PATHOPHYSIOLOGICAL EFFECTS OF CRUDE OIL ON RODENTS

S.N. Gashev [a,1], A.V. Elifanov [a], T.I. Moiseenko [b]

[a]*Tyumen State University Semakova street 10, Tyumen, 625003, Russia*

[b] *V.I.Vernadsky Institute of Geochemistry and Analytical Chemistry of Russian Academy of Sciences Kosygin Street 19, Moscow, GSP-1, 119991 , Russia*

Abstract. We conducted experimental research into the effects of crude oil pollution on laboratory rodents. It was found that crude oil causes stress reactions in nonspecific small mammal organisms. Increases in metabolic intensity were noted, as were increases in intensity of energy metabolism, hematogenesis process malfunction, lymphocyte migration in blood flow, the share of pyknomorth cells in diverse tissues (indicating the direct toxicological effect of the oil and the general growth of the stress conditions), organism adaptation (variable between species, and between different gender-age groups within one species) as is actively supported by hypothalamic-pituitary-ardenic system.

Keywords: ecology; pollution; crude oil; mammals; rodents; toxicology; pathophysiology

1. Introduction

The increasing global need for raw hydrocarbon products has led to the intensive growth of oil extraction in regions and countries. According to official Russian sources, Russia possesses more than 10% of the world's oil supplies,

[1]*Corresponding author. Fax: +7(3452) 25-52-78*

E-mail address: gsn-61@mail.ru (S.N. Gashev).

occupying the second place in the world for confirmed supplies. Western Siberia maintains the leading position among Russian regions for the oil and gas extraction: more than 6% of the world's oil production is concentrated in this region. In the near future, oil extraction will be sufficiently extended geographically. Russia is facing the expansion of large oil reconnaissance and exploration projects in Western Siberia and in the Far East, in the regions of the Extreme North; a new system of oil and gas transportation is being planned at the Caucasus, Kamchatka, North-Western Region and the Okhotsk Sea. A serious extension of oil and gas works on the Far East and Arctic shelf are being mapped out. But areas of oil and gas reconnaissance, extraction and processing, as well as transportation pipelines, are often a source of water and ground pollution to ecosystems.

The toxic properties of crude oil are widely known and there are many descriptive works devoted to the toxic influence of crude oil on water species, especially marine (Ottaway, 1971; Cherkashin, 2005 etc.). The effects of oil spills on non-marine mammals are much less studied than those upon birds and water species. There is little published research on the influence of oil spills on mammals. However data connected with the physiological influences of different oil components and oil products have been collected in the laboratory since the end of the 19th century (Kulyabko, Ovsyannikova, 1899). It has been found that paraffin hydrocarbons cause narcosis and convulsions; aromatic and naphthenic hydrocarbons influence blood and hematopoietic organs. Under the chronic influence of volatile fractions of non-sulfurous oil, the following have been observed: functional changes in the central nervous system, low blood pressure, slowdown of pulse rate, and indicators of liver affection as well as a high inclination to various diseases. Under the influence of highly sulfurous oil some differed responses are noted, such as weakened sense of smell, liver and thyroid body malfunction, effects upon the mucous tunic, the occurrence of chronic conjunctivitis, and the ruination of the normal flow of embryogenesis (Panov et al., 1986; Cherkashin, 2005; Draft Toxicological Intake Values..., 2010 etc.). The toxicity and oncogenicity of oil hydrocarbons has been sufficiently studied (Coomes, Hazer, 1984; Holland, Frome, 1984; Lewis et al., 1984; Rahimtula et al., 1984; Carcinogenic substances, 1987; CCME, 2008 etc.). There are data on mutagenicity of some hydrocarbons.

Separate studies have been devoted to benzo[a]pyrene. These include those on the influence of heavy oil fractions on the reproductive system in mammals (Nishimoto et al., 2008, 2009). The naphthalene acids found in oil were also under investigation (Abdullayev, Beybutova, 1965, Karayev, 1965, Sabirova, Man'kovskaya, 1966). Further, there can be found some methodological works devoted to the interpretation of hydrocarbon pollution in wild animal tissue (Hall Russel, Coon Nancy, 1988). A synthesis publication on the toxicity of separate crude oil components in mammals, as well as the guidance for all the studies of this kind, is "Guidance on the application of Globally Harmonized System (GHS) criteria to petroleum substances" (2010). The most recent complex studies of crude oils of various composition and their toxic influence on rabbits and rats were carried out by the American Petroleum Institute (Test plan crude oil category, 2003), and related literature can be found there. Of great interest are earlier works exploring biochemical peculiarities of rodents under the influence of crude oil (Ayalogu et al., 2001). The work of V.M. Shaposhnikov (1980) – one of few available in Russia, reveals the results of laboratory experiments on mice under the influence of crude oil.

