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The Interaction of Tritium with Some Types of Aquatic Plants

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Abstract

Tritium is the least toxic radionuclide. The main contribution into the total tritium content into ecosystems is made by technogenic tritium which is due to the operation of nuclear fuel cycle enterprises. The tritium content in the ecosystem of the River Yenisei is connected with its background values as well as with tritium entering the water ecosystem as a result of the operation of the Mining and Chemical Combine, MCC Rosatom. Presented here are the investigations of the possible transformation of tritium interacting with certain species of aqueous plants – submerged macrophyte *Elodea canadensis* and aqueous plant, floating on the surface of water reservoirs, *Lemna minor*. Elodea sampling was made in a real water reservoir – the River Yenisei, while lemna was grown in the laboratory conditions. The experiments show that with the chronic exposure of young elodea shoots to tritium, the latter transforms from free tritiated water (HTO) to organically bound tritium (OBT). Optimal conditions were also obtained for the maximum transformation of tritium ~35 % from the total content: at 25 ^{0}C and the light period 6/18 (day/night). In the lemna experiments the number of rosettes in the medium was higher than in the control experiment under the same conditions.

Keywords: Tritium; Elodea Canadensis; Lemna minor; Accumulation; Transformation

Introduction

Aquatic plants provide important food for many animals. Ducks and geese eat the seeds, leafy parts, and tubers of plants such as pondweeds (*Potomogeten* spp.), watershield (*Brasenia schreberi*), arrowhead (*Sagittaria latifolia)*, water pepper (*Polygonum* sp.), and duckweed (*Lemna* sp.). Songbirds use fluff from cattails (*Typha* sp.) as nest material and eat the seeds of many emergent plants. Otter, beaver, muskrats, turtles, and moose will also graze on a variety of aquatic plants. Historically humans have also utilized aquatic plants as a food source. Cattails have edible shoots and roots and even the pollen has been used in making biscuits. Watercress (*Rorippa nasturtium-aquaticum*) has many historic medicinal uses and its spicy vegetation continues to be used in salads and garnishes. Water lily roots are a common source of food in many parts of the world and have historic medicinal value. Even the submersed plant, coontail (*Ceratophylum demersum*) has been used for

medicinal purposes [1].

Aquatic plants provide important living space for small animals such as aquatic insects, snails, and crustaceans, which in turn supply food for fish and waterfowl. Many studies have shown that vegetated areas support many times more of these tiny creatures than do unvegetated areas. Aquatic plants form a vital part of the complex system of chemical cycling in a waterbody. They can also influence the supply of oxygen in the water. Recently aquatic plants have received a lot of attention for their ability to absorb pollutants from contaminated water. They utilize nutrients that would otherwise be used by algae, thereby improving water clarity. Increasing attention is being paid toward their possible use as indicators of water quality [1].

^{3}H is attributed to long-lived radionuclides and can pollute the biosphere not only on the local scale (directly in the area of the source location), but also on the regional and global scales [2]. Tritium occupies a special place in radiological studies both due to the necessity of controlling the radioactive contamination of the environment and the possibility by using tritium to track the pathways of the contaminated water transport, to experimentally verify theoretical models of forecasting radioactive pollution of water areas and those with the potential sources of radioactive accidents (nuclear power stations, coastal bases and repair facilities for Nuclear Navy, fuel cycle facilities and radioactive waste storages of different hazard levels) [3,4].

Having the energy of β-particles which is the smallest among all the isotopes, ^{3}H leads to a significant density of the tissue ionization (the number of the ion pairs formed by a charged particle per its distance unit) [3,4]. The most dangerous impact is the radionuclide being the source of internal irradiation when entering the organism. Tritium toxicity is largely determined by the chemical form of a compound entering the organism. For example, tritium oxide is less toxic than tritiated organic compounds. The latter are bound with organic structures of a tissue and are long retained in them, irradiating radiosensitive parts of its cells [5].

The source of tritium entering into the River Yenisei has been so far the operation of the Mining and Chemical Combine MCC Rosatom whose last reactor was shut down in 2010. In the earlier published papers [6-11] the data are given on the tritium content in the water of the River Yenisei of the nearest impact zone of MCC and one of the right-bank tributaries as well as the estimation results of the total tritium content in several components of the Yenisei River ecosystem. However, the published papers do not consider the tritium interaction with aqueous plants.

