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ACCEPTED MANUSCRIPT

## Co-exposure to internal and external radiation alters cesium biokinetics and retention in the mouse

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CO-EXPOSURE TO INTERNAL AND EXTERNAL RADIATION ALTERS CESIUM BIOKINETICS AND  
RETENTION IN THE MOUSE

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Keyword: Coexposure; External exposure; Cesium-137; Biological effects

**Abstract:**

Exposures in post-accidental situations are complex and include both external exposure and internal contamination with several radionuclides. However, *in vivo* and *in vitro* studies generally use simplified exposures, while a recent study suggested that combined external irradiation and internal contamination may induce more severe biological effects as compared to single exposures. In an attempt to test the hypothesis of potential non-additive effects of multiple radiological exposures, we used a mouse model of combined external X rays irradiation at 1 and 5 Gy and internal contamination with injection of 20 KBq  $^{137}\text{Cs}$ . Results showed a differential kinetics of  $^{137}\text{Cs}$  elimination in irradiated animals as compared to sham-irradiated,  $^{137}\text{Cs}$  injected animals. Moreover, changes in plasma potassium and in relative testis weight were observed 38 days after irradiation and injection in co-exposed animals as compared to  $^{137}\text{Cs}$  injection alone. These results demonstrate that an external exposure combined with an internal contamination may lead to unexpected changes in biokinetics of radionuclides and biological effects as compared to single exposures.

**Introduction:**

Population exposure during the early phase of a major nuclear accident is mainly due to two components, the external exposure due to radionuclides (RN) dispersion in the atmosphere and internal exposure due to inhalation. On the long term, these two components change to an external irradiation due to the integration of RNs in all components of the environment and to internal exposure due to the presence of RNs in foodstuffs and feedstuffs (Bernhardsson *et al.*, 2011; Cooper *et al.*, 1992; Handl *et al.*, 2003). The relative contribution of these two components of post-accidental exposure changes with time, typically with a trend of increased contribution of internal exposure (Bernhardsson *et al.*, 2011). However, in most studies (either epidemiological or not) on the health effect of exposure on human populations in post accidental situation, it is quite difficult to make a distinction between the effect of external exposure and the effect of internal exposure, although an evaluation of the relative contributions of internal exposure and external exposure can be made. In most of the post Chernobyl studies, the exposure is reconstructed based mainly on the contamination level of the place of residence, assimilating external and internal exposure in a single aggregated parameter. A recent study showed that, in a group of 590 children aged less than 18 and born after the Chernobyl accident, a decrease in the number of red blood cells was correlated with  $^{137}\text{Cs}$  whole body counting (WBC) but not with the  $^{137}\text{Cs}$  contamination level of soils from the place of residence (Lindgren *et al.*, 2013), suggesting that the use of contamination level of soils is not a good surrogate for estimating overall exposure.

Laboratory *in vivo* studies utilize highly controlled exposure modes consisting of either external irradiation at low doses and low dose rates (Vieira Dias *et al.*, 2018; Ebrahimian *et al.*, 2018) or internal contamination with a single radionuclide,  $^{137}\text{Cs}$  (a gamma ray emitter) in most cases (Grison *et al.*, 2012; Le Gallic *et al.*, 2015) and  $^{90}\text{Sr}$  (a beta ray emitter) less frequently (Nikula *et al.*, 1995; Synhaeve *et al.*, 2016). A variety of biological effects have been demonstrated in such single radionuclide exposure studies, for example a reduction in arterial pressure (Gueguen *et al.*, 2008) or a decrease in inflammatory status of atherosclerotic lesions (Le Gallic *et al.*, 2015) after internal exposure to  $^{137}\text{Cs}$ .

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3 These results on  $^{137}\text{Cs}$ -induced changes in cardiovascular physiology appear somewhat in contradiction  
4 with results obtained in human populations showing either an increase in cerebrovascular diseases in  
5 liquidators (Ivanov *et al.*, 2006) or an absence of effect in children living on contaminated territories  
6 (Jourdain *et al.*, 2018). An absence of effect of  $^{137}\text{Cs}$  was also observed on the immune system in a  
7 mouse model (Bertho *et al.*, 2011), while numerous effects were described in human populations living  
8 on contaminated territories (UNSCEAR, 2000; Chumak *et al.*, 2001; Kuzmenok *et al.*, 2003; McMahon  
9 *et al.*, 2014).

