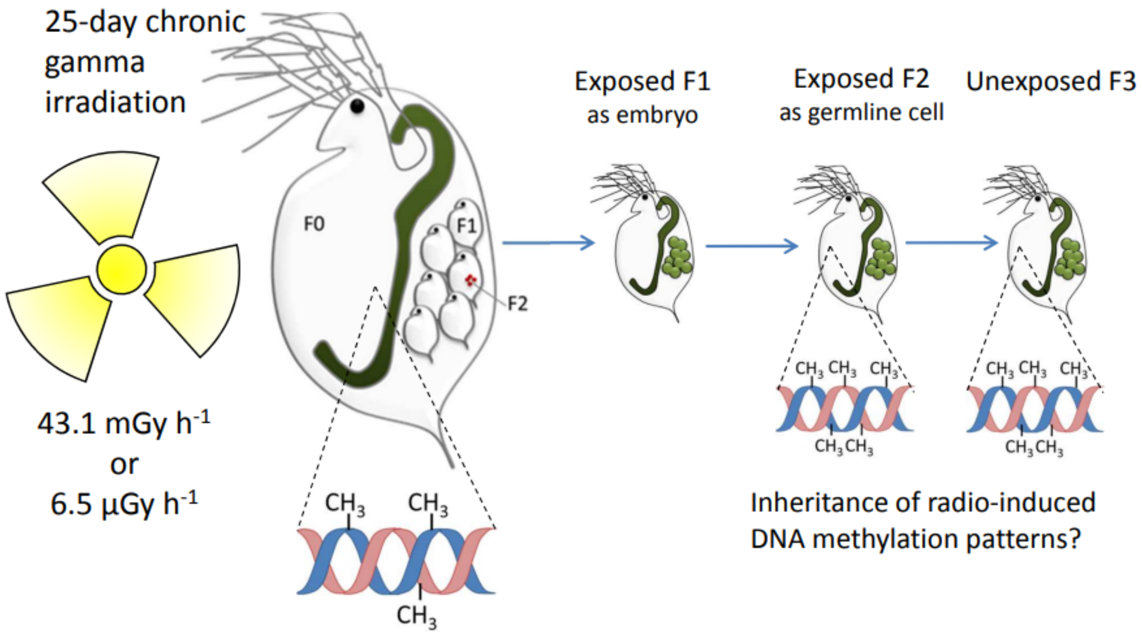
Involved in several **biological processes (development, specialisation ex : bees), and diseases** (prostate and colorectal cancers, diabete, cardiovascular diseases, obesity…)

Most studied epigenetic mechanisms :

1. **DNA methylation**
2. Histone modifications
3. miRNAs

Epigenetic changes in invertebrates

- After parental exposure of microcrustaceans to 6 µGy/h and ~40 mGy/h, **common methylation changes were detected between generations F2 and F3 showing that epigenetic modifications can be transmitted to unexposed generations. Highlighted differentially methylated genes have a function in radiation effect defence (HSP70, rpL28)**. Effects on reproduction in F0 but not in F1 to F3.

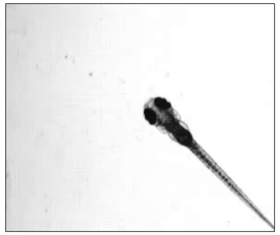


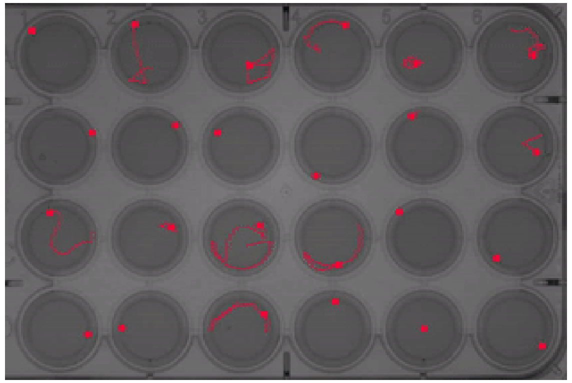
Trijau et al., 2018

Similar studies in C. elegans, with methylated DNA, histone modifications and miRNAs (Guédon R, 2022)

Epigenetic changes in vertebrates during developement

- In zebrafish embryos exposed from 0.005 to 50 mGy/h, a hypomethylation of DNA in the promoter of genes that colocalised with both **H3K27me3 and H3Kme4 histone marks** was observed and this correlated with changes in transcriptional activity. **DNA methylation changes occurred in the promoter of important developmental genes, including morphogenesis of the ectoderm and mesoderm** (Murat et al., 2020). These molecular effects were linked to neuromuscular impairments and larval motility defects in 5 days old larvae (Murat et al., 2019).





