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SIMULATION OF CONDITIONS FOR EXTERNAL AND INTERNAL EXPOSURE OF HUMAN BLOOD TO LOW DOSES OF ^{137}Cs RADIONUCLIDE *IN VITRO* TO STUDY ITS GENOTOXICITY

Proposed are the models for external exposure to and contamination with ^{137}Cs (imitation of internal exposure) of human blood samples *in vitro* in doses up to 0.53 Gy for 1.75 h. Exposure conditions and experimental devices, as well as models for the calculation of the absorbed doses under external irradiation and estimation of the activity of the radioisotope needed to obtain the desired absorbed dose under internal exposure of blood cells, are presented. The created experimental models allow conduction of comparative *in vitro* studies of the ^{137}Cs genotoxicity at external and internal exposure of human blood.

Keywords: ^{137}Cs, *in vitro* radiation simulation, external exposure, internal exposure, human blood, absorbed dose calculation.

1. Introduction

^{137}Cs is one of the main doses of contributors to radiological accidents [1, 2]. In the case of external exposure to ^{137}Cs, its damaging effects are caused predominately by gamma rays. In the case of the radionuclide uptake into a human body, the effects are imposed by a prolonged combined effect of gamma rays and beta particles, with the latter accounting for up to 90 % of the total absorbed energy, depending on the thickness of the absorber [3]. Effectiveness of the external exposure to ^{137}Cs is evaluated via a cytogenetic analysis, based on the level of induced radiation-specific chromosome markers in T-lymphocytes in the peripheral blood. The results of the cytogenetic analysis are interpreted using the dose-effect dependencies, which are experimentally determined under acute external exposure of blood *in vitro* [4 - 6]. In the case of internal exposure by ^{137}Cs, the information about the relationship between the absorbed dose and the yield of cytogenetic effects in the contaminated human blood *in vitro* is extremely limited in the available literature [7, 8]. Note that literature on the induction of chromosome damages by beta-emitters is generally scarce [9 - 11]. Available results suggest that there may be differences in the effectiveness of gamma rays and beta particles, although the radiation weighting factor for both types of the radiation is equal to one, in accordance with [12].

Taking into account the above considerations, it was deemed reasonable to perform a comparative study of the cytogenetic effectiveness of ^{137}Cs under the conditions of the external and internal irradiation of human blood in low doses *in vitro*, and to find the dose-effect dependencies for the induced chromosome damage. The latter data, obtained for the case of blood radionuclide contamination, may be useful for the development of a cytogenetic dosimetry method for the internal exposure to ^{137}Cs. Since prolonged exposure to radioisotopes is characteristic for the aftermath of an emergency, causing simultaneous processes of induction and reparation of damaged cells, we believe that the above comparative study *in vitro* would make sense to be conducted for conditions approaching *in vivo.* For this purpose, it was necessary, first of all, to ensure prolonged exposure *in vitro* to low doses of ^{137}Cs from an external source at normal human body temperature and radionuclide contaminated blood. Moreover, for experimental research involving low doses, high requirements are posed to the accuracy of absorbed dose calculations and measurements.

The goal of this work was to model conditions for a comparative study of cytogenetic effectiveness of ^{137}Cs in human lymphocytes in case of external and internal exposure of blood samples *in vitro* under conditions approaching *in vivo* and calculation of doses absorbed in the blood with health physics support.

2. Blood sample exposure conditions

The plan was to expose blood samples, contaminated with ^{137}Cs chloride solution during incubation, and an external source of ^{137}Cs for several hours. Samples of heparin-treated blood from a conditionally healthy donor, prepared in sterile conditions, were

exposed under the temperature of 37.0 ºC in air bath ovens. Integrated into the ovens were special holders for test tubes containing blood samples and, for external exposure, also for the source and dosimetry equipment. During the exposure, process holders were rotated by an electric motor (RD-09), located outside the oven. The rotation was carried out around a conventional axis at the rate of 12 revolutions per minute. This ensured a continuous and effective mixing of blood cells inside the test tubes. This was especially important considering radionuclide contamination of the blood. We believe that under such conditions the ^{137}CsCl solution would distribute evenly between blood cells in plasma. In the above-mentioned studies [7, 8], blood samples were exposed under room temperature without motion.

