Effects of internal exposure to neutron-activated $^{56}$MnO$_2$ powder on locomotor activity in rats

Keiko Otani$^{1,*}$, Megu Ohtaki$^{1,2}$, Nariaki Fujimoto$^3$, Darkhan Uzbekov$^4$, Ynkar Kairkhanova$^4$, Aisulu Saimova$^4$, Nailya Chaizhunusova$^4$, Dariya Habdarbaeva$^4$, Almas Azhimbekhanov$^5$, Kassym Zhumadilov$^6$, Valeriy Stepanenko$^7$ and Masaharu Hoshi$^{1,2}$

$^1$The Center for Peace Hiroshima University, 730-0053, Hiroshima, Japan
$^2$Hiroshima University, 739-8527, Japan
$^3$Research Institute for Radiation Biology and Medicine, Hiroshima University, 734-8553, Hiroshima, Japan
$^4$Semeny State Medical University, Semey, 071400, Kazakhstan
$^5$Eurasian National University named after L.N. Gumilyov, Kazakhstan, 010008, Kazakhstan
$^6$Tsyb Medical Radiological Research Center, Obninsk, 2490031, Russian Federation
$^7$Corresponding author. The Center for Peace Hiroshima University, Hiroshima, Japan; Higashisenda-machi 1-1-89, Naka-ku, Hiroshima, 730-0053, Japan. Email: ohtani@hiroshima-u.ac.jp

(Received 13 November 2021; revised 10 January 2022; editorial decision 22 January 2022)

ABSTRACT

At the detonation of the atomic bombing in Hiroshima and Nagasaki, a significant amount of radionuclides was produced by the neutron induced activation. The residual radiation from the explosion is crucial to the health risk of the people who entered these cities after the bombing and might have inhaled these radioactive materials. Because $^{56}$Mn is one of the major radionuclides produced in soil and have not been studied until now, we had conducted a series of experiments using rats to investigate the biological impacts of exposure of $^{56}$MnO$_2$ particles. In these experiments, the rats’ spontaneous locomotor activity was also assessed to examine the possible effects of $^{56}$Mn on their behavior. However, the locomotor activity data obtained from an individual experiment failed to identify radiation effects due to the large variation among animals and the small sample size. In the present study, all available data from our previous studies on $^{56}$MnO$_2$ exposure (0.02–0.15 Gy of whole-body doses) as well as $^{60}$Co-γ exposure (at 2–5 Gy of whole-body doses) were pooled. Our statistical method, which takes into account individual differences and daily fluctuations, successfully identified a decrease in locomotor activity caused by $^{56}$MnO$_2$ exposure, where the changes were gradual and reached the maximum reduction around 2 weeks after exposure. In contrast, exposure to $^{60}$Co-γ rays produced the highest decline of activity within one day. These results suggest that internal exposure to $^{56}$Mn at whole-body doses of even less than 0.15 Gy may have a long-lasting impact on locomotor activity.

Keywords: fluctuations; individual differences; internal exposure; linear mixed effect model; locomotor activities; $^{56}$MnO$_2$ microparticles

INTRODUCTION

While the immediate radiation from the atomic bombing in Hiroshima and Nagasaki had a large, negative impact on the populace, residual radiation may have played a role in radiation injuries as well. However, the health effects of exposure to residual radiation have not been investigated in previous radiation effect studies. For example, the health effects study of people exposed to radiation in Hiroshima and Nagasaki by the Radiation Effects Research Foundation did not take into account exposure from residual radiation [3,4]. People who entered the city after the explosion of the atomic bomb, i.e. early entrants, are thought to have been exposed to the residual radioactivity. Therefore, the study of residual radiation is essential for evaluating the risks to those who entered these cities early after the bombing and suffered from various acute radiation syndromes [5,6] or late effects like solid cancer [7,8].
The residual radiation sources include neutron-activated radionuclides on the ground and radioactive fallout from the bombs. The former is the neutron-induced activation of Japanese houses and soil on the ground surface, while the latter is the radioactivity produced by nuclear fission. Typical radionuclides for the former include $^{56}$Mn and $^{24}$Na which were produced by the neutron activation from the stable $^{55}$Mn and $^{23}$Na in soil elements, and $^{137}$Cs and $^{90}$Sr for the latter. There are not so many reports about the former as those about the latter, but not on Hiroshima and Nagasaki in particular, but there are some reports on the general health effects of internal exposure [9,10]. The residual radioactivity within a few kilometers of the hypocenter in Hiroshima and Nagasaki was mainly the radioactivity activated by neutrons, while the radioactivity from fission rose more than 10 km and was carried away by the wind, and hardly fell into the city [11]. Therefore, it was necessary to study the effects of residual radioactivity, especially the radioactivity produced by neutron activation.

