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Review

Low-intensity Exposures via Luminescent Bioassays of Different Complexity: Cells, Enzyme Reactions and Fluorescent Proteins

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Abstract: Current paper reviews applications of luminescence bioassays for monitoring results of low-intensity exposures, which produce a stimulative effect. Impact of radioactivity of different types (alpha, beta and gamma) and bioactive compounds (humic substances and fullerenols) are under consideration. High rates of luminescence response can provide (1) a proper number of experiments under comparable conditions and, therefore, proper statistical processing, with this being highly important for 'noisy' low-intensity exposures; (2) non-genetic, i.e. biochemical and physicochemical mechanisms of cellular response in short-term exposures. Bioassays based on luminous marine bacteria, their enzymes, and fluorescence coelenteramide-containing proteins were used to compare results of low-intensity exposures at cellular, biochemical and physicochemical levels, respectively. Results of the cellular exposures were discussed in terms of hormesis concept, which implies low-dose stimulation and high dose inhibition of physiological functions. Dependencies of the luminescence response on exposure time and radionuclide' concentration/gamma-radiation dose rate, as well as on concentration of the bioactive compounds were analyzed and compared for the bioassays of different levels.

Keywords: luminescence bioassays; bacterial cells, enzymes; fluorescent protein; low-intensity factors; hormesis; radiation; bioactive compounds; antioxidant activity

1. Introduction

All biological objects on the Earth are exposed to low-intensity factors – radioactive, chemical, and electromagnetic. Currently, accumulation of evidence of the inhibitory and activating effects of these factors is ongoing. Activation of physiological functions of organisms is associated with term "hormesis", which implies a favorable biological response to the low impact of toxins and other stressors. This term was introduced by Southam and Ehrlich in 1943 [1-2]. Over the past decades, an exponential increase in citation for hormesis in the biomedical community has been observed [2-5]. Hormesis model is based on non-linear dependence of effect on dose of toxic compounds, as shown schematically in Figure 1. Hormesis is considered as the basic model; two other models (linear and threshold) can be considered as particular cases of hormesis [2,5].

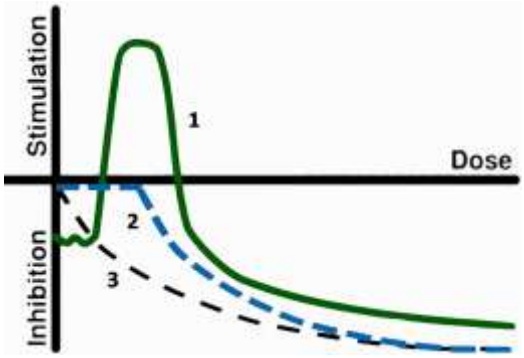

Figure 1. Scheme of dose-effect models: 1 – hormesis, 2- threshold, 3- linear.

There exist evidences that hormesis is highly generalized phenomenon; it does not depend on the level of biological organization (cells, organs, or organisms). Understanding and prediction of the responses of organisms to low-intensity exposures is of high importance for practical applications. This understanding should be based on molecular mechanisms of these effects, which are intensively studied now [1-6], but not clear yet. We suppose that simple bioassay systems, such as cells and enzymes, will allow understanding the low-intensity effects at cellular and biochemical levels, respectively. The next stage of investigation should be concerned with the level of physicochemical processes (energy, electron or hydrogen transfer) and application of the simplest bioassay systems.

Current review considers application of luminescence bioassay systems of different level organization (cells, enzyme reactions, and fluorescent proteins) for study mechanisms (cellular, biochemical, and physicochemical, respectively) of low-intensity exposures.

In general, bioassays are a basis of toxicological investigations. The term "toxicity" is of biological origin; it means a suppression of organism's physiological functions (Ilyin LA, Kutsenko SA, Savateev NV, Sofronov GA, Tiunov LA (1990) Toxicological problems in mitigation strategies of chemical industries. J All-Union Mendeleev Chemical Society. 35:440–447). In general, bioassays are used to monitor toxicity for centuries. Classic bioassays use mice, frogs, fish, crustaceans, plants, microorganisms, and others (Tigini V, Giansanti P, Mangiavillano A, Pannocchia A, Varese GC (2011) Evaluation of toxicity, genotoxicity and environmental risk of simulated textile and tannery wastewaters with a battery of biotests. Ecotoxicol Environ Saf. 74:866–873; Rizzo L (2011) Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. Water Res. 45:4311–4340; Petukhov VN, Fomchenkov VM, Chugunov VA, Kholodenko VP (2000) Plant Biotests for Soil and Water Contaminated with Oil and Oil Products. Appl Biochem Microbiol. 36:564–567; Donnelly K, Chen J, Huebner H, Brown K (1997) Utility of four strains of white-rot fungi for the detoxification of 2,4,6-trinitrotoluene in liquid culture. Environ Toxic Chem. 16:1105–1110). Their physiological functions monitored in the bioassay procedure are: respiration, life-time, rates of growth or movement, as well as the bioluminescence intensity. The main feature of all bioassays is integral response. This means that a lot of processes of different organization level (cellular, biochemical, physicochemical) are integrated in change of definite physiological function – growth, respiration, lifetime, movement, bioluminescent intensity, etc. Bioassays are based on monitoring of these functions and widely used in toxicological investigations. Traditionally, the bioassays are applied as "signal' assays, their results point to necessity of more detailed and expensive analyses, chemical or biochemical. More detailed characteristics of biological assays and their correlations with chemical analyses are discussed in [N.S.; Tarasova, A.S. Pollutant toxicity and detoxification by humic substances: Mechanisms and quantitative assessment via luminescent Biomonitoring. Environ. Sci. Pollut. Res. Int. 2015, 22, 155–167, doi:10.1007/s11356-014-3459-6]

Luminescence feature of the bioassays provide a proper registration of biological responses. Luminescence intensity is a testing physiological parameter of these bioassays. The advantages of the luminescence are high rates of analysis (down to 1-3 min), ease of use, high sensitivity, and availability of instruments and reagents [10-12 Катя, здесь уже не по порядку]. Since the luminescent type of registration is not time consuming, it provides a bundle of experimental results

under comparable conditions, and hence, proper statistical processing. This advantage is important as biological analyses are usually characterized by lower reproducibility than chemical or radiometric assays. In addition, this advantage is of particular importance for low-intensity exposures that are usually can be described in terms of "stochastic effects" [6]. Besides, quick response can contribute to the investigation of non-genetic mechanisms of low-intensity exposures.

The most known luminescent cellular bioassay is based on luminous marine bacteria. Bacterial luminescence is sensitive to toxic compounds; this is a reason why the marine bacterium has been used for several decades to assess environmental toxicity [7-13].

Current tendency to simplification of bioassay systems resulted in development of enzymatic assays. Enzymatic assays estimate rates of biochemical reactions under toxicant influence. Enzymatic bioluminescence assay based on the bacterial enzymes, progresses from early 90-s [14-17]. Solid immobilized enzymatic and bacterial preparations develop now as a basis for bioluminescent biosensors [14,17-20]. Effects of toxic compounds on the bioluminescence enzymatic system was classified in [21]; the classification was developed later in [22-25]. This classification describes (1) physicochemical, (2) chemical, and (3) biochemical processes in the bioluminescence assay systems at exposure to toxic compounds.

Advanced experience in study the toxic effects on bioluminescence of bacteria and their enzymes is one more advantage of their application for evaluation the effects of low-intensity exposures.

Biochemical and physicochemical approaches contribute to non-genetic aspect of toxic and adaptive effects. These approaches are applicable in frames of novel "exposome" concept, where "exposome complements the genome and encompasses the totality of environmental non-genetic exposures" [26-28]. Exposome concept was originated as a challenge in molecular epidemiology [29] and concerned with human exposures. Application of simple model organisms and biochemical systems might provide human exposure sciences with fundamental support basing on molecular, physicochemical, biochemical, and cellular investigations.

This review analyzes results of application the simplest luminescence bioassays (luminous marine bacteria, their enzymes, and fluorescent coelenetamide-containing proteins) to study (i) the effects low-dose radiation of alpha, beta and gamma type, and (ii) antioxidant effects of bioactive compounds of natural and artificial origination – humic substances and fullerenols, respectively.

Next section of the review, Section 2, justifies an application of the coelenteramide-containing fluorescent protein as a simplest multicolor bioassay based on physicochemical process in the protein complex. Section 3 discusses application of luminescence bioassays in study the low-dose radiation effects. Section 4 presents low-concentration antioxidant effects of bioactive compounds.

2. Coelenteramide-Containing Fluorescent Protein as a Simplest Multicolor Fluorescent Bioassay for low-intensity exposures

Main structural components of fluorescent proteins are polypeptide and aromatic fluorophore; the latter is responsible for light emitting. The most important representative of fluorescent proteins is Green Fluorescent Protein (GFP). It was isolated in 1962 from jellyfish *Aequorea victoria* by American scientist O. Shimomura. In 2008 Simimura has got a Nobel Prize for the discovery and development of GFP. A series of fluorescent proteins of different color, homologues to GFP, are known now [30-31]. They are widely used in medical and biological research for labeling individual molecules, intracellular structures, living cells, organs and whole organisms in order to visualize intracellular processes [32-33].

