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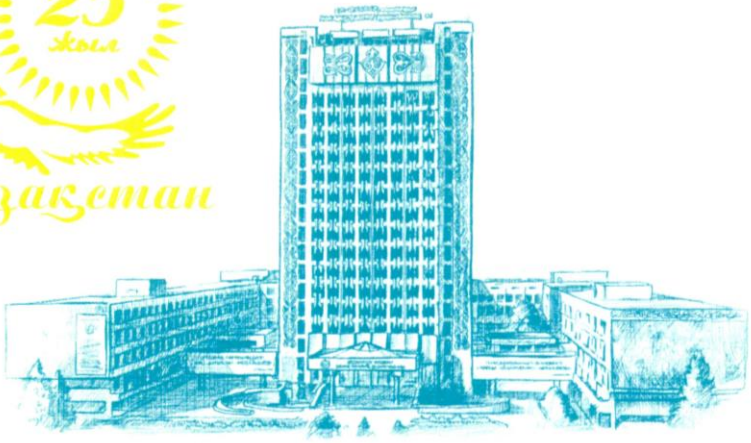

ӘЛ-ФАРАБИ атындағы ҚАЗАҚ ҰЛТТЫҚ УНИВЕРСИТЕТІ
КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ УНИВЕРСИТЕТ имени АЛЬ-ФАРАБИ
AL-FARABI KAZAKH NATIONAL UNIVERSITY

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Detoxification and antioxidant function of liver of the marsh frog (Rana ridibunda) intoxicated with oil from Kenkiyak oilfield

Detoxification and antioxidant functions of liver of marsh frog (Rana-ridibunda) exposed to water-soluble fraction of oil from Kenkiyak oilfield in concentrations of 0.05, 0.5 and 1% during 30 days were studied. Dose-dependent increase of cytochrome P450 content (1.5-2 times) in comparison to intact animals (control), indicating the activation of detoxification processes in liver of intoxicated frogs was observed. It was shown that the growth of monooxigenases was accompained by enchancement of lipid peroxidation processes: the content of MDA in liver of intoxicated marsh frogs also rose dose-dependently 1.5-2 times(P≤0,05)compared with control. Herewith, the content of reduced glutathione, one of the key substances of antioxidant and detoxification systems of the body, decreased 1.3-2 times (P≤0,05) as a result of almost the same induction ofglutathione-S-transferase (1.3-2.1 times, P≤0,05). It was found that activation of lipid peroxidation in liver of intoxicated marsh frogs was caused by supression ofactivity of catalase (1.2-1.5 times) and superoxide dismutase (1.1-1.4 times). On the basis of obtained results a conclusion about activation of detoxification functions and inhibition of antioxidant defence in liver of frogs, leading to strengthening of oxidative stress in studied animalsas a result of oil intoxication, was made. Accumulation of lipid peroxides in liver led to disruption of adaptive reactions of intoxicated animals and development of destructive and necrobioticchanges of hepatocytes.

Key words: marsh frog, liver, oil, cytochrome P450, malondialdehyde, reduced glutathione, glutathione-S-transferase, catalase, superoxide dismutase.

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Кеңқияқ мұнай шығу орнында уланған көлбақа (Rana ridibunda) бауырының детоксикациялық және антиоксиданттық қызметі

Кеңқияқ мұнай шығу орнынан алынған суда еритін мұнай фракциясының 0,05; 0,5 және 1% концентрацияларымен 30 тәулік бойы көлбақаға (Rana ridibunda) әсер етіп, бауырдың детоксикациялық және антиоксиданттық қызметі зерттелінді. Мөлшерге тәуелділігін арттырғанда (1,5-2 есе) цитохром Р450 мөлшерінің бақылау жануарларымен салыстырғанда артқаны байқалынды, яғни уланған бақалардың бауырындағы детоксикация процесінің белсенділігі көрінді. Монооксигеназдың артуы ЛАТ процесінің күшеюімен қатар жүрді: уланған көл бақалардың мөлшерге тәуелділігін 1,5-2,5 есе (P≤0,05) арттырғанда бауырдағы МДА мөлшері бақылау жануарларымен салыстырғанда артқаны көрінді. Бірақ организмнің антиоксидантты және детоксикациялық жүйесінің кілтті байланыстарының бірі – қайта қалыпына келген глутатионның мөлшері 1,3-2,0 есеге (P≤0,05) төмендеп, нәтижесінде глутатион-S-трансферазаның (в 1,3-2,1 есе P≤0,05) индукциясы да төмендеді. Уланған көл бақа бауырындағы ЛАТ белсенділігі каталаза (1,2-1,5 есе) және супероксиддисмутаза (1,1-1,4 есе) белсенділіктерінің баяулауының нәтижесінде жүрді.

