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DELIVERABLE 5:
ANNEX B
Experiments on chronic exposure to radionuclides and induced biological effects on two invertebrates (earthworm and daphnid). Results and discussion

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ERICA will provide an integrated approach to scientific, managerial and societal issues concerned with the environmental effects of contaminants emitting ionising radiation, with emphasis on biota and ecosystems. The project started in March 2004 and is to end by February 2007.

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Forward

The dissemination of this Annex is temporarily restricted to partners of the ERICA project, because the authors intend to submit the experimental work for publication in peer-reviewed journals. Scientific editors generally impose the publication of original unpublished data and the transfer of copyrights to the editor for exclusive distribution, thus the dissemination of data has been restricted until the publication of corresponding manuscripts. The authors believe that the publication of results in scientific articles will allow a broader diffusion to the scientific community. This type of publication will also strengthen the PhD and post-doctoral works performed within the ERICA project.

Primary data (tables of results) are publicly available in the D5 manuscript, as well as the following executive summary

Executive Summary

This Annex details the experimental works performed within the WP2 of the ERICA project. The aim of the experimental work was to illustrate types of data and associated mathematical treatment that might be utilised within a risk assessment and management framework. Within D5, the experimental data have been used to address two extrapolation issues: external vs internal irradiation and individual vs population. In this respect, the experiments have been conceived to illustrate the application of methodologies (1) to derive relevant alpha to gamma weighting factors on reproduction endpoints and chronic low-level exposures, and (2) to acquire a robust set of data to allow individual to population extrapolation on a science-based reasoning.

Two recognised ecotoxicological test organisms were chosen: one short-lived aquatic invertebrates (waterflea *Daphnia magna*) and one soil invertebrates (earthworm *Eisenia fetida*). External gamma exposure rates were designed from 0.4 µGy/h to 31 mGy/h for daphnids (at IRSN) and from 0.19 µGy/h to 42 mGy/h for earthworms (at UMB), and alpha exposure of daphnids was performed with ^{241}Am (at IRSN). For both internal and external exposure a variety of reproduction endpoints (*e.g.* number of offspring, survival and growth of offspring, sexual maturation) were followed over different life-stages and generations. This Annex gives a detailed description of the specific measured parameters for the two organisms (daphnids and earthworms) and the to exposure pathways (internal alpha and external gamma).

The waterflea *Daphnia magna* was continuously exposed to external gamma irradiation from a ^{137}Cs source and internal alpha irradiation from ^{241}Am during a large part of the life cycle, from <24h neonates to 23-day adults, during this period 5 to 6 broods occurred). Adult F0 reproduction capacity (i.e number of eggs and neonates produced) and F1 survival to starvation were measured over a 21 day exposure period, at 3 dose rates (external gamma: 0.4, 4.2 and 31 mGy/h and internal alpha: 0.01, 0.07 and 0.80 mGy/h). External irradiation was easier to set up than the internal exposure scenario for which several uncertainties could not be solved simply (*eg.* accumulation and depuration biokinetics measurement; contribution of the trophic way of bioaccumulation; choice of the geometrical models and experimental constraints: maximum 6000 Bq of ^{241}Am can be manipulated each time). During these experiments, survival, growth in dry mass and reproduction investment were examined.

Results showed a reduction in body dry mass at the alpha dose rate of 0.80 mGy/h, whereas duration of instars and moulting frequency was not affected. The mass-specific respiration rate was significantly higher at 0.80 mGy/h than in the control, as a result of combined high oxygen consumption and low body mass. Fecundity did not differ significantly between exposure conditions, but egg dry mass was affected. This reduction in egg dry mass had a slight influence on the total mass of eggs produced per daphnid over the 23-day period. Therefore, concomitant reductions in daphnid individual mass and total mass of eggs produced resulted in unchanged mass-specific fecundity rates between exposure conditions. Furthermore, the dry mass of neonate differed between exposure conditions, with a significantly smaller weight at the dose rate of 0.80 mGy/h than in the control. This reflected differences in the amount of energy reserves invested per egg and had potentially strong consequences for larval resistance to starvation. In conclusion, the most sensitive endpoints examined in the current study were linked to biomass investment in reproduction.

