FEDERAL BIOMEDICAL AGENCY

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TO: V.V. Uybe, Head of the Federal Microbiological Agency of Russia

[hw:] 07/31/2013 Our Ref [hw:] 32-024/46

RE: Testing

Following your instructions, the A. I . Bumazyan Federal Medical Centre hereby delivers to you the results of the testing conducted by the Radiology Safety Department (Department No.3) for your perusal and in order for a decision to be made.

CEO,
AI BURYAZAN FEDERAL
MEDICAL BIOPHYSICAL CENTRE,
[seal:] [illegible] [signature] K.V. Kotenko

Head,
Radiology Safety Department
M.D. [signature] N.K. Shandala

APPROVED:
CEO,

AI BURNAZNAN MEDICAL BIOPHYSICAL CENTRE

[signature]
K.V. Kotenko
[seal:] [illegible]

EXPERT OPINION
On the results of measurements of specific activity of Lead-210 and Polonium-210 in biological samples presented for examination

SUBJECT UNDER EXAMINATION:

The following biological samples have been submitted for examination to Doctor of Science V.N. Yatsenko, Director of the Laboratory of Radiometric and Spectroscopic Human and Environmental Research (Lab No.15) of the Radiology Safety Department, A. I. Burnazyan Medical Biophysical Centre,: 4 (four) samples of bones of the skull and extremities (in sizes up to 1.08 x 1.34) acquired in the result of an exhumation performed in accordance with Special Message No. 1326/01-24-2013/SS of the Ministry of Foreign Affairs of the Russian Federation. In the course of disinterment, general inspection of the remains has been carried out; identification of personality has been performed. The remains are of Subject No.3187/01 of the Index File (Mohammed Abdel Rahman Abdel Raouf Arafat al-Qudwa *al-Husseini [arabic script]).*

METHODS OF MEASUREMENT:

In accordance with the Federal Medical Biological Agency Listing of Methods for Measurements and Calculations (List No R 17.4-2006.2010), inclusive of Supplement No.1 "Method for the measurement of specific activity of lead-210 and polonium-210 in environmental specimens and biological material", according to Measurement Methods List No 1 attested by Certificate No. 797/02 of the D.I. Mendeleyev Russian Research Institute of Metrology Federal State Unitary Enterprise (Mendeleyev Institute of Metrology) ". Approved by the Director of the Research Institute of Industrial and Marine Medicine Federal State Unitary Enterprise", the radiometric measurement method has been used..

Measured value: activity, in Bq/g; lead-210 and polonium-210

Limit (range) of measurements: Lead-210 activity (0.3-300) Bq/g; lead-210 specific activity (60-$6x10^4$) Bq/kg; polonium-210 specific activity ($4x10^5$) Bq/kg

Error description: Consistent error 17%, random error for α-radiation – 5%, for β-radiation – 6%.

Equipment: α – β radiation detector of type UMF-2000 or any other with specifications not worse than following:

- Background count rate – not exceeding 0.05 imp/min for α-channel and 2.3 imp/min for β-channel;
-137Cs β-ray registration efficiency – not less than 3.6 imp/(min x Bq), and for 90Sr (90Y) – not worse than 7.5 imp/(min x Bq), accordingly;
- Efficiency of registration of α-rays of electrochemical countable samples with active spot diameter of 26 mm – not worse than 16.2 imp/(min x Bq);
- Error rate in determining activity levels of countable samples: – not exceeding 10% at $\rho = 0.95$.

Benchmark radionuclide sources and reference solutions:

Benchmark specimen OSK-210 (210Po+210Bi) with activity from 10 to 50 Bq at measurement error not exceeding 6%.

REGULATORY REFERENCES

The conduct of the present study is underpinned by the following recommendations and requirements:

- The Decree of the President of the Russian Federation of 11.10.2004 No. 1304 "On the Federal Medical and Biological Agency".

- The Resolution of the Government of the Russian Federation of 17th July 2004 No. 294:. *The Rules on the Federal Agency for Technical Regulation and Metrology.*

- Direction No 32-024/206 dated 09.12.1998 of the Federal Directorate "Medbioekstrem" "Conducting analyses of the state of the metrological level of measurements and tests in the system of institutions and enterprises of the Federal Directorate "Medbioekstrem".

- STATE STANDARD 1.5-2001. The Inter-State System of Standardisation. Inter-State standards, rules and recommendations for Inter-State standardisation. General requirements for structure, exposition, drawing up, content and designation.

