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AQUATIC TOXICOLOGY

Effect of the Insecticide Tanrec® on Reproduction and Vital Activity of *Daphnia magna* Straus in a 15-day Test

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Abstract—The effect of the insecticide Tanrec® at concentrations of 3.0×10^{-7}, 3.0×10^{-2}, and 3.0×10^{-1} mg/L (as of imidacloprid) on *Daphnia magna* Straus has been studied. An acute toxic effect of this insecticide at a concentration of 3.0×10^{-1} mg/L and a depressive effect at concentrations of 3.0×10^{-2} mg/L and 3.0×10^{-7} have been revealed. A damaging effect of Tanrec was revealed during the stage of early development of studied crustaceans. This effect was manifested in the inhibition of the growth of oocytes, abnormal functioning of the intestine, retardation of body growth, and pathological changes in tissues.

Keywords: Tanrec®, imidacloprid, *Daphnia magna*

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INTRODUCTION

Modern technologies of agriculture necessitate the use of highly-efficient pesticides. Many new pesticides on the market possess extremely high activity and are used at very low doses. It is noted in the registration certificate of insecticide Tanrec® (active compound imidacloprid) that, to prepare 10 l of working solution, only 1–5 mL of the commercial compound is necessary; 5–10 L of such solution is sufficient to treat 100 m^2 of crops [4]. The studies on the toxicity of imidacloprid are focused mainly on the development of new preparations based on this compound [5] and on the specification of doses of preparation to effectively combat agricultural pests [16] and parasites of livestock [19]. A series of articles in the domestic and foreign literature is devoted to the problem of protecting honey bees from the harmful effects of imidacloprid [3]. Despite the long-term use of imidacloprid-based pesticides in agriculture, its toxic effects on aquatic animals have not being studied yet.

The goal of this paper is to study the effect of Tanrec on the reproduction and vitality of *Daphnia magna* Straus, 1829 (Daphniidae, Cladocera, Crustacea).

MATERIALS AND METHODS

Tanrec is a water-soluble imidacloprid at a concentration of 200 g/L belonging to the chemical class of neonicotinoids. The ingredients enhancing the activity of the active compound or simplifying the application of the insecticide are indicated neither in the manual nor on the package. The insecticide is registered in Russian Federation and is marketed by the company Avgust.

Biotesting followed the methodical guidelines of the State Committee on Nature Preservation of the Russian Federation [18]. The duration of experiments was 15 days. The parthenogenetic culture of daphnia raised in laboratory conditions was used for the experiments. The groups of 70 genetically uniform daphnias (age <24 h; body length, 0.84 ± 0.014 mm) were placed in four 2.5-L vessels. Settled tap water saturated with oxygen was used in the control. The solutions of insecticide (as of amidocloprid) at concentrations of 3.0×10^{-1} mg/L, 3.0×10^{-2} mg/L (corresponding to Maxima Acceptable Concentration in ambient waters [1]), and 3.0×10^{-7} mg/L were prepared using the same water.

The crustaceans were fed daily with *Chlorella vulgaris* Beyer. cultivated in laboratory conditions. Following 6 days of exposure, the number of survivors was calculated; the body length (from the top of the head to the base of caudal needle) was randomly measured under a binocular microscope at ×8 magnification. General conditions of specimens, as well as the status of intestine and oocytes were analyzed using an Olympus CX31 microscope at a magnification of ×40.