In contrast to alpha exposure, gamma radiation caused no significant reduction in body dry mass or change in moulting frequency up to the dose rate of 31 mGy/h. The mass-specific respiration rate was significantly lower at 31 mGy/h than in the control, as a result of greater body mass. Compared to results obtained with ^{241}Am, daphnids started producing eggs at the same age but broods 3 to 5 were deposited earlier than in the control and fecundity was significantly lowered However, egg and neonate dry mass was not significantly affected. As a consequence, the combination of early brood deposition and decreased fecundity resulted in a smaller total mass of eggs produced per daphnid at 4.2 mGy/h and 31 mGy/h than in the control. Even if dry mass of neonate did not differ between gamma exposure conditions in a dose-dependent way, a smaller resistance to starvation was observed

The earthworm experiments focused on providing data on the effects of ionising radiation on reproduction parameters relevant for population dynamics.The earthworm *Eisenia fetida* was continuously exposed to gamma irradiation from a ^{60}Co source during different stages of the life cycle, in 2 generations (F0 and F1). Adult F0 reproduction capacity (*i.e.* number of cocoons produced, hatchability and number of F1 hatchlings) was measured over a 13 week exposure period, at 5 dose rates (0.18, 1.7, 4, 11 and 42 mGy/h). The total accumulated doses were 0.37, 3.6, 8.6, 23 and 85 Gy at the respective dose rates. Survival, growth and maturation of F1 hatchlings, produced during the last period of adult F0 exposure, were examined for 11 weeks, at 4 dose rates (0.18, 1.7, 4, 11 mGy/h). This was followed by 13 weeks' exposure of the F1 as adults, for registration of their reproduction capacity. At the end of the maturation and reproduction studies, the total accumulated doses in F1 were approximately, 0.64, 6.1, 15 and 40 Gy, at the various dose rates, respectively.

Results showed that at the highest dose rate (42 mGy/h) the hatchability of cocoons produced during the first 4 weeks of adult F0 exposure was reduced to 60 %, compared to 98 % in controls. Effects were even more pronounced at longer exposure times. At the highest dose rate none of the cocoons produced between 5 and 13 weeks hatched. At 11 mGy/h a pronounced effect on cocoon hatchability was observed only after 13 weeks of adult F0 exposure, when hatchability was reduced to 25% of that in controls. Exposure to 4 mGy/h gave a slight, but not significant, reduction in the hatchability and the numbers of hatchlings emerging from each cocoon. However, at the end of the 13 week period, the total number of F1 hatchlings produced was significantly lower than in the controls. For adult F1, a significant reduction in hatchability of cocoons was observed at 11 mGy/h over the entire 16 week exposure period, ranging from 45 – 69% of the controls. However, and in contrast to the results observed for F0, hatchability increased with time, possibly indicating adaptation of the F1 organisms Dose rates below 4 mGy/h gave no observable effect on the reproduction capacity of either adult F0 or adult F1 worms. In conclusion, the most sensitive endpoints examined in the current study were the hatchability of the cocoons and the number of hatchlings emerging from each hatched cocoon. The likely mechanism behind the reduction in hatchability is that damage is induced in male germ cells (i.e spermatogenic cells) or that there is accumulation of damage in the testes and seminal vesicles, resulting in reduced sperm production or infertile sperm. The results confirm that reproduction is a more sensitive endpoint as compared to mortality (approximately 30 times), but also illustrates that the standard OECD reproduction test for *Eisenia fetida* (4 weeks of exposure) is not applicable to the assessment of chronic radiation effects. The test would have seriously underestimated the radiation effects on this species. This underlines the need for extended exposure periods, including during more than one generation, when radiation effects on population dynamics are to be analysed. Since the delay between exposure and observation of effects in individuals is linked to underlying mechanisms in spermatogenesis, this need for sufficient exposure and monitoring periods would apply to any species tested for reproduction effects.

In conclusion, experiments on both daphnids and earthworms illustrated a method of providing a robust set of data to be used for the extrapolation of effects from the individual to the population on a science-based reasoning. Future studies on daphnids could be supplemented by the measurement of energy reserves and subsequent dynamic energy budget modelling. The studies also showed that obtaining data for the reduction of uncertainties related to the use of the RBE on non-human species would be possible and useful. But this issue cannot be solved simply, and the only way to improve the robustness of datasets (and consequently of RBE) would be to obtain a full dose(rate)-effect relationship (*i.e.* from no effect to maximal effect), both from alpha and gamma exposure, on each individual endpoint.