Түйін сөздер: көлбақа, бауыр, мұнай, цитохром Р450, малонды диальдегид, қайта қалпына келген глутатион, глутатион-S-трансфераза, каталаза, супероксиддисмутаза.

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Детоксикационная и антиоксидантная функция печени озерной лягушки (Rana ridibunda) при интоксикации нефтью месторождения Кенкияк

У озерной лягушки (Rana ridibunda) исследована детоксикационная и антиоксидантная функция печени при воздействии на них в течение 30 суток водорастворимой фракции нефти месторождения Кенкияк в концентрации 0,05; 0,5 и 1%. Установлено дозозависимое увеличение (1,5-2 раза) содержания цитохрома Р450 по сравнению с интактными животными (контроль), свидетельствующее об активации процессов детоксикации в печени интоксицированных лягушек. Показано, что увеличение монооксигеназ сопровождалось усилением процессов ПОЛ: содержание МДА в печени интоксицированных озерных лягушек также дозозависимо увеличивалось в 1,5-2,5 раза (P≤0,05) по сравнению с контролем. При этом содержание восстановленного глутатиона – одного из ключевых соединений антиоксидантной и детоксицирующей системы организма, снижалось в 1,3-2,0 раза (P≤0,05) в результате практически такой же индукции глутатион-S-трансферазы (в 1,3-2,1 раза, P≤0,05). Установлено, что активация процессов ПОЛ в печени интоксицированных озерных лягушек происходит в результате подавления активности каталазы (1,2-1,5 раза) и супероксиддисмутазы (1,1-1,4 раза).

Ключевые слова: озерная лягушка, печень, нефть, цитохром Р450, малоновыйдиальдегид, восстановленный глутатион, глутатион-S-трансфераза, каталаза, супероксиддисмутаза.

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DETOXIFICATION AND ANTIOXIDANT FUNCTION OF LIVER OF THE MARSH FROG (RANARIDIBUNDA) INTOXICATED WITH OIL FROM KENKIYAK OILFIELD

Introduction

The oil industry of Kazakhstan, on the one hand, is the main economic component of the country and, on the other hand, is one of the most powerful anthropogenic sources of pollution. Developingoilfields in Aktobe region are not exception. They make a significant contribution to the growing economy of the Republic:about 10% of proven reserves and 30% of natural resources of hydrocarbons of Kazakhstan are concentrated on the territory of the region. Those areKenkiyak, Zhanazhol, Urikhtau, Karatyube, Kumsay, Mortuk, Akzhar and other oilfields. The area around the petrochemical complex is contaminated with oil and oil products, and in most cases can not be used for the national economy [1]. Despite the introduction of innovative technologies in the oilfields of Aktobe region, there is a need for constant monitoring of oil pollution, the state of ecosystem, biota and morbidity of the population assessment. Bioindication is one of the most informative and promising methods for assessment of the environment quality, it also allows to reveal the extent and intensity of the effects of pollutants and trace the dynamics of ecosystem degradation in time and space [2, 3]. Herewith, it is advisable to carry out bioindicativeresearch using representatives of ground and ground-water faunawhich are sensitive to anthropogenic impacts [4, 5]. Among the ground-water fauna amphibians are the perfectbioindication object because of their prevalence, ecological plasticity, the multiplicity and accessibility [6].