The groups of 30 specimens survived in control and toxicant solutions at concentrations of 3.0×10^{-2} mg/L and 3.0×10^{-7} mg/L and 12 specimens at 3×10^{-1} mg/L were preserved for histological examination. Ten specimens from the solutions with concentrations of 3.0×10^{-2} mg/L and 3.0×10^{-7} mg/L and six specimens from 3×10^{-1} mg/L were individually placed into 250-mL beakers in order to register the first litter in

Changes in the biological parameters of *Daphnia magna* in control and in Tanrec solutions

Toxicant concentration, mg/L	The proportion of surviving specimens by the sixth day of experiment, %	Body length of specimen by the sixth day of the experiment, %	Time of the first hatching of newborns, days
Control	97.1	2.46 ± 0.044	7.8 ± 0.20
3.0×10^{-7}	88.6	2.45 ± 0.052	**9.9 ± 0.28**
3.0×10^{-2}	74.3	**1.91 ± 0.023**	**12.8 ± 0.44**
3.0×10^{-1}	25.6	**1.06 ± 0.032**	–

The parameters significantly different from control (Student's test, $p \leq 0.05$) are given in boldface; (–) no litter produced. The means and their errors are given.

each daphnia. The hatching of the litter was recorded daily.

The effect of the toxicant was assessed by the extent of ovary development (which in *Daphnia magan* stretch as paired tubes along the sides of the intestine and open with short oviducts into the brood chamber [21]) and status of intestine and adipose body. The crustaceans were preserved in a Bouins fixative for one day. The thickness of paraffin cross sections is 7 μm. The mounts were stained with ferrous hematoxylin according to Heidenheim. Polystyrene was used instead of cover glasses according to method proposed by D.S. Sarkisov [15]. The photographs were taken using an Olympus CX31 digital microscope coupled with a JVC TK-C1481BEG video camera. The number of specimens preserved for histological examination was 30 in the control, 22 at a concentration of 3×10^{-7} mg/L, 20 at 3×10^{-2} mg/L, and 4 at 3×10^{-1} mg/L.

RESULTS

Since first days of exposure, the intensive mortality of daphnias was recorded in the insecticide solutions at a concentration of 3.0×10^{-1} mg/L; at concentrations of 3.0×10^{-2} and 3.0×10^{-7} mg/L the mortality was less pronounced. After 6 days of exposure at concentrations of 3.0×10^{-1} mg/L and 3.0×10^{-2} mg/L, the linear sizes of crustaceans were significantly lower than in the control. The coloration of the control specimens was more intensive; the color of crustaceans exposed to toxicant solutions was light gray. The hatching times of first litter in the specimens exposed to 3.0×10^{-2} mg/L and 3.0×10^{-7} mg/L significantly differed from the control. At a concentration of 3.0×10^{-1} mg/L no progeny was produced for 15 days of exposure (see Table).

The effect of the toxicant following 6 days of exposure was assessed by the extent of development of ovaries and the status of intestine and adipose body (Fig. 1). The crustaceans exposed to the toxicant solutions at low concentrations considerably differed from the control specimens. In large number of control crustaceans, brood chambers contained well-developed mobile embryos of the first litter ready for hatching form the brood chamber; in addition, the oocytes were clearly visible in the ovaries (Fig. 1a). In a small number of control animals, the oocytes moved from the ovaries to the brood chamber and the next portion of oocytes start to accumulate yolk (Fig. 1b). The effect of the insecticide on the reproduction of daphnias was noted already at the stage of oogenesis. The ovaries in most specimens exposed to the toxicant at concentrations of 3.0×10^{-7} mg/L contained oocytes at the terminal stage of yolk accumulation (Fig. 1d) ready to move to the brood chamber; the least number of animals contained developing oocytes at the stage of yolk accumulation (Fig. 1e). In solitary specimens the oocytes moved to the brood chamber (Fig. 1e) (individual reaction of specimens). The ovaries in all surviving crustaceans exposed to the insecticide at a concentration of 3.0×10^{-1} mg/L were empty (no oocytes were visible) (Fig. 1j); i.e., in these animals oogenesis was completely blocked. In terms of the development of ovaries, the specimens exposed at concentrations of 3.0×10^{-2} mg/L were intermediate. In most crustaceans only the chain of oocytes that had not accumulated yolk yet was visible (Fig. 1g). In some specimens the eggs of the first litter that transferred to the brood chamber were observed; the next portion of oocytes was not ripened (Fig. 1h). In solitary specimens, the oocytes in the ovaries were not developing at all (Fig. 1i).