As the main radionuclides produced in the soil, $^{56}$Mn and $^{24}$Na were the ones with the largest exposure doses [3,12]. Furthermore, people who entered the city within three days after the bombing had a higher risk of death due to solid cancer [7,13], so radionuclide $^{56}$Mn with a half-life of 2.6 h is supposed to be one of the main neutron activated emitter contained in residual radiation.

The early signs of acute radiation syndrome include nausea, emesis, fever, anorexia, fatigue, and tiredness. Atomic bomb survivors in Hiroshima and Nagasaki and the population exposed to the radiation from the Semipalatinsk Nuclear Test Site commonly suffered from tiredness and fatigue [14,15], although these have been generally considered late psychological effects [16,17]. Spontaneous locomotor activity is a simple measure to evaluate fatigue in animal experiments [18]. In a mouse study, locomotor activity was reduced 35% by 6 hours after gamma-irradiation at 0.5 Gy and was recovered by 24 hours [19].

We had conducted a series of experiments using rats to investigate their changes in behavior and biochemical or pathological changes by exposing laboratory rats to $^{56}$MnO$_2$ particles from 2014 to 2016. Their effects on blood chemistry, lungs, small intestine and testes have been reported already [20–24]. We measured the spontaneous locomotor activity of rats after exposure to $^{56}$MnO$_2$ by an infrared-based continuous recording system. However, we did not find any statistically significant changes resulting from exposure to $^{56}$MnO$_2$ due to the small number of rats examined and the large variation in data among animals in each experiment. In the present study, all our previously collected locomotor activity data were combined. A linear mixed model [25] was applied to compensate for individual differences, daily fluctuations, and systemic differences due to experimental numbers when studying locomotor activity.

### MATERIALS AND METHODS

#### Experimental design

**Animals and irradiation**

Although the data in the present study were original, the animal experiment setups summarized in Table 1 were previously reported [23, 24, 26]. Briefly, Experiments 1 and 2 used 5-month-old male Wistar rats obtained from Karaganda State Medical University, Kazakhstan. Rats were divided into four groups in each experiment: $^{56}$MnO$_2$, $^{55}$MnO$_2$, $^{60}$Co, and control. The $^{56}$MnO$_2$ and $^{55}$MnO$_2$ groups were exposed to $^{56}$MnO$_2$ and non-radioactive $^{55}$MnO$_2$, respectively. The $^{60}$Co group received 2 Gy of external $^{60}$Co γ-ray irradiation. Experiments 3 and 4 used 10-week-old male Wistar rats purchased from Kazakh Scientific Center of Quarantine and Zoonotic Diseases, Almaty, Kazakhstan. In experiment 3 rats were divided into four groups: $^{56}$MnO$_2$-H, $^{56}$MnO$_2$-L, $^{60}$Co, and control groups. $^{56}$MnO$_2$ groups were exposed to two different levels of radioactive $^{56}$MnO$_2$ and the $^{60}$Co group received 2 Gy of external $^{60}$Co γ-rays. Experiment 4 consisted of four groups exposed to external $^{60}$Co γ-rays at 0, 2, 3.5, and 5 Gy.

**Exposure to $^{56}$MnO$_2$ and the corresponding internal dose estimation** have been described previously [27]. $^{56}$MnO$_2$ was obtained by neutron activation of $^{55}$MnO$_2$ powder using the Baikal-1 nuclear reactor at the National Nuclear Center in Kurchatov, Kazakhstan, and air-sprayed into a box containing the rats. Whole-body γ-ray irradiation was performed with a $^{60}$Co-γ-ray irradiator, Teragam K-2 unit (UJP Praha, Praha-Zbraslav, Czech Republic).

The animal experiment was approved by the Animal Experiment Committee of Semey Medical University, Republic of Kazakhstan (Protocol No. 5 dated 04/16/2014), and conducted following the Institutional Guide for Animal Care and Use.

#### Recording of locomotor activities

The spontaneous locomotor activities of the rats were continuously recorded by infrared sensors (Model NS-AS01; Neuroscience, Inc., Tokyo, Japan) placed on top of the animal cage, with one rat per cage. A change in the strength of infrared rays emitted from the animal was counted as one movement. The activities of 16 animals were independently recorded from day 2 to day 30 post-exposure.

