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ASSESSING NEUROTOXICITY RISK THROUGH ELECTRORETINOGRAPHY WITH REDUCED LIGHT IRRITATION INTENSITY

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Summary. *The article is devoted to questions of the risk assessment of human neurotoxicity caused by the negative influence of free radicals of nanostructures, using electrophysiological methods of research - electroretinography with low intensity of light irritation. It has been established that the negative influence of toxins (chemical compounds of industrial and household purposes, nanomaterials as a source of free radicals) leads to changes in the parameters of electroretinosignal (ERS) in the early stages of detection of neurotoxicity. The use of advanced electroretinography (by decreasing the intensity of light irritation) and the use of a low intensity stimulation semiconductor source is substantiated. The ERS was obtained in the required range of values of light irritation, and morphological parameters were determined for further detection of ERS in admixture with noise, and for evaluating the characteristic change of the form of ERS under the influence of neurotoxicity.*

Keywords. *Electroretinography, electroretinosignal, low intensity of light irritation, neurotoxicity, evaluation of risks.*

Statement of the problem. The article presents the results of the study of electroretinosignal with a decrease in the intensity of light irritation to detect and assess the risk of neurotoxicity of the human body. The received ERS in the range of intensity of light irradiation from 0.1 to 10 $mcd \cdot s$, confirms the Weber-Fechner's empirical-psychophysical law, and allows the use of electroretinography with a reduced intensity of light irritation, to assess the risk of neurotoxicity of the human body due to the negative effects of free radicals of nanostructures.

Analysis of the available investigation results. The development, creation and use of new materials leads to increased risks of environmental pollution and human organism, which requires timely diagnosis and treatment [1]. Among the diseases, a special place is occupied by neurotoxicity caused by chemical factors. Industrial chemicals that affect the nervous system include: metallic mercury, manganese, arsenic compounds, tetraethyl lead, etc. [1], [2] Neurotoxicity is a toxic encephalopathy, in which neurological disorders are dominated by a progressive insufficiency of peripheral hemodynamics.

Syndrome of toxic-hypoxic encephalopathy is considered as a functional failure of the central nervous system, as a result of the combination of metabolic, hemodynamic, morphological changes in the brain tissues with chemical lesion. It develops under the influence of neurotoxins – chemical compounds that are in the body in an amount that is capable of breaking the functions of vital organs and creating a danger to life. Intoxication, or poisoning, leads to a pathological condition caused by a violation of chemical homeostasis due to the interaction of the toxin with the organism. Substances that may cause intoxication are divided into: 1) industrial poisonous substances; 2) poisonous chemicals (pesticides); 3) medicines; 4) household chemicals; 5) biological, plant and animal poisons; 6) fighting poisonous substances, etc..

In addition, toxicants can selectively affect any system of the body or organs. Toxic damage to the nervous tissue can occur as a result of exposure: a) heavy metals; b) nanomaterials (as sources of free radicals); c) organic compounds of phosphorus; g) gasoline; e) carbon monoxide; e) methyl and ethyl alcohol. Since, under the influence of these toxins, the brain is primarily affected, the spread of acute and chronic neurotoxicosis is a major problem as clinical toxicology, occupational medicine, neurology, and medicine as a whole.

Objective and subjective methods of research are used to study the state of retina as an indicator of the influence of toxins on the human body. To subjective include psychophysical tests: the Purkinje test, laser interference, Medoc’s sticks, recognition of two points, etc. However, subjective tests do not provide enough information to diagnose a patient's condition. Among the objective methods of research complementary to psychophysical tests, distinguish between ultrasound and electrophysiology. The basic methods of ultrasound diagnostics of the eye include: one-dimensional echography (A-method), two-dimensional echography (acoustic scanning, B-method), ultrasound three-dimensional echography, energy dopplerography and ultrasound duplex scanning. However, even with low energy of ultrasound radiation, there is an additional invasiveness and a negative effect on the patient.

Therefore, for the reliable diagnosis and localization of the causes of the disease, electrophysiological methods based on the analysis of bioelectric signals from the studied parts of the visual system are the most promising ones [3] and [4]. Among the electrophysiological methods of the study are the following methods for assessing the functional state of the visual analyzer: the determination of electrical sensitivity of the optic nerve, electrooculography (EOG), electroencephalography (together with the registration of visual stimulated brain cortical potentials) and electroretinography.