In our experiments, blood samples were exposed inside plastic test tubes, as plastic is a sufficiently tissue-equivalent material to allow modelling exposure in the human body [4]. Tube size: internal diameter and height, respectively, 11 mm and 32 mm; wall thickness 0.90 mm. The volume of a blood sample inside the tube was 1.1 ml.

3. Blood samples exposure to external source

A ^{137}Cs source with diameter 6 mm was fixed inside a metal vial with the external diameter of 8 mm. Source activity: $(1.79 \pm 0.15)\cdot 10^{10}$ Bq. The source and the tubes with blood samples and monitor dosimeters were located in slots of the holder made of polystyrene foam. A magnet inserted in the holder was used to fix the source.

For a cytogenetic calibration of the dose received during a prolonged exposure at human body temperature, the determining factor is the duration of exposure rather than the dose rate. This is related to the origin of the endocellular reparation process sequence [4]. Therefore, to achieve various blood exposure doses for the same time, the slots holding blood sample tubes were positioned at different distances from the source, depending on the planned absorbed doses. Midpoints of the tubes were levelled with the source.

The lithium fluoride monocrystal TLD dosimeters model KDT-02M were used to monitor the doses received by the blood samples during exposure. Three crystals (diameter 4.5 mm, height 1.0 mm, mass 0.048 ± 0.002 g) inside a polyethylene tube were placed in test tubes similar to those containing blood samples and at the same distances from the source.

Based on the dosimeter calibration results in the units of exposure dose from reference ^{137}Cs sources the expanded uncertainties for doses 8.07 and 40.46 R were 9.5 and 9.6 %, respectively (p = 0.95). The assessment was performed by SE «Ukrmetrteststandart» according to the guide [13]. The air-to-blood dose conversion factor for the low-LET radiations is close to 1 (0.96) [14]. Based on the results of dosimetry survey, the uncertainty of measurement of absorbed dose inside the tubes in the holder at various distances from the source is, according to [13], 9.53 - 9.76 %, p = 0.95. The number of measurements at different test points ranged from 11 to 13.

An example arrangement of the radiation source and test tubes with blood samples and dosimeters in the holder is shown in Fig. 1. The oven with the holder for external exposure of blood samples to ^{137}Cs is shown in Fig. 2. The oven was placed inside a hot cell. After blood samples and dosimeters had been placed in the holder, the radiation source was put in place using manipulators operated from the hot cell control panel, and the electric motor was started.

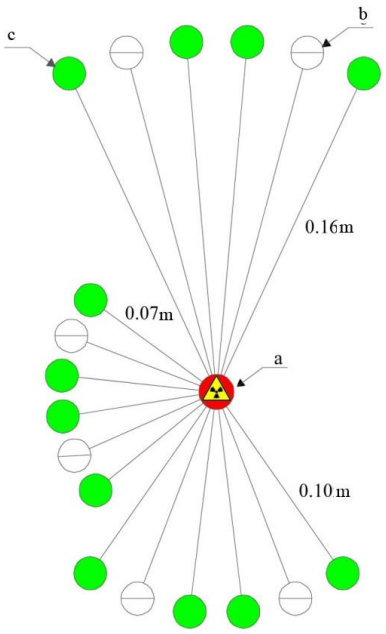

Fig. 1. Scheme of the holder for blood samples exposure to external ^{137}Cs source: *a* – ^{137}Cs source; *b* – dosimeter; *c* – blood sample.

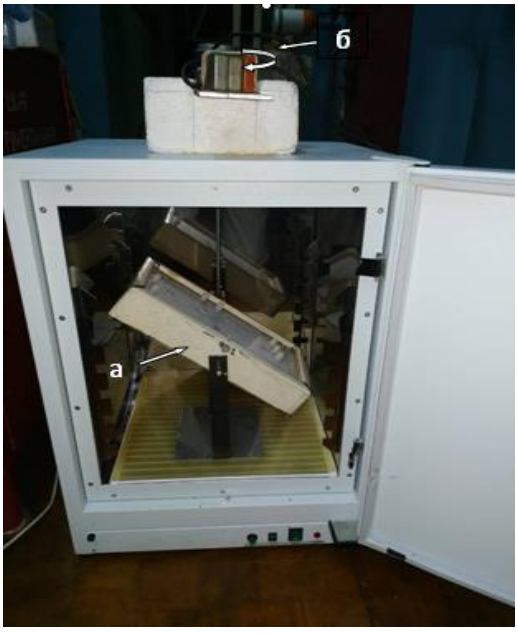

Fig. 2. Air bath oven with holder for exposure of blood samples to external ^{137}Cs source: *a* – holder with the radiation source, blood samples and dosimeters; *b* – electric motor.