### Table 1. Experimental groups

| Exposure                | Whole-body dose (Gy) | Number of rats |
|-------------------------|----------------------|----------------|
| Experiment 1 (Jun 2014) |                      |                |
| $^{56}$MnO$_2$          | 0.15                 | 4              |
| $^{55}$MnO$_2$          | 0.0                  | 4              |
| $^{60}$Co-γ             | 2.0                  | 4              |
| Control                 | 0.0                  | 4              |
| Experiment 2 (Apr 2015) |                      |                |
| $^{56}$MnO$_2$          | 0.02                 | 4              |
| $^{55}$MnO$_2$          | 0.0                  | 4              |
| $^{60}$Co-γ             | 2.0                  | 4              |
| Control                 | 0.0                  | 4              |
| Experiment 3 (Feb 2016) |                      |                |
| $^{56}$MnO$_2$-H        | 0.15                 | 3              |
| $^{56}$MnO$_2$-L        | 0.11                 | 2              |
| $^{60}$Co               | 2.0                  | 4              |
| Control                 | 0.0                  | 2              |
| Experiment 4 (Aug 2016) |                      |                |
| $^{60}$Co-γ-2           | 2.0                  | 4              |
| $^{60}$Co-γ-3.5         | 3.5                  | 4              |
| $^{60}$Co-γ-5           | 5.0                  | 4              |
| Control                 | 0.0                  | 4              |
Statistical analyses

Elapsed time trend of daily activities

Daily activity was defined by the cumulative number of movements of the rat between 18:00 to 6:00 as previously described [26]. Then, we conducted a statistical analysis by merging all the data in the four experiments. Regardless of their radiation doses, all the data of the $^{55}$MnO$_2$ groups and the $^{60}$Co-$\gamma$ groups were pooled and designated as $^{55}$MnO$_2$ and $^{60}$Co, respectively. A linear mixed model was applied [25], where individual differences in the rats and daily environmental changes were treated as random effects, while differences in experimental setups and exposure types were treated as fixed effects. The linear mixed model is expressed as follows:

$$ Y_{it}^{(k)} = \mu + \beta_1^{(k)} \alpha(t) + \gamma \Theta + \delta_i^{(k)} + \eta_i^{(k)} + \epsilon_{it}^{(k)}, $$

where $\mu$ is the intercept, $\beta_1^{(k)}, \beta_2^{(k)}, \beta_3^{(k)},$ and $\beta_4^{(k)}$ denote unknown parameters showing log-activity of $^{55}$MnO$_2$, $^{55}$MnO$_2$, and $^{60}$Co groups against the control group at the elapsed time $t \in \{-1, 0, 1, \ldots, 30\}$, respectively (note that $\beta_1^{(k)} \equiv 0$ is an offset variable). The term $\mu$ denotes the log-transformed mean of the activities of rats belonging to the control group in Experiment 1. Terms $\alpha(t)$ denote unknown parameters to be estimated showing the difference of the mean of the experiments against the first experiment, respectively (note that $\alpha(t) \equiv 0$ is an offset variable), which are treated as fixed effects. Terms $\delta_i^{(k)}, \eta_i^{(k)},$ and $\epsilon_{it}^{(k)} (i = 1, \ldots, 4, k = 1, 4 ; t = 1, 4, t = -1, 0,1, \ldots, 30$) are random terms having independent normal distributions with mean zero and variance. Terms $\Psi^2, \Phi^2,$ and $\sigma^2$ represent individual differences in rats, daily fluctuations, and measurement errors, respectively.

Trends of daily activities in three stages

In order to characterize time-dependent change in each exposure group, the days elapsed from the day of exposure were divided into three stages: an early stage (from the day of exposure to 12 days), a middle stage (13 days to 24 days), and a late stage (after 25 days, in which the effects of exposure were considered to disappear). A time $t$ in days after exposure is expressed with two dummy variables having inclusive relationship as shown below:

$$ I_1(t) = \begin{cases} 1 & (t \in [0, 24]) \\ 0 & (\text{otherwise}) \end{cases}, \quad I_2(t) = \begin{cases} 1 & (t \in [13, 24]) \\ 0 & (\text{otherwise}) \end{cases}. $$