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11 A possible explanation for these discrepancies is that the exposure profile is not sufficiently well  
12 assessed in human studies and overly simplified (i.e. not sufficiently representative of real exposure)  
13 in laboratory studies. For instance, laboratory studies using a mixed exposure, both internal and  
14 external, are very sparse. A series of studies was conducted by the end of the 60's by Nishio (Nishio,  
15 1968, 1969, 1971; Nishio *et al.*, 1968) in a mouse model of  $^{137}\text{Cs}$  +  $^{90}\text{Sr}$  chronic, long term internal  
16 exposure. This author demonstrated a reduced life expectancy and a reduction of reproductive  
17 efficiency. However, in these studies, there was no comparison between exposure with a single  
18 radionuclide and the mixture of the two radionuclides (Nishio, 1968, 1969, 1971; Nishio *et al.*, 1968).  
19 This absence of study about the effect of combined exposure is probably linked to the hypothesis of  
20 additivity of health effects as absorbed dose are considered as additive, too. However, a recent study  
21 (Calvi *et al.*, 2019) showed that the elimination of  $^{137}\text{Cs}$  after i.p. injection was slowed in animals  
22 concomitantly irradiated at 6 Gy as compared to unirradiated controls. This slowdown of  $^{137}\text{Cs}$   
23 elimination induced an increase in the absorbed dose due to  $^{137}\text{Cs}$ , which in turn induced an increase  
24 in the death rate of co-exposed animals. These results suggests that, even if the absorbed doses from  
25 external irradiation and internal exposure are purely additive, the resulting biological effects of mixed  
26 exposures are possibly very different from what can be expected from single exposures.  
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56 In order to test this hypothesis, we used a male mouse model exposed at 1 or 5 Gy external X rays  
57 irradiation together with internal exposure through i.p. injection of 20 KBq of  $^{137}\text{Cs}$ . Since the exposure  
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3 to  $^{137}\text{Cs}$  through either inhalation or ingestion results in a rapid transfer of  $^{137}\text{Cs}$  to the blood with  
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5 almost 100% efficacy (Leggett et al, 2003, ICRP 2017), we assume that an i.p. injection of  $^{137}\text{Cs}$  is  
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7 equivalent to an acute exposure through either inhalation or ingestion. This exposure schedule is not  
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9 expected to induce the death of animals. However, tissue effects are expected to appear mainly in the  
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11 hematopoietic system and in the testis, two of the most radiosensitive organs with this respect.  
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13 Animals were then followed for  $^{137}\text{Cs}$  elimination through whole body counting (WBC) and  $^{137}\text{Cs}$  organ  
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15 content. Animals were then tested for several biological endpoints, including hematopoietic and testis  
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17 end-points, since these two organs are the most radiosensitive to tissue effects. Moreover, cesium is  
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19 considered as a physiological analog of potassium (Leggett et al, 2003, ICRP 2017) which is tightly  
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21 regulated in the blood, as well as other ions such as chloride, sodium and calcium playing major roles  
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23 in physiology both at the molecular cellular and tissue level. It thus appeared interesting to look at  
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25 potential modifications of ion concentration in the plasma.  
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**Material and methods:**

*Animals and experimental groups:* BALB/C JrJ male mice (Janvier labs, Le Genest Saint Isle, France) were housed by groups of 6 in standard cages and kept at constant room temperature ( $21^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ) with a 12h daylight cycle. Animals received ad libitum acidified water and commercial rodent chow (R03-type chow; Safe, Epinay-sur-Orge, France). The animal care committee of the Institut de Radioprotection et de Sûreté Nucléaire (IRSN) reviewed and approved all animal experiments under the agreement number APAFIS#10405-2017062716552009 v3. These animal experiments were conducted in accordance with the French regulations for animal experimentation (Ministry of agriculture act No 2001-464, May 2001).

After arrival, animals were trained for restraint by staying for increasing times (5 min up to 30 min) once a day in plastic restrain tubes (Bioseb, Vitrolles, France) for two consecutive weeks. Mice were then randomly distributed into six experimental groups: Control group (sham irradiated and saline injection),  $^{137}\text{Cs}$  injection alone, total body (TBI) external irradiation 1 Gy or 5 Gy, and TBI 1 Gy or 5 Gy plus  $^{137}\text{Cs}$  injection (n=6 per group).

*Irradiation and  $^{137}\text{Cs}$  injection:* TBI or sham irradiation were done using an Elektra Synergy platform (Elektra SAS France, Boulogne, France) delivering 4 MV X-rays at  $2.8 \text{ Gy}\cdot\text{min}^{-1}$ . Two radiation doses were used, 1 Gy and 5 Gy. 30 minutes after irradiation, animals were injected i.p. with either saline solution (NaCl 0.9%, Osalia, Paris, France) or with 20 kBq  $^{137}\text{CsCl}$  (mass activity  $1040 \text{ kBq}\cdot\text{g}^{-1}$  in a  $10 \mu\text{g}\cdot\text{ml}^{-1}$  solution of stable CsCl, CERCA-LEA, Pierrelatte, France) diluted in saline with pH adjusted to 7.0. The resulting quantity of cesium ( $^{137}\text{Cs}$  + stable) injected per animal was thus  $2.28 \times 10^{-9}$  mole.

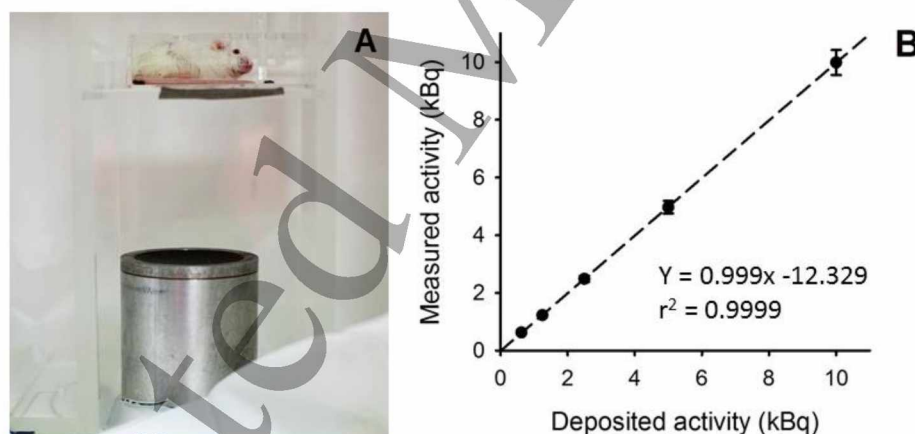
*Follow-up of  $^{137}\text{Cs}$  internal contamination:* Total activity of  $^{137}\text{Cs}$  in animals was followed by whole body measurement using a Germanium detector (Model GR4020, Canberra, Olen, Belgium) and a Genie 2000 spectrum analysis software. The active part of the detector and the animals were inside a low-background lead cell. Animals were placed 10 cm above the center of the detector entrance window in a restrain cage (Figure 1A). The holder for the restrain cage was fixed for all the counting campaign.