Thus, the aim of the present work is to study the process of the tritium accumulation by the aqueous plants *Elodea canadensis, Lemna minor* and to estimate the contribution of organically bound tritium content (OBT) into the total radionuclide amount in the aqueous plant *Elodea canadensis*, as a result of the simulation experiments.

Materials and Methods

Experimental plants

To carry out the experiments on the accumulation and transformation of tritium in the biomass structures, the most common species of the submerged aqueous plant *Elodea canadensis* Michx. (elodea) was used. The Canadian Elodea –*Elodea canadensis = Lagarosiphon major*. Perennial plant. The whole plant is submerged into water. The root system is poorly developed. Branched shoots can be up to 100 cm long. Simple fine and thin leaves are located on the stems in whorls, in groups of three [12].

The lemna minor (*Lemna minor* L.) – a floating species was also used. Lemna represents the family, which, as a result of its hydrophilic evolution, significantly simplified its own structure. The vegetative body represents a shoot which is not differentiated into the stem and the leaf. Since this shoot apparently looks like a leaf (which is not the case), it is called a frond [13].

Experimental procedure

The elodea shoots were sampled just before carrying all series of the experiments. The samples were taken in several experimental plots: 1) upstream the Krasnoyarsk city and 2) in the control area, the village Yesaulovo, located at a distance of 46 km downstream the Krasnoyarsk city.

After sampling the plants were carefully washed with a large quantity of water. They were sorted out according to the biomass quality. Apical shoots from 3 to 6 cm in length from the whole biomass were used for the study. Before the start of the experiment the biomass was placed into an aquarium with water in a climate room with the constant temperature $t \sim 18$ °C and light regime 12/12 h.

The washed-elodea biomass was used for accumulating tritium and estimating HTO and OBT in the control point (the initial point). For this purpose the initial biomass was divided into two parts. In one of them a weight of 50 g was taken from which tritium was extracted, the latter being in the form of HTO. The amount left (400 g) was weighted and dried (for 72 h at t = 60 °C). The dried biomass was weighted and used to extract tritium in the form of OBT ($m \sim 30$ g). The second part of the plants was used in the experiments. The lemna samples were grown in a climatostat for a week using the Steinberg medium [14].

The algae cultures were grown in the cultivator KB-05 designed in the Siberian State University. A transparent bottle made of colorless glass 400 cm^3 in volume was used as a reactor. An algae suspension, 125±10 cm^3 in volume, was poured into the reactor. To provide carbon dioxide the container with the suspension was rotated around its longitudinal axis. The constant temperature of the medium was equal to 36.0±0.5 °C.

Experimental methods for tritium accumulation

For the experiment on the tritium accumulation by elodea, the plant shoots (~ 5 cm) were placed in the aquarium and poured over by tritium-containing water until the plants were completely submerged (250 g of the plants were placed into 2500 ml tritiated water). A photoperiod of ~ 12 h was set. The duration of the experiment was 11 days. The water samples (the aliquot volume was equal to 15 ml) to control the tritium content were taken after 24, 72, 96 h, and further after 168, 216 and 264 h. At the end of the experiment the biomass was weighted. The length of the shoots was measured. From the whole biomass 50 g were taken to estimate the HTO. The biomass left was dried down to a constant weight (for 72 h at t = 45°C) and used for estimating OBT. The original water of the river Yenisei with the background tritium value of 4±1 Bq/l was used as a control.

For the experiment on the tritium accumulation by lemna the culture samples were taken, choosing three-leaf rosettes looking similar. To prepare the control solution, 4 ml of 100% Steinberg medium were poured into a measuring cylinder and made up to 200 ml with distilled water. The obtained solution was poured out into 4 flasks, 50 ml each. For the experiment with the water from the Yenisei, an analogous series of the control solution was prepared, where the distilled water was replaced by that from the Yenisei River.

For each of the solutions being tested, 4 ml of 100% Steinberg medium were poured into a measuring cylinder and made up to 200 ml with distilled water or with the water from the River Yenisei. In the first tested solution the following was introduced: 20 Bq (100 Bq/l), into the second - 60 Bq (300 Bq/l), into the third - 100 Bq (500 Bq/l), and into the fourth - 200 Bq (1000 Bq/l) of tritium. Four rosettes of lemna were placed into each of the flasks with the solutions prepared. The flasks were placed into the cassette of the Climatostat chamber, where the constant light and temperature of 27-28 °C were maintained.

