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DERMAL AND ORAL TOXICITY OF MALATHION IN RATS

Sabina Toś-Luty, Daniela Obuchowska-Przebirowska, Jadwiga Latuszyńska, Małgorzata Tokarska-Rodak, Agnieszka Haratym-Maj

Department of Pathomorphology, Institute of Agricultural Medicine, Lublin, Poland

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Abstract: The aim of the study was the evaluation of dermal and oral toxicity of malathion based on the results of histopathologic and ultrastructural tests. The standard of the pesticide - IPO 460 Malathion was used in the study. The preparation was suspended in oil emulsion. The study was conducted on Wistar rats. Dermal toxicity was examined in 2 groups of experimental rats. The animals were applied 8 mg (1/100 LD_{50}) and 16 mg (1/50 LD_{50}) of the preparation on the tail skin for 4 hours daily for a period of 28 days. In the case of oral toxicity, a dose of 1/50 LD_{50} malathion was used. The amount of 1 ml (11.2g) of the preparation was administered intragastrically by stomach tube for 28 days. In both experiments the control animals were administered only the emulsion used for suspending the pesticide. The following organs were subject to histopathologic and ultrastructural evaluation: liver, kidneys, heart and lungs. The histopathologic and ultrastructural changes observed showed various degrees of intensity according to the route of malathion administration and the size of the dose applied. Dermal application of the pesticide in a smaller dose did not cause histopathological changes in the organs of the animals, while the administration of a higher dose resulted in changes only in the liver. Changes on the ultrastructural level occurred in all organs and were dose-dependent. After oral administration of malathion, both histopathologic and ultrastructural changes observed in all organs were more intensified than after dermal application.

Address for correspondence: Prof. Sabina Toś-Luty, PhD, Head of the Department of Pathomorphology, Institute of Agricultural Medicine, P.O. Box 185, 20-950 Lublin.

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INTRODUCTION

Organophosphorous pesticides show neurotoxic effects directly associated with cholinesterase inactivation [5]. They also possess mutagenic and carcinogenic properties and show organ-specific toxicity with relation to the heart, kidneys and other organs [2, 3, 7].

Malathion is an insecticide of the group of organophosphorus pesticides showing strong insecticidal properties accompanied by low toxicity for vertebrates [5]. Despite the commonly accepted small hazardous effect of this preparation for mammals (hence the wide application of malathion, even for therapeutic purposes - anti-pediculosis preparations) there are scientific reports concerning its harmful effect. Cholinesterase inhibition in rats' blood and inhibition of this enzyme, as well as its genotoxic effect in mice fed with cereals treated with malathion was confirmed [1, 8, 11].

The metabolism of malathion in bodies of vertebrates and invertebrates is complex. As a result of metabolic changes, with the contribution of phosphatases and carboxyesterases, many metabolites are produced (malaoxon) of varied toxicity, which may be reflected by the level of cholinesterase inhibition.

Studies with the use of 14C malathion showed an existing relationship between its distribution in the internal organs

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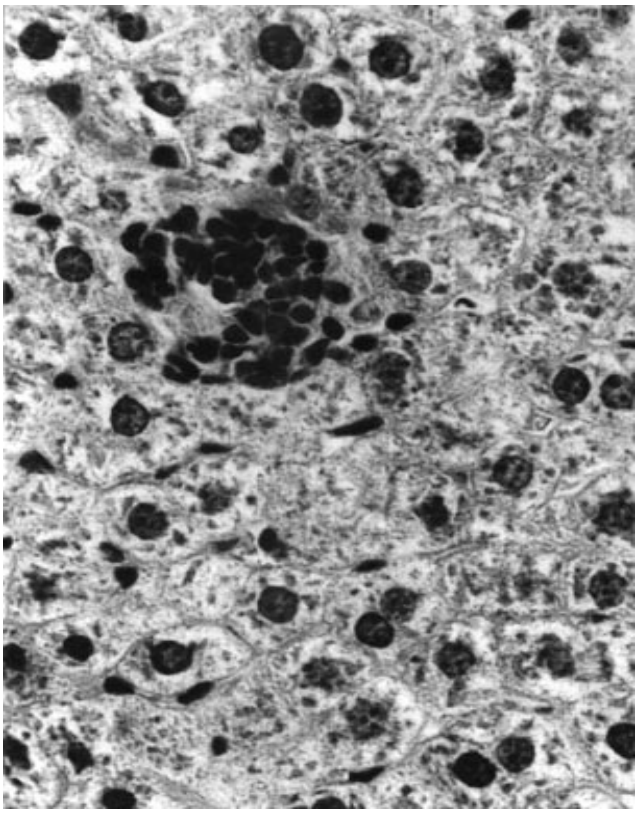

