

**THE INFLUENCE OF SOME ENVIRONMENTAL POLLUTANTS ON THE  
HISTOGENESIS OF TEETH IN EXPERIMENTAL RATS**

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**ABSTRACT**

The purpose of the work was to identify the features of embryonic development of rats as a whole, as well as embryonic and postnatal development of their dentoalveolar system under conditions of intrauterine exposure to some chemicals polluting the environment. Pesticides, hexachlorane and fosalone, as well as sulfur dioxide and nitrogen dioxide, which are the main components of harmful emissions from oil refineries, have been selected as polluting chemicals. The experiments were performed on non-matured mature white female rats with a body weight of 170-180 g. It is found that prenatal exposure of toxic substances (pesticides, sulfur and nitrogen dioxides) leads to impaired fetal and postnatal development of offspring, which are shown to the maximum extent in combined toxicity of pesticides and dioxides. It was also revealed that combined intrauterine intoxication substantially interferes with the tooth tissue differentiation and jaw bones of the fetus, resulting in the delayed eruption and abnormal development of the teeth in the postnatal period. Combined intrauterine intoxication significantly violates the processes of tissue differentiation of dental germ and ossification of the jaw bones of the fetus, which result in delayed eruption and abnormal development of teeth in the postnatal period.

**KEYWORDS:** pesticides, dioxins, embryonic development, developmental dental toxicity, dental histogenesis, environmental toxicants.

**INTRODUCTION**

Pollution of the environment by harmful substances, mainly pesticides and industrial emissions, can lead to a disturbance of the ecological balance and a real danger to public health. The organism is most vulnerable to the influence of various harmful environmental factors in the prenatal and early postnatal periods of life, when practically all organs and systems are becoming established.<sup>[1; 2; 3]</sup> It has been established that in the most contaminated regions the incidence of respiratory, digestive and nervous diseases, physical and mental development disorders and the likelihood of congenital malformations in children is much higher than in ecologically safe regions.<sup>[4; 5; 6]</sup> Organs and tissues of the oral cavity are one of the first to come into contact with unfavorable environmental factors and therefore the earliest changes in the body can be localized precisely in the dentoalveolar system of children.<sup>[6; 7; 8]</sup> Unfortunately, up to the present time, the influence of the isolated and combined effects on the organism of pesticides and industrial chemical emissions (sulfur dioxide, nitrogen, ammonia, etc.) on the embryonic and postembryonic development of the organism as a whole, and the dentoalveolar system in particular, has not been sufficiently studied.

**The purpose** of the work was to identify the features of embryonic development of rats as a whole, as well as

embryonic and postnatal development of their dentoalveolar system under conditions of intrauterine exposure to some chemicals polluting the environment.

**MATERIAL AND METHODS**

Pesticides, hexachlorane and fosalone, as well as sulfur dioxide and nitrogen dioxide, which are the main components of harmful emissions from oil refineries, have been selected as polluting chemicals. Experiments were carried out on non-matured mature white female rats with a body weight of 170-180 g. Within 2 weeks prior to the beginning of the experiments, all rats were evaluated for estrous cycle condition, and in the absence of any deviations, the animals were selected for experiments. Depending on the nature and mode of exposure of toxic substances to the body, all animals were divided into 3 groups of 20 females in each. The first group of animals, which was not exposed to toxic effects, served as a control. A combination of pesticides of hexachloran and fosalon was administered to the second group of females during the entire pregnancy by the intragastric method. A third group of animals, along with the preparation of this combination of pesticides, was exposed to sulfur dioxide and nitrogen dioxide through inhaled air. The dose and concentration of inputs were selected in such a way that they were close to the actual conditions of environmental pollution in the

