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STUDY OF THE EFFECT OF THE XENOBIOTIC HORMONE ON THE REPRODUCTIVE FUNCTION OF RATS

N. Kononenko, A. Minukhin, V. Chikitkina

The aim is to study the effect of the xenobiotic of Nonylphenol on functional and morphological indices of the reproductive system of males of rats.

Materials and methods. Experimental modelling of gonadotoxic effects of the xenobiotic was performed by inhalation of Nonylphenol at a concentration of 500 mg/m³ for 30 days, 5 days a week, 5 hours a day in conditions of the seed chamber. Male rats ejaculate were obtained by the method of electrostimulation seed tubercle in the mucous membrane of the rectum. The number of sperm in the ejaculate and their mobility were determined in the Hemocytometer 1, 7, 14 and 30 days after the start of the experiment. Macroscopic study of the testes included the assessment of external signs of damage, the determination of the mass and length of the testes.

Results. It is established that the introduction of Nonylphenol to rats males leads to a gradual dynamical reduction of the total number and the number of mobile forms of sperm cells compared to intact animals. At the end of the experiment the number of mobile forms of sperm was 2.1 times below the original data. Long-term effect of Nonylphenol has also led to a significant decrease of body weight growth, which confirms its high toxicity. In the study found that intoxication in Mature rats Nonylphenol were significant changes in the reproductive system, which is primarily characterized by decreased weight of the testes and the appendages of the testes, and changes in linear dimensions of the bodies downward. These violations indicate a feminizing effect of this hormone-like xenobiotic and its role in the development of male infertility.

Conclusions. Quantitative and qualitative parameters of the ejaculate of sexually mature male rats can serve as a valid criterion for adaptive and maladaptive processes occurring in the organism under the influence of the xenobiotic. In conditions of prolonged exposure to Nonylphenol (within 30 days) the changes in mass of the testes and reduce their linear dimensions

Keywords: Nonylphenol, reproductive system, gonads, spermatogenesis

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1. Introduction

Millions of years living organisms seen only with the «familiar cues» of the environment, as the basis on which in evolution formed their functional relationships with the outside world, and between organs and cells of the organisms themselves were the chemical and physical factors that are strictly conform to the composition and level of specific terrestrial environment: chemical composition of seawater, where life began, atmospheric gases, temperature fluctuations, light, atmospheric pressure, levels of natural radiation, etc. At the same time, on its basis, mechanisms of neutralization and excretion of a substance foreign to it were formed. All this ensured the effective functioning and reproduction of organisms and their adaptation to the changing conditions of existence due to fluctuations in climate and other factors, the
changing composition of food etc. With the advent of a person who is capable of «transforming impact on the environment», the situation began to change.

Until the mid-twentieth century «anthropogenic pressure» on the biosphere has become a global concern, which was accompanied by massive entry into the environment of industrial, agricultural and household waste. The resulting changes in physical and chemical environmental parameters, effective adaptation of which has not been developed in the process of evolution, began to pose a real threat to human health. It should highlight the consequences of adverse environmental impacts on the reproductive system because of reproductive disorders affect the health of not only existing but also future generations [1].

Among the harmful external factors lately dedicated a group of so-called reproductive toxicants and, above all, hormone-like xenobiotics (HLX), among them, non-ionic surfactants that are used in industrial and household preparations, in the first place, nonylphenol ethoxylate [2]. The processes of destruction of nonylphenol ethoxylates in the environment lead to the formation of Nonylphenol (NF) – 2, 6-dimethyl-4-heptylphenol (C\(_9\)H\(_{19}\)) – final degradation product of non-ionic surfactants.

NF in a free form in the environment is a product of synthesis and thus referred to the group of xenobiotics. For the first time, NF was synthesized in the 40-ies of the last century and since then its production is increasing annually. About 80 % of the produced NF is used to obtain industrial surfactants and liquid detergents and for the production of rubber, plastics and oils. NF is contained in pesticides, cosmetics, office equipment, such as correction fluid (correctors), ink, etc. In addition, NF is a component of pharmaceutical preparations, insecticides, bactericides, chemical stabilizers.

The presence of NF in the environment is solely a consequence of anthropogenic influence: getting into the environment mainly from industrial wastewater, he is in water, soil, groundwater and sediments. In connection with the widespread use of NF in various industrial processes for the production of household and technological means possible revenues of the water system of the environment, as confirmed by the detection of NF in water of rivers, lakes and coastal waters. Revealed the presence of NF in varying concentrations in ambient air. In humans and animals NF may enter by inhalation, through the mouth and the skin [2].