In 10 out of 22 specimens exposed to the toxicant solution at a concentration of 3.0×10^{-7} mg/L, oocytes of the older generation disintegrated (Fig. 2e); in others, no torphoplasmatic growth of oocytes (as was observed in the control, Fig. 2b) was observed. In the specimens exposed at concentration of 3×10^{-1} mg/L, the oocytes did not grow and the ovary was represented by oogonia (Fig. 3b).

In the control specimens, the walls of the intestine were clearly pronounced and the intestine was completely filled with food (Fig. 1a–1c); abundant aggregations of the adipose-body cells were noted in the body of animals (Fig. 1c). In the animals exposed to toxicant solutions along with an increase in concentrations, the deformation of the intestine walls, unclear borders (Figs. 1g–1i), and uneven filling of the gut with food (Fig. 1j) were observed. Adipose-body

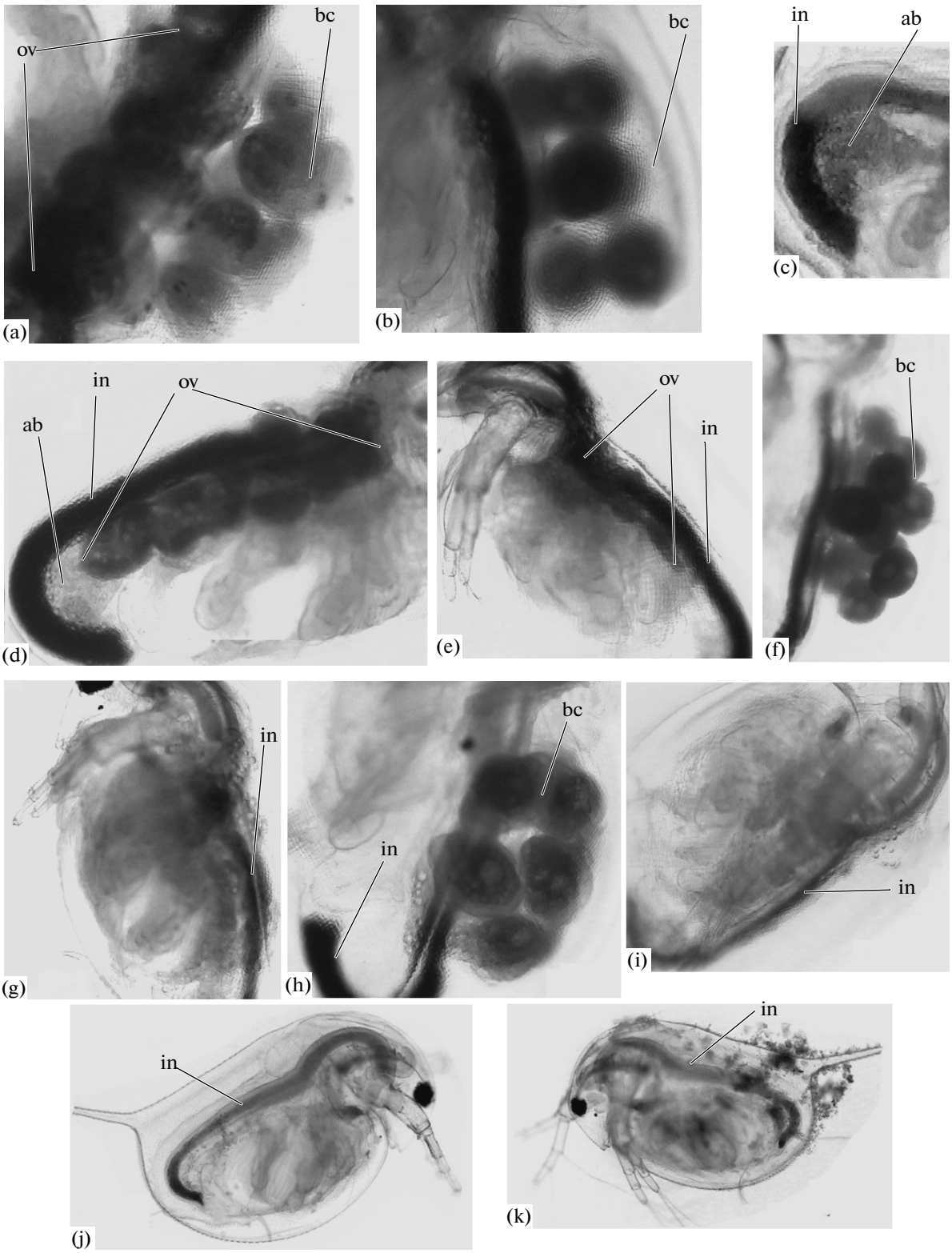

Fig. 1. Changes in the organs of *Daphnia magna* following 6 days of exposure to Tanrec solutions. Insecticide concentration, mg/L: (a–c) control; (d–f) 3.0×10^{-7}; (g–i) 3.0×10^{-2}; (j, k) 3.0×10^{-1}; (ov) ovary; (in) intestine; (bc) brood chamber; and (ab) adipose body.

