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Elimination Percentage and Dose Load from Radioisotope on Critical Organs of Laboratory Animals when using Sorbents

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ABSTRACT

One of the most urgent problems of modern medicine and veterinary medicine is the search for effective methods of prevention and nosotropic therapy for radiation damage to humans and animals. The first stage was carried out on 30 laboratory mice. According to hematological studies of animal blood, all blood parameters were within the normal range. The second stage included the evaluation of the effectiveness of sorbents in rats by determining the percentage of radioisotope elimination from the animal body when using the sorbent and determining the dose load from the radioisotope over time (after 5, 15, 30, and 60min) on critical organs (gastrointestinal tract) and its elimination from the rat body. In one group, the rats were monitored without using the sorbent enriched with Shungite and bentonite. In contrast, the other group consisted of rats that received the sorbent with the feed (administered twice with an interval of 8-12 hours). The difference in the accumulation of radiopharmaceutical preparations in the area of interest (gastrointestinal tract) between the two groups was 4.48% after 5min (5min), 1.39% after 15min (20min), 1.35% after 30min (50min), and 1.04% after 60min (110min). The study revealed significantly higher initial RPP accumulation in the GI tract of the first group (4.48% at 5min), with differences gradually decreasing to 1.04% by 60min, suggesting variations in absorption/metabolism between groups. Shungite-enriched bentonite demonstrated high efficacy as a radioprotective sorbent, reducing radionuclide-induced damage when administered twice at 8–12-hour intervals and maintaining chemical stability. Its use enhanced adaptive regulatory mechanisms for accelerated radionuclide elimination, lowering risks of radiation-induced pathologies.

Key words: Radioisotope, Animals, Radioactive contamination, Veterinary medicine, Sorbent.

INTRODUCTION

Radiological contamination, whether caused by nuclear accidents, industrial emissions, or medical applications, poses a significant threat to human and animal health. The accumulation of radionuclides in living organisms can lead to long-term health complications (Stawkowski 2020; Hachinohe et al. 2021; Sarsembayeva et al. 2021; Lundquist et al. 2023).

In addition to natural background radiation, animals can be exposed to radiation as a result of accidents at nuclear power plants, medical diagnostics/treatment and human activities. With the development of the nuclear industry and the increasing use of radioactive materials, the concentration of various pollutants, including radioactive substances, such as uranium, cadmium, cesium, and cobalt, in the environment has increased over the past few decades. These radioactive materials eventually circulate in the biosphere and enter the air, drinking water, vegetables and grasses. Countermeasures to reduce radionuclide contamination of food animals and their products usually include cultivating land used for growing feed crops or grazing livestock, changes in animal husbandry, administering adhesive agents or similar substances to animals and delayed slaughter of animals (Howard 2021; Rosnovskaya et al. 2023; Kashparov et al. 2024; Zhang et al. 2024). Options that can be implemented in animal husbandry systems include permanent placement and provision of uncontaminated feed (especially in situations of exposure after a nuclear accident), pasture management (for example, cultivation of forage species with low radionuclide absorption potential), and selective grazing (Chen 2023; With et al. 2023; Hatvani-Nagy et al. 2024; Sweeck et al. 2024).

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Environmental protection from radiation depends primarily on the dose assessment for non-human biota. Most of these dose estimates combine measured or predicted radionuclide concentrations in soil or water with concentration ratios (CR) to estimate concentrations in animals and plants (Goulet et al. 2022; George et al. 2024). However, CR data is scarce compared to the many potential taxa and radioactive pollutants in the environment and their taxon-specific ecosystems. Because there are many taxa, each with different behaviors and biology, and because there are many potentially bioavailable radionuclides, CR can vary by orders of magnitude, as is often observed in published data. Given the diversity of taxa, the International Commission on Radiological Protection (ICRP) has selected 12 species of non-human biota as reference animals and plants (RAP), while the U.S. Department of Energy (DOE) uses non-specific taxonomic categories of terrestrial, coastal, and aquatic animals. Part of the question we are considering here is whether these RAP and categories are sufficient to adequately protect all species, given the diversity of animals in the region. To investigate this issue, we use the allometric kinetic (AK) model to calculate the CR of radionuclides for general classes of animals, further divided into herbivorous, omnivorous, carnivorous and invertebrate detritivores (Kryshev et al. 2022; Kryshev et al. 2023; Puchkov et al. 2023; Whicker et al. 2023). CR comparisons between animal classes showed only minor differences, but differences of several orders of magnitude were noted between herbivores, carnivores, and detritivores for many radionuclides of particular interest. These results show that the ICRP, RAP and DOE categories are appropriate. However, their accuracy can be improved by including subcategories related to animal ecology and food biology. Finally, comparing the CR predicted by the AK model with the published CR shows differences in the order of magnitude, justifying further studies of assimilation of radionuclides in animals, environmental conditions, and animal classes (Guillén et al. 2020; Kundu et al. 2024; Zheng et al. 2024).