Figure 1. Liver of rat after dermally absorption of malathion (1/50 LD_{50}). Parenchymatous degeneration of hepatocytes with slight infiltration. H+E, × 160.

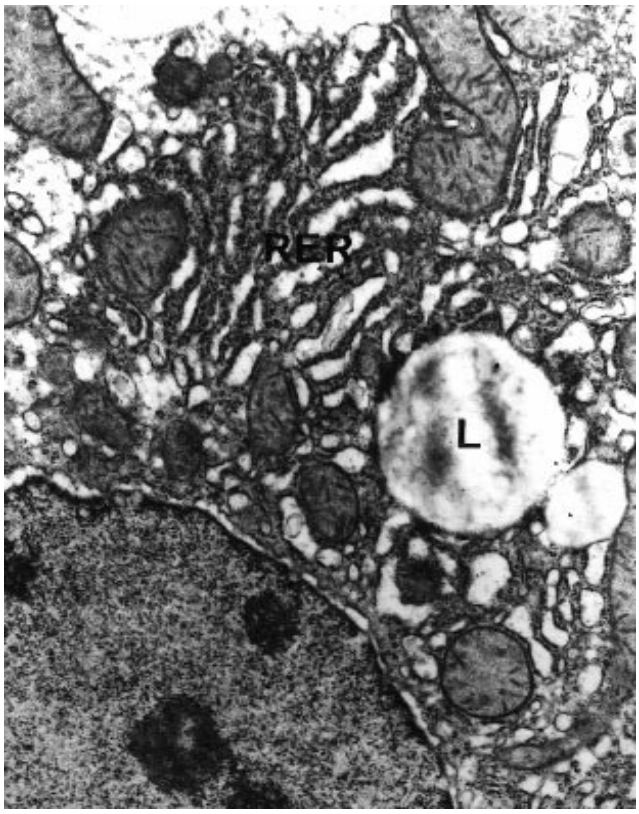

Figure 2. Liver of rat after dermally absorption of malathion (1/50 LD_{50}). Dilation of cisternae of the ergastoplasma (RER) and normal lipid droplet (L). EM, × 10 000.

and the route of administration. The greatest accumulation of the pesticide in the liver and kidneys was noted after intravenous administration, followed by an oral and dermal application [6].

Subcellular examinations with the use of 14C malathion administered *per os* showed its highest activity in the cytosol of liver cells and excretion mainly through the kidneys, followed by the lungs and intestines [10].

MATERIALS AND METHODS

Pesticide standard - IPO 460 Malathion 99% was applied (Prochem Co. Ltd., Warsaw, Poland). The preparation was suspended in an emulsion of arabic gum, olive oil and water at the proportion 1:2:1.5.

Studies were conducted on Wistar rats; at the beginning of the experiment the body weight of animals ranged from 200–230 gm. The rats were fed with standard feed LSM [4] and provided with water *ad libitum*.

Studies of dermal toxicity were carried out on 2 experimental groups, 10 animals in each group. The preparation was applied on the tail skin of rats of surface area 9 cm^2, in the doses of 8 mg (1/100 LD_{50}) and 16 mg (1/50 LD_{50}) for 4 hours daily for a period of 28 days. During the period analogous to the experimental, rats of the control group (5 animals) were applied only the emulsion used for suspending the pesticide.

In order to investigate oral toxicity a dose of 1/50 LD_{50} malathion was used. The preparation was administered intragastrically by stomach tube in the amount of 1 ml containing 11.2 mg malathion, daily for 28 days. The animals of the control group (10 rats) were administered intragastrically only the emulsion used for suspending the pesticide. For the whole period of the experiment, body mass of the rats was controlled at weekly intervals.

