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TOXICITY OF DERMALLY ABSORBED DICHLORVOS IN RATS

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Abstract: Toxicity of dermally absorbed dichlorvos was studied in rats, based on its effects on internal organs, and on phagocytic and bactericidal activity of the neutrophile system. The studies were conducted on 30 female rats of Wistar strain. The animals were divided into three groups, two of which were experimentally exposed to dermal absorption of dichlorvos (37.5 mg/kg - 1/2 LD_{50} or 7.5 mg/kg - 1/10 LD_{50}), and one control group which was exposed to dermal absorption of the solvent. The animals were exposed to dermal absorption for 4 hours daily for a period of 4 weeks. After 28 days, the rats were anaesthetized, blood was drawn from the heart to evaluate the activity of the neutrophilic system, and the internal organs excised for histological and ultrastructural studies. Dermally absorbed dichlorvos caused histopathological changes in lungs, lymphatic glands and thymus, as well as histopathological and ultrastructural changes in liver, kidneys and heart muscle. Dichlorvos stimulated the bactericidal and phagocytic activity of neutrophils.

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Key words: Dermal toxicity, dichlorvos, histopathology, ultrastructure, neutrophile activity.

INTRODUCTION

Dichlorvos (2,2 - dichlorovinyl dimethyl phosphate), an organophosphorous compound, is an insecticide applied in order to control ectoparasites in domestic animals.

The acute oral LD_{50} dose is 80 mg/kg for male rats and 56 mg/kg for female rats, while the dermal dose is 107 mg/kg and 75 mg/kg respectively [11].

Dichlorvos, dermally applied for 4 hrs in three concentrations (0.30%; 0.62%; 1.5%), as a single dose, penetrated into the rat tail skin. The amounts of substance in the region of direct exposure of the skin (9 cm^2) were different. For the dermally applied dichlorvos doses of 7.90 mg/g, 13.48 mg/g and 27.83 mg/g of rat tail skin, the following amounts of dichlorvos in the skin were observed: 0.2302 mg/g, 0.2728 mg/g, and 0.6230 mg/g, respectively. In all cases the amount of dichlorvos which penetrated into the rat tail skin ranged from 2.03% to 2.91% of the dermally applied solution [22].

The mechanism for the toxicity of organophosphorous compounds is mainly by blocking of acetylcholinesterase - an enzyme which decomposes acetylcholine [6, 10]. Immobilisation of this enzyme results in an accumulation of excessive amounts of acetylcholine in nervous tissue and muscular motor plates, as well as in symptoms of endogenic poisoning by this neurohormone.

Dichlorvos also causes disturbances in the permeability of cellular membranes and in the flow of ions through these membranes by the inhibition of enzymes which regulate this flow [5, 18]. Enzyme inhibition is a reversible reaction, but the dynamics of binding is considerably quicker and stronger than decomposition [1].

The absorption of xenobiotics through the skin is of importance with respect to occupational poisonings,

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especially by pesticides. Dichlorvos is highly permeable and despite quick decomposition it exerts an inhibitory effect on cholinesterase activity in rats, both after administration of single and multiple doses [4, 8]. Dermatitis was observed in dogs and cats wearing anti-flea collars impregnated with dichlorvos, and cases of dermatitis and dermal allergy have also been described in workers occupationally exposed to dichlorvos [7].

Tungul *et al*. [23], who studied the effect of dichlorvos on keratinocyte cultures from mice epidermis, confirmed the relatively rapid dermal permeability of dichlorvos. They observed that this pesticide may induce an increase in micronucleus test (MN) values.

Studies *in vitro* in the tissues of rabbits and rats indicate that metabolism of dichlorvos begins with the splitting of the ester bond linked to the dichlorovinyl or methyl group. Rabbit and rat livers are most prominent in hydrolysis activity [24].

Studies conducted *in vivo* on rats, cattle and goats confirmed that the metabolites obtained *in vitro* are also created in the living organism. In mammals, dichlorvos undergoes a quick hydrolysis and is excreted in the form of ionised products. In a cow which was administered 20 mg/kg of dichlorvos in fodder, 70% of this dose was excreted within 32 hours, and after 128 hrs - 91% had been excreted [24].

Plestina *et al*. [15] reported morphological changes observed in the eyes of agricultural workers and pilots involved in spraying pesticides. These changes are associated with irritation and functional weakness of sight.

Studies performed on bone marrow cells in rats, where oral doses of 1/100, 1/75 and 1/50 LD_{50} of three organophosphorous insecticides (dimetoate, dichlorvos and methylparathion) were administered for 6 weeks, confirmed a mutagenic effect of dimetoate and dichlorvos based on numerical and structural aberrations of chromosomes [13].

Intoxication with pesticides may dramatically change the course of infection. In rats intoxicated per os for 3 weeks with chlorphenvinphos, carbaryl and with the mixture of both substances before their intraperitoneal infection with *Toxoplasma gondii* RH strain, the acute form of toxoplasmosis occurred very often. It appeared in 40% of animals poisoned with chlorphenvinphos before infection, in 30% of animals after poisoning with carbaryl, while in rats not intoxicated - only infected with *T. gondii* - the infection had a chronic form [21].

