Review
Tritium: Doses and Responses of Aquatic Living Organisms (Model Experiments)

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Abstract: Tritium is a byproduct of many radiochemical reactions in the nuclear industry, and its effects on aquatic organisms, particularly low-dose effects, deserve special attention. The low-dose effects of tritium on aquatic microbiota have been intensively studied using luminous marine bacteria as model microorganisms. Low-dose physiological activation has been demonstrated and explained by the signaling role of reactive oxygen species through the “bystander effect” in bacterial suspensions. The activation of microbial functions in natural reservoirs by low tritium concentrations can cause unpredictable changes in food chains and imbalances in the natural equilibrium. The incorporation of tritium from the free form into organically bound compounds mainly occurs in the dark and at a temperature of 25 °C. When tritium is ingested by marine animals, up to 56% of tritium is accumulated in the muscle tissue and up to 36% in the liver. About 50% of tritium in the liver is bound in non-exchangeable forms. Human ingestion of water and food products contaminated with background levels of tritium does not significantly contribute to the total dose load on the human body.

Keywords: tritium; living organisms; dose-forming effects

1. Introduction

Tritium, 3H, is classified as a long-lived radionuclide and it can pollute the biosphere on local, regional, and global scales [1]. Tritium is one of the most biologically significant radionuclides. As a constituent of water molecules, tritium is an ideal label for studying the exchange processes between the stratosphere and the troposphere when studying air-mass transport and water cycles in nature. Tritium is present in many organic compounds, including those which are biologically important.

The global air pollution by tritium is largely a result of nuclear and thermonuclear weapons tests. The release of industry-related tritium into the environment began with the launch of industrial reactors and radiochemical production facilities intended for the development of weapons-grade plutonium in the United States. By the year 1945 (the beginning of nuclear weapons tests), the situation dramatically changed, and the content of 3H in rainwater increased by several orders of magnitude. By November 1952 (following the first thermonuclear explosion), the amount of tritium released into the Earth’s atmosphere exceeded its natural level by more than 60–190 times [2–6].

Currently, the main source of industry-related tritium is the nuclear industry. There are more than 500 large reactors operating in 35 countries around the world. Tritium is produced during the operation of nuclear reactors of all types. Its sources include fission reactions of heavy nuclei and the interactions of fast neutrons with boron, lithium, and deuterium nuclei [7]. According to [8,9], tritium discharges through liquid waste during the operation of various types of reactors, amounting to 5–130 TBq·year⁻¹. Nuclear
fuel recycling plants annually release about 600 TBq of tritium in the form of gaseous waste and about 15 PBq of tritium in the form of liquid waste into the environment. The rate of tritium formation varies in different types of reactors. For example, the fast neutron reactor at the Beloyarsk Nuclear Power Plant (NPP) releases 740 GBq of tritium per MWe per year. After 10 years of operation of the heavy water nuclear reactor, an average of 700 GBq of tritium per MWe per year was formed [10]. Tritium discharges from the VVER-1000 reactor at the Novovoronezh NPP amount to 15 ± 1.9 GBq·MWe\(^{-1}\) per year [11]. According to the data presented in [12,13], in other countries, the radionuclide release into the environment from nuclear power plants is 1.9 to 38 GBq·MWe\(^{-1}\) per year.

Several studies have indicated a local increase in the tritium content in the areas where large nuclear power centers are located. In the Russian Federation, within the Ural region, the Beloyarsk and the Mayak NPPs are major sources of industry-related tritium pollution. In addition to atmospheric radionuclide emissions, increased tritium concentrations have been recorded in the aquatic ecosystems adjacent to these plants. Thus, in the Olkhovskaya marsh–river ecosystem in the area of the Beloyarsk NPP, the tritium content in the water varies from thousands to tens of thousands becquerels per liter [14,15]. The tritium activity concentration of 1–4 Bq·L\(^{-1}\) in the water of industrial reservoirs of the Mayak NPP are not at a high level. Low concentrations of tritium (2–3 Bq·L\(^{-1}\)) can be found in precipitation in the northern hemisphere during late spring and early summer (Global Network of Isotopes in Precipitation, GNIP-IAEA) [16].

Periodic increases in tritium concentrations in waterways from the Krasnoyarsk Mining and Chemical Combine, and in some aquatic ecosystems of the Chernobyl NPP area, were recorded as compared with the technogenic background level, but did not reach the level of intervention established for drinking water by the radiation safety standards NRB: 99–7700 Bq·L\(^{-1}\) [17–19]. It is worth noting that the Mining and Chemical Combine (MCC), located by the Siberian Yenisei River, has engaged in the production of weapons-grade plutonium for several decades.

Data analysis presented in the literature regarding the radiation–chemical state of the Yenisei River identified several potential sources of tritium that contribute to the presence of tritium in the water-collecting area of the Yenisei River, namely [20,21]:

(a) Nuclear weapons tests;
(b) MCC aerosol releases;
(c) Water discharge from reactor cooling systems;
(d) Tritium migrating from open reservoirs of sedimentation tanks at the MCP industrial site;
(e) Tritium migrating from the subsurface horizons of the Severny landfill sites. It is known that up to 330,000 Bq·L\(^{-1}\) of tritium has been pumped into the subsurface horizons of the landfill site over many years.

Therefore, during the operational period of MCP nuclear reactors, there have been six potential sources of tritium entering the Yenisei River. This created a unique situation, prompting the investigation of a variety of tritium migration routes in the environment.

The term ‘tritium problem’ has increasingly been used in the contemporary literature on radiation exposure risk assessments. Radioactive beta decay of tritium leads to the disruption of molecular structures and intermolecular bonds under the action of its own beta radiation [22]. Due to the presence of tritium in surface and underground water, there is a risk of \(^3\)H migration in air and water flows. This may contribute to the entry of \(^3\)H into several trophic levels of the food chain.