industrial and agricultural zones of the region studied by us. The most optimal dose of hexachlorane was 0.08 mg / kg, fosalone 0.008 mg / kg, concentration of sulfur dioxide 0.12 mg / m<sup>3</sup>, nitrogen dioxide 0.08 mg / m<sup>3</sup>. Poisoning of females began with the first day of pregnancy, the onset of which was determined by the presence of spermatozoa in the vaginal smears. For the histological study of developing dental rudiments, 10 females from each group were sacrificed shortly before birth, on the 20th day of pregnancy, by decapitation under light ether anesthesia. Isolated pieces of the jaw section of the fetal skull (in 4-5 fetuses from 5-6 females) and fixed in 10% neutral formalin. After dehydration, the pieces were poured into paraffin, sections were prepared with a thickness of 7-10 µm, which was stained with hematoxylin and eosin. To obtain sufficiently complete information on the embryotoxic and teratogenic effects of the substances studied, the number of live fetuses per rat was determined, the total intrauterine mortality (in%), the body weight (in g), and the cranio-caudal fruit size (in mm) were calculated. In addition, the frequency of occurrence of external anomalies, abnormalities of internal organs, skeleton and dentoalveolar system of the fetus was detected. The second half of the females (10 females in each group) were left until the completion of natural childbirth in order to study the features of the dynamics of postnatal development of the offspring in conditions of combined intrauterine action of toxic environmental factors. On the 1st, 7th, 14th, 30th and 60th days after birth, offspring (in 5-6 rats from 6-8 females) measured the mass and length of the body, as well as the timing of teething, the eruption of the auricles, the opening of the eyes and the appearance of the hair cover. All the received digital data were statistically processed using the Microsoft Excel software package with the calculation of the Student's criterion. Differences satisfying  $P < 0.05$  were considered reliable.

## RESEARCH RESULTS AND DISCUSSION

The study of embryonic material showed that the most unfavorable, mediated through the organism of a pregnant female, is the combination of pesticides in combination with sulfur dioxide and nitrogen dioxide in the fetal development of the fetuses. In this (III) group of animals, the total intrauterine mortality was the highest and amounted to  $21.3 \pm 2.5\%$  with a control of  $15.2 \pm 1.8\%$  ( $P < 0.05$ ). Accordingly, the number of live-born fetuses per female in this group was significantly less than the control level and was  $8.2 \pm 0.8$  with a control of  $11.4 \pm 1.3$  ( $P < 0.05$ ). It was also found that in the fetuses obtained from the females of group III, the average body weight was reliably low and was  $2.01 \pm 0.2$  g versus control  $2.96 \pm 0.3$  g ( $P < 0.01$ ). Similarly, in the fetuses of this group, a significant decrease in the mean cranio-caudal size was revealed in comparison with the control group (up to  $27.2 \pm 0.2$  mm with a control of  $29.8 \pm 0.4$  mm,  $P < 0.01$ ). And finally, only in this group up to 3.9% of the fruits external anomalies of development (short tail, subcutaneous hemorrhages) and anomalies of

internal organs (micrognathia, delay of ossification of bones) were observed. Anomalies of development were practically absent in offspring from the females of group II, exposed only to pesticides. The fetuses of this group had only a slight decrease in body weight and fetal size, as well as a slight increase in fetal mortality. However, all these indicators were statistically unreliable ( $P > 0.05$ ).

Thus, the combined intrauterine effect of pesticides in combination with sulfur dioxide and nitrogen dioxide leads to significant disturbances in the embryonic development of the offspring of experimental animals.

Intrauterine exposure to environmental pollutants also contributed to some disturbances in the process of postnatal ontogeny of offspring, the extent of which depended on the mode of action. In group II, rats born from females with intoxication with pesticides alone, during the first month there was a moderate, but unreliable, lag in the growth of body length in comparison with the control group. At the same time, the body weight of the rats remained significantly low, from the very beginning of the experiments up to 30 days. Only on the 60th day these rats began to gain weight. Hypotrophy of the rat was accompanied by a slowdown in the growth of the body's hair and a delay in the appearance of other signs of postnatal growth. In the rats of this group, the auricles shook off and the eyes opened 1-2 days later than in the animals of the control group.

The most pronounced changes in the postnatal period of development were observed in group III of rats born from females with combined intoxication during pregnancy with pesticides and dioxides. Thus, the weight and body length indices of these rats remained reliably low in comparison with the control throughout the experiment. Similarly, a marked late appearance of signs of postnatal growth in these rats has been noted. The auricles shook off 3-4 days after the control, the opening of the eyes and the growth of the hair cover also occurred with a delay of 2-3 days compared to the control.

