Determination of chronic toxicity of glyphosate by biotesting in laboratory mice

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Abstract. Glyphosate is the most promising herbicide currently used throughout the world. Glyphosate-containing preparations are also used as a desiccant in the pre-harvest period. Repeated processing of agricultural plants during the growth period and immediately before harvesting contributes to the accumulation of the herbicide in residual amounts in crops. The article presents the results of chronic intoxication of laboratory mice. It has been shown that glyphosate in residual amounts of 7, 14, 28 mg/kg is capable of causing quantitative and qualitative changes in formed blood cells, inhibits the functions of the reproductive system of experimental animals, and promotes changes in the structure of parenchymal organs. Experimental data indicate chronic toxicity of glyphosate at low concentrations.

1. Introduction
The use of herbicides is an integral practice in agriculture [1-3]. Changes in climatic conditions, natural communities of weeds and the introduction of transgenic cultivated plants into agriculture have led to the large-scale use of herbicides, an expansion of their range and the search for new active substances [4-6]. Residual amounts of herbicides are found in environmental objects, feed, food, animal tissues and human biomaterial [5]. Analysis of literature data showed that residual amounts of glyphosate and its metabolite aminomethylphosphonic acid were identified not only in grain, but also in its processed products (flour, bran). Residual amounts of the herbicide at a level of 0.12 mg / kg or less were found in the tissues of animals that were fed grain treated with glyphosate as a desiccant [1-7]. Failure to comply with the rules for the use of herbicides causes diseases and deaths [8].

Chemical methods of analysis make it possible to determine the quantitative content of some chemical substances, but do not make it possible to draw a conclusion about the toxic effects of herbicides on warm-blooded animals and humans, and for many of them maximum permissible concentrations have not been established. Biotesting makes it possible to determine the impact of pollutants on environmental objects. For this purpose, many test organisms and their responses have been studied [9]. Fish and invertebrates [10-12], earthworms [13-15], plants (Lemna minor L.) [16, 17] are used as test objects in biotesting.

The widespread study of acute and chronic toxicity of herbicides for mammals is associated with the possible similar effect of xenobiotics on the human body. The presence of glyphosate in residues in food and animal feed explains the relevance of this study.

The aim of this work is to study the chronic toxicity of the herbicide during biotesting in white mice.
2. Methods and materials
To assess chronic toxicity, laboratory mice were used, the animals were obtained from the vivarium of an accredited laboratory of the Federal State Budgetary Institution "Tver Interregional Veterinary Laboratory". The animals were kept in accordance with the requirements of GOST 33216-2014 “Guidelines for the maintenance and care of laboratory animals. Rules for the maintenance and care of laboratory rodents and rabbits ", Order of the Ministry of Health of the USSR of August 12, 1977 N 755 "On measures to further improve organizational forms of work with the use of experimental animals."

The study was carried out on 48 white laboratory mice, 12 males and 36 females at the age of 2 months with an average standard weight of 25-30 g. All animals were healthy; their condition was assessed visually. Water was supplied from stationary drinkers, feeding was carried out once a day without restriction.

Intoxication to laboratory mice was simulated by feeding animal oat grains treated with glyphosate. The concentration of glyphosate in the grain was 7 mg/kg, 14 mg/kg, 28 mg/kg. The experiment was carried out in two stages.

The first stage involved keeping males and females separately. The animals were divided into 16 groups, and were placed in individual cages as follows: four cages of 3 males, twelve cages of 3 females. Duration of feeding the treated grain to mice at separate keeping was 1 month. Feeding and keeping of animals is presented in table 1.

| Cell number | Content schema animal | Feeding scheme |
|-------------|-----------------------|----------------|
| 1           | Males                 | Untreated oat grain |
| 2           | Males                 | Oat grain treated with the drug (7 mg/kg) |
| 3           | Males                 | Oat grain treated with the drug (14 mg/kg) |
| 4           | Males                 | Oat grain treated with the drug (28 mg/kg) |
| 5-7         | Females               | Untreated oat grain |
| 8-10        | Females               | Oat grain treated with the drug (7 mg/kg) |
| 11-13       | Females               | Oat grain treated with the drug (14 mg/kg) |
| 14-16       | Females               | Oat grain treated with the drug (28 mg/kg) |

The second stage of the study included the formation of families from sexually mature laboratory animals, the family consisted of 3 females and 1 male. White mice were divided into 12 groups (families) and placed in individual cages, the feeding scheme is shown in table 2.

To observe the dynamics of the herbicide effect, part of the laboratory animals was sacrificed by decapitation after 3 months, the other part 5 months after keeping the animals according to scheme No. 2. Blood sampling was carried out for morphological analysis, visual examination of internal organs during postmortem examination, and pathological material was taken for the preparation of histological preparations.

| Cell number | Content schema animal | Feeding scheme |
|-------------|-----------------------|----------------|
| X(1)        | 1 male, 3 females     | Unprocessed oat grain with glyphosate |
| X(2)        | 1 male, 3 females     | Unprocessed oat grain with glyphosate |
| X(3)        | 1 male, 3 females     | Unprocessed oat grain with glyphosate |
| 7(1)        | 1 male, 3 females     | Oat grain, herbicide content 7 mg/kg |
| 7(2)        | 1 male, 3 females     | Oat grain, herbicide content 7 mg/kg |
| 7(3)        | 1 male, 3 females     | Oat grain, herbicide content 7 mg/kg |
| 14(1)       | 1 male, 3 females     | Oat grain, herbicide content 14 mg/kg |
| 14(2)       | 1 male, 3 females     | Oat grain, herbicide content 14 mg/kg |
| 14(3)       | 1 male, 3 females     | Oat grain, herbicide content 14 mg/kg |
| 28(1)       | 1 male, 3 females     | Oat grain, herbicide content 28 mg/kg |
| 28(2)       | 1 male, 3 females     | Oat grain, herbicide content 28 mg/kg |
| 28(3)       | 1 male, 3 females     | Oat grain, herbicide content 28 mg/kg |
3. Results and discussion

During the experiment, a daily visual inspection of the condition of the animals was carried out. The criteria for chronic toxicity were changes in behavioral reactions, reproduction of offspring, the number of individuals in the offspring and the viability of cubs, the number of dead animals and the timing of their death, pathological changes in tissues and organs.