This article assesses the level of the tritium impact on living organisms, including the trophic chain of water–bacteria–aquatic plants–fish, and to model the processes of human exposure to tritium-containing fish and water.

2. Effects of Tritium on Living Organisms

The radiological impact of tritium is a product of the characteristics and behavior of the radionuclide. On one hand, tritium absorption is similar to that of other hydrogen isotopes and has considerable biological significance [23]. On the other hand, some features of
tritium impart low-level radiotoxicity. Indeed, low-energy beta particles emitted by tritium have a maximum free path in water or tissue, ranging within 6 microns, which reduces risks of external exposure. Therefore, tritium is associated with radiological risk only if the human body absorbs it, especially after the ingestion of tritiated organic molecules [1,22,23].

The behavior of tritium in plants is of particular interest, because photosynthesis is a necessary step in the production of organic matter, which moves throughout the environment along food chains to its potential human consumers [3,24–27]. The radiological effects of tritium can be evaluated from observations of irradiated cells or animals. On the other hand, there are convincing data on the biological effects of tritium on plants [28–30]. No noticeable effect on biomass production was observed in experiments performed to assess the impact of tritium on various vegetables [31–35]. However, it can be assumed that DNA mutations and plant cell death may occur at very high levels of tritium impact, as seen in animals.

The results of animal experiments cannot be directly transferred to humans. However, the biological effectiveness of weak beta emissions under different irradiation conditions can also be considered in order to assess the radiotoxicity of tritium in relation to humans.

3. Effects of Tritium on Marine Bacteria

Microorganisms are the basic and the simplest organisms of aquatic ecosystems; they contribute significantly to the ecosystem equilibrium. Metabolic products of aquatic microorganisms may influence all water inhabitants.

Luminous marine bacteria constitute a suitable bioassay system for radiotoxicity monitoring in different multicomponent media. This bioassay is widely utilized in ecotoxicological monitoring [36–38]. It applies bioluminescence intensity as a physiological test parameter, which can be measured with simple devices, easily and quickly. The advantages of the bioassay are its simplicity and the high rates of assay procedure (1–10 min) [39,40]. This enables the possibility of numerous sample analyses and proper statistical processing. The high rates ensure a large number of measurements for comparable conditions, and hence, enable robust statistical processing, which is extremely important for low-dose exposures, usually described in terms of “stochastic effects” [41]. Furthermore, the rapid luminescence response is supposed to indicate the non-genetic mechanism of low-intensity exposures [42,43].

Previous studies [44–48] have demonstrated both the activation and inhibition effects of tritium on marine bacteria (Figure 1A), as well as the absence of a monotonic dependence of the luminescence response on tritium concentration at chronic low-dose exposure (<0.03 Gy), in a wide range of tritium radioactivity from 0.0001 to 200 MBq·L⁻¹ (Figure 1B). Intact and lyophilized bacterial suspensions were studied and exhibited similar results. The results obtained were explained in terms of the ability of the bacterial cells to adapt to the low-dose radiation, based on the “hormesis” model. The term “hormesis” was introduced by H. Schulz and R. Arndt at the end of the 19th century [49]. The current development of the hormesis concept is attributed to E. Calabrese [50–54]. The term “radiation hormesis” was suggested by Luckey [55]. The phenomenon of radiation hormesis was intensively studied in [52,56,57].

An increase in the bacterial luminescence intensity in the presence of tritiated water, HTO, was demonstrated in a series of experiments where bi-phasic dependence (activation + inhibition) was found in [44,46], whereas mono-phasic dependence (activation only) was shown in [42,58,59]. The results were analyzed in reviews [47,48].

The mechanism of the activation effect of tritium is of special interest. The first hypothetical mechanism is based on DNA damage repair [60–62]. The involvement of non-genetic mechanisms in low-dose chronic radioactive effects in bacteria was demonstrated in [42,59].

In addition, it was shown that tritium activated bacterial growth at the activity levels of 10–10⁴ kBq·L⁻¹ and suppressed this growth at higher activities (>10⁵ kBq·L⁻¹) [45].
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etc.

through the reaction between $O_2$ and electron donors such as metal centers, dihydroflavin, etc. [63,64]. It is known that peroxide derivatives of flavin molecules are native intermediate compounds of the bacterial bioluminescence reaction [65,66]. It is suggested that the oxygen-detoxifying function directed the emergence of many bioluminescent systems, including bacterial bioluminescence [67]. This suggestion provides an additional reason to study the ROS balance in radioactive bacterial environments.

Previously, the authors demonstrated that low-intensity tritium exposure did not noticeably increase the content of reactive oxygen species (ROS) in bacteria-free media [46]; hence, the non-biological production of ROS could hardly be responsible for this effect. Subsequently, it was confirmed that the rates of ROS production were low in highly diluted tritiated water in the absence of bacteria [58]. This result can be explained by the low-energy radioactive decay of tritium. However, the exposure of marine bacteria to chronic low-dose tritium irradiation in highly diluted tritiated water (<0.08 Gy) considerably increased the ROS content in the bacterial environment (up to 300%). A comparison of the low-dose effects of tritium (0.03, 4.0 and 500 MBq·L$^{-1}$) on the luminescence of marine bacteria and ROS content in a cellular water suspension was performed in [58]. It was demonstrated that an increase in the ROS concentration correlated with the intensification of the physiological bioluminescence process in the bacteria during the bacterial lifetime.