The measurement consists in recording the net count rate ( $\tau$ , in counts/s) of the  $^{137}\text{Cs}$  photoelectric peak (at 662 keV) which is related to the total body activity ( $A$ , in Bq) through the efficiency ( $\varepsilon$ ):

$$A = \frac{\tau}{\varepsilon}$$

The efficiency was calculated using 5 mouse phantoms. They were made of 30 ml polystyrene Sterilin Tubes (Thermo Fischer Scientific, Cergy Pontoise, France) filled with 25 ml 30 % polyacrylamide gel containing graded quantities of  $^{137}\text{Cs}$  between 625 Bq and 10 kBq. Counting was performed until the net count under the photoelectric peak was greater than 10 000 events so that the counting uncertainty was less than 1%. Averaging over the 5 measurements with 5 phantoms with increasing activity the efficiency was obtained:  $\varepsilon = (3.33 \pm 0.03) 10^{-3}$  counts/s/Bq (Figure 1B). The *in vivo* monitoring protocol used for animals (Hinton *et al.*, 2019; Ishigure *et al.*, 1994) follows the same analysis procedure that is used for human subjects (Beaumont *et al.*, 2017; Griffith *et al.*, 2016).



**Figure 1:** **A:** Animal positioning in the restrain tube over the Ge detector. **B:** Correlation between  $^{137}\text{Cs}$  deposited activity in the phantoms and measured activity with the Ge detector. Results are presented as mean  $\pm$  SD of three successive measurements. The resulting linear regression parameters and correlation coefficient are indicated in the figure.

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3 *Follow-up of animals, blood sampling and organs treatment:* During the experiment, animals were  
4 weighted once a week. A blood sample was obtained once a week (starting 3 days before irradiation  
5 and injection) by puncture at the facial vein (allowing to collect a maximal volume of 100 µl of blood  
6 per week), without anesthesia, in agreement with the animal care committee. On day 38 post-  
7 irradiation, i.e. after a complete cycle of spermatogenesis (which last for 35 days in the mouse)(Ray et  
8 al, 2015), animals were anesthetized by isoflurane inhalation and blood was drawn by intracardiac  
9 puncture on EDTA-coated tubes (BD biosciences, Le pont de Claix, France). After cervical dislocation,  
10 central nervous system (CNS), liver, spleen, kidneys, heart, lungs, digestive tract, striated muscles (left  
11 and right), femur (left and right), skin and carcass were harvested, weighted and frozen for later use.  
12 Reproductive organs i.e. testis (left and right), epididymis (left and right) and seminal vesicle were  
13 removed and carefully cleaned from adipose tissue before being weighted. Plasma was isolated from  
14 blood immediately after sampling by centrifugation 10 min at 800 g and frozen for subsequent use.

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30 *<sup>137</sup>Cs measurement in organs:* <sup>137</sup>Cs measurements were done using a Cobra II gamma counter (Perkin-  
31 Elmer, Courtabœuf, France) equipped with a 3-inches NaI detector, by placing organs in 14 ml counting  
32 tubes (Thermo Fischer Scientific) without any preparation. The calibration coefficient of the detection  
33 at 661 keV was determined with a reference curve of serial dilutions of <sup>137</sup>Cs. Counting time was 60  
34 min for most organs, except for organs with low <sup>137</sup>Cs content for which a 120 min counting time was  
35 applied. Detection limit (DL) was calculated according to the following formula:

$$DL = [4\sqrt{2 \times B \times T}] \div T$$

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where B is the background in counts per minutes (cpm) (obtained by averaging counts of 10 empty  
tubes) and T the time of counting. The DL was in the range of 0.07 to 0.2 Bq per sample, depending on  
the size and the nature of the organ measured. As a control, 6 samples of all organs from control  
animals were counted and gave a <sup>137</sup>Cs content lower than the detection limit.

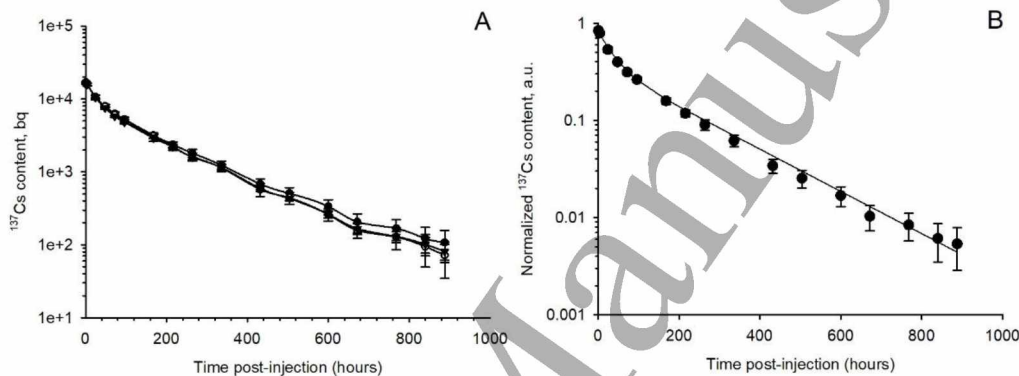
*Flt3-ligand measurements:* Concentration of plasma Flt3-ligand (FL) was measured by ELISA according  
to manufacturer's recommendations (Biotechne Ltd, Lille, France).

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3 *Measurement of plasma ions:* Potassium (K<sup>+</sup>), sodium (Na<sup>+</sup>) and chloride (Cl<sup>-</sup>) ions were measured in  
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5 plasma samples with an automated spectrophotometric system (Konelab 20i, Thermo Fischer  
6  
7 Scientific) using potentiometry. Calcium (Ca<sup>2+</sup>) was measured with specific reagents and according to  
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9 manufacturer's instructions (Thermo Fischer Scientific).

