TOXICITY OF DERMALLY APPLIED ALPHA-CYPERMETHRIN IN RATS

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Abstract: The aim of the study was to assess the immunotoxic effect of dermally
applied alpha-cypermethrin in rats based on phagocytic and bactericidal activity of
neutrophils of peripheral blood, and the general toxic effect based on histological and
ultrastructural examination of internal organs. The preparation was dermally applied in
doses of 50 mg/kg and 250 mg/kg. It was administered to the tail skin of female Wistar
rats, 4 hours daily for 28 days. After the experiment, the animals were anaesthetized and
heart blood was taken in order to evaluate the activity of granulocyte system. The
following organs were taken for histological examinations: brain, lung, heart, liver,
spleen, kidneys, thymus and lymphatic nodes. Lung, liver, kidney and heart were used
for ultrastructural studies. The results of the study showed that bactericidal and
phagocytic activity of neutrophils was stimulated after administration of 50 mg/kg
alpha-cypermethrin. Dermal application of the preparation resulted in slight histological
changes in liver, kidney, lung and brain. Pathological changes in heart were observed
only on the level of ultrastructure.

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INTRODUCTION

Pyrethroids are highly active insecticides in considerably
lower quantities (up to 100 kg/ha), compared to other
insecticides. Therefore, their contribution to the contamination
of the natural environment is smaller than that caused by
other groups of pesticides [14]. Good solubility of
pyrethroids in fats facilitates their absorption, spread in an
organism and penetration to the nervous tissue. Pyrethroid
insecticides show neurotoxic effect which is manifested
by an increased excitatory effect of central and peripheral
nervous systems [3, 11, 22, 32].

Acute toxic effect in humans and animals is relatively
small. Low toxicity for mammals is explained by their swift
biotransformation and discharge from the organism in the
form of non-active metabolites, mostly in the urine [15].

The compounds of this group show allergic and immuno-
suppressive effects [4, 5, 7, 10, 14, 16, 17, 18, 19, 20, 24, 27].
Hoellinger *et al.* [7] described a highly sensitizing effect
of bioresmethrin, cismethrin, and deltamethrin. Allergic reac-
tions were observed among workers exposed to fenvalerate
and permethrin [10]. The mitogenic response of lymphocytes
stimulated by concavalin A and lipopolysaccharides was
inhibited by allethrin, cypermethrin and permethrin [27].

Alpha-cypermethrin is an active pyrethroid which
intensively controls a wide range of pests in agriculture
and animal breeding. It is available on the market in the
form of a concentrate in suspension, or mixed with other
insecticides (Nurella EC, Cymbush, Cyperkill, Mastor,
Politrin, Ripcord, Sherpa).

Feeding rats with diets containing 200 and 180 mg/kg
body mass for 5 and 13 weeks caused neither clear

histopathological nor hematologic changes. Higher doses led to the symptoms of poisoning associated with changes in the nervous system, decrease of the body mass or increase in the mass of liver and kidneys. Similar results were obtained in the studies in dogs [34].

Cypermethrin administered to female rats during pregnancy and lactation periods resulted in changes on the level of cerebral neurotransmitter in infants and might cause a delay in brain development [2, 21]. Sensitivity of infant rats to the toxic effect of cypermethrin is significantly higher, compared to adult rats [2]. Cypermethrin administered per os to adult rats, unlike permethrin, increases acetylcholinesterase activity in all regions of the brain (cortex cerebri, cerebellum, striatum, hippocampus, hypothalamus) [25]. Cypermethrin induces chromosome aberrations and the exchange of sister chromatids *in vivo* in the spleen and bone marrow in mice, and *in vitro* in cell culture [1]. Single epidermal LD_{50} doses of alpha-cypermethrin for mice and rats range from 100–500 mg/kg body mass.

Human dermal exposure to alpha-cypermethrin occurs during such activities as preparation of the working solution, spraying and washing of equipment. No metabolites of alpha-cypermethrin were observed in the urine of people exposed, and only slight dermal changes were noted [34].