The conditions above ensured the absorbed doses of 0.09 Gy to 0.53 Gy after exposure of blood samples for 1.75 h (6,300 s) at the 0.16 0.07 m distances from the source, respectively.

4. Exposure of blood samples contaminated with ^{137}Cs

Radionuclide contamination of blood was achieved by administration of ^{137}Cs chloride solution (100 μl) in the sterile environment directly in test tubes with blood samples (1.0 ml).

Test tubes with blood samples were placed one by one in polystyrene foam containers fixed to a shaft horizontally located inside the oven and passing through the centre of four lead blocks. Containers were inserted in clearances between the blocks. Lead blocks dimensions were 100×100×50 mm. Their thickness was sufficient for the reduction of the flux of gamma-rays by two orders of magnitude [3], thereby mitigating the cross-exposure of the samples to an acceptably low level.

The blood samples exposure time was 1.75 h, as in the case of external exposure.

The air bath oven with contaminated blood sample exposure device is shown in Fig. 3.

Fig. 3. Air bath oven with the device for exposure of ^{137}Cs contaminated blood samples: *a* – containers with samples; *b* – protective lead blocks.

5. Calculation of doses absorbed in blood from external and internal exposure to ^{137}Cs *in vitro*

Calculation of doses absorbed in blood subjected to external and internal exposure was performed by the custom-designed exposure modelling software developed based on the GEANT-4 library [15]. This library was specially designed to model nuclear physics experiments.

It contains a set of algorithms and data for modelling particle transport through different components of an experimental setup considering their layout, elemental composition and geometries. The developed software models interactions that occur during the transport of a source particle and simulates the emission of all secondary particles based on the user-specified properties of materials and geometry. The events are generated by the Monte Carlo method. The software supports graphical representation of the interaction processes

6. Analysis model for external exposure dose absorbed in blood

The model used to predict the absorbed dose resulting from the external exposure of a blood sample to the ^{137}Cs source is shown in Fig. 4.

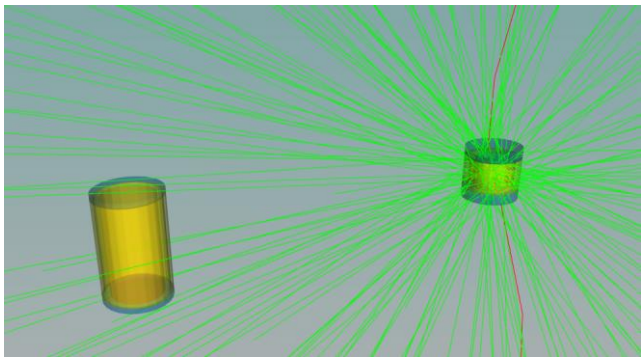

Fig. 4. Model of the blood sample exposure to an external ^{137}Cs source *in vitro*.

The model includes a ^{137}Cs source in the form of a synthetic zeolite cylinder 6 mm in diameter, containing radioactive caesium and having an external 1 mm thick stainless steel shell; a polyethylene test tube with 11 mm internal diameter, 32 mm height and 0.90 mm wall thickness, containing 1.1 ml of blood sample; a polystyrene foam sheet (density 0.034 g/cm^3) holding the source and the sample.

The elemental composition and density data for the materials used in our models, including elemental composition and density of the blood, were taken from the GEANT-4 internal libraries (ICRP). Although radionuclide genotoxicity is determined based on the presence of radiation specific markers in T-lymphocytes of peripheral blood, the dose *in vitro* effect dependence is built based on the assessment of the dose absorbed in a whole blood sample [4]. Since the specific weight of blood lymphocytes (1.03 g/ml) is close to the specific weight of the whole blood (1.06 g/ml), the dose absorbed in lymphocytes is approximately equal to the dose averaged over the volume of a blood sample. Thus, the calculations assumed that the blood was a homogenous medium with a specified density.

In an external exposure model, it was assumed that the radioactive decay occurred inside the source material with equal probability across the entire source volume. The simulation of the radioactive decay involved emissions of β-particles and γ-rays, as well as internal conversion electrons, characteristic X-rays and Auger electrons. All charged particles and soft characteristic X-rays were absorbed in the source shell, whereas gamma-rays with the energy of 662 keV were emitted isotropically. During the transport of the γ-rays through the source and surrounding medium, all relevant processes of interaction with matter were taken into account.