Coelenteramide-containing proteins are the other group of fluorescent proteins. "Discharged aequorin", a representative of this group, was isolated and studied by Prof. Shimomura too, simultaneously with GFP. He called it Blue Fluorescent Protein (BFP). This group differs from the GFPs with fluorophore formation: the fluorophore of GFPs is formed by amino acid chain residuals Ser65-Tyr66-Gly67 as a result of their specific cyclization [[C.W. Cody, D.C. Prasher, W.W. Wester, F.G. Prendergast, W.W. Ward Chemical structure of the hexapeptide chromophore of the Aequorea green-fluorescent protein, Biochemistry 32 (1993) 1212-1218. M. Ormö, A. B. Cubitt, K. Kallio, L. A.

Gross, R. Y. Tsien, S. J. Remington, Crystal structure of the *Aequorea victoria* green fluorescent protein, Science 273 (1996) 1392-1395]; while the fluorophore of BFP is a CLM molecule (Figure 2) which is non-covalently bound inside the hydrophobic cavity of the apoprotein, forming CLM-aporotein complex. As opposed to GFPs, the coelenteramide-containing proteins are not widely used, and their potential as color fluorescent biomarkers is not evaluated yet.

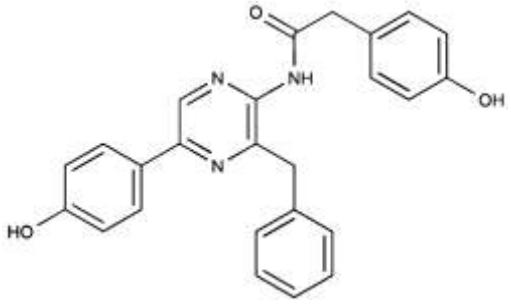

Figure 2. Chemical structure of CLM molecule (neutral and ionized forms) and scheme of photophysical and photochemical processes in CLM and CLM-containing fluorescent proteins [O. Shimomura, F.H. Johnson, Calcium binding, quantum yield, and emitting molecule in aequorin bioluminescence, Nature 227 (1970) 1356-1357]

CLM-containing proteins are known to be products of bioluminescent reactions of marine coelenterates. The reactions are Ca^{2+}-dependent, and this is a basis for their biomedical application [34-35]. Biochemical and photophysical mechanisms of the bioluminescence reactions [36-41] and spectral characteristics of their products - coelenteramide-containing proteins [42-49] are under intensive investigations now.

We suggested application of coelenteramide-containing proteins as simplest bioassay system basing on their ability to change fluorescence color under exposure to chemical toxicants, radiation, and other destructive factors [50]. Peculiarities of this bioassay are concerned with photobiophysical properties of the protein complex. CLM molecule is a photochemically active compound; its photoexcitation initiates a proton transfer out (Figure 2); His22 is an acceptor of the proton in the CLM-apoprotein complex. Fluorescence color of neutral and ionized forms differ (violet and green spectral regions, respectively) (Figures 3,4).

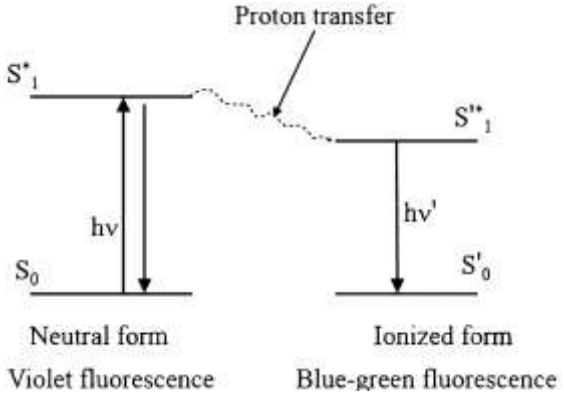

Figure 3. Chemistry in electron-excited states in coelenteramide molecule. Jablonslki diagrams of two forms of colenteramide.

Any destructive exposures can decrease the efficiency of the photochemical reaction (proton transfer out) in the protein complex, change contributions of fluorescence components, and hence, fluorescence color. Therefore, CLM-containing protein based bioassay provides relations of a toxic effect with a primary physicochemical process – proton transfer. Figure 4 shows schematically a result of the destructive exposures: chemical or radioactive exposures can change contributions of colored components to the fluorescence spectra of CLM-containing protein.

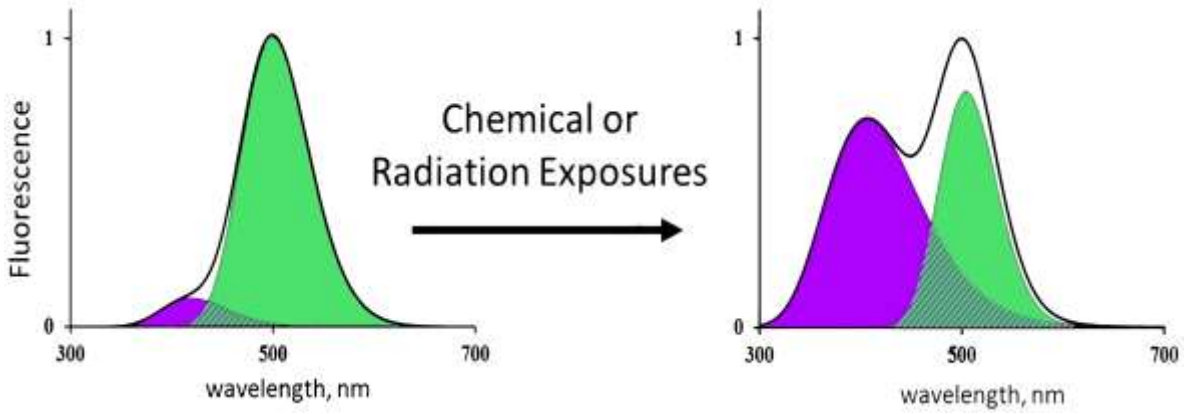

Figure 4. Change of fluorescence spectra of CLM-containing fluorescence proteins exposed to chemical agents or radiation.

Figure 5 presents fluorescent component contributions to CLM-containing protein spectra with glycerol taken as an example of chemical agent.

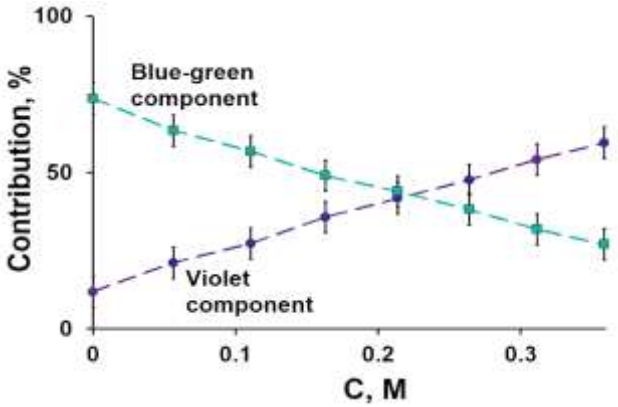

Figure 5. Contributions of spectral components to fluorescence spectra of CLM-containing protein at different concentrations of glycerol, C [51].

Therefore, variations of fluorescence color of CLM-containing proteins are the result of destructive protein exposures; toxicity monitoring, in this case, is concerned with the changes in protein structure; the toxicity evaluation takes place via efficiency of primary photochemical processes of proton transfer. Low-intensity exposures of CLM-containing proteins to radiation or chemical agents are of fundamental interest.

3. Luminescence bioassays as tools for study low-dose radiation effects

The intensity of studying radiobiological low-dose effects has been growing since the 70s [52-56], including effects on microorganisms [57-59]. Radiation hormesis is intensively discussed. The first radiation hormesis tutorial was written by Luckey in 1980 [60].

Luminous marine bacteria have been applied to monitor low-dose radiation effects for about one decade [13,15]. For this period, effects of alpha- and beta- emitting radionuclides americium-241, uranium-235+338, and tritium, as well as gamma radiation were investigated [13,15-16,61-63]. It was shown that the bacterial bioluminescence response to radionuclides americium-241 and tritium includes three stages: (1) threshold, (2) activation, and (3) inhibition. We have chosen these two radionuclides for presentation here due to their radioecological significance: both radionuclides are accumulated in environment now. Tritium is a by-product of a lot of radiochemical reactions in nuclear industry; americium-241 is a by-product of a plutonium decay with high radiation lifetime (432,6 years).

Bacterial bioluminescence kinetics in solution of americium-241, alpha-emitting radionuclide of high specific radioactivity, is presented in Figure 6.

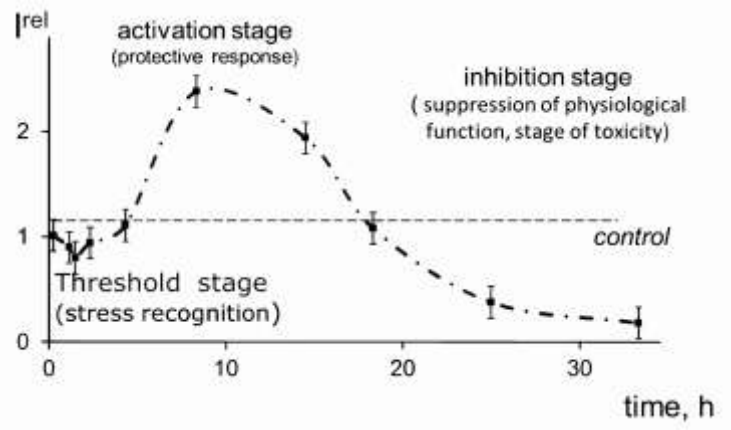

Figure 6. Bioluminescence kinetics of bacteria in solution of americium-241, 3 kBq/L.

Similar kinetic changes were obtained at exposure to beta-emitting radionuclide, tritium [16,63]. Figure 7A reveals the same three stages in bacterial luminescence response, with activation included. Hence, activation is a main peculiarity of the response of bacteria to low-dose radiation of alpha and beta emitting radionuclides - americium-241 and tritium, respectively. The responses can be discussed in terms of "radiation hormesis", as well as "protective response of organisms".