The study aims to evaluate the potential of natural sorbents, in particular bentonite and Shungite, as effective means to reduce radionuclide contamination of animals.

Bentonite clay is widely known in gamma radiation protection because it is a natural, cheap, readily available, common material with excellent protective properties. Bentonite clay was thermally activated at 900, 1,000, and 1,100°C to obtain ceramic properties with the desired porosity. Heat treatment of bentonite clay leads to weight loss due to dehydration processes. The elemental composition of burnt bentonite ceramic samples was determined using X-ray fluorescence. The structure of bentonite ceramic samples was characterized by X-ray diffraction, particle size determination and density measurement, and scanning electron microscopy clarified the morphology of the sample surface; gamma radiation shielding measurements of various sintered bentonite ceramic samples were investigated using a sodium iodide detector and narrow beam method, as well as two point sources (Cs-137 and Co-60) emitting at 662, 1,173, and 1,332 keV (Kerr et al. 2015; Hoshi 2022; Alafer et al. 2024; Stojković et al. 2024). Shungite is a filler for composite materials. It has a natural composition of carbon nanoparticles with various micro and nanoscalemineral additives that give it high permeability and shielding properties of electromagnetic radars. The radar absorption and shielding characteristics of a composite material based on fine-grained Shungite and urea-formaldehyde resin were studied in the frequency range from 500 MHz to 4 GHz. The effect of the sample thickness on the electromagnetic properties of the composition material under study was determined (Tanaka et al. 2008; Stepanenko et al. 2017).

In Japan, the genetic effects and radioactive contamination of large mammals, including wild boar, were studied due to the release of radionuclides at the Fukushima-1 nuclear power plant in 2011. Such studies have generally shown a downward trend in the measured amount of radiocesium and stress on the body in nature. Assessing the effects of radiation on wildlife is important to understand the potential long-term effects. The radiation exposure of 307 wild boars living in radioactively contaminated areas (50-8,000 kBq m-2) in Fukushima Prefecture from 2016 to 2019 was assessed here, and genetic markers were examined to assess possible germline mutations caused by multiple chronic radiation exposures. Naturally occurring concentrations of cesium activity in wild boars (Stepanenko et al. 2020; Hoshi 2021) remained high in the areas surrounding the power plant, with a peak concentration of 54 kBq/kg measured in 2019. The total dose rate for wild boars ranged from 0.02 to 36 micrograms/hour, mainly due to external radiation. The radiation exposure and dose rate in the maximally irradiated animals exceeded the total non-irradiated control level by 10mcg/h. Depending on the estimated age of each animal, lifetime radiation doses ranged from <0.1 to 700mGy. Despite chronic exposure, genetic analysis did not show a significant accumulation of mutations (Shichijo et al. 2020; Fujimoto et al. 2021; Otani et al. 2022). Since wild boar is not commonly found in the human diet in Japan, an effective dose for humans from contaminated wild boar meat consumption was calculated. With a per capita pork consumption of 12.9kg/year, the hypothetical consumption of contaminated wild boar meat in the contaminated areas of Fukushima will result in an average annual effective dose of 0.9mSv/year, which is below the annual oral dose limit of 1mSv/year-1. Furthermore, the consumption of the most contaminated meat in this study, about 1.4kg/year, does not exceed the annual consumption limit (Fujimoto et al. 2020a, 2020b; Ruslanova et al. 2021). The dynamics of behavioral parameters of male rats were studied in an "open field" test. Total gamma radiation (absorbed dose of 5Gy) decreased all types of motor and exploratory activity and increased emotional distress. Treatment of animals with chitin/protein and carotene-tocopherol complexes (0.35 and 0.6.67g/kg, respectively) before and after irradiation, due to the antioxidant and enterosorbent properties of these complexes, significantly mitigated the negative effects of radiation and provided a clear trend towards normalization of measured behavioral parameters (with a follow-up period of 30 days) (Stepanenko et al. 2022).