Dermal absorption of pesticides is especially important in occupational poisonings. The most frequently reported symptoms of human occupational exposure consist of paraesthesia of the skin, which is considered to be a result of repetetive firing of sensory nerve endings and should be a warning signal for overexposure. Systemic signs of poisoning affect mainly the CNS in the form of dizziness, headache, disturbance of consciousness, muscular fasciculation, convulsive attacks and coma. Poisonings were due to inappropriate handling with pyrethroids [24].

Le Quesne *et al.* [13] described transient facial sensory symptoms among 23 workers exposed to cypermethrin, fenopathrin, fenvalerate, and permethrin in the laboratory or in field trials. Sensations were noted as tingling, burning, like "coming in from the cold", nettle rash or sunburn. There were no abnormal neurological signs.

He *et al.* [6] reviewed 573 cases of acute pyrethroid poisoning, including 344 cases of accidental and 229 cases of occupational poisoning, reported in Chinese medical literature during 1983-1988. Most of the cases of poisoning were caused by deltamethrin (167 accidental, 158 occupational) followed by fenvalerate (133 accidental, 63 occupational) and cypermethrin (39 accidental, 6 occupational).

The study by Wachowiak *et al.* [33] shows that the best method of investigating dermal absorption is the Massman tail method, which is connected with the more intense blood supply in the tail skin than in the skin of the dorsum. They concluded that the absorptive properties of p-toluidine from the water solution are 95 times higher in tail than in patch method. In the case of administration of the compound in ointment, the absorptive properties in tail method were twice as high.

Dermal uptake is strongly dependent on the carrier solvent. Rat epidermal membranes *in vitro* were more than 20 times more permeable to cypermethrin than human epidermal membranes, indicating that cypermethrin would be less readily absorbed in humans then in rats. *In vivo* only 1% of the applied ^{14}C-cypermethrin was absorbed following 8 hr application of 3 ml of the concentrate formulation through the skin of rats [26].

In available literature concerning immunotoxicity of alpha-cypermethrin, there are only few data pertaining to the effect of this preparation on leukocytes of the peripheral blood.

Neutrophils constitute one of the defence elements of the body and are of great importance for immunological response. Biological activity of granulocytes directed against antigens consists of several phases: chemotaxis, phagocytosis and intracellular distribution of phagocytised material. An increase in the bactericidal activity of neutrophils is stimulated mainly by the oxygen-dependent system of myeloperoxidase. In the presence of oxygen-independent mechanisms the bactericidal activity of granulocytes significantly decreases. The defence activity of granulocytes may be changeable due to intracellular lesions of enzymatic mechanisms caused by various factors [8, 9].

The aim of this study was to evaluate the effect of alpha-cypermethrin on phagocytic and bactericidal activity of neutrophils in peripheral blood, and to assess the toxicity of the preparation based on histological and ultrastructural examinations of selected organs.

MATERIALS AND METHODS

Alpha cypermethrin [(R)-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate] - produced by the Chemical Plant in Jaworzno, Poland, was used in the study. The acute toxic epidermal LD_{50} dose for rats is 500 mg/kg body mass (b.m.). The liquid was applied in the form of 20% water-alcohol solution. Alpha-cypermethrin for dermal application was used in doses: 50 mg/kg b.m.- 1/10 LD_{50} and 250 mg/kg b.m.- 1/2 LD_{50}.

The study was conducted on female Wistar rats aged 3 months, in good condition, with no microscopically detected changes of the tail skin. The animals were fed with standard feed LSM [12] and watered *ad libitum*. The initial body mass of the rats was 200 ± 20g.

Experiments were conducted on 3 groups of rats, 10 animals in each. Experimental groups received dermally 50 mg/kg b.m. or 250 mg/kg b.m.of alpha-cypermethrin for 4 weeks, except for Saturdays and Sundays.

The examined preparation was applied on the tail skin of rats with the use of absorptive fabric FPP-15, isolated from the environment with aluminium foil [29]. The time of exposure was 4 hours daily. The animals of the control group were exposed to the dermal absorption of the

Table 1. Results of the nitroblue-tetrazolium reduction test (NBT) and of the phagocytosis latex test (PLT) in rats exposed to dermal absorption of alpha-cypermethrin.