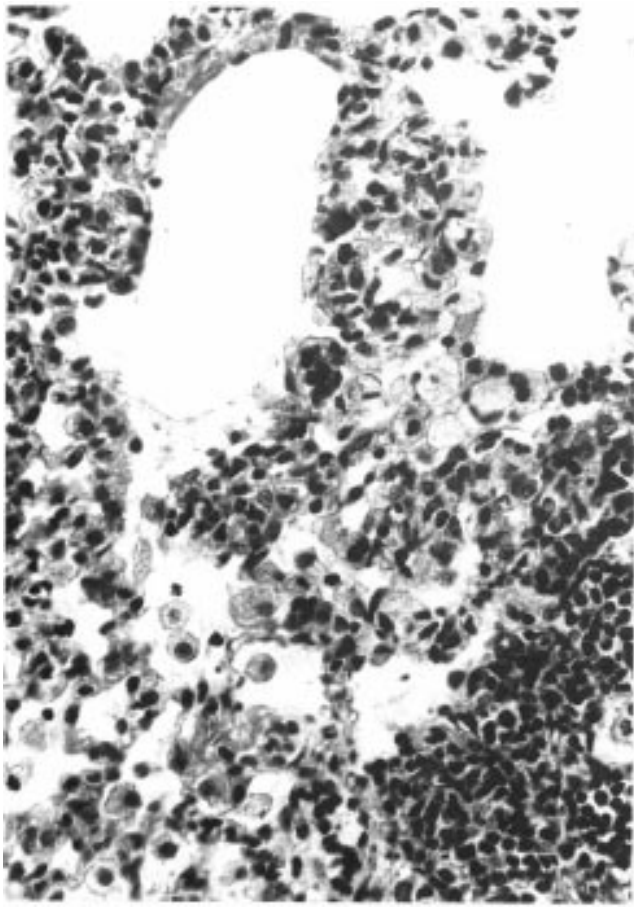

Figure 1. Lung of rat exposed to dermal absorption of dichlorvos (7.5 mg/kg). Widened intracellular septa with histiocytes of foamy cytoplasm. H-E, × 125.

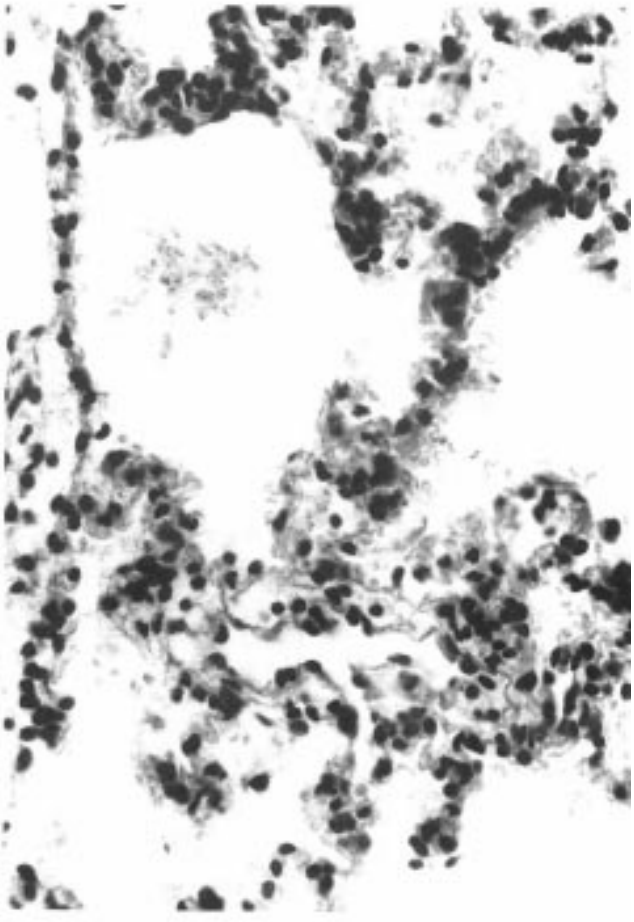

Figure 2. Lung of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Hyperemic foci of interalveolar septa and extravasation to the lumen of lung alveoli. H-E, × 125.

There are few studies concerning dichlorvos immunotoxicity. However, it is known that pesticides, like other pharmacologically active substances, may exert an effect on the immunological system. According to Sterzl [19], this effect may be immunotoxic, immunosuppressive or immunostimulatory - depending on the dose administered.

The aim of the study was to evaluate the effect of dichlorvos on phagocytic and microbicidal activity of peripheral blood neutrophils and to assess the toxicity of the preparation based on histological and ultrastructural examinations of selected organs.

MATERIALS AND METHODS

Dichlorvos standard: 2,2-dichlorovinyldimethyl-phosphate, DDVP, produced by the Institute of Organic Industry in Warsaw, was dermally applied in the following doses: 7.5 mg/kg - 1/10 LD_{50} and 37.5 mg/kg - 1/2 LD_{50}. The application liquid was a 20% water-alcohol solution.

