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Review Article

The overview of neutron-induced ^{56}Mn radioactive microparticle effects in experimental animals and related studies

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ABSTRACT

Investigation into the risks associated with radiation exposure has been carried out on those exposed to radiation in Hiroshima and Nagasaki, Semipalatinsk and other parts of the world. These risks are used as a guidance standard for the protection for radiation workers and the general public when exposed to radiation, and it sets upper regulatory limits for the amount of radiation exposure. However, the effects of internal exposure to radioactive microparticles have not been considered in these studies. These effects cannot be ignored since the exposure dose increases are inversely proportional to the square of the distance to the vicinity of the particles and can exceed tens of thousands of mGy. So far, only retrospective studies of people who have been exposed to radiation have been conducted, therefore we hypothesized that animal experiments would be necessary to investigate these effects. As a result, we found specific effects of radioactive microparticles. One particularly noteworthy finding was that internal exposure to radioactive microparticles resulted in pathological changes that were more than 20 times greater than those caused by the same level of external exposure. In contrast, there were other results that showed no such effects, and the reasons for this discrepancy need to be clarified. We also conducted RNA expression experiments and found that there was a difference between external exposure to ^{60}Co gamma rays and internal exposure to ^{56}Mn microparticles. In the future, we will need to study the mechanisms behind these findings. If the mechanism can be confirmed, it is expected to lead to the development of protective and therapeutic methods.

Keywords: effects of radioactive microparticles; internal exposure; neutron activation of ^{56}Mn; Hiroshima and Nagasaki atomic bombings; residual radioactivity; rat

INTRODUCTION

The level of risk from radiation to human health, such as carcinogenesis, has been determined almost primarily based on epidemiological studies of people exposed to radiation in Hiroshima and Nagasaki. After discussions at the International Commission on Radiological Protection (ICRP), these risks have been codified in the national laws of each country. In Japan, for example, the Radiation Injury Prevention Law defines the limits of radiation exposure for radiation workers and for the public.

The studies in Hiroshima and Nagasaki have been conducted mainly by the Radiation Effects Research Foundation (RERF), where health data on A-bomb survivors are collected every two years, and risks analyzed using dose data from the Dosimetry System 2002 (DS02) [1]. The general formula for calculating risk is shown in equation 1. RERF evaluates the radiation risk for each organ based on sex and age (age at exposure and attained age). This includes the incidence of cancer of the lungs, stomach, intestines and blood, as well as the association of cancer induction and mortality.

$$\frac{\text{Excess Health Effects of Exposed People}}{\text{Radiation Dose (DS02)}} = \text{Risk} \qquad (1)$$

This risk is used to estimate the effects from other radiation exposures, such as radiation accidents and medical exposure. If the dose to a person or cohort can be estimated, it is possible to calculate an estimate of the 'Excess Health Effects of this Person or Cohort' due to the

Table 1. Induced radionuclides * [18], ** [19]

Radionuclide	**Initial exposure dose rate*** (mGy/h)	**Cumulative dose* Tissue kerma** (mGy)
^{28}Al	5800**	310**
^{56}Mn	67	250
^{24}Na	20	430
^{46}Sc	0.001	10
Total (Rounded)	5900	1000

exposure, such as how much cancer will be induced in the future. These estimates can be used as a guide for regulation, compensation and to suggest further health monitoring and care for the exposed.

In contrast, the risk assessment based on the DS02 Hiroshima–Nagasaki survey only considered external exposure from gamma rays and neutrons and did not take internal exposure into account. The risk assessment of internal exposure is important because the exposure of the public and radiation workers is internal as well as external.

There have been recent dose estimations from the nuclear bomb explosions [2–7]. Using such precise methods of the estimation, it is desirable to assess the health effects of internal exposure using a cohort of exposed people such as those living around the Semipalatinsk nuclear test site.

Internal exposure includes inhalation through the lungs, uptake through the gastrointestinal tract, and uptake from dust and food. Some radioactivity is absorbed in soluble form due to its chemical nature, and some is taken in as microparticles. In the case of microparticles, the exposure dose is highest when in close proximity to the microparticles, while in the case of solutions it is considered to be distributed, the same as external exposure [8,9]. However, the effects have only been briefly studied [10–12].

Also, much earlier work has not pinpointed the importance of the 2.5 micron aerosol fraction in general environment studies, but it is now receiving research attention and is emphasized in this article.