In the waters of Aktobe region marsh frog (Ranaridibunda) is the most common species ofamphibians [7]. However, in recent years, there are many waterbodies in Aktobe region contaminated by oil and oil products, which can lead to a wide range of developmental abnormalities, a shift in sex ratio, and reduced population size of marsh frog. In this regard, it is necessary to conduct studies of toxic effects of the mentioned pollutants on this species firstly in the experiment, and then under natural conditions. Herewith, it is appropriate to investigate the level of inducible detoxification enzymes - cytochrome P450 and glutathione-S-transferase in the liver of marsh frog as markers of toxic effects [8]. It is also necessary to study the processes of lipid peroxidation (LP) and the level of antioxidant defense enzymes in the liver of the amphibians since it

was shown that the basis for the toxic effect of oil and oil products is the activation of lipid eroxidation [9-11].

Thus, the aim of this study was to investigate the effect of water-soluble fraction of the oil from Kenkiyakoilfieldon detoxification and antioxidant functions of marsh frog(Ranaridibunda) in the laboratory.

Materials and methods

To conduct experiments on the effects of oil onamphibians 24 marsh frogs (Ranaridibunda) captured from the pure waters of Aktobe region, weighing 50-80 g, were used. The animals were divided into 4 groups of 6 animals each: I - intact animals (control); II- frog exposed to water soluble fraction of crude oil at a concentration of 0.05%; III-frog exposed to water soluble fraction of crude oil at a concentration of 0.5%; IV - frog exposed to water soluble fraction of crude oil at a concentration of 1%. Exposure continued for 30 days. The crude oil from the Kenkiyakoilfield(Temir District, Aktoberegion) was mixed with water at the rate of 1:9 (100 ml of oil to 900 ml of water). Next, the mixture was stirring in the dark for 48 hours with use of a magnetic stirrer. To isolate the water-soluble fraction from the mixture it was left at room temperature for 12 hours [12]. Further, water soluble fraction was separated withseparatory funnel and then was stored at -70°C (Plarinum 500 V, AngelantonyIndustrie). Before use, the water-soluble fraction of oil was held at room temperature until complete dissolution and increase of the temperature to 24-26°C. The water in aquaterrariums was exchanged every two days, and then the water-soluble fraction of oil in respective concentrations was reintroduced into the water.

Liver samples were taken and frozen in liquid nitrogen foranalysis of biochemical indicators of the state of antioxidant system in studied animals. The contentofcytochromeP450,malondialdehyde(MDA), reduced glutathione, and activity of glutathione-S-transferase (GST), superoxide dismutase (SOD), and catalase were defined using biochemical methods. Isolation of microsomes was performed according to the modified method of Shenkman and Cynthia [13]. The content of cytochrome P450 was determined withOmura and Sato method [14]. To determine the content of malondialdehyde in supernatant of liver homogenate method with thiobarbituric acid was used [15]. Determination of reduced glutathione was performed by measuring the intensity of fluorescence of buffered supernatant with excitation light wave with length of 350 nm, and absorption of 420 nm. Determination of glutathione-S-transferaseactivity was carried out by measuring the conjugation of 1-chlor-2,4-dinitrobenzyl with reduced glutathione, indicating activity of GST in the liver samples [14]. SOD activity was determined according to Guengerich method [15] based on the quantitative determination of red formazan in the supernatant of liver homogenate. Catalase activity was determined using the method of H.Luck [16]. The results of quantitative research were subjected to statistical analysis. Mean values and the error of average values were determined in all cases. The significance of differences of mean values was evaluated by Student t-test. Differences were considered significant at a confidence level equal to 0.95.

Results and discussion

Figure 1 shows the results of the biochemical determination of cytochrome P450 in the liver of marsh frogs in norm and after intoxication with oil from Kenkiyakoilfield (Aktobe region). It can be seen that the contents of key enzyme of monooxygenase system of the body - cytochrome P450 - increased depending on the concentration of oil-soluble fraction: 1.5 times at 0.05%; 1.8 times at 0.5%; 2 times at 1% ($p \leq 0.05$) compared with the control. This evidenced not only the activation of detoxification processes in the liver ofintoxificated frogs, but also a dose-dependent nature of the process. The level of basal enzyme ofbiotransformation of endogenous and exogenous substances - cytochrome P450- is known to determine the status of organism detoxification, since the activity of this system plays a key role in protecting cells against the damaging effects of various factors [17].