For histologic and ultrastructural studies, the liver, kidneys, heart and lungs were taken. The organs for histological examinations were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with H+E. For ultrastructural studies, organs were fixed in 5% glutaraldehyde buffered to a pH 7.2-7.4 with 0.1 sodium cacodylate, and post-fixed with 1% solution O_s0_4 in water. The material was embedded in Poly/Bed 812 medium (Polysciences, Inc., Warrington, PA, USA). Ultrathin sections were observed and photographs taken using a Tesla BS 500 electron microscope.

RESULTS

Evaluation of dermal toxicity of malathion. In histopathological studies, after the dermal application regressive changes were observed in the liver only after the administration of a higher dose of malathion (1/50 LD_{50}). These changes occurred in the livers of 10% of animals in the form of parenchymatous degeneration in hepatocytes (Fig. 1).

Examinations of specimens of the liver by an electron microscope showed widening of ergastopasma tubules and slightly swollen mitochondria in hepatocytes after

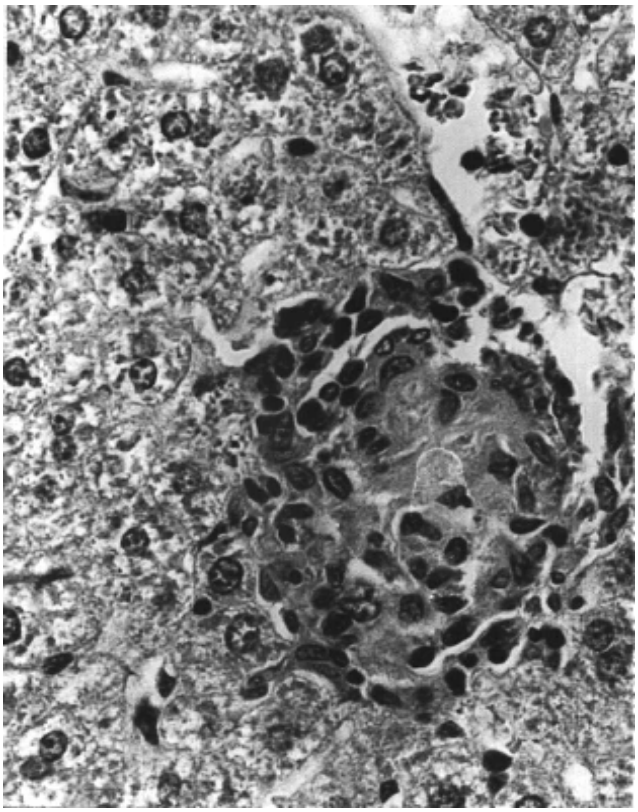

Figure 3. Liver of rat after *per os* application of malathion (1/50 LD_{50}). Parenchymatous degeneration of hepatocytes and fine infiltration between hepatocytes. H+E, × 160.

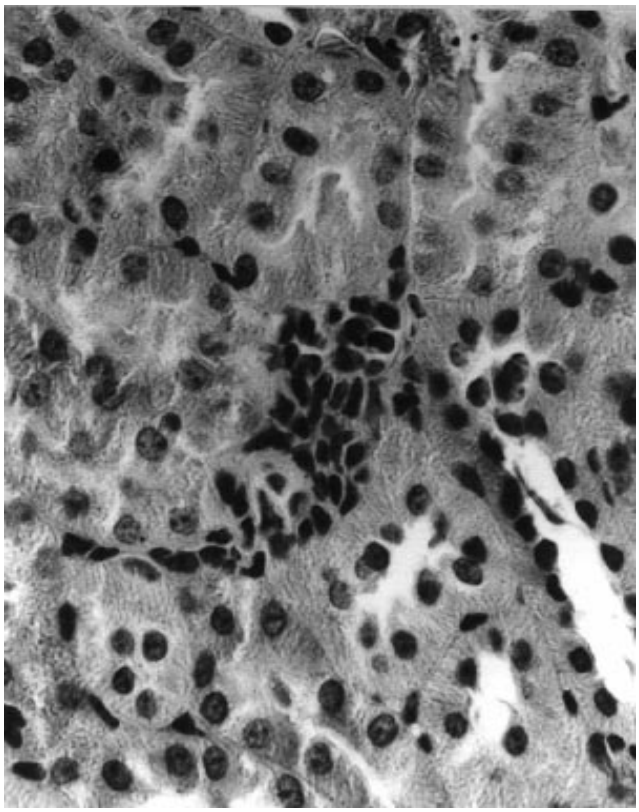

Figure 5. Kidney of rat after *per os* application of malathion (1/50 LD_{50}). Parenchymatous degeneration of cells of renal tubules and infiltration between the proximal tubules. H+E, × 160.

