

FATTY ACID PROFILE OF THE LIVER LIPIDS UNDER ACUTE FUNGICIDE ACTION AND INTAKE OF A BIOLOGICALLY ACTIVE PREPARATION IN RATS

S. V. KHYZHNYAK[✉], S. V. MIDYK, A. O. VELINSKA,
O. V. ARNAUTA, L. H. KALACHNIUK

National University of Life and Environmental Sciences of Ukraine, Kyiv;
[✉]e-mail: khyzhnyaks@gmail.com

Received: 18 August 2022; **Revised:** 24 October 2022; **Accepted:** 04 November 2022

The widespread use of fungicides in agriculture leads to a negative consequences for both humans and the environment. The acute effect of fungicides containing tebuconazole alone or in combination with triadimefon on fatty acid (FAs) composition of the total lipids in the liver of rats was studied. FAs content was analyzed by gas chromatography with the use of flame ionization detector. The obtained results indicate that the acute effect of triazole fungicides one-time oral administration to rats is characterized by toxic reactions and is accompanied by the total liver lipids FAs profile redistribution, similar for the studied fungicides. In particular, the reduction in the total content of monounsaturated FAs (by 26% – 37%) and in the $\omega 3/\omega 6$ ratio was observed. It was demonstrated that the subsequent two-week introduction of the preparation based on Milk thistle seeds oil extract had a positive effect and improved the FAs profile of lipids in dynamics after fungicide intake.

Key words: fatty acids, rat liver lipids, fungicides, triazoles, Milk thistle seeds extract.

Pesticides are the versatile group of chemicals which are widely used as plant protection agents. However, this increases the environmental and health risks associated with their use [1]. Pesticides, in particular, can accumulate in agricultural products with which they enter human and animal organisms. Similarly, pesticide-contaminated water and air have adverse effects on humans. The most numerous and important group of modern fungicides – are triazoles, systemic pesticides, used to protect plants from a wide range of fungal diseases. However, representatives of this group, in particular tebuconazole, are often detected in elevated concentrations in the environment.

Current data indicate diverse toxic effects of tebuconazole in vertebrates. In fact, the search for potential biochemical targets of pesticide toxicity is relevant today [2]. Thus, the study of the nature and mechanisms of biochemical action of tebuconazole fungicides is important as a typical example for assessing toxicity to vertebrates, including humans. Hematotoxic action is shown in acute intoxication of rats with tebuconazole [3]. Hepatotoxicity is also observed with signs of hepatocyte dystrophy [4]. In addition, studies of tebuconazole accumulation in

HepG2 cells and associated lipid metabolic disorders led to suggestion that human exposure to tebuconazole may be a risk factor for the development of non-alcoholic fatty liver disease [5].

It is therefore important to study the composition of lipid fatty acids (FAs) in various tissues to understand the mechanism of tebuconazole toxicity, in particular, which is related to its effect on lipid metabolism. Lipid composition plays an important role in controlling the direction and functioning of various cellular processes. Moreover, modification of lipid FAs profile can accompany and characterize the effect of exogenous factors on organism [6], including pesticides [7]. On the other hand, the study of the possibility of regulating the FAs profile of rat liver lipids under the influence of pesticides in combination with a biologically active preparation contributes to the correction of metabolic abnormalities, which is important in the development of a set of measures to prevent the negative impact of pesticides on public health.

The aim of the current research was to study the toxic manifestations and fatty acid composition of the liver lipids in rats under acute exposure to triazole fungicides containing tebuconazole separately

and in combination with triadimefon and the possibility of correction with biologically active preparation "OVA+".

