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Contamination of terrestrial gastropods *Helix aspersa Maxima* with ^{137}Cs, ^{85}Sr, ^{133}Ba and ^{123M}Te by direct and trophic pathways

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Abstract. Contaminations of the terrestrial gastropods *Helix aspersa maxima* by direct deposition or labelled food ingestion of ^{137}Cs, ^{85}Sr, ^{133}Ba and ^{123m}Te were carried out under laboratory conditions. The aim of this study was to compare the two contamination pathways: direct and trophic, in terms of individual mortality and radionuclide uptake, depuration and tissular distribution. A first group of 30 snails (2-years old) was exposed to radioactive aerosols during a twenty-hour period. These aerosols were assumed to be representative of those that would be released during a nuclear accident occurring in a PWR. A second group of 40 snails (same age) was submitted to an ingestion of food contaminated by the same aerosols, twice-a-week for 10 days (flour at a feeding rate of about 0.2g). During the 21 day-observation period, a comparison between the two groups and the reference group (not contaminated by radionuclides) was performed. No significant difference between the three groups was observed in the growth or in the mortality. One day after deposition, cesium was the most bioavailable element, distributed rather homogeneously through the whole body (from 13 to 28% of the total Cs in organs other than the digestive system and the muscle, respectively). Strontium accumulated in the shell (about 70%). Barium was found in the muscle (20%) and the shell (65%). Tellurium was mainly present in the shell (70%) and in the digestive system (20%). After a few days, this element was mostly present in the faeces. As regards contamination by ingestion, it was mainly accumulated in the digestive system.

1. INTRODUCTION

In the terrestrial trophic systems, invertebrates are of great ecological importance in terms of pollution transfer. They are directly subject to the effects of air pollution by direct contamination, by direct contact with the ground or the plants, or by ingestion of radionuclides taken up by the plants. Gastropods were also selected as biological models. Moreover, they are recognized as adequate bioindicators, because of their ability to accumulate elements. They are all the more interesting as they are more active in rainy weather: when most of the elements are in solution, they are more assimilable by the various tissues. If gastropods were used within the context of research relating to heavy metal pollution [1], they are seldom used in the field of radioecology [2]. A first exploratory phase of research has been undertaken in order to determine the response of gastropods to a contamination by direct deposition or by labelled food ingestion of ^{137}Cs, ^{85}Sr, ^{133}Ba and ^{123m}Te following a nuclear accident.

2. MATERIALS AND METHODS

2.1 Samples

The studied snails were obtained from a breeding farm, which allowed us to control the food that they had consumed at all the stages of their development cycle. The gastropods were *Helix aspersa maxima* specy (Figure 1) with initial mean individual weights of 19.88 ± 2.63 g and about two years old at the time of the experiments. They were fed with a mixture of vegetable flour whose composition was as follows: oilcake soya bean (20%), Maize (27%), Manioc (10%), Maize gluten (7%), Carbonate calcium (28%), Salt (0.4%), Oligo elements (0.1%), Vitamines (0.5%), Phosphates bicalcic (4%), "solubles de distillerie" (3%). The flour (contaminated or not) was given at a feeding rate of about 0.2g per individual, twice-a-week. Simultaneously, rainfalls with an intensity of 8mm.h^{-1} were applied for 10 minutes. Rainfalls were produced by a rain simulator, supplied with demineralised water. Throughout the experiment, the contaminated snails and the reference group were placed in individual PVC boxes in a greenhouse in a temperate climate with a temperature change between day and night (the observation period was spread over 2 months: from mid-May to mid-July). The extreme values of the temperature reached 8°C for the minimum and 27°C for the maximum in May, 11°C for the minimum and 30°C for the maximum in June and 15°C for the minimum and 32°C for the maximum in July.

Figure 1. *Helix aspersa maxima* species and experimental (set up).

2.2 Contamination

The IRSN has developed a special device (POLYR) enabling the simulation of a core melting accident followed by the release of aerosols into the atmosphere (S3 source term) [3 and 4]. Aerosols were produced by placing 15 elements, in the form of powder, in a graphite crucible, and heating the mixture to 2800°C by induction in a water-saturated atmosphere. The 15 elements represent the structural materials, the zircalloy cladding and control rod components, and the fission products (i.e. matter present in a 900 MWe pressurised water reactor). The POLYR device was used to contaminate gastropods and their food (Figure 2) by radioactive aerosols (^{137}Cs, ^{85}Sr, ^{133}Ba and ^{123m}Te). The deposition on the snails and on their food lasted for 20 hours.