In addition, there has not been an investigation of radioactive particulates activated by neutrons produced during the explosion of an atomic bomb. There are two types of atomic bomb-derived radioactivity: (i) fission product, such as ^{137}Cs and ^{90}Sr and fuel component, such as Pu; and (ii) induced radioactivity, such as ^{28}Al, ^{56}Mn and ^{24}Na, which are produced by neutron irradiation of soil (Table 1). The former, such as fission and fission-derived radioactivity such as Pu, has been well reviewed by Dagle [13] for example, but the latter, has not been investigated. This neutron-induced radioactivity is produced by the activation of soil particles and urban environments by neutrons from nuclear explosions, and the half-lives of the most significant radionuclides are short. Recently, the International Atomic Energy Agency has published a special issue as a coordinated study on radioactive microparticles [14], but these studies do not directly investigate radioactivity by neutron activation but focus on environmental dynamics and translocation into the body. Therefore, it is not a study of biological effects.

In the photo of the mushroom cloud in Hiroshima, the spreading of dust on the ground can be seen [15]. At that time, the area around the epicenter in Hiroshima was densely populated with Japanese houses, but most of the wooden houses within a 2 km radius of the hypocenter collapsed due to the bombing. A large amount of clay was used for the walls and attics of these Japanese houses, and the shock wave and blast of the explosion sent a large amount of dust up from the clay into the air. There are many testimonies from people were exposed to the bombing who said that for a while after the explosion, the area was completely dark (due to the high density of dust) and they could not see anything.

The spread of the dense dust mass on the ground was about 5 km in diameter. Over this spread, Imanaka *et al.* [16] present the results of the Manhattan Engineering District Survey measurements, which show that the soil activation exposure rate peaked at four locations (the hypocenter, about 1.3 km south, 2.3 km north and 3.2 km west). Also, Egbert *et al.* [17] showed excess doses compared with DS02 by using the analysis of the thermoluminescence dosimetry (TLD) and suggested additional doses were from ground-activated radioactive fallout. These indicate that the neutron-activated soil dust was spread by the blast within the 5 km diameter area, but not uniformly. If we can understand the relationship between the exposure location and the health effect on the exposed people, we may be able to consider the effect of radioactive dust.

Some of the elements in this dust were activated by neutrons from the atomic bomb. The significant radionuclides are ^{28}Al, ^{56}Mn and ^{24}Na, which were produced from ^{27}Al, ^{55}Mn and ^{23}Na, which are part of the main constituents of the soil. According to the Dosimetry System 1986 Report (DS86), doses from ^{28}Al, ^{56}Mn and ^{24}Na, which were in the ground, are estimated to contribute 310 mGy, 250 mGy and 430 mGy, respectively, as the integrated value of exposure dose immediately after the end of the explosion at the Hiroshima hypocenter (Table 1) [18,19]. Most of this exposure dose was received in the first day. This is far above the 1 mSv per year standard of radiation protection for the public. It also exceeds the permissible limit for radiation workers of 20 mSv per year, and the boundary values of the evacuation zones at Chernobyl and Fukushima. The lofted radioactive dust is thought to have been taken into the body through contact and adhesion to the skin or by inhalation. However, studies conducted by the RERF and others over the years have not yet investigated the health effects of this radioactive dust, or radioactive microparticles.

Furthermore, in Semipalatinsk, where the former Soviet nuclear test site in Kazakhstan was located, clouds (plumes) containing radioactive particulates passed through villages, especially during atmospheric nuclear tests, exposing people to radiation and causing health problems [20,21]. In one of these villages, Kainar, it is known that people exposed to the radioactive cloud which passed after the explosion report symptoms such as strong fatigue, subsequently this was named Kainar syndrome [22]. Symptoms include hair loss and complaints of intense malaise. The same thing happened in Hiroshima and Nagasaki, where people were so exhausted that they were unable to work, this exhaustion is known as 'Genbaku-Burabura-Byou' (Severely tired and unable to work). Similar health problems have also been reported by American soldiers who served in the Gulf War, which may be connected with depleted uranium munitions [23].

The common factor is that the calculated external dose to the exposed people from gamma residual radiation is low at survivors' locations at the time of the bombing, at most less than about 10 mGy [24]. Therefore, this mode of radiation exposure has not been considered to be the cause of significant radiation injury. On the other hand, in

Hiroshima, hair loss was observed in people exposed at 2 km at the time of bombing [25], where the initial and residual radiation dose was very low. To date, no scientific explanation has been given for these findings.