Material and Methods

Systemic fungicides of various pesticide formulations Azimuth and Azimuth Classic (manufactured by "Frandes Ukraine") in preparative form - emulsifiable concentrate (EC), which are registered in Ukraine and used to protect agricultural crops, were studied. Their stock solutions contained active ingredients in the following concentrations in Azimuth: tebuconazole, 125 kg/m³ + triadimefon, 100 kg/m³ and in single-component fungicide Azimuth Classic: tebuconazole 250 kg/m³. The active ingredients of fungicides belong to the chemical class of triazoles. The patented preparation "OVA+" (production of NULES Ukraine) is obtained according to [8] by extracting substances from the seeds of Milk thistle (*Silybum marianum*) with corn oil, which are collected from an annual plant. Milk thistle seeds are a source of various biologically active substances: flavolignans, flavonoids, as well as vitamins, tannins, macro- and microelements, etc. It has a potential choleric effect, stabilizes the functioning of the liver and digestive system, promotes the elimination of toxins from the body.

Female Wistar Han rats weighing 210-240 g, used in the studies, were kept under standard vivarium conditions of the State Institution "Institute of Occupational Medicine of the National Academy of Medical Sciences of Ukraine" under conventional temperature and light conditions. Rats were divided into four groups: control (Group I) and 3 experimental (Groups II, III, IV) of 7 animals each. Animals of Group II were given via gavage once orally 1200 mg/kg of an aqueous emulsion of the Azimuth fungicide (contained tebuconazole in combination with triadimefon). Animals of Groups III and IV were administered via gavage once orally 1200 mg/kg of an aqueous emulsion of the Azimuth Classic fungicide (contained tebuconazole). The control group (Group I) received an equivalent volume of distilled water. The rats of Group IV were orally given 4 drops (about 0.1 ml) of oily liquid of the "OVA+" preparation twice a day for each animal over the next 13 days. The used fungicide dose (1200 mg/kg), which is 1/4 LD₅₀, was calculated from the LD₅₀ values for the active ingredients (reference data).

Toxicological studies were conducted in accordance with the principles and criteria set out in

[9]. Toxic manifestations in animals were visually evaluated during the entire period of research, in particular, toxic reactions were determined and the beginning of the recovery period was noted. After 14 days the animals were decapitated under light ether anesthesia and the liver was used in research. Animal manipulations were carried out following the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), according to the general ethical principles of experiments on animals, adopted by the First National Congress of Ukraine on Bioethics (2001).

The liver tissue was homogenized and the lipids were subsequently extracted with a chloroform-methanol mixture to determine the fatty acid composition of total lipids. Hydrolysis and methylation of lipid FAs carried out according to [10]. Gas chromatographic analysis of FAs methyl esters was performed on a Trace GC Ultra gas chromatograph (USA) with a flame ionization detector. Experiment conditions: column temperature 140-240°C, detector temperature 260°C, analysis duration 65 min. Fatty acids were identified using a Supelco 37 Component FAME Mix standard. To quantify individual FAs the method of internal normalization was used and the relative content of FAs was expressed as a percentage of their total amount. Besides, the total quantities of saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), FAs of the ω3 and ω6 families were calculated.

The data were analyzed by computer software Origin 6.0 and Excel (Microsoft, USA) using Student's *t*-test. Differences were considered significant when *P* < 0.05.

Results and Discussion

Studies on rats are carried out to assess the toxicity of chemicals used in industry and agriculture, as well as to establish the mechanisms of their toxic action [11]. With acute exposure to fungicides of the triazole group, toxic manifestations in rats were observed from the first hours after administration, which were most intense in the first four hours after administration, partially persisted until the second day and disappeared by the end of the study. Among the manifestations of toxic effects on animals reduced activity, depression, disheveled hair, ataxia, humpback posture, untidy appearance, constriction of the eye, salivation, respiratory failure

re, decreased appetite, etc were noted. The obtained results indicate a pronounced toxic effect of fungicides containing tebuconazole both individually and in combination with triadimefon at their acute action at a dose of 1200 mg/kg.

The fungicide toxic action is mediated by the processes of absorption and metabolism in the organism, which determines the involvement of the lipid component of cells. Important cellular structural and energy compounds - FAs play a significant role not only in metabolic processes, but also mediate protective reactions of the body under the action of exogenous factors [6, 12].