	N	Index of NBT	Index of PLT
Rats exposed to 50 mg/kg b.m.	10	17.2 ± 8.1[*]	81.8 ± 8.9[**]
Rats exposed to 250 mg/kg b.m.	8	15.9 ± 8.6	65.5 ± 7.5
Control group	10	12.1 ± 3.9	69.2 ± 6.6

Values are mean ± SEM (standard error of the mean); [*]$p < 0.05$, [**]$p < 0.01$ compared to control group.

solvent at the same time and under the same conditions. During the entire experiment, the body mass of all rats was controlled once a week.

After 28 days of the experiment, the animals were anaesthetized and blood taken from the heart in order to evaluate the activity of granulocytic system. For evaluation of phagocytic properties of neutrophils, phagocytic reaction with Bacto-Latex (Difco, USA) was conducted [28]. The bactericidal activity of neutrophils was investigated by nitroblue-tetrazolium test (NBT) [23]. In both tests full peripheral blood was used. In each test, 100 cells were counted. In phagocytic test, the cells which contained at least 3 latex grains were considered as positive. In NBT test, cells containing formazan deposits of at least the size of one lobe of nucleus were recorded as positive. The numbers of positive cells per 100 cells analysed were determined as indices of the tests applied.

The following organs were taken for histological examinations: lung, liver, heart, kidney, brain, thymus, spleen and lymphatic nodes. The brain was perfused with a solution of methanol, formalin and glacial acetic acid, embedded in paraffine and dyed by Nissel method [35]. The remaining organs were fixed in 10% neutral buffered formalin, embedded in paraffine and dyed with H+E.

For ultrastructural studies, heart, liver, kidney and lung were taken. The material was fixed in 4% glutaraldehyde buffered to pH of 7.2-7.4, with 0.1 M sodium cacodylate, and postfixed with 1% water solution of OsO_4. Dehydration was carried out by ethyl alcohol in a concentration up to absolute. The material was embedded in Epon 812. Ultrathin specimens were observed and photographs taken by Tesla BS 500 electron microscope.

The results were presented as mean ($\bar{x}$) ± standard error (SEM) and subjected to statistical analysis by the parametric t-Student test.

RESULTS

Body mass, general condition. During 4 weeks of observations, no clear differences were observed in the increase of the body mass between the experimental groups exposed to alpha-cypermethrin absorption and the control group. No clinical signs of toxic effects were observed in the rats exposed to alpha-cypermettrin.
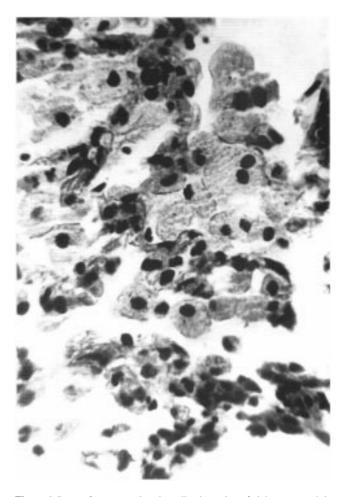

Figure 1. Lung of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Presence of macrophages in the alveolar septa. H-E, × 160.

Study of immunotoxicity. The results are shown in Table 1. Alpha-cypermethrin dermally applied in the dose of 50 mg/kg b.m. (1/10 LD$_{50}$) caused a significant increase in the phagocytic activity of neutrophils (p<0.01).

After administration of 50 mg/kg b.m. (1/10 LD$_{50}$) of alpha-cypermethrin an elevated bactericidal activity of neutrophils was noted which significantly differed from that in the control group (p<0.05). An exposure to a higher dose of the preparation induced only a slight increase in the bactericidal activity.

Histological and ultrastructural studies. No gross-morphology changes of any internal organ were observed during necropsy. No histological changes were observed in the spleen, thymus and lymphatic nodes.