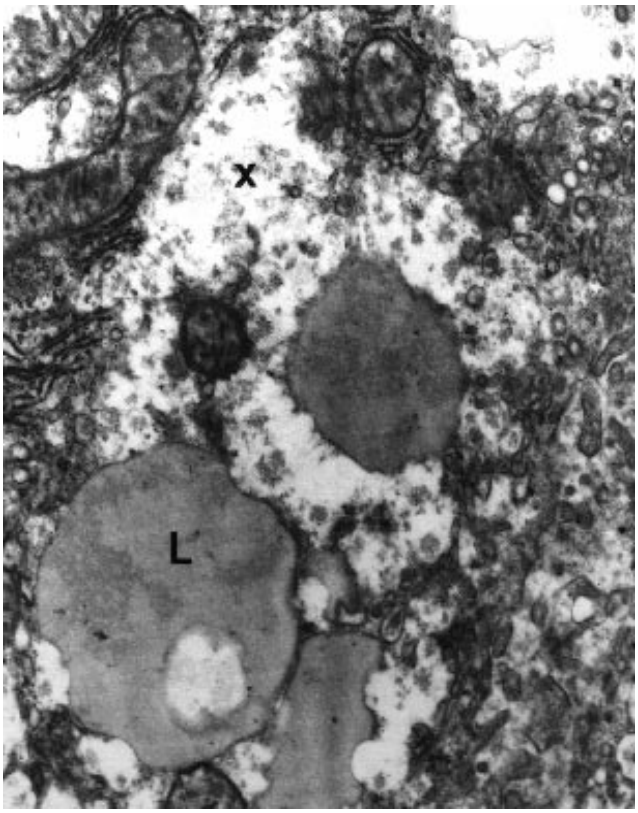

Figure 4. Liver of rat after *per os* application of malathion (1/50 LD_{50}). Lucent area of cytoplasm consist of residual organelles (X) and lipid-vacuoles (L). EM, × 12 000.

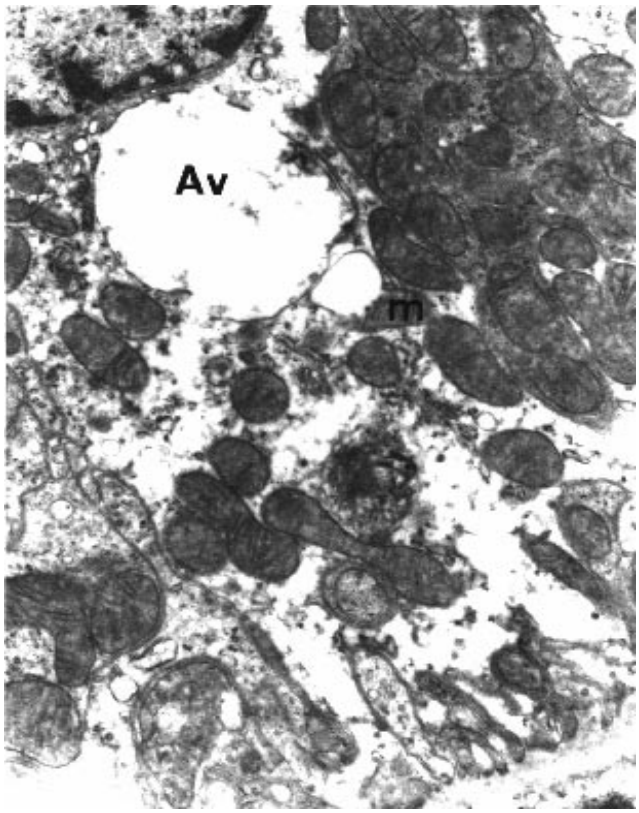

Figure 6. Kidney of rat after *per os* application of malathion (1/50 LD_{50}). Autophagic vacuoles shows disruption of membrane (Av) and swollen mitochondria (M). EM, × 10 000.