Studies were conducted on female Wistar rats aged 3 months that were in good condition, with no macroscopic changes observed in the tail skin. The animals were fed with standard fodder LSM [9] and watered ad libitum. The body mass of rats at the beginning of an experiment ranged from 190-250 g.

The rats were placed in 3 groups with 10 animals in each group. Two experimental groups were administered dermally 7.5 mg/kg or 37.5 mg/kg dichlorvos daily for 4 weeks, except Saturdays and Sundays. During the dermal exposure to dichlorvos the animals of experimental groups were placed in specially constructed cages.

Dichlorvos was dermally applied to the tail skin according to Massmann's method in own modification [22]. The exposure time was 4 hrs daily .

Animals of the control group were dermally exposed to the sole solvent, exposure time and conditions being similar to those for experimental animals.

After 28 days, the rats were anaesthetised and blood was taken from heart in order to evaluate the function of granulocyte system. Phagocytosis Bacto-Latex test (Difco, USA) according to Ślopek's method [20], was applied to investigate the phagocytic properties of whole blood neutrophils. The bactericidal function of whole blood neutrophils was examined by the nitrobluetetrazolium reduction test (NBT), according to Park's method [14].

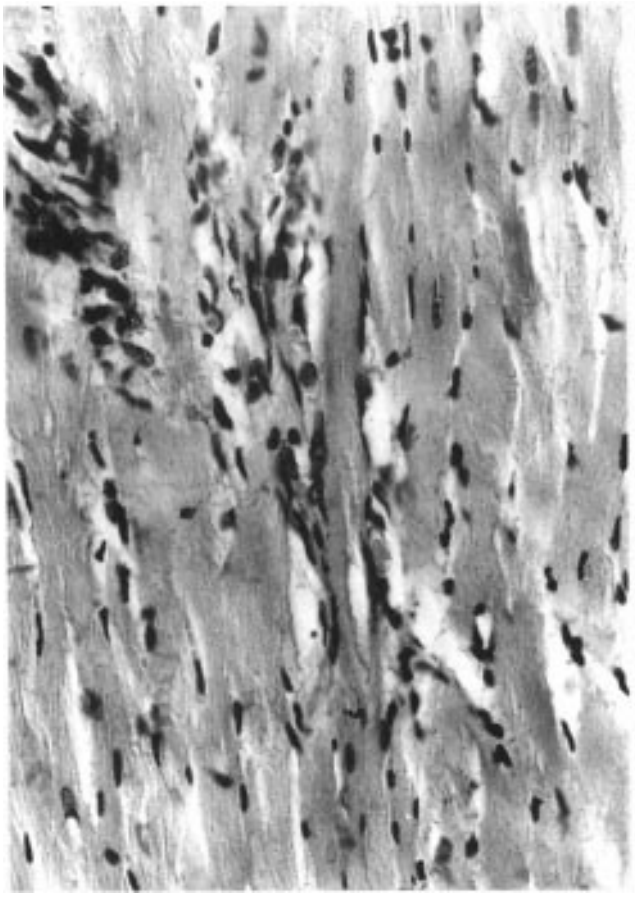

Figure 3. Heart of rat exposed to dermal absorption of dichlorvos (7.5 mg/kg). Infiltration of lymphocytes between cardiomyocytes. H-E, × 125.

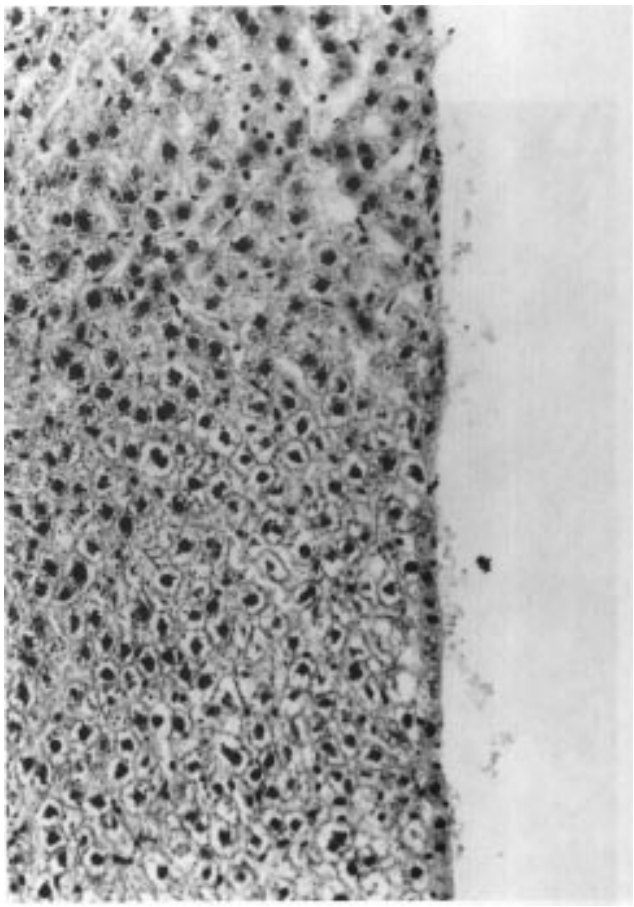

Figure 4. Liver of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Cytoplasm more porous in subcapsular hepatocytes. H-E, × 50.