Summarizing the daily activities into these three stages, model (1) is rewritten as follows:

$$ Y_{it}^{(k)} = \mu + \gamma^{(0)} I_1(t) + \gamma^{(1)} I_2(t) + \alpha^{(k)} + \delta_i^{(k)} + \eta_i^{(k)} + \epsilon_{it}^{(k)}, $$

where $\mu + \gamma^{(0)}, \gamma^{(1)},$ and $\alpha^{(k)}$ are fixed effects parameters to be estimated ($\gamma^{(0)} = \gamma^{(1)} = \alpha^{(k)} \equiv 0$ are offset variables) and $\delta_i^{(k)}, \eta_i^{(k)},$ and $\epsilon_{it}^{(k)}$ are random terms defined as in model (1). Thus, the magnitude of activities in each of the three stages by each exposure group ($^{55}$MnO$_2$, $^{55}$MnO$_2$, $^{60}$Co, and control group) were obtained as terms of fixed effects in Model [2].

Trends of daily activities using optimal model

For each exposure type, the optimal model representing the time dependency of behavioral changes due to exposure was obtained by selection variables in the model [2] using AIC criterion [28].

RESULTS

Daily activities in individual rat

The observed daily activities in log transformed value by an individual rat at an elapsed time from the day of exposure by control, $^{55}$MnO$_2$, $^{55}$MnO$_2$, and $^{60}$Co groups in experiments 1–4 are illustrated in Figure 1. Figure 2 shows the estimated mean trend of daily activities by $^{55}$MnO$_2$, $^{55}$MnO$_2$, and $^{60}$Co groups, which is the result of analysis using model [1]. The locomotor activity in the $^{60}$Co group decreased early after exposure, while the decrease appeared at the later stage in the $^{55}$MnO$_2$ group.

Further, the analysis shows that the estimated variance of random effects due to individual differences, daily variation, and measurement error random effect parameters were ($\Psi^2, \Phi^2, \sigma^2$) = (0.0030, 0.0068, 0.0044), which account for 21%, 48%, and 31% of the total variance, respectively.

Activities by exposure groups in 3 divided elapsed days

Using the mixed effect Model [2], we obtained the estimates of the locomotor activity by exposure groups in 3 divided elapsed days as fixed effect parameters. Table 2 shows the locomotor activities increased 32% (= $e^{0.277}$), 50% (= $e^{0.433}$), 56% (= $e^{0.687}$) in Experiment 2, Experiment 3, and Experiment 4 compared with that of Experiment 1, respectively. The estimates for fixed effects other than between-experiment differences are visualized in Figure 3. This figure shows the estimated locomotor activities in the early stage (from the day of exposure to 12 days) and in the middle stage (from 13 days to 24 days) with their 95% confidence intervals, where the horizontal dotted line indicates the mean locomotor activities in the late stage (after 25 days, in which the effects of exposure were considered to disappear).

Figure 3 shows that the estimated locomotor activities of the $^{55}$MnO$_2$ group and the $^{60}$Co group in the early and middle stage were significantly lower than those in their original state, while the estimated locomotor activity of $^{55}$MnO$_2$ group in each stage was not significantly lower than that in their original state. The estimated locomotor activities of the $^{55}$MnO$_2$ group in the middle stage was lower than those in the early stage, while the estimated locomotor activities of the $^{60}$Co group in the middle stage were significantly higher than those in the early stage ($P$ value = 0.04).

Further, this analysis shows that the variance of the estimated random effects is due to individual differences, diurnal variation, and measurement error($\Psi^2, \Phi^2, \sigma^2$) = (0.0032, 0.0065, 0.0045), which accounted for 23%, 46%, and 32% of the total variance, respectively, and all of them show random variation of substantial magnitude.
Fig. 1. Changes in daily locomotor activity of each rat in experiments 1–4. The logarithm of daily activity (Y-axis) was plotted against days after exposure. Doses shown in Gy in the notations above each figure refer to the whole-body doses of $^{60}$Co and $^{64}$MnO$_2$ in Table 1.
Table 2. Estimated fixed effects parameters with 95% confidence intervals