However, unfortunately, we note that few works are devoted to the investigation of the complex influence of crude oil on the animal organism, though in real life we deal with such integrated effects more often. In connection to this it is very important to understand the mechanisms of mammals pathophysiological processes, and how these develop under the influence of crude oil.

Small mammals are a model group of organisms in ecological research, as an important element of water and coastal ecosystems, satisfying the requirements for an indicator species (high population, high rate of generation change). In this respect small mammals are useful for the experimental exposure of separate pollutants (i.e.: crude oil) response biotesting, including the estimating the quality of the environment in ecological monitoring (Test plan crude oil category, 2010; Moiseenko et al., 2010).

The aim of our work was to denote the morphophysiological, histological and cytological influence of crude oil on rodents as whole organisms, as applied via food and water in laboratory conditions.

2. Material and research methods

The investigation of crude oil on small mammals in the laboratory conditions (subacute experiment) was held in accordance with “International recommendations….” (1993).

As test animals we used non-pedigreed white mice (*Mus musculus* var alb.) and rats (*Rattus norvegicus* var alb.). We used mice aged 2.5 months with an average body weight of 20 grams, and rats aged 3 months with a body weight of 200 grams.

As the dietary supplement for white mice we used South-Balyk oil (average light, low-viscous, sulfurous, pitch-asphalt) for 2 months. Into the diet of experimental white rats we included the crude oil of the North Hohryakov deposit (light, low-viscous, low-sulfurous, paraffin, with a low concentration of aromatic and many toxic monocyclic hydrocarbons) (Table 1). Both deposits are located in the mid-taiga zone of Western Siberia, in the territory of the Chanty-Mansyisk Autonomous Region of the Tyumen Region.

The control group of animals (16 non-pedigreed white mice and 10 non-pedigreed white rats) was fed the same fodder of equivalent amount and proportions, but without the addition of oil.

Over the course of the experiment, crude oil was systematically added to the dietary intake of the experimental groups of animals (16 white mice and 18 white rats) every third day. The fodder was mixed with oil in the proportion 1:0.01 by weight – a weak oil concentration of about 1%; in drinking water, a oil concentration of 0,001% was made. Thereby, given their daily ration, the mice received crude oil in the dose of 3.5 g/kg/day; and rats – 5.85 g/kg/day.

The experiment was conducted for 60 days. During the experiment we denoted animal deaths, reproductive and behavioral peculiarities in both groups. At the end of the experiment all the animals were slaughtered and examined thoroughly; involving morphophysiological, histological and cytogenetic analyses according to the standard methods (Shvarts et al., 1968; Kononsky, 1976; Darlington, La Cur, 1980 etc.).

In accordance with the method of morphophysiological indicators we used as indicators the relative body weight (body weight to body length cube ratio), and indices relating to the primary organs (heart, liver, kidneys, adrenal, spleen), recorded as organ's weight to body weight ratio. Using hematological characteristics, we measured the amount of hemoglobin in the rodent's blood. All the histological analyses were conducted at the laboratory, according to conventional methods (Bucke, 1994). The organs were taken immediately from the slaughtered animals, cut into blocks of 1 cubic centimeter, and placed into fixing solution (Bouin's fluid). Histopathological changes were studied with the help of a microscope (450x). The study of cytogenetic peculiarities was conducted via pyknotic test: all the types of nucleus degeneration related to the chromatinic net detachment or the detachment of some of its components from nuclear membrane and it's concentration into homogenous paste were taken into account.

3. Results and discussion

In the mice control group were noted 3 generations of new-born individuals (3.4 and 7 rodents in each). Of the animals slaughtered at the end of the experiment, 50% of control females were pregnant (general number of embryos was 17). In the oil-fed group of mice, all reproductive processes were absent. The death-rate in the control group (without taking into account those born during the course of the experiment) was 12.5%, and in the experimental group – 37.5% (Fig. 1).