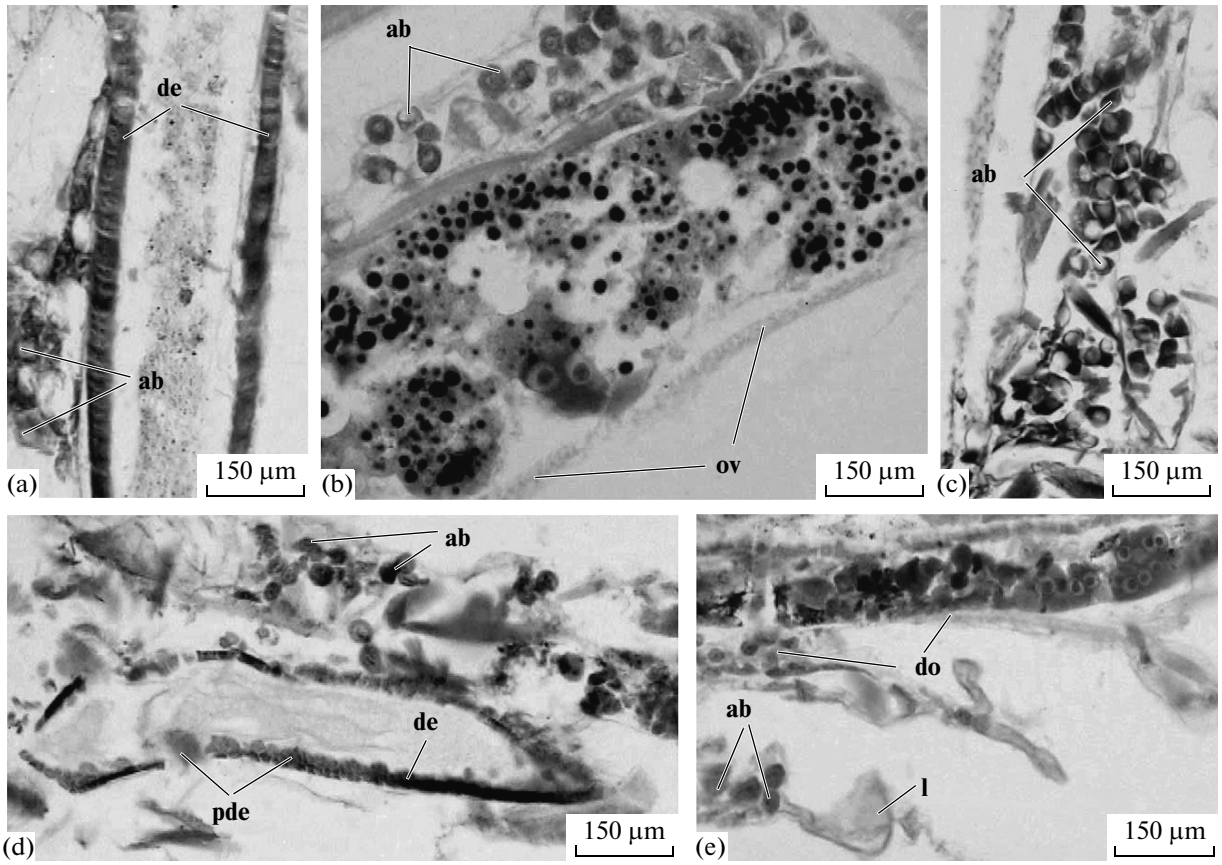

Fig. 2. Region of the tissue from the cross section of the *Daphnia magna* body from control (a–c) and Tanrec solution of 3.0×10^{-7} mg/L (d, e): (a) mesenteron; (b) ovary of the parthenogenetic female and diffuse adipose body; (c) diffuse adipose body; (d) mesenteron, angular cross section; (e) ovary of the parthenogenetic female with disintegrating oocytes of the older generation and diffuse adipose body; (de) digestive epithelium; (pde) diseased thickened portion of digestive epithelia; (do) disintegrating oocytes of older generation; (l) leg. Other designations are the same as in Fig. 1.

cells were present, but were disintegrated and did not form aggregations (Fig. 1d), unlike in control. The walls of the intestine in the crustaceans exposed to the toxicant at a concentration of 3.0×10^{-1} mg/L had a loose structure; there were no adipose body cells (Fig. 1j). Some surviving specimens were covered by algae (Fig. 1i), presumably due to low locomotor activity.

The histological examination of daphnias has shown that the epithelium of mesenteron by the sixth day of exposure to Tanrec at three concentrations (Figs. 2d, 3a, 4a, 4c and 4d) was thinner compared to the control (Fig. 2a). At concentrations of 3.0×10^{-7} mg/L and 3.0×10^{-2} mg/L in some specimens, the thickening of the digestive epithelium (Fig. 2d) related to the swelling of the cells was observed. In some specimens exposed to the toxicant at a concentration of 3.0×10^{-2} mg/L, the delamination of the epithelium from the basal membrane was observed (Figs. 4c, 5). The epithelial cells disintegrated from the basal membrane were in the gut cavity (Fig. 4d). Upon exposure at a concentration of 3.0×10^{-2} mg/L, the digestive epithelium was normal (Fig. 4a). The adipose-body cells in control specimens (Figs. 2a, 2b) and in the animals exposed to the toxicant at concentrations of 3.0×10^{-7} mg/L (Figs. 2d, 2e) and 3.0×10^{-2} mg/L (Figs. 4a–4d) were larger than in the daphnias exposed at concentrations of 3.0×10^{-1} mg/L; cells of daphnias at the magnification used were not visible (Figs. 3a, 3b).

DISCUSSION

The xenobiotics entering ambient waters may both negatively affect the aquatic organisms and stimulate them, influencing various parameters of their vitality, including survival, body size, and fecundity. In some cases the toxicants stimulate the vitality processes [17, 23]; in other cases they suppress [13], damage [19], or even block [12] these processes. For instance, in various regions differing in levels of water trophy and receiving the pulpmill wastewaters (in which sodium lignosulfonate is the main component), the increase in the rate of gain of *Daphnia cristata* Sars. population number was observed. In the experiments, sodium lignosulfonate caused a significant increase in the body sizes and fecundity of *D. magna* [17]. Some