MATERIALS AND METHODS

Research location and ethical authorization

The study was conducted at the Kazakh National

Agrarian Research University in the laboratory of the Department of Veterinary Sanitation and at the laboratory of the Kazakh Research Institute of Oncology and Radiology. The Bioethics Commission of the Kazakh National Agrarian Research University on November 10, 2022, discussed and approved the research methods. Manipulations with experimental animals were carried out following the provisions of the Helsinki Declaration on Humane Treatment of Animals (Guiding Principles for Research, 2002).

Experiment design

Experiment 1: Assessing the Impact of Sorbents on General Health, Weight Changes and Hematological Parameters in Mice

For the experiment, two groups of 30 mice identical in age and weight were formed, where one group was selected as the control group.

The animals were kept in the vivarium of the Department of Biological Safety of the Veterinary Faculty of the Kazakh National Agrarian Research University at 20-22°C, humidity no more than 50%, in standard plastic cages with fine wood shavings. Keeping and feeding were carried out according to the Rules for working with experimental animals. After admission and a two-week medical examination, they were included in the experiment. During the experiment, the animals were kept in standard conditions (vivarium, recommended diet, free access to drinking water, and natural light). Several experiments were conducted to study radiosorbents designed to remove radionuclides from the animal body. The first stage was carried out on laboratory mice.

Each group was given a specific sorbent. The control group included mice kept under the same conditions, but not receiving the sorbent. The sorbent (12g/1kg of feed) was administered daily according to the schedule in Table 1.

The animals were given two doses of stable sorbent, for example, in the morning and evening, with an interval of 12 hours between doses. The first and second portions of feed contained 6g of sorbent.

Shungite (33.2kg), bentonite (14.2kg) and sucrose (2.6kg) were thoroughly mixed in a mixer. The resulting powder was fed to animals in a dose of 6g per 1kg of feed.

Blood was taken from mice for hematological analysis to study the toxic effects of sorbents on the body. A general blood test allows one to identify the development of an infection in the body and determine a metabolic disorder or a malfunction of the internal organs of the animal.

During the experiment, the following parameters were measured: white blood cells (WBC), eosinophils (Eo.), basophils (Bas.), red blood cells (RBC), platelets (PLT), hemoglobin (HGB), hematocrit (HCT), plateletcrit (PCT), as well as the following RBC parameters: mean corpuscular volume (MCV), mean corpuscular HGB (MCH), mean corpuscular HGB concentration (MCHC), etc.

Calculated parameters were lymphocytes (LYM) %, monocytes (MON) %, neutrophils (NEU) %, Bas. %, Eo. %, etc. The analysis was performed on an automatic MS 4/5 hematology analyzer (France).

Experiment 2: Evaluating Sorbent's Effectiveness in Reducing Radionuclide Accumulation in the Gastrointestinal (GI) Tract and the Whole Body.

To substantiate the effectiveness of the preparation as a sorbent to reduce the content of radionuclides in the animal body, a series of experiments on rats using radioactive rays was performed.

The experimental study was conducted in the Laboratory of Radionuclide Diagnostics of the Department of Radiology and Nuclear Medicine of the Kazakh Scientific Research Institute of Oncology and Radiology.

16 Wistar rats were used for the study. The subjects were divided in advance into two groups of eight animals, depending on the addition of the sorbent to the food, for further study of its role in reducing the radioactivity of the preparation Technetium-99m: 169-485g. Each laboratory animal was weighed on an OHAUS laboratory scale, and the animal weight data is shown in Table 3.

One group of rats was monitored without receiving the sorbent, while the other group was given the sorbent as part of their feed. The rats received feed with bentonite in powder form enriched with Shungite (2/1), with a dose of 6g per 1kg of feed. They were fed twice with an 8-12-hour interval, during the period of immediate threat of radionuclide damage, with the following ratio of components by weight/%: Shungite: 59, starch: 10, bentonite: 30, purified water: 1.

The main objective of the experiment was to determine the percentage of radioisotope elimination from the animal body using various sorbents and to determine the dose load from the radioisotope over time (after 5, 15, 30, and 60min) on critical organs (the GI tract) and its elimination from the rat body as a whole using sorbents.

