Impact of Tritium on Living Organisms

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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.

tritium living organisms dose-forming effects

1. Introduction

Tritium, ^{3}H, 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 ^{3}H 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][3][4][5][6].

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 [7]. 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][7][8].

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][9][10][11][12]. 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 [13][14][15]. No noticeable effect on biomass production was observed in experiments performed to assess the impact of tritium on various vegetables [16][17][18][19][20]. 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 [21][22][23]. 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) [24][25]. 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” [26]. Furthermore, the rapid luminescence response is supposed to indicate the non-genetic mechanism of low-intensity exposures [27][28].

Previous studies [29][30][31][32][33] have demonstrated both the activation and inhibition effects of tritium on marine bacteria, 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 $MBq \cdot L^{-1}$. 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

[34]. The current development of the hormesis concept is attributed to E. Calabrese [35][36][37][38][39]. The term “radiation hormesis” was suggested by Luckey [40]. The phenomenon of radiation hormesis was intensively studied in [37][41][42].

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 [29][31], whereas mono-phasic dependence (activation only) was shown in [27][43][44].

The mechanism of the activation effect of tritium is of special interest. The first hypothetical mechanism is based on DNA damage repair [45][46][47]. The involvement of non-genetic mechanisms in low-dose chronic radioactive effects in bacteria was demonstrated in [27][44].

In addition, it was shown that tritium activated bacterial growth at the activity levels of $10–10^4$ kBq·L^{-1} and suppressed this growth at higher activities (>10^5 kBq·L^{-1}) [30].

Previously, researchers demonstrated that low-intensity tritium exposure did not noticeably increase the content of reactive oxygen species (ROS) in bacteria-free media [31]; 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 [43]. 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 [43]. 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 O2 and electron donors such as metal centers, dihydroflavin, etc. [48][49]. It is known that peroxide derivatives of flavin molecules are native intermediate compounds of the bacterial bioluminescence reaction [50][51]. It is suggested that the oxygen-detoxifying function directed the emergence of many bioluminescent systems, including bacterial bioluminescence [52]. 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 [43]. 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 released into the environment can serve as specific signaling molecules for other cells (the so-called "bystander effect").

It was shown [53][54][55] 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 [56][57]. 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 physico-chemical approach to understanding the metabolic functioning of bacteria and their interactions with aqueous environments, which can affect other water inhabitants [27][28][29][30][31][32][33][43][44]. 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 [56] show the accumulation of tritium in phytoplankton populations of *Dunaliella tertiolecta* and *Nodularia spumigena* and mussels *Mytilus edulis*.

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 [58][59], whereas gold nanoparticles can additionally suppress physiological functions of the microorganism (so called, radio-sensitizing effect) [60].

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 [13]. 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 [13][56][61][62][63].

Previously, a study was carried out on the assimilation and transformation of tritium in the aquatic plants Elodea canadensis and Lemna minor [64].

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 [10].

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 $kBq \cdot L^{-1}$ 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 $kBq \cdot L^{-1}$) of tritium were introduced into the first tested solution, 60 Bq (300 $kBq \cdot L^{-1}$) into the second, 100 Bq (500 $kBq \cdot L^{-1}$) into the third, and 200 Bq (1000 $kBq \cdot L^{-1}$) 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 [62][63].

The tritium content in each of the biological samples (plants or fish) under study was measured using a liquid scintillation spectrometer, Quantuluse-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.

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, and illumination. A large proportion of TFWT transformation into OBT was obtained in the light/shadow mode—6/18 h.

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.

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