The clinical picture in mice did not show signs of poisoning, changes in the behavior of the animals were not observed.

The fertility of mice in the control and experimental subgroups differed significantly. The birth of the first offspring in the control subgroups was registered in the period from 20-23 days after the beginning of the second stage of the experiment. The number of cubs ranged from 6 to 9 individuals, the survival rate of the offspring was 100%, except in cases of cannibalism, which is a natural behavior in a stressful situation in which the female is found.

In the course of the study, a decrease in the fertility and viability of the offspring of the experimental animals was noted in comparison with the control ones. In a subgroup of laboratory animals, which were fed with oat grain containing the herbicide 7 mg/kg, one female reproduced the offspring. The number of cubs in the offspring was recorded 6 individuals, the life expectancy was from 1 to 3 days, the period to reproduction of the offspring was 68 days, with a delay of 48 to 46 days compared to the control subgroup of laboratory animals.

When observing laboratory animals, which were fed with oat grain containing 14 mg/kg glyphosate, the following data were recorded. The offspring was reproduced by one female, the number of cubs in the offspring was 7 individuals, the life span was from 1 to 4 days, the period to reproduction of the offspring was 80 days, with a delay of 60 to 58 days compared to the control subgroup of laboratory animals.

In the course of further research, autopsy revealed 7 embryos in the horns of the uterus of the female, which was fed with oat grain containing 28 mg/kg glyphosate. The dissection of this animal was carried out 5 months after the beginning of the second stage of the experiment.

The results of the experiment showed a quantitative change in the formed cells of the peripheral blood in the experimental animals as compared with the control ones. The average decrease in the number of leukocytes in the blood in the group of animals fed with grain with a herbicide content of 7 mg/kg was 30% and 66% after 4 and 6 months, respectively, in animals that were fed with grain with a herbicide content of 14 mg/kg, amounted to 30% and 62% after 4 and 6 months, respectively, in animals, which were fed with grain with a herbicide content of 28 mg/kg, was 38% and 73% after 4 and 6 months, respectively.

A quantitative decrease in blood erythrocytes in experimental animals compared to control animals was also observed during the entire experiment. The average decrease in the number of erythrocytes in the blood in the group of animals fed with grain with a herbicide content of 7 mg/kg was 23% and 38% after 4 and 6 months, respectively, in animals that were fed with grain with a herbicide content of 14 mg/kg, was 36% and 47% after 4 and 6 months, respectively, in animals, which were fed with grain with a herbicide content of 28 mg/kg, was 43% and 59% after 4 and 6 months, respectively.

In the study of cytological blood preparations, the following results were obtained. Most erythrocytes are normochromic, have the same shape, a central zone of enlightenment. At the same time, qualitative changes in erythrocytes were observed in blood smears of experimental animals. Anisocytosis and poikilocytosis were expressed in changes in the size and shape of erythrocytes.

In the pathological examination of laboratory animals, the greatest changes were found in the liver, small and large intestines. In laboratory animals of the control experiment, parenchymal organs corresponded to a healthy animal. In all experimental animals, visual examination showed a change in color, consistency of the liver and intestines. As a result of chronic intoxication, the liver is slightly enlarged, flabby, light brown in color. The walls of the thin and thick parts of the intestine are gray, the consistency is flabby, the mucous membrane is light brown, circular folds are not visible, in places the mucous membrane is exfoliated. Pathological changes in the intestinal wall are most pronounced in mice
eating grain with a herbicide concentration of 28 mg/kg, and more severe injuries appear: punctate hemorrhages under the mucous membrane of the small intestine.

Microscopic examination of liver preparations revealed the greatest changes in experimental animals eating grain with a herbicide concentration of 28 mg/kg. In histological preparations of animal organs, which were fed with grain containing the herbicide of 7 mg/kg, 14 mg/kg, there was an expansion of sinusoidal capillaries, single lymphocytes were seen in the ducts. The beam-radial structure of the hepatic lobules is preserved, the polygonal shape of cells of natural size is clearly traced. In histological preparations of animal organs, which were fed with grain with an herbicide content of 28 mg/kg, a significant amount of leukocytes was observed in the capillary ducts, the beam-radial structure of the hepatic lobules is not clear, it can be traced, the form of hepatocytes is changed - polygonal, elongated, the cells are enlarged.

4. Conclusion
As a result of the studies performed, it was found that glyphosate at concentrations of 7, 14, 28 mg/kg causes inhibition of the reproductive system of experimental animals, negatively affects the viability of the offspring.

When analyzing cytological blood preparations of experimental animals, a decrease in the number of uniform cells, both leukocytes and erythrocytes, was recorded. In the course of chronic intoxication, the negative effect of glyphosate on the morphological parameters of blood was revealed.

At postmortem examination of laboratory animals, changes in the liver, small and large intestines were observed. The study of histological preparations of the liver showed that the herbicide at various concentrations causes a violation of the structure of the organ tissue.

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