The preparation is characterized by high chemical stability during storage, which increases the effectiveness of radionuclide damage prevention, expands the range of preventive measures, and reduces the level of pathologies caused by exposure to high doses of the technetium isotope.

The rats were fixed with soft bandages on a wooden plate. Technetium-99m (^{99m}Tc), a daughter product of the beta disintegration of the radioisotope molybdenum–99 (^{99}Mo), was used as a radionuclide isotope. To produce ^{99m}Tc, a transported ^{99}Mo/^{99m}Tc gel generator is used in medical laboratories. The device is "charged" with highly active ^{99} Mo, produced at a pressurized water reactor (PWR) at the Institute of Nuclear Physics (INP) (Almaty) using a nuclear neutron capture reaction with a stable isotope of molybdenum-98 (^{98}Mo). A radiopharmaceutical preparation (RPP), sodium pertechnetate ^{99m}Tc, was obtained in KazNIIOiR JSC using a ^{99}Mo/^{99m}Tc generator by elution with 0.9% sodium chloride (NaCl) solution. The half-life of ^{99m}Tc is 6.04 hours. During disintegration, gamma rays with an energy of 140keV are emitted with a yield of 90%.

Table 1: Experiment design (sorbent intake)

No.	Number of mice	Sorbent was administered twice a day, in the morning and evening, with an interval of 12 hours between doses
1	15	Control group
2	5	Bentonite was administered daily from the second day of the experiment
3	10	Bentonite and Shungite were administered daily from the second day of the experiment.

Table 2: Biologically active feed additive in the form of a powder of the following composition

Item No.	Ingredients	Content, g	Content, %
1	Shungite	33.2	68
2	Bentonite	14.2	26.3
3	Sucrose	2.6	5.7

Table 3: Data from the studied rats

No.	Group	Identification of the subject	Weight (grams)
1	1	1–1	350
2	1	1–2	485
3	1	1–3	354
4	1	1–4	396
5	1	1–5	294
6	1	1–6	238
7	1	1–7	358
8	1	1–8	335
9	2	2–1	349
10	2	2–2	369
11	2	2–3	226
12	2	2–4	389
13	2	2–5	277
14	2	2–6	213
15	2	2–7	306
16	2	2–8	169

The activity of the administered dose of ^{99m}Tc in the body depends on the type of study, in our case (a study of the entire body of the animals) it was equivalent for humans, based on the animal's body weight. The activity of the administered dose was measured using a Capintec CRC-15R ionization chamber (Fig. 1).

Fig. 1: Exterior view of the Capintec CRC-15R ionization chamber.

Next, a syringe with a calculated dose of radiopharmaceutical preparation (RPP) for each animal was scanned on a gamma camera, after which the prepared dose was administered to the laboratory animals. After administration, the syringe with the remaining amount of RPP was re-scanned on a gamma camera to determine the percentage of RPP directly injected into the animal, and the residual activity in the syringe was re-measured using the Capintec CRC-15R ionization chamber. These actions make it possible to automatically calculate the percentage accumulation of RPP in the body of a laboratory animal. The diagnostic dose of RPP was 6-8MBq of activity per 1kg of animal body weight. The correspondence of RPP activity to the effective equivalent dose (EED) is shown in Table 4.

Table 2 shows that the range of the introduced RPP activity was from 1.3 to 3.4MBq. This variation in the administered dose of RPP is explained by the difference in the weight of the animals (from 169 to 485g). The obtained EED was calculated based on the fact that 1MBq equals 0.013mSv.

Table 4: Indicators of the introduced activity of RPP and EED in animals

Identification of the subject	Introduced RPP activity (MBq)	EED (mSv)
1–1	2.8	0.036
1–2	3.4	0.044
1–3	2.7	0.035
1–4	2.8	0.036
1–5	1.9	0.025
1–6	1.68	0.022
1–7	2.63	0.034
1–8	2.5	0.033
2–1	2.66	0.035
2–2	2.54	0.033
2–3	1.6	0.021
2–4	2.91	0.038
2–5	2	0.026
2–6	1.7	0.022
2–7	2.3	0.030
2–8	1.3	0.017

RPP was injected into the caudal vein of rats with preheating of the tail in a water bath (Fig. 2). The accumulation of ^{99m}Tc in the subjects' bodies was visualized using a Philips Forte single-photon emission computed tomography scanner (SPECT) (Fig. 3) in planar mode using a LEGP collimator (Fig. 4).