Electroretinography is one of the most informative methods of early diagnosis of many diseases of the visual analyzer, as indicated by the study. The founder of the clinical electrophysiology of the organs of vision is the Swedish ophthalmologist G. Karpe, who used contact lenses for research in 1945. His work was based on the development of Riggs. A large amount of work in the direction of fixing electroretinography and study of electroretinosignal, which is a graphical representation of the electrical reaction of the set of cellular elements of the retina of the eye to external light irritation, was carried out by the Swedish physiologist R. Granit [5] and [6].

It is known that to date, precise methods for diagnosing toxic encephalopathy and neurotoxicity have not been developed, therefore it is important to improve the electroretinography study to assess the risk of neurotoxicity.

The objective of the paper. The object of the study is to receive electroretinosignal with a reduced intensity of light irritation to assess the risk of neurotoxicity.

Statement of the problem. It is known that human body changes in neurotoxicity are most rapidly manifested in deviations of the processes of the retina [7]. In particular, it was found that mercury intoxication shows a decrease in the thickness of the retina in the area of the fovea, which indicates the development of dystrophic changes in it. As a result of the research, inhibition of retinal bioelectric activity was detected in the form of increasing the threshold of electrical sensitivity, lengthening the latent recovery time, and reducing the morphological parameters (Fig. 1).

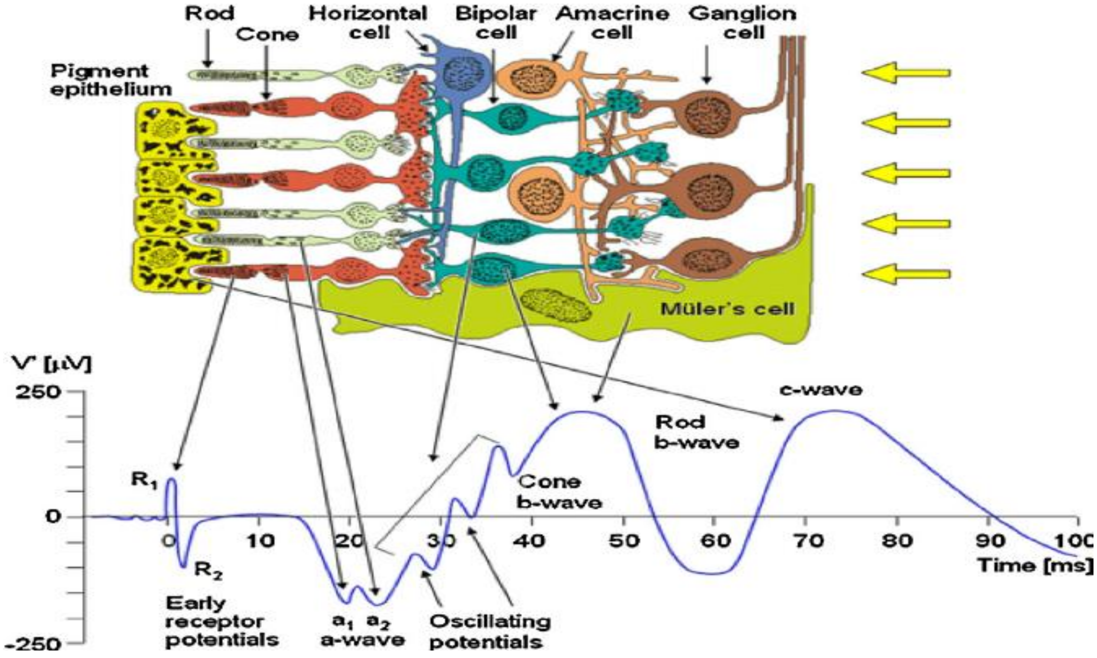


Figure. 1. Retina structure and sources of potential of ERS [8]

From studies of the group of applied physics doc. Nicolas Gisina, Doc. Shamshinova A.M. etc., found that the b-wave of electroretinogram (ERG) is the most sensitive to toxins and the value of the parameters of the ERS, depends on the intensity of the light stimulus. Therefore, when the intensity of light irritation decreases, there is a greater chance of accurately registering the b-wave. To determine the intensity range of light irritation, the following criteria for the registration of ERG is a prerequisite:

I. threshold value of the logarithm of intensity of irritation, which improves the results of ERG registration;

II. determining the maximum value of the amplitude of the b-wave.

Accordingly, the level of irritation of the visual system (retina) increases in proportion to the logarithm of the intensity of light. In this case, the accuracy and resolution (Fig. 2) of the registered electroretinosignal increases (Weber-Fechner's law) [9].