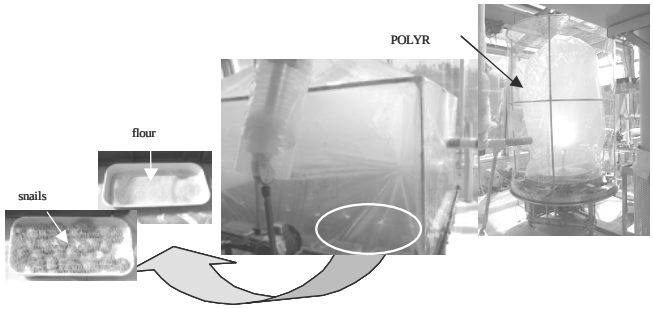

Figure 2. The contamination device with the POLYR furnace (on the right) and the trays of contaminated flour and snails.

The experimental protocol of snail contamination was structured as follows:

- Direct exposure (30 snails): about 1-day of direct deposition of radioactive aerosols. By direct exposure, each snail received about (± 20%): 1200 Bq of ^{85}Sr, 1400 Bq of ^{123m}Te, 1400Bq of ^{137}Cs and 1600Bq of ^{133}Ba. Then, the snails were subjected to 11-days of depuration (not contaminated food, of about 0.2g (± 5%) per individual, twice-a-week).
- Trophic way (40 snails): 11-days of labelled food (4 meals). Each snail ingested contaminated flour of: 80 Bq.g^{-1} of ^{85}Sr, 120 Bq.g^{-1} of ^{123m}Te, 82 Bq.g^{-1} of ^{137}Cs and 150 Bq.g^{-1} of ^{133}Ba at a feeding rate of about 0.2g (± 5%) per individual, twice-a-week. The weight of one becquerel is 1.13 10^{-12} mg for ^{85}Sr, 3.05 10^{-12} mg for tellurium, 3.10 10^{-10} mg for cesium and 1.06 10^{-10} mg for ^{133}Ba. These values give the following food concentrations: 9.04 10^{-8} µg.g^{-1} for strontium, 3.66 10^{-7} µg.g^{-1} for tellurium, 2.54 10^{-5} µg.g^{-1} for cesium and 1.59 10^{-5} µg.g^{-1} for baryum.

At the end of the ingestion period, the snails were subjected to 11-days of depuration (not contaminated food, of about 0.2g (± 5%) per individual, twice-a-week).

2.3 Sampling, analysis and data treatment

The animals were killed by freezing (-20° C) after eating nothing for 2 days; later, they were thawed and dissected in order to separate the shell, the muscle, the digestive system, the remains of organs and to recuperate the physiological fluid. Each piece was weighed and analysed by γ spectrometry using a high-resolution germanium co-axial detector (EGPC 20-180-R). All measured values were corrected for physical decay from the day of aerosols deposition.

The fraction of the received radioactivity (either by direct deposition or by ingestion) retrieved at a given date in a snail tissue was obtained, for each radionuclide, by normalizing the snail piece concentration (Bq) from the total radioactivity (Bq) received by each gastropod. For the trophic way, the bioaccumulation factor was evaluated by calculating the ratio between the concentration in the whole body (or in the shell or the soft tissues) following the radioactive food ingestion and the food concentration (Bq.kg $_{\text{snail fresh weight}}^{-1}$ by Bq.kg $_{\text{flour fresh weight}}^{-1}$). The retention factor was evaluated by calculating the ratio between the tissue concentration at a given date during the depuration and the mean concentration of the same tissue just at the beginning of the depuration (Bq.kg $_{\text{snail fresh weight}}^{-1}$ by Bq.kg $_{\text{snail fresh weight}}^{-1}$).

Comparisons between the different results were made by performing the ANOVA ($P \leq 0.05$) variance analysis tests using Sigma Stat 2.03.

3. RESULTS AND DISCUSSION

3.1 Effect on the growth or on the mortality

Sampling showed that, whatever the series, all the samples had lost weight; regardless of their initial weight. Moreover, the snails with the highest weight at the beginning of the experiment did not lose more than the others (expressed as a percentage of their initial weight). This result seems to indicate that the weight loss was not due to a lack of food. However, as the reference group presented an identical decrease in weight, there was presumably a direct relationship with the change in way of life (habitat). The low mortality among the different contaminated series on the two first days of the experiment

(1 individual for direct deposition, 2 for the trophic pathway, and 0 for the reference group) did not allow us to see any influence of the contamination. This mortality may be related to the age of the snails (about 2 years), since the average mortality age is about 3 years.