Chromatograms of FAs of rat liver total lipids were obtained in our researches. In the studied samples 21 short-, medium- and long-chain FAs with carbon chain length from C8 to C22 were identified. The main FAs and their relative content in total lipids of rat liver are shown in Table 1.

Minor components, the content of which did not exceed 0.1 %, are not presented in the Table. SFAs are mainly represented by palmitic (C16:0) and stearic (C18:0) acids, which are precursors of long-chain FAs. UFAs are heterogeneous, in particular, palmitoleic (C16:1) and oleic (C18:1 ω 9) have one saturated bond each, and such as arachidonic (C20:4 ω 6), eicosapentaenoic (20:5 ω 3) and docosahexaenoic (C22:6 ω 3) are polyunsaturated.

The FAs composition of total lipids in rat liver tissue after fungicide administration did not differ qualitatively from the control. However, there was a similar redistribution of the content of both SFAs and UFAs for fungicides containing tebuconazole both individually and in combination with triadimefon (Table 1). Opposite changes in SFAs content, especially in the major palmitic (C16:0) and stearic (C18:0) acids, should be noted. Thus, the content of palmitic acid decreased by 28% and 27%, and the

Table 1. Fatty acid content of total lipids (% of total fatty acids) in rat liver tissues after acute peroral fungicide administration and preparation "OVA+" ($M \pm m$, $n = 7$)

Fatty acid	Group I (control)	Group II	Group III	Group IV
C10:0 Capric acid	0.20 $\pm$ 0.02	0.14 $\pm$ 0.01*	0.09 $\pm$ 0.01*	0.20 $\pm$ 0.02
C14:0 Myristic acid	0.62 $\pm$ 0.05	0.28 $\pm$ 0.04*	0.33 $\pm$ 0.02*	0.62 $\pm$ 0.02
C15:0 Pentadecanoic acid	0.27 $\pm$ 0.02	0.23 $\pm$ 0.02	0.30 $\pm$ 0.03	0.25 $\pm$ 0.01
C16:0 Palmitic acid	21.79 $\pm$ 1.61	15.62 $\pm$ 1.10*	15.92 $\pm$ 1.21*	18.63 $\pm$ 1.32
C16:1 Palmitoleic acid	0.97 $\pm$ 0.07	0.42 $\pm$ 0.03*	0.30 $\pm$ 0.02*	0.59 $\pm$ 0.02
C17:0 Margaric acid	0.69 $\pm$ 0.04	0.62 $\pm$ 0.06	0.68 $\pm$ 0.05	0.57 $\pm$ 0.03
C17:1	0.12 $\pm$ 0.02	0.12 $\pm$ 0.02	0.14 $\pm$ 0.02	0.13 $\pm$ 0.02
C18:0 Stearic acid	16.74 $\pm$ 1.41	22.58 $\pm$ 1.76*	21.62 $\pm$ 1.88*	19.61 $\pm$ 1.12
C18:1 ω 9 Oleic acid	8.69 $\pm$ 0.41	6.72 $\pm$ 0.32*	5.80 $\pm$ 0.28*	7.35 $\pm$ 0.52
C18:2 ω 6 Linoleic acid	15.23 $\pm$ 1.21	15.20 $\pm$ 1.11	14.09 $\pm$ 1.04	14.93 $\pm$ 1.01
C20:0 Arachidic Acid	0.94 $\pm$ 0.06	0.27 $\pm$ 0.02*	0.32 $\pm$ 0.02*	1.03 $\pm$ 0.03
C18:3 ω 3 Linolenic acid	0.64 $\pm$ 0.04	0.52 $\pm$ 0.03*	0.56 $\pm$ 0.03	0.43 $\pm$ 0.02*
C21:0 Heneicosanoic acid	1.02 $\pm$ 0.04	0.76 $\pm$ 0.05*	0.52 $\pm$ 0.04*	0.90 $\pm$ 0.03
C22:0 Behenic acid	0.40 $\pm$ 0.02	0.54 $\pm$ 0.02*	0.64 $\pm$ 0.03*	0.43 $\pm$ 0.02
C20:3 ω 6 Eicosatrienic acid	1.08 $\pm$ 0.09	1.46 $\pm$ 0.06*	1.40 $\pm$ 0.03*	0.98 $\pm$ 0.05
C20:4 ω 6 Arachidonic acid	18.36 $\pm$ 1.11	23.57 $\pm$ 1.21*	25.94 $\pm$ 1.12*	21.00 $\pm$ 1.02
C20:5 ω 3 Eicosapentaenoic acid	0.90 $\pm$ 0.05	0.80 $\pm$ 0.03	0.63 $\pm$ 0.04*	1.00 $\pm$ 0.03
C22:5 ω 3 Docosapentaenoic acid	0.71 $\pm$ 0.03	0.67 $\pm$ 0.04	0.65 $\pm$ 0.02	0.67 $\pm$ 0.03
C22:6 ω 3 Docosahexaenoic acid	10.57 $\pm$ 0.91	9.37 $\pm$ 0.9	10.0 $\pm$ 0.89	10.60 $\pm$ 0.88