Marine luminous bacteria are appropriate bio-objects for studying the biological effects of radiation, and particular attention should be paid to their metabolites, particularly ROS, because they can suppress or activate physiological functions of marine microorganisms. It is known that marine bacteria form ROS endogenously in aerobic environments through the reaction between $O_2$ and electron donors such as metal centers, dihydroflavin, etc. [63,64]. It is known that peroxide derivatives of flavin molecules are native intermediate compounds of the bacterial bioluminescence reaction [65,66]. It is suggested that the oxygen-detoxifying function directed the emergence of many bioluminescent systems, including bacterial bioluminescence [67]. This suggestion provides an additional reason to study the ROS balance in radioactive bacterial environments.

HTO molecules can easily penetrate a cell membrane. Therefore, products of tritium radioactive decay, electrons and ions of helium-3, may affect the bacterial structure, from the outer membrane to the enzymes and their substrates inside the cells. Thus, the stimulating effect of $^3$H can be associated with the process of electron/ion transfer in intracellular bacterial structures, leading to increased rates of bioluminescent enzymatic reactions. The same processes inhibit the luminescence function of bacteria at longer exposure times.

An important issue is the interaction of tritium decay products with bacterial cells. Considering the concentration of cells and the number of ion pairs formed during the decay of tritium (up to 200 ions and/or excited molecules), the number of ion pairs per cell was calculated: 3.33, 0.0266, and 0.0002 ion pairs·(cell·s)$^{-1}$ for the tritium activity concentrations of 500, 4, and 0.03 kBq·mL$^{-1}$, respectively [58]. This low value of ion pairs per cell suggests a specific mechanism underlying the effect of tritium on the cells. It is likely that tritium decay products can serve as “triggers” for enhancing the metabolic oxygen-dependent processes in cells, which result in ROS increase within the cell. On the other hand, ROS

![Figure 1. Effect of tritiated water on the bioluminescence of bacteria [47,48]. (A) Bioluminescence kinetics of bacteria in tritiated water, 2 MBq L$^{-1}$; the red arrows denote the times of exposure (20 and 50 h); (B) bioluminescence intensity vs. activity concentration of tritiated water at 20 h (black) and 50 h (green) exposures.](image-url)
released into the environment can serve as specific signaling molecules for other cells (the so-called “bystander effect”).

It was shown [68–70] that for human cells, the bystander effect can be induced even by one cell in a thousand-cell population, and it does not depend on the number of the initially induced cells (one cell or 50% of cells). Additionally, it should be noted that the absence of dose–response dependence is in accordance with the concept of the “stochasticity” of low-dose radiobiological effects [71,72]. This concept assumes the involvement of free radicals and ROS in radiobiological responses.

The results of studying tritium effects on luminous marine bacteria present a physicochemical approach to understanding the metabolic functioning of bacteria and their interactions with aqueous environments, which can affect other water inhabitants [42–48,58,59]. Based on the rapid luminescence bioassay, the studies considered only short-time exposures and did not reveal mutagenic effects or the cellular accumulation of tritium. The results of another study [71] show the accumulation of tritium in phytoplankton populations of *Dunaliella tertiolecta* and *Nodularia spumigena* and mussels *Mytilus edulis*. The paper considers the scenarios where tritium may be concentrated or transferred in biota relevant to Baltic coastal communities.

Hence, the activation of microbial functions and accumulation of tritium in marine organisms introduced into food chains are highlighted in the scientific literature. The processes mentioned may result in an imbalance in the natural equilibrium in water ecosystems.

It is important that molecular surroundings in natural aquatic ecosystems might change the effects of tritium on microorganisms. It was reported that humic substances, products of natural transformation of organic matter in water sediments, mitigate activating and inhibiting effects of tritiated water on marine bacteria [73,74], whereas gold nanoparticles can additionally suppress physiological functions of the microorganism (so called, radio-sensitizing effect) [75].

4. Effects of Tritium on Aquatic Plants

Aquatic organisms incorporate tritium as a fraction of TFWT (tissue-free water tritium) contained in organic compounds as a result of isotopic exchange or enzyme-catalytic reactions [28]. In exchange reactions, tritium binds to oxygen, sulfur, phosphorus, and nitrogen atoms to form hydroxides, thiols, phosphides, and amines, respectively. Usually, this produces exchangeable, organically bound tritium (OBT), which is in equilibrium with TFWT in the studied plants or animals and behaves similarly to HTO. Tritium binds to the carbon skeleton of organic molecules during enzymatically catalyzed reactions. In this case, non-exchangeable tritium is formed. This is a form of tritium that remains in dry biological matter even following repeated washes with light water.

It is necessary to have a clear definition of OBT formed from TFWT in living systems through natural ecological or biological processes. This has been accomplished within the framework of the International Atomic Energy Agency (IAEA) in the Environmental Modelling of Radiation Safety (EMRAS) programm [28,71,76–78].

Previously, a study was carried out on the assimilation and transformation of tritium in the aquatic plants *Elodea canadensis* and *Lemna minor* [79].

Branched shoots of *Elodea canadensis* can reach lengths of up to 100 cm long. They were sorted out according to the biomass quality. Apical shoots from 3 to 6 cm in length from the entire biomass were used for the study.

The *Elodea* biomass washed was used for accumulating tritium and estimating TFWT and OBT in the control point (the initial point). For this purpose, the initial biomass was divided into two parts. In one of them, tritium (in HTO form) was extracted from an aliquot of 50 g taken from one part of the biomass. TFWT can be measured by azeotrope distillation of the fresh sample, then liquid scintillation spectrometry–total OBT (exchangeable and non-exchangeable) combustion in oxygen atmosphere of dry sample, purification of combustion water, liquid scintillation spectrometry [25].
The *Lemna* samples were grown in Climatostat apparatus for one week using Steinberg medium (ISO/DIS 20079, 2005). The algae cultures were grown in a cultivator, KB-05, designed at the Siberian State University (Krasnoyarsk, Russia). 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.