Among white rats reproduction was not noted: neither in the control group, nor in the experimental one. The death-rate by the end of the experiment was 22.2% in the experimental group and 0% in the control group.

3.1. Behavior and death dynamics

The death dynamics in both groups of crude oil fed animals indicates a direct relationship with exposure to the crude oil diet, and with a dependency that is non-linear in character: such a concentration of oil did not lead to death during the first month, but by the end of the second the death-rate had increased dramatically. After a month of crude oil consumption in white mice, significant differences from biochemical indicators in the control group were noted (Ayalogu et al., 2001).

Also it should be mentioned that mice have a higher death-rate, even when fed food with a lower concentration of toxic matter than rats.

Moreover, we denoted a significant difference in oil-fed animals' behavior in comparison to control animals: they could be characterized as being less active, listless, as a rule interacting weakly with the other individuals, and not making attempts to hide from the observer. Consequently, if in the control group all the animals gathered in one nest in low temperatures, this effect was not observed in the experimental group; the mice stayed separate, or (occasionally) two individuals were observed in the same nest.

In conducting an external examination of the experimental animals, we noted some significant morphological differences from the control group animals. The hair became thinner (skin could easily be seen through it), spine hairs partially stuck together, the general coloration became darker, despite the animals cleaning their fur regularly. Half of the animals had tumors and ulcers up to 10 mm in diameter throughout their bodies, and 3% of the animals had tail tissue necrosis.

3.2. Viscera indices and histopathology

When we compared the morphophysiological indices of crude-oil-fed white mice (exposed for 2 months) and control mice, we noted a significant difference over the majority of analyzed indicators for both males and females (Table 2).

This included: increased heart, liver, kidney, and spleen indices; the tendency for an increased adrenal index; significant decreases of hemoglobin in experimental animals' blood compared to control animals. Some individuals suffered from intestinal epithelium ulceration. Taking into account the differences in oil pollution administered via food and the demographic characteristics of the experimental group of animals, these findings correspond with data obtained during the laboratory experiments of other authors (Shaposhnikov et al., 1980).

We should note additionally that females demonstrated higher sensitivity to oil pollution than males.

<u>Liver.</u> Histological analysis of the livers of white mice showed that 20% of the experimental mice had cirrotic changes in liver tissue, in some cases ulcers and non-typical areas of green color were noted.

During microscope analysis we noted changes in nucleus size and hypatocyte size: the average hypatocyte nucleus size in the experiment was 139915±28007.1 cubic micrometers, or 156% in comparison to the control group (89475±26053.7 cubic micrometers); the average number of hypatocytes on the experimental group was 56% of the average in the control group; all differences are significant at $P<0.01$. Similar results were obtained for white rats after 2 months of a crude oil diet: the average hypatocyte nucleus size was 107479±586.5 cubic micrometers, or 158% of that in the control group (67758.9±514.8 cubic micrometers); and, the average cell count in the experimental group was 59% of that in the control group. These phenomena were also observed by other authors (Vermel, 1935; Magloliocea, 1959; Hesinн, 1967; Ayalogu et al., 2001 etc.) under the influence of various poisonous substances and oil components – indicating functional hypatocyte activity. This also could be observed by the increased liver index during the experiment, as the induction of microsomal monooxidase is often accompanied by the forming of new smooth membranes in the endoplasmatic reticulum, and the increased liver weight (Meldolesi, 1967; Schulte-Hermann et al., 1968; Böhn, Moser, 1976).

Kidneys. Feeding white rats with food slightly polluted by oil resulted in an increase in kidney weight by 1,8 times, with increased proximal (1,6 times) and distal (1,7 times) sectional areas of nephron tubules. The renal corpuscle lumen of experimental animals became less discernible as a result of the overflow of capillary loops with regular blood elements. The tubules lumen was poorly observed because of a fuzzy brush border. Cell’s nucleus size was increasing: 635503±1.2 cubic micrometers in control animals, compared to 741231±2.1 cubic micrometers in experimental animals. This could indicate a non-specific organ reaction by the functional increase on those without any signs of acute toxic influence. Basic pathological changes are presented in Figure 2.