Considering the above, we focused on radioactive dust particles as a common exposure factor not only in Hiroshima and Nagasaki but also for events in other parts of the world. In this animal experiment, we paid particular attention to microparticles of ^{56}Mn, which are about 2.5 microns (μm) in diameter. It was found that these microparticles can cause bleeding and inflammation when they adhere to the alveoli and irradiate the alveolar cells. This is because the radiation dose rate increases inversely proportional to the square of the distance of the tissue to the radioactive microparticles. Calculations show that the dose rate can exceed tens of thousands of mGy in the vicinity of the microparticles [10–12], while the average dose is usually less than a few mGy.

Based on the epidemiological and theoretical findings described above, we decided to conduct animal experiments using rats and mice to empirically understand the biological effects of exposure to radioactive microparticles. In this special issue, we summarize the history and the current research on animal experiments to investigate the actual health effects of radioactive microparticle exposure, and to provide materials for future studies on the mechanisms.

MATERIALS AND METHODS

Animal experiments

Rats and mice were used in the experiments. Manganese dioxide powder containing a microparticle size of 3 μm was irradiated with neutrons from a nuclear reactor to produce ^{56}Mn, which was then sprayed on the animals for about one hour. The animals were male Wistar rats and male mice, strains C57BL and BALB/c, and the biological effects of internal irradiation were compared with those of external irradiations with ^{60}Co gamma rays. For two months following the internal radiation dose, we investigated potential pathological changes, locomotion changes, and differences in gene expression in each organ. To investigate the chemical toxicity, an equivalent exposure to non-radioactive manganese dioxide was also performed for comparison.

The amount of radioactivity was directly measured for each organ immediately after irradiation. Internal doses were determined by Monte Carlo methods based on animal models [26,27]. External doses were also determined by survey meter measurements and precise measurements using glass dosimeters [28]. These results are described by Stepanenko *et al.* in this issue [29]. All of them were about 20 mGy or less. In addition, external doses were measured using ESR dosimetry on rat teeth, and the trend was similar [30].

Outline of each research

In this study, we collected and analyzed data on: (i) dosimetry; (ii) locomotion changes; (iii) pathological damage; and (iv) gene expression through experiments. The following is a description of each.

Dosimetry

In these experiments, rats and mice were irradiated with manganese dioxide powder, which was activated by neutrons from a nuclear reactor. The diameter size was a few micrometers and the powder was sprayed on the rats and mice for approximately one hour. Immediately after this internal exposure, the amount of radioactivity was directly measured for each organ (liver, heart, kidney, tongue, lung, esophagus, stomach, small intestine, large intestine, trachea, eye, blood and skin) by autopsy. Using these values, internal doses for each animal were calculated by Monte Carlo methods. The results are described in detail by Stepanenko *et al.* [29] in this special issue.

During the exposure, external radiation doses were also measured. The measurements were made by using survey meters and glass dosimeter tips [28]. All the doses were about 20 mGy or less, which is not as large as the internal dose. In addition, external radiation doses were measured using rat teeth and obtained similar results [30].

Locomotor activity of rats

We monitored the locomotion of rats using an infrared sensor. The infrared sensor detects infrared light from the rats and was measured continuously during the observation period as one count when the rat moved once. The cage contained one infrared sensor and one rat, and four groups were usually set up: $^{56}MnO_2$ (4 rats), Mn(stable)O_2 (4 rats), ^{60}Co 2000 mGy (4 rats), and control (4 rats), for a total of 16 individuals observed. The experiments were conducted several times from 2014 to 2016. According to the analysis of Otani *et al.* [31] in the experiment to investigate the effect of ^{60}Co external irradiation on the locomotion of rats, a dose-dependent decrease in locomotion of rats was observed immediately after irradiation, and the effect disappeared in about one week. In these experiments to investigate the effects of exposure to $^{56}MnO_2$, the effect of decreased locomotion was strongest at about 2 weeks post-exposure and lasted for over 3 weeks [32]. Therefore, in contrast the effect appeared later and lasted longer than that of ^{60}Co external irradiation. No effects on locomotion changes were observed for the control and Mn(stable)O_2 exposure control animals.