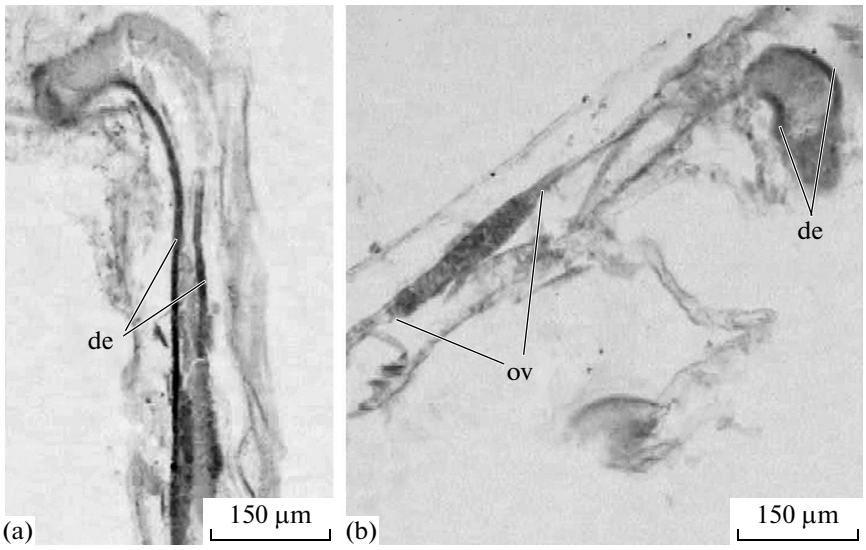

Fig. 3. Cross sections of *Daphnia magna* tissues from Tanrec solutions of 3.0×10^{-1} mg/L: (a) longitudinal cross section of mesenteron; (b) angular. Designations same as in Figs. 1 and 2.