The exposure duration was 120 hours. After that, the morphological changes in the rosettes and increment of the lemna area were analyzed. To study the changes in the increment of the lemna area, the photocamera SONY-A580 was used to take pictures employed in estimating the area with the program ImageJ. The tritium accumulation in the experiments with lemna was estimated by the tritium reduction in the water medium taken after 12, 24, 48, 120 hours after the start of the experiment in the quantity of 10 ml from each vessel.

Transformation process from H*TO* into *O*B*T* in Elodea canadensis

The processes of the HTO transformation into OBT in the plant biomass were studied upon changing the ambient temperature and light regime. For this purpose the apical shoots of the green plants (3–4 cm) were used, which were preliminarily washed with running water, the remaining water was removed using absorbent paper. The prepared plants were placed in the cylinders containing the same amount of tritium. The shoot weight was equal to 200 g. The water volume was – 1600 ml. The content of the tritium introduced amounted to 1 kBq/l. The ambient temperature was changed using a thermostat. The light regime was provided by using special chambers equipped with lamps. The experiment duration was 14 days. At the end of the experiment the content of tritium in the form of HTO and OBT was estimated.

Estimating tritium in biological samples

To estimate the total tritium in the samples it was necessary to eliminate all liquid from the samples. A weight of about 50 g dry weight was taken from the previously prepared sample. This weight was placed into a round-bottomed flask, where it was mixed with toluene chosen for stripping the azeotropic mixture. The mixture obtained was kept in a corked flask for 12 h. Then, the flask was placed into a flask heater. A special device was put onto the flask neck to strip the azeotropic mixture and separate aqueous and organic phases. This device was designed by the paper's author, L.G. Bondareva.

Stripping was performed at t ~ 70°C for 4 hours. After the separation the aqueous phase was either immediately mixed with a scintillation cocktail and prepared for the measurements or, when necessary, purified from organic impurities by distilling it with $KMnO_4$ [15] until a transparent and colorless liquid was obtained. After that an aliquot of the solution was mixed with the cocktail and prepared for the measurements.

To separate organically bound tritium (OBT) a weight of 100–150 g dry weight was used. The weight of the prepared elodea samples was placed into a round-bottomed flask to be mixed with toluene. It was kept in the flask for 8 h at room temperature.

At further sample preparation for the tritium estimation it turned out to be necessary to perform distillation 5-6 times with the addition of $KMnO_4$ until a transparent liquid sample was obtained. This was due to the fact that when stripping

the azeotropic mixture from the dry biological samples the liquid fraction being stripped was highly colored and contained a large amount of organic admixtures (for example, carotenoids) which were either formed when heating the mixture and extracted along with toluene or were present in the initial samples.

Measurement of tritium activity

The method described below is intended for measuring the volumetric activity of tritium in liquid samples by liquid-scintillation spectrometry (LSS) using a scintillation cocktail where the sample under study is dissolved. The cocktails UltimaGold AB were used. When using UltimaGold AB the ratio «sample – cocktail» can vary in a wide range, which is important for estimating tritium in ultrasmall quantities, with the quenching index being tSIE = 320 and the efficiency of tritium estimation equal to 37.7%.

Before the measurements the scintillation cocktail (V ~ 10 ml) was poured into clean vials, afterwards the cocktail was cooled down to the given temperature in the absence of light. Then, the necessary volume of the sample under study (V ~ 10 ml) was taken using a pipette and introduced into the vial. The vial was sealed and shaken until the sample was completely mixed with the scintillation cocktail. Before the measurements the mixture was kept in a dark and cool place for 24 h to stabilize the luminescence.

The standard and the background samples were prepared simultaneously with the main samples to minimize the measurement errors. The duration of the sample measurement was 8-24 hours. The tritium content in the samples under study was measured using a liquid scintillation spectrometer Quantuluse-1220, USA (The Joint Center of the Krasnoyarsk Scientific Center, SB RAS).

Calculation of OBT in plant biomass

The % portion of OBT was estimated using the equations given in [16]:

$$(\%)\ (DS) = 100 \times [m_{dry} / m_{wet}]\ (1)$$

$$(\%)\ (HTO) = 100 \times [\{^3H_{wet} - {}^3H_{dry} \times \%CB / 100\} / {}^3H_{wet}]\ (2)$$

Then,

$$OBT\ (\%) = 100 - HTO\ (\%)\ (3),$$

where DS is the content of the dry substance (%), m_{dry} is the weight of the dry substance (kg), m_{wet} is the weight of the wet substance (kg), $^3H_{wet}$ is the tritium content in the wet weight (Bq/kg), $^3H_{dry}$ is the tritium content in the dry weight (Bq/kg).

