Exposure of the nematode worm *Caenorhabditis elegans* during its whole life cycle to radionuclides (e.g. uranium) or external irradiation (gamma radiation) for the study of toxic effects on growth and reproduction kinetics

1- Organism husbandry

The wild-type N2 strain of *C. elegans* (provided by the Caenorhabditis Genetics Center, MN, U.S.A.) is maintained at 20 °C, 80% relative humidity in darkness, on Nematode Growth Medium (NGM)-agar, using *Escherichia coli* strain OP50 as food source (Stiernagle, 2006). *Escherichia coli* strain OP50 is grown in L-Broth medium at 37 °C overnight. NGM plate is seeded with saturated OP50 culture and is then exposed to UV for 20 min (l = 254 nm; intensity = 200 µW m^{-2}) to kill bacteria (figure 1). The UV exposure time was chosen in accordance to its capacity to suppress the colony forming unit ability (this was checked by inoculating UV exposed bacteria on LB-agar petri dishes and incubating them at 37°C overnight).

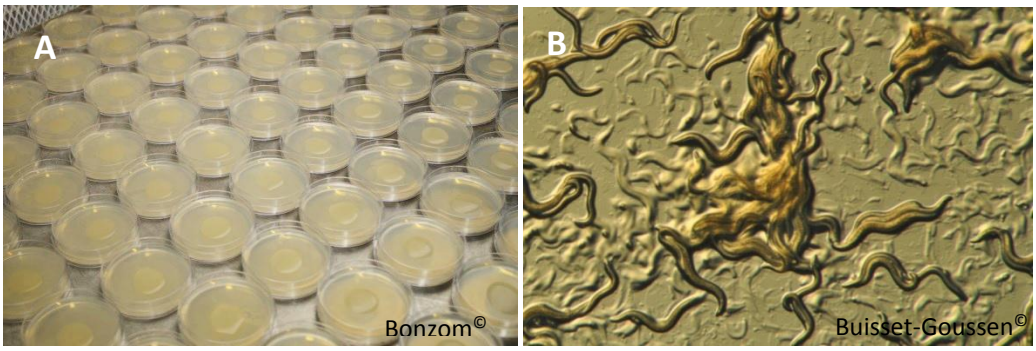

Figure 1: (A) Petri dishes that contained NGM-agar seeded with OP50 culture, (B) *C. elegans* population on NGM-agar plate.

2- Organism synchronisation

Gravid worms are randomly selected from the stock population and are placed on a new NGM-agar plate at t = 0h. After 1 hour gravid worms are removed and laid embryos are considered to be age-synchronized. Three embryos are placed into new plate (containing NGM seeded with OP50 *E. coli*) to ensure one larvae per plate. The next day, the supernumerary larvae are removed.

3- External gamma irradiation

Organisms are exposed to external gamma radiation from embryo stage to young adult (about 65 hours at 20°C). Each experimental unit (petri plate containing three embryos) are placed in containers perpendicular to the source to allow homogeneous dose rate at the surface of each experimental unit (figure 2, Buisset-Goussen et al., 2014). Dose rates are determined by the distance between the source and the organism. These dose rates are first determined by Monte Carlo N-Particle Model (MCNP). Before the experiment, radio photo

luminescent (RPL) dosimeters are placed on petri plate to validate the model. During the test, RPL dosimeters are placed on the back of the container to check the stability of the dose during the experiment. Temperature and moisture are continuously measured using data logger.