Adrenals. A reliable increase of adrenal weight in experimental white rats compared to control ones (16.7±0.2 and 14.3±0.3 me) was found. This reaction of animals under unfavorable conditions was noted previously by many researchers (Kovrizhko, Oleynik, 1984; Kirillov, 1994 etc.), however, during the course of our experiment reactions of strong stress such as hemorrhage, cells destruction, and wearing in vessel walls were not observed; though changes of morphometric indices such as nuclei sectional area, adrenal width zones, dilated capillary part, nuclei-plasmatic ratio and kariokynetic index (Table 3) (which directly detects the activation of adrenals function and it’s hormone’s role in organism adaptation to the stress factors) were.

3.3. Immune and hematopoietic systems

The oil pollution of food even during a 60-day period caused for white rats substantial changes in morphology of spleen lymphatic follicle: the significant increase of concentration of lymphoid cells on control area in all investigated zones of white pulp. In the periarterial thymus-dependent area the number of lymphoid cells was 21.3% lower than that of the control group; in thymus-independent mantle area the result was 62,9% in marginal area, whilst the destruction reached 18,2% in comparison to the control group. According to Kryzhakovsky (1985) destruction of lymphatic follicles is the result of intensified migration of T- and B- lymphocytes, conducting the “emergency” role in tissue homeostasis maintenance and in the elimination of damaged and degenerative cells. These cells play an important role in tissue regeneration. We speak of trophic and plastic stimulations of regenerating parenchymatous cells, of providing them with extra or needed plastic material of nucleic acids and proteins in the stress conditions by lymphocytes.

During the experiment we observed the blood-making process malfunction. So the white rats which were crude-oil-fed for a month had the number of erythrocytes lowered to $5.98 \pm 0.30 \times 10^{12}$ cells/l (intact animals), from $8.26 \pm 0.16 \times 10^{12}$ cells/l; this way hemoglobin concentration was reduced to 103.17±2.4 from control level 134.83±1.9 g/l - or by 76,5%. Similar results were obtained for the white mice subjected to 2 months exposure. By the end of the 60-day exposure, the white rats had reticulocytosis (100.59±1.04‰ reticulocytes to the erythrocytes number in the peripheral blood flow, and 43.38±0.86‰ in the control group), indicating a significant increase in the last stages of blood-making process reactivity. Evaluating the medullar substance blood-making condition we noted the following peculiarities: the number of proerythoblasts were: 77.3% compared to the control, basophilic and polychromatophilic erythroblasts, polychromatophilic and oxyphyle normoblasts – 89.9; 98.1; 106.5; - 22.0% compared to the control group (significant difference).

The results obtained demonstrate an active reaction of bone marrow erythron system to the oil pollution, and indicate an inhibiting influence of oil upon proliferation of blood-making cells, a reactivity increase of the last stages of blood-making, and a fast transition of the last generation myelocariocytes (oxyphyle normoblasts) to retilocyte raw.

Both control and experimental white mice had an unequal distribution of Gomory-positive neurosecretory material of the back neurohypothysis with it’s concentration around capillary. However, speaking of those animals, we should note that in this case such concentration is shown more vividly and the concentration of neurosecretory material of the back neurohypothysis is lower (2 points out of 5), than in the control group (4points). Back hypothysis capillary in the experimental group were mostly dilated and contained regular blood elements (Elifanov, 1991). The lowering of Gomory-positive neurosecretory material in the middle eminence and the back lobe of hypothysis were noted by E.G. Berlinger and others in 1991, in the case of lead intoxication. All these facts give weight to the hypothesis that, in organism adaptation processes under conditions of prolonged but low degree oil pollution, the hypothalamic nona peptides exuded from the back hypothysis into blood-flow play an important role.

3.4. Genetoxicological effects

The gene toxic influence of crude oil data evaluated by pyknotic test is of great interest. Z.Brazhe (1969), A.I. Atabekova and E.I. Ustinova (1987) discuss the increasing of the pyknosis part under the influence of some non-specific factors. Nowadays the pyknotic (micronuclear) test is widely used for exploring genetoxic agents (Pshenichnov, 1991; Liu Yongchang et al., 1991 etc.). In our experiments we use the pyknotic index of intestinal epithelium and liver in non-pedigreed white rats. In both cases the reliable rise of pyknomorph cells and their part was noted among animals fed by crude oil for more than a month (Tables 4 and 5).

As in the case with morthophysiological indices of 60-day crude-oil-fed mice, we can note that female rats demonstrated a higher sensitivity to the oil-pollution than male rats.