The changes in the lung covered very small areas and were manifested by the widening of interalveolar septa and the presence of lung macrophages (Fig. 1). In submicroscopic studies, changes in the lung covered the thinning of the endothelium of capillary vessels and its detachment from the basal lamina in some sections (Fig. 2). After the administration of a higher dose of the preparation, apart from the above-mentioned changes, microtraumas in the pneumocytes type I were observed.
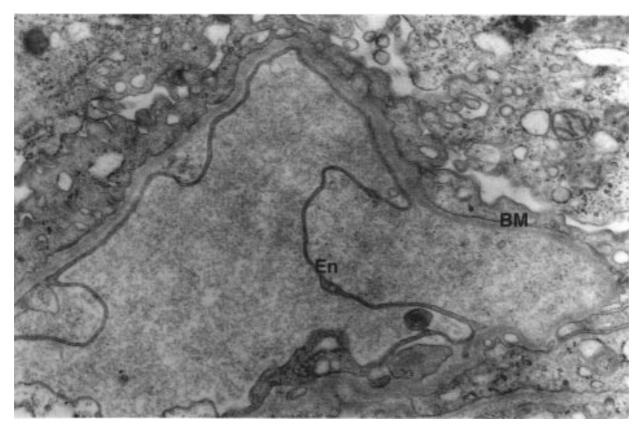
Figure 2. Lung of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg).Thinned endothelium (En) of a capillary vessel of the lung and its detachment from the basal membrane (BM). EM, × 20 000.
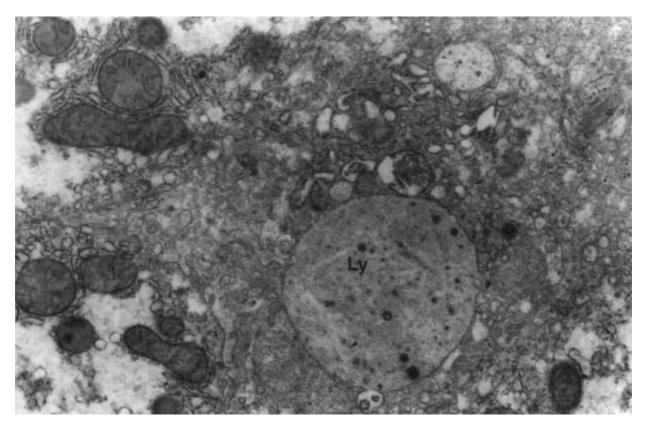

Figure 3. Liver of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Secondary lysosomes (Ly) of varying size and internal structure in hepatocytes. EM, × 15 000.
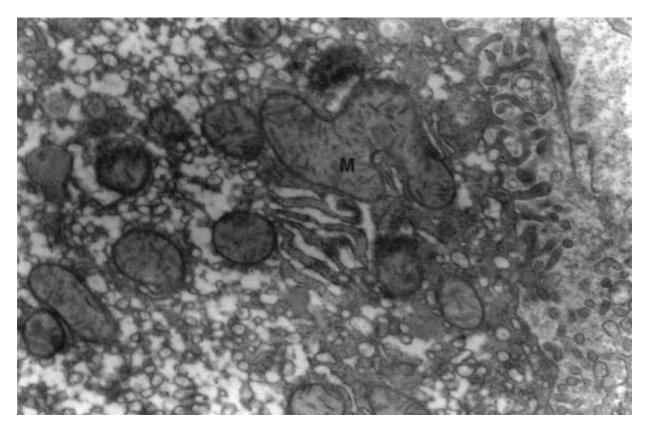
Figure 4. Liver of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Mitochondria (M) in hepatocytes enlarged and of irregular shape. EM, × 20 000.
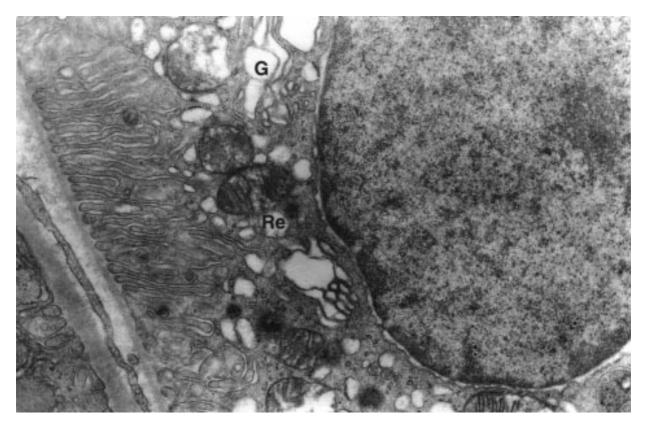