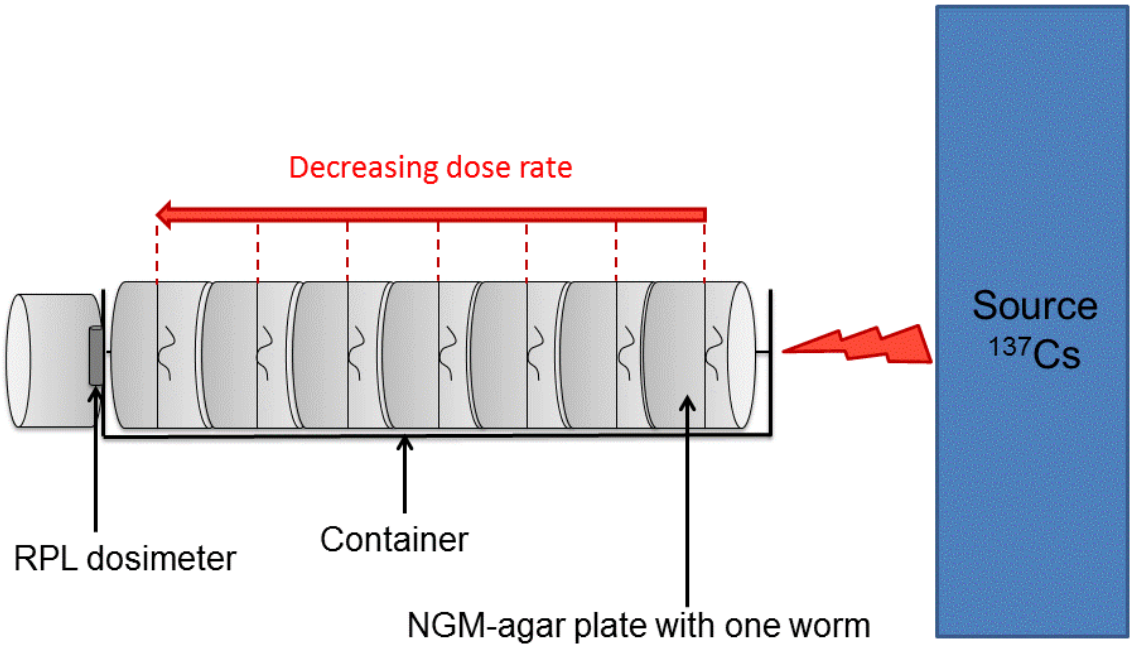

Figure 2: Experimental setup for *C. elegans* exposure to gamma radiation. Plate surface is perpendicular to the source in order to exposed *C. elegans* to a uniform dose rate. The distance to the source provides a range of dose rates. RPL dosimeter allows an accurate measurement of the dose to the organisms.

4- Exposure to uranium

Worms are exposed to uranium on plates that contained modified NGM-agar in which KPO4 buffer is replaced by HEPES buffer to avoid U precipitation (Dutilleul et al., 2013). Stock solutions of uranyl nitrate in demineralized water (62 mM) are used to spike NGM-agar before pouring it in plates. Extra additions of NO_3^- and acidification of medium due to uranyl solution is compensated with additions of $NaNO_3$ and NaOH. Plates were then seeded with *E. coli* solution and exposed to UV as detailed previously. Plates are prepared maximum

24 h before exposure and are kept at 20 °C. Samples of NGM-agar contaminated with uranium are kept for later chemical analysis.

5- Endpoints measurement

5.1- Reproduction

Single worms from exposed and control plates are transferred daily into new plates (that contain NGM + OP50 lawn) following spawning (figure 3A). Plates containing the eggs are placed at 20°C overnight to allow hatchability. The hatched progeny and unhatched eggs are counted twice (difference between two counts should be below 10%) the day after each transfer (figure 3B). By using this method, the measured endpoints are cumulative brood size and hatchability.