Fig. 2: The RPP introduction process.

Fig. 3: Philips Forte SPECT.

Fig. 4: The research process.

RESULTS

According to the results of the weekly weighing of mice from experimental group 1 and control group 2, a difference in weight was observed. On the 7th day, the experimental group gained an average of 20% in weight. Mice that were kept under the same conditions but didn't receive the sorbent showed no significant changes in weight. The results of the experiment on mice are shown in Table 5 and 6.

According to the results of the weekly weighing of mice from experimental group 1 and control group 2, a difference in weight was observed. On the 7th day, the experimental group gained an average of 20% in weight. Mice kept under the same conditions but not receiving the sorbent did not show change in weight.

According to the results of an external measurement of body weight, mice in group 1 that received sorbent exceeded the mice from group 2 by 15% in terms of weight. Hematological analysis of the control group showed the following: WBC, n/mm3: 8.2; LYM, %: 73.3; MON, %: 12.1; RBC, n/mm3: 8.0; HGB, g/l: 125; PLT, n/mm3: 272. Hematological analysis of the experimental group showed the following: WBC, n/mm3: 8.3; LYM, %: 73.5; MON, % -: 3.9; RBC, n/mm3: 9.6; HGB, g/l: 165.7; PLT, n/mm3: 295. According to hematological studies of animal blood, all blood parameters were within the normal range.

The results of the experiment in the second stage are an assessment of the effectiveness of sorbents in rats. The received data was processed using JETStream Workspace 3.0 software and the Thyroid analytic processing tool. To determine the accumulation of RPP, planar scintigrams of each rat were performed 4 times, with an interval of +5min, +15min, +30min, +1 hour from the moment of RPP administration (that is, 5min, 20min, 50min, 110min after administration). The measurement data of each animal is presented on an individual registration card.

As a result of processing the scintigram data of each animal, the percentage of accumulation of ^{99m}Tc, depending on time, was demonstrated throughout the body and separately in the GI tract of the subjects (Table 7).

It follows from Table 3 that the change in the accumulation of RPP in animals over time from the moment of its introduction naturally changes, gradually accumulating more in the GI tract. A graphical representation of the average accumulation value of ^{99m}Tc as a function of time and organ is shown in Fig. 5 and 6.

Due to the movement of the limbs and tail of the studied rats and extravasation of blood vessels during the administration of RPP, the obtained data may contain errors of up to 5% in terms of the total accumulation level, while the accumulation level in the GI tract (ROI1) has a measurement error of only 0.2%.

Table 5: Results of weekly weighing of the mice from experimental group 1 and control group 2

Mouse number	Control group 2 before the experiment	Control group 2 after the experiment	Experimental group 1 before the experiment	Experimental group 1 after the experiment
1	26.4	26.5	27.2	30.1
2	25.1	25.3	28.5	33.2
3	27.1	27.2	26.5	32.4
4	26.2	26.3	29.5	34.2
5	25.3	25.1	26.5	33.5
6	26.4	26.3	31.4	32.1
7	27.2	27.3	26.8	30.4
8	29.3	29.3	29.2	31.6
9	28.6	28.6	25.2	34.3
10	26.4	26.5	26.7	28.6
11	27.4	27.3	28.2	33.2
12	28.2	28.2	27.6	29.3
13	29.2	29.2	28.5	30.3
14	24.2	24.5	26.3	28.9
15	27.3	27.4	29.3	30.5