| Parameter | Explanation | Estimate | 95%LCB | 95%UCB | P-value |
|-----------|-------------|----------|--------|--------|---------|
| $\mu$     | Intercept   | 3.883    | 3.836  | 3.929  | 0.000***|
| $\alpha^{(1)}$ | Exp1       | 0 (ref)  | -      | -      |        |
| $\alpha^{(2)}$ | Exp2 (vs. Exp1) | 0.277    | 0.212  | 0.342  | 0.000***|
| $\alpha^{(3)}$ | Exp3 (vs. Exp1) | 0.410    | 0.341  | 0.480  | 0.000***|
| $\alpha^{(4)}$ | Exp4 (vs. Exp1) | 0.443    | 0.379  | 0.507  | 0.000***|
| $\gamma^{(1)}$ | 1st stage in Control | 0 (ref)  | -      | -      |        |
| $\gamma^{(2)}$ | 1st stage in $^{56}$MnO$_2$ | -0.043   | -0.069 | -0.017 | 0.001**|
| $\gamma^{(3)}$ | 1st stage in $^{55}$MnO$_2$ | 0.031    | -0.005 | 0.067  | 0.091  |
| $\gamma^{(4)}$ | 1st stage in $^{60}$Co | -0.056   | -0.076 | -0.037 | 0.000***|
| $\zeta^{(1)}$ | 2nd stage in Control | 0 (ref)  | -      | -      |        |
| $\zeta^{(2)}$ | 2nd stage in $^{56}$MnO$_2$ | -0.059   | -0.084 | -0.034 | 0.000***|
| $\zeta^{(3)}$ | 2nd stage in $^{55}$MnO$_2$ | 0.018    | -0.016 | 0.053  | 0.299  |
| $\zeta^{(4)}$ | 2nd stage in $^{60}$Co | -0.021   | -0.040 | -0.001 | 0.038*  |

***P < 0.001, **P < 0.01, *0.01 ≤ P < 0.005

**Time dependency of each exposure using optimal model**

Time dependency of rats' locomotor activities by each exposure using the optimal model obtained by selection variables is shown in Figure 4, which is a summary of Figure 3. It was suggested that the exposure of $^{56}$MnO$_2$ did not affect rats' activities, that of $^{55}$MnO$_2$ decreased rats' activities in the early and middle stages, and that of $^{60}$Co decreased rat' activities in the early stage, but recovered to its original state in the middle stage.

**DISCUSSION**

The external dose of the residual radiation from Hiroshima A-bombing was estimated to be less than 30 mGy, which has been regarded as a negligible level [29, 30]. The biological effects of internal exposure could be significant but have not been investigated yet. The inverse square law of distance increases the exposure dose in the vicinity of radioactive particles, and when radioactive particles adhere to the cells of living organisms, there
The analysis found a significant impact of $^{56}$MnO$_2$ on the animal’s activity, even though the highest whole-body dose was 0.15 Gy. The locomotor activity gradually slowed for around 2 weeks after exposure to $^{56}$MnO$_2$, while it immediately decreased after a $^{60}$Co-irradiation. The latter is consistent with a previous finding in mice exposed to $^{60}$Co-γ rays, showing a decline of locomotor activity 6 hours post exposure [18]. Our results suggest that internal irradiation with $^{56}$Mn induces a long-lasting reduction in activity (i.e. fatigue) after irradiation.

CONCLUSION
Our statistical analysis of the spontaneous locomotor activity of rats demonstrates that internal exposure to $^{56}$MnO$_2$ powder significantly reduces locomotor activity for more than 2 weeks at less than 0.15 Gy of the whole-body doses. The duration of the effect of irradiation with $^{56}$MnO$_2$ is longer than that of external irradiation, suggesting that the effect of internal irradiation with radioactive particles is long-lasting.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

FUNDING
This work was supported by JSPS KAKENHI Grant Numbers 26257501 (April 2014–March 2018) and 19H01149 (April 2019–March 2023).

SUPPLEMENT FUNDING
This work was supported by JSPS KAKENHI Grant Number JP19H01149.

REFERENCES
1. Imanaka T, Endo S, Tanaka K et al. Gamma-ray exposure from neutron-induced radionuclides in soil in Hiroshima and Nagasaki based on DS02 calculations. Radiat Environ Biophys 2008;47:331–6.
2. Kerr GD, Egbert SD, 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.
3. Roesch C (ed). US-Japan Joint Reassessment of Atomic Bomb Radiation Dosimetry in Hiroshima and Nagasaki Final Report – Dosimetry System 1986 (DS86). Hiroshima: Radiation Effects Research Foundation, 1987.
4. Young R, Kerr G. Reassessment of the atomic bomb radiation dosimetry for Hiroshima and Nagasaki – dosimetry system 2002 (DS02). Radiation Effects Research Foundation 2002.
5. 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.
6. Sutou S. Rediscovery of an old article reporting that the area around the epicenter in Hiroshima was heavily contaminated with residual radiation, indicating that exposure doses of A-bomb survivors were largely underestimated. J Radiat Res 2017;58:745–54.
7. Matsuura M, Hayakawa N, Shimokata H. Survival analysis of atomic bomb survivors in Hiroshima prefecture, Japan, 1962-1982. – kasr mortality risk among early entrants. *J Hiroshima Medical Association* 1995;44:29–38.