Additionally, independence of bioluminescence bacterial response on tritium activity concentration was found for low-dose exposures. To demonstrate this peculiarity, the time of exposure to tritium was fixed 20 and 50 hours, corresponding to activation and inhibition stages of bioluminescence (Figure 7A). The bioluminescence intensity at different concentrations of tritium is presented in Figure 7B for 20 and 50 h exposure times. It is seen here that the monotonic dependence is absent in a wide interval of activity concentrations of tritium – five orders. The result can be explained in terms of adaptation ability of the bacterial cells. It should be paid attention that the conventional limit of low-dose interval (0.1 Gy) was not exceeded in this experiment.

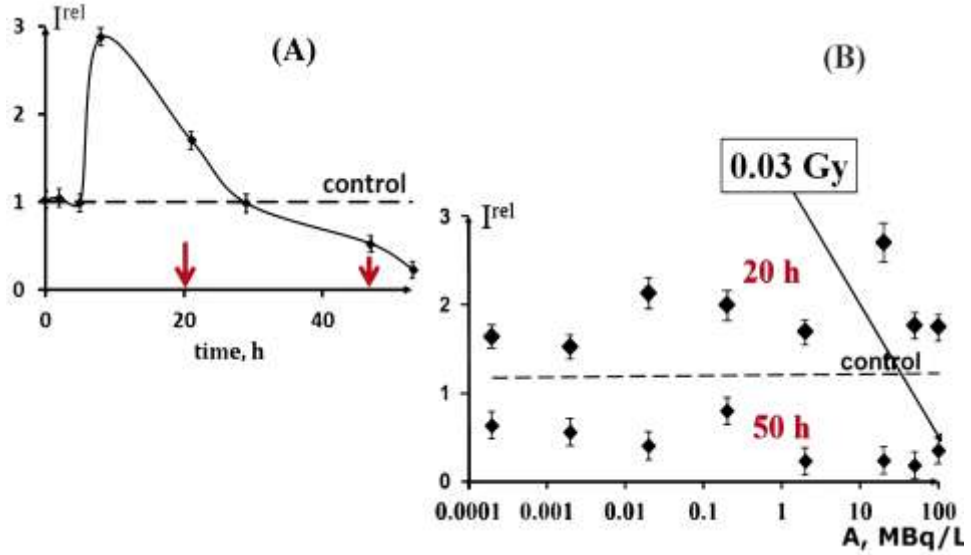

Figure 7. Effect of tritiated water on bioluminescence of bacteria. (A) Bioluminescence kinetics of bacteria in tritiated water, 2 MBq/L; (B) bioluminescence intensity vs. activity concentration of tritiated water, A, at 20 and 50 h exposures.

Activation of the bacterial bioluminescence by tritium was demonstrated in a series of experiments. Bi-phasic dependence (activation+ inhibition) was found in [16,63]; and mono-phasic dependence (only activation) was found in [64-65].

To date, there are two hypothetical mechanism that describe radiation hormesis: this phenomenon is associated with either DNA damage or membrane processes [52,55-56,66-67]. Original approach was applied in [64] to test involvement of genetic changes to activation of the bacterial bioluminescence by tritium: using tritium labeled films as a solid source of tritium radiation, the authors demonstrated that tritium activates bacterial luminescence without penetration to the cells. Additionally, no mutations were found in bacterial DNA (16S rRNA gene responsible for vital functions of bacterial cells [68] under the low-intensity irradiation of alpha, beta, and gamma type [65]. A conclusion was made that bioluminescence response is not associated with mutations in the tested gene. Alternative mechanism of biological regulation should be considered, which is related to cell membrane processes, water media ionization, and formation of Reactive Oxygen Species (ROS) under low-dose radiation exposures. Recent results [69] demonstrated that the exposure of marine bacteria to low-intensity irradiation of tritium increases ROS content in the bacterial environment considerably; and a rise of the ROS content correlates with intensification of bacterial bioluminescence intensity. These correlations were explained with "trigger" function of products of tritium decay, signaling role of ROS, and "bystander effect" in the bacterial suspension.

Previously [62], effect of americium-241 on luminous bacterium was attributed to ROS generated in aqueous solutions as secondary products of the radioactive decay. The effects of americium-241 and tritium on luminous bacteria were compared in [63] at comparable radiation doses, higher impact of alpha-irradiation of americium-241 was found. The result was related with different energy of radioactive decay of americium-241 and tritium (5637.8 and 18.6 keV [70], respectively) and much higher ROS concentration in americium-241 water solutions as compared to tritiated water. The authors discuss [62] a biological role of ROS generated in aqueous solutions at low-dose exposures.

Diffuse reflectance fourier-transform infrared (FTIR) spectroscopic studies [71] showed that the alpha-radioactivity effect of americium-241 "to be transmitted by live cells mainly to the bacterial bioluminescence enzyme system, with negligible structural or compositional changes in cellular macrocomponents."

For the first time, bioluminescence of bacteria was used to monitor toxicity of gamma-rays in [72]; effects of average and high doses were under investigation. Exposure of luminous bacteria to low-dose (≤ 250 mGy) gamma radiation was studied in [61]. Dose-effect dependencies were of stochastic character here, however, the dependencies: exposure time-effect were evident (Figure 8A). Bioluminescence activation was not found under low-dose gamma-radiation exposure; the bioluminescence kinetics corresponded threshold model (Figure 8A), which is supposed as a particular case of hormesis model [2,5]. Probably, lower ionization ability of gamma rays (as compared with alpha and beta particles) is responsible for reducing the bacterial adaptive response.

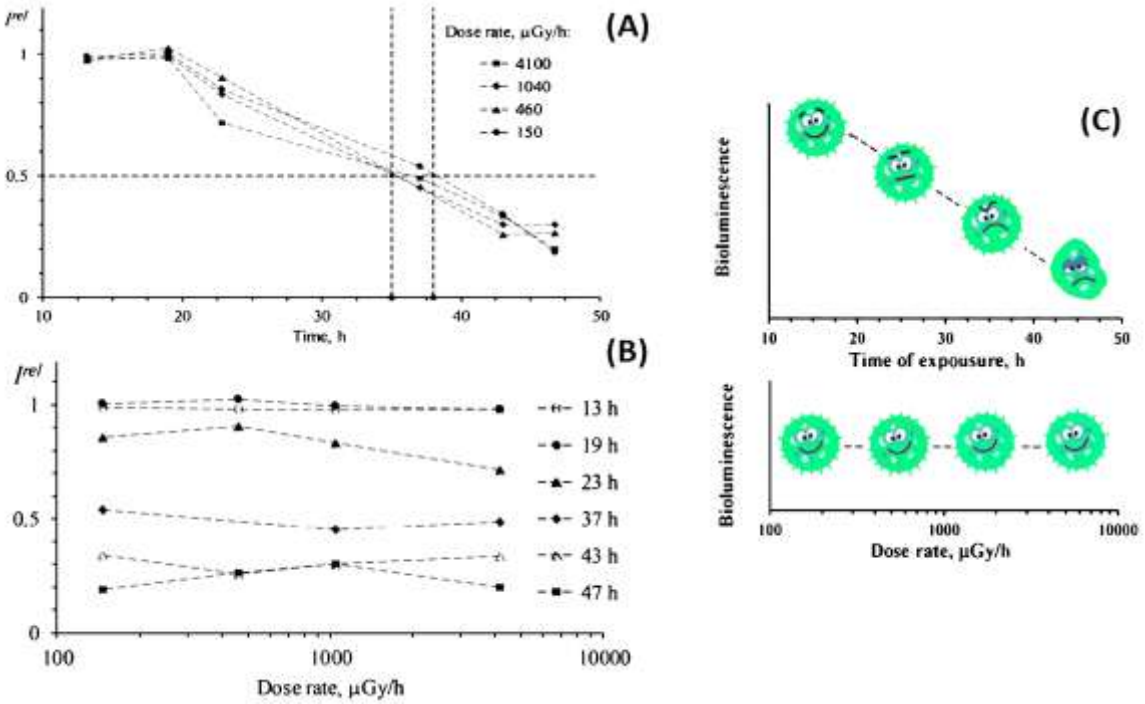

Figure 8. Bioluminescence intensity (I^{rel}) of *P. phosohoreum* exposed to gamma radiation, ^{137}Cs, 20ºC. An error for I^{rel} was 10% [61]. (A) Bioluminescence intensity vs. exposure time; (B) bioluminescence intensity vs. dose rate; (C) schematic presentation of the dependence of bioluminescence intensity on exposure time and dose rate.

Independence on dose rate of the gamma irradiation was found at the experimental conditions, Figure 8B, similar to low-dose effect of tritium (Figure 7B).

One more finding for gamma radiation effects was demonstrated in [61]: lowering temperature (from 20ºC down to 10ºC and 5ºC) decreased sensitivity of the bacteria cells to low-dose gamma-radiation; bioluminescence inhibition was not observed at 10ºC and 5ºC. This result was generally explained by the temperature dependence of metabolic processes including radiation-induced ones.

Hence, experiments with bacterial cells demonstrated independence of their bioluminescence response on intensity of irradiation (activity concentration for alpha/beta radionuclides americium-241/tritium, and dose rate for gamma radiation) under low-dose exposures; however, time dependence was evident corresponding to hormesis (for alpha/beta radionuclides americium-241/tritium) or threshold (for gamma radiation) models. Independency of the response on irradiation intensity (1) and hormesis/threshold type of response vs. time (2) can be considered as a basis for "cellular adaptive response".