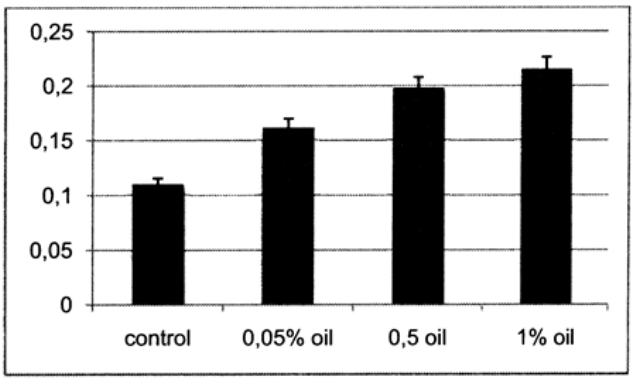

Figure 1 – The content of cytochrome P450 in the liver in marsh frogs in normal and after exposure to water-soluble oil fractions ofoil from Kenkiyakoilfield (Aktobe region), mmol / cm, M ± m

Earlier, we, as well as a number of authors have shown that the cytochrome P450 can be used as a biomarker serving as an indicator of exposure to chemical pollutants in the body and allows assessing the extent and the possible risks of environmental pollution [17-19]. A number of researchers have also demonstrated that the enhancement of catalytic activity ofmonooxygenasescan be accompanied by activation of oxidative stress [18]. As known, the oxidative stress is a universal mechanism involved in the development of most of the pathological changes in the cells, and may play a crucial role in the violations and damage of cell structures [18]. The most dangerous processes run by oxidative stress are the reaction of the chain oxidation of lipids - lipid peroxidation. Therefore, determining the level of lipid peroxidation products plays a crucial role in assessing the damage in a cell. One of the secondary lipid peroxidation products is malondialdehyde (MDA). Therefore, determination of levels of MDA may be an indicator of oxidative stress in the liver cells.

Figure 2 shows the results of the biochemical definition of MDA content in the liver of marsh frogs in normal and after intoxication with water-soluble fractions of oil from Kenkiyakoilfield. It can be seen that the MDA content in the liver of marsh frogs exposed to different concentrations of water-soluble oil fractions is increased significantly: 1.5 times at 0.05%; 2 times at 0.5%; 2.5 times at 1% ($p \leq 0.05$) in comparison to intact animals. As in the case of cytochrome P450, a dose-dependent increase of MDA content was observed.

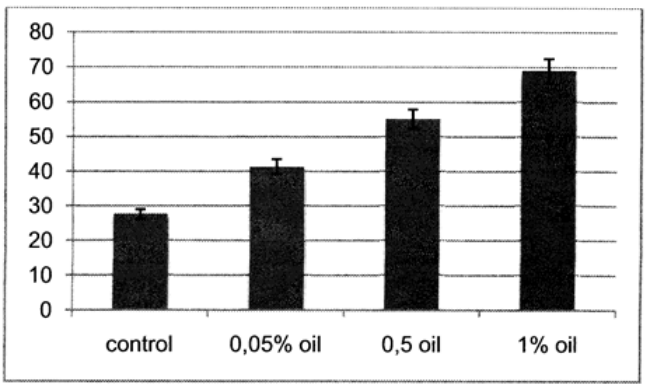

Figure 2 – The content of malondialdehyde in liver of marsh frogs in normal and after exposure to water-soluble fractions ofoil from Kenkiyakoilfield (Aktobe region), mg / ml, M ± m.

It is known, that there is an antioxidant system in the body of animals, hindering the development of oxidative stress, which works for their survival or adaptation to stress factors [17]. For example, usually conjugation of metabolites with various endogenous substances - glutathione, glucuronic acid, and others takes place during the second stage of the biotransformation of xenobiotics. [17]. Reduced glutathione, as an important component of antioxidant and detoxifying systems of the body, plays an important role in the removal of toxic products of metabolism of xenobiotics [19]. Therefore, biochemical determination of the reduced glutathione content in the liver of marsh frogs exposed to water-soluble fraction of crude oil deposits Kenkiyak at the same concentrations was conducted in our study. The results are shown in Figure 3.