- STATE STANDARD 8.417-2002 (State Measurement Standard). Units of measurement.

- STATE STANDARD 27451-87 Means of measurement of ionising radiation. Nomenclature of indicators.

- STATE STANDARD R 1.0-2004 Standardisation in the Russian Federation. General provisions.

- STATE STANDARD R 1.5-2004 GSS. National Standards of the Russian Federation. Rules on structure, exposition, drawing up, content and designation.

- STATE STANDARD R 1.12-2004. Standardisation in the Russian Federation. Terminology and definitions.

- STATE STANDARD R 8.563-2009 (State Measurement Standard). Methodologies (methods) of measurement.

- STATE STANDARD R 8.594-2002 GSS. Metrological support for the monitoring of radiation. Main provisions.

- R 1.1.003-96 -1.1 General matters. General requirements for the structure, exposition and drawing up of regulatory and methodological documents of the State Sanitary and Epidemiological [Public Health] Standard Setting System. Moscow, 1998.

- Methodological Direction of the Federal Biomedical Academy of Russia 4.4.19-2008. 4.4. General questions of methods of monitoring "Relative measurements, Radiometry. Requirements for methodologies of measurement of the activity of specimens of biological subjects, environmental subjects and foods (with assessment of error and uncertainty of measurements)".

DEVICES FOR MEASUREMENT OF MASS AND VOLUME USED IN PREPARATION OF CALCULATION SPECIMENS:

- analytical scales ADV-200, STATE STANDARD 24104-80;

- technical scales VRL-1, STATE STANDARD 19491-74;

- set of scales, STATE STANDARD 73228-82;

- measuring pipettes, class 2, 1.5 and 10 ml, STATE STANDARD 20292-74;

- measuring flasks, class 2, 100, 250 and 1000 ml, STATE STANDARD 1770-78;

- laboratory beakers, 50, 200, 250, 300, 800, 1000 and 2000 ml, STATE STANDARD 10394-72.

ANCILLARY EQUIPMENT:

- electric hob, STATE STANDARD 14919-83;

- distillation system, D-4 TU 64-1-1640-78;

- electric furnace PM-8 with thermostatic regulator up to 900°C;

- chromatographic columns made of glass, with inside diameter of 10 mm;

- backing vacuum pump (VN-461, VN-2M) maintaining working pressure in the vacuum chamber not greater than 1333 Pa (10 mm Hg);

- portable unit for electrolytic deposition of radionuclides on the stainless steel substrate 34 mm in diameter, which includes an electrolytic cell with anodic and cathodic electrodes;

- regulated DC power supply unit with max. current up to 3 A and max. voltage up to 30 V;

- nickel discs (diameter 34 mm, thickness 0.6 mm), fabricated from a nickel strip, grade NP-2 (0.6x250), STATE STANDARD 2170-73;

- fine grit sandpaper;

- universal test-paper, pH 1-10, TU MKhPORU 76-56;

- set of density meters;

- standard sieves;

- 25, 50 and 100 ml porcelain bowls and crucibles, STATE STANDARD 9147-80;

- 50 ml quartz bowls, STATE STANDARD 10973-64;

- porcelain mortar;

- watch glass;

- glass rods, 33 mm in diameter;

- spatula;

- glass funnels, 5, 7.5, 10 and 15 mm in diameter, STATE STANDARD 8613-75;

- round bottom flask K-1-2000-29/32, STATE STANDARD 25336-82;

- cone flask Kn-2-1000-34, TU 92-891.029-91;

- straight condenser KhPT-1-600-14/23-14/23, STATE STANDARD 25336-82;

- substrates made of grade 1Kh18N9T, 12Kh18N10T, 08Kh18N10T stainless steel or similar, STATE STANDARD 4543, STATE STANDARD 5632, 34 mm in diameter, 0.7-1.0 mm thick;

- aluminium foil, 81 g/cm^2, thickness 3 mm, grades A5, A6, A7, ADO, AD31, STATE STANDARD 15175-89;

- teflon support for electrolytic deposition;

- filter paper, STATE STANDARD 12026-66;

- 250-500 W infrared lamp;

- rubber gloves;

- rags, cotton wool.