Table 6: Studies of hematological parameters of blood by clinical analysis, n=15

Indicators	Standard	Control groups	Experimental groups
WBC, n/mm^3 White blood cells	6.0-13.0	8.3±0.02	8.28±0.01
LYM, % Lymphocytes	60 -78	77.2±0.01	73.5±0.02
MON, % Monocytes	2 - 5	12.0±0.03	3.8±0.03
NEU, % Neutrophils	0	1.7±0.02	0.08±0.01
Eo. Eosinophils, %	0 - 4	1.72±0.02	2.3±0.01
Bas. Basophils, %	0 - 2	0.5±0.01	1.27±0.02
RBC, n/mm^3 Red blood cells	8.0-11.0	7.9±0.03	9.57±0.01
MCV (mean corpuscular volume), FL	-	48.1±0.02	48.21±0.03
HCT, % Hematocrit	40 - 60	41.3±0.01	53.93±0.01
MCH (mean corpuscular HGB), pg	-	14.8±0.03	12.02±0.01
MCHC (mean corpuscular HGB concentration), g/dl	-	29.8±0.02	24.08±0.02
HGB, g/l Hemoglobin	140-180	125.8±0.02	165.7±0.01
PLT, n/mm^3 Platelets	200-400	277.3±0.01	256.9±0.02
MPV (mean PLT volume), FL	-	6.3±0.02	5.78±0.03
PCT, % Plateletcrit	0.4 - 0.8	0.154±0.01	0.56±0.01
PDW (PLT distribution width), fl	-	7.7±0.03	6.6±0.02

Table 7: Accumulation of RPP as a function of time

Identification of the subject	Percentage of RPP accumulation (%)							
	5min (5min)		15min (20min)		30min (50min)		60min (110min)	
	Body	GI tract	Body	GI tract	Body	GI tract	Body	GI tract
Group 1								
1–1	85±0.1	16.1±0.1	82.1±0.2	22.3±0.2	82.1±0.2	24±0.3	77.2±0.1	29.5±0.4
1–2	69±0.2	7.2±0.2	72.2±0.3	13.9±0.1	79.8±0.3	15.3±0.2	78.8±0.3	15.6±0.5
1–3	78.3±0.3	18.4±0.3	74.1±0.1	16.8±0.2	78±0.4	27±0.5	78.3±0.2	38.4±0.4
1–4	60.1±0.2	9.3±0.2	85.1±0.2	12.6±0.4	73.4±0.2	11.9±0.1	74.5±0.1	19.1±0.3
1–5	79.4±0.1	6.9±0.4	82.3±0.4	11.8±0.1	80.5±0.5	18.6±0.2	75.8±0.2	18.4±0.1
1–6	84.4±0.2	21.9±0.1	88.8±0.2	31.4±0.3	86.4±0.1	40.3±0.3	87.2±0.4	44.3±0.2
1–7	87.8±0.4	16.9±0.2	84.8±0.1	20.6±0.5	81.2±0.2	24.9±0.4	71.5±0.2	24.7±0.4
1–8	71.7±0.1	9.6±0.1	71.6±0.5	12.9±0.2	69.3±0.5	17.6±0.2	71.9±0.1	21.5±0.2
Average value	76.96±0.2	13.29±0.4	80.13±0.1	17.79±0.3	78.83±0.1	22.45±0.2	76.90±0.3	26.44±0.4
Group 2								
2–1	78.7±0.3	2.1±0.1	83.5±0.2	9.3±0.2	83.3±0.3	19±0.2	84.1±0.3	31.4±0.1
2–2	88.6±0.1	17.2±0.5	83.8±0.4	22.4±0.1	88.5±0.2	23±0.5	86.6±0.1	30.8±0.4
2–3	87.2±0.2	8.4±0.4	86.9±0.2	15.3±0.4	89.3±0.5	24.2±0.1	88.9±0.2	26.2±0.5
2–4	74.3±0.4	9.3±0.3	75.6±0.3	13.9±0.3	77.1±0.2	20.2±0.5	78.4±0.5	21.7±0.3
2–5	76.7±0.3	7.6±0.2	65.5±0.2	19.4±0.1	72.4±0.1	18.3±0.5	73.2±0.4	20.2±0.2
2–6	51.1±0.2	1.5±0.1	68.2±0.5	10.6±0.2	70.6±0.2	12.5±0.3	66.8±0.2	14.7±0.4
2–7	24.6±0.1	2.3±0.2	58.6±0.4	15.4±0.2	76±0.3	25.5±0.2	77.7±0.1	27.9±0.2
2–8	89.5±0.4	22.1±0.3	87.2±0.2	24.6±0.5	85.5±0.4	26.3±0.4	85.3±0.2	29.9±0.3
Average value	71.3±0.2	8.81±0.2	76.2±0.3	16.4±0.2	80.3±0.1	21.1±0.3	80.1±0.4	25.4±0.1

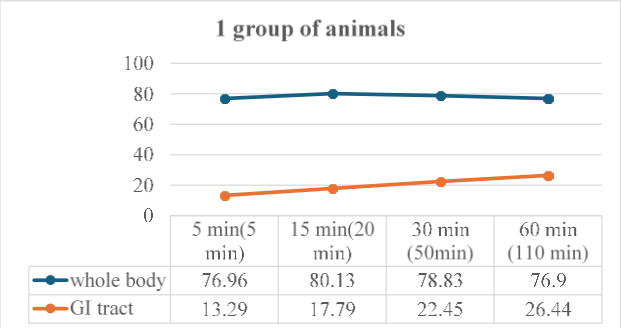

Fig. 5: Accumulation of ^{99m}Tc in group 1.