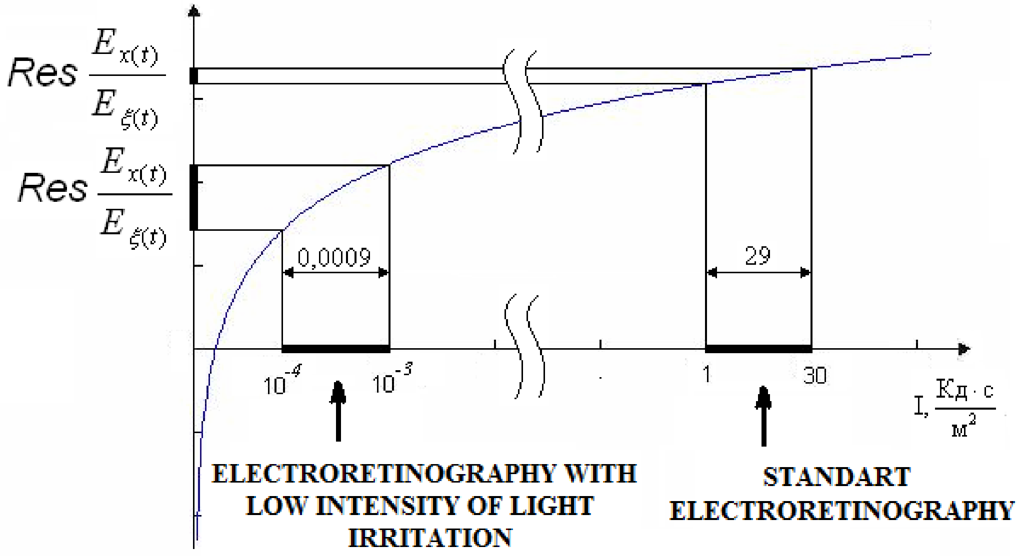


Figure.2. Dependences of the ratio of the energy of the ERS and the energy of noise, on the intensity of light irritation (*Res* – resolution)

However, the essential disadvantage of electroretinography examination (Fig. 3) is the patient's discomfort caused by the "standard" high intensity of light irritation (which manifests itself as a feeling of a foreign body, tear and tears, etc.), therefore, the problem of selecting the characteristics of a light stimulus with minimal discomfort on the retina of the patient's eye and getting the most informative.

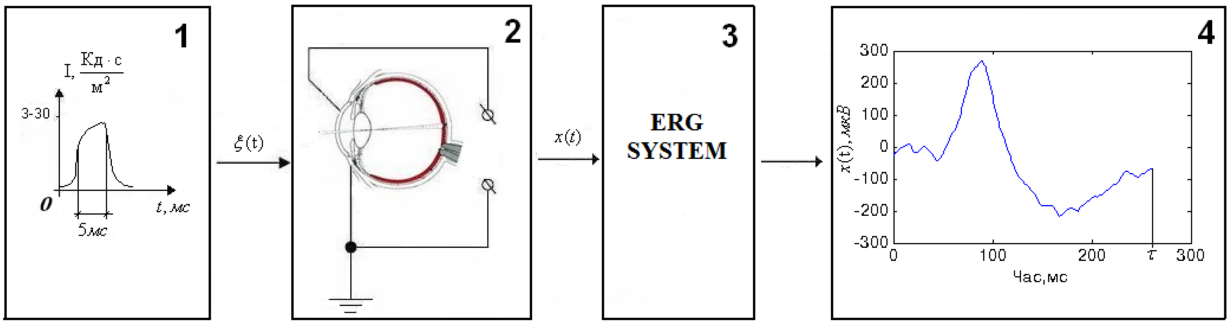


Figure.3. Stages of electroretinography selection of the signal: 1 – a source of light irritation; 2 – bioobject; 3 – electroretinography system; 4 – is a typical electroretinosignal

The ERS is recorded by measuring the electric potential of the retina of the eye relative to the reference point (ear lobes, points on the forehead) that arise in response to stimuli (flashes of light of different wavelengths, intensity, frequency of repetition). By way of obtaining, the ERS is divided into two types: the general (the entire surface of the retina is illuminated), local (the effect of light irritations on separate zones of the retina).

A typical ERS consists of a negative extremum - wave "a", which is generated by photoreceptors; a positive extreme - wave b ", which is caused by the action of Bulls, Müller's ganglion cells and other secondary nerve structures that perform the prior processing of receptor signals before they are transmitted to the brain.