SCIENTIFIC RATIONALE OF THE STUDY

Based on the assumption that polonium-210 entered the body under examination, taking into account the fact that since the moment of death (presumably in the first ten-day period of November 2004) and the moment of first set of tests (first ten-day period of 2012), mathematically expected amount of ^{210}Po left in the body under examination shall be 1/10^6 (21 half-life periods). ^{210}Po is excreted from the body mainly through the gastrointestinal tract and kidneys, 0.9 of ^{210}Po is excreted with faeces and only 0.1 is excreted with urine. Values of T_b are 37±6 and 35.7±4 days, respectively. Average T_{eff} of ^{210}Po for the whole body is 37±6 days.

Taking into account the generalised research data for radiotoxicity of polonium, it is assumed that in case of ingestion of the substance with food, the conventional value for degree of ^{210}Po adsorption from food into blood is about 10%.

Based on the research data of E. V. Erleksova, after subcutaneous or intravenous injection, or oral administration of ^{210}Po nitrate solution (neutralised with alkali to pH = 6.0 – 6.5 in the presence of mannitol),

most of the radionuclide in the body of a mammal, within first five days after the injection, is contained in the brain matter, and after 1<*missing text*> and later – in the cortex of the kidneys. E. V. Erleksova observed deposition of ^{210}Po in endosteum and connective tissue membrane, which lines the foramens of the compact bone that transmit blood vessels, and in the bone marrow. In the bone tissue, ^{210}Po is localised in the organic matrix, that is, in the intercellular part of the bone tissue with its high concentration of calcium salts. The main mineral component of the bone matrix is a complex salt, hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$). Its crystals are deposited along the collagen fibres. The organic component of the matrix in human body amounts to 30% of its weight. Intercellular substance consists of organic part (25%), inorganic part (50%), and water (25%). Average radioactivity concentration of ^{238}U in bone tissues is 150 mBq/kg, ranging from 20 to 200 mBq/kg.

For this study, evaluation and estimation of uncertainty for dose calculation is performed by mathematical modelling of the radionuclide admission and excretion process in standard human body, and Monte Carlo method is used for determining the distribution of E magnitude estimation and its parameters.

DETAILS OF ESTIMATION

Due to low penetration power of α-particles, special low-background equipment is required for detection of ^{210}Po. In the process of radioactive decay, ^{210}Po also produces penetrating γ-radiation, but its intensity is very low (one gamma quantum for each 80 thousand of α-decays), which makes its detection extremely difficult against the background of natural radiation in the environment; therefore it is not suitable in view of the conditions mentioned above.

Radionuclide	T1/2	Type of decay	Average energy of radiation, MeV/(Bq-s)			Daughter radionuclide (output)
			α-radiation and recoil nucleus	Characteristic γ- and annihilation radiation	β-radiation, conversion electrons and Auger electrons	
^{210}Po	138.38 days	α	5.40	8.50×10^{-6}	8.18×10^{-6}	^{206}Pb stable

Radiometric method is the main method for determining the presence of polonium, it is based on detection of ^{210}Po α-radiation. Amounts of polonium above 1 μg may be detected by using g-counter ($11\text{-}10^{-3}$% of ^{210}Po decay, $E_\gamma = 0.803$ MeV) or calorimetric method (specific heat liberation of ^{210}Po is 140 W/g).

RADIOCHEMICAL EXTRACTION OF 210PO AND 210BI (210PB)

The method for identifying polonium is based on the electrochemical sedimentation of radioactive nuclide on a nickel disc in chloride solutions. The method is called electrolytic substitution or no-current electrolysis.

In identifying polonium, measures have been taken to prevent its sorption on glass; solutions: 0.7n - for hydrochloric acid and 0.5 - for 1% citric acid.

In all cases of sedimentation of polonium on a nickel disc only hydrochloric acid was used, and not the nitric acid, since the latter dissolves nickel (especially intensively when heated).

To exclude the influence of iron, ascorbic acid was added as it reduces the ferric iron to a ferrous one which does not interfere with the electrochemical sedimentation of polonium. The volume of the solution should not exceed 100 ml.