Taking into account the independent indicative data of some parameters variation indices, it is necessary to point out that, in analyzing the adrenal indices of female animals in the control and experimental groups (white mice), we didn’t mention any significant differences; however, according to the variation character of these differences, females in the experimental group differed considerably from females in the control group: variation indices were 87.4±27.6 and 33.9±9.8 (differences according to t-criterion of Student for sigma and F-criterion of Fisher for dispersions, reliable at $P<0.05$ and $P<0.01$). Taking into account the fact that in the experimental group we had no pregnant female animals, which are characterized by a increased adrenal index during that period (and in the control group 50% were pregnant) we can conclude that the great variability of this index during the experiment was fully connected with the stress caused by the crude-oil-polluted food.

Also the decrease of the variational factor of liver cells pyknotic index during the course of the oil diet (white mice) is understandable: females had the lowest as possessing the highest physiological and functional loads, having the tendency to its increase in intestinal epithelium cells in the role of “consumables”.

The key to the solution of the question of different animals group heterogeneity is in the study of organism part correlations over the course of the size enlargement of individuals – so-called “allometry growth”. During the course of our investigation into the dependence of adrenals weight on body weight for female animals in the experimental and control groups, some allometric equations were deduced (Elifanov et al. 1996), which made it possible to mark differently directed correlation character in both cases: experiment $Y=506.6X^{-0.440}$, and control $Y=0.003X^{0.737}$.

The values distribution character of one or the other indicator, the distribution deviation degree – are also very significant. As an example we can take a distribution criterion of average body weight for the animals in the control and experimental groups (almost the same average size): if in the first case this distribution doesn’t deviate from the normal one, then in the second the negative excess takes place ($Ex=-1.3\pm0.75$ at $t_{Ex}=-1.73$), characterized by a platykurtic distribution. The biological interpretation of this phenomenon could be connected with the presence of disturbing factor, which couldn’t be limiting for this indicator according to its value.

Comparing the effects arising from the influence of crude oil on animal organisms in the laboratory with research data on the influence of oil pollution the on morphophysiological peculiarities of small mammals in the natural biogeocenosis of Middle Ob Region, we can see the correspondence of reactions in both cases. V.M. Shaposhnikov et al. (1980) consider that such a standard reaction of rodents in oil-production areas and under laboratory conditions demonstrates the direct effects of oil pollution, and the changes revealed are caused by the accompanying oil-production factors (noise, chemical reagents, level changes of subterranean waters, clearing – as the result of wood cutting etc.).

Taking all this into account we can come to a conclusion that changes in morphophysiology of small mammals occupying oil-polluted territories are caused in the first instance by the direct influence of crude oil and accompanying chemical substances.

4. Conclusion

Comparison of the outcomes of laboratory experiments to the results obtained over the course of field research of oil-polluted territories (Gashev, 1992), suggests the conclusion that it is crude oil that causes a non-specific reaction of small mammals to pollution.

During the experiment, the crude-oil-fed rodents underwent an increase in metabolic intensity, leading to a rise in heart and kidney indices. The intensity of energy metabolism revealing in liver hypertrophy by means of glycogen redundancy was also noted, normal for the stress conditions, which was confirmed by the increase in adrenals index for the experimental group of animals.

Toxic effects were confirmed by the simultaneous growth of spleen index and decrease of hemoglobin level in the crude-oil-fed animals’ blood. Malfunctions of blood-making processes were also noted, as well as the growth of lymphocytes migration to the blood stream, the enlargement of pyknomorph cells part in various tissues indicating the direct toxic effect of crude oil.

In general we can speak about increasing of the stress over the course of oil diet, and of animals' adaptation, specific for different rodents and sex and age groups of the same species. In the course of adaptation the hypothalamic-pituitary-adrenal system takes an active part.

REFERENCE

1. Ayalogu, O.E., Igboh, N.M., Dede E.B., 2001. Biochemical Changes in the Serum and Liver of albino rats exposed to Petroleum Samples (gasoline, kerosene, and crude Petroleum). J. Appl. Sci. Environ. Vol. 5(1), 97-100.

2. Abdullaev, M.D., Beybutova, T.S., 1965. To the antitoxic influence of petroleum bios. Petroleum fertilizers and stimulators in farming: Proceedings of the Conference, 22-25 January 1963, Baku, 547-549 (in Russian).