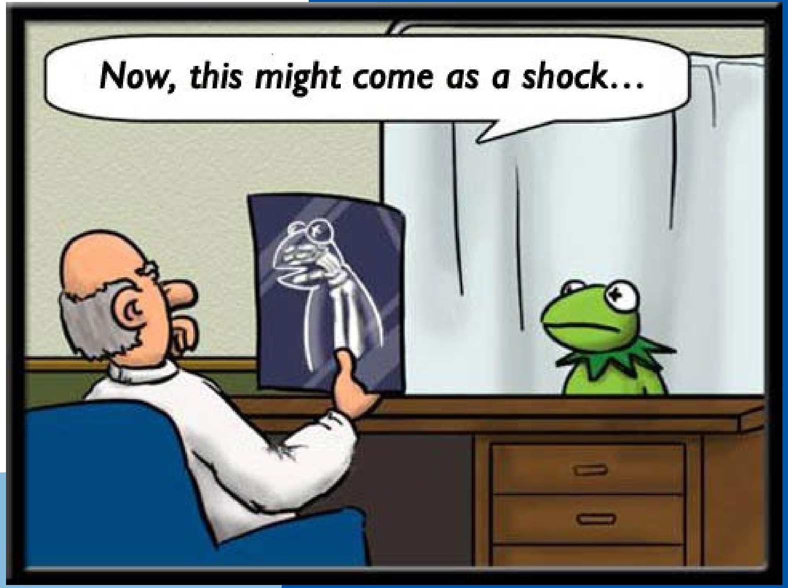
Epigenetic changes in vertebrates during transgenerational exposure

- DNA methylation was assessed in F1, F2 and F3 zebrafish embryos following parental exposure to 8.7 mGy/h for 27 days. **Differentially methylated regions were observed up to F3 generation and were related to pathways such as development, apoptosis and cancers, which could be linked to previous observed developmental defects and genomic instability in the offspring** (Kamstra et al., 2018). -> Monitoring DNA methylation could serve as a biomarker to provide an indication of ancestral exposures to ionizing radiation.
- Direct exposure of zebrafish embryos to gamma radiation (10.9 mGy/h for 3h) induced **histone modification at genes of embryonic development and cancer induction** (*hnf4a*). Same result was obtained for the same genes in ovaries of adult zebrafish irradiated during gametogenesis (8.7 and 53 mGy/h for 27 days) and in F1 embryos of the exposed parents, while these modifications were almost negligible in F2 embryos. **These results suggest that ionizing radiation can affect chromatin structure and organization, and that these changes can be detected in F1 offspring, but not in subsequent generations** (Lindeman et al., 2019)

Environmental perspective

- Are there ecologically relevant molecular effects impacting populations ?
- How to take into account the historical dose ? How many generations should be studied/monitored ? Are these changes reversible ?
- To what extend are these findings general for all biota or even for human ? (zebrafish and *C. elegans* have ~70% genetic similarity to humans)

Thank you!



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Other potential mechanisms

- **Persistence in tissues and transfer of contaminants (from F0 to F1) : uranium can be maternally transferred** to zebrafish offspring (Simon et al., ,Env. Toxicol. Chem, 2011 ; Gombeau et al., Aquat. Toxicol., 2017). Moreover, **elimination of U through reproduction (detoxication)** explains why the reproductive output is severely impacted in zebrafish (Augustine et al., 2012).

- **Changes in energy allocation :** mechanistic Dynamic Energy Budget model shows an effect of IR on the number of eggs and **on the cost for growth and maturation** in *C. elegans* exposed up to 27 mGy/h (Lecomte-Pradines et al., 2017).

- **Transfer of biological factors?** High levels of messenger RNA encoding metallothioneines in oocytes and in newly hatched F1 larvae were observed, conferring resistance to Cd (Lin et al., 2000). Transfer of small ncRNAs transmitted through the spermatozoa are the carriers of a paternal epigenetic memory for obesity (Barouki et al., 2018). What about ionizing radiation ?