The method for 210Po extraction can be summarized as follows: the filtrates unionized after the sedimentation of sulphates (filtrate 1, filtrate 2 and solution [text cut-off] of sediment 3) were evaporated down to 600-800 ml and then a CO2-free ammonia [text cut-off] pH=9 was used to deposit ferric hydroxide, where co-deposits of isotopes of uranium, thorium, polonium, lead, bismuth, and others were obtained. On settling, the residual matter was filtered by using the ashless "red ribbon" filter of 12.5 (11.0) cm in diameter; the resulting filtrate then was flushed with hot distilled water and dissolved in 70 ml of 1n HCL with the addition of 500-700 mg of citric acid [text cut-off] ascorbic acid until the solution is decolorized. The solution was then brought to 100 ml by adding water. A disc made of nickel foil was placed in the solution, fixed on a teflon support to ensure that 210Po is deposited only on one side of the disc. The contents of the beaker were heated in a boiling-water bath for 6 hours, stirring occasionally. The volume of the liquid remained 100 ml at all times as distilled water was added to the tested solution to compensate for the evaporation of water. After 6 hours, the disc was removed from the teflon support, rinsed with distilled water and ethanol, and left to naturally dry. The time of dissociation of 210Bi from 210Pb was registered.

When electrolytic sedimentation of 210Po and 210Bi on the nickel disc was complete, the counting rate of the obtained specimen via α and β channels was determined. The activity of the preparation was measured 14 hours after the disengagement of radionuclides, because, apart from 210Po and 210Bi, the preparation might have contained also short-lived α-active (218Po, 214Po, 216Po, 212Po, 215Po, 211Po, 211Bi) and β-active (214Bi, 212Bi) radionuclides. The holding of the preparation for 14 hours prior to measurements was necessary to allow for the decay of these short-lived radionuclides in order to eliminate their interference.

Specific activity of 210Po was calculated as per following formula:

$A_{Po\text{-}210} = n \times K_{Po\text{-}210}/m \times \rho$, *Bq/g,*

Where:

n – counting rate of impulses from the countable specimen subtracting the α- channel background, in *imp/min*;

$K_{Po\text{-}210}$ – association ratio for 210Po, *Bq/ (imp/min)*, obtained, when using a benchmark specimen;

m - test specimen mass, in *g*;

ρ – chemical yield of 210Po.

The specific activity of 210Bi, which is assumed to be equal to the specific gravity of 210Pb, was calculated as per following formula:

$A_{Bi\text{-}210} = A_{Pb\text{-}210} = n_1 \times K_{Bi\text{-}210}/m \times \rho_1 \times e^{-\lambda Bi\text{-}210 \times t}$, *Bq/g,*

Where:

n_1 – counting rate of impulses from the countable specimen subtracting the β- channel background, in *imp/min*;

$K_{Bi\text{-}210}$ – association ratio for 210Bi, *Bq/ (imp/min)*, obtained, when using a benchmark specimen;

m - test specimen mass, in *g*;

ρ_1 – chemical yield of 210Bi;

t - time from the electrochemical extraction of 210Bi to the measurement of its activity, in *hours*;

$\lambda Bi\text{-}210$ – 210Bi decay constant.

The chemical yield of both 210Po and 210Bi was observed while developing the method using the corresponding standard solutions:

$\rho_{Po\text{-}210} = 0.9$; $\rho_{1Bi\text{-}210} = 0.85$;

Corrections for 210Bi decay ($T_{1/2}$=120 hours) are presented in Table 1.

Table 1

Time dependent 210Bi ($e^{-\lambda Bi\text{-}210 \times t}$) decay corrections

210Bi decay time , in hours (*t*)	$e^{-\lambda Bi\text{-}210 \times t}$
12	0.93
16	0.91
20	0.89
24	0.87
28	0.85

32	0.83
40	0.81

In employing this method, the major problem was the relevancy of self-attenuation corrections since in case of low-energy gammas the attenuation ratio is highly dependent on the elemental composition of the specimen. In order to solve this problem, standard materials with elemental composition similar to that of the tested specimen, external normalization and the Monte Carlo method were used.

To identify 210Po in the same specimens, gamma-ray spectrometry was carried out using HPGe detectors with lead shielding (10cm) with the following specifications: 25% efficiency, resolution for 1.33 MeV (60Co)-1.9 keV, cumulative background in the range of 25 keV-2MeV that equals to 1.5 c-1. A 20.9 g sample weight of the specimen in a Petrie dish was placed directly on the detector and measured for 24 hours. As a reference, a certified standard material of suitable elemental composition and very low radioactivity was chosen; the material contained certain amounts of uranium, thorium, and potassium. The material had gamma-ray density and absorption parameters identical to those of the sediments. Variation factors in two different methods were the same; average values – 11.8% (α method) and 12.9% (γ method). Besides, the values were obtained in the result of the analysis of specimens with low 210Pb content.