Pathological damage

Shichijo *et al.* [33–35] examined the pathological changes at 3 days, 2 weeks, 2 months, after irradiation. At these time points the liver, heart, kidney, trachea, lung, tongue, esophagus, stomach, small intestine, eye, spleen, testis, thymus, and skin were dissected, fixed in 10% formalin, and embedded in paraffin. Sections of 4 μm thickness were prepared and stained with hematoxylin and eosin (HE). For scoring of mitotic cells in the intestinal crypt, a good longitudinal section of the crypt, which was aligned clearly with the other crypt and contained the lumen of the crypt, was selected. At least 30 cryptic foci per rat were observed by light microscopy as described above. Pathology of lung tissue was evaluated for hemorrhage, emphysema, inflammation (number of inflammatory cells), development of lymphatic follicles and enlargement of alveolar walls from '−' to '++'.

As a result, we found a significant effect of internal exposure to ^{56}Mn compared to external exposure to ^{60}Co (2000 mGy), especially for lung tissue (Shichijo *et al.* [33–35]). Although the internal exposure to the lungs at that time was only 100 mGy, pronounced hemorrhage and emphysema were observed. On the other hand, no effect was observed at an external exposure of 2000 mGy to ^{60}Co which means that the effect was at least 20 times greater than that of the 100 mGy internal exposure. However, later studies by Fujimoto *et al.* [39,40] reported that such strong differences of the pathological effects were

not observed. Shichijo *et al.* reported a higher absorbed dose of 51–110 mGy, which were very different from the results of Fujimoto *et al.* On the other hand, both groups found no difference in the thickening of alveolar walls and collagen deposition associated with fibrosis, which are commonly observed in radiation injury. In addition, elastin expression, another pathological indicator of fibrosis, was higher in the internally exposed group and is proposed as a 'new pathological indicator' of internal exposure. Since molecular pathological image analysis was performed using a microscope with imaging software, more in-depth analysis may be required to confirm internal exposure. In the report by Fujimoto *et al.*, elastin mRNA expression was high, but the difference was not significant, so further examination of gene expression will be necessary. These results are summarized in Shichijo *et al.* [35].

Gene expression

The biological impact of irradiation could be detected by changes in gene expression in the target tissues [36,37]. The mRNA levels of several marker genes were determined to examine the effects of the exposure to $^{56}MnO_2$ in the lung, the testis, the prostate as well as liver.

In our earlier rat study, Kairkhanova *et al.* [38] investigated the mRNA expression of hyaluronan synthase 2 in the lung of rats exposed to two different levels of $^{56}MnO_2$. The absorbed doses were 55 and 110 mGy in the lung. The hyaluronan synthase 2 mRNA levels were significantly reduced 3 days post-exposure, then showed signs of recovery when measured on days 14 and 60, suggesting a significant adverse effect in the lung. Unfortunately, the control groups were set up after the $^{56}MnO_2$ exposure experiment due to a limitation with animal supply. With a lack of proper control groups, the results were inconclusive. Recently, we conducted new experiments in rats exposed to $^{56}MnO_2$ with the appropriate control groups to further examine the effects of $^{56}MnO_2$ on the lungs by determining the gene and protein expression changes [39]. Absorbed doses from internal irradiation of the lungs ranged from 25 to 65 mGy for animals exposed to $^{56}MnO_2$ and from 41 to 100 mGy for the whole body [39]. Animals were examined on days 3 and 61 post-irradiation, where no significant pathological changes associated with exposure to $^{56}MnO_2$ microparticles were observed. However, aquaporin 5 mRNA and protein expression were significantly increased in lung tissue on day 3 post-exposure in the $^{56}MnO_2$ group (1.6- and 2.9-fold, respectively, in the highest dose group); Smad7 mRNA expression was also significantly increased by 30% in the highest dose group of those exposed to $^{56}MnO_2$. These results indicate that internal irradiation with $^{56}MnO_2$ induces significant biological responses, including changes in gene expression in the lung, while external irradiation with 2000 mGy of ^{60}Co gamma radiation does not.