The question is whether these two peculiarities are inherent in a biological system of lower level of organization – enzymatic reactions. Effects of alpha- and beta- emitting radionuclides (americium-241 and tritium) on bioluminescence system of coupled enzyme reactions catalyzed by bacterial luciferase and NADH:FMN-oxidoreductase were studied in [15-16]. Bioluminescence activation and inhibition were observed. Monotonic dependence on concentration of radionuclides was found, too. An example of this dependence is shown in Figure 9.

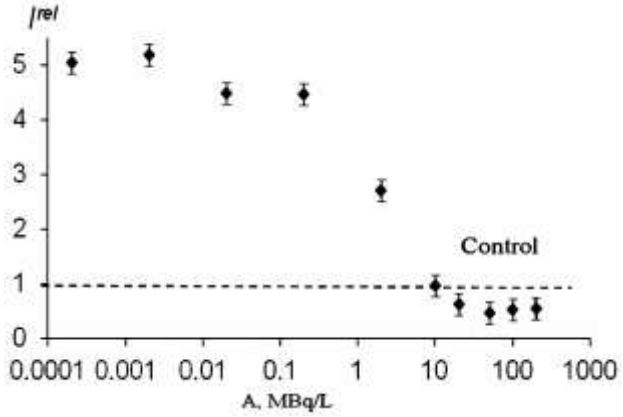

Figure 9. Bioluminescent intensity of enzyme system, I^{rel}, vs. specific radioactivity of tritiated water, A [16].

Paper [63] compared rates of redox processes exposed to americium-241 and tritium: the NADH auto-oxidation rates were much higher in highly-diluted americium-241 solutions, thus contributing to inhibition of NADH-dependent enzymatic processes. This result was compared to ROS concentration in these solutions; a conclusion was made about ROS involvement to the redox transformations of biological low-weigh molecules under the low-intensity radiation exposures.

The simplest luminescent bioassay based on CLM-containing protein ("discharged obelin" from *Obelia longissima*) did not demonstrate even activation stage under low-intensity exposures. Figure 10 presents time-courses of colored fluorescence contributions under exposure to beta (A) and gamma (B) irradiation according to [73-74]. The increase of contributions of violet component in both cases is an evidence of proton transfer inhibition in the excited CLM-apoprotein complex, due to the destructive effect of the low-intensity radiation (see Section 2).

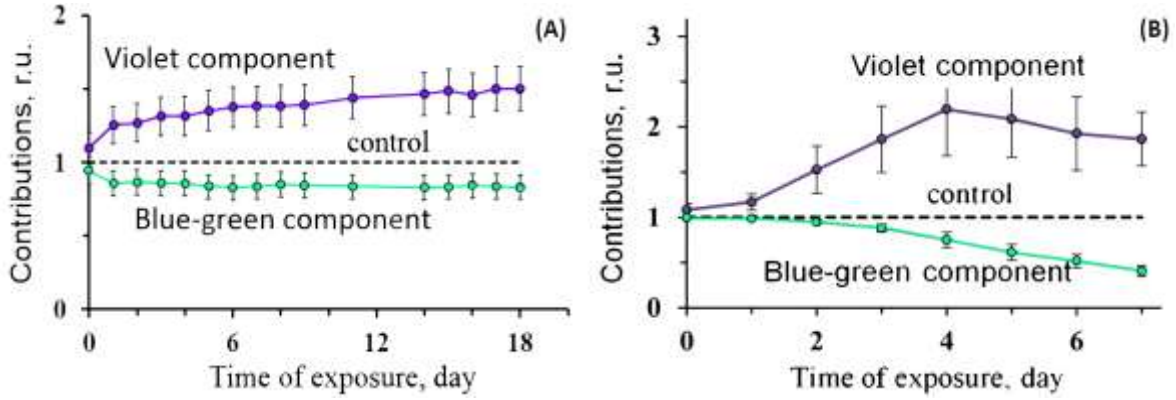

Figure 10. Relative contributions of spectral components to fluorescence spectra of CLM-containing protein ("discharged obelin" from *Obelia longissima)* exposed to (A) tritiated water, 200 MBq/L [73]; (B) gamma radiation, ^{137}Cs, 2 mGy/h, 20°C [74].

Dependence of the response of CLM-containing protein to low-intensive gamma radiation on temperature was demonstrated in [74] as well.

Hence, simplest bioassay systems (based on enzymatic reaction and physicochemical processes in protein complex) show lower ability for adaptive response to low-dose radioactive exposures. In contrast to bacterial cells, they can demonstrate dependence on intensity of irradiation or absence of the activation stage in the course of chronic exposure. However, a lack of experimental results does not allow making definite conclusions. The study should be developed in this direction.

4. Low-concentration effects of bioactive compounds: antioxidant activity via bioluminescence bioassays

Investigation of low-concentration effects of bioactive compounds has been developed from 60th of the previous century [52,75-77] Hormesis concept progresses to describe intensification of physiological functions of organisms under low-concentration exposures [2-4,76-79].

Bioluminescence bioassays based on luminous marine bacteria and their enzymes are excellent tools for study low-concentration effects due to their features mentioned before:

1. high rates of test procedure which
   - provide statistical reliability of the bioassay results and
   - exclude genetic level of analysis and appeal to biochemical, chemical and physicochemical processes in cells;
2. possibility to compare effects of bioactive compounds at different organization levels – cellular and enzymatic.

The bioassays based on luminous marine bacterium and bacterial enzymes were used to study antioxidant properties of the bioactive compounds of natural and artificial origination. Humic substances and fullerenols were chosen as examples of these compounds, Figure 11. Humic substances are products of natural oxidative transformation of organic matter in soils and sediments, attenuators of toxicity in natural water bodies and soils. Fullerenols are specific allotropic form of carbon, nanosized polyhydroxylated water-soluble derivatives of fullerenes, bioactive compounds, and perspective pharmaceutical agents. Hypothetical structures of humic substances and fullerenol are presented in Figure 11. Antioxidant activity of humic substances was studied in [80-84] and fullerenols – in [84-87].

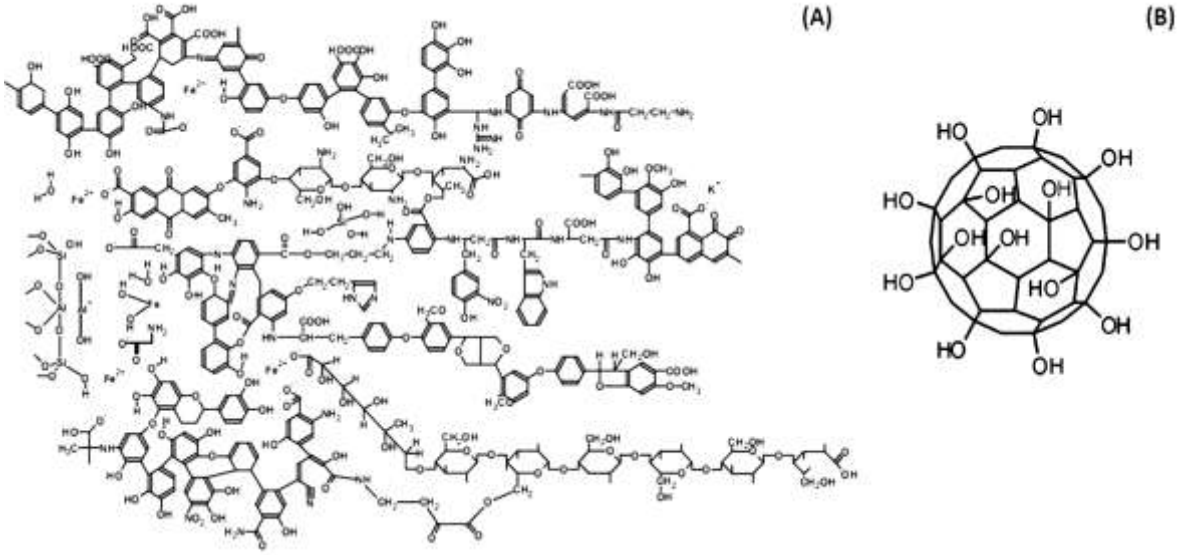

Figure 11. Hypothetical structure of bioactive compounds: A –fragment of humic substances [88], B – fullerenol C-60.

Cellular and enzymatic assays were used to evaluate a General Toxicity (GT) under conditions of the oxidative stress; this stress was modeled in the solutions of exogenous organic or inorganic oxidizers (1,4-benzoquinone or potassium ferricianide, respectively). Additionally, enzymatic bioassay was shown to be specific to oxidizers [25,89]; it can be used to monitor an oxidative toxicity (OxT) of the solutions. This toxicity type characterizes the redox properties of toxic compounds, while the other type of toxicity, general toxicity (GT) mentioned before, integrates all the interactions of toxic compounds with bioassay system: redox reactions, non-polar and polar interactions, so on. The GT is concerned with suppression of maximal bioluminescence intensity, while the OxT uses a specific kinetic parameter, induction period of bioluminescence. The latter appears in the presence of

exogenous oxidizers, depending on their redox potential and concentration [25]. Justification for GT and OxT application to evaluate toxicity of bioactive compounds is presented in [80-87].

Principle of antioxidant efficiency evaluation is presented in Figure 12 for bacteria-based (upper line) and enzyme-based (lower line) bioassays; humic substances are chosen here as an example of an antioxidant agent.