In both tests 100 cells were counted. In the phagocytosis test, cells which contained at least 3 latex granules 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 defined the indices of the tests applied.

The following organs were used for histological studies: lungs, liver, heart, kidneys, brain, thymus, spleen and lymphatic nodes. Brain was perfused with a solution of methanol, formalin and glacial acetic acid and dyed according to the Nissel method [25]. The remaining organs were fixed in formalin diluted 1 : 9 and dyed H+E. Resorcin-fuchsin, according to Weigert method, was applied to stain the elastic fibres.

Heart, liver and kidneys were taken for ultrastructural studies. The material was fixed in 4% glutaraldehyde buffered to pH 7.2–7.4, with 0.1 M cacodylate buffer and refixed with 1% OsO4 acid water solution. Dehydration was performed in graded concentrations of ethyl alcohol increasing up to absolute, followed by embedding in Epon 812. Ultrathin specimens were observed and photographed using an electron microscope Tesla BS 500.

Statistical analysis. The obtained results were analysed statistically by the Student's t-test.

RESULTS

Body weight. No evident differences in body weight were observed in the groups of rats dermally exposed to dichlorvos, compared to the control group.

Histological and ultrastructural changes. In the lungs, after administration of 7.5 mg/kg of dichlorvos, perivascular infiltrations and widened intracellular septa with histiocytes of foamy cytoplasm were observed (Fig. 1). After administration of 37.5 mg/kg of dichlorvos, hyperemic foci of interalveolar septa and extravasation to the lumen of lung alveoli were observed (Fig. 2). In focally concentrated lung tissue, infiltrates of mononuclear lymphatic cells and lung macrophages were noted.

In the heart of some animals, after exposure to 7.5 mg/kg (1/10 LD_{50}) of dichlorvos, infiltration of lymphocytes was observed in the muscle of the right ventricule and around the small blood vessels (Fig. 3). After administration of a higher dose of dichlorvos, foci of cardiocytes with basophilic cytoplasm and concentrated nuclei were noted in the heart muscle. Ultrastructural changes in the heart muscle occurred after administration of 37.5 mg/kg (1/2 LD_{50}) of dichlorvos, manifested by widened spaces between cardiomyocytes. In these spaces a significant amount of fibres of connective tissue was present (Fig. 5). No changes were observed in the internal structure of the cardiomyocytes.