For the gamma-rays transported inside a test blood sample and interacting with the sample material, the programme calculates the energy loss by secondary particles for each gamma-ray to obtain the energy spectrum and accumulates the emitted energy to obtain the integral value of the absorbed energy for all ^{137}Cs decay events. After $1 \cdot 10^8$ simulations of caesium decay, we arrived at the average absorbed dose in a blood sample per decay. This value was calculated as (Table 1):

$$D = k \cdot A \cdot t,$$

where D is the absorbed dose, Gy; k is the average absorbed dose per decay, Gy/decay; A is the source activity, Bq; t is the exposure time, s.

Table 1. **Results of calculation of the dose absorbed in blood samples under external exposure to ^{137}Cs**

Source activity, Bq	Distance to the source, m	k, Gy/decay	Exposure time, s	Absorbed dose, Gy	
				calculated	measured
$01.79 \cdot 10^{10}$	0.07	$4.74 \cdot 10^{-15}$	6300	0.534	0.533 ± 0.051
	0.085	$3.50 \cdot 10^{-15}$		0.395	0.400 ± 0.038
	0.10	$2.46 \cdot 10^{-15}$		0.277	0.283 ± 0.027
	0.11	$1.83 \cdot 10^{-15}$		0.206	0.200 ± 0.020
	0.16	$7.55 \cdot 10^{-16}$		0.085	0.100 ± 0.010

The calculated and empirical values of the absorbed dose differ by less than the uncertainty of the experimental data received.

7. Analysis model for ^{137}Cs activity calculation in blood samples to find planned absorbed doses of internal exposure

The model of internal exposure of blood samples to ^{137}Cs is shown in Fig. 5.

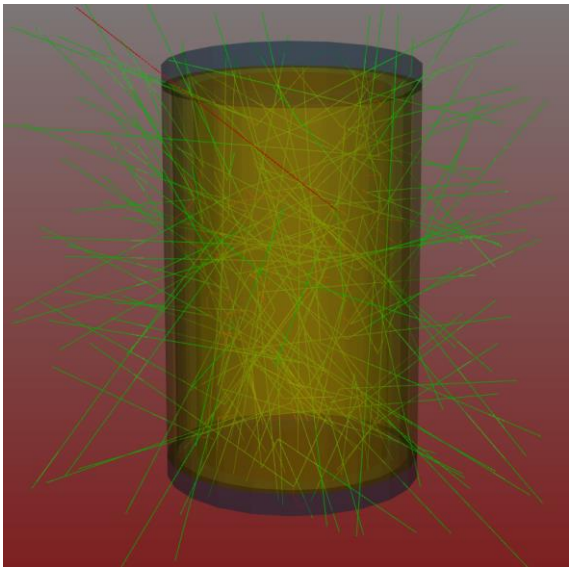

Fig. 5. Model of internal exposure of blood samples to ^{137}Cs.

For modelling of the internal exposure, we used the same geometry of a test tube filled with blood as in the case of the external exposure. The only difference was that the irradiation source – ^{137}Cs radionuclide – was evenly distributed inside the blood sample volume.

The decay of radioactive caesium occurred uniformly across the sample volume. Due to the absence of any absorbing layers between the source and the sample, the entire energy of the emitted β-particles and electrons were absorbed in the blood, except for thin boundary layers, from which charged particles could escape thereby leaving only part of their energy in the blood.

Due to the low probability of interaction of the high-energy γ-rays with the sample matter, mostly composed of light elements, only a small part of the gamma-rays contributed to the total absorbed dose.

After $1 \cdot 10^7$ simulations of caesium decay, we obtained the average absorbed dose value in a blood sample per decay. Using this result, the ^{137}Cs activity could be calculated (Table 2) by the equation

$$A = D/(k \cdot t),$$

where A is the activity of ^{137}Cs in a blood sample, Bq; D is the absorbed dose, Gy; k is the average absorbed dose per decay, Gy/decay; t is the exposure time, s.