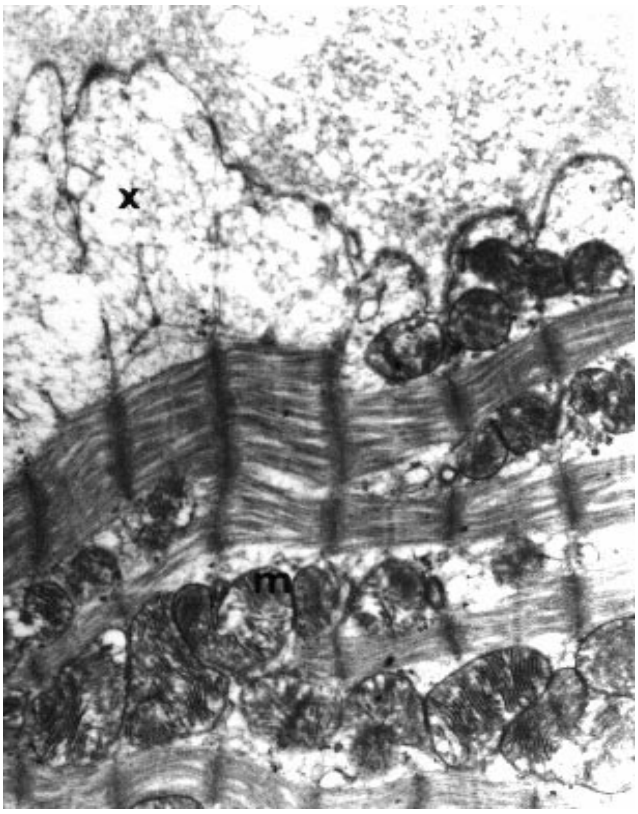

Figure 7. Heart of rat after *per os* application of malathion (1/50 LD_{50}). Swelling of sarcoplasma (X) and mitochondria (M). EM, × 8 000.

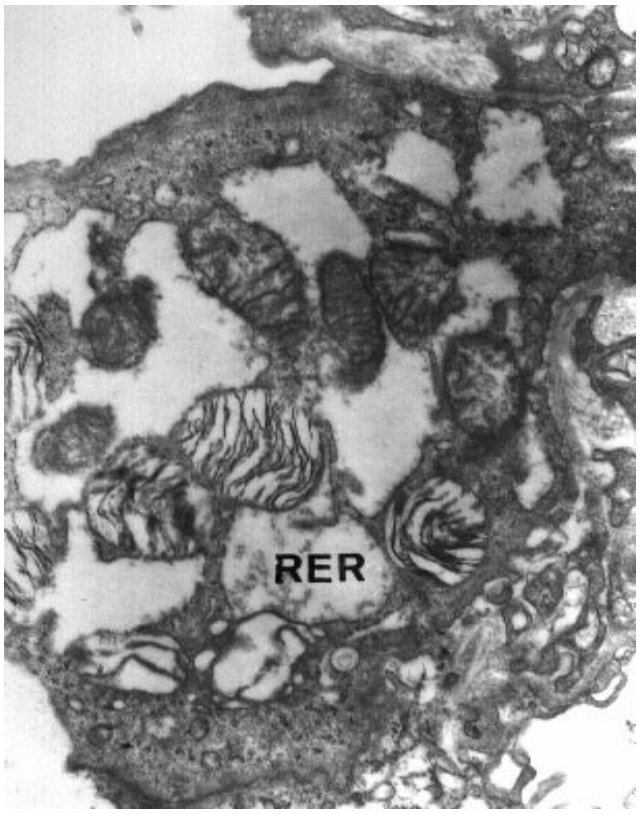

Figure 9. Lung of rat after *per os* application of malathion (1/50 LD_{50}). A strong widening of the ergastoplasma canals in the pneumocyte type II (RER). EM, × 10 000.