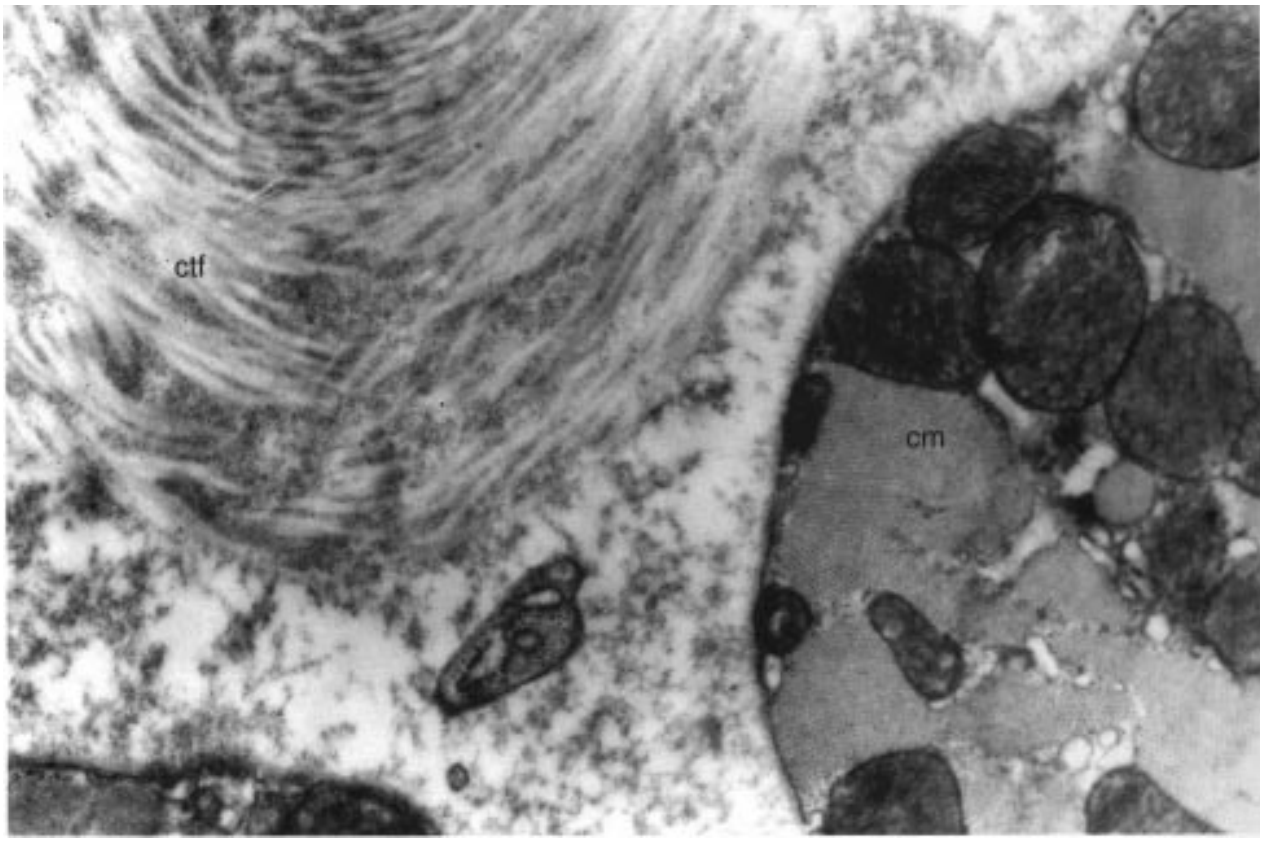

Figure 5. Heart of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Significant amount of connective tissue fibres (ctf) between cardiomyocytes (cm). EM, × 20000.

Table 1. Results of nitrobluetetrazolium test (NBT) and of the phagocytosis latex test (PLT) with whole blood neutrophils in rats exposed to dermal absorption of dichlorvos.

	N	Index of NBT	Index of PLT
Rats exposed to 7.5 mg/kg	10	18.30 ± 2.60**	50.50 ± 2.01
Rats exposed to 37.5 mg/kg	10	36.20 ± 3.43***	66.90 ± 2.58*
Control group	10	10.50 ± 0.90	56.70 ± 3.35

Values are mean ± SEM (standard error of the mean); * $p < 0.03$, ** $p < 0.01$, *** $p < 0.0001$, compared to control group.

In the liver, cytoplasm was more porous in subcapsular cells, and focal subcapsular hyperemia was observed in animals of both experimental groups (Fig. 4). In the cytoplasm of hepatocytes, ultrastructural examinations showed vacuoles of low electron density of various sizes and shapes. Moreover, in some cells, high concentrations of lipid-like bodies were observed (Fig. 6).

Changes in the kidneys in both experimental groups consisted exclusively of lymphocytic infiltrations of the paraglomerular region and in the outlet part of the kidney (Fig. 7). This was confirmed by ultrastructural studies of the kidney which showed considerably widened spaces between the convoluted tubules infiltrated with cells, mainly lymphocytes (Fig. 9). In the cells of proximal tubules, clarifications of the basic cytoplasm were observed, and sporadic, large, electron light vacuoles were noted. Slight changes were also observed in peroxysoma in proximal tubules and mitochondria in distal tubules. These organellae were characterised by an evident irregularity in shape.

Changes in the brain were only noted in animals which were dermally exposed to 37.5 mg/kg of dichlorvos. Gradual concentration of cytoplasm in the neurocytes of the brain cells was evident in the stratum pyramidale subiculum and in the pyramidale and granular part of the parietal cerebral cortex. Pyknosis affected mainly pyramidal cells. Small foci of concentrated neurocytes occurred in CA 1 layer of hippocampus (Fig. 8). Single Purkinje cells were also concentrated, as well as neurons of nucleus pontis.

No changes were noted in the spleen, apart from an insignificant outgrowth of the connective tissue in the peripheral part of lymphatic follicles.

Foci of blood extravasations in the thymus were observed in the lower part of lobule and were placed subcapsularly in those rats which were exposed to the higher dose of dichlorvos.

Widening of the marginal sinuses in lymph nodes in rats poisoned with 7.5 mg/kg of dichlorvos could be evidence of their slight oedema. In the group of animals exposed to the higher dose of dichlorvos in sinuses and