3. Atabekova, A.I., Ustinova, E.I., 1987. Plant cytology. Moscow, 244 pp. (in Russian).

4. Böhn, N., Moser, R., 1976. Reversible Hyperplasie und Hypertrophie der Mänseleber unter funktioneller Belastung mit Phenobarbital. Beitr. Pathol. Bd 157, N 3.

5. Berlinger, E.G., Danilova, O.A., Chekunova, M.P., 1991. The influence of the lead intoxication on hypothalamic-petuitary neurosecretory complex of rats. Materials of IV national conference "Endocrine system of organism and harmful factors of the environment". Leningrad (in Russian).

6. Brazhe, Zh., 1960. Biochemical cytology. Moscow, 516 pp. (in Russian).

7. Bucke, D., 1994. Histology. In: Austin, B., Austin, D.A. (Eds.), Methods for the Microbiological Examination of Fish and Shellfish. Ellis Horwood Ltd., Chichester, 69–97.

8. Carcinogenic substances, 1987. Reference book, ed. V.S. Turusov. Moscow, 334 pp. (in Russian).

9. CCME (Canadian Council of Ministers for the Environment), 2008. Canada-wide Standard for Petroleum Hydrocarbons (PHC) in Soil: Scientific Rationale. Supporting Technical Document. Canadian Council of Ministers for the Environment, 383 pp.

10. Cherkashin, S.A., 2005. Some aspects of oil hydrocarbons influence on fish and crustaceans. Bulletin of Russian Academy of Science. 3, 83-91 (in Russian).

11. Conaway, C.C., Schreiner, C.A., Cragg, S.T., 1984. Mutagenicity evaluation of petroleum hydrocarbons. Appl. Toxicol. Petrol. Hydrocarbons, Princeton, N.Y., 89-107.

12. Coomes, R.M., Hazer, K.A., 1984. Statistical analyses of crude oil and shale oil carcinogenic test data. Appl. Toxicol. Petrol. Hydrocarbons, Princeton, N.Y., 167-186.

13. Darlington, S.D., La Kur, L.F., 1980. Chromosomes. Work methods, 183 pp. (in Russian).

14. Draft Toxicological Intake Values for Priority Contaminants in Soil, 2010.Wellington: Ministry for the Environment, 168 pp.

15. Elifanov, A.V., Kurochkina, O.I., Gashev, S.N., 1991. The influence of oil pollution to the condition of back neurohypothysys of white mice. Proceedings of

IV National Conference "Endocrine system of organism and harmful factors of the environment", 84 (in Russian).

16. Gashev, S.N., 1992. The influence of oil spills on fauna and ecology of small mammals of Middle Ob. Ecology. 2, 40-48 (in Russian).
17. Gashev, S.N., Elifanov, A.V., Il'uk, E.S., 1996. About some adaptation mechanisms of small mammals to the petrol pollution. Scientific bulletin of TSU (biology), 76-79 (in Russian).
18. Gashev, S.N., Elifanov, A.V., Solov'ev, V.S., Gasheva, N.A., 1994. The influence of crude oil on morphofunctional organism condition of non-perigreed white mice and rats. 6, 23-29 (in Russian).
19. Guidance on the application of Globally Harmonized System (GHS) criteria to petroleum substances, 2010. Version 1. 17 June, 27 pp.
20. Hall, Russel J., Coon, Nancy C., 1988. Interpreting residues of petroleum hydrocarbons in wildlife tissues. US Dep. Inter., Fish and Wildlife Serw. 15, 1-7.
21. Hesin, E., 1967. Functional morphology of nuclei (in Russian).
22. Holland, J.M., Frome, E.L., 1984. Statistical evaluations in the carcinogenesis bioassay of petroleum hydrocarbons. Appl. Toxicol. Petrol. Hydrocarbons. Princeton, N.Y., 151-166.
23. International recommendations to involving animals medico-biological research rendering, 1993. Lanimalogy. 1, 29 pp.
24. Karaev, A.N., 1965. Mechanism of influence of petroleum bios on animal organism. Petroleum fertilizers and stimulators in farming: Proceedings of the Conference, 22-25 January 1963, Baku, pp. 21-29 (in Russian).

25. Kirillov, O.I., 1994. Stress hypertrophy of adrenals. Moscow (in Russian).

26. Kononsky, A.I., 1976. Histochemistry. Kiev, 280 pp. (in Russian).

27. Kovrizhko, N.M., Oleinik, N.K., 1984. Adrenal functional morphology under the serdatives' stress. Morphology, 9 (in Russian).