Note: in this and next table rats received fungicides: Group II – Azimuth (contained tebuconazole in combination with triadimefon), Groups III, IV – Azimuth Classic (contained tebuconazole) and preparation "OVA+" (Group IV). * $P < 0.05$ vs control

content of stearic acid increased by 35 and 29% relative to the control in Groups II and III, respectively. At the same time, the total content of SFAs did not change significantly, and saturation index (SFAs/UFAs), which in control was 0.74, decreased to 0.68 and 0.70 (Table 2).

Moreover, analysis of FAs profile of total lipids indicates the decrease in the MUFAs content. In particular, the content of oleic acid (C18:1 ω 9), which can exhibit antioxidant properties [13], is reduced on average by 23% in Group II and 33% in Group III compared to control (Table 1). At the same time, MUFAs total content decreased significantly by 26% in Group II and 37% in Group III relative to the control (Table 2). It is known that ω 9 FAs can partially replace those of the ω 3 and ω 6 families in lipids during the action of stress factors [12].

On the other hand, the PUFAs redistribution occurred in liver total lipids under acute fungicide action. In this case their total content increased insignificantly (Table 2). In ω 3 FAs family the tendency to a decrease is observed in the content of C18:3 ω 3 and biologically active long-chain ω 3 FAs (C20:5 ω 3 and C22:5 ω 3), which are characterized by the functions of an adaptive stabilizer of the cellular lipid bilayers [14].

As for the ω 6 FA family, there was a clear upward trend in their total content, which increased by 16 and 20% compared to the control group in

Groups II and III, respectively (Table 2). A significant differences were shown for a number of the ω 6 family representatives. For the main constituent of the ω 6 family, arachidonic acid (C20:4 ω 6), a significant increase by 28% and 41% was also found in groups II and III, respectively (Table 1). The accumulation of this ω 6 FA, which, along with other reasons, is due to interaction abnormalities of the of FAs with membrane protein complexes, with proteins of exo- and endocytosis resulted from the modification in acute toxicity conditions, in turn can lead to an increase in the content of prostaglandins in cells [15].

Thus, fungicide delivery into the rat organism (Groups II and III) led to a redistribution of the relative content of FAs of the ω 3, ω 6 and ω 9 families in total lipids of liver tissues. The revealed decrease in ω 3/ ω 6, ω 9/ ω 3 and ω 9/ ω 6 ratios (Table 2) specifies the direction of FAs metabolism of liver lipids in view of the interconnection of these processes and the existence of competitive synthesis pathways in the metabolic process [16]. It is important to maintain the ratio ω 3/ ω 6, since fatty acids of the ω 6 and ω 3 families are endogenous bioregulators, and therefore physiologically important for the animal organism [15]. Derivatives of ω 6 FAs are highly active regulators of cellular functions that enhance membrane permeability and cause inflammatory processes. On the other hand, ω 3 FAs metabolites which are antiag-