For "wave b", other local extrema is recorded: negative wave b1, which is the potential aftereffect; positive potential is "wave c1", and "wave d", which arises when the light irritation is stopped. Additional studies of the last two waves, which have not yet been sufficiently studied, are required, and therefore their analysis has not been used in diagnostics and clinical practice.

Such studies did not have a prospect for selecting a standard electrolyte signal when conducting research with high energy of light irritation (in the range from 3 to 30 $cd \cdot s \cdot m^{-2}$) [10]. High energy of light irritation allows to detect significant violations of the functioning of the visual analyzer, but it has a number of significant disadvantages:

1) the eye rejuvenates considerably;
2) the need for multiple ERS registrations (for the application of coherent filtration);
3) as a result, the length of the procedure increases significantly;
4) there is no possibility of displaying small morphological changes in the ERS, which is important for modern medicine.

Therefore, to reduce the negative effects of standard electroretinography, the decrease in the intensity of light irritation is used [11]. Possibility of electroretinography with a significantly reduced energy of light irritation due to the peculiarity of the structure of the eye. In the works [12], [13] and [14], the authors point out that to obtain the maximum light sensitivity at a wavelength of 505-510 nm (blue-green light). For the reaction to light it takes from 43 to 48 quanta of light, but since the light flux passes through the optical environment of the eye, for the emergence of a low intensity electroretinosignal it is necessary that much more quanta be reached on the retina - up to 50-150 quanta of light [12] and [13].

In view of this, in medical practice, the use of electroretinography with ultra-low energy of light irritation has been substantiated. The main advantages of quantum electroretinography are:

a) early diagnosis of changes in the functional state of the human body (in particular, in neurotoxication);

b) low energy of light irritation (from 10^{-3} to 10^{-4}) leads to decrease of invasiveness;

c) shortening the duration of the procedure, due to a significant reduction in eye rejuvenation time.

However, this will require the study of light stimulation (photostimulants) to reduce the intensity or exposure of light irritation, and the experimental reception of low-intensity ERS. The standards of ISCEV indicate the intensity of light stimuli for electroretinography studies. However, these standards are based on the use of gas-discharge xenon lamps (Grass PS-22, lamp PST-2100), while units of light stimulation measurement are adapted to the technical features of gas-discharge xenon lamps.

The development of the elemental base leads to the improvement of the apparatus part of the electroretinosignal study. The emergence and widespread use of modern semiconductor corrosive sources of irritation leads to the adequate use of LED photostimulators in electroretinography. To reduce the intensity of light irritation used (Fig. 4) [15].

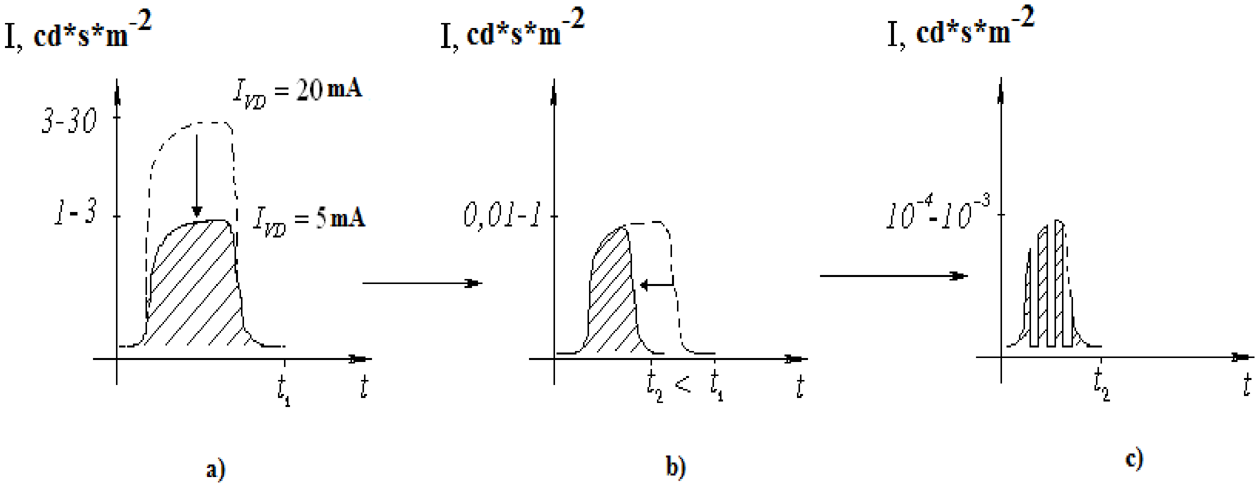


Figure.4. Ways of reducing the intensity of the light stimulus (irritation):
a) reducing the electrical power supply parameters of the LED (supply current);
b) reducing the duration of the light flash (reducing the exposure);
c) using the modulation of the power LED.