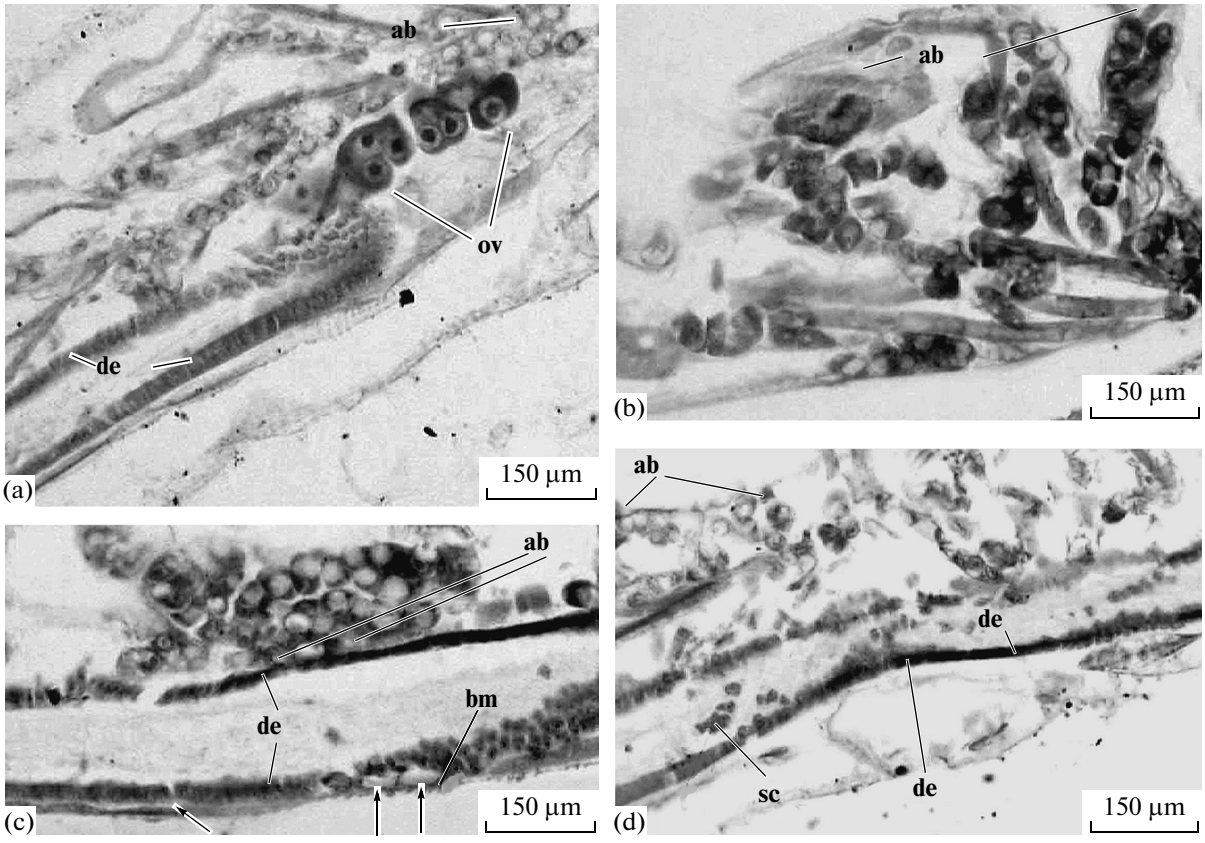

Fig. 4. Region of the tissue from the cross section of *Daphnia magna* body from Tanrec solution of 3.0×10^{-2} mg/L: (a) mesenteron and ovary, angular cross section; (b) diffuse adipose body; (c, d) mesenteron and diffuse adipose body, longitudinal cross section; (bm) basal membrane of mesenteron epithelium; and (sc) cells of digestive epithelium separated from basal membrane. Arrows denote the spaces formed as a result of the delamination of the mesenteron epithelium from basal membrane. Other designations are same as in Figs. 1 and 2.

chemical compounds within a narrow interval of ultralow concentrations affecting the endocrine and immune systems increase the longevity and stimulate reproduction [23]. The solution of the herbicide Roundup at sublethal concentrations of 25 mg/L and 50 mg/L (as glyphosate) negatively affects the reproduction and linear sizes of *D. magna* [13], as well as morphological parameters, which becomes evident starting from the fourth generation and is expressed in the appearance of pathomorphological deviations in the structures of several organ systems of crustaceans [14].

The Tanrec solution at all studied concentrations decreased the intensity of body coloration in exposed animals. A similar effect was observed in experiments with the herbicide Roundup. Both sexually mature specimens and newborns in the toxicant solutions visually appeared discolored compared to the intensively colored control [13]. This phenomenon relates to the sharp decrease in the number of the adipose body cells and in its size. It is believed that the decrease in the adipose body relates to the insufficient amount of food [7]. At the initial stage of adaptation to the intensive external impact, the urgent but imperfect set of protective–compensatory reactions is realized, which makes it possible to sustain the adequate vitality at the expense of the enhanced use of functional reserves [6].

In the present experiment, the amount of food was sufficient. Consequently, the reason for the abovementioned phenomenon is different. Tanrec is characterized by acute contact-intestinal action. The compound actively affects the nervous system of insects, blocking the nicotinergic receptors of the postsynaptic nerve. The toxicant blocks fast the transduction of signals via central nervous system, which, at high toxicant concentrations, results in paralysis and death in a few hours or minutes, depending on the concentration. It is likely that in the experiment the functioning of complex filtering apparatus of daphnias was impaired and the animals received insufficient amounts of food for successful vital activity. As a result of the decrease or vanishing (depending on the concentration) of the adipose-body cells, the accumulation of yolk in the oocytes was either declined or blocked and the growth of crustaceans was either retarded or stopped (3.0×10^{-7} mg/L and 3.0×10^{-2} mg/L). At high concentrations (3.0×10^{-1} mg/L), this resulted in mass mortality. Exposure to imidacloprid at 3.0×10^{-2} mg/L (corresponding to the maximal acceptable concentration in ambient waters) was not completely harmless for daphnias, because the first hatching of newborns at this concentration took place on average 5 days later than in the control. The specimens had smaller body sizes and light gray bodies, also indicating the unfavorable state of the animals.