In conclusion: No significant difference between the groups of contaminated snails and the reference group was observed; neither in the growth nor in the mortality under these experimental conditions. Similar results have been found following heavy metal contamination (cadmium for example) [5 et 6].

3.2 Distribution of the radionuclides through the whole body

Following the two contamination pathways, the radionuclides were found to have distributed differently, depending on whether they had been contaminated by direct or by trophic pathway (Figure 3). In the case of a direct pathway, though as expected, most radionuclides were found on the shell, it can be observed that the whole body was contaminated and that the cesium content tended to distribute uniformly throughout the whole body. At the end of the contamination by the trophic pathway, the target elements are: the shell for strontium, the digestive system for tellurium and cesium, the muscle for barium and cesium. At the end of the depuration period, the tendencies are similar (Figure 4); however, a greater percentage of strontium can be found in the shell for both contamination pathways. This result reflects the entry of strontium into the shell from the soft tissues whatever the contamination pathway.

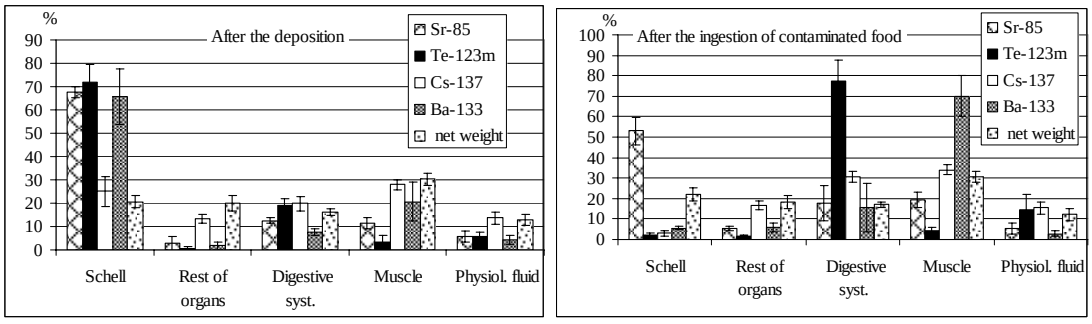

Figure 3. Radionuclide distribution in the total body just after deposition (20 h) and after ingestion of contaminated food (11 d).

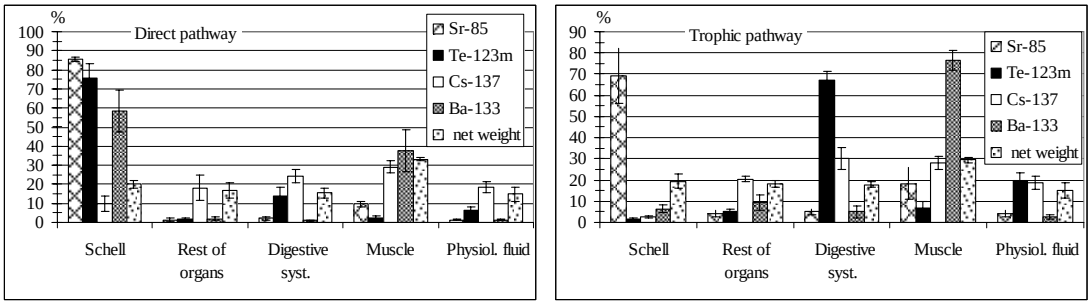

Figure 4. Distribution of the radionuclides through the whole body following the depuration period.

Table 1. Distribution of radionuclides during the observation period (Bq in a snail piece by total Bq received by the snail).