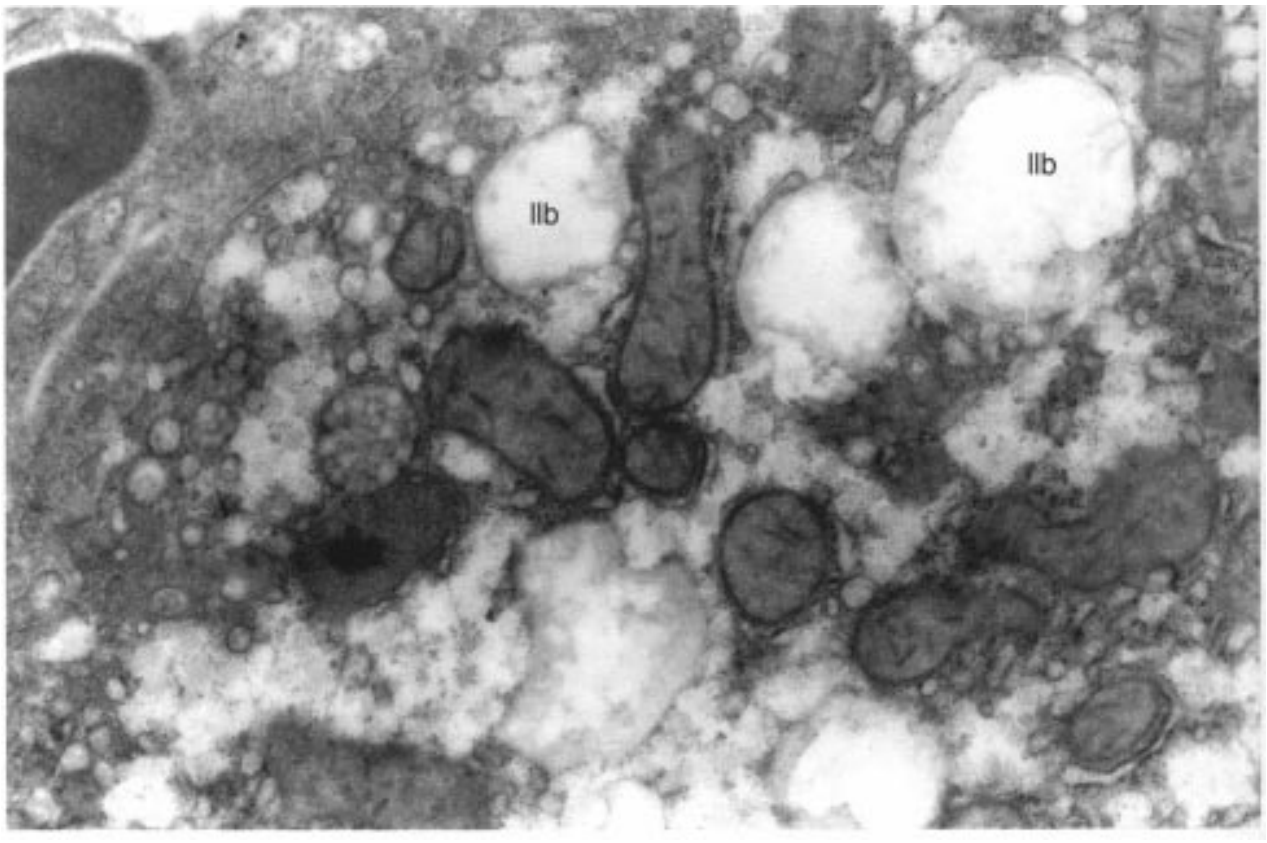

Figure 6. Liver of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). High concentrations of lipid-like bodies (llb). EM, × 25000.

the essential parts of the nodus an increased amount of histiocytes and mast cells were present. Macrophages were filled with hemosiderin.

Study of immunotoxicity. In the rats under study, dermally absorbed dichlorvos resulted in an increased bactericidal function of neutrophils in both experimental groups, which was significantly greater compared to the control group. Dichlorvos, when applied dermally in the dose of 7.5 mg/kg (1/10 LD_{50}), caused an increase of NBT index to 18.3 ($p < 0.01$). After dermal administration of 37.5 mg/kg (1/2 LD_{50}) the NBT index increased to 36.2 ($p < 0.0001$) (Tab. 1).

In rats dermally exposed to dichlorvos in the dose 7.5 mg/kg, the phagocytosis test did not differ significantly from control. However, the phagocytosis index was higher in the group of rats which were exposed to 37.5 mg/kg ($p < 0.03$) (Tab. 1).

DISCUSSION

The US EPA has published its intent to revoke the food additive registration of dichlorvos (DDVP) [12]. The basis for the Agency action is the National Toxicology Program (NTP) - toxicology and carcinogenesis study of DDVP in rats and mice.

Mennear [12] assessed the predictive validity of the results related to potential human impact and concluded that DDVP creates neither mutagenic nor carcinogenic risks in humans exposed under normal conditions of use.

In our studies, dermal exposure to dichlorvos resulted in the appearance of mononuclear cell infiltrates in the lungs, liver, kidneys and heart. After administration of a higher dichlorvos dose, changes in the lungs were manifested as widened interalveolar spaces infiltrated with macrophages and lymphatic cells, as well as by hyperemia. Similar histological changes in the lung of rats exposed to dermal absorption of a single dose of dichlorvos for 4 hours were observed earlier. In these animals the total amounts of leukocytes and lymphocytes were significantly higher when compared to the control group [22]. Infiltrations observed in the heart muscle, liver and kidneys confirmed the results of studies by other authors [7]. Cunningham *et al*. [2], however, did not note the proliferation of liver and kidney cells after 2 weeks per os administration of dichlorvos in rats.