NF has a negative impact on neuroimmunoendocrine system that is mediated by its binding to the estrogen receptors, resulting in the occurrence of estrogen-like effects [3]. Probably, the occurrence of estrogen-like effects can be explained by common structures of NF and hormone estradiol.

Therefore, NF can cause disorders of the endocrine functions of the body through various mechanisms. In particular, NF is able to simulate the functions of sex steroid hormones – estrogens and androgens manifested through the ability of NF to bind to their natural receptors, or by altering the synthesis and breakdown of natural hormones in the body. Reproducing, modifying or blocking the action of endogenous hormones, NF can affect the mechanisms of regulation of reproductive function, so they are referred to endocrine destructors [4]. In connection with the foregoing, it can be argued that the emergence in the biosphere of the Earth hormone-like xenobiotics fundamentally changed «the ecological situation on the planet».

Thus, the NF has the ability to mimic the effects of natural biologically active compounds, even in relatively low doses and disrupt different functions in living organisms, leading often to the development of chronic toxic effects.

Therefore, the increase in the external environment of NF that have estrogenic and anti-androgenic effect, had an impact primarily on men's organisms [5]. This obviously explains the appearance of signs of «exhaustion» of the modern men reproductive system with the «main world sensation of the twentieth century» – a progressive reduction in the volume of ejaculate and concentration of sperm in it and, accordingly, increase in the incidence of male infertility.

So, when studying spermiogram candidates in donor semen in Belgium [6], they noted that, if prior to 1980, characteristics of subnormal semen was detected only in 5 % of patients, in subsequent decades – in 40 %. «Screening» candidates to sperm donors as «unpromising» increased in 17 years from 13 % to 54 %. According to calculations, the speed of decrease of the concentration of sperm cells in the ejaculate of men of Europe is approximately 2 % per year.

In favour of communication of deterioration in the condition of the reproductive system of males from the influence of anthropogenic pollutants show evidence that the described trends are taking place predominantly in men in industrialized countries of Europe and America, and less characteristic of men in Asia and Africa [6]. This was also indicated by the results of the study of spermatogenic function in different regions. Most pronounced they were in men who work in chemical plants that live in industrialized regions or in agricultural areas with intensive «pesticide load».

As you know, the ability of sperm to fertilize reflects male fertility is, to some extent, «threshold character», and by reducing the concentration of sperm below «critical» level (currently it is 20 million/ml) fertility falls sharply. The result of the continued deterioration of spermatogenesis in humans was a significant decrease in the so-called «margin of safety» – the value of the concentration of sperm cells, which exceeds the lower threshold level [7]. Now the total average concentration of sperm in humans is greater than the threshold level in 3–4 times [8]. At the same time, the males of other mammals, particularly in laboratory rodents, this difference amounts to hundreds and thousands of times. In the case of a continuing trend of continued deterioration of spermatogenesis mankind may step over the threshold beyond which the problem of male infertility will take the nature of a global catastrophe, and the biblical predictions of the «end of the world» will become a reality.
The aim of our study was to study the effect of the xenobiotic of Nonylphenol on functional and morphological indices of the reproductive system of males of rats.

2. Materials and methods

The studies were carried out on the basis of the Central Research Laboratory of Kharkiv National Medical University from September to October 2019.

The experiments were performed on 30 non-linear mature rats in males in the age from 7 months to 1 year, which corresponds to the reproductive (18–30 years) and mature early (31–44 years) periods of human life [9]. The body weight of animals was 180–200 g. The animals were kept in standard vivarium conditions at a temperature of 20–22 °C, humidity 60–70 %, the volume of air (exhaust-supply) 8/10, light mode day/night in standard aluminium cages, no more than 5 animals in each (Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes).

Pharmacological studies conducted in the Central research laboratory of the National University of pharmacy that is certified by the State expert center the Ministry of health of Ukraine as a basis for research in experimental pharmacology (certificate N 058/15 from 08.12.2015). During work with animals was adhered the international code of medical ethics (Venice, 1983), «The European Convention for the protection of vertebrate animals used and experimental and other scientific purposes» (Strasbourg, 1986), «General ethical principles of animal experimentation» adopted by the First national Congress on bioethics (Kyiv, 2001), Directive 2010/63/EU of the European Parliament and Council on the protection of animals used for scientific purposes and the law of Ukraine «On protection of animals from cruelty» No. 3477-IV of 21.02.2006.