Contamination	Period of depuration (d)		Sr-85			Te-123m			Cs-137			Ba-133		
Direct pathway	5	shell	0.84	±	0.25	0.58	±	0.19	0.08	±	0.02	0.55	±	0.16
	5	soft tissues	0.18	±	0.06	0.22	±	0.08	0.78	±	0.18	0.34	±	0.09
	5	faeces	0.06	±	0.04	0.17	±	0.11	0.03	±	0.004	0.07	±	0.06
	21	shell	0.85	±	0.37	0.50	±	0.14	0.07	±	0.02	0.47	±	0.12
	21	soft tissues	0.14	±	0.06	0.16	±	0.06	0.71	±	0.31	0.36	±	0.15
	21	faeces	0.16	±	0.04	0.42	±	0.20	0.28	±	0.05	0.15	±	0.06
Trophic pathway	5	shell	0.36	±	0.12	0.005	±	0.002	0.02	±	0.01	0.02	±	0.01
	5	soft tissues	0.17	±	0.04	0.26	±	0.07	0.66	±	0.07	0.31	±	0.09
	5	faeces	0.50	±	0.06	0.68	±	0.06	0.16	±	0.05	0.50	±	0.05
	11	shell	0.41	±	0.10	0.003	±	0.0003	0.02	±	0.003	0.02	±	0.003
	11	soft tissues	0.18	±	0.06	0.20	±	0.04	0.62	±	0.08	0.34	±	0.06
	11	faeces	0.52	±	0.08	0.76	±	0.09	0.20	±	0.04	0.48	±	0.03

Following the depuration period, the distribution of the radionuclides between the shell, the soft tissues, and the faeces - elimination during the whole observation period (contamination and depuration) - is shown in **Table 1.** The contamination pathway appears to strongly influence the elimination of strontium, tellurium and barium: the best elimination occurs after contamination by the trophic pathway. Furthermore, the elimination of strontium and that of barium are comparable for one contamination mode. Tellurium is the element that best eliminates in the faeces, whatever the contamination pathway. As regards cesium, the contamination mode appears to have little influence on its elimination.

3.3 Evolution of the radioactivity in the different parts of the snail

For the trophic pathway, the bioaccumulation factor (BAF) is given in **Table 2** and shows that strontium is accumulated in the shell, whereas tellurium, cesium and baryum are found in the soft tissues. The highest values are obtained in the shell for strontium and in the soft tissues for cesium, tellurium and baryum come next. These values are difficult to compare with other elements such as cadmium because the food concentrations are much lower than those given in the literature [7]. It can however be noted that our BAF values are very low: literature values are in the range of 200-400 for cadmiun and the *Helix aspersa* soft body [7].

Table 2. Bioaccumulation factor (Bq.kg $_{\text{snail fresh weight}}^{-1}$/Bq.kg $_{\text{food fresh weight}}^{-1}$).

	Sr-85	Te-123m	Cs-137	Ba-133
Shell	$5.2 \cdot 10^{-2} \pm 1.5 \cdot 10^{-2}$	$1.2 \cdot 10^{-3} \pm 1.7 \cdot 10^{-4}$	$3.6 \cdot 10^{-3} \pm 8.2 \cdot 10^{-4}$	$3.6 \cdot 10^{-3} \pm 9.3 \cdot 10^{-4}$
soft tissues	$1.3 \cdot 10^{-2} \pm 2.7 \cdot 10^{-3}$	$1.7 \cdot 10^{-2} \pm 4.2 \cdot 10^{-3}$	$3.4 \cdot 10^{-2} \pm 4.2 \cdot 10^{-3}$	$1.7 \cdot 10^{-2} \pm 3.1 \cdot 10^{-3}$
total body	$2.1 \cdot 10^{-2} \pm 2.6 \cdot 10^{-3}$	$1.3 \cdot 10^{-2} \pm 3.4 \cdot 10^{-3}$	$2.7 \cdot 10^{-2} \pm 3.7 \cdot 10^{-3}$	$1.4 \cdot 10^{-2} \pm 2.7 \cdot 10^{-3}$

Table 3. Evolution of the retention factor (Bq.kg $_{\text{snail fresh weight}}^{-1}$/Bq.kg $_{\text{snail fresh weight}}^{-1}$).