For the experiment on the tritium accumulation by *Elodea*, the plant shoots (~5 cm) were placed in an aquarium and completely submerged in tritium-containing water (250 g of the plants were placed into 2500 mL HTO). A photoperiod of ~12 h was set. The duration of the experiment was 11 days, followed by measurement of the shoot length. Water from the Yenisei River with the background tritium levels of 4 ± 1 kBq·L$^{-1}$ was used as a control.

For the experiment on tritium accumulation by *Lemna*, culture samples were taken by picking similar three-leaf rosettes. For each of the solutions being tested, 4 mL of 100% Steinberg medium was poured into a measuring cylinder and filled to 200 mL with distilled water or with water from the Yenisei River. Thus, near 20 Bq (100 kBq·L$^{-1}$) of tritium were introduced into the first tested solution, 60 Bq (300 kBq·L$^{-1}$) into the second, 100 Bq (500 kBq·L$^{-1}$) into the third, and 200 Bq (1000 kBq·L$^{-1}$) into the fourth. Four rosettes of *Lemna* were placed into each flask with the prepared solutions. The flasks were placed into the cassette of the Climatostat chamber, where conditions of constant light and a temperature of 27–28 °C were maintained.

The processes of the TFWT 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 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$^{-1}$. The ambient temperature was changed using a thermostat. The light regime was provided by 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 TFWT and OBT was estimated.

To estimate the total tritium in the samples, it was necessary to eliminate all the liquid. An aliquot of about 50 g dry weight was taken from the previously prepared sample. This aliquot 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. To separate OBT, an aliquot of 100–150 g dry weight was used. The aliquot of the prepared *Elodea* samples was placed into a round-bottomed flask to be mixed with toluene [77,78].

The tritium content in each of the biological samples (plants or fish) under study was measured using a liquid scintillation spectrometer, Quantulus-1220, USA (The Joint Center of the Krasnoyarsk Science Center, SB RAS), using a scintillation cocktail, UltimaGold AB, where the sample under study was dissolved. The background determined for the prepared tritium-free water samples ranged between 0.926 CPM and 1.002 CPM, and the counting efficiency, using the internal standard method (ISO 9698:2010), was between 25.3% and 26.1% for the maximum figure of merit.

Table 1 shows data on the content and distribution of tritium according to the occurrence forms (tissue-free water tritium (TFWT) and organically bound tritium (OBT)) in the initial biomass and after the experiment with *Elodea*.

These results confirm the fact that during the chronic interaction of tritium with aquatic plants, processes occur which are associated with the intensive accumulation and retention of tritium in the biological structures of living organisms.

When conducting model experiments to study the influence of environmental parameters on tritium transformation, a dependence of OBT content on ambient temperature was obtained. It was found that the proportion of tritium in the form of OBT in the *Elodea* shoots
strongly depended on the ambient temperature, optimal temperature (~25 °C; Figure 2), and illumination. A large proportion of TFWT transformation into OBT was obtained in the light/shadow mode—6/18 h (Table 2).

Table 1. Results of tritium content estimation and its distribution with regard to the forms of binding to the Elodea biomass (n = 10, p = 0.95).

<table>
<thead>
<tr>
<th>Plants</th>
<th>Average Length, cm</th>
<th>Average Weight, g</th>
<th>Tritium Concentration, Bq·kg⁻¹ (% of Tritium Accumulated in the Biomass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>8.0 ± 0.5</td>
<td>0.59 ± 0.3</td>
<td>TFWT: 5.5 ± 1.1 (97 ± 1); OBT: 0.20 ± 0.05 (3 ± 1)</td>
</tr>
<tr>
<td>Following exposure</td>
<td>15 ± 1</td>
<td>0.75 ± 0.9</td>
<td>TFWT: 141 ± 2 (85 ± 1); OBT: 23 ± 2 (15 ± 1)</td>
</tr>
</tbody>
</table>

Table 2. OBT content (% of the total tritium content) in the plant biomass as a function of the illumination mode (n = 5, p = 0.95).

<table>
<thead>
<tr>
<th>Light/Dark, Hours</th>
<th>24/0</th>
<th>18/6</th>
<th>16/8</th>
<th>12/12</th>
<th>6/18</th>
<th>0/24</th>
</tr>
</thead>
<tbody>
<tr>
<td>OBT, %</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>15</td>
<td>35</td>
<td>22</td>
</tr>
</tbody>
</table>

Figure 2. Dependence of the proportion of the OBT content (percentage from the tritium activity accumulated in the biomass) in the plant biomass on the ambient temperature.

Figure 3 shows the initial duckweed samples (a) and samples at the end of the experiments: control (b), samples with the maximum tritium activity in the media with distilled water (c) and those with the Yenisei River water (d).

Figure 3. Appearance of the duckweed: initial (a); at the end of the experiment: control (b), 1000 Bq·L⁻¹: distilled water (c); water of the Yenisei River (d).

No necrosis, chlorosis, or other physiological changes were found. There was an increase in the number of leaves: instead of six leaves as in the control, there were eight leaves in each system with the maximum tritium content, both with the water of the Yenisei River and with distilled water. An increase in the surface area of leaves in the systems
with the introduced tritium activities was observed as compared with the control systems, especially in the systems with the water of the Yenisei River (tritium activity ~4 Bq·L\(^{-1}\)).