By reference to the hypothesis suggested by the group of researchers, it is impossible to establish the gamma emission since the intensity of this type of radiation for 210Po upon its ingress into the body is extremely low and equals to one gamma-quantum per 80 thousand α-decays that makes its registration exceedingly complicated against the background of natural ambient radiation.

TENTATIVE CONCLUSION

Ref. No.	Sample	Series of tests 210Po (mBq/g)		
		No.1	No. 2	No.3
3187/01-01	Skull bone fragment	1.4	1.2	1.1
3187/01-02	Skull bone fragment	0.9	0.7	-
3187/01-03	Extremity bone fragment	0.3	0.2	-
3187/01-04	Extremity bone fragment	0.5	0.4	-

(The research results on types of radiation are presented in Appendices 1A, 1B, and 1C)

Considering the half-decay period and other objective factors present during the actual study of the sample, the qualifying level of activity of the radioactive source (1 decay every 1.090 seconds), and having analyzed the number of impulses, their activity and level of tolerance in the first and second series of tests, it has been impossible to come to a decisive conclusion regarding the presence of the radioactive background in the provided samples. The results of the undertaken research are tentative and might be associated with instrumental errors and natural background. Series 3 tests were carried out on sample 3187/01-01 only. The obtained result does not constitute unambiguous evidence of the presence of 210Po in the system of the subject under examination.

INTER-DISCIPLINARY MODELING

Commonly, an adaptation of pharmacokinetic models – toxicokinetic modeling - is employed for the analysis of the processes of poisoning. These models make it essentially impossible to simulate processes that led to death since they can be adequately employed only at small amounts of poison, when homeostasis of the affected system

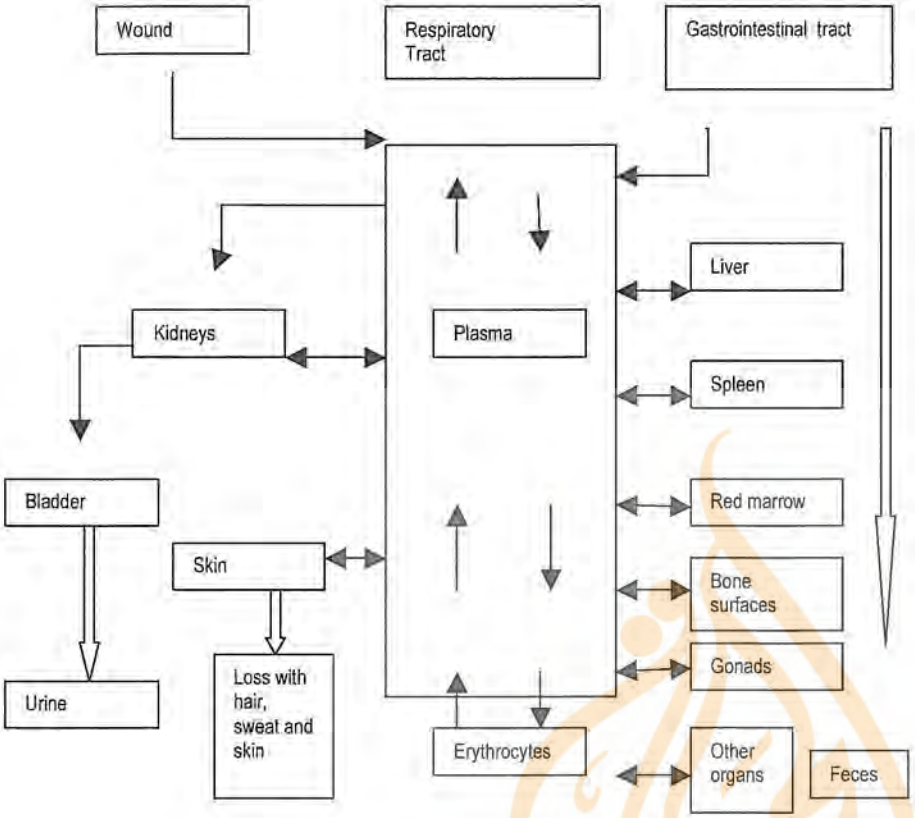
has been preserved. At large amounts, the poison afflicts the system, while the system is trying to overcome this affliction. Depending on which of these actions turns out to be the strongest, the subject either dies or recovers.