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12 *Statistical analysis:* All results are presented as mean ± standard deviation (SD) from 6 animals per  
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14 experimental group. Absorbed radiation doses were estimated by summing <sup>137</sup>Cs activities from all  
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16 measured organs and carcass and using the RODES software (Locatelli *et al.*, 2017; Miloudi *et al.*, 2017).  
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18 Relative organ weight was calculated as the percent of organ weight relative to body weight. Statistical  
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20 and regression analyses were made using Sigmaplot software (SPSS, Paris, France). Comparisons  
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22 between groups were done using one or two way analysis of variance (ANOVA) or ANOVA on ranks  
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24 when the Shapiro-Wilk test for normality failed. A Holm-Sidak pairwise multiple comparison procedure  
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26 including a Bonferroni correction was then used for group comparison. Differences were considered  
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28 statistically significant for p<0.05.  
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## Results:

Follow up of  $^{137}\text{Cs}$  elimination: Control animals and animals injected with  $^{137}\text{Cs}$  (either externally irradiated or not) were measured for  $^{137}\text{Cs}$  content starting 1 hour post injection until the end of experiment on day 38 post irradiation and injection. Results showed (Figure 2A) a rapid decrease of  $^{137}\text{Cs}$  content in all animals. The elimination of  $^{137}\text{Cs}$  was significantly increased in irradiated animals as compared to non-irradiated animals (Two way ANOVA,  $F_{(2, 255)} = 6.82$ ,  $p = 0.001$ , Figure 2), clearly visible after 2 weeks post-irradiation when the retention in the irradiated group was lower than in the contaminated group.



**Figure 2: A:** Follow-up of  $^{137}\text{Cs}$  content in animals either sham-irradiated (closed circle) or irradiated at 1 Gy (open circles) or 5 Gy (closed triangles). Results are the mean  $\pm$  SD of 6 animals per experimental group. Animals injected with saline gave results under the detection limit (not shown). A significant difference between the three exposure groups is observed (Two way ANOVA,  $F_{(2, 255)} = 6.82$ ,  $p = 0.001$ ) although no time specific difference was observed. **B:** Curve fit analysis on normalized  $^{137}\text{Cs}$  content in animals exposed to  $^{137}\text{Cs}$  injection only. The curve fit was obtained with a sum of three exponential decays.

A curve fit analysis was made on each of the three experimental groups receiving  $^{137}\text{Cs}$  whatever the external radiation dose, using  $^{137}\text{Cs}$  contents ( $R$ ) normalized to the quantity of  $^{137}\text{Cs}$  injected (20 KBq per animal) and a sum of three exponential decays, with the following equation:

$$R(t) = a \times e^{(-\lambda_1 \times t)} + b \times e^{(-\lambda_2 \times t)} + c \times e^{(-\lambda_3 \times t)}$$

Where a, b and c are the respective contribution of each of the exponential component so that the sum of these three parameters is equal to 1 and  $\lambda_1$ ,  $\lambda_2$  and  $\lambda_3$  are the decay constants of each exponential component. Result of the curve fit for the group receiving  $^{137}\text{Cs}$  alone is shown in figure 2B and values of the parameters for each of the experimental group receiving  $^{137}\text{Cs}$  are indicated in table 1. The effective half-live ( $T_{\text{eff}}$ ), corresponding to a retention of 0.5 (Bq/Bq injected), can be calculated from the fit parameters and is also given in table 1.

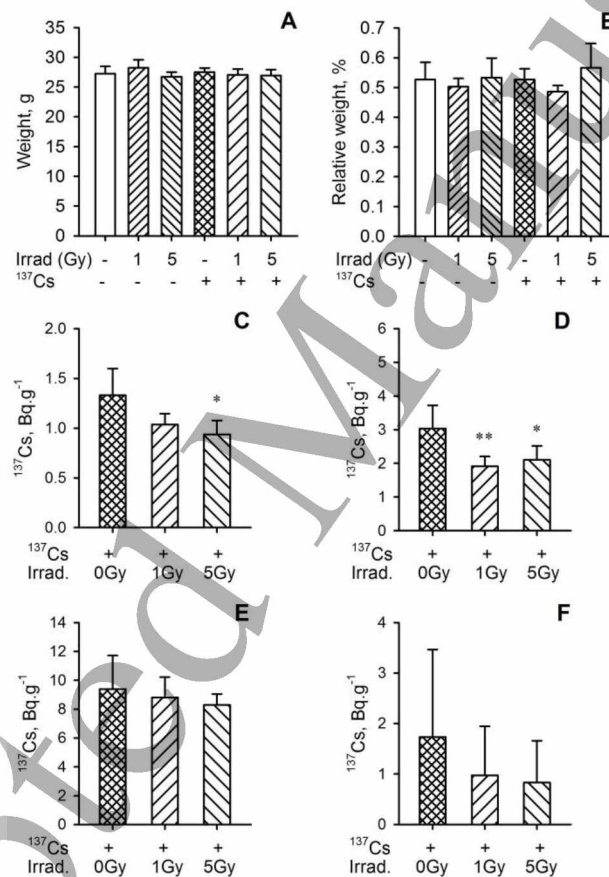
**Table 1:** value of parameters of the curve fit for each of the experimental group.

group	$^{137}\text{Cs}$ alone	$^{137}\text{Cs} + 1 \text{ Gy}$	$^{137}\text{Cs} + 5 \text{ Gy}$
a	0.17	0.14	0.15
$\lambda_1$ (hours $^{-1}$ )	2.2	3.25	5.7
b	0.46	0.47	0.49
$\lambda_2$ (hours $^{-1}$ )	0.028	0.033	0.031
c	0.37	0.39	0.35
$\lambda_3$ (hours $^{-1}$ )	0.0052	0.0056	0.0055
$T_{1/2}$ effective (hours)	32.6	30.5	29.5

Results indicate that the  $T_{\text{eff}}$  of  $^{137}\text{Cs}$  is shorter in animals receiving an external irradiation dose associated to  $^{137}\text{Cs}$  injection as compared to animals receiving  $^{137}\text{Cs}$  alone. This shortening of  $T_{\text{eff}}$  confirms the observed difference in  $^{137}\text{Cs}$  elimination between groups (Figure 2A). When looking more precisely to the curve fit results, it appears that the parameter values for the second and the third exponentials are very close for all experimental groups. The main difference appeared for the first exponential, describing the short time retention, with an increase of the value of  $\lambda_1$  for animals receiving  $^{137}\text{Cs}$  + external irradiation. This suggests that the observed decreased retention of  $^{137}\text{Cs}$  in

externally irradiated animals is mainly due the rapid elimination component of  $^{137}\text{Cs}$  biokinetics during the first hours after  $^{137}\text{Cs}$  injection.