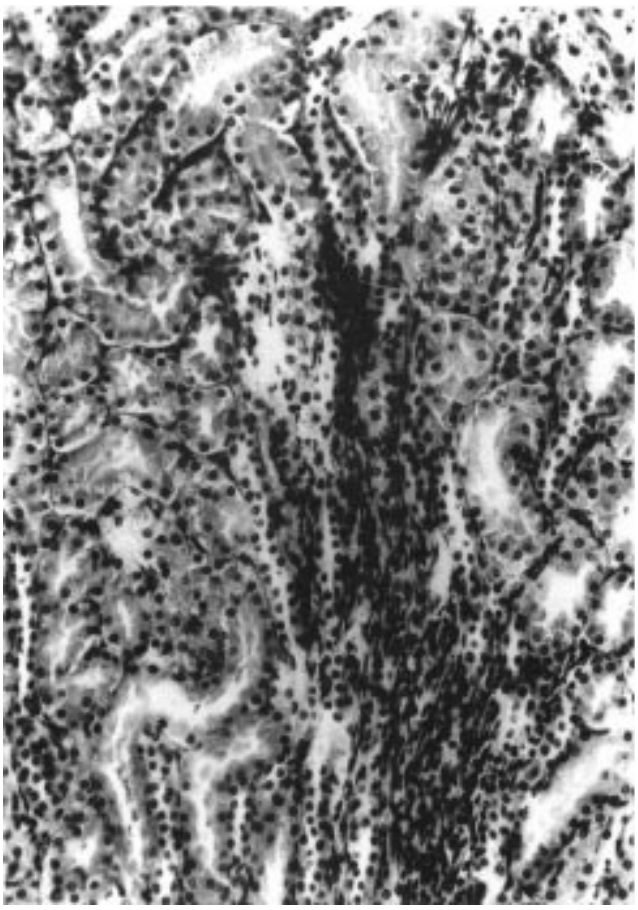

Figure 7. Kidney of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Lymphocytic infiltrations located between cortex tubules. H-E, × 125.

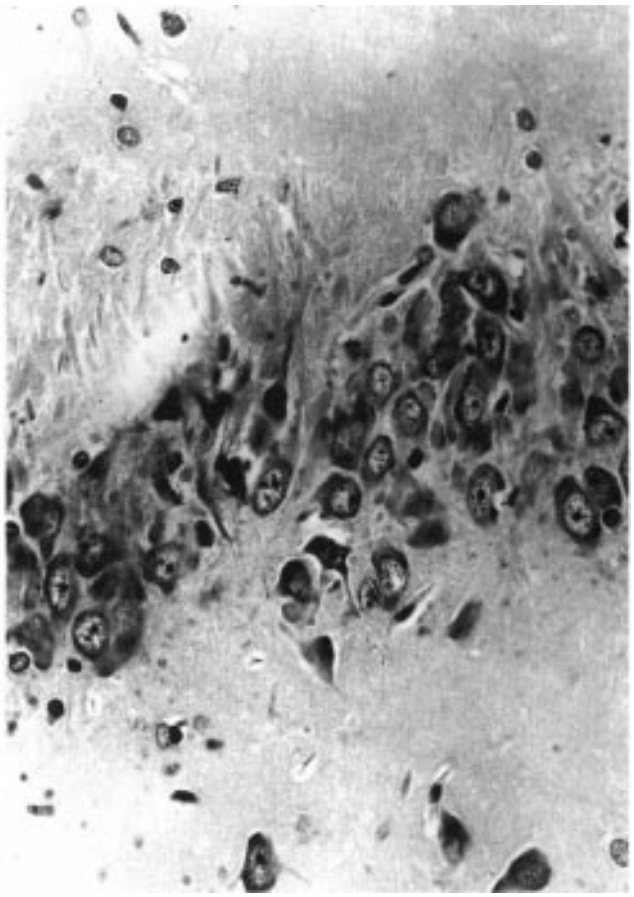

Figure 8. Brain of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Pyknosis of pyramidal cells in CA 1 layer of hippocampus. Dyed according to Nissel method. H-E, × 125.