Thus, the exposure of toxic environmental pollutants to the maternal organism during pregnancy leads to the development of a number of general disturbances in the process of embryonic and postembryonic growth and the formation of the organism. With intoxication only with pesticides, they are relatively moderately expressed, the degree of their severity increases significantly when pesticides are combined with sulfur dioxide and nitrogen dioxide. This pattern fully applies to embryonic and postembryonic histogenesis of the dentoalveolar system. In the rats of group II, subjected to intrauterine exposure to pesticides alone, moderate morphological changes in the forming dental rudiments were found. In the majority of embryos, differentiated enameloblasts and odontoblasts were determined. The phenomena of destruction of enameloblasts and vacuolization of the cytoplasm of odontoblasts were rarely detected. In some cases, microcirculatory disturbances were found in the

forming pulp of the tooth in the form of capillary and venous stasis of blood. Often, thinning and weak mineralization of the formed bone trabeculae of the jaw bones are often found, which is probably due to insufficient differentiation and destructive changes in osteoblasts. Nevertheless, these changes in embryonic histogenesis of dental rudiments were sufficient to slow the rate of dentition. In the rats of group II, cutting of incisors occurred only on the 10th -12th day, whereas in control animals this process was observed already on the 8th -9th day after birth.

The most pronounced disturbances in the histogenesis of the dentoalveolar system as a whole were observed in group III of animals subjected to combined intrauterine exposure to pesticides with dioxides. The histological study of the dentofacial tissues of the fruits of this group revealed an even more significant delay in the processes of differentiation of dental rudiments than in group II. Often found rudiments that contain an insufficiently formed enamel organ. Often there were enamel organs of small size, with pronounced deformations and asymmetrical edges. The pulp of the enamel organ was often represented by a narrow strip of closely spaced stellate cells. In the outer layer of the enamel organ, epithelial cells in some areas underwent destructive changes and lysis with the formation of large vacuoles. Internal cells of the enamel organ were characterized by a low degree of differentiation, an uneven appearance of characteristic for enameloblast characters was noted. The layer of enameloblast consisted of cells with different levels of differentiation. Enameloblasts with various disturbances of the process of "inversion", that is, moving the nucleus from the basal to the apical pole of the cell, were often found. The number of such cells with a slowing down of the process of changing the "polarity" of cells varied in different dental organs, which indicated the asynchrony of the differentiation of enameloblast and enamel formation. In most dentition, a thin, poorly discernible layer of organic enamel base with irregular contours was traced. Often there were signs of destruction and vacuolization of the cytoplasm of enameloblast. Simultaneously with the changes in the process of enamel formation, signs of a violation of histogenesis and other tissues of dental rudiments were found in this group of animals. At the tops of the dental papillae, among the layer of preodontoblasts and odontoblasts, destructively altered and vacuolated cells were often observed. Against this background, in some areas, in connection with the violation of differentiation of enameloblast and odontoblast, the line of the organic matrix of enamel was represented by a formlessly weakly eosinophilic substance with indistinct boundaries. As a result of destructive changes in odontoblast, asynchronous deposition of the organic matrix of predentine was noted with the formation of voids in it. Such voids were also retained in mineralized areas of dentin. Certain changes were found in the central part of the dental papilla, which forms the basis of the future pulp of the tooth and its microcirculatory

bed. The pulp was represented by slightly differentiated cells with oval and polygonal nuclei, among which were blood vessels. Attention was drawn to the significantly higher frequency of detection of dilatation of venous vessels and stasis of blood in the capillaries of the fruits of this group compared with Groups I and II. In our opinion, such a high degree of changes in the microcirculatory bed of the tooth rudiment is one of the factors contributing to the development of more pronounced disorders of the histogenesis of enamel and dentin in this group of animals. In addition to these changes, these fruits showed a significant slowing in the rate of formation of the jaw bone trabeculae. The surrounding tissue was in most cases represented by strips of hyaline cartilage, with weak signs of ossification and mineralization. All this in general had a more pronounced negative effect on the timing of teething in the postnatal period. In the rats of group III, cutting of the incisors was observed only on the 13th -15th day after birth (in the II group by 10-12, in control - on the 8th-9th day). It should also be noted that in most rats the crown part of the cut incisors was thinned. Often in the crown, the appearance of uncharacteristic for healthy teeth multiple yellow spots or grayish-matt strips. In some cases, there was a close and anomalous location of incisors and chewing teeth.

Thus, exposure to toxic pollutants of the environment during pregnancy has an adverse effect on both fetal development in general, and on fetal and postnatal histogenesis of the dentoalveolar system.

## CONCLUSIONS

1. Intrauterine exposure to environmental pollutants (pesticides and sulfur dioxide and nitrogen dioxide) leads to disturbances in the embryonic and postnatal development of the offspring, which are manifested to the greatest extent by combined intoxication with pesticides and dioxides.
2. Combined intrauterine intoxication significantly violates the processes of tissue differentiation of dental germ and ossification of the jaw bones of the fetus, which result in delayed eruption and abnormal development of teeth in the postnatal period.

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