Figure 5. Kidney of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Widening of endoplasmic reticulum (Re) and Golgi apparatus (G) in the epithelial cells of proximal tubule. EM, × 20 000.
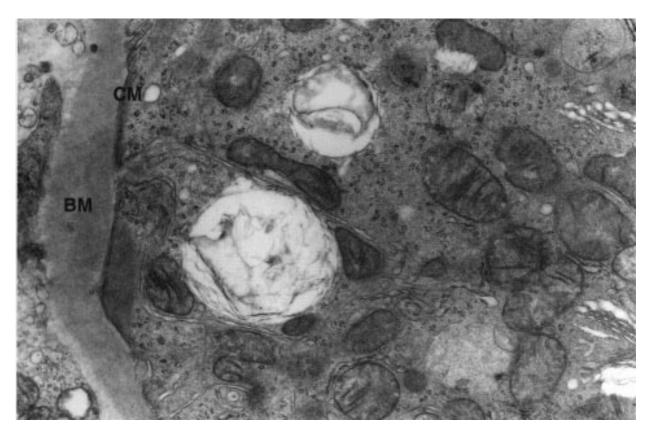
Figure 6. Kidney of rat exposed to dermally absorption of alpha-cypermathrin (250 mg/kg). The lack of invagination of the cell membrane (CM) and thickening of the basal membrane (BM) of proximal tubule. EM, $\times$ 20 000.

Changes in the liver occurred only after administration of a higher dose of the preparation (250 mg/kg b.m.) and were confined to the increased porosity of the cytoplasm of hepatocytes situated under the capsule of the organ (Fig. 7). Submicroscopic changes were manifested by an increase in the quantity of electron dense bodies which occurred primarily in the form of secondary lysosomes of varying size and internal structure (Fig. 3). After administration of a higher dose of alpha-cypermethrin, changes were observed mainly in endoplasmic reticulum. In the majority of hepatocytes a widening of the tubuli of rough endoplasmic reticulum, and in some cases, also the overgrowth of smooth endoplasmic reticulum was noted. Some mitochondria were enlarged and of irregular shape (Fig. 4).

No changes were observed in kidney after the administration of a lower dose of the preparation, whereas a higher dose caused parenchymatous degeneration in single cells in the proximal tubuli. Ultrastructural studies showed widening of endoplasmic reticulum and Golgi apparatus in the epithelial cells of proximal tubuli (Fig. 5). In these cells, swollen mitochondria with brightened matrix were noted. In some cells, large, electron light structures filled with a small quantity of membranous material were present. Sporadically, the lack of invagination of the cell membrane, as well as thickening of the basal lamina of proximal tubules, were observed (Fig. 6). After administration of 1/2 LD_{50} (250 mg/kg b.m.) of alpha-cypermethrin, the above-mentioned changes were considerably greater.

In the heart muscle no changes were noted on the level of light microscope, whereas in ultrastructural studies an oedema and enlightment of the matrix in some mitochondria were observed after the administration of a higher dose of alpha-cypermethrin.

In the brain, after administration of 250 mg/kg b.m. of alpha-cypermethrin for 4 weeks, there occurred a focal concentration of the neurocytes' cytoplasm of the stratum granulosum as well as of single neurocytes of the CA 3 hippocampus layer, and of neurocytes in the hypothalamus and in the cortex cerebrum. In addition, a pyknosis of the Purkinje cells was noted in the cerebellum, and some of these cells disappeared (Fig. 8).

DISCUSSION

Studies by Tulinska *et al*. [31] showed that supercypermethrin administered intragastrically caused an elevated humoral and cellular response in Wistar rats in the subacute experiment (28 days) after administration of 1/40 LD_{50} of the preparation, while higher doses (1/20 and 1/14 LD_{50}) had a suppressive effect. Other studies showed that alpha-cypermethrin administered per os in rats in the 28-day experiment slightly affected the immunological system. Desi *et al*. [4] described the suppressive effect of orally applied cypermethrine on the humoral and cellular immunological response in rats and rabbits.
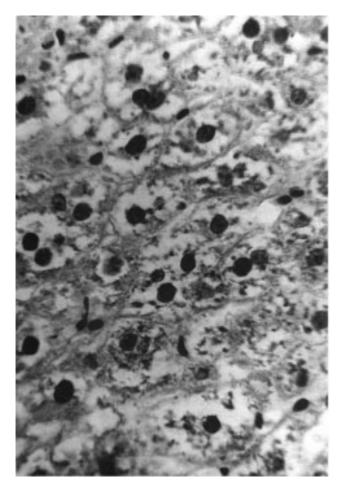
Figure 7. Liver of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Increased porosity of the cytoplasm hepatocytes. H-E, × 160.