Based on the experimental results, it was concluded that the OBT proportion depended on the mode of illumination. The TFWT transformation into OBT occurred mainly as a result of physiological processes associated both with photosynthesis and plant growth. During the day, plants use energy from the sunlight to convert carbon dioxide into sugars. The latter (including starch) are subsequently consumed by the plant itself, supplying energy for cell division, the assembly of biological macromolecules, maintenance of physiological processes, etc. This embeds the incoming tritium atom into the plant structures.

5. Studying the Effect of Tritium on the Physiological Functions of the Submerged Aquatic Plant *Elodea canadensis*

To study the toxic effect (via phototaxis) of tritium on shoots of the submerged macrophyte *Elodea canadensis* [79], a sample of plant shoots was used. Before studying the phototaxis of chloroplasts, glasses containing *Elodea* shoots were kept in the dark for 15–30 min. Phototaxis was observed on the upper surface cells of the leaves under the light microscope “Mikmed-2” (LOMO, Russia) at 600\( \times \) magnification. A leaf was separated from the shoot, placed under a cover slip, and exposed to a lower illumination lamp for 3–5 min on the microscope slide table. Under intense illumination, the chloroplasts adopted a parastrophic position (perpendicular to the incident light rays), shifted to the cell edges, they encircled the cell. The proportion of active chloroplasts was calculated as a ratio of the number of chloroplasts in the parastrophic position shifted to the cell edges, to the total number of chloroplasts in the cell. For each concentration, ten randomly selected cells from one leaf were examined, in one or three replicates. To measure chlorophyll epifluorescence, an *Elodea* leaf was placed under a cover slip and a drop of simazine herbicide (C\(_7\)H\(_{12}\)ClN\(_5\), 10\(^{-5}\) M) was introduced to block the non-cyclic electron transport between the two photosystems. Epifluorescence was measured using a Lumam-I and FMEL microscope (LOMO, Russia), excited by light with a wavelength of 410 nm in a circle with a diameter of 45 \(\mu\)m and with an average cell size of 60 \(\mu\)m \(\times\) 70 \(\mu\)m. On each leaf, ten fields were measured with random movement of the preparation, in one or three replicates. The results are presented as mean values or weighted mean values. The significance of the differences in the mean values was calculated according to the Student’s t-test.

The following aqueous media were used: water from the Yenisei River, to which tritium with different activity was introduced, and water collected from Atomic Lake (Semipalatinsk test site (STS), Kazakhstan).

Reference: Atomic Lake. The power of all excavating explosions at STS with soil displacement was 140 kt; 99% of the power of these explosions occurred in a single explosion performed in Well 1004 on January 15, 1965, at the confluence of the Shagan and Aschisu rivers, at a depth of 178 m. As a result of the explosion, long-lived radionuclides were accumulated: \(^{239,240}\text{Pu—8.5 Ci}, \quad ^{137}\text{Cs—800 Ci}, \quad ^{60}\text{Co—80 Ci}, \quad ^{152}\text{Eu—120 Ci}, \quad ^{90}\text{Sr—400 Ci}, \quad ^{3}\text{H—4·10}^5\text{ Ci}\) [80]. Between 30% and 40% of the accumulated number of radionuclides entered the bulk soil.

As a result of soil displacement during the explosion, a funnel with a diameter of 430 m along the initial surface was formed; the height of the bulk ridge was 20–35 m, and the bulk width from the funnel ridge was 400 m. The volume of the visible funnel was more than 10 million \(\text{m}^3\) from the bulk ridge and 6 million \(\text{m}^3\) from the initial surface. With spring flood waters filling the funnel, two large reservoirs were formed: the inner one in the funnel with the volume of 7 million \(\text{m}^3\) and water surface area of 0.5 km\(^2\), and the outer one in the floodplains with the volume of 10 million \(\text{m}^3\) and water surface area of 3.5 km\(^2\). The reservoir waterfront was formed by an earth-and-rockfill dam, a rockfill dam with a bottom discharge and a surface flood spillway. The constructed reservoir was the first hydroelectric facility of this kind, and it was considered to be a hydrotechnical test site.
We determined the metal content by ICP-MS, and the anion content by ionic liquid chromatography. To estimate the tritium content in the samples under study we used the procedures described above.

The tritium content in the water was $\sim 5.6$ kBq·L$^{-1}$. In addition, the water contained (mg·L$^{-1}$): Na—1100; Cl—1700; Mg—160; S—350; Ca—94; Sr—6.4; K—4; Br—1.7; Si—0.58; B—0.55, and (µg/L): Cu—87; I—52; Ba—22; U—16; Al—12; Mo—9.2; P—7.6; Ti, Ni, As, Se ~3; R—1.1. The main anions in the water from Atomic Lake were as follows (mg·L$^{-1}$): HCO$_3^-$—214; Cl$^-$—1430; SO$_4^{2-}$—1290.

Figure 4 shows the images obtained during the experiments on the effect of tritium on the photosynthesis of *Elodea*.

![Figure 4](image-url)

**Figure 4.** Results of the tritium action on the movement of chloroplasts of the *Elodea* leaves in the experiments with water from the Yenisei River—14 days, (a-c): (a) control (~4 Bq·L$^{-1}$), (b) +100 Bq·L$^{-1}$, (c) +2900 Bq·L$^{-1}$; and with water from Atomic Lake—5 days, (d): tritium content of 5600 Bq·L$^{-1}$ in the mixture with other radionuclides.

It was found that when the system with the water from the Yenisei River contained tritium, a lethal effect occurred at a tritium concentration of $\sim 3000$ Bq·L$^{-1}$, 29 days after the beginning of the experiment. In addition, significant damage to the integrity of the cell membranes was observed, followed by leakage of the intracellular fluid (Figure 3). Despite the external well-being of the shoots used, it was observed under a microscope that at a tritium content of $\sim 100$ Bq·L$^{-1}$, the movement of chloroplasts decreased by 70% in 29 days, and by 25% at a content of $\sim 10$ Bq·L$^{-1}$.