Results

Experiments with the Elodea canadensis

To estimate the tritium accumulation ability of elodea, special laboratory experiments were carried out. The main loss of tritium was observed to occur within the first 72 hours after the beginning of the experiment (~ 25%) in all the experiments. Then, tritium was additionally gained due to the biomass. The total loss of tritium amounted to ~ 44%. Table 1 presents the tritium content and distribution according to the forms of existence (HTO and OBT) in the initial biomass and after the experiment.

Table 1. The results of estimating the tritium content and its distribution according to the forms of binding with the biomass in all the experiments (n=9).

	Mean shoot length, cm	Mean shoot weight, g		Tritium content, Bq/kg (% from the total)	
		wet	dry	HTO	OBT
Initial shoot	8.0 ± 0.5	0.59 ± 0.03	0.054 ± 0.005	5.5 ± 0.1 (97 ± 1)	0.20 ± 0.05 (3 ± 1)
Shoots after the experiment	15 ± 1	0.75 ± 0.9	0.047 ± 0.009	141 ± 2 (85 ± 1)	23 ± 2 (15 ± 1)

As is seen from the presented results, during the experiment the length of the shoots increased, resulting in the natural increase of the mean wet weight. Besides the natural increase of the tritium content in the form of HTO, the actual increase of tritium content in the form of OBT was also observed: from 3 to 15%. Due to the physiological processes connected with the plant growth the interaction of tritium with biological macro-molecules is likely to occur, resulting in the transformation of tritium into the non-exchangeable form.

Thus, it is proved that at the chronic interaction of tritium with the biological samples there exist processes connected with the intensive accumulation and considerable retention of tritium in the biological structures of an organism. To reveal the possible transformation of tritium and to investigate the possible mechanisms of the transformation of HTO into OBT, experiments were carried out at different light regimes and ambient temperatures. As a result of the experiments, the dependences of the OBT content on the ambient temperature were obtained. The results are presented in Table 2.

Table 2. The results of the OBT content dependence on the ambient temperature (n=5).

T, ºC	9	15	18	20
OBT, %	2.5±0.5	7±1	14±2	24±2
T, ºC	22	25	27	30
OBT, %	29±2	32±2	21±1	15±1

The tritium content in the form of OBT in the Elodea shoots was found to strongly depend on the ambient temperature. The

optimal temperature was ~ 25 ºC (Table 2). The experiments on the influence of the light regime on the OBT content in the plants show the following (Table 3).

Since the obtained optimal conditions for the tritium transformation from HTO into OBT are not the actual ones for the investigated area of the River Yenisei, the obtained OBT content in the real biological objects is considerably lower than the one obtained in the simulation experiments.

Table 3. The dependence of the OBT content (% from the total tritium content) in the plant biomass on the light regime (n=5).

Day/night, h	24/0	18/6	16/8	12/12	6/18	0/24
OBT, %	3	6	9	15	35	22

Lemna experiments

In the experiments to study lemna the maximum loss of radionuclide was found to occur after 24 hours after the beginning of the experiments in all the introduced activities and amounted to 35 %. During the remaining time the activity was additionally gained. At the end of all the experiments in the water medium the tritium content amounted to: 8 Bq (40 Bq/l), 25 Bq (125 Bq/l), 45 (220 Bq/l) and 115 Bq (575 Bq/l), which corresponded to 40 %, 42 %, 45 % and 57.5 % from the introduced amounts of the radionuclide. Figure. 1 presents the initial samples of lemna (a), the samples at the end of the experiments: those of the control system (b) and with the maximum tritium activity in the media with the distilled water (c) and water from the River Yenisei (d).

maximum tritium content, both with the water from the River Yenisei and with the distilled water. Besides, the increase of the frond area was found in the systems with the introduced tritium activities in comparison with the control systems, especially, in the systems with the water from the River Yenisei (Table 4).

In the system with the water of the River Yenisei the area increase was higher as compared to the controls than when using the distilled water. This is likely to be connected with the dissolved substances being present in the river water, which contribute to more intensive plant growth.

Table 4. The change of the lemna frond area in the simulation systems, mm^2 (% of the area increase with respect to the control).