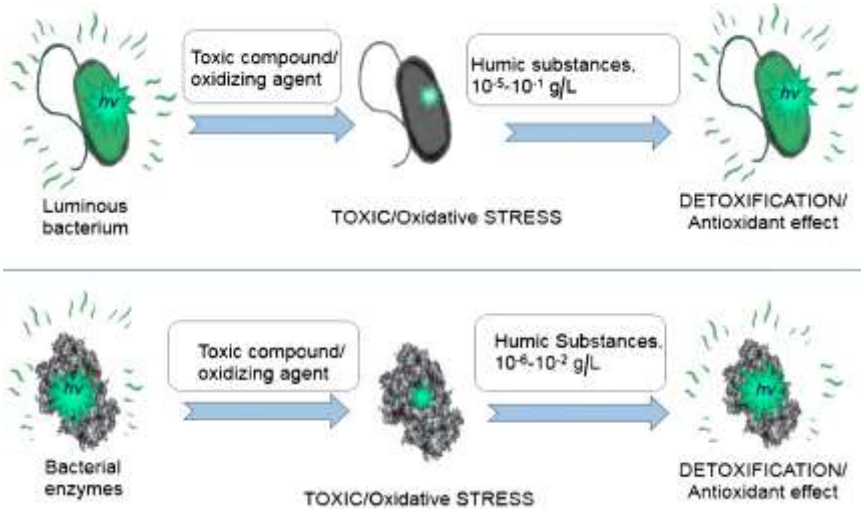

Figure 12. Principle of antioxidant efficiency evaluation using bacteria-based (upper line) and enzyme-based (lower line) bioluminescent assays.

Antioxidant efficiency of bioactive compounds was characterized by antioxidant coefficients D_{GT} or D_{OxT}, corresponding to GT or OxT monitoring. Values of D_{GT} >1 or D_{OxT} >1 revealed antioxidant effects of the bioactive compounds.

Paper **[**86] compares biological activity of carbon nano-structures of natural and artificial origination, i.e. humic substances and fullerenols. The representative of the group of fullerenols, $C_{60}O_y(OH)_x$ where y+x =20–22, was chosen. Both bioassays were used to monitor toxicity and antioxidant activity of the bioactive compounds. Toxic concentrations of humic substances and fullerenol, inhibiting bioluminescence of the assay systems were determined and excluded from further antioxidant experiments. Antioxidant coefficients of the bioactive compounds and ranges of their active concentrations were determined in solutions of model oxidizers. Figure 13 presents an example of dependencies of antioxidant coefficients D_{OxT} on concentration of fullerenol, *F* (A) and humic substances, *HS* (B).

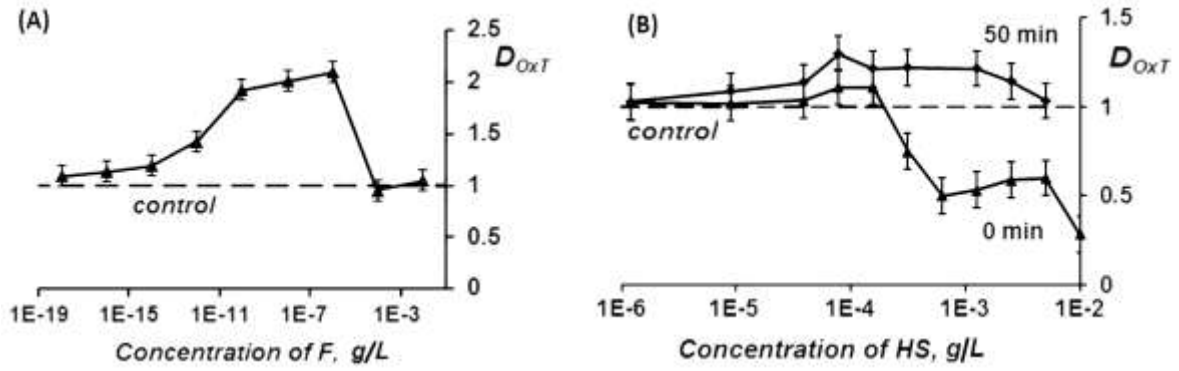

Figure 13. Antioxidant coefficients D_{OxT} of fullerenol, *F* (A), and humic substances, *HS* (B) in model solution of organic oxidizer (1,4-benziquinone) [86]. Time of incubation of humic substances with oxidizer (0 min and 50 min) is indicated in Figure (B). Enzymatic assay.

Both *HS* and *F* demonstrated low-concentration antioxidant activity (D_{OxT} >1); however, quantitative antioxidant characteristics were different: fullerenol' D_{OxT} -values were higher, its antioxidant activity covered wider concentration range as seen from Figure 13. Antioxidant activity of *HS* was found to be time-dependent, Figure 13(B), while the *F*' antioxidant effect showed independency on time. The *HS*' antioxidant effect did not depend on amphiphilic characteristics of the environment (D_{OxT}-values were 1.3 in the solutions of inorganic and organic oxidizers), while D_{OxT} of *F* was maximal in solutions organic oxidizer (D_{OxT} = 2.0). The difference in effects of bioactive compounds in solutions of organic and inorganic oxidizers can be concerned with their hydrophobic interactions in enzymes or cellular membranes. Changes in fluidity and structural organization of lipid bilayers in hydrophobic fragments of membranes by fullerenol F-60 were previously reported in [90].

In **[**86], the differences in toxic and antioxidant effects of fullerenol and humic substances were attributed to the structure of these compounds. Non-rigidity of humic macromolecules determines their diffusion restrictions, which result in higher toxicity and time-dependence of their antioxidant coefficients. The ability to decrease ROS content in water solutions probably contributes to higher toxicity of humates, as well. Non-rigidity and polyfunctionality can be responsible for unification of humate' properties in solutions of oxidizers of different hydrophilic/hydrophobic characteristics. Low-concentration antioxidant activity was explained by catalytic redox activity of π-fragments of the bioactive structures.

Antioxidant property of highly diluted solutions of fullerenols was attributed [85] to hormesis phenomenon. The bacteria-based and enzyme-based assays demonstrated similar peculiarities of the antioxidant processes: "(1) ultralow concentrations of fullerenols were active (*ca* 10^{-17}–10^{-4} and 10^{-17}–10^{-5} g/L, respectively), (2) no monotonic dependence of detoxification efficiency on fullerenol concentrations was observed, and (3) detoxification of organic oxidizer solutions was more effective than that of the inorganic oxidizer". The antioxidant properties were concerned with adaptive cellular response under low-dose exposures. The sequence analysis of 16S ribosomal RNA was carried out for long-term exposures; mutations in bacterial DNA were not revealed. The conclusion was suggested that hydrophobic interactions might be involved to the antioxidant mechanism.

Biological efficiency of low and ultralow concentrations of hydrated fullerenes was studied and discussed earlier in [91-92]. This effect was attributed to the fullerene' ability to regulate dynamic structure of aqueous media and to adjust "redox processes (especially those involving oxygen) in aqueous systems". Earlier [93], a role of aqueous media in fullerenol' antiradical activity was discussed. Probably, ROS in aqueous media are able to contribute to the antioxidant effect of fullerenols.

In [84,87] the antioxidant activity of fullerenols was related with modification of their surface by oxygen substituents, as well as presence of exo- or endo-hedral metal atoms. Difference in the antioxidant activity of these fullerenols was explained through their electron donor/acceptor properties and different catalytic activity.

Paper [81] studied combined influence of humic substances (HS) and salts of aliovalent metals on the bioluminescent assay systems, the rates of biochemical reactions and bacterial ultrastructure were analyzed. The detoxifying/antioxidant effects of HS were explained [81-83] by: (1) "a decrease of free metal content in water solutions under metal–HS binding; (2) increase of biochemical reaction rates in a bioluminescent assay system under HS effect; (3) enhancement of mucous layers on cell surface as a response to unfavorable impact of toxicants. Detoxifying mechanisms (2) and (3) reveal the active role of bioassay systems in detoxification processes".

As an outlook:

1. Bioactive compounds can produce toxic (inhibiting) and antioxidant effects. Toxic effect of humic substances was found at higher concentrations (>0.1 g/L for bacterial assay and >0.01 g/L for enzymatic assay), an antioxidant effect was found at lower concentrations. All fullerenols showed toxic effects at higher concentrations: from >0.01 to >0.001 g/L; their antioxidant effect was found at low and ultralow concentrations: *ca* 10^{-17}-10^{-4} and 10^{-17}-10^{-5} g/L for bacteria-based and enzyme-based

assays, respectively. The range of active fullerenol' concentrations might correspond to several tens of molecules per liter.

2. No monotonic dependencies of antioxidant coefficients on concentration of bioactive compounds were found in a wide concentration range (up to 15-17 orders for fullerenols).

3. Both bacterial and enzymatic assays demonstrated antioxidant effect of bioactive compounds. This result reveals role of biochemical and physicochemical processes in low-concentration antioxidant effects of bioactive compounds.

4. Cellular and enzymatic bioluminescent assays showed that detoxification of solutions of organic oxidizers was more effective than inorganic oxidizers, with this indicating the importance of hydrophobic interactions in the antioxidant mechanism.

Hence, similar to low-dose radiation effects, antioxidant effects of bioactive compounds (fullerenols and humic substances) showed (a) positive response of the luminous bacteria and their enzymes to low-concentration exposure and (b) absence of linear concentration-effect dependencies.

5. Conclusions

Luminescence bioassay systems can provide non-genetic approach to low-intensity exposures due to high rate of registration of luminescence response. Possibility to study biochemical, chemical and physicochemical (polar, apolar, hydrophobic) processes using luminescence signaling is a contribution to exposomic concept [28-31].

