In: Fukushima Nuclear Accident
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*Chapter 13*

# Commentary 1: Biokinetics of $^{137}$Cs and Estimation of Internal Radiation Dose; Physiological Effects in Descendant Mice After the Every Generational Low Dose-Rate Internal $^{137}$Cs Radiation Exposure, As the Fukushima Simulation Experiment

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## Abstract

To investigate the transgenerational effects of chronic low dose-rate internal radiation exposure after the Fukushima Nuclear Power Plant accident in Japan, every generations of mice were maintained in the radioisotope facility with free access to drinking water containing $^{137}$CsCl (0 and 100 Bq/ml).

Mice of the tenth generation ($F_{10}$) were assessed γ-H2AX foci in hepatic cell, micro nuclei test, chromosome aberration analysis, tumorigenicity of lung, oxidative stress, and metabolomic analysis in heart and liver.

Suppression of the tumor growth, increasing of the γ-H2AX focus number, and the metabolite of oxidative stress were observed in $^{137}$Cs drinking mice, however, no significant difference was observed in the analyses of micro nuclei test, chromosome aberration, and tumor incidence.

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## INTRODUCTION

The health effects of radioactive contamination on people living in contaminated areas such as Belarus, Russia, and Ukraine have been studied in various ways in the nearly 30 years since the Chernobyl Nuclear Power Plant accident. Among these studies have been reports on acute and late radiation sickness due to high-dose exposure in people involved in decontamination and fire control efforts immediately after the accident [1 ,2]. In addition, childhood exposure to radioactive iodine reportedly has a clear effect on the occurrence of pediatric thyroid cancer [3, 4]. However, various reports on the health effects on residents of radioactively contaminated areas, including other cancers, developmental deformities, and genetic effects, have yet to be confirmed.

The current absence of conclusive evidence is due to the time required for indices, such as cancer onset and effects on later generations, to occur. In addition, the influence of low-dose radiation exposure on genetic effects and carcinogenesis cannot be ignored, despite the difficulty of accurately determining dose, variable exposure doses, and the potentially confounding effects of chemical substances from environmental pollution and living conditions.

Amid unresolved concerns over the health effects of low-dose radiation exposure on residents of contaminated areas, the Fukushima No.1 Nuclear Power Plant accident, caused by the tsunami of the Great East Japan Earthquake, occurred in 2011, 25 years after the Chernobyl nuclear accident. Low-dose radiation exposure has become a real concern to many residents in Fukushima as well as Chernobyl, which has led to various studies in Japan on the effects of low-dose radiation exposure.

$^{137}$Cs, with a half-life of 30.2 years, is a major nuclide that persists in contaminated areas. Early reduction of radioactive contamination due to natural purification is expected, but the purification rate of $^{137}$Cs in undisturbed soil is relatively slow. In addition, internal exposure by organisms living in such environments, including humans, can be expected to continue for multiple generations, much longer than the initial external exposure. However, various studies of Hiroshima and Nagasaki atomic bomb survivors have found no significant differences in genetic mutations between exposed and non-exposed groups [5-7]; studies on decontamination workers in Belarus and their children have also reported no significant next-generation effects thus far [8-10]. Because these investigations can only study the $F_1$ generation, the presence or absence of accumulated transgenerational effects is unclear. However, even if accumulated transgenerational effects occur at exposure doses lower than the average Hiroshima and Nagasaki doses, it will take several generations and potentially several hundred years until they become apparent. Therefore, new methods other than epidemiological studies must be developed for risk assessment of the health effects on descendants of those exposed to low-dose radiation caused by radioactive contamination from the Fukushima and Chernobyl accidents.

This study used inbred A/J strain mice, which have shorter generation times and more uniform genetic backgrounds than humans. A simulation experiment in a radioisotope facility that reproduced Chernobyl's $^{137}$Cs environmental pollution was initiated in 2007 to examine the transgenerational health effects caused by low-dose radiation exposure.

This study has bred many generations of mice administered 100 Bq/mL $^{137}$CsCl water ad libitum as drinking water and investigated the carcinogenic, genetic, and physiological effects in at least 10 generations of offspring to predict the health effects of low-dose radiation on humans.

## 1. EXPERIMENTAL METHODS

Two mouse pairs were selected from among A/J strain littermates bred in the radioisotope facility. One pair was assigned to the control group and bred while offered drinking water ad libitum; the other pair was administered a low-dose internal radiation exposure of $^{137}$CsCl water (100 Bq/mL). Harvested offspring from both pairings were sibling-mated for more than 10 generations, corresponding to a human generation turnover of approximately 300 years. Both 10$^{th}$ generation groups were analyzed for the following:

1) $^{137}$Cs distribution in each organ after long-term ad libitum intake of $^{137}$CsCl water (10, 100 Bq/mL).
2) γ-H2AX focus number as an indicator of DNA double-strand break repair initiation in all cells due to radiation exposure.
3) Detection of chromosomal abnormalities common to all cells by multi-color FISH and micronucleus tests.
4) Whole genome sequencing to detect base sequence mutations in germ cells that could accumulate in non-coding regions in each generation.
5) Quantification of naturally occurring and urethane-induced lung tumor incidence and mean tumor volume at 10 months of age to investigate the effects of low-dose radiation on carcinogenesis.
6) The effects of low-dose radiation on myocardium and blood vessel cell structures.
7) Metabolomic analysis of heart and liver tissues in the $^{137}$Cs water and control groups.
8) Balance of oxidative and anti-oxidative stress in the body.