The histological examination has shown that exposure of daphnias to Tanrec triggered both the protective reaction of normal ontogenesis and a pathological response. The normal ontogenesis reactions were

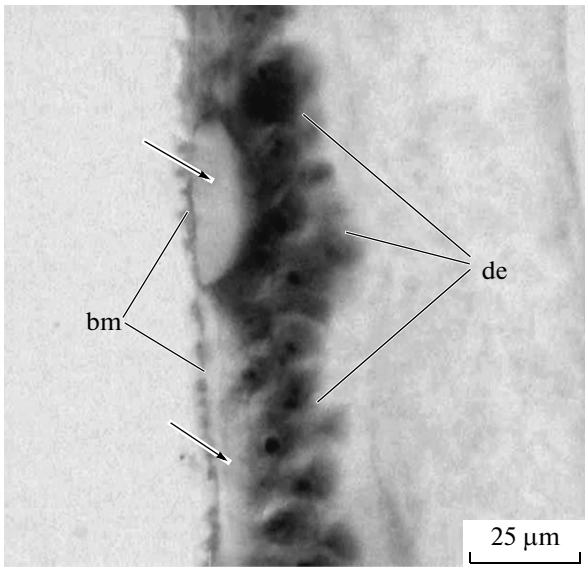

Fig. 5. Region of mesenteron at the initial stage of delamination of mesenteron epithelium from basal membrane. Designations are same as in Figs. 2 and 4.

those providing healthy daphnias with the ability to adapt to a shortage of food in the natural environment. Sudden food deficiency results in a deficit of energy for vital functions of crustaceans. To sustain the energetic balance of the organism, they limit the energy expenditures and use the internal sources of the energy. The decrease in the energy expenditures is provided by the retardation or stopping of oogenesis; the use of internal energy sources comes from the resorption of oocytes and shrinking of the cells of the mesenteron epithelium and adipose body. The yolk of the resorbed oocytes and the cytoplasm of the shrinking cells become sources of additional energy. By impairing the metabolism in daphnias, exposure to Tanrec at sufficient amounts of food resulted in a shortage of the energy necessary for life. In response to this shortage, the oogenesis either retarded or stopped; the oocytes resorbed and the epithelium cells of mesenteron and adipose body shrank.

In contrary to the responses of normal ontogenesis, the pathological reactions are atavistic reactions. Using these reactions, the remote ancestors of daphnias sustained the energetic balance of the organism during normal ontogenesis. If an organism is unable to adapt to environmental changes by the responses of normal ontogenesis, it uses the pathological reactions. The price of the use of the latter is much higher than the price of the reactions of normal ontogenesis [11]. The erosion of digestive epithelium was a pathological reaction of daphnias. This phenomenon is described in the cladocerans exposed to various toxicants in laboratory conditions [8, 20, 22] and inhabiting the polluted waters of the Rybinsk Reservoir and Gulf of Fin-

land [9, 10]. The pathological reactions are also adaptive [2]. The destruction of the digestive epythelium is a common reaction of many metazoans to impairments of various natures. It is not clear yet how or why this reaction appeared during the process of evolution.

CONCLUSIONS

The insecticide Tanrec in solutions with concentration of 3.0×10^{-1} mg/L (as of imidacloprid) acutely toxically affects *Daphnia magna*; at concentrations of 3.0×10^{-2} mg/L and 3.0×10^{-7} mg/L, the effect is suppressed. The latter is expressed in a decrease or vanishing of vacuoles in the adipose-body cells, the destruction of oocytes, the blocking of ooegenesis, and a retardation in the growth rate of crustaceans during early ontogenesis. Similar reactions are observed upon a shortage of food. Histological examination has shown that exposure to Tanrec erodes the epithelium of mesenteron in daphnias.

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