The first two methods have a significant disadvantage, due to the fact that the reduction of electrical parameters can only be to a certain limit. Further, the reduction of their light flux is impossible due to the internal structure and peculiarities of the physics of the transition of electrons through the p-n-junction. Therefore, it is promising to use PWM-modulation of the power supply of the LED.

In order to obtain an ERS with a reduced intensity of light irritation in a wide range of values, the ophthalmology system DCSO-01 has been improved, with 250 samples with a quantization period Tq = 2.336 ms (sampling rate fs = 428 Hz) and a predetermined number of registrations.

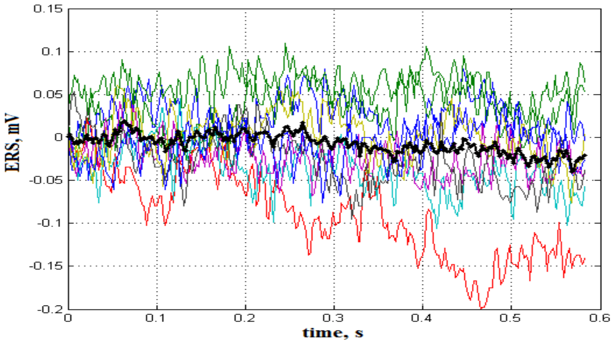
ERS is selected from the eye of a rabbit in a wide range of stimulus energies. As a source of irritation, a white LED (smd 3528) was used, the energy (E) of the radiation (stimulus) of which was given by three components: pulse cleft (D), magnitude and duration of direct current.

Table1

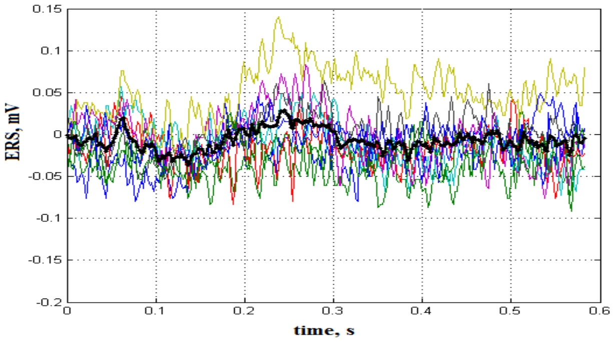
Summary table of parameters of the LED photo stimulator
on the basis of the PWM modulator of power LED 3528

Switch position on PWM modulator	0	1	2	3	4	5	6	7	8	9	10
E(lux)	859	1847	4904	9044	14044	27276	24522	29808	35573	40732	41847
t_i(ms)	–	2,83	5,03	6,93	–	10,6	–	–	–	–	16,60
D, %	1	10	20	30	40	50	60	70	80	90	97
H($lux \cdot s$)	–	0,522	4,933	18,802	–	144,562	–	–	–	–	673,82

Such a method of forming the energy of the stimulus allows to provide a wide range of values $mcd \cdot s$ (Fig.5.a – c).



a)



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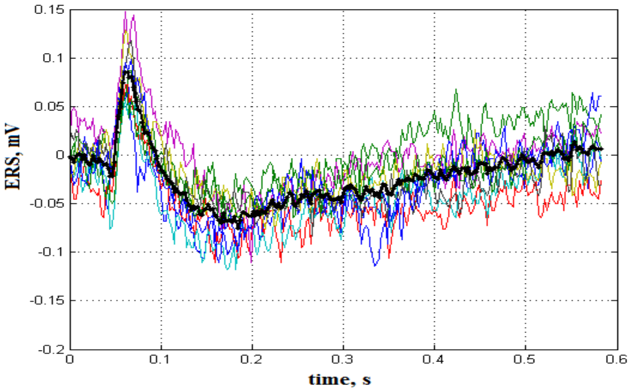

c)

Figure.5 ERS at different levels of intensity of light irritation:
a) I_{st} = 0.1 $mcd \cdot s$; b) I_{st} = 1.9 $mcd \cdot s$; c) I_{st} = 10.5 $mcd \cdot s$.