Introduced tritium content, Bq/l				
0 - control	100	300	500	1000
Yenisei River water				
2.1 ± 0.1 (0)	2.4 ± 0.1 (14.0 ± 0.6)	2.6 ± 0.1 (23.8 ± 0.9)	2.7 ± 0.1 (29 ± 1)	2.9 ± 0.2 (38 ± 2)
Distilled water				
2.0 ± 0.1 (0)	2.1 ± 0.1 (5.0 ±0.2)	2.1 ± 0.1 (5.0 ± 0.2)	2.2 ± 0.1 (10.0 ± 0.5)	2.4 ± 0.1 (20.0 ± 0.8)

Discussion

Tritium is known to be present in aqueous plants as a mixture of tritium water and tritium-containing organic substances (OBT) [17]. A part of tritium entering in the form of tritiated water is transformed from HTO into OBT as a result of the following processes. These are: 1) the incorporation of

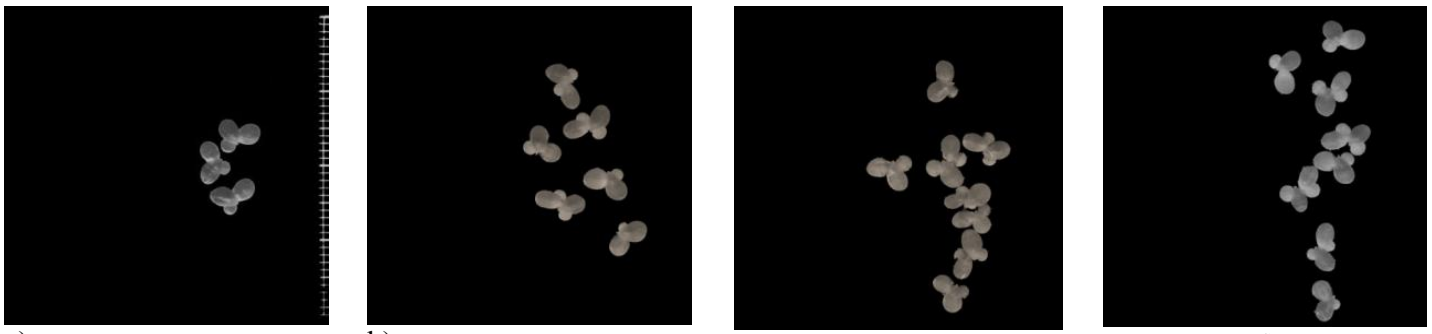

Figure. 1. Appearance of duckweed: the original (a); at the end of the experiment: Control (b); 1000 Bq / l: distilled water (c), the Yenisei River water (d)

As is seen from the presented results, despite the accumulation of tritium by the plant all the introduced activities did not influence the external appearance of lemna. No necrosis, chlorosis and other physiological changes were found. Though, the increase of the number of fronds was observed, instead of 6 in the control, there were 8 in each of the systems with the

HTO with carbohydrates into the plant biomass resulting from photosynthesis and plant growth in the presence of light; 2) metabolic processes; 3) the exchange of the mobile hydrogen atoms in organic molecules with the tritium atoms [5]. Moreover, up to 30% of tritium which is present in the form of free tritiated water in the plant biomass tissues can be transformed into OBT [5,18]. In the experiments carried

out with elodea the statement presented in the information sources was confirmed.

Besides, based on the results of the experiments with Elodea a conclusion is made that the OBT content depends on the light regime. The transformation of HTO into OBT is likely to occur predominantly in the night period due to the physiological processes connected also with the plant growth. During the day, plants transform carbon dioxide into sugars using the energy obtained from the sunlight. The sugars (including starch) are consumed by the plant itself, supplying energy for the cell division, assembly of biological macromolecules, maintaining physiological processes etc., thus incorporating incoming tritium atoms into the plant structures [19].

Since the lemna biomass is not sufficient to estimate the tritium content in it, and, moreover, to isolate and assess tritium in the form of OBT; in this case it is rather difficult to reveal any regularities concerning the influence of the temperature change and light regime on the transformation of tritium from HTO into OBT. However, it can be assumed that, as in the case with elodea, a greater portion of tritium exists in the form of HTO. Moreover, tritium binding occurs exactly in the process of the plant growth and multiplication. On the other hand, the tritium content contributes into more intensive division of fronds and their development, which is observed in the given experiments.

According to the results of the present study, the following conclusions are made:

The experiments on the tritium accumulation by the aqueous plants under study, namely, by *Elodea canadensis* and *Lemna* show the maximum radionuclide decrease to occur after the first day of the experiment. The maximum tritium absorption by the aqueous plants amounts to ~60 % from the introduced amount.