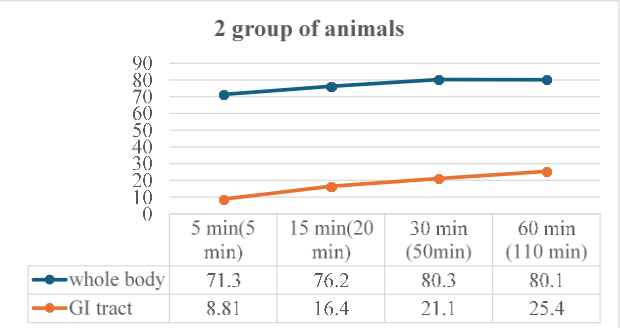

Fig. 6: Accumulation of ^{99m}Tc in group 2.

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Table 8: The EDR of the animals after the end of the study

Indicators	Individual EDR							
Group 1, identification of the subject by number								
Animal numbers	1–1	1–2	1–3	1–4	1–5	1–6	1–7	1–8
EDR (mSv/hr)	4	5	3	3	2	1.5	1.5	4
Group 2, identification of the subject by number								
Animal numbers	2–1	2–2	2–3	2–4	2–5	2–6	2–7	2–8
EDR (mSv/hr)	5	5	5	4	3	4	5	6

Table 9: Summary data for the study of groups 1 and 2, n=8

No.			Group 1	Group 2
Weight (grams)			351.3±0.1	287.3±0.2
Entered activity RPP (MBq)			2.55±0.2	2.13±0.1
EED (mSv)			0.033±0.2	0.028±0.1
Percentage of RPP accumulation (%)	5min (5min)	Body	76.96±0.1	71.3±0.2
		GI tract	13.29±0.2	8.81±0.2
	15min (20min)	Body	80.13±0.1	76.2±0.1
		GI tract	17.79±0.2	16.4±0.1
	30min (50min)	Body	78.83±0.3	80.3±0.2
		GI tract	22.45±0.2	21.1±0.1
	60min (110min)	Body	76.90±0.1	80.1±0.1
		GI tract	26.44±0.1	25.4±0.2
EDR (mSv/hr)			3±0.1	4.6±0.1

To further monitor the elimination of ^{99m}Tc after the last scan (scintigraphy), the equivalent dose rate (EDR) of each rat was also measured using aminirad-1000 dosimeter No. C002619. The measurement result was in the range of 2-6mSv/h (Table 8). All data on the subjects and the results are presented in Table 9.

The study showed differences in the accumulation of RPP between the two groups of test animals. In the first group, the percentage of accumulation throughout the body and in the GI tract increased over time, reaching a maximum of 30-60min after administration of the preparation. In the second group, similar dynamics were observed, but the accumulation values were slightly lower compared to the first group at all-time intervals, except at some measurement points. The difference in the accumulation of RPP in the area of interest (GI tract) between the two groups was 4.48% after 5min (5min), 1.39% after 15min (20min), 1.35% after 30min (50min), and 1.04% after 60min (110min). Thus, the measurement data in the first group demonstrated a higher overall level of RPP accumulation immediately after introducing RPP and the most rapid redistribution (accumulation) of RPP in the GI tract throughout the study period. These results indicate potential differences in the absorption or metabolism of the preparation in this organ between the two groups. Regarding the use of sorbents, combinations of the two components have obvious advantages over separate use, due to the specifics of their metabolism, and these advantages have a positive effect on the body's condition after radiation, which is especially important. Their combination forms the most effective sorbent for the absorption and elimination of isotopes.