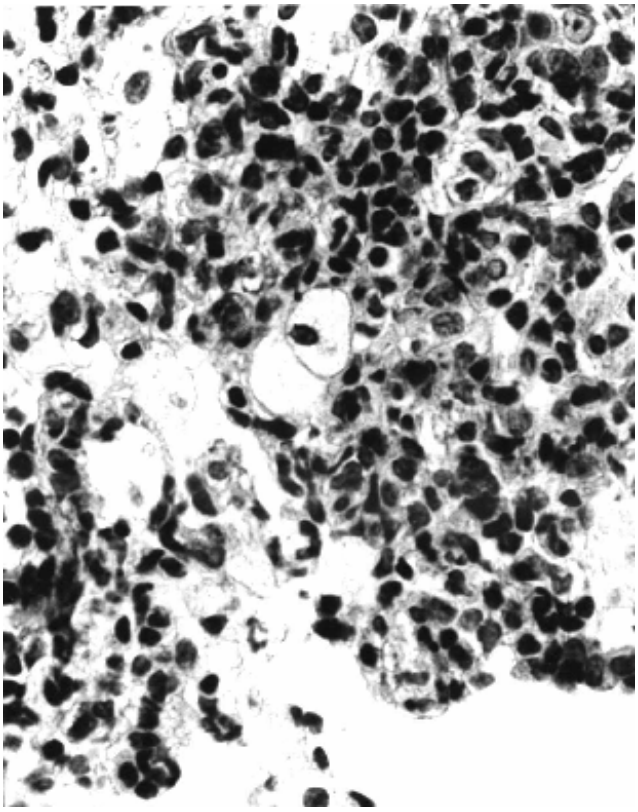

Figure 8. Lung of rat after *per os* application of malathion (1/50 LD_{50}). A single pulmonary phagocytes in widened interalveolar septa. H+E, × 160.

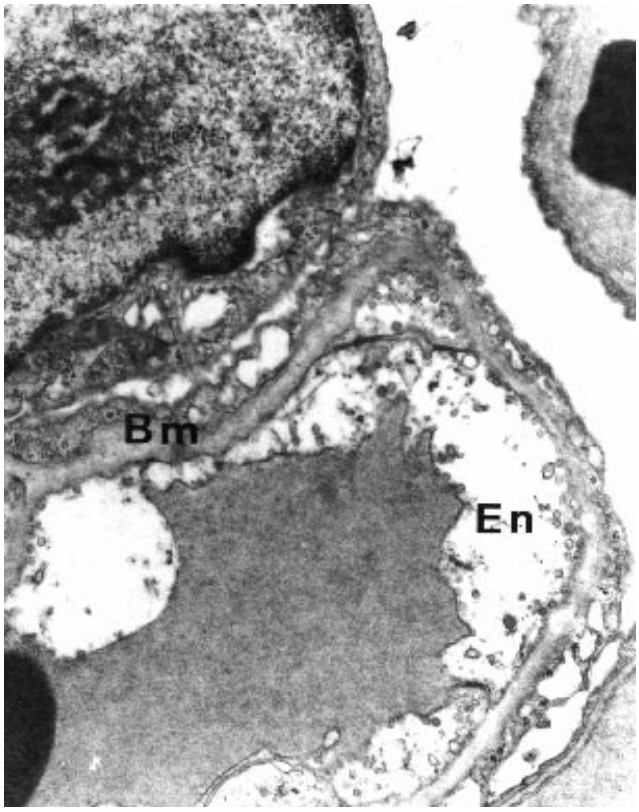

Figure 10. Lung of rat after *per os* application of malathion (1/50 LD_{50}). Swelling of vascular endothelial cells (En) and irregular thickening of basal membrane (Bm). EM, × 10 000.

administration of 1/100 LD_{50}, whereas after administration of 1/50 LD_{50} a considerably greater widening of ergastoplasma canals was noted (Fig. 2.).

In histopathologic studies of the lungs in animals which were applied a higher dose of the preparation widening of interalveolar septa was observed, with the presence of pulmonary phagocytes. In submicroscopic studies, after administration of both doses, the endothelium of pulmonary alveoli was swollen.

No histopathologic changes were noted in the kidneys and heart in both experimental groups. However, in the ultrastructure of the cells of renal proximal tubules an increased number of autophagous vacuoles was observed as well as the widening of spaces between the external and internal lamina of the nuclear membrane. Swollen mitochondria were noted in cardiomyocytes. The degree of the intensification of submicroscopic changes was of a dose-related character.