28. Krizhanovsky, G.N., 1985. Stress and immunity. Bulletin Academy of Medicine USSR. 8, 2-12 (in Russian).

29. Kulyabko, A.A., Ovsyannikova, F.V., 1899. About physiological influence of oil and oil products. Bulletin of the Imperial Academy of Science, vol. VII, 9, 1-19 (in Russian).

30. Lewis, S.C., King, R.W., Cragg, S.T., Hillman D.W., 1984. Skin carcinogenic potential of petroleum hydrocarbons: crude oil, distillate fractions and chemical class subfractions. Appl. Toxicol. Petrol. Hydrocarbons. Princeton, N.Y., 139-150.

31. Liu, Guocheng, 1991. How can the complexity result in the stability in ecosystem. J. North-East Forest. Univ., 19. N 6, 9-14 (in Chinese).

32. Magloliocea, R.M., 1959. Sperim. T.36.

33. Meldolesi, I., 1967. On the significance of the hypertrophy of the smooth endoplasmic reticulum in liver cells after administration of drugs. Biochem. Pharmacol. vol.16, 125-129.

34. Moiseenko, T.I., Gashev, S.N., Petuhova, G.A., 2010. Biological methods of water quality evaluation: Part 2. Biotesting. Bulletin of TSU. 7, 39-50 (in Russian).

35. Nishimoto, S., Yamawaki, M., Akiyama, K., Kakinuma, Y., Kitamura, S-I., Sugahara, T., 2009. Severe abnormalities in the reproductive organs of mice

caused by chemical substances contained in heavy oil. The Journal of Toxicological Sciences. Vol. 34. N 2, April, 239-244.

36. Nishimoto, S., Yamawaki, M., Kitamura, S.-I., Akiyama, K., Kakinuma, Y., Sugahara, T., 2008. Risk Assessment of Heavy Oil on Terrestrial Mammals. Interdisciplinary Studies on Environmental Chemistry. Biological Responses to Chemical Pollutants, 269-274.
37. Ottway, S.M., 1971. The comparative toxicity of crude oils. The ecological effects of oil pollution on littoral communities. Institute of Petroleum, London, 172-180.
38. Panov, G.E., Petryashin, L.F., 1986. Environmental protection on the oil and gas production plants. Moscow, 244 pp. (in Russian).
39. Pshenichnov, R.A., 1991. Contemporary test-systems of environmental mutagen revealing. Sverdlovsk (in Russian).
40. Rahimtula, A.D., O’Brien, P.J., Payne, J.F., 1984. Induction of xenobiotic metabolism in rats in exposure to hydrocarbon-based oils. Appl. Toxicol. Petrol. Hydrocarbons. Princeton, N.Y., 71-79.
41. Rosmarie, A.F., 1994. Toxicity summary for benzo[a]pyrene. Oak ridge reservation environmental restoration program, pp. 10.
42. Sabirova, G.V., Man’kovskaya, N.K., 1966. The production of petroleum bios. Kiev (in Russian).
43. Schulte-Hermann, R., Thom, R., Sohlisht, I., Kozansky, W., 1968. Zahl und Ploidiegrad der Zellkerne der Leber unter dem Einfluss körperfremder Stoffe.

Naunyn-Schmiedebergs Arch. Pharmacol. Und Experimental. Pathol., Bd 261, H.1.

44. Shaposhnikov, V.M., Kirushkina, M.N., Simonova, G.P., Blinov, S.P., 1980. Rodents as possible indicators of oil pollution of environment. Rodents. Proceedings of V International Conference, Moscow, pp. 462-464 (in Russian).

45. Shvarts, S.S., Smirnov, V.S., Dobrinsky, L.N., 1968. Morphophysiological indicator method in the ecology ground vertebrates, Sverdlovsk, 387 pp. (in Russian).

46. Test plan crude oil category / High production volume (HPV) chemical challenge program, 2003. November 21, 2003. Crude Oil Consortium Registration #1100997.

47. Vermel, E.M., 1935. The size, reproduction and growth of cells. Animal growth. Moscow, pp. 107- 163 (in Russian).

The authors declare that there are no conflicts of interest.

The captions to figures

Fig. 1. Crude-oil-fed white mice and rats death-rate dynamics.

Fig. 2. Basic pathological changes in renal corpuscle of experimental animals (b) in comparison with control (a).