Table 2. The level of fatty acid saturation of total lipids (% of the total fatty acids) in the rat liver tissues after acute oral administration of fungicides and preparation "OVA+" ($M \pm m$, $n = 7$)

SFAs	Group I (control)	Group II	Group III	Group IV
Σ SFAs	42.6 $\pm$ 3.2	41.0 $\pm$ 3.1	40.4 $\pm$ 3.3	42.2 $\pm$ 3.4
Σ UFAs	57.3 $\pm$ 4.1	58.9 $\pm$ 4.1	59.5 $\pm$ 4.2	57.7 $\pm$ 3.9
SFAs /UFAs	0.74	0.70	0.68	0.73
Σ MUFAs	9.8 $\pm$ 0.8	7.3 $\pm$ 0.5*	6.2 $\pm$ 0.4*	8.1 $\pm$ 1.1
Σ PUFAs	47.5 $\pm$ 3.2	51.6 $\pm$ 4.1	53.3 $\pm$ 4.2	49.6 $\pm$ 3.2
$\Sigma\omega$ 6	34.7 $\pm$ 1.8	40.2 $\pm$ 2.1	41.8 $\pm$ 2.0*	36.9 $\pm$ 1.9
$\Sigma\omega$ 3	12.8 $\pm$ 0.9	11.4 $\pm$ 1.0	11.8 $\pm$ 1.1	12.7 $\pm$ 1.0
$\Sigma\omega$ 3/ $\Sigma\omega$ 6	0.37	0.28	0.29	0.34
C20:4 ω 6/C18:2 ω 6	1.21	1.55	1.84	1.41
C22:6 ω 3/C18:3 ω 3	16.5	18.0	17.9	24.7
ω 9/ ω 3	0.68	0.59	0.53	0.59
ω 9/ ω 6	0.28	0.18	0.15	0.22

Note: FAs – fatty acids, SFAs – saturated fatty acids, UFAs – unsaturated fatty acids, MUFAs – monounsaturated fatty acids, PUFAs – polyunsaturated fatty acids. * $P < 0.05$ vs control

gregants, anti-inflammatory substances, contribute to the membrane stabilization [12].

It should be noted that modulation of the lipid PUFAs content can be associated with the processes of desaturation and elongation of fatty acids. It is known that adaptive changes in the degree of FAs unsaturation ($\omega 3$ and $\omega 6$) can occur through the participation of acyl-lipid $\Delta 3$ - and $\Delta 6$ -desaturases [17]. Changes in their activity can be seen by changes of the unsaturation indices (ratios C22:6 $\omega 3$ /C18:3 $\omega 3$ and C20:4 $\omega 6$ /C18:2 $\omega 6$). The increase in the C20:4 $\omega 6$ /C18:2 $\omega 6$ ratio, which characterizes the activity of acyl-lipid $\Delta 6$ -desaturase, is shown for Groups II and III (Table 2).

Thus, after administration of tebuconazole-containing fungicides, both individually and in combination with triadimefon, an imbalance in the PUFAs ratio is observed in total lipids of rat liver, which may reflect the direction of FAs metabolism.

Subsequently, the preparation "OVA+" was used to correct changes in the FAs content after tebuconazole action, which, along with other biologically active compounds, includes FAs [8]. Under experimental conditions (Group IV), the content of most individual FAs was stable or approaching the control level (Table 1). In particular, the content of the main SFAs: palmitic (C16:0) and stearic (C18:0) did not differ significantly from the control, as did the content of monounsaturated oleic (C18:1 $\omega 9$) acid. The content of FAs of the $\omega 3$ and $\omega 6$ families stabilized at the control level. No differences were observed in the total content of SFAs, UFAs, FAs of the $\omega 3$ and $\omega 6$ families compared to the control (Table 2). Simultaneously, the decrease in the content of linolenic acid (C18:3 $\omega 3$) and corresponding increase by 1.5 times of the ratio C22:6 $\omega 3$ /C18:3 $\omega 3$ compared to the control reflect the activation of FAs metabolism of $\omega 3$ family in Group IV. Thus, the consumption of the preparation "OVA+" by animals that received the fungicide contributed to the normalization of the FAs composition of total liver lipids.