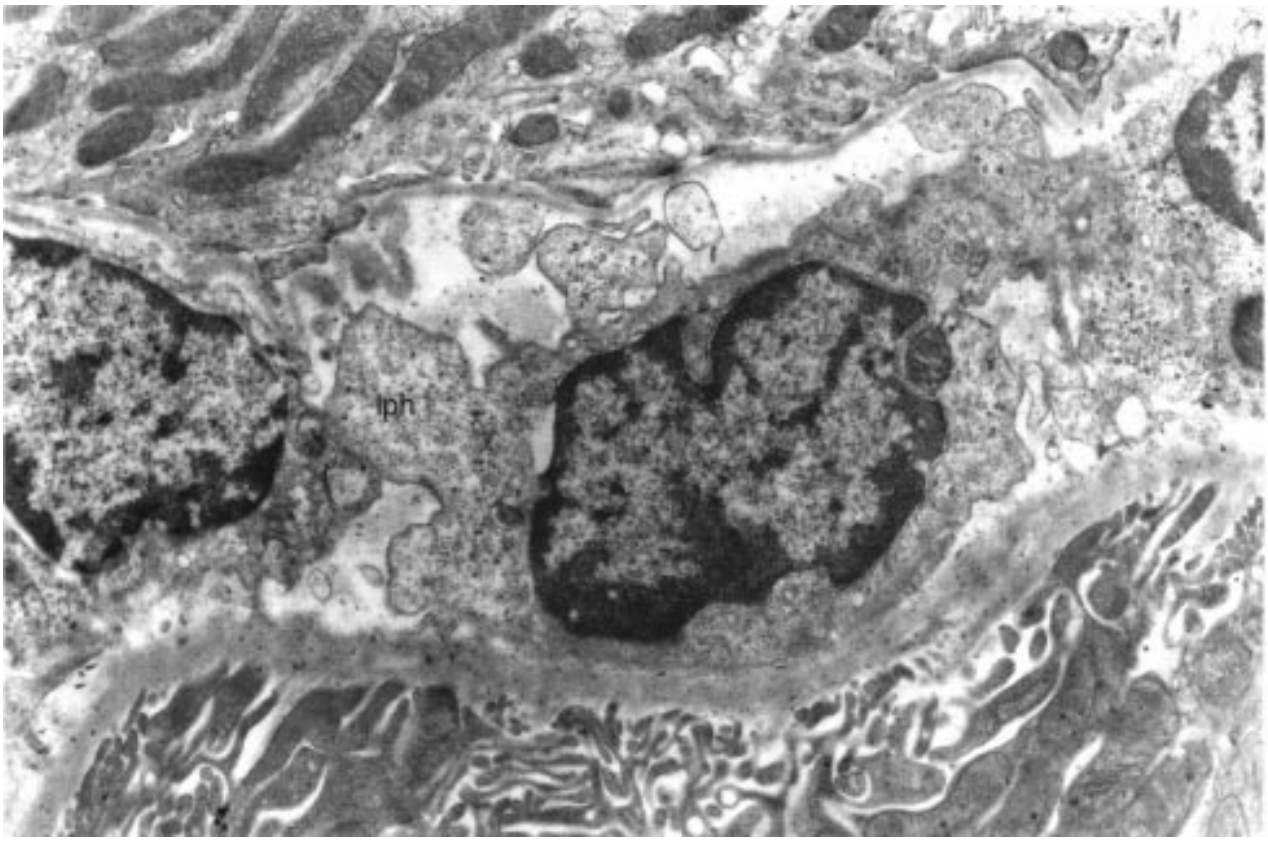

Figure 9. Kidney of rat exposed to dermal absorption of dichlorvos (37.5 mg/kg). Considerably widened spaces between proximal tubules infiltrated with lymphocytes (lph). EM, × 25000.

Ultrastructural changes in the heart muscle were observed after administration of a higher dichlorvos dose and were reflected by the occurrence of fibres in widened spaces between cardiomyocytes. Staining the fibres confirmed that their number increased slightly only in the vicinity of blood vessels.

The porous cytoplasm of liver cells observed in histological sections was confirmed by ultrastructural studies. Empty spaces were filled with vacuoles of various sizes and low electron density, as well as with lipid-like bodies. Similar results were obtained by other authors who noted lipid infiltration of liver cells after dichlorvos administration at doses of 2.5 and 12.5 mg/kg (IARC Working Group, [7]).

The studies by Dambska & Maślińska [3] on the effect of dichlorvos on the developing brain in rats show that cholinesterase inhibition leads to pathological changes in all neuroectodermal structures. This is especially harmful for developing cells which undergo differentiation. In our studies, changes in brain appeared in the form of pyknosis, mainly of pyramidal cells.

Studies *in vitro* by Podstawka [17], aimed at the evaluation of the toxic dichlorvos effects on neutrophils in human peripheral blood, confirmed that neutrophils showed an intensified phagocytosis activity. This was not, however, accompanied by an elevated level of nitrobluetetrazolium reduction, which remained close to control values.

In our studies the dermal exposure to dichlorvos resulted in an increased activity of both parameters examined: phagocytosis and bactericidal function of neutrophils. The increase in cellular function of both parameters is a reaction contrary to that observed in cultures of neutrophils exposed to chloroorganic pesticides (fenarimol and lindane), where a significant decrease in phagocytic activity of these cells was observed [16].

Different results in tests for phagocytic and bactericidal activity of neutrophils may be due to different mechanisms of both tests, which are affected by a number of parameters such as character of pesticides, their doses, penetration way and time of exposure.

The direction of changes and intensity of metabolic activity of neutrophils may vary similar as histopathological changes in rat tail skin according to the type of the applied pesticide. However, it did not correlate with their oral toxicity [22].

CONCLUSIONS

1. Dermally absorbed dichlorvos caused histopathological changes in lungs, lymphatic glands and thymus, as well as histopathological and ultrastructural changes in liver, kidneys and heart muscle.
2. Dichlorvos stimulated the bactericidal and phagocytic functions of neutrophils.