After incubation in the air bath oven, the samples were moved to centrifugal test tubes with culture medium RPMI-1640 (5.0 ml in each tube) and were subjected to centrifuge spinning for 7 min at 200 g. Each sample was spinned in a separate centrifuge (OPN-3.02). Individual centrifuges were separated from one another using lead blocks. The actual sample

Table 2. **Results of calculations of ^{137}Cs activity in a blood sample for obtaining of the planned absorbed doses**

Absorbed dose, Gy	*k*, Gy/decay	Exposure time, s	Activity, MBq
0.085	$3.28 \cdot 10^{-11}$	6300	0.411
0.200			0.968
0.277			1.340
0.395			1.911
0.534			2.584

exposure time was measured from the moment of the addition of radionuclide to the moment of transferring of the samples in the centrifuge test tube with the culture medium.

The additional dose received by blood cells during their centrifugal settling was estimated as follows. For the calculation of the average absorbed dose per decay we used the model in which the activity was located in 6.1 ml of solution, whereas settled cells were contained inside the volume of approximately 0.5 ml. Thus, cells were exposed to the activity located in the sediment itself, as well as to the activity of the supernatant fluid. The calculated *k* value was found to be $2.76 \cdot 10^{-12}$ Gy/decay. Therefore, at the activity of $2.584 \cdot 10^{6}$ Bq and spinning time of 7 min the cells would get the additional dose of $2.98 \cdot 10^{-3}$ Gy, which constituted 0.56 % of the main absorbed dose.

Settled blood cells were subjected to triple washing in the culture medium to remove radioisotope residues. Radionuclide activity in the supernatant fluid was detectable on the gamma spectrum.

8. Conclusions

Experimental capabilities were created for external and internal exposure to low doses of ^{137}Cs radionuclide of blood samples *in vitro* under conditions approaching in vivo. Computational models with the use of the Monte Carlo method were developed and validated for the assessment of the exposure doses absorbed in blood samples. The discrepancy between the calculated and empirical values of the adsorbed dose was found to be less than the uncertainty of the obtained experimental data.

We believe that the proposed models for the exposure of blood samples to ^{137}Cs radionuclide *in vitro* are usable for comparative studies of its effectiveness under external and internal exposure during several hours.

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МОДЕЛЮВАННЯ УМОВ ЗОВНІШНЬОГО І ВНУТРІШНЬОГО ОПРОМІНЕННЯ КРОВІ ЛЮДИНИ РАДІОНУКЛІДОМ ^{137}Cs У МАЛИХ ДОЗАХ *IN VITRO* ДЛЯ ДОСЛІДЖЕННЯ ЙОГО ГЕНОТОКСИЧНОСТІ

Запропоновано моделі зовнішнього опромінення та забруднення ^{137}Cs (імітація внутрішнього опромінення) зразків крові людини *in vitro* в дозах до 0,53 Гр протягом 1,75 год. Представлено умови експозиції та експериментальні пристрої, а також моделі для обчислення поглинених доз при зовнішньому опроміненні та оцінки активності радіоізотопу, необхідної для отримання бажаної поглиненої дози при внутрішньому опроміненні клітин крові. Створені експериментальні моделі дають змогу проводити порівняльні дослідження *in vitro* генотоксичності ^{137}Cs при зовнішньому та внутрішньому впливі на кров людини.

Ключові слова: ^{137}Cs, моделювання опромінення *in vitro*, зовнішнє опромінення, внутрішнє опромінення, кров людини, розрахунок поглиненої дози.

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МОДЕЛИРОВАНИЕ УСЛОВИЙ ВНЕШНЕГО И ВНУТРЕННЕГО ОБЛУЧЕНИЯ КРОВИ ЧЕЛОВЕКА РАДИОНУКЛИДОМ ^{137}Cs В МАЛЫХ ДОЗАХ *IN VITRO* ДЛЯ ИССЛЕДОВАНИЯ ЕГО ГЕНОТОКСИЧНОСТИ

Предлагаются модели внешнего воздействия и загрязнения ^{137}Cs (имитация внутреннего облучения) образцов крови человека *in vitro* в дозах до 0,53 Гр в течение 1,75 ч. Представлены условия воздействия и экспериментальные устройства, а также модели для расчета поглощенных доз при внешнем облучении и оценки активности радиоизотопа, необходимой для получения желаемой поглощенной дозы при внутреннем облучении клеток крови. Созданные экспериментальные модели позволяют проводить сравнительные исследования *in vitro* генотоксичности ^{137}Cs при внешнем и внутреннем воздействии на кровь человека.

Ключевые слова: ^{137}Cs, моделирование облучения *in vitro*, внешнее облучение, внутреннее облучение, кровь человека, расчет поглощенной дозы.

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