## 2. RESULTS

### 2.1. *In Vivo* Dynamics and $^{137}$Cs Dose

Figure 1a shows the accumulation of $^{137}$Cs over time in each organ in the first generation of A/J strain male mice after continuous 8-month ad libitum intake of drinking water supplemented with an aqueous 100 Bq/mL cesium chloride solution. An average daily water consumption of 4.5 mL corresponded to 450 Bq $^{137}$Cs intake per mouse per day and average intake of 16 Bq per 1 g body weight for a 28-g mouse. The accumulated level of $^{137}$Cs increased slowly to approximately 10 times the average daily intake per 1 g body weight (16 Bq) in muscles by 3 weeks; levels in other organs reached an equilibrium concentration less than half that of muscles. As shown in Figure 2, a single oral dose of $^{137}$Cs resulted in a peak muscle tissue concentration similar to intake concentration levels per 1 g body weight; however, chronic and continuous dosing resulted in an accumulated concentration approximately 10 times the intake concentration per 1 g body weight. Figure 1b compares accumulation in 10 and 100 Bq/mL water concentrations.

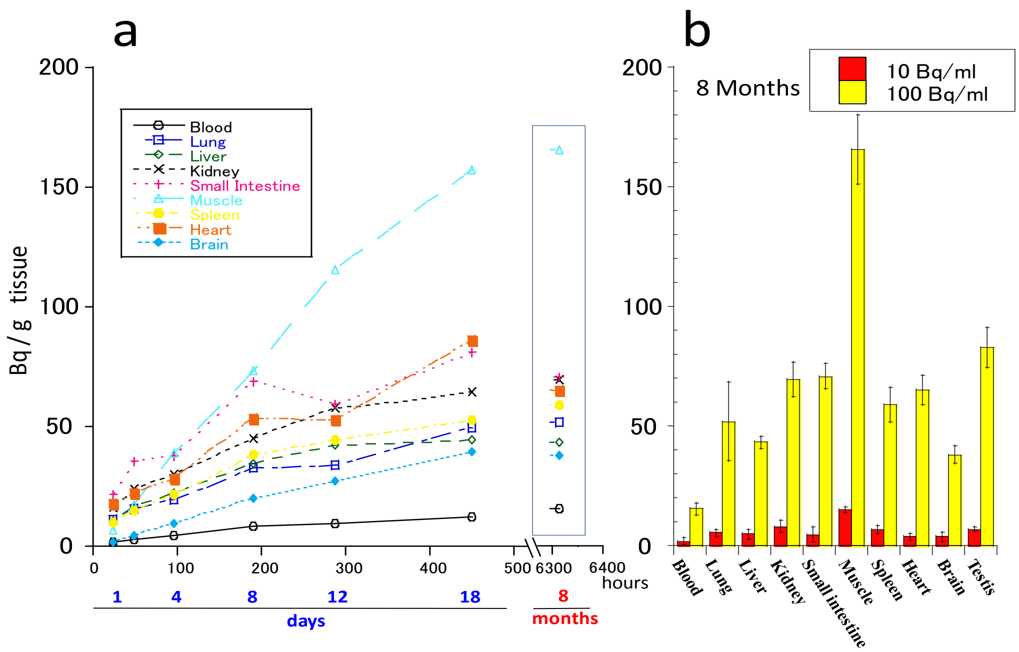


Figure 1. Accumulation of $^{137}$Cs in mouse organs. a: Organ-specific temporal changes in accumulated $^{137}$Cs levels with ad libitum $^{137}$Cs water (100 Bq/mL). b: Body distribution of $^{137}$Cs after 8 months of ad libitum $^{137}$Cs water (10 Bq/mL or 100 Bq/mL).