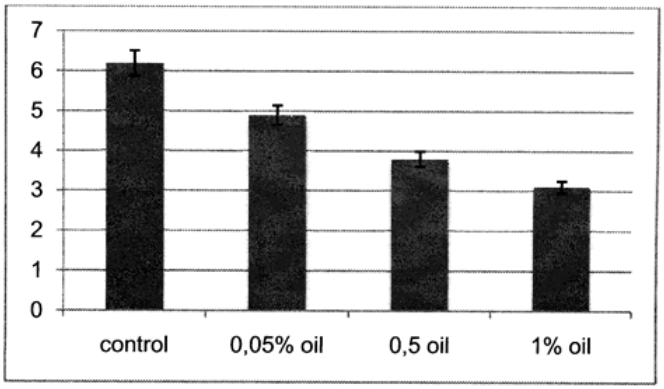

Figure 3 – The content of reduced glutathione in the liver marsh frogs in normal and afterexposure to water-soluble fractions of oil from Kenkiyakoilfield (Aktobe region), mm / r, M ± m

It was found that in contrast to enhance of cytochrome P450 and MDA contents after exposure of the frogs to different concentrations of water-soluble oil fractions a significant dose-dependent reduction of glutathione in the liver of animals takes place: 1.3 times at 0.05%; 1.6 times at 0.5%; 2.0 times at 1% ($p \leq 0.05$) compared with the control. Reduction of glutathione in the liver can be caused by increase of its consumption for metabolic processes (protection of cells from oxygen and peroxy radicals, intensification of lipid peroxidation, oxidative modification of proteins), as well as for the reactions catalyzed by glutathione transferases, which are involved in utilization of hydrogen peroxide, lipid peroxides and xenobiotics [18]. One of the main enzymes in the second phase of the biotransformation of endogenous and exogenous substances in the liver is glutathione-S-transferase. It is known that glutathione-S-transferase uses reduced glutathione for xenobiotic transformation [18]. Strengthened glutathione

transferase activity increases the body's ability to adapt to the growingenvironmental pollution. Therefore, we carried out biochemical tests ofglutathione-S-transferase activity in the liver of marsh frogs exposed to different concentrations of water-soluble fraction of crude oil fromKenkiyakoilfield (Aktobe region). The results of those tests are shown in Figure 4.

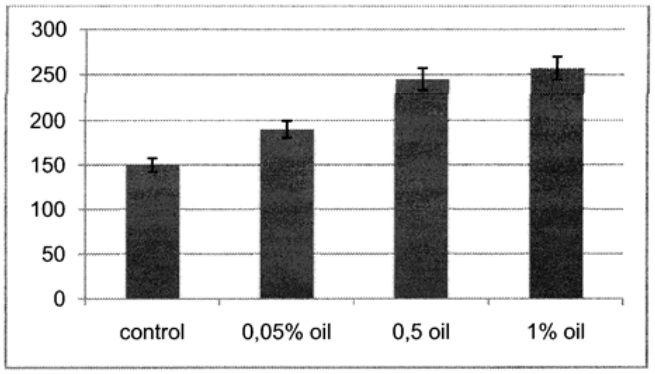

Figure 4 – The activity of glutathione-S-transferase in liver of marsh frogs in normal and after exposure to water-soluble fractions of oil from Kenkiyakoilfield(Aktobe region), nmol/min·mg

As represented on the figure, the activity of glutathione-S-transferase in the liver of animals exposed to water-soluble oil fraction, increases dose-dependently: 1.3 times at 0.05%; 1.6 times at 0.5%; 2.1 at times 1% ($p \leq 0.05$), compared with the control. At the same time, the activity of enzymes detoxifying reactive oxygen -catalase and superoxide dismutase, conversely, decreased. The results of thatbiochemical study are given in Figures 5-6.

The results indicate that the water-soluble oil fraction leads to a dose-dependent decrease in the activity of antioxidant enzymes - superoxide dismutase and catalase 1.2 and 1.1 timesin concentration of 0.05%; 1.3 and 1.4 times in concentration of 0.5%; 1,5 and 1,4 times in concentration of 1%, respectively.