With the chronic influence of tritium in the form of HTO on the young elodea shoots the transformation of HTO into OBT occurs, as a result of the experiments on the tritium accumulation by elodea the tritium content in the form of OBT increased from 3 to 15 % from the total amount of the isolated radionuclide.

The maximum portion of tritium in the form of OBT was found in the course of the experiments carried out at ~25°C and the light regime – 6/18 (day/night). In this case the OBT portion in the plant biomass is 35 % from the total tritium content.

Upon the introduction of tritium the number of rosettes in the lemna at the end of the experiment was 8, with only 6 ones in the controls. Under the influence of tritium on the lemna fronds the increase of the frond area was found to increase by 38 % in comparison with the control in the case of using the water from the Yenisei River as the medium and by 20 % when using the distilled water.

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Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Assessment of freshwater fish seed resources for sustainable aquaculture. Edited by Melbo G Bondad-Reantaro. Food and agriculture organization of the United Nations. Rome, 2007, 630 p.

2. Strack S, Kirchmann R, Luttk'e A , Bonotto S. Selective Accumulation of Organically Bound Tritium in the Marine Unicellular Algae Dunaliella bioculata and Acetabularia mediterranea. Appl Radiat Isot. 1983, 34(5), 965-869.

3. Balanov MI. Dosimetry and rationing of tritium/ Moscow: Energoatomisdat, 1983. p. 193-230. (in Russian)

4. Report 2002. DOE: the Tritium Systems Test Assembly at the Los Alamos National Laboratory, 2002.

5. Sepall O, Mason SG. Hydrogen exchange between cellulose and water. I. Measurement of accessibility. Can J Chem.1961, 39(10): 1934-1943.

6. Bolsunovsky AY, Bondareva LG. New data on the tritium content in one of the tributaries of the Yenisei River. Doklady Biological Sciences. 2002, 385(1-6): 714-717.

7. Bolsunovsky AY, Bondareva LG. Tritium in surface waters of the Yenisei River basin. J Environ Radioact. 2003, 66(3): 285-294.

8. Bolsunovsky AY, Bondareva LG. Tritium in the waters of the Yenisei River basin in the zone of influence of the mining and chemical plant Minatom. Ecology. 2005, 5: 59-63.

9. Bondareva LG, Pomozova NV. Study the influence of different types of extinguishing the efficiency measurement of tritium in the environment. Journal of Siberian Federal University. Chemistry. 2009, 2 (1): 56–60.

10. Bondareva LG. Mechanisms of transport of tritium in freshwater ecosystems. Vestnik of the National Nuclear Center of Kazakhstan. 2011, 1, 10–23.

11. Bondareva L. Natural Occurrence of Tritium in the Ecosystem of the Yenisei River. Fusion Science and Technology. 2011, 60(4):1304–1307.

12. Simpson PS, Eaton JW. Comparative studies of the photosynthesis of the submerged macrophyte Elodea canadensis and the filamentous algae Cladophora glomerata and Spirogyra sp. Aquatic Botany.1986, 24 (1): 1-12.

13. Takhtajan AL. Life of plants (Lemnaceae). V.6. Moscow: Prosveshcheniye Publishing house, 1982: 493-500.

14. ISO/DIS 20079. Water quality – Determination of the toxic effect of water constituents and waste water to duckweed (Lemna minor) – Duckweed growth inhibition test. Reference number ISO 20079), 2005.

15. Grasshoff K, Ehrhadt MG, Kremling K. Methods of seawater analysis,. Chapter 13. The analysis of natural radionuclides in seawater/ 3rd edition. Weinheim, Germany: Verlag Chemie, 1999. 39 p.

16. McCubbin D, Leonard KS, Bailey TA, Williams J, Tossell P. Incorporation of organic tritium (3H) by marine organisms and sediment in the Severn Estuary/Bristol Channel (UK). Mar Poll Bull. 2001, 42(10), 852–863.

17. Murphy CE. Tritium Transport and Cycling in the Environment, Health Phys. 1993, 65(6): 683-697.

18. Pointurier F, Baglan N, Alanic G, Chiappini R. Determination of organically bound tritium background level in biological samples from a wide area in the south-west of France. J Environ Radioact. 2003, 68(2):171-189.

19. Bray D. Protein molecules as computational elements in living cells. Nature.1995, 376(6538): 307-312.