In the experiments with the water samples from Atomic Lake, the movement of chloroplasts decreased by 40% from the initial value five days after the start of the experiment. In this case, the lethal effect would occur much earlier because the radiation–chemical composition of water is quite complex and diverse.

Chlorophyll fluorescence is widely used for biotesting as an activity indicator of the photosynthetic apparatus of aquatic plants [79,81–83]; epifluorescence microscopy has previously been used for detecting chlorophyll complexes with heavy metals in vivo [82,83]. It is assumed that the decrease in chlorophyll fluorescence under the action of tritium probably occurs as a result of the substitution of a fraction of the protium atoms in the chlorophyll molecule “a” with tritium atoms.

In contrast to fluorescence, the phototaxis reaction of chloroplasts is rarely used for biotesting, although it is often used for duckweed [81–84].

The movement of chloroplasts in duckweed cells in light was experimentally shown to not only occur as a result of the light effect on photosynthesis and respiration [81]. The movement of chloroplasts in cells involves the participation of actin-myosin proteins, ATP, photosynthesis, and other processes [82–84]. Therefore, as compared with epifluorescence, phototaxis is a more integral reaction. Our experiments show that a certain decrease in the epifluorescence signal under the action of tritium occurs faster than the inhibition of chloroplast phototaxis. One of the main advantages of the phototaxis reaction in terms of biotesting appears to be the absence of the need for control. Under favorable conditions, the phototaxis of chloroplasts should be 100%. In contrast, the lethal effect is accompanied by the complete extinction of phototaxis.
Consequently, the effect of tritium on the biological functions of *Elodea* with the chronic radionuclide intake is observed at a tritium content of $2900 \text{ Bq L}^{-1}$, which is several times lower than the intervention level established by NRB-99 ($7700 \text{ Bq L}^{-1}$).

6. Effects on the Development of Phytophagous Freshwater Prussian carp

Among living organisms in polluted reservoirs, fish are the most suitable objects of study due to their biological peculiarities that make it possible to assess the medium-term transformation processes of water reservoirs. Fish are sensitive to a wide variety of direct impacts and integrate the adverse effects of the entire range of different impacts, including impacts on other components of the aquatic ecosystem (habitat, macroinvertebrates, primary products, etc.). The dose loads on fish bodies are formed due to external radiation (from water and bottom sediments) and, internal radiation (from incorporated radionuclides) [5,85–88].

The embryonic period of development does not end when the embryo exits the shell; it includes a period of time after hatching, while the sac fry, with a number of embryonic peculiarities related to the structure of respiratory, circulatory and digestive organs, goes through the final stages of embryonic development.

To estimate the tritium content in the samples under study, we used the procedures described above. In the experiments, we utilized water from the Yenisei River (Table 3), which differs in terms of the level of added tritium activity, and the water from Atomic Lake (Table 3). At the end of the study, the number of dead eggs, number of fries with developmental abnormalities, and the number of fries with the approximately normal physiological development were calculated in each experimental system (Table 3).

### Table 3. Effects of the tritium water on the development of carp eggs 25 days after starting the experiment ($p = 0.95$).

<table>
<thead>
<tr>
<th>Tritium Concentration, Bq L$^{-1}$</th>
<th>Initial Eggs, Units</th>
<th>Dead Eggs, Units (%)</th>
<th>Eggs with Abnormal Development Units (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water from the Yenisei River</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>3500 ± 150</td>
<td>280 ± 20 (9)</td>
<td>250 ± 15 (7)</td>
</tr>
<tr>
<td>500 ± 10</td>
<td>7600 ± 200</td>
<td>850 ± 15 (11)</td>
<td>750 ± 20 (10)</td>
</tr>
<tr>
<td>5000 ± 18</td>
<td>3500 ± 100</td>
<td>700 ± 10 (20)</td>
<td>1100 ±100 (30)</td>
</tr>
<tr>
<td>50,000 ± 57</td>
<td>4400 ± 200</td>
<td>950 ± 30 (20)</td>
<td>1400 ±50 (30)</td>
</tr>
<tr>
<td>Water from Atomic Lake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5600 ± 28</td>
<td>5800 ± 250</td>
<td>3900 ± 200 (67)</td>
<td>1500 ±170 (26)</td>
</tr>
</tbody>
</table>

According to the assessment of external physical characteristics, at the level of the tritium impact approximately equal to $5000 \text{ Bq L}^{-1}$ or exceeding (6.5 times) the level of intervention ($7700 \text{ Bq L}^{-1}$), a clear radiation effect was manifested only at the stage of egg development. In this case, the proportion of the dead eggs and fry with abnormal development in the systems with $5000 \text{ Bq L}^{-1}$ and $50,000 \text{ Bq L}^{-1}$ was comparable.

The system with the water from Atomic Lake stood out to a large extent. In this case, the number of the dead eggs reached 67%, while 26% of the eggs showed abnormal development.

Most fry from the system with the addition of $50,000 \text{ Bq L}^{-1}$ were placed in a clean medium (clean water, pure feed), where they remained for breeding and subsequent use. The fry from the model systems with the additions of $500 \text{ Bq L}^{-1}$ and $5000 \text{ Bq L}^{-1}$ were not used in further studies.

The remaining fry from the system with the water from Atomic Lake were also placed in a clean system for nursery purposes. However, all the fry died within ten days.

Table 4 shows the results of changes in the length of the fries under nursery conditions in clean water where pure feed was used as food. In such conditions, the fry were kept for 150 days before entering the third stage of the experiment.
Table 4. Changes in the length of the fish fry in relation to the control system \((p = 0.95)\).