Thus, as a result of the research, it was found that intoxication of marsh frog with water-soluble fraction of oil from Kenkiyak oilfield leads to induction of a key enzyme of monooxygenase system - cytochrome P450 - in the liver, acting independently of the pollutant concentration. Perhaps the increase in detoxification status of the animals allowed them to survive in conditions of laboratory testing of different concentrations of studied oil for 30 days. However, a dose-dependent growthof the content of end product of lipid peroxidation (MDA) in the liver of theanimals was also found, that indicated an increased activation of lipid peroxidation. Herewith, the content of reduced glutathione and activity of antioxidant enzymes - superoxide dismutase and catalase, inhibiting lipid peroxidation in membranes, was depleted, indicating an increase in the susceptibility of animals to the cytotoxic effects of oil. This is also evidenced by the destructive processes observed in the liver of marsh frog, particularly expressed when exposed to water-soluble oil fraction in concentrations of 0.5% and 1% (Figure 7-8). In the marsh frogs exposed to water-soluble fraction of oil in a concentration of 0.5%, tubular structure of the liver wasviolated, as well asexpansion of the Disse spaces, degenerative and necrobioticchanges of hepatocytes, numerous melano-macrophage accumulation and activation of Kupffer cells were observed (Figure 7).

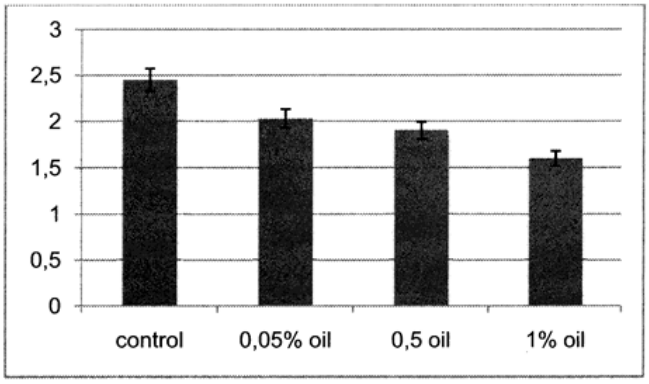

Figure 5 – The activity of catalase in the liver of marsh frogs in normal and after exposure to water-soluble fractions of oil from Kenkiyakoilfield (Aktobe region), U / g.

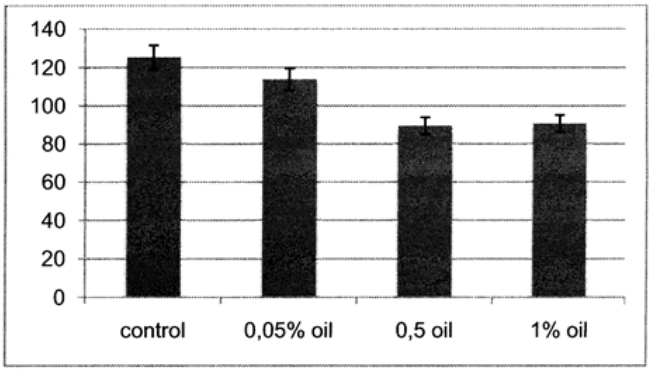

Figure 6 – Superoxide dismutase activity in the liver of marsh frogs in normal and after exposure to water-soluble fractions of oil from Kenkiyakoilfield (Aktobe region), U / mg.

After exposure to water-soluble fraction of oil in a concentration of 1% microcirculatory bed disorders,