Hence, when the analysis of severe poisoning is required - as is the case under examination- a more complex, cross-disciplinary method was employed. A cross-disciplinary model consists of two disciplinary models (a model of natural body technologies and a model of poison). Exposure to low-level doses of polonium (0.5 Gy) results in prodromal syndrome, while exposure to high-level doses (103 Gy) causes damage to bone marrow, kidneys and liver.

Without LD-50 ~2.5 Gy medical assistance, the major cause of death is considered to be the bone marrow dysfunction. The details are poorly studied.

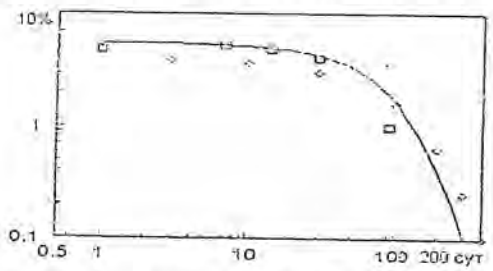
The biokinetic systemic model of distribution, retention and excretion of 210Po, absorbed in blood in the result of indigestion, inhalation or through an open wound, is based on the data acquired on humans and laboratory animals; the model is presented in the figure below.

The modelling of the polonium ingress into the system to the effect of the lethal outcome allowed for the adjustment of the tentative data regarding the absorbed amount. The dependency of the life duration of a human or animal following the poisoning has been modelled. 210Po is a radionuclide with even distribution (multiple organ distribution) and its excretion from the system is a slow process (half-decay time -138.8 days; half-elimination time – 50-80 days), which means that it has a long-term impact on the system and causes DNA mutations and neoplasms.



Modeling based on different assumptions (bone marrow lesion only, damage to bone marrow and kidneys, damage to bone marrow, kidneys and liver) showed that at doses less than 1.1 GBq a significant divergence from the experimental data is observed. Hence, the analysis should account for other factors that might have influenced the death of the subject under examination, considering also the factor of the administered treatment, which is impossible to account for in this research due to the instructions provided for the independent study.

Besides, the results obtained by the Federal Medical Center regarding the distribution of the radionuclide in the skeleton prove that after 200 days the level of 210Po content in the skeleton is next to 0% from the initial systemic burden. It has been established through the experiments that polonium-210 doses from 100 to 300 MBq (3-10 m-curie), absorbed in blood, can cause death of an adult of average weight 70 kg within one month. While in cases of indigestion of polonium-210,



the same corresponds to 1-3 MBq (30-100 m-curie) since only 10% of the radioisotope is absorbed by blood from the polonium-210 contaminated food. Polonium is a toxin exclusively due to its α-radiation. 1 megabecquerel activity corresponds to 1 microgram of polonium chloride. Considering the results of radiochemical extraction within the range of the assumed error, the modeling provides **no grounds for a decisive positive statement that the ingress of polonium into the system of the subject could have constituted the cause of the subject's death** (*Modeling results are presented in Appendix 2*)

MATHEMATICAL MODEL

As a part of the study, we checked dependence of indirect measurements on any number of initial direct measurements in form of algorithm including logical elements without linearization. Error of a result was directly dependant on the number of tests, which modelled the error for the initial direct measurements within the working range of values. **As a result of (i) the calculation of the error distribution law for the results of indirect measurement (ii) the point estimation of this error and (iii) its evaluation in terms of a confidence interval, it has been shown that the obtained result of measurement falls outside the limits of the confidence interval, and therefore, it cannot be accepted as mathematically correct.**

CONCLUSION

After studying the samples, only one of the four provided fragments (skull bone, sample 3187/01-01) was found to have radioactive background, which can, with low probability,

– within the range of mathematical error– can conditionally evidence that the system body of the subject, who this sample belonged to, contained a certain amount of ^{210}Po substance (^{210}Po level assessment result ambiguity exceeds 49%). According to the requirements of Measurement and Calculation Methodology R 17.4 – 2006.2010 of the Federal Biomedical Agency of Russia, the degree of certainty of the result does not fall within the acceptable limit. The radioactivity level of the rest of the samples is within the natural background framework.

Considering that within the framework of mathematical, cross-disciplinary inter-disciplinary modelling the results were re-verified and included into the artificial modules structure and the working hypothesis was not confirmed, the working hypothesis regarding the subject's death being caused by penetration of ^{210}Po into his body was recognized as unsubstantiated. However the result of the study may also be connected with the long time, which has passed from the moment, when ^{210}Po penetrated the subject's organism and the time of the study.

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