Evaluation of oral toxicity of malathion. Histopathologic changes in the liver were observed in 80% of animals. These changes concerned the presence of fine subcapsular infiltrations, diffused parenchymatous degeneration of single hepatocytes, and the presence of fine foci constructed of plasmatic cells and histiocytes located between hepatic plates (Fig. 3).

In the submicroscopic structure of hepatocytes there occurred lucent areas of cytoplasm containing the residues of cell organellae and lipid vacuoles. Mitochondria were usually swollen, showed a clearance of the matrix and destruction of crists (Fig. 4).

Histopathological changes in the kidneys occurred in all animals. These changes covered parenchymatous degeneration of the cells of renal tubules and hyperemia of the cortical part of the kidney, especially of renal glomeruli, as well as infiltrations were noted (Fig. 5). In the ultrastructure of the cells of renal proximal tubules, vacuoles with damaged external membrane were observed, as well as swollen and pleomorphic mitochondria (Fig. 6).

Histopathological changes in the heart covered focal parenchymatous degeneration of cardiomyocytes and the presence of single basophils. In the submicroscopic studies of cardiomyocytes, swollen mitochondria and sarcoplasma especially under sarcolemma were noted (Fig. 7).

In the lungs, there infiltrations sporadically occurred, and widening of interalveolar septa with the presence of single pulmonary phagocytes (Fig. 8). In ultrastructure of type II pneumocytes a significant swelling of ergastoplasma cisterns was noted (Fig. 9). In the walls of pulmonary alveoli there occurred a strong oedema of the endothelium of capillars and irregular thickening of the basal lamina (Fig. 10).

DISCUSSION

Changes observed on the level of light and electron microscopes showed a varied scope of occurrence and different degree of intensity according to the malathion dose and method of application.

The administration of the same dose of malathion (1/50 LD_{50}) *per os* caused degenerative changes in the liver in the form of parenchymatous degeneration in 80% of animals, while following the dermal application changes of this type were observed in 10% of rats.

Ultrastructural changes in hepatocytes following the oral administration of the pesticide were significantly more intensified, compared to dermal application. The areas of cytoplasm were observed to be deprived of normal cellular organellae, with an increased number of lipid vacuoles. These changes confirm the focal degeneration of the cytoplasm, whereas after the dermal application only widening of ergastoplasma canals and swollen mitochondria were observed.

In the kidneys, in histopathologic studies, no changes were noted in dermal poisoning, while following oral administration the changes were noted in all animals. These changes were manifested by parenchymatous degeneration of the cells of renal tubules and hyperemia of the cortical part, especially of the renal glomeruli. Moreover, in 10% of animals the stimulation of the reticular-endothelial system was observed.

Ultrastructural studies of the cells of renal proximal tubules from animals poisoned dermally with malathion showed an increased number of autophagous vacuoles and widening of perinuclear space. In oral poisoning, however, a focal degeneration of the cytoplasm was observed in tubular cells, manifested by the presence of autophagous vacuoles with damaged external membrane. Changes of this type are irreversible and are undoubtedly associated with the destruction of the protein-lipid structure of intracellular membranes and lysis of cytoplasm.

In studies *in vitro* concerning the effect of organophosphorous pesticides, including malathion, on protein-lipid membranes in mammals it was shown that these compounds change physical and chemical properties of the membranes. According to the authors, this is a manifestation of the toxic effect on cells, because the integrity of protein-lipid membranes ensures the normal functioning of the cells [9].

In the heart, histopathological changes were observed following poisoning *per os.* Ultrastructural studies revealed a considerable intensification of changes after oral administration, expressed by a large oedema, not only of mitochondria, but also of the sarcoplasm. This may be evidence of disorders in the processes of penetration through membranes.

In the lungs, histopathologic changes were similar in both dermal and oral poisonings, and were manifested by the widening of interalveolar septa and the presence of pulmonary phagocytes. In the ultrastructure of the lungs considerable changes in the blood-air barrier were observed, especially after poisoning *per os.* Swelling of the endothelium of the vessels was noted, as well as irregular thickening of the basal lamina and changes in pneumocytes.

The majority of the changes observed in our studies, especially on the ultrastructural level, confirm that higher doses of malathion applied dermally, and especially orally,

exert a damaging effect on the intracellular structure of the liver, kidneys, heart and lungs in rats.

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