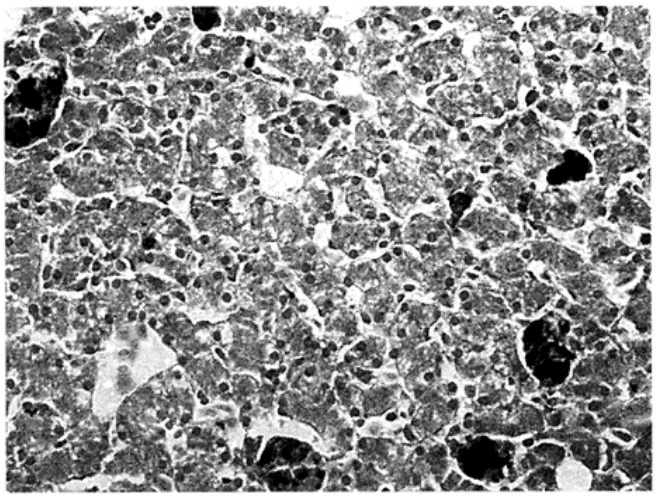

Figure 7 – Histological structure of the liver of marsh frog under the influence of water-soluble fraction of oil from Kenkyakoilfieldin aconcentration of 0.5%. Extensions of Dissespaces, degenerative andnecrobioticchanges of hepatocytes, melano-macrophage accumulation. Stained with hematoxylin and eosin, x 200

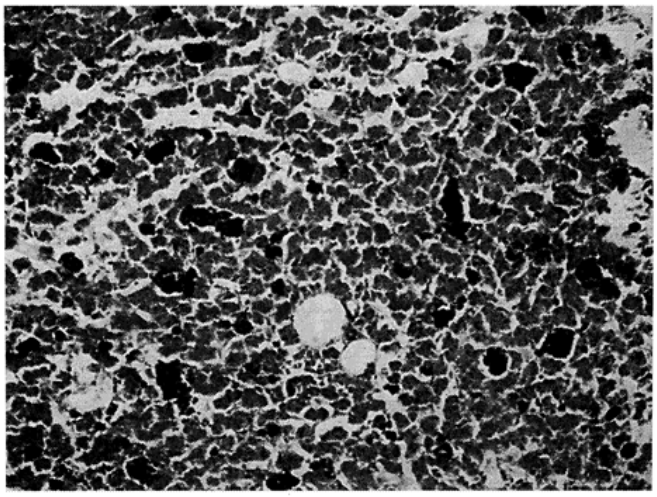

Figure 8 – Histological structure of the liver of marsh frog under the influence of water-soluble fraction of oil from Kenkyak oilfield in a concentration of 1%. Perivascular edema and multiple foci of necrosis of hepatocytes. Stained with hematoxylin and eosin, x 200

perivascular oedema, multiple fociof necrosis of hepatocytes were observed in the liver of the marsh frogs (Figure 8). The boundaries of the cells were blurred; the tubular structure of the liver parenchyma was impaired. Dystrophic and necrobiotic processes were accompanied by diffuse inflammatory infiltration. It should be noted that exposure to oil, along with dystrophic and necrotic changes in the liver parenchyma and stroma, is characterizedby mild inflammatory infiltration. Moreover, unlike mammals, amphibians, particularly in marsh frog exposure to oil causes inflammation represented byneutrophilia and eosinophilia [20].

Based on the data, it must be concluded that the exposure to water-soluble oil fraction in concentrations of 0.05; 0.5 and 1% for 30 days activates the detoxification function of the liver and inhibits the body antioxidant protection, enhancing lipid peroxidation processes leading to violations of histostructure of liver in the marsh frog. That is, despite the 100% survival of animals under those conditions, there is a disruption of adaptive responses to oil. It should be assumed that the effect of large doses of oil and/or a long stay ofthe marsh frogsin conditions of oil pollution of water environment can lead to mass death of animals.

References

1 Kaiser MJ, Pulsiphera AG (2007) A review of the oil and gas sector in Kazakhstan, Energy Policy, 35(2):1300-1314. DOI: 10.1016/J.ENPOL.2006.03.020.

2 Diusenov ZT (2001) Petrochemical pollution of soils of the Caspian region [Neftekhimicheskaiazagriaznennost' pochvPrikaspiiskogoregiona] // VestnikKazGNU. Ser. ekologicheskaia.1(8):70-75 (in Russian)

3 Moore MJ, Mitrofanov IV, Valentini SS, Volkov VV, Kurbskiy AV, Zhimbey EN,Eglinton LB, Stegeman JJ (2003) Cytochrome P4501A expression, chemical contaminants and histopathology in roach, goby and sturgeon and chemical contaminants in sediments from the Caspian Sea, Lake Balkhash and the Ily RiverDelta,Kazakhstan, Marine Pollution Bulletin, 46(1):107-119.