In order to reduce the noise of the ERS, the ERS ensemble was registered from 16 implementations with fixed energy of the stimulus followed by its averaging:

$$x_c\left(nT_q\right) = \frac{1}{16}\sum_{i=1}^{16} x_i\left(nT_q\right),$$

where $x_i(nT_q)$ — implementation of ERS, $x_c(nT_q)$ — averaged ERS for the given stimulus energy, nT_q — discrete time, i — implementation number.

The results are shown in Fig. 5, where the bold curve is depicted $x_c(nT_q)$, but thin — $x_i(nT_q)$.

During the experiment, more than 5000 ERS implementations were registered at different intensities of the light stimulus, but the resulting ERS when reducing the intensity of light irritation requires further elaboration and further research.

Conclusions. The analysis showed that in conditions of increasing the negative influence on the human body, the accuracy and resolution of standard electroretinography is not sufficient to detect the risks of neurotoxicity (detection of neurotoxicity, evaluation of their type, quantitative and qualitative characteristics, etc.). Therefore, in order to diagnose the state of the visual analyzer, the most promising is the use of electroretinography with a reduced intensity of light irritation. The following results are obtained:

1) The functioning of the visual analyzer and the manifestation of sensations from the effects of stimuli (light stimulus) is explained by the Weber-Fechner empirical psychophysical law, therefore, to increase the accuracy and resolution (to detect the risk of neurotoxicity) reduce the energy of light irritation.

2) A pilot model for reducing the intensity of the light stimulus based on the PWM modulation of the LED power supply was created. This allowed to reduce the level of light irritation to 0.522 $lux \cdot s$, compared to the standard stimulation of electroretinography (Grass PS-33) – 59 $lux \cdot s$.

3) Experimentally, an ERS with a reduced intensity of light irritation has been obtained in a wide range of values $mcd \cdot s$, and the need for further elaboration of ERS for assessing the risk of neurotoxicity has been established.

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ОЦІНЮВАННЯ РИЗИКУ НЕЙРОТОКСИКАЦІЇ ЗА ДОПОМОГОЮ ЕЛЕКТРОРЕТИНОГРАФІЇ ЗІ ЗМЕНШЕНОЮ ІНТЕНСИВНІСТЮ СВІТЛОВОГО ПОДРАЗНЕННЯ

Резюме. *Стаття присвячена питанням оцінювання ризиків нейротоксикації організму людини, викликаної негативним впливом вільних радикалів наноструктур, з використанням електрофізіологічного методу дослідження – електроретинографії з низькою інтенсивністю світлового подразнення. Дослідження електроретиносигналу при зменшенні інтенсивності світлового подразнення підтверджується законом Вебера-Фехнера, і є перспективним при застосуванні ЕРС у виявленні нейротоксикації. Проблема нейротоксикації стає актуальною через зростаюче використання нових матеріалів, що можуть негативно впливати на навколишнє середовище та здоров'я людини. Особливо це стосується промислових хімічних речовин, таких як металева ртуть, марганець, сполуки миш'яку та інші. Встановлено, що негативний вплив токсинів (хімічних сполук промислового та побутового призначення, наноматеріалів як джерела вільних радикалів) призводить до зміни параметрів електроретиносигналу (ЕРС) на ранніх етапах виявлення нейротоксикації. Традиційні методи діагностики нейротоксикації є обмеженими та інвазивними, тоді як ЕРС може надати об'єктивну та неінвазивну оцінку стану зорової системи. Обґрунтовано використання вдосконаленої електроретинографії (шляхом зменшення інтенсивності світлового подразнення) та застосування фотостимулятора на основі напівпровідникового джерела низької інтенсивності (LED). Отримано ЕРС у необхідному діапазоні значень світлового подразнення та визначено морфологічні параметри для подальшого виявлення ЕРС у суміші з шумом та оцінювання характерної зміни форми ЕРС під впливом нейротоксикації. Дослідження вказує на переваги використання електроретинографії для виявлення нейротоксикації та надає підстави для подальших досліджень у цьому напрямі. Отримані результати сприяють розвитку методів діагностики та оцінювання нейротоксикації, що може бути корисним у медицині та токсикології.*

Ключові слова. *Електроретинографія, електроретиносигнал, низька інтенсивність світлового подразнення, нейротоксикація, оцінка ризиків.*