As we assume, the biochemical, chemical and physicochemical processes have not attract, until now, much attention in frames of hormesis conception. However, compensatory effects can be strictly studied in terms of chemical equilibrium, by analyzing the individual equilibrium constants in complex systems of coupled chemical reactions. The compensatory effects can be considered, in this case, as a result of equilibrium disturbing followed by concentrational reconstruction of the whole system, streaming to the new equilibrium with the new equilibrium concentrations. While streaming to the new balance, fluctuations of concentrations are possible, as well as involvement of new processes to the system of coupled reactions, with this providing "over-compensative" response to the exposures.

The results presented in this review reveal the dependences of the biological response on time of exposure to low-intensity factors, as well its independencies on radioactivity or concentration. Uncertainty of dose-response relations was paid attention, too. Differences in dependencies of luminescence responses on time and exposure intensity should be analyzed in detail at molecular level in further experiments.

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Abbreviations

CLM	Coelenteramide
F	Fullerenol
FTIR	Fourier-transform infrared
GT	General Toxicity
HS	Humic Substances
NADH	Nicotinamide adenine dinucleotide
OxT	Oxidative Toxicity
ROS	Reactive oxygen species

References

1. Southam, C.M.; Ehrlich, J. Effects of extracts of western red-cedar heartwood on certain wood-decaying fungi in culture. *Phytopathology* **1943**, *33*, 517-524.
2. Calabrese, E.J. Hormesis: Path and Progression to Significance. *Int. J. Mol. Sci.* **2018**, *19*, 2871-2886, doi:10.3390/ijms19102871.
3. Calabrese, E.J. Hormetic mechanisms. *Crit. Rev. Toxicol*. **2013**, *43*, 580–606, doi:10.3109/10408444.2013.808172.
4. Calabrese, E.J. Hormesis: a fundamental concept in biology. *Microb. Cell* **2014**, *1*, 145-149, doi:10.15698/mic2014.05.145.
5. Iavicoli, I.; Leso, V.; Fontana, L.; Calabrese, E.J. Nanoparticle exposure and hormetic dose–responses: An update. *Int. J. Mol. Sci.* **2018**, *19*, 805, doi:10.3390/ijms19030805.
6. Vasilenko, I.Ya.; Vasilenko, O.I. Radiation risk when exposed to small doses is negligible. *Atomic Energy Bulletin* **2001**, *12*, 34-37 (In Russian).
7. Bulich, A.A.; Isenberg, D.L. Use of the luminescent bacterial system for rapid assessment of aquatic toxicity. *ISA Trans.***1981**, *20*, 29–33.
8. Abbas, M.; Adil, M.; Ehtisham-Ul-Haque, S.; Munir, B.; Yameen, M.; Ghaffar, A.; Shar, G.A.; Asif Tahir, M.; Iqbal, M. Vibrio fischeri bioluminescence inhibition assay for ecotoxicity assessment: A review. *Sci. Total Environ.* **2018,** *626*, 1295–1309, doi:10.1016/j.scitotenv.2018.01.066.
9. Fedorova, E.; Kudryasheva, N.; Kuznetsov, A.; Mogil'naya, O.; Stom, D. Bioluminescent monitoring of detoxification processes: Activity of humic substances in quinone solutions. *J. Photochem. Photobiol. B* **2007**, *88*, 131–136, doi:10.1016/j.jphotobiol.2007.05.007.
10. Girotti, S.; Ferri, E.N.; Fumo, M.G.; Maiolini, E. Monitoring of environmental pollutants by bioluminescent bacteria. *Anal. Chim. Acta* **2008**, *608*, 2–29, doi:10.1016/j.aca.2007.12.008.
11. Kudryasheva, N.; Kratasyuk, V.; Esimbekova, E.; Vetrova, E.; Nemtseva, E.; Kudinova, I. Development of bioluminescent bioindicators for analyses of environmental pollution. *Field Anal. Chem. Tech.* **1998**, *2*, 277–280, doi:10.1002/(SICI)1520-65212:5<277::AID-FACT4>3.0.CO;2-P.
12. Roda, A.; Pasini, P.; Mirasoni, M.; Michchelini, E.; Guardigli, M. Biotechnological application of bioluminescence and chemiluminescence. *Trends Biotech.* **2004**, *22*, 295–303, doi:10.1016/j.tibtech.2004.03.011.
13. Kudryasheva, N.S.; Rozhko, T.V. Effect of low-dose ionizing radiation on luminous marine bacteria: radiation hormesis and toxicity. *J. Environ. Radioact*. **2015**, *142*, 68-77, doi:10.1016/j.jenvrad.2015.01.012.
14. Kratasyuk, V.A.; Esimbekova, E.N. Applications of luminous bacteria enzymes in toxicology. *Comb. Chem. High Throughput Screen.* **2015**, *18*, 952–959, doi:10.2174/1386207318666150917100257.
15. Rozhko, T.V.; Kudryasheva, N.S.; Kuznetsov, A.M.; Vydryakova, G.A.; Bondareva, L.G.; Bolsunovsky, A.Ya. Effect of low-level α-radiation on bioluminescent assay systems of various complexity. *Photochem. Photobiol. Sci.* **2007**, *6*, 67–70, doi:10.1039/B614162P.
16. Selivanova, M.A.; Mogilnaya, O.A.; Badun, G.A.; Vydryakova, G.A.; Kuznetsov, A.M.; Kudryasheva, N.S. Effect of tritium on luminous marine bacteria and enzyme reactions. *J. Environ. Radioact*. **2013**, *120*, 19–25, doi:10.1016/j.jenvrad.2013.01.003.
17. Esimbekova, E.N.; Kondik, A.M.; Kratasyuk, V.A. Bioluminescent enzymatic rapid assay of water integral toxicity. *Environ. Monit. Assess*. **2013**, *185*, 5909–5916, doi:10.1007/s10661-012-2994-1.
18. Efremenko, E.N.; Maslova, O.V.; Kholstov, A.V.; Senko, O.V.; Ismailov, A.D. Biosensitive element in the form of immobilized luminescent photobacteria for detecting ecotoxicants in aqueous flow-through systems. *Luminescence* **2016**, *31*, 1283-1289, doi:10.1002/bio.3104.
19. Ismailov, A.D.; Aleskerova, L.E. Photobiosensors containing luminescent bacteria. *Biochem.* **2015**, *80*, 733–744, doi:10.1134/S0006297915060085.
20. Ranjan, R.; Rastogi, N.K.; Thakur, M.S. Development of immobilized biophotonic beads consisting of Photobacterium leiognathi for the detection of heavy metals and pesticide. *J. Hazard Mater.* **2012**, *225–226*, 114–123, doi:10.1016/j.hazmat.2012.04.076.
21. Kudryasheva, N.S. Bioluminescence and exogenous compounds: Physicochemical basis for bioluminescence assay. *J. Photochem. Photobiol. B* **2006**, *83*, 77–86, doi:10.1016/j.jphotobiol.2005.10.003.
22. Kirillova, T.N.; Kudryasheva, N.S. Effect of heavy atom in bioluminescent reactions. *Anal. Bioanal. Chem.* **2007**, *387*, 2009–2016, doi:10.1007/s00216-006-1085-y.