We used a concentrated Nonylphenol ethoxylate (company “Novokhim”, Kharkiv, Ukraine).

Rats were divided into 2 groups (15 animals each): group 1 – intact animals; group 2 – rats that were subjected to inhalation exposure of Nonylphenol at a concentration of 500 mg/m² for 30 days, 5 days a week, 5 hours a day. Conditions were created using a special setup, which consisted of the seed chamber and the chamber in which was maintained the necessary concentration of the active substance; sensor Nonylphenol and accessories [8].

The study was conducted in 2 stages: at the first stage studied the ejaculate of male rats, on the second – conducted a macroscopic study of the testes. To obtain the ejaculate using the method of electrostimulation seed tubercle in the mucous membrane of the rectum. The number of sperm in the ejaculate and their mobility were determined in the Hemocytometer 1, 7, 14 and 30 days after the start of the experiment. On the 30th day after a single 4-hour exposure of animals under mild chloroform anesthesia, they were killed by simultaneous decapitation, the testes were removed and their appearance was assessed in order to identify pathological abnormalities (blood filling, inflammatory changes, atrophy, etc.). In addition, the testes were weighed on an analytical balance of Radwag AS 220.R2 (Poland) and the ratio of the weight of the testes to body weight was calculated. The volume and length of the testes were also determined [10]. Then they photographed the resulting organs for subsequent macroscopic morphometric analysis. The data obtained were processed using Student’s t-test.

3. Results

Semen analysis of the intact group of animals showed that in the ejaculate, there were 11.2±0.48 million sperm, of which is 9.65±0.41 million was mobile, which accounted for 86.2 % (Table 1).

On the first day after exposure to Nonylphenol was observed insignificant decrease in the total number of spermatozoa to 10.6±0.51, however, the number of motile forms was 67.5 % of the total number of cells. That is, the findings suggest that already in the first days of action of Nonylphenol, the number of motile forms was significantly reduced in 1.3 times.

A week after exposure to Nonylphenol was observed a significant decrease in the number of sperm 1.7 times in comparison with intact animals, the number of motile forms was 48.4 %.

On the 14th day of the experiment, it was observed insignificant increase in sperm count (7.81±0.65 million) compared to the same indicator on the 7th day of the study, and the number of motile forms was significantly decreased to 4.26±0.64 million.

On the 30th day of the experiment the total number of sperm in the group of Nonylphenol increased to 9.26±0.16 million sperm and number of motile forms reached minimum values during the experiment and amounted to 36.9 per cent.

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Indicators of semen analysis, mil/ml</th>
<th>The day of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>7 day</td>
</tr>
<tr>
<td>Intact group</td>
<td>Total sperm count</td>
<td>11.2±0.48</td>
</tr>
<tr>
<td></td>
<td>Number of movable forms</td>
<td>9.65±0.41</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>Total sperm count</td>
<td>10.6±0.51</td>
</tr>
<tr>
<td></td>
<td>Number of movable forms</td>
<td>7.15±0.08               *</td>
</tr>
</tbody>
</table>

Note: * – p<0.05 relative to the intact group of animals; ** – p<0.05 compared with the indicator of the Nonylphenol group on the 1st day of the experiment.
Consequently, obtained data indicate that analysis of seminal fluid from male rats can reflect the depth of pathological changes in sexual development in terms of the effects of Nonylphenol.

The macroscopic study determined that the indicator (30 days after start of experiment) of the body weight of mature male rats that were exposed to Nonylphenol at the end of the experiment amounted to 223.8 g, significantly lower than in intact rats by 14 %. The weight gain of the rats in this group were of 81.7 g (Table 2).

### Table 2

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>The state of the body weight of rats, g</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial data</td>
<td>30th day</td>
</tr>
<tr>
<td>Intact group</td>
<td>178.6±10.1</td>
<td>260.3±15.4</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>186.4±11.8</td>
<td>223.8±10.3*/**</td>
</tr>
</tbody>
</table>

Note: * – p<0.05 relative to the baseline data of the nonylphenol group at the beginning of the experiment; ** – p<0.05 relative to the intact group at the end of the experiment

The average absolute weight of the right testis of rats of the experimental group was 1406.8 mg, which was significantly lower in this indicator in the intact group of animals. The absolute weight of the average was 628 mg/100 g of animal’s body. The absolute weight of the left testicle in the group of intact animals was significantly higher in the group that modelled the control condition. So, the difference was 11.8 %. The relative weight of the left testis was on average equal to 564.4 mg/100 g of body weight (Fig. 1). The average length of the right testis of rats, which in the experiment were exposed to Nonylphenol, on the first day after the termination of the specified adverse factors was 19.01 mm, which is lower than in animals of the intact group by 9.4 %.