*$^{137}\text{Cs}$  concentration in organs:* In order to confirm the difference in  $^{137}\text{Cs}$  content of animals according to the external irradiation status, all harvested organs at the end of experiment were weighted and were measured for  $^{137}\text{Cs}$  concentration. No significant difference was observed in the body weight of animals (Figure 3A) or in the relative weight of heart (Figure 3B), CNS, liver, spleen, kidney, digestive tract and femurs (not shown) between the experimental groups. By contrast, when looking at the  $^{137}\text{Cs}$  concentration in organs, a significant decrease was observed in the femurs (Figure 3C) and in the hearts



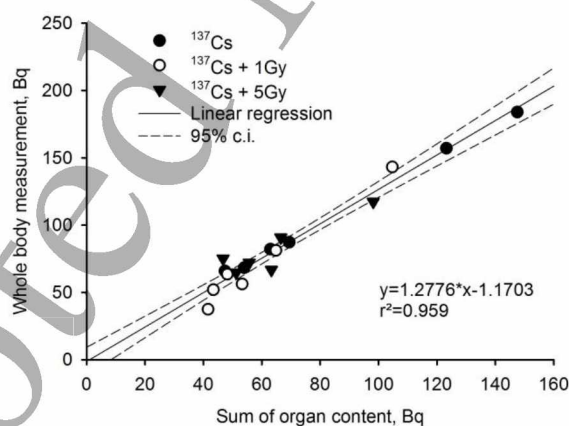
**Figure 3:** Whole body mass (A) and heart mass relative to the body mass (B) at the end of experiment.

No significant variation of organ mass was observed.  $^{137}\text{Cs}$  concentration in heart (C), femurs (D), striated muscles (E) and whole body (F) of mice subjected to combined external and internal exposures. Results are expressed as mean  $\pm$  SD of 6 animals per group. Significant differences were observed between exposure groups in the femurs (One way ANOVA,  $F_{(2, 17)} = 8.97$ ,  $p < 0.005$ ) and in the

heart (ANOVA on ranks,  $H_{(2)} = 7.83$ ,  $p < 0.05$ ) but not in other organs. Differences as compared to the sham-irradiated group are indicated for \*:  $p < 0.05$  and \*\*:  $p < 0.001$ .

(Figure 3D) of irradiated animals as compared to sham-irradiated and  $^{137}\text{Cs}$  injected animals. One has to note that femurs were measured unflushed, i.e. with the intact bone marrow in the femurs. Decreases, although statistically non-significant, were also observed in all tested organs, including striated muscles (Figure 3E) and whole body (Figure 3F). The heart, the kidneys and the striated muscles were already shown to be the most contaminated tissues in previous rat and mouse biokinetic experiments (Bertho *et al.*, 2010; Tournalonias *et al.*, 2010).

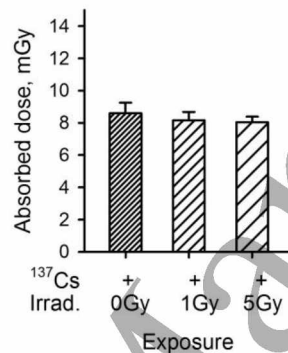
In order to confirm these results, a correlation analysis was made between whole body counting with the Ge detector and the sum of  $^{137}\text{Cs}$  organ content as measured on day 38. Results showed a very good correlation between the two datasets (Figure 4), confirming the agreement between the two methods of measurements used in this study. This also confirmed the observed decrease in  $^{137}\text{Cs}$  whole body content in animals exposed to external irradiation and internal exposure as compared to animals exposed to  $^{137}\text{Cs}$  internal exposure only.



**Figure 4:** Correlation between whole body content in  $^{137}\text{Cs}$  and the sum of measured  $^{137}\text{Cs}$  activity in all organs by the end of experiment. Each symbol represents a single animal. Closed circles:  $^{137}\text{Cs}$  + sham irradiation; open circles:  $^{137}\text{Cs}$  + 1 Gy irradiation; closed triangles:  $^{137}\text{Cs}$  + 5 Gy.