23. Kirillova, T.N.; Gerasimova, M.A.; Nemtseva, E.V.; Kudryasheva, N.S. Effect of halogenated fluorescent compounds on bioluminescent reactions. *Anal. Bioanal. Chem.* **2011**, *400*, 343–351, doi:10.1007/s00216-011-4716-x.
24. Nemtseva, E.V.; Kudryasheva, N.S. The mechanism of electronic excitation in bacterial bioluminescent reaction. *Russ. Chem. Rev.* **2007**, *76*, 91–100, doi:10.1070/RC2007v076n01ABEH003648.
25. Vetrova, E.V.; Kudryasheva, N.S.; Kratasyuk, V.A. Redox compounds influence on the NAD(P)H:FMN-oxidoreductase-luciferase bioluminescent system. *Photochem. Photobiol. Sci*. **2007**, *6*, 35–40, doi:10.1039/b608152e.
26. Rappaport, S.M.; Smith, M.T. Epidemiology. Environment and disease risks. *Science* **2010**, *330*, 460–461, doi:10.1126/science.1192603.
27. Wild, C.P. The exposome: from concept to utility. *Int. J. Epidemiol.* **2012**, *41*, 24–32, doi:10.1093/ije/dyr236.
28. Siroux, V.; Agier, L.; Slama, R. The exposome concept: a challenge and a potential driver for environmental health research. *Eur. Respir. Rev.* **2016**, *25*, 124-129, doi:10.1183/16000617.0034-2016.
29. Wild, C.P. Complementing the Genome with an "Exposome": The Outstanding Challenge of Environmental Exposure Measurement in Molecular Epidemiology. *Cancer Epidemiol. Biomark. Prev.* **2005**, *14*, 1847–1850, doi:10.1158/1055-9965.EPI-05-0456.
30. Zubova, N.N.; Bulavina, A.Y., Savitsky, A.P. Spectral and physicochemical properties of green (GFP) and red (drFP583) fluorescent proteins. *Usp. Biol. Khim.* **2003**, *43*, 163–224 (In Russian).
31. Stepanenko, O.V.; Verkhusha, V.V.; Kuznetsova, I.M.; Turoverov, K.K. Fluorescent proteins: physical-chemical properties and application in cell biology. *TSITOLOGIYA* **2007**, *49*, 395-420.
32. Kumar, A.; Pal. D. Green fluorescent protein and their applications in advance research. *Res. J. Appl. Sci. Eng. Tech.* **2016**, *1*, 42-46.
33. Remington, S.J. Green fluorescent protein: a perspective. *Protein Sci.* **2011**, *20*, 1509–1519, doi:10.1002/pro.684.
34. Frank, L.A. Ca^{2+}-Regulated photoproteins: effective immunoassay reporters. *Sensors* **2010**, *10*, 11287–1130, doi:10.3390/s101211287.
35. Krasitskaya, V.V.; Burakova, L.P.; Pyshnaya, I.A.; Frank, L.A. Bioluminescent reporters for identification of gene allelic variants. *Russian Journal of Bioorganic Chemistry* **2012**, *38*, 298–305, doi:10.1134/S1068162012030090.
36. Shimomura, O.; Teranishi, K. Light-emitters involved in the luminescence of coelenterazine. *Luminescence* **2000**, *15*, 51-58, doi:10.1002/(SICI)1522-7243(200001/02)15:1<51::AID-BIO555>3.0.CO;2-J.
37. Li, Z.-S.; Zhao, X.; Zou, L.-Y.; Ren, A.-M. The Dynamics Simulation and Quantum Calculation Investigation. About Luminescence Mechanism of Coelenteramide. *Photochem. Photobiol.* **2013**, *89*, 849–855, doi:10.1111/php.12073.
38. Malikova, N.P.; Stepanyuk, G.A.; Frank, L.A.; Markova, S.V.; Vysotski, E.S.; Lee, J. Spectral tuning of obelin bioluminescence by mutations of Trp92. *FEBS Letters* **2003**, *554*, 184-188, doi:10.1016/S0014-5793(03)01166-9.
39. Belogurova, N.V.; Kudryasheva, N.S.; Alieva, R.R. Activity of upper electron-excited states in bioluminescence of coelenterates. *J. Mol. Struct.* **2009**, *924*, 148–152, doi:10.1016/j.molstruc.2008.11.014.
40. Sharifian, S.; Homaei, A.; Hemmati, R.; B. Luwor, R.; Khajeh, K. The emerging use of bioluminescence in medical research. *Biomed. Pharmacother.* **2018**, *101*, 74-86, doi:10.1016/j.biopha.2018.02.065.
41. Lee, J. Perspectives on Bioluminescence Mechanisms. *Photochem. Photobiol.* **2017**, *93*, 389–404, doi:10.1111/php.12650.
42. Chen, S.-F.; Ferre, N.; Liu, Y.-J. QM/MM Study on the Light Emitters of Aequorin Chemiluminescence, Bioluminescence, and Fluorescence: A General Understanding of the Bioluminescence of Several Marine Organisms. *Chem. Eur. J*. **2013**, *19*, 8466–8472, doi:10.1002/chem.201300678.
43. Alieva, R.R.; Tomilin, F.N.; Kuzubov, A.A.; Ovchinnikov, S.G.; Kudryasheva, N.S. Ultraviolet fluorescence of coelenteramide and coelenteramide-containing fluorescent proteins. Experimental and theoretical study. *J. Photochem. Photobiol. B* **2016**, *162*, 318-323, doi:10.1016/j.jphotobiol.2016.07.004.
44. Min, C.-G.; Li, Z.-S.; Ren, A.-M; Zou, L.-Y; Guo, J.-F.; Goddard, J.D. The fluorescent properties of coelenteramide, a substrate of aequorin and obelin. *J. Photochem. Photobiol. A* **2013**, *251*, 182–188, doi:10.1016/j.jphotochem.2012.10.028.
45. van Oort, B.; Eremeeva, E.V.; Koehorst, R.B.M.; Laptenok, S.P.; van Amerongen, H.; van Berkel, W.J.H.; Malikova, N.P.; Markova, S.V.; Vysotski, E.S.; Visser, A.J.W.G.; Lee, J. Picosecond Fluorescence Relaxation

Spectroscopy of the Calcium-Discharged Photoproteins Aequorin and Obelin. *Biochem.* **2009**, *48*, 10486–10491, doi:10.1021/bi901436m.

46. Belogurova, N.V.; Kudryasheva, N.S.; Alieva, R.R.; Sizykh, A.G. Spectral components of bioluminescence of aequorin and obelin. *J. Photochem. Photobiol. B* **2008**, *92*, 117-122, doi:10.1016/j.jphotobiol.2008.05.006.
47. Belogurova, N.V.; Kudryasheva, N.S. Discharged photoprotein Obelin: Fluorescence peculiarities. *J. Photochem. Photobiol. B* **2010**, *101*, 103–108, doi:10.1016/j.jphotobiol.2010.07.001.
48. Alieva, R.R.; Belogurova, N.V.; Petrova, A.S.; Kudryasheva N.S. Fluorescence properties of Ca^{2+}-independent discharged obelin and its application prospects. *Anal. Bioanal. Chem*. **2013**, *405*, 3351-3358, doi:10.1007/s00216-013-6757-9.
49. Gao, M.; Liu, Y.-J. Photoluminescence Rainbow from Coelenteramide—A Theoretical Study. *Photochem. Photobiol*. **2018**, *95*, 563-571, doi:10.1111/php.12987.
50. Alieva, R.R.; Kudryasheva, N.S. Variability of fluorescence spectra of coelenteramide-containing proteins as a basis for toxicity monitoring. *Talanta* **2017**, *170*, 425-431, doi:10.1016/j.talanta.2017.04.043.
51. Alieva, R.R.; Belogurova, N.V.; Petrova, A.S.; Kudryasheva, N.S. Effects of alcohols on fluorescence intensity and color of a discharged-obelin-based biomarker. *Anal. Bioanal. Chem*. **2014**, *406*, 2965-2974, doi:10.1007/s00216-014-7685-z.
52. Burlakova, E.B.; Konradov, A.A.; Maltseva, E.X. Effect of extremely weak chemical and physical stimuli on biological systems. *Biophysics (Moscow*) **2004**, *49*, 522-534.
53. Feinendegen, L.E.; Pollycove, M.; Neumann, R.D. Whole-body responses to low-level radiation exposure: New concepts in mammalian radiobiology. *Exp. Hematol.* **2007**, *35*, 37-46, doi:10.1016/j.exphem.2007.01.011.
54. Feinendegen, L.E. Evidence for beneficial low level radiation effects and radiation hormesis. *Br. J. Radiol.* **2005**, *78*, 3-7, doi:10.1259/bjr/63353075.
55. Mothersill, C.; Seymour, C. Implications for human and environmental health of low doses of ionising radiation. *J. Environ. Radioact.* **2014**, *133*, 5-9, doi:10.1016/j.jenvrad.2013.04.002.
56. Jo, E.-R.; Jung, P.-M.; Choi, J.; Lee, J.-W. Radiation sensitivity of bacteria and virus in porcine xenoskin for dressing agent. *Radiat. Phys. Chem.* **2012**, *81*, 1259-1262, doi:10.1016/j.radphyschem.2011.08.016.
57. Mesquita, N.; Portugal, A.; Piñar, G.; Loureiro, J.; Coutinho, A.P.; Trovão, J.; Nunes, I.; Botelho, M.L.; Freitas, H. Flow cytometry as a tool to assess the effects of gamma radiation on the viability, growth and metabolic activity of fungal spores. *Int. Biodeter. Biodegr.* **2013**, *84*, 250-257, doi:10.1016/j.ibiod.2012.05.008.
58. Paul, J.; Kadam, A.A.; Govindwar, S.P.; Kumar, P.; Varshney, L. An insight into the influence of low dose irradiation pretreatment on the microbial decolouration and degradation of Reactive Red-120 dye. *Chemosphere* **2013**, *90*, 1348-1358, doi:10.1016/j.chemosphere.2012.07.049.
59. Xavier, M.P.; Dauber, C.; Mussio, P.; Delgado, E.; Maquieira, A.; Soria, A.; Curuchet, A.; Márquez, R.; Méndez, C.; López, T. Use of mild irradiation doses to control pathogenic bacteria on meat trimmings for production of patties aiming at provoking minimal changes in quality attributes. *Meat Sci.* **2014**, *98*, 383-391, doi:10.1016/j.meatsci.2014.06.037.
60. Luckey, T.D. *Hormesis with Ionizing Radiation*; Publisher: CRC Press, Incorporated Boca Raton, Florida, 1980; 225 pp.
61. Kudryasheva, N.S.; Petrova, A.S.; Dementyev, D.V.; Bondar, A.A. Exposure of luminous marine bacteria to low-dose gamma-radiation. *J. Environ. Radioact.* **2017**, *169-170*, 64-69, doi: 10.1016/j.jenvrad.2017.01.002.
62. Alexandrova, M.; Rozhko, T.; Vydryakova, G.; Kudryasheva, N. Effect of americium-241 on luminous bacteria. Role of peroxides. *J. Environ. Radioact.* **2011**, *102 (4)*, 407-411, doi:10.1016/j.jenvrad.2011.02.011.
63. Selivanova, M.A.; Rozhko, T.V.; Devyatlovskaya, A.N.; Kudryasheva, N.S. Comparison of chronic low-dose effects of alpha-and beta-emitting radionuclides on marine bacteria. *Cent. Eur. J. Biol.* **2014**, *9*, 951-959, doi:10.2478/s11535-014-0331-0.
64. Rozhko, T.V.; Badun, G.A.; Razzhivina, I.A.; Guseynov, O.A.; Guseynova, V.E.; Kudryasheva, N.S. On mechanism of biological activation by tritium. *J. Environ. Radioact.* **2016**, *157*, 131–135, doi:10.1016/j.jenvrad.2016.03.017**.**
65. Rozhko, T.V.; Guseynov, O.A.; Guseynova, V.E.; Bondar, A.A.; Devyatlovskaya, A.N.; Kudryasheva, N.S. Is bacterial luminescence response to low-dose radiation associated with mutagenicity? *J. Environ. Radioact.* **2017**, *177*, 261–265, doi:10.1016/j.jenvrad.2017.07.010.
66. Albers, R.W. Biochemical aspects of active transport. *Annu. Rev. Biochem.* **1967**, *36*, 727-756.