The maximum length of the right testicle in the experimental group of animals was recorded at the level of 21.8 mm, the average width of the right testis after termination of Nonylphenol were equal to the average 13.04 mm, which is less than the intact group of rats.

It is established that the average length of the left testicle in the first day after the termination of the specified adverse factor was 17.42 mm, lower than the intact group. The average width of the left testicle was 9.25 mm. The minimum width of the left testis of the animals were recorded at the level of 8.12 mm.

In the study increased the absolute weight of right epididymis in rats of the control pathology group found that there is a significant difference between the specified rate and similar in the group of intact control. Thus, the absolute weight was 468.4 mg, while in the intact – 540.8 mg, the difference to 72.4 mg. The average absolute weight of the left epididymis of mature rats was recorded at 451.2 mg and 515.6 mg, respectively.

The maximum length of the right epididymis of mature rats subjected to the action of Nonylphenol in the first day after the termination of this factor was 44.4 mm, the average length of the right testis in this group was 41.7 mm, which are lower than in intact group of animals.

In the result of the study is established that inhalation intoxication of mature rats Nonylphenol resulted in significant changes in the reproductive system, which is primarily characterized by decreased weight of the testes and the appendages of the testis, and changes in linear dimensions of the bodies downward.

![Fig. 1. Influence of Nonylphenol on the absolute (mg) and relative mass of the right and left testicles:](image-url)

* – p<0.05 relative to the intact group at the end of the experiment
4. Discussion

In our time the reproductive system of men is exposed to many adverse factors, particularly exposure to chemicals, which leads to depletion of the functional capacity of the organism, reduction in the generative functions of the testes and infertility.

Nonylphenol, which is widely used in various fields of industrial production and human life, revealed in many environments environment and is considered by modern scholars as a powerful “destroyer” of the endocrine system.

Most experimental studies devoted to the investigation of the effect of Nonylphenol in the use of drinking water contaminated by this xenobiotic. In our work we presented the results of the effect of Nonylphenol on the reproductive system of male rats during inhalation exposure.

Study of parameters of semen of animals showed that exposure to Nonylphenol for 30 days resulted in a gradual dynamic reduction of the total number and the number of mobile forms of sperm cells compared to intact animals. Significant violation of sperm motility at the end of the experiment 2.1 times in the group of animals treated with Nonylphenol, compared with the original data indicates a decline in their functional activity and ability of the xenobiotic to accumulate if it enters through the respiratory system.

Long-term effect of Nonylphenol has also led to a significant decrease of body weight growth, which confirms its high toxicity.

The negative effects of Nonylphenol on the neuroendocrine system is manifested by its binding to estrogen receptors, causing estrogen-like effects, and prolonged exposure of the developing endocrine imbalance and male infertility [11].

The results of determining the morphometric parameters of gonads testified about the possible feminizing hormone-like effect of the xenobiotic, which caused a significant decrease in absolute and relative testes mass and linear dimensions.

Study limitation. The study reflects only quantitative and macroscopic changes in sperm and gonads and does not include the study of the functional state of sperm.

Prospects for further researches. In the future we plan to study the deep mechanisms of the influence of Nonylphenol on the reproductive system of male rats in inhalation introduction.

5. Conclusions

1. In conditions of prolonged exposure, the toxic properties of Nonylphenol were confirmed, as evidenced by a decrease in the weight gain of rats.

2. Established a pronounced gonadotoxic effect of Nonylphenol when it administered by inhalation for 30 days, which is manifested by a significant decrease in the absolute and relative mass of testes and their linear dimensions, the total number and number of motile forms of spermatozoa compared with intact animals.

3. Quantitative and qualitative parameters of changes in the ejaculate of sexually mature male rats can serve as a reliable criterion for adaptive and maladaptive processes occurring in the body under the influence of xenobiotics (Nonylphenol).

Conflicts of interest

The authors declare that they have no conflicts of interest.

References


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