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3 Solid line represents the linear regression of all data and dotted lines the 95% confidence  
4 interval (c.i.).  
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10 *Absorbed radiation doses induced by  $^{137}\text{Cs}$ :* To evaluate the additional absorbed radiation dose due to  
11  $^{137}\text{Cs}$  injection, we used the RODES software (Miloudi *et al.*, 2017; Locatelli *et al.*, 2017) with the  
12 individual whole body measurements of  $^{137}\text{Cs}$  during the experiment. Results (Figure 5) showed that  
13 the range of additional dose due to  $^{137}\text{Cs}$  injection estimated in each individual animal was between  
14 7.4 mGy and 9.3 mGy without any significant difference according to the group of external irradiation.  
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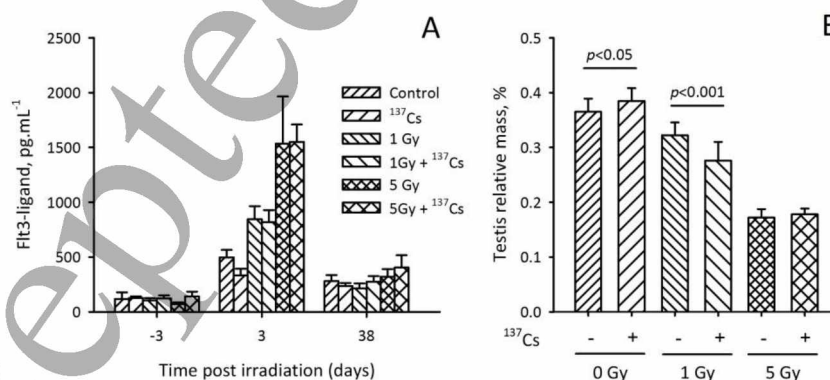
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33 **Figure 5:** Additional absorbed doses due  $^{137}\text{Cs}$  injection estimated using individual data from Figure 2  
34 and the RODES software (Locatelli *et al.*, 2017; Miloudi *et al.*, 2017). Results are mean  $\pm$  SD  
35 of 6 animals per experimental group. Differences between groups are non-significant (One  
36 way ANOVA,  $F_{(2, 15)} = 1.953$ , n.s.).  
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45 *Differential biological consequences of co-exposure:* Although the added absorbed radiation dose may  
46 not induce significant biological changes as compared to the effect of high dose external irradiation,  
47 we examined biological endpoints for the two of the most radiosensitive physiological systems for  
48 tissue effects, the hematopoietic system and the testis. We also measured plasma ions since  $\text{Cs}^+$  is a  
49 physiological analog of  $\text{K}^+$  (Leggett *et al.*, 2003), which is essential to cardiovascular and muscular  
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The hematopoietic system was evaluated using plasma Flt3-ligand (FL) concentration as a bio-indicator of radiation-induced damage to the hematopoietic system (Prat *et al.*, 2006; Bertho *et al.*, 2010). FL was measured three days before exposure (d-3), 3 days after exposure (at the beginning of the hematopoietic syndrome, d3) and at the end of experiment (d38). Results indicated that there was a significant change in plasma FL concentration depending on the external radiation dose at day 3 post-exposure ( $F_{(2, 28)} = 87.0$ ,  $p < 0.001$ ) and by the end of experiment ( $F_{(2, 29)} = 11.3$ ,  $p < 0.001$ ) (Figure 6A). This is in line with the radiation induced bone marrow aplasia induced by external irradiation, as previously described (Prat *et al.*, 2006). By contrast,  $^{137}\text{Cs}$  co-exposure did not influence the external radiation dose-dependent increase in FL regardless of time post exposure. These results showed that the  $^{137}\text{Cs}$  internal exposure did not induce a change in the effect of external exposure on the hematopoietic system, irrespective of the external radiation dose.

With respect to testis, we measured the testis weight as a surrogate of the number of cells (and especially the number of germ cells) present in each of the testis by the end of the experiment. Results (Figure 6B) showed a significant dose dependent decrease in testis weight according to the external radiation dose ( $F_{(2, 65)} = 459$ ,  $p < 0.001$ ). It is well known that spermatogenesis is severely impaired by high dose external irradiation (De Felice *et al.*, 2019; Meistrich *et al.*, 1978). Consistent with this, a significant decrease in testis weight was evident at the lowest external dose used, 1 Gy (Pairwise



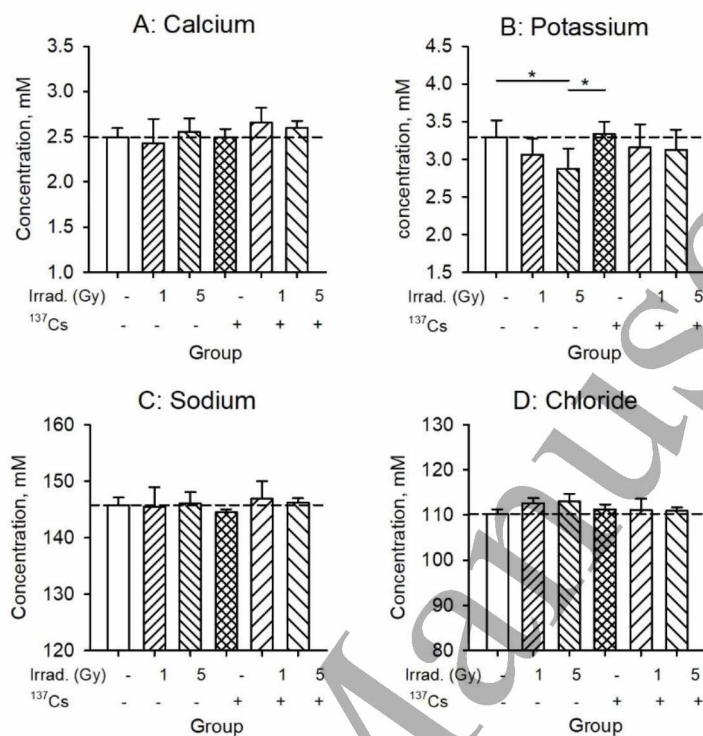
**Figure 6: A:** Plasma FL concentration according to the time post irradiation. Data are presented as mean  $\pm$  SD of 3 to 5 animals per group. Significant changes were observed after the external radiation exposure for d3 ( $F_{(2, 28)} = 87.0$ ,  $p < 0.001$ ) and d38 ( $F_{(2, 29)} = 11.3$ ,  $p < 0.001$ ), but not

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3 after the  $^{137}\text{Cs}$  exposure (d-3:  $F_{(1, 14)} = 4.1$ , n.s.; d3:  $F_{(1, 28)} = 0.7$ , n.s.; d38:  $F_{(1, 29)} = 2.25$ , n.s.). **B:**  
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5 Relative testis weight in different exposure groups (n=6 per group). Significant changes were  
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7 observed after the external radiation dose ( $F_{(2, 65)} = 459$ ,  $p < 0.001$ ) and after the  $^{137}\text{Cs}$  exposure  
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9 ( $F_{(1, 65)} = 4.75$ ,  $p = 0.033$ ).