<table>
<thead>
<tr>
<th>System</th>
<th>Age of Fish, Days</th>
<th>Units</th>
<th>Length, mm</th>
<th>Average (Difference in Relation to Control, %)</th>
<th>Range, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 50,000 Bq L(^{-1})</td>
<td>35</td>
<td>800</td>
<td>17 ± 1</td>
<td>16.8–17.4</td>
<td>17.6–18.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1050</td>
<td>18 ± 1</td>
<td>(1.1%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.3 ± 2.8</td>
<td>27.8–28.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.4 ± 2.4</td>
<td>28.0–28.9</td>
<td></td>
</tr>
<tr>
<td>Control 50,000 Bq L(^{-1})</td>
<td>76</td>
<td>500</td>
<td>65.7 ± 6.6</td>
<td>65.0–67.0</td>
<td>69.6–77.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>70.6 ± 6.7</td>
<td>(9.7%)</td>
<td></td>
</tr>
<tr>
<td>Control 50,000 Bq L(^{-1})</td>
<td>125</td>
<td>206</td>
<td>80.3 ± 8.6</td>
<td>88.3–92.2</td>
<td>70.1–93.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>203</td>
<td>81.7 ± 7.9</td>
<td>(2.2%)</td>
<td></td>
</tr>
<tr>
<td>Control 50,000 Bq L(^{-1})</td>
<td>150</td>
<td>75</td>
<td>17 ± 1</td>
<td>16.8–17.4</td>
<td>17.6–18.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>97</td>
<td>18 ± 1</td>
<td>(1.1%)</td>
<td></td>
</tr>
</tbody>
</table>

The maximum difference in fry length was detected only on day 125 (9.7%); however, on day 150, this difference diminished by several times.

After 150 days, the carp fry were placed in large aquariums, with clean filtered water from the Yenisei River. At this stage of the experiment, the accumulation and distribution of tritium that entered the body with food were studied. The pre-used feed had been enriched with tritium during the course of the previous experiments on the accumulation of tritium by aquatic plants [74,84]. Previously, the tritium-containing plants (~1.5 kBq kg\(^{-1}\)) were dried at ~45°C in a nitrogen atmosphere to prevent the tritium exchange with the laboratory atmosphere. The anhydrous concentrates obtained were mixed with dry fish fodder (1:1), and the mixture was pressed into 0.3 g granules. The tritium amount in each granule was ~50 Bq, and the total amount of radionuclide fed daily in each experimental system was up to 600 Bq. The maximum amount of the introduced tritium was up 300 kBq per individual [78,89].

Table 5 shows the data on the changes in the morphological parameters of the studied fish during the last stage of the study.

Table 5. Changes in the morphological parameters of the studied carp specimens \((n = 10, p = 0.95)\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial</th>
<th>250 Days</th>
<th>550 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass, g</td>
<td>120 ± 17</td>
<td>500 ± 55</td>
<td>800 ± 110</td>
</tr>
<tr>
<td>Length, cm</td>
<td>17 ± 3</td>
<td>35 ± 5</td>
<td>60 ± 6</td>
</tr>
</tbody>
</table>

From the results presented, it is evident that the fish developed quite well. This is indicated by the increase in the weight and length of the specimens.

Due to the fact that the welfare conditions for the fish were more favorable (sufficient food, optimal lighting and temperature, and water circulation in the aquariums) compared with natural conditions, a fairly rapid weight gain was observed [90,91].

The methods from [92] were used for the complete ashing and isolation of lipids and proteins from the fish. The main muscle mass components (%; \(n = 10\)) were: proteins—16 ± 1%, water—70 ± 2%, fat—13 ± 1%, ash—1%.

Following the conclusion of experimentation, we obtained the following results: the muscle tissue accumulated a large proportion of the tritium absorbed by the whole fish (up to 56%), although the proportion of the accumulated tritium did not depend on the feed consumed by the fish. In both cases, about 35% of the total tritium accumulated by the fish was bound to the liver.
The distribution of OBT in the parts of the fish body was considered in a number of studies [93–96]. However, our research deals with the total tritium content, i.e., the total sum of TFWT and OBT [74,86]. These experiments are presented in detail in prior research [77,78,89]. The consumption of fish products is increasing all over the world; the quantity and quality of this product depends on the type of fish and on its origin (growing in natural or artificial conditions). Quantitatively, the content of the muscle tissue of each specimen varies within 40–50% of the total weight of the fish [92]. Therefore, when fish is consumed by humans, the main dose-forming part is the muscle tissue.

The obtained results did not reveal any differences in the radionuclide accumulation in the systems with duckweed and hornweed. Hence, it was concluded that both of these plants were equally digested in the digestive organs of carp intentionally fed with pellets containing plants with tritium.

In the course of the studies, it was revealed that tritium accumulated in the liver, mainly binding to adipose tissues which included lipids (~39%). A slightly lower amount of tritium was bound to proteins (~19%). The smallest amount of tritium was found in the form of free water (~3% of the total tritium content in the liver) [85]. The unrecorded tritium content accumulated by the liver was bound to unidentified substances in the liver.

The liver has a very high level of metabolic activity. Food lipids are re-esterified in the liver and are carried to other tissues and fat depots by the blood. The lipids that are mobilized from the depot are again transferred to the liver, which is the main site of their oxidation and synthesis [30]. An increase in the level of the lipid-bound tritium in the muscle mass of fish, up to 39% of the tritium content in the whole fish, and a greater contribution of lipids included in the liver due to the accumulation of tritium (~39% of the total tritium content in the liver), were found.