Analyzing the obtained results of the effect of triazole fungicides on rats, it should be noted that the greatest toxic manifestations occur in the first two days after their administration at a dose of 1200 mg/kg. Moreover, it is established that the administration of fungicides containing tebuconazole alone or

together with triadimefon leads to similar modifications in the relative content of FAs in total lipids in the liver of rats. Taking into account the obtained results and scientific data [4], indicating the morphological manifestations of tebuconazole intoxication in female rats, characterized by signs of hepatocyte dystrophy, in particular, fatty degeneration and the appearance of signs of hepatic parenchyma necrosis, the own results may reflect the development of inflammatory or destructive processes. In this case, the impairment of FAs metabolism in the lipids of the liver tissue obviously acts as a causal mechanism for the formation of the syndrome of endogenous intoxication in the period after the fungicidal load of the animal organism. The established increase in the content of pro-inflammatory FAs of the $\omega 6$ family relative to anti-inflammatory fatty acids of the $\omega 3$ family in our researches can affect the manifestation of pathological changes in liver tissues under the fungicide load of animals, in particular due to changes in the functional activity of cell membranes [18].

The use of the preparation "OVA+", rich in biologically active substances, can cause an effect on FAs metabolism and have a positive effect in maintaining the FAs profile of lipids in dynamics after fungicide intake. Besides, the increase in $\omega 3$ FAs metabolism, occurred under these conditions, possibly prevents the involvement of $\omega 9$ acids in the synthesis of lipids (the content of C18:1 $\omega 9$ is close to that in animals of the control group) due to inhibition of gene transcription of other enzymatic proteins in the liver [12].

Thus, studying the role of fatty acids in tissue lipids is important for understanding the biochemical mechanisms of adverse effects of pesticides on mammals and for finding ways to protect and repair tissues under pesticide load.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The research is carried out within the framework of scientific research work (SRW) No. of state registration 0117U002548, funded by the Ministry of Education and Science of Ukraine.

ЖИРНОКИСЛОТНИЙ ПРОФІЛЬ ЛІПІДІВ ПЕЧІНКИ ЩУРІВ ЗА ГОСТРОГО ВПЛИВУ ФУНГІЦИДІВ ТА ВВЕДЕННЯ БІОЛОГІЧНО- АКТИВНОГО ПРЕПАРАТУ

С. В. Хижняк✉, С. В. Мідик,
А. О. Велинська, О. В. Арнаута,
Л. Г. Калачнюк

Національний університет біоресурсів
і природокористування України, Київ;
✉e-mail: khyzhnyaks@gmail.com

Широке використання фунгіцидів у різних сферах життєдіяльності людини призводить до негативних наслідків як для людини, так і довкілля. Метою роботи було дослідити жирнокислотний склад загальних ліпідів тканин печінки щурів за гострого впливу двох фунгіцидів: однокомпонентного, що містить тебуконазол та двокомпонентного – тебуконазол разом із триадимефоном. Аналіз метилових естерів жирних кислот (ЖК) проводили на газовому хроматографі з полум'яно-іонізаційним детектором. Одержані результати свідчать, що гостра дія триазольних фунгіцидів за одноразового перорального введення характеризується токсичними реакціями і супроводжується перерозподілом жирнокислотного профілю загальних ліпідів печінки, подібним для досліджуваних фунгіцидів. Встановлено зниження сумарного вмісту мононенасичених ЖК (на 26-37%) та співвідношення $\omega 3/\omega 6$. Показано, що подальше введення протягом двох тижнів препарату на основі олійного екстракту насіння розторопши плямистої покращувало жирнокислотний профіль ліпідів за дії фунгіцидів.

Ключові слова: жирні кислоти, ліпіди печінки щурів, фунгіциди, триазоли, екстракт плодів розторопши плямистої.