contamination	period of depuration (d)		Sr-85	Te-123m	Cs-137	Ba-133
direct pathway	5	shell	1.09 ± 0.13	0.71 ± 0.19	0.28 ± 0.05	0.74 ± 0.14
	5	dig. system	0.23 ± 0.08	0.74 ± 0.42	0.73 ± 0.08	0.25 ± 0.17
	5	muscle	0.80 ± 0.29	0.45 ± 0.25	0.75 ± 0.08	1.20 ± 0.36
	21	shell	1.27 ± 0.62	0.70 ± 0.22	0.28 ± 0.11	0.72 ± 0.19
	21	dig. system	0.14 ± 0.04	0.51 ± 0.19	0.84 ± 0.33	0.09 ± 0.02
	21	muscle	0.70 ± 0.36	0.45 ± 0.18	0.68 ± 0.32	1.35 ± 0.59
trophic pathway	5	shell	1.35 ± 0.36	0.72 ± 0.35	1.01 ± 0.33	1.18 ± 0.18
	5	dig. system	0.38 ± 0.14	0.64 ± 0.18	0.85 ± 0.08	0.30 ± 0.11
	5	muscle	1.16 ± 0.33	0.96 ± 0.29	0.98 ± 0.09	1.18 ± 0.36
	11	shell	1.54 ± 0.22	0.55 ± 0.31	0.94 ± 0.22	1.22 ± 0.20
	11	dig. system	0.29 ± 0.10	0.47 ± 0.12	0.83 ± 0.14	0.27 ± 0.16
	11	muscle	1.05 ± 0.42	1.09 ± 0.44	0.76 ± 0.08	1.11 ± 0.25

The evaluation of the retention factor for each radionuclide shows that there is no significant change in the shell over time (Table 3). Moreover, the values obtained for strontium are the highest, whatever the contamination ways. These results are in agreement with those obtained with the evaluation of the distribution of strontium over time. For the direct pathway, cesium and barium have the lowest values. These elements are very soluble in rainwater, and after depositing onto the shell, they are washed off by rainfall.

In the muscle, radionuclide retention factors do not change over time for a contamination by direct pathway; cesium values decrease for a trophic pathway: this result indicates a migration of this element from the muscle to another part of the body. Concerning tellurium and cesium, at the end of 5-day-depuration, purification is more efficient for the direct pathway.

In the digestive system, the retention factors do not change over time for every radionuclide, but cesium and tellurium have the highest values for both pathways. These results are in agreement with those obtained in the shell and the muscle.

4. CONCLUSIONS

During the 21-day-observation, there was no significant difference between the 2 groups of contaminated snails and the reference group; neither in the growth nor in the mortality of the samples. Following a contamination by the radionuclides, the distribution through the total body of snails depends on the radionuclides and on the exposure way. For the 2 exposure pathways, cesium was mainly present in the soft tissues and strontium in the shell. The target tissues for barium and tellurium were: the shell for a direct exposure and the soft tissues for a trophic pathway (digestive system for tellurium and muscle for barium). The faeces reduced the elements differently: around 25 % for cesium whatever the contamination pathway, around 15 % (direct pathway) to 50% (trophic pathway) for strontium and barium and last, about 45% (direct pathway) to 75% (trophic) for tellurium.

References

[1] Gomot A. (1997) 'Effets des métaux lourds sur le développement des escargots. Utilisation des escargots comme bio-indicateurs de pollution par les métaux lourds pour la préservation de la santé de l'homme' *bull. Acad. Nattle Méd.* : 1997, 181, n°1,59-75, séance du 28 janvier 1997.

[2] Aragon Del Valle A. (2003) 'Radiecologia de transuranidos: caracterizacion y comportamiento de particulas de combustible nuclear en suelos afectados por el accidente de Palomares.' Editorial CIEMAT, Madrid, ISBN: 84-7834-460-8.

[3] Manesse D. & Fermandjian J. (1985). "Projet RESSAC. Proposition d'un terme source représentatif des formes physico-chimiques des produits de fission relâchés lors d'un accident grave sur un REP". *Note CEA*, SAER 85/456.

[4] Madoz-Escande C., Bréchignac F., Colle C., Dubois E., Hugon J., Jouglet H., Moutier M., Rongier P., Sanchez A., Schulte E.H., Zanon R. (1999). "Experimental installations for radioecology research on defined ecosystems subjected to contamination in controlled conditions." Nuclear Science and Engineering 133: 178-191.

[5] Lazkowski R. and Hopkin S. P. (1996) ' Effect of Zn, Cu, Pb, and Cd on fitness in snails (Helix Aspersa).' *Ecotoxicology and Environmental Safety* : 34, 59-69, article n°0045.

[6] Russel L. K., DeHaven J.I. and Botts R.P. (1981), 'Toxic effects of cadmium on the garden snail (helix aspersa).' *Bull. Environ. Contam. Toxicol.* : 26, 634-640.

[7] Scheifler R., Gomot de Valfleury A., Badot P.M. (2002) 'Transfer of cadmium from plant leaves and vegetable flour to the snail Helix Aspersa: bioaccumulation and effects' *Environmental Research* : 53 (B), 148-153.