Figure 8. Brain of rat exposed to dermally absorption of alpha-cypermethrin (250 mg/kg). Focal concentration of the neurocytes cytoplasm of the CA 3 hippocampus layer. Dyed according to Nissel method, × 160.

In our studies, alpha-cypermethrin applied dermally in lower doses (50 mg/kg b.m.) for 28 days caused a significant increase in the bactericidal and phagocytic activity of neutrophils. This is an evidence of the stimulation of the receptor system of these cells. A higher dose (250 mg/kg b.m.) did not cause a significant change compared to the control group.

Materials by the WHO group [34] concerning the studies of alpha-cypermethrin indicated that this preparation administered in the diet in various doses and over various periods of time led to the symptoms of poisoning associated with changes in the nervous system. High doses caused a decrease in the liver and kidney mass. Based on our studies, alpha-cypermethrin applied dermally, especially in high dose (250 mg/kg b.m.), caused slight histological changes in the lung, liver, kidney, heart and brain. This was probably due to the small amount of pyrethroid which penetrated the skin, responding to about only 1% of dermally applied dose.

Toukhy and Girgis [30] described an inhibitory effect of cypermethrin on the activity of the total ATP-ase in rat liver, which may disturb the active transport of Na+, K+ and Mg+ ions and result in pathological changes in liver cells. In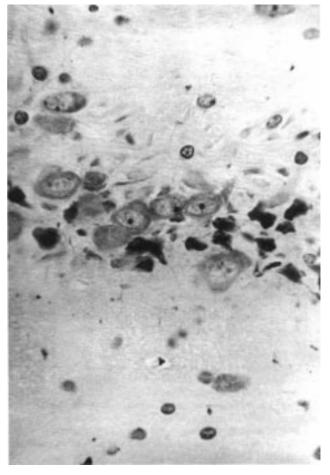 our studies an increased porosity of cytoplasm of some liver cells was observed. In the majority of hepatocytes the tubules of the rough endoplasmic reticulum were widened, and in some of them the overgrowth of the smooth endoplasmic reticulum was noted. Changes were observed also in mitochondria.

Low toxicity of pyrethroid insecticides for mammals is explained by their rapid biotransformation and discharge in urine. Studies of cypermethrin effect on the renal function and metabolism by Łukowicz-Ratajczak and Krechniak [15] showed that cypermethrin applied intragastrically had no nephrotoxic effect. The changes observed in our studies concerned parenchymatous degeneration of single cells in renal proximal tubules. Ultrastructural studies showed in these cells swollen mitochondria, widened endoplasmic reticulum and widened Golgi apparatus. A considerable number of autophagocytic vacuoli was noted.

Pyrethroid insecticides may show neurotoxic properties. Cypermethrin administered per os increases the activity of acetylcholinesterase in the cerebral cortex, cerebellum, striatum, hypothalamus and in the area of the hippocampus [25]. The present studies showed changes concerning the Purkinje cells in the cerebellum, concentrations of the cytoplasm of single pyramidal cells of CA3 hippocampus

layer, and a focal pyknosis of the neurocytes of nuclei lateralis hypothalami and the cerebral cortex

CONCLUSIONS

1. Alpha-cypermethrin exerted a stimulatory effect on the phagocytic activity of neutrophils after dermal application of a lower dose (50 mg/kg b.m.) of the preparation.

2. The bactericidal activity of neutrophils increased after dermal administration of 50 mg/kg b.m. of alpha-cypermethrin.

3. Slight histopathological changes were observed in the brain, liver, kidney and lung of the rats exposed to higher dose (250 mg/kg b.m.) of alpha-cypermetrin.

4. Ultrastructural changes occurred in the liver, kidney, lung and heart of the rats exposed to both doses of alpha-cypermethrin.

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