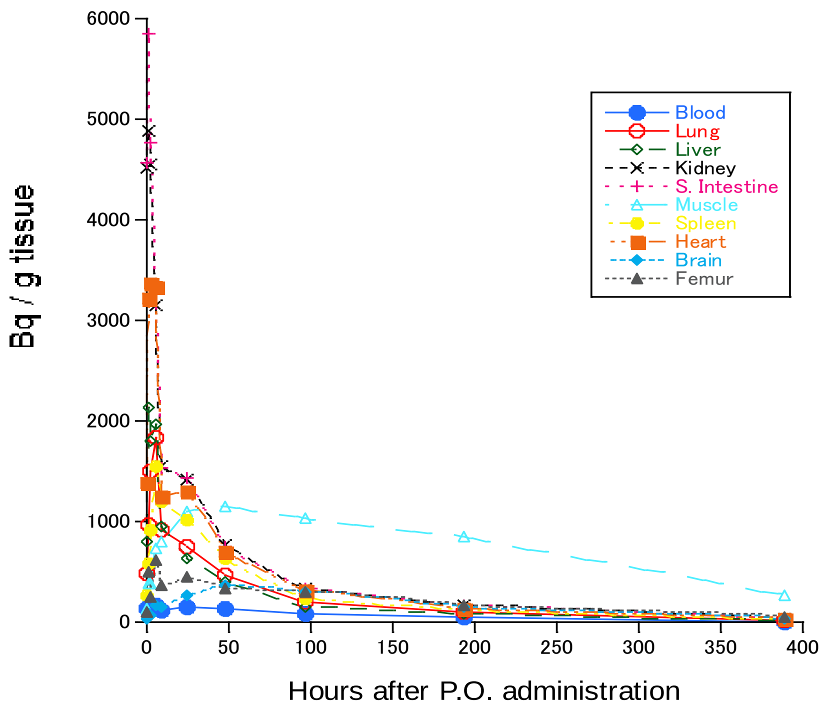


Figure 2. Organ-specific temporal changes in $^{137}$Cs levels after administration of a single oral dose of $^{137}$Cs (1,000 Bq/g body weight).

The equilibrium concentration was one-tenth for a corresponding one-tenth water concentration. Thus, if $^{137}Cs$ is ingested continuously, the accumulated levels will peak depending on the organ tissue and intake amount (Figure 2). Based on this observation, fixed internal exposure doses of 160 Bq/g and 80 Bq/g in the muscles and other organs, respectively, have been continuously administered for at least 15 mouse generations.

### 2.2. Genomic Effects

Increased levels of phosphorylated histone H2AX (γH2AX), a marker for initiation of DNA double-strand break repair, were observed in mice ingesting 100 Bq/mL water, suggesting chronic stress on the genome. Although DNA base sequence mutations were likely, there were no differences in micronucleus test results between the first and tenth generations. Similarly, multi-color FISH tests for chromosomal aberrations in bone marrow cells showed no differences between the control group and the tenth generation of mice. These results suggest that almost all damage was repaired or that limited genome damage occurred. Because small genomic mutations could occur, be inherited, and accumulate in each successive generation in non-coding genomic regions (introns and intergenic) and have no effect on survival, we compared levels of accumulated base mutations between the offspring exposed to continuous low-dose radiation and control mice of the same generation that started from a littermate mouse.

### 2.3. Carcinogenesis Effects

No difference in lung tumor incidence per mouse was observed between the two groups. However, the average tumor volume of naturally occurring or urethane-induced tumors at 10 months of age was significantly smaller in the $^{137}Cs$ water group compared to control group, suggesting an inhibition of tumor proliferation by $^{137}Cs$. These findings suggest that low-dose internal $^{137}Cs$ exposure does not induce lung tumorigenesis in mice. Even more importantly, the result possibly suggests the expression of hormesis or the adaptive response.

### 2.4. Physiological Effects

A significant increase in oxidative stress was noted in the $^{137}Cs$ water group, but there was no significant difference in the total antioxidation capacity, and the oxidative stress of mice living in these experimental conditions fell within the expected range. We are currently creating a detailed report addressing points 2.1 to 2.4.

## CONCLUSION

Until now, the low sensitivity of detection systems has prevented detailed studies on the biological effects of low-dose radiation exposure. There are also various barriers to this type of study, such as the complexity of animal experiments to determine the effects of long-term low-dose internal exposure, the challenge of obtaining clear results, and the relatively low impact of these findings in the research community.

However, animal experiments and individual evaluations are crucial for understanding the true effects of exposure to low-dose radiation. Regardless of the adverse or beneficial effects of low-dose radiation exposure on genes, cells, and tissues, it is ultimately most important to understand the potential range of effects on individual life expectancy. Studying the experimental effects on individual animals allows a full survey of all harmful and beneficial physical effects.

The results of these Fukushima and Chernobyl simulation experiments are informative whether or not biological effects are identified. While novel discoveries are generally considered positive research findings, negative results in this study can also contribute to a sense of security for people living in contaminated areas. Studies that offer useful results with either positive or negative results are less likely to have observational bias. However, researchers are reluctant to pursue this field of study, as evidenced by the low impact index of academic papers on this topic in recent years. Although this is a research area that lacks a research leader, increased interest in the effects of low-dose radiation exposure on individuals is anticipated. The author hopes that many scientists are interested in this field.

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