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14 multiple comparison procedure,  $p < 0.001$ ). This external radiation induced reduction of testis weight  
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16 was further reduced if 1 Gy irradiation was combined with  $^{137}\text{Cs}$  exposure ( $F_{(1, 65)} = 4.75$ ,  $p = 0.033$ ). This  
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18 suggests that the combination of external irradiation with internal  $^{137}\text{Cs}$  exposure induced a slowdown  
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20 of testis physiology recovery after high-dose radiation damage. Such an effect was not observed after  
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22 5 Gy irradiation. One may suggest that at this high radiation dose, the effect of  $^{137}\text{Cs}$  internal exposure  
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24 might be masked by the strong effect of external irradiation. Consistent with this, we observed a  
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26 seminal vesicle weight reduction after 5 Gy exposure (data not shown) confirming the tissue effect of  
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28 5 Gy external exposure on testis physiology. Surprisingly, an increase in testis weight was observed in  
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30 animals exposed to  $^{137}\text{Cs}$  alone as compared to the unexposed control group (Pairwise multiple  
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32 comparison procedure,  $p < 0.05$ ). One has to note that animals were sacrificed at day 38 post  
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34 irradiation, i.e. after the completion of a complete cycle of spermatogenesis which last for 35 days in  
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36 the mouse (Ray *et al.*, 2015). This result suggests that the  $^{137}\text{Cs}$  exposure may induce a modification  
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38 during the cycle of spermatogenesis.

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42 Results of ion measurements showed no significant change in  $\text{Ca}^{2+}$  (Figure 7A) and  $\text{Na}^{+}$  (Figure 7C)  
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44 concentrations, irrespective of treatment group. By contrast, a significant change was observed for  $\text{K}^{+}$   
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46 (Two way ANOVA,  $F_{(5, 35)} = 3.149$ ,  $p = 0.021$ , Figure 7B) and  $\text{Cl}^{-}$  concentrations ( $F_{(5, 35)} = 2.675$ ,  $p = 0.047$ ,  
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48 Figure 7D). A significant decrease (12.6%) in  $\text{K}^{+}$  concentration was observed for the 5 Gy group as  
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50 compared to the control, whereas there was no significant difference between 5 Gy +  $^{137}\text{Cs}$  group and  
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52 control group and between  $^{137}\text{Cs}$  group and control group. A slight decrease (7.1%) in  $\text{K}^{+}$  concentration,  
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54 although not significant, was also observed for the 1 Gy group with a return to control level in the  
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presence of  $^{137}\text{Cs}$ . This suggested that external irradiation induced a decrease in  $\text{K}^+$  concentration which returned to control values in the presence of  $^{137}\text{Cs}$ .



**Figure 7:** Measurement of calcium (A), potassium (B), Sodium (C) and Chloride (D) in the plasma of animals at day 38 post irradiation. Two way ANOVA analysis showed a significant change for potassium ( $F_{(5, 35)} = 3.149$ ,  $p = 0.021$ , figure 7B) and chloride ( $F_{(5, 35)} = 2.675$ ,  $p = 0.047$ , figure 7D). Differences between groups are indicated as \*:  $p < 0.05$ .

**Discussion:**

The present study demonstrates that a co-exposure to external irradiation and internal contamination with  $^{137}\text{Cs}$  induces differential effects as compared to single exposure, either external or internal. In particular, for both external radiation doses used, this resulted in an accelerated elimination of  $^{137}\text{Cs}$  at short times after injection and consequently a reduction of retained  $^{137}\text{Cs}$  over the duration of follow-up, as demonstrated by the curve fit analysis of biokinetics data. Interestingly, the best fit was obtained taking into account three exponential components, suggesting the existence of three main compartment of  $^{137}\text{Cs}$  retention in the mouse model. The nature of these three different compartments remains elusive, since a detailed analysis of  $^{137}\text{Cs}$  content in various organs was not made during the course of the experiment in the present study. However, this model is compatible with the currently admitted biokinetics of  $^{137}\text{Cs}$  as described by others (ICRP 2017). This curve fit analysis showed that a decrease in  $T_{eff}$  was observed when an external irradiation was added to the  $^{137}\text{Cs}$  injection. This decrease in  $T_{eff}$  was mainly due to an increased elimination rate of  $^{137}\text{Cs}$  during the first hours after irradiation. Thereafter, the rate of  $^{137}\text{Cs}$  elimination was similar in the three experimental group. This may be explain by a possible radiation-induced increased permeability of the endothelial barrier (Gabrys *et al.*, 2007) or to the modification of intestinal barrier integrity, which in turn induces a loss of electrolytes at high doses (Dublineau *et al.*, 2007) during the early period after irradiation.

These results are somewhat inconsistent with a recent study (Calvi *et al.*, 2019) showing that animals co-exposed to 6 Gy external irradiation plus  $3.7 \times 10^6$  Bq of  $^{137}\text{Cs}$  demonstrated a reduction of  $^{137}\text{Cs}$  elimination. The consequence of this slowdown of  $^{137}\text{Cs}$  elimination was an increase in absorbed dose due to  $^{137}\text{Cs}$  injection from 2.5 Gy up to 3.2 Gy. Thus animals receiving both external irradiation and internal contamination received a total absorbed dose estimated to 9.2 Gy, as compared to the  $\text{LD}_{50/30}$  of approximately 8 Gy in the C57BL/6J mouse strain as stated by the authors (Calvi *et al.*, 2019). Thus it is conceivable that co-exposed animals showed a 40% death rate as well as increased long-term defects in hematopoiesis in surviving animals with a total absorbed dose dependence (Calvi *et al.*,

2019). By contrast, in our study using lower radiation doses and  $^{137}\text{Cs}$  quantities, the absorbed dose due to  $^{137}\text{Cs}$  injection is very low, between 7.4 and 9.3 mGy, i.e. less than 1% of the 1 Gy external radiation dose. The faster elimination of  $^{137}\text{Cs}$  induced a slight but non-significant reduction of absorbed dose due to  $^{137}\text{Cs}$  injection. Thus the additional absorbed dose due to  $^{137}\text{Cs}$  injection was unlikely to be responsible for the observed biological effect. However, both studies are consistent in demonstrating that a combination of an external irradiation and an internal contamination give rise to unexpected biological effects.