In the same study, the mRNA expression of testicular marker protein genes and prostate secretory protein genes were quantified by Q-RT-PCR [40]. Gene expression of steroidogenesis-related enzymes, Cyp17a1 and Hsd3b1, was decreased in testes exposed to $^{56}MnO_2$ on day 3 post-exposure. The mRNA levels of germ cell specific Spag4 and Zpbp were also decreased. On the 61st day after exposure, the expression of Cyp11a1 gene was significantly decreased in the testes of the group exposed to the highest dose of $^{56}MnO_2$, and the mRNA level of another steroidogenesis-related StAR gene was decreased in the ^{60}Co gamma radiation group. In contrast there was no difference in Spag4 and Zpbp mRNA levels between groups at day 61. No histopathological changes were observed in the testes after exposure in either group. The expression of prostate protein genes, including CRP1, KS3, and PSP94, was significantly decreased on day 61 after exposure in the $^{56}MnO_2$ exposure group and the ^{60}Co gamma radiation group. These data suggest that internal exposure to $^{56}MnO_2$ powder of less than 100 mGy significantly affects gene expression in testis and prostate, with effects more pronounced than external irradiation of 2000 mGy.

Hepatic gene expression changes were also determined in our study [41]. Although no histopathological changes were observed in the liver, the mRNA expression of a p53 related gene, cyclin-dependent kinase inhibitor 1A, increased in $^{56}MnO_2$ as well as γ-ray irradiated groups on postexposure days 3 and 61. The expression of a stress-responsive gene, nuclear factor κB, was also increased by $^{56}MnO_2$ exposure and γ-rays on post exposure day 3. However, the expression of cytokine genes (interleukin-6 or chemokine ligand 2) or fibrosis related TGF-β/Smad genes (TGF-β1, Smad3, or Smad4) was not altered by the exposure. The data showed that the internal exposure to $^{56}MnO_2$ microparticles at less than 100 mGy significantly affected the gene expressions in a similar manner to liver exposed to 2000 mGy of external γ-irradiation. Rather than being adverse, these changes may be adaptive responses because there were no changes in cytokine or TGF-β/Smad gene expressions. The changes in the hepatic gene expression may be involved in our previous finding of an effect on blood chemistry, in which the ALT level was decreased on day 3 and increased significantly on day 61 in the $^{56}MnO_2$ exposed group [42].

THE OTHER RELATED STUDIES

Otani *et al.* [43,44], in a cohort study of early entrants to the city in the immediate aftermath of the Hiroshima bombing reported that the risk of death from malignant neoplasms other than leukemia was statistically significantly higher among those who entered the city on the day of the bomb explosion (Day 1: 6 August 1945), 7 August and 8 August, compared with those who entered the city on or after 9 August. In addition, there was a significant increase in risk among males aged 20 years or older at the time of the bombing, which can be explained by the fact that they spent a long time in the contaminated area, mainly men who were physically strong, searching for and rescuing their family members and acquaintances (Table 2).

This suggests the possibility of health effects due to exposure to radioactive microparticles, which has not been considered until now, as a common factor of so-called 'Genbaku-BuraBura-Byo disease' (Severely tired and unable to work). Therefore, we thought that animal experiments were necessary in addition to the epidemiological studies. The radionuclides with the high exposure doses to early entrants are ^{56}Mn (half-life: 2.6 h) and ^{24}Na (half-life: 15 h) which are listed in Table 1. In view of the high mortality risk observed specifically in the Day 1 entrants (6 August), we decided to conduct animal experiments focusing on ^{56}Mn with a half-life of 2.6 h. Otani *et al.* [43,44] reported an in-depth analysis of the health effects of residual radiation in early entrants to the city after the Hiroshima bombing.

Stepanenko *et al.* [45] studied the external exposure at 1.3 km from the epicenter using quartz-containing tile samples from Hiroshima. It was shown that measurements using the method of optically stimulated luminescence from single quartz grains inclusions in very thin layers of the samples, confirm the radiation exposures to beta rays as a component of irradiation from residual radioactivity in Hiroshima and Nagasaki.

Table 2. Health effects of early entrants who entered after the explosion of the Hiroshima atomic bomb: excess mortality risk who enter the city on 6, 7 and 8 August, compared with entrants who entered the city after 9 August [Otani *et al.* 43,44]

		Male			Female		
		The day of entrance			The day of entrance		
		6 August	7 August	8 August	6 August	7 August	8 August
Age at exposure	[0, 10)	—	↑	↑	—	—	—
	[10,20)	—	↑	—	—	—	—
	[20,30)	↑	—	—	—	—	—
	[30,40)	↑	—	—	—	—	—
	[40,50)	↑	—	—	↑	—	—

Zhumadilov *et al.* [46] measured the teeth of people exposed to radiation in the black rain area of Hiroshima (about 9.5 km), and found that one of the cases tested had external exposure exceeding 100 mGy. This result also strongly suggests that airborne radioactive microparticles travelled over long distances. From the results in this section, it can be assumed that people in Hiroshima were exposed to radioactive microparticles, and it is suggested that there is room for reconsideration of the conventional exposure system of DS02 to include residual radiation and microparticle effects.

SUMMARY

The results of our animal experiments have shown that there are significant effects of radioactive microparticles. Notably, we found that internal exposure to radioactive microparticles resulted in pathological changes that were more than 20 times greater than those caused by an equivalent level of external exposure [15,33–35,47]. However, other studies report conflicting findings, which therefore require further investigation. It was also found that there was a difference in RNA expression between external exposure to ^{60}Co gamma rays and internal exposure to ^{56}Mn [39–41]. Research should continue on gene expression. Many factors including environmental ones are now known to influence gene expression, and this complexity may account for some of the findings which are erratically reproducible. Future studies should aim to elucidate the mechanisms behind these findings. Clarification of these mechanisms is expected to lead to the development of treatment and protection methods. Furthermore, as explained in '3. The other related studies,' there are additional modes of exposure that could have occurred in Hiroshima that cannot be fully explained by conventional DS02 [48], Therefore, we recommend that the possibility of exposure to radioactive microparticles should be further examined.

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CONFLICT OF INTEREST

The author declares that they have no conflicts of interest.

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REFERENCES

1. Young R, Kerr G (eds). *Reassessment of the Atomic Bomb Radiation Dosimetry for Hiroshima and Nagasaki - Dosimetry System 2002 (DS02)*. Radiation Effects Research Foundation: Hiroshima Japan, 2005.
2. Simon S, André B, Beck H et al. Dose estimation for exposure to radioactive fallout from nuclear detonations. *Health Phys* 2022;122:1–20.
3. Beck H, Bouville A, Simon S et al. A method for estimating the deposition density of fallout on the ground and on vegetation from a low-yield, low-altitude nuclear detonation. *Health Phys* 2022;122:21–53.
4. Bouville A, Beck H, Anspaugh L et al. A methodology for estimating external doses to individuals and populations exposed to radioactive fallout from nuclear detonations. *Health Phys* 2022;122:54–83.
5. Anspaugh L, Bouville A, Thiessen K et al. A methodology for calculation of internal dose following exposure to radioactive fallout from the detonation of a nuclear fission device. *Health Phys* 2022;122:84–124.

6. Melo D, Bertelli L, Ibrahim S et al. Dose coefficients for internal dose assessments for exposure to radioactive fallout. *Health Phys* 2022;122:125–235.
7. Thiessen K, Hoffman FO, Bouville A et al. Parameter values for estimation of internal doses from ingestion of radioactive fallout from nuclear detonations. *Health Phys* 2022;122:236–68.
8. H. SMITH, ICRP, Didcot, Oxfordshire (eds). ICRP. *Dose Coefficients for Intakes of Radionuclides by Workers*. Oxford: ICPR Publication 68, Annals of the ICRP 24, 1994, Elsevier, 1994, ISSN 0146-6453.
9. Ishigure N. Doses used for internal exposure protection. *Radioisotopes* 2013;62:465–92.
10. Stepanenko V, Rakhypbekov T, Kaprin A et al. Irradiation of laboratory animals by neutron activated dust: development and application of the method - first results of international multicenter study. *Rad Risk* 2016;25:111–25.
11. Stepanenko V, Zhumadilov K, Hoshi M et al. Preliminary assessment of dose distribution on the spatial micro level for internal exposure of alveolar epithelium of rats by ^{56}Mn. *Bull Karaganda Univ Ser Phys* 2019;95:59–63.
12. Stepanenko V, Kaprin A, Ivanov S et al. Microdistribution of internal radiation dose in biological tissues exposed to ^{56}Mn dioxide microparticles. *J Radiat Res* 2022;63(S1):i21–25.
13. Dagle GE, Sanders CL. Radionuclide injury to the lung. *Environ Health Perspect* 1984;55:129–37.
14. Salbu B, Ulanowski A, Fesenko S (eds). IAEA: coordinated research on radioactive particles. *J Environ Radioactivity*, Elsevier Ltd., 2021; 216:106160,106127,106111,106073. ISSN: 0265-931X. https://www.sciencedirect.com/journal/journal-of-environmental-radioactivity/special-issue/10TXGWSCBJL.
15. Hoshi M. Radioactive microparticle effects found in animal experiments. The innovation platform ISSUE 5. *Innovation news. Network* 2021;274:186–8. https://www.innovationnewsnetwork.com/effects-of-radioactive-microparticles-found-in-animal-experiments/9639/.
16. Imanaka T. Radiation survey activities in the early stages after the atomic bombing in Hiroshima. In: Aoyama M, Oochi Y (eds). *Revisit the Hiroshima A-Bomb with a Database - Latest Scientific View on Local Fallout and Black Rain (HisoF) Hiroshima City*. Springer: Berlin, Heidelberg, 2011, 69–81 ISBN 978-4-9905935-0-6.
17. Egbert S, Kerr G. Gamma-ray thermoluminescence measurements: a record of fallout deposition in Hiroshima. *Radiat Environ Biophys* 2012;51:113–31.
18. Okajima S, Fujita S, Harley J. Radiation doses from residual radioactivity. In: Roesch WC (ed). *US–Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki, Final Report, Vols 1 and 2*. Hiroshima: Research Foundation, 1987, 205–26.
19. Imanaka T. *20th Hiroshima International Symposium at Miyajima on 10*, 11 January 2016. http://www.rri.kyoto-u.ac.jp/NSRG/etc/16-1-11Miyajima_imanaka.pdf.
20. Gusev B, Rosenson R, Abylkassimova Z. The Semipalatinsk nuclear test site: a first analysis of solid cancer incidence (selected sites) due to test-related radiation. *Radiat Environ Biophys* 1998;37:209–14.
21. Bauer S, Gusev B, Pivina L et al. Radiation exposure due to local fallout from soviet atmospheric nuclear weapons testing in Kazakhstan: solid cancer mortality in the Semipalatinsk historical cohort, 1960-1999. *Radiat Res* 2005;164:409–19.
22. Rozenson R, Apsalikov K, Zhumambayeva S et al. The Kainar syndrome: history and modern understanding. *IPSHU English Research Report Series* 2012;28:144–50. http://doi.org/10.15027/33638, ipshu_en_28_144.pdf.
23. National Academies of sciences, Engineering and Medicine 2016. *Gulf War and Health, 10 Update of Health Effects of Serving in the Gulf War*, 2016. Washington, DC: The National Academies Press. https://doi.org/10.17226/21840.
24. Imanaka T, Endo S, Kawano N et al. Radiation exposure and disease questionnaires of early entrants after the Hiroshima bombing. *Radiat Prot Dosim* 2012;149:91–6. https://doi.org/10.1093/rpd/ncr370.
25. Preston D, Mabuchi K, Kodama K et al. Relationship of epilation to distance from hypocenter. *J Nagasaki Med Assoc* 1998;73:251–3.
26. Stepanenko V, Rakhypbekov T, Otani K et al. Internal exposure to neutron-activated ^{56}Mn dioxide powder in Wistar rats: part 1: dosimetry. *Radiat Environ Biophys* 2017;56:47–54.
27. Stepanenko V, Kaprin A, Ivanov S et al. Internal doses in experimental mice and rats following exposure to neutron-activated $^{56}MnO_2$ powder: results of an international multicenter study. *Radiat Environ Biophys* 2020;59:683–92.
28. Stepanenko V, Sato H, Fujimoto N et al. External dose estimates of laboratory rats and mice during exposure to dispersed neutron-activated ^{56}Mn powder. *J Radiat Res* 2022;63(S1):i16–20.
29. Stepanenko V, Kaprin A, Ivanov S et al. Overview and analysis of internal radiation dose estimates in experimental animals in a framework of international studies of the sprayed neutron-induced ^{56}Mn radioactive microparticles effects. *J Radiat Res* 2022;63(S1):i8–i15.
30. Todaka A, Toyoda S, Fujimoto N et al. ESR tooth enamel doses of rats and mice exposed to radioactive $^{56}MnO_2$ dust. *Adv ESR Appl* 2021;37:13–8.
31. Otani K, Ohtaki M, Fujimoto N et al. Quantitative analysis of the effects of a single ^{60}Co gamma ray point exposure on time-dependent change in locomotor activity in rats. *Int J Environ Res Public Health* 2020;17:5638. https://doi.org/10.3390/ijerph17165638.
32. Otani K, Ohtaki M, Fujimoto N et al. Effects of internal exposure to neutron-activated $^{56}MnO_2$ powder on locomotor activity in rats. *J Radiat Res* 2022;63(S1):i38–i44.
33. Shichijo K, Fujimoto N, Uzbekov D et al. Internal exposure to neutron-activated ^{56}Mn dioxide powder in Wistar rats -part 2: pathological effects. *Radiat Environ Biophys* 2017;56:55–61.
34. Shichijo K, Takatsuji T, Abishev Z et al. Impact of local high doses of radiation by neutron activated Mn dioxide powder in rat lungs: protracted pathologic damage initiated by internal exposure. *Biomedicine* 2020;8:171. https://doi.org/10.3390/biomedicines8060171, https://www.mdpi.com/2227-9059/8/6/171.

35. Shichijo K, Takatsuji T. Pathological observation of the effects of exposure to radioactive microparticles on experimental animals. *J Radiat Res* 2022;63(S1):i26–i37.
36. Kabacik S, MacKay A, Tamber N et al. Gene expression following ionizing radiation: identification of biomarkers for dose estimation and prediction of individual response. *Int J Radiat Biol* 2011;87:115–29.
37. Paun A, Lemay A, Haston CK. Gene expression profiling distinguishes radiation-induced fibrosing alveolitis from alveolitis in mice. *Radiat Res* 2010;173:512–21.
38. Kairkhanova Y, Saimova A, Uzbekov D et al. Effects of exposure to radioactive 56 mno2 powder on hyaluronan synthase 2 in the lungs of rats. *Georgian Med News* 2017;9:120–4.
39. Fujimoto N, Ruslanova B, Abishev Z et al. Biological impacts on the lungs in rats internally exposed to radioactive $^{56}MnO_2$ particle. *Sci Rep* 2021;11:11055. https://doi.org/10.1038/s41598-021-90443-9.
40. Fujimoto N, Amantayeva G, Chaizhunussova N et al. Low-dose radiation exposure with $^{56}MnO_2$ powder changes gene expressions in the testes and the prostate in rats. *Int J Mol Sci* 2020;21:4989. https://doi.org/10.3390/ijms21144989, https://www.mdpi.com/1422-0067/21/14/4989.
41. Ruslanova B, Abishev Z, Chaizhunussova N et al. Hepatic gene expression changes in rats internally exposed to radioactive $^{56}MnO_2$ particle at low doses. *Curr Issues Mol Biol* 2021;43:758–66.
42. Fujimoto N, Baurzhan A, Chaizhunusova N et al. Effects of internal exposure to $^{56}MnO_2$ powder on blood parameters in rats. *Eur J Med* 2020;52:52–6.
43. Otani K, Tonda T, Satoh K et al. Mortality risk analysis among early entrants in Hiroshima. *J Nagasaki Med Accoc* 2012;87:261–4.
44. Otani K, Ohtaki M, Yasuda H. Solid cancer mortality risk among a cohort of Hiroshima early entrants after the atomic bombing, 1970-2010: implications regarding health effects of residual radiation. *J Radiat Res* 2022;63(S1):i45–i53.
45. Stepanenko V, Kaprin A, Ivanov S et al. Estimation of 'dose-depth' profile in the surface layers of a quartz-containing tile from the former HiroshimaUniversity building indicates the possible presence of beta-irradiation from residual radio activity after A-bombing. *J Radiat Res* 2022;63(S1):i54–i60.
46. Zhumadilov K, Ivannikov A, Stepanenko V et al. Tooth enamel ESR dosimetry for Hiroshima 'black rain' zone residents. *J Radiat Res* 2022;63(S1):i61–i65.
47. Hoshi M. Radiation exposure and health effects of the residents around the former soviet union nuclear test site in Kazakhstan -a new perspective: radioactive microparticles. *Impact* 2020;2020:70–2.
48. Kerr G, Egbert S, Al-Nabulsi I et al. Workshop report on atomic bomb dosimetry - review of dose related factors for the evaluation of exposures to residual radiation at Hiroshima and Nagasaki. *Health Phys* 2015;109:582–600.