The quantity of phospholipids in the tissues, as compared with other fractions, was lower as related to the physiological state of the fish. It did not depend on feeding habits. The welfare conditions for all the fish were the same, and the content of phospholipids had the least variability, which also affected the proportion of tritium binding by this fraction, the maximum being 7.9%.

The use of cholesterol in biosynthesis processes and in the regulation of membranes motivates assessing the proportion of tritium binding. In the experiments conducted, cholesterol had the greatest contribution to the accumulation of tritium (~30%). Considering cholesterol esters, the proportion of tritium binding reached 50% (in terms of the dry weight) of all the tritium accumulated by the liver.

During the period of intensive weight uptake, lipids are accumulated in the depot in the form of triacylglycerols [32,92]. The feeding and welfare conditions for the fish specimens did not change for the entire duration of the experiment; therefore, the content of triacylglycerols did not decrease, in contrast to the fish in natural conditions, and continued to accumulate. This was indicated by the overall increase in the mass of the fish muscle tissue, especially by the increase in lipid content. This pattern also manifested in the liver. Therefore, the increase in the tritium content in triacylglycerols has a direct relationship with the content of lipids in the liver.

Thus, the accumulation of tritium in the liver occurs due to the formation of non-exchangeable organically bound forms (more than 50% of the total tritium accumulated by the liver).

An increase in the proportion of non-exchangeable organically bound tritium, both in the liver and in the whole body, increases the radiotoxicity of tritium as compared with tritium water (TFWT).

Another important parameter is the determination of the biological half-life of tritium for carp, including the consumption of food enriched with tritium. For this purpose, three fish specimens were placed in clean water after 250 days from the tritium accumulation experiment. For 300 days, the fish were fed with pure tritium-free food. At the end of the experiment, the fish were also dissected. The tritium content was determined at each
stage. According to the recommendations given in the paper by Melintescu et al. [26], the half-lives of tritium were calculated for fish organs.

The results obtained showed that the decrease in tritium varied from 27% to 65%, depending on the organ under study (Table 6). The average value for the half-life for the whole fish can be assumed to be 175 days, whereas the half-life for the liver is 550 days.

Table 6. Determination of the half-life period of tritium from the model fish (n = 3, p = 0.95).

<table>
<thead>
<tr>
<th>Organ</th>
<th>250 Days</th>
<th>550 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>1.0 ± 0.1</td>
<td>&lt;MDA</td>
</tr>
<tr>
<td>Bones + head</td>
<td>&lt;MDA</td>
<td>&lt;MDA</td>
</tr>
<tr>
<td>Skin + fins + scale</td>
<td>2.0 ± 0.4</td>
<td>&lt;MDA</td>
</tr>
<tr>
<td>Liver</td>
<td>6.0 ± 0.4</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Intestine with contents</td>
<td>8.0 ± 0.6</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>Stomach with contents</td>
<td>9.0 ± 1.0</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>Muscle tissue</td>
<td>74.0 ± 3.0</td>
<td>33.0 ± 4.0</td>
</tr>
</tbody>
</table>

Thus, the accumulation of tritium in the liver occurs due to the formation of non-exchangeable forms strongly bound to organic compounds (more than 50% of the total amount of tritium accumulated by the liver). An increase in the proportion of non-exchangeable organically bound tritium, both in the liver and in the whole body, increases the radiotoxicity of tritium as compared with tritium water (TFWT).

It is known that the effects of tritium and its compounds on the human body are characterized by a number of peculiarities, one of which is its ability to be incorporated in the composition of biologically active molecules. Tritium can replace hydrogen in DNA molecules, which may lead to an increase in its biological half-life and, accordingly, to an increase in the risk of the long-term effects of radiation including carcinogenic risk [97–100].

To calculate the radiation doses, we considered the following: eggs with the introduction of the maximum tritium content (50,000 Bq L⁻¹) and fish with the longest exposure interval (550 days). Both external and internal radiation was calculated, according to the IAEA recommendations [85–87] (Table 7). Specifically, we used the values of the total tritium content in the eggs and fish according to the recommendations provided in the literature.

Table 7. Radiation doses for the eggs and fish.

<table>
<thead>
<tr>
<th>Types of Irradiation</th>
<th>Eggs</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\mu$Gy hour⁻¹</td>
<td>$\mu$Gy 25 Days⁻¹</td>
</tr>
<tr>
<td>Internal</td>
<td>$5.36 \times 10^{-7}$</td>
<td>$3.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>External</td>
<td>$4.82 \times 10^{-6}$</td>
<td>$2.9 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

7. Conclusions

Tritium is a by-product of many radiochemical reactions in the nuclear industry, and its effects on water organisms, particularly low-dose effects, deserve special attention. In the past few decades, the low-dose effects of tritium on aquatic biota have been studied in detail using luminous marine bacteria as model microorganisms, as well as during the transformation of fresh tritium water to organically bound tritium. The efficiency of activation and inhibition processes has been demonstrated and studied using cellular and enzymatic systems. Low-dose activation can be explained by the “trigger” function of the products of tritium decay for bacterial metabolic oxygen-dependent processes and by the signaling role of reactive oxygen species (ROS) in the “bystander effect” in bacterial suspensions. ROS are supposed to serve as intercellular messengers in the latter process.

Briefly, despite the low energy of tritium decay, its influence on living organisms in water environments may be considerable. The activation of microbial functions in natural
reservoirs, due to the low content of tritium, may result in unpredictable changes in food chains and an imbalance of natural equilibria.

The accumulation of tritium in the liver occurs mainly due to the formation of organically bound tritium, and, for the most part, these compounds are non-exchangeable. This leads to the fact that the half-life of tritium in the liver is the longest and reaches 550 days.

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