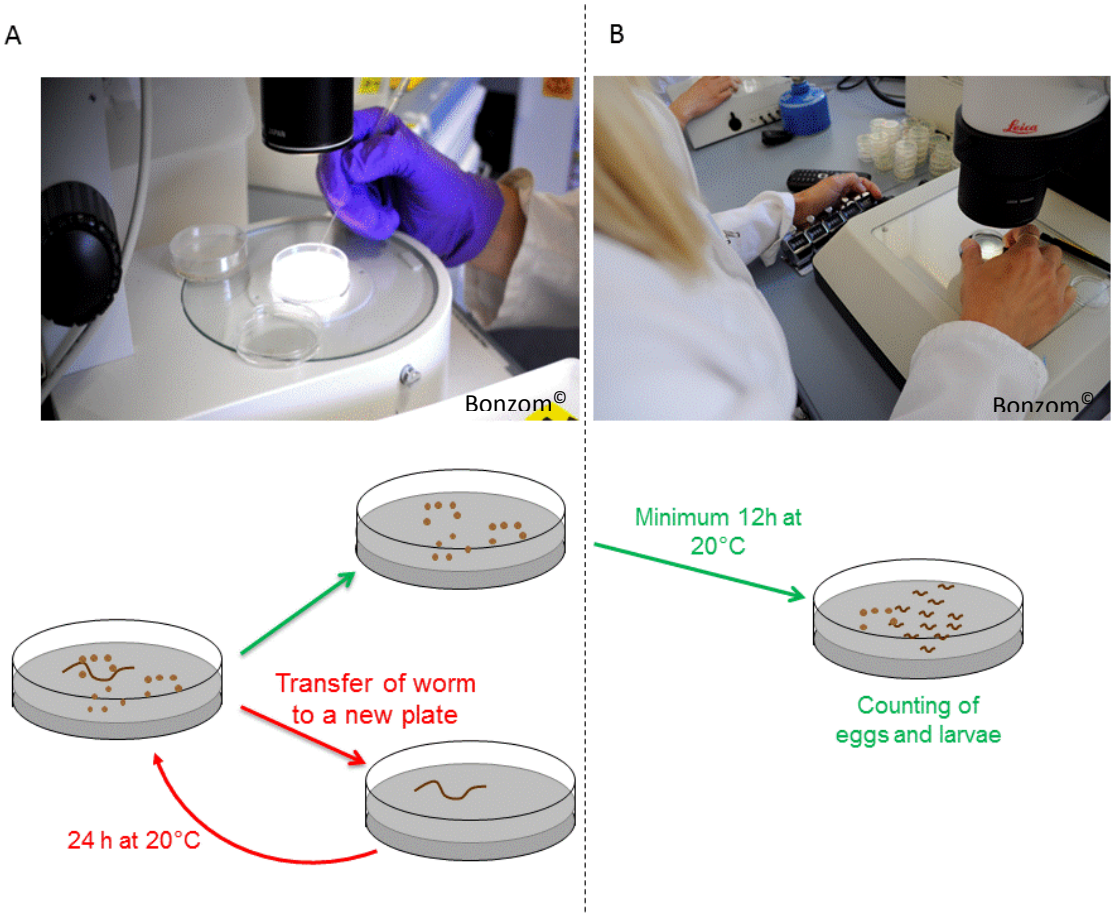

Figure 3: Schematic of the method to measure *C. elegans* total brood size and hatchability. (A) *C. elegans* are daily transferred to a new plate and (B) the progeny is counted the day after.

5.2- Growth

Worms are photographed (using a stereomicroscope coupled with a computer connected camera) twice a day from hatching to maturity then once a day until maximal size. Body length is measured by analysing the picture using the software Image J® and a micrometer

scale measure (figure 4). Length increase was determined by the calculation of the difference between measured length and the average measured length at hatching.

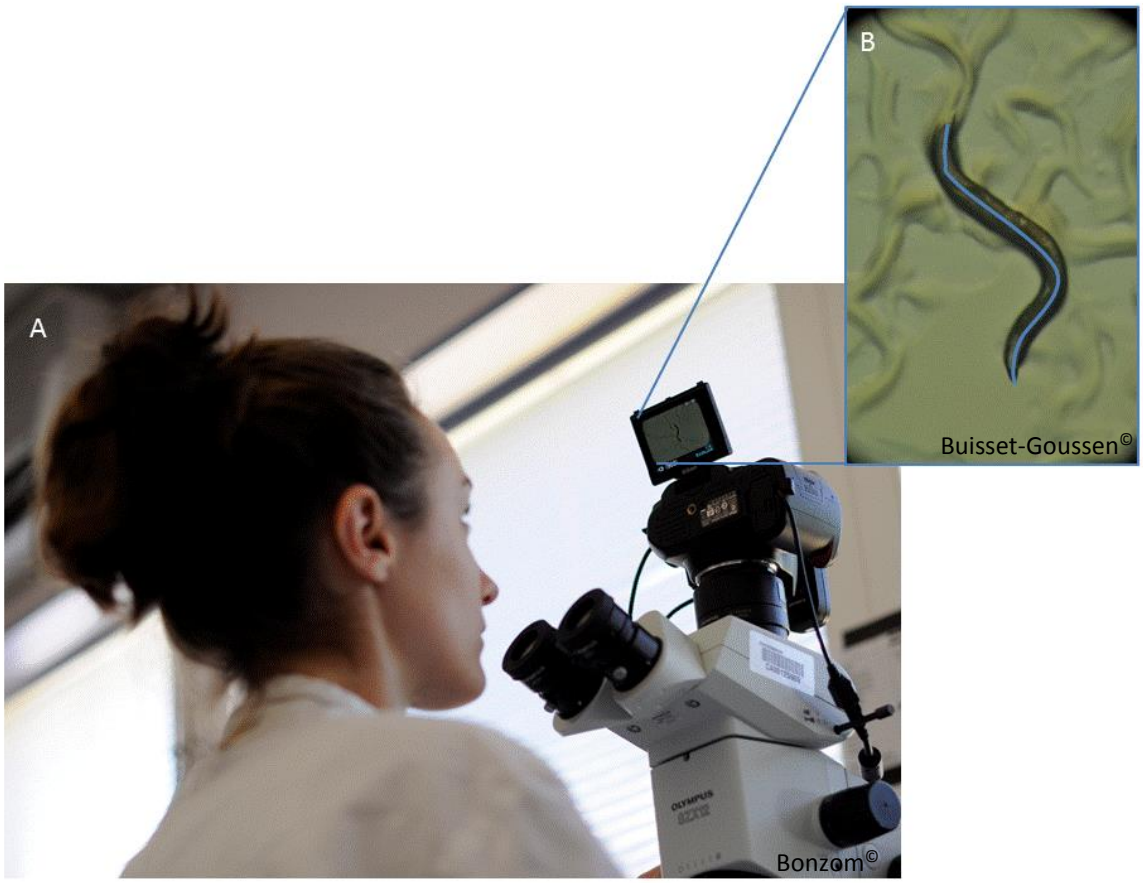

Figure 4: Schematic of *C. elegans* size measurement. (A) Worm is photographed daily using a stereomicroscope coupled to a camera and (B) the picture is analysed using Image J® software.

References:

Buisset-Goussen et al., 2014. J. Environ. Radioact., 137 : 190-194.

Dutilleul et al., 2013. Ecotoxicology, 22:862-868.

Stiernagle, 2006. Wormbook: the online review of C. elegans biology, pp:1-11.

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