4 Mora S, Sheikholeslami MR, Wyse E, Azemard S, Cassi R(2004) An assessment of metal contamination in coastal sediments of the Caspian Sea, Marine Pollution Bulletin,48(1-2):61-77 DOI: 10.1016/S0025-326X(03)00285-6

5 Zoriy P, Ostapczuk P, Dederichs H, Höbig J, Lennartz R, Zoriy M (2010) Biomonitoring of environmental pollution by thorium and uranium in selected regions of the Republic of Kazakhstan, Journal of Environmental Radioactivity, 101(5):414-420. DOI:10.1016/j.jenvrad.2010.02.014

6 Kosarev AN (2005) The Caspian Sea Environment.Springer, Germany.ISBN 978-3-540-31505-6

7 DuisebaevaTN, BerezovikovNN, BrushkoZK, KubykinRA, KhromovVA (2005) Marsh Frog (Ranaridibunda Pallas, 1771) in Kazakhstan: habitat change in the XX century and the present distribution of the species [Ozernaialiagushka (Ranaridibunda Pallas, 1771) v Kazakhstane: izmeneniareala v XX stoletiiisovremennoerasprostranenievida],Sovremennaiagerpetologiia. 3(4):29–59 (in Russian)

8 USAID. Biodiversity Assessment for Kazakhstan. Almaty, Kazakhstan, 2001

9 Bannikov AG, Darevsky IS, Ishchenko VG, Rustamov AK, Shcherbak NN(1977) Guide to the USSR amphibian and reptile fauna [Opredelitel' zemnovodnykhipresmykaiushchikhsiafauny SSSR].Prosveshchenie, Moscow, Russia (in Russian)

10 Borkin LJ (1999) Distribution of amphibians in North Afri-ca, Europe, Western Asia, and the former Soviet Union. In: DuellmanWE (Ed.). Patterns of Distribution of Amphibians: a Global Perspective. The Johns Hopkins University Press, Baltimore and London

11 Marsili L, Casini S, Mori G, Ancora S, Bianchi N, D'Agostino A, Ferraro M, Fossi MC (2009) The Italian wall lizard (Podarcissicula) as a bioindicator of oilfield activity, Science of The Total Environment, 407(11):3597-3604. DOI: 10.1016/j.scitotenv.2009.01.035

12 Lowry LK (1995) Role of biomarkers of exposure in the assessment of health risks, Toxicology Letters, 77(1-3):31-38

13 Pradnya S Walawalkar, Pooja S Serai, Iyer KR (2006) Isolation and catalytic competence of different animal liver microsomal fractions prepared by calcium-aggregation method, Indian journal of pharmaceutical sciences. 68(I. 2):262-265

14 Herbig WJ, Pabst MJ, Jacoby WB (1974) Glutathione S-transferase, the firdt enzymatic step in mercapturic acid formation, J. Biol. Chem., 249:7139-7147

15 Lam PK, Gray JS (2003) The use of biomarkers in environmental monitoring programs, Marine Pollution Bulletin, 46(2):182-186.

16 Guengerich FP (1994) Analysis and characterization of enzymes. In: Principles and methods of toxicology, Hayes A.W (eds). 3rd Edn., Raven Press, New York. P. 1259-1313

17 McFarlane M, Price SC, Cottrell S, Grasso P, Bremmer JN, Bomhard EM, Hinton RH (1983) Petrol exposure and hepatic enzyme activity, Food and Chemical Toxicology, 21(5):763-767

18 Bolognesi C (2010) The micronucleus test as a biomarker of genomic damage: The validation process in environmental animals, Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology, 157:12-13. DOI: 10.1016/j.cbpa.2010.06.031

19 Brookes P (1977) Mutagenicity of polycyclic aromatic hydrocarbons, Mutation Research/Reviews in Genetic Toxicology,39(3-4):257-283.

20 20.Karmazin AP (2010) Biomonitoring of oil pollution the mouth of the Don River with aquatic vertebrates [Biomonitor ingneftianogozagriazneniiaust'iareki Don sispol'zovaniemvodnykhpozvonochnykh]. Krasnodar: Dissertation of applicant for the candidate of biological sciences, 23 p.

Section 3
Actual problems of biodiversity conservation