DISCUSSION

The findings of this study demonstrate the efficacy of Shungite-enriched bentonite as a natural sorbent for counteracting radionuclide contamination in laboratory animals (*in vivo*) and potentially farm animals. This study aligns with recent trends focused on finding natural adsorbents for heavy metals and radionuclides (Novikau and Lujaniene 2022). In addition, several studies have highlighted the effectiveness of Shungite and bentonite as natural sorbents for absorbing radionuclides (Surkova et al. 2022; Muslim et al. 2022). In comparison to the study on saponite by Krykhtina et al. (2021), the Shungite-bentonite mix offers a more efficient means and requires a lesser dose. By comparing the elimination rates and dose load from the radioisotope on critical organs, this study provides substantial evidence that Shungite-based sorbents can reduce radioactive contamination more effectively than conventional methods. The findings on weight gain correspond with the results obtained in the studies of Wlazło et al. (2023) and Lykhach et al. (2022), where they observed average weight gain in farm animals after administering sorbents. The results indicate minimal to no variations in the recorded blood parameters, and this finding agrees with the works of Nowakowicz-Dębek et al. (2025) and Wlazło et al. (2023), who also recorded minimal favorable variations and no negative influence on hematologic characteristics when sorbents were integrated into feed systems. The comparative study of ^{99m}Tc uptake in the gastrointestinal (GI) tract of the two groups indicated that the participants who were administered the Shungite-rich bentonite sorbent exhibited decreased accumulation rates over time. The differences observed in accumulation percentages indicate that the sorbent plays a significant role in decreasing radionuclide retention in the organism. This is because bentonite and Shungite have high adsorption capacities and absorb radionuclides, promoting their elimination (Skrypnik et al. 2021; Marouf et al. 2021). The implications of these findings are beyond laboratory practices but possess potential for practical applications in environmental remediation, veterinary medicine, and human health protection. In the future, research studies should aim to optimize the Shungite-bentonite formula for peak adsorption efficiency safely and cost-effectively. Future studies should focus on examining the long-term biological consequences of sorbent administration, specifically under conditions of prolonged radiation exposure. Further research with various animal models and in conjunction with human trials would validate the broader applicability of these results. It is also necessary to understand the influence of external factors such as environmental conditions (temperature, humidity) and feed quality (nutrient composition, contamination level, etc.) on the efficacy of natural sorbents in feed mixtures. Further research is also necessary to ascertain the possible effects of long-term consumption of these sorbents and if side effects become probable. Advances in nanotechnology of materials will further improve natural sorbents' efficiency. The addition of Shungite-based sorbents to other nanostructured materials, as revealed by recent research, can enhance their adsorption and radiation-shielding characteristics (Alham et al. 2021). The present research validates the effectiveness of Shungite-supplemented bentonite as a sorbent in decreasing radionuclide contamination in experimental animals. These results offer new information on the use of natural sorbents in radiation protection. The inhibition of radionuclide absorption in the gastrointestinal tract and preservation of standard hematological indices in the present study support the effectiveness of Shungite-based sorbents as a promising solution for radiation

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exposure management. Future studies must persist in investigating and developing these materials further to broaden their utility within more general environmental and medical contexts.

Conclusion

The difference in the accumulation of RPP in the area of interest (GI tract) between the two groups was 4.48% after 5min (5min), 1.39% after 15min (20min), 1.35% after 30min (50min), and 1.04% after 60min (110min). Thus, the measurement data in the first group demonstrated a higher overall level of RPP accumulation immediately after introducing RPP and the most rapid redistribution (accumulation) of RPP in the GI tract throughout the study period. These results indicate potential differences in the absorption or metabolism of the preparation in this organ between the two groups. When added to feed and administered twice at 8–12-hour intervals during periods of radionuclide exposure, Shungite-enriched bentonite enhances the prevention of radionuclide damage. It expands available protective measures, reduces radiation-induced pathologies caused by high doses of the technetium isotope, and remains chemically stable during storage. The results of studies involving the preparation made of bentonite and Shungite showed the following results: - the evolutionarily developed genetic and biochemical ability of animal body tissues to metabolize an exogenous preparation; - the high efficiency of the bound form of bentonite and Shungite as a radio-excretory sorbent; - the practicability of using the preparation as a sorbent, which allows the body to implement adaptive regulatory mechanisms for rapid elimination of radionuclide.

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Data Availability: All the data is available in the article.

Ethical Statement: The experiments were carried out following the Bioethics adopted at the University and the provisions of the European Convention for the Protection of Vertebrates Used for Practical and Scientific Purposes.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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