592 *67.* Lloyd, D.C.; Edvards, A.A.; Leonard, A.; Deknut, G.L.; Verschaeve, L.; Natarajan, A.T.; Darrudi, F.;
593 Obe, G.; Palitti, F.; Tanzarella, C.; Tawn, E.J. Chromosomal aberrations in human lymphocytes induced in
594 vitro by very low doses of X-rays. *Int. J. Radiat. Biol.* **1992**, *61*, 335-343, doi:10.1080/09553009214551021.
595 68. Clarridge, J.E. Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical
596 Microbiology and Infectious Diseases. *Clin. Microbiol. Rev.* **2004**, *17*, 840–862,
597 doi:10.1128/CMR.17.4.840-862.2004.
598 *69.* Rozhko, T.; Nogovitsyna, E.; Badun, G.; Lukyanchuk, A.; Kudryasheva, N. Reactive Oxygen Species and
599 Low-Dose Effects of Tritium on Bacterial Cells. *J. Environ. Radioact., under revision.*
600 *70.* Audi, G.; Wapstra, A.H.; Thibault, C. The AME2003 atomic mass evaluation (II).Tables, graphs, and
601 references. *Nucl. Phys. A* **2003**, *729*, 337–676, doi:10.1016/j.nuclphysa.2003.11.003.
602 *71.* Kamnev, A.A.; Tugarova, A.V.; Selivanova, M.A.; Tarantilis, P.A.; Polissiou, M.G.; Kudryasheva, N.S.
603 Effects of americium-241 and humic substances on *Photobacterium phosphoreum*: Bioluminescence and
604 diffuse reflectance FTIR spectroscopic studies. *Spectrochim. Acta A: Mol. Biomol. Spectrosc.* **2013**, *100*,
605 171-175, doi:10.1016/j.saa.2012.06.003.
606 *72.* Min, J.; Lee, C.W.; Gu, M.B. Gamma-radiation dose-rate effects on DNA damage and toxicity in bacterial
607 cells. *Radiat. Environ. Bioph.* **2003**, *42*, 189-192, doi:10.1007/s00411-003-0205-8.
608 *73.* Petrova, A.S.; Lukonina, A.A.; Badun, G.A.; Kudryasheva, N.S. Fluorescent Coelenteramide-Containing
609 Protein as a Color Bioindicator for Low-Dose Radiation Effects. *Anal. Bioanal. Chem*. **2017**, *409*, 4377–4381,
610 doi:10.1007/s00216-017-0404-9.
611 74. Petrova, A.S.; Lukonina, A.A.; Dementyev, D.V.; Bolsunovsky, A.Ya.; Popov, A.V.; Kudryasheva, N.S.
612 Protein-based fluorescent bioassay for low-dose gamma radiation exposures. *Anal. Bioanal. Chem*. **2018**,
613 *410*, 6837-6844, doi: 10.1007/s00216-018-1282-5.
614 *75.* Selye, H. Changing distress into eustress: voices theories on stress. *Tex. Med.* **1980**, *76*, 78-80.
615 *76.* Wang, C.-R.; Tian, Y.; Wang, X.-R.; Yu, H.-X.; Lu, X.-W.; Wang, C.; Wang, H. Hormesis effects and
616 implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings.
617 *Chemosphere* **2010**, *80*, 965-971, doi:10.1016/j.chemosphere.2010.05.049.
618 *77.* Baldwin, J.; Grantham, V. Radiation hormesis: historical and current perspectives. *J. Nucl. Med. Technol.*
619 **2015**, *43*, 242-246, doi:10.2967/jnmt.115.166074.
620 *78.* Kaiser, J. Hormesis: sipping from a poisoned chalice. *Science* **2003**, *302*, 376–379,
621 doi:10.1126/science.302.5644.376.
622 *79.* Calabrese, E.J.; Baldwin, L.A. The frequency of U-shaped dose responses in the toxicological literature.
623 *Toxicol. Sci.* **2001**, *62*, 330–338, doi:10.1093/toxsci/62.2.330.
624 80. Tarasova, A.S.; Stom, D.I.; Kudryasheva, N.S. Effect of humic substances on toxicity of inorganic oxidizer
625 bioluminescent monitoring. *Environ. Toxicol. Chem*. **2011**, *30*, 1013–1017, doi:10.1002/etc.472.
626 81. Tarasova, A.S.; Kislan, S.L.; Fedorova, E.S.; Kuznetsov, A.M.; Mogilnaya, O.A.; Stom, D.I.; Kudryasheva,
627 N.S. Bioluminescence as a tool for studying detoxification processes in metal salt solutions involving
628 humic substances. *J. Photochem. Photobiol. B* **2012**, *117*, 164–170, doi:10.1016/j.jphotobiol.2012.09.020.
629 82. Kudryasheva, N.S.; Tarasova, A.S. Pollutant toxicity and detoxification by humic substances: Mechanisms
630 and quantitative assessment via luminescent Biomonitoring. *Environ. Sci. Pollut. Res. Int*. **2015**, *22*, 155–167,
631 doi:10.1007/s11356-014-3459-6.
632 83. Tarasova, A.S.; Stom, D.I.; Kudryasheva, N.S. Antioxidant activity of humic substances via bioluminescent
633 monitoring in vitro. *Environ. Monit. Assess*. **2015**, *187*, 89, doi:10.1007/s10661-015-4304-1.
634 84. Kudryasheva, N.S.; Kovel, E.S.; Sachkova, A.S.; Vorobeva, A.A.; Isakova, V.G.; Churilov, G.N.
635 Bioluminescent enzymatic assay as a tool for studying antioxidant activity and toxicity of bioactive
636 compounds. *J. Photochem. Photobiol.* **2017**, *93*, 536–540, doi:10.1111/php.12639.
637 85. Sachkova, A.S.; Kovel, E.S.; Churilov, G.N.; Guseynov, O.A.; Bondar, A.A.; Dubinina, I.A.; Kudryasheva,
638 N.S. On mechanism of antioxidant effect of fullerenols. *Biochem. Biophys. Rep*. **2017**, *9*, 1–8,
639 doi:10.1016/j.bbrep.2016.10.011.
640 86. Sachkova, A.S.; Kovel, E.S.; Churilov, G.N.; Stom, D.I.; Kudryasheva, N.S. Biological activity of carbonic
641 nano-structures — Comparison via enzymatic bioassay. *J. Soils Sediments* **2018**,
642 doi:10.1007/s11368-018-2134-9.
643 87. Kovel, E.S.; Sachkova, A.S.; Vnukova, N.G.; Churilov, G.N.; Knyazeva, E.M.; Kudryasheva, N.S.
644 Antioxidant Activity and Toxicity of Fullerenols via Bioluminescence Signaling: Role of Oxygen
645 Substituents. *Int. J. Mol. Sci.* **2019**, *20*, 2324, doi:10.3390/ijms20092324.

646 *88.* Kleinhempel, D. Ein beitrag zur theories des huminstoffzustandes. *Arch. Agron. Soil Sci.* **1970,** *14*, 3-14,
647 doi:10.1080/03650347009412655.
648 89. Kudryasheva, N.; Vetrova, E.; Kuznetsov, A.; Kratasyuk, V.; Stom, D. Bioluminescent assays: Effects of
649 quinones and phenols. *Ecotoxicol. Environ. Saf.* **2002**, *53*, 221–225, doi:10.1006/eesa.2002.2214.
650 90. Brisebois, P.P.; Arnold, A.A.; Chabre, Y.M.; Roy, R.; Marcotte, I. Comparative study of the interaction of
651 fullerenol nanoparticles with eukaryotic and bacterial model membranes using solid-state NMR and FTIR
652 spectroscopy. *Eur. Biophys. J.* **2012**, *41*, 535-544, doi: 10.1007/s00249-012-0809-5.
653 91. Voeikov, V.L.; Yablonskaya, O.I. Stabilizing effects of hydrated fullerenes C_{60} in a wide range of
654 concentrations on luciferase, alkaline phosphatase, and peroxidase in vitro. *Electromagn. Biol. Med*. **2015**,
655 *34*, 160–166, doi:10.3109/15368378.2015.1036077.
656 92. Yablonskaya, O.I.; Ryndina, T.S.; Voeikov, V.L.; Khokhlov, A.N. A paradoxal effect of hydrated
657 C60-fullerene in an ultralow concentration on the viability and aging of cultivated Chinese hamster cells.
658 *Moscow Univ. Biol. Sci. Bull.* **2013**, *68*, 63-68, doi: 10.3103/S0096392513020107.
659 93. Bensasson, R.V.; Bretteich, M.; Frederiksen, J.; Gottinger, H.; Hirsch, A.; Land, E.J.; Leach, S.;
660 McGarvey, D.J.; Schonberger, H. Reactions of e-aq, CO_2^-, HO^-, O_2^- and $O_2(^1Dg)$ with a dendro[60]fullerene
661 and $C_{60}[C(COOH)_2]n$ (n = 52-6). *Free Radic. Biol. Med*. **2000**, *29*, 26-33, doi:10.1016/s0891-5849(00)00287-2.

662