References

- Cunningham M. Use of pesticides: benefits and problems associated with pesticides. Retrieved from: <https://study.com/academy/lesson/use-of-pesticides-benefits-and-problems-associated-with-pesticides.html>. Accessed Dec 23, 2014.
- Kaplia AA, Midyk SV, Khyzhnyak SV. Assessment of the effect of monohydroxy alcohols, unsaturated fatty acids, organophosphate compounds on the enzymatic ATP-hydrolysis in the cell membranes of the smooth muscle of the rat colon *Ukr Biochem J.* 2018; 90(4): 64-73.
- Usenko TV, Shulyak VG, Prodanchuk MG. Changes of hemopoiesis in wistar han rats bone marrow and spleen as a mechanism of hematotoxic action of tebuconazole. *Med Clin Chem.* 2018; (3): 33-42.
- Kornuta NO, Reshavs'ka OV. Morphological studies in the study of the impact of pesticides on the body of pregnant females and fetal development. *Bull Probl Biol Med.* 2011; 2(2): 132-135. (In Ukrainian)
- Kwon HC, Kim DH, Jeong CH, Kim YJ, Han JH, Lim SJ, Shin DM, Kim DW, Han SG. Tebuconazole Fungicide Induces Lipid Accumulation and Oxidative Stress in HepG2 Cells. *Foods.* 2021; 10(10): 2242.
- Khyzhnyak SV, Midyk SV, Sysoliatin SV, Voitsitsky VM. Fatty acids composition of inner mitochondrial membrane of rat cardiomyocytes and hepatocytes during hypoxia-hypercapnia. *Ukr Biochem J.* 2016; 88(3): 92-98.
- Khyzhnyak S, Midyk S, Polishchuk S, Velinskaya A. Effect of triazole fungicides on fatty acid content in *Eisenia fetida*. *Polish J Nat Sci.* 2020; 35(3): 275-286.
- Pat. 147929 UA. Way of production of preparation for veterinary purpose "OVA+/" Arnauta O.V., Mikhayliuk M.M., Kalachniuk L.H., Fedyshyn P.M., Pavliuk O.V. Publ. 23.06.2021, Bul. No 25.
- OECD Guideline for Testing of Chemicals. Acute Oral Toxicity – Acute Toxic Class Method. 2001. No 423.
- ISO 12966-2:2017. Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids.
- Gupta RC. (Ed.). Biomarkers in toxicology. Academic Press, 2019. 1246 p.
- Kang JX. The importance of omega-6/omega-3 fatty acid ratio in cell function. The gene transfer of omega-3 fatty acid desaturase. *World Rev Nutr Diet.* 2003; 92: 23-36.
- Perona JS, Arcemis C, Ruiz-Gutierrez V, Catalá A. Effect of dietary high-oleic-acid oils that are rich in antioxidants on microsomal lipid peroxidation in rats. *J Agric Food Chem.* 2005; 53(3): 730-735.
- Bell MV, Dick JR, Porter AE. Biosynthesis and tissue deposition of docosahexaenoic acid

- (22:6n-3) in rainbow trout (*Oncorhynchus mykiss*). *Lipids*. 2001; 36(10): 1153-1159.
15. Smolyanyanov KB, Paranyak RP, Yunovich VG. Biological role of polyunsaturated fatty acids. *Animal Biol*. 2002; 4(1-2): 16-31. (In Ukrainian).
 16. de Roos B, Mavrommatis Y, Brouwer IA. Long-chain n-3 polyunsaturated fatty acids: new insights into mechanisms relating to inflammation and coronary heart disease. *Br J Pharmacol*. 2009; 158(2): 413-428.
 17. Los DA, Murata N. Membrane fluidity and its roles in the perception of environmental signals. *Biochim Biophys Acta*. 2004; 1666(1-2): 142-157.
 18. Dlyaboga YuZ, Rivis YF. Fatty acid composition of phospholipids of blood plasma, liver and skeletal muscles of rats under experimental hypercholesterolemia and the influence of fish oil. *Studia Biologica*. 2011; 5(2): 73-85. (In Ukrainian).