Interestingly, not only the elimination of  $^{137}\text{Cs}$  was modified by external irradiation, but also the homeostasis of  $\text{K}^+$  was modified in 5 Gy irradiated animals, and returned to control values in co-exposed animals. Also, a slight, although non-significant, decrease in  $\text{K}^+$  was observed after 1 Gy irradiation. This result suggests that external irradiation at high doses modify the homeostasis of some ions in the blood, possibly due to the radiation-induced increased permeability of the endothelial barrier (Gabrys *et al.*, 2007) or to the modification of intestinal barrier integrity, which in turn induces a loss of electrolytes at high doses (Dublineau *et al.*, 2007). Such mechanisms may also explain the lower retention of  $^{137}\text{Cs}$  in heart and femur, two organs that were previously shown to accumulate  $^{137}\text{Cs}$  (Tourlonias *et al.*, 2010; Bertho *et al.*, 2010). One have to note that cesium is a physiological analog of potassium (ICRP 2017) and that potassium concentration in the blood is tightly regulated. However, the mechanism by which the exposure to a low quantity of  $^{137}\text{Cs}$  correlates with a return to normal levels of potassium in the blood remains elusive. Unfortunately, we were unable to test for a longitudinal follow-up of ion homeostasis in our animals, since the volume of blood drawn during the course of this experiment was limited. It should be of interest to verify if such changes in ion homeostasis have occurred earlier after exposure.

Interestingly, the exposure to  $^{137}\text{Cs}$  alone also induced an increase in testis weight, one of the most radiosensitive organs for tissue effects of ionizing radiation. We previously showed that  $^{137}\text{Cs}$  accumulated in the testis during chronic contamination (Tourlonias *et al.*, 2010). The presence of  $^{137}\text{Cs}$  in the testis in the absence of external exposure may explain the observed change in testis physiology.

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3 By contrast, the decrease in testis weight observed after a combination of 1 Gy external irradiation and  
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5  $^{137}\text{Cs}$  exposure is more intriguing. In fact, the added radiation dose due to  $^{137}\text{Cs}$  exposure is limited to  
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7 approximately 1% of the dose due to external exposure while the observed testis weight decrease is  
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9 about 15 % as compared to the external irradiation only. Thus the observed decrease in testis weight  
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11 is not proportional to the absorbed radiation dose due to  $^{137}\text{Cs}$ . The underlying mechanisms remain  
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13 elusive, but this result confirms that, at least in our specific controlled conditions, the addition of an  
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15 external exposure with an internal contamination with radionuclides may induce not only a change in  
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17 the biokinetics of internalized radionuclides, but also unexpected biological effects.

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21 Experimental data on combined internal contamination plus external radiation exposures are very  
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23 sparse, and are mainly limited to very early studies with a combination of  $^{137}\text{Cs}$  and  $^{90}\text{Sr}$ , but without  
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25 any comparison with single radionuclide exposure (Nishio, 1968, 1969, 1971; Nishio *et al.*, 1968). It is  
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27 thus difficult to compare our study of combined exposures with work from others, even with the study  
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29 by Calvi *et al.* (Calvi *et al.*, 2019). In this later study, the effect of the combined exposure is clearly due  
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31 to the addition of external plus internal high dose radiation exposure, while in our study, the observed  
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33 biological effects appear as independent from the radiation dose. Our results could be explained only  
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35 partly by the chemical effect of cesium, known to act on potassium channels in muscles (Cecchi *et al.*,  
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37 1987). The quantity of cesium injected in the animals corresponding to 20 KBq, estimated to  $2.28 \times 10^{-9}$   
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39 mole per animal including the stable cesium used as a carrier, is well below the  $\text{LD}_{50}$  in the mouse  
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41 model (ATSDR, 2004). In chemical toxicology studies, such differential effects of exposures to mixtures  
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43 of chemical toxicants were already described. For instance, a study testing different mixtures of  
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45 pollutants showed that in some cases, supra-additive effects of the mixtures were observed as  
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47 compared to what was expected from the exposure to individual pollutants (Crepet *et al.*, 2013).  
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49 Another study using two mixtures of 14 or 19 pollutants showed that these mixtures induced toxic  
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51 effects in various *in vivo* and *in vitro* models while used at concentrations below their known toxic  
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53 threshold (Carvalho *et al.*, 2014). Considering the results of these chemotoxicological studies, it would  
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55 be reasonable to suggest that combined internal and external radiation exposures produce biological  
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3 effects that are not obvious to predict. Our model of co-exposure to external irradiation and internal  
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5 contamination is clearly relevant of the exposure situation of populations living on a contaminated  
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7 territory, although the external radiation dose is much higher than what could be encountered in a  
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9 contaminated territory (Bertho et al, 2019). Therefore, it should be of interest to study the biokinetics  
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11 and the biological effects of mixed exposures, combining external irradiation at relevant  
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13 environmental dose rates and mixtures of radionuclides such as  $^{137}\text{Cs} + ^{90}\text{Sr}$ , the two main radionuclides  
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15 remaining in the environment on the long term after a nuclear accident.  
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