8. Otani K, Tonda T, Satoh K et al. Mortality risk analysis among early entrants in Hiroshima. *Nagasaki Medical Journal* 2012;87:261–4 (in Japanese).

9. ICRP. *Dose Coefficients for Intakes of Radionuclides by Workers; ICRP Publication 68; Annalsof the ICRP 24*. UK: Elsevier Science Ltd. Oxford OX5 1GB, 1994, ISSN 0146-6453.

10. Ishigure N. Doses used for internal exposure protection. *Radioisotopes* 2013;62:465–92.

11. Shizuma K, Iwatani K, Hasai H et al. Fallout in the Hypocenter area of the Hiroshima atomic bomb. *Health Phys* 1989;57:1013–6.

12. Hoshi M. The overview of the effects neutron-induced $^{56}$Mn radioactive microparticles in experimental animals and related studies. *this issue* 2021.

13. Otani K, Ohtaki M, Yasuda H et al. Solid cancer mortality risk among a cohort of Hiroshima early entrants after the atomic bombing, 1970-2010: implications regarding health effects of residual radiation. *this issue* 2021.

14. Christensen DM, Iddins CJ, Parrillo SJ et al. Management of ionizing radiation injuries and illnesses, part 4: acute radiation syndrome. *J Am Osteopath Assoc* 2014;114:702–11.

15. Daino K, Ichimura S, Nenoi M. Early induction of CDKN1A (p21) and GADD45 mRNA by a low dose of ionizing radiation is due to their dose-dependent post-transcriptional regulation. *Radiat Res* 2002;157:478–82.

16. Semenova Y, Pivina L, Manatova A et al. Mental distress in the rural Kazakhstani population exposed and non-exposed to radiation from the Semipalatinsk nuclear test site. *J Environ Radioact* 2019;203:39–47.

17. Yamada M, Izumi S. Psychiatric sequelae in atomic bomb survivors in Hiroshima and Nagasaki two decades after the explosions. *Soc Psychiatry Psychiatr Epidemiol* 2002;37:409–15.

18. Landauer MR. Radiation-induced performance decrement. *Mil Med* 2002;167:128–30.

19. York JM, Blevins NA, Meling DD et al. The biobehavioral and neuroimmune impact of low-dose ionizing radiation. *Brain Behav Immun* 2012;26:218–27.

20. 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.

21. Fujimoto N, Baurzhan A, Chaizhunussova N et al. Effects of internal exposure to $^{56}$MnO$_2$ powder on blood parameters in rats. *Eur Asian J Med* 2020;52:52–6.

22. 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;11:1–8.

23. Kaikhanova Y, Saimova A, Uzbekov D et al. Effects of exposure to radioactive $^{56}$MnO$_2$ powder on hyaluronan synthase 2 in the lungs of rats. *Georgian Med News* 2017;9:120–4.

24. 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.

25. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and S-PLUS*, 2nd edn. New York: Springer, 2000, 1–197.

26. Otani K, Ohtaki M, Fujimoto N et al. Quantitative analysis of effects of a single60 co sublethal point exposure on time-dependent change in locomotor activity in rats. *Int J Environ Res Public Health* 2020;17:1–11.

27. 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* 2020;56:47–54.

28. Akaike H. *Information Theory and an Extension of the Maximum Likelihood Principle. Proceedings of the 2nd International Symposium on Information Theory*, Petrov, B. N., and Caski, F. (eds.), Akadimiai Kiado, Budapest. 1973;267–281.

29. Atomic Bomb Casualty Commission. Mortality and radiation dose, October 1950-September. *Life Span study Report* 1966;5:61–5.

30. Radiation Effect Research Foundation. Mortality from causes other than cancer among atomic bomb survivors. *Life Span Study Report* 1981;9:1950–78.

31. Stepanenko V, Kaprin A, Ivanov S et al. Microdistribution of internal dose in biological tissue exposed by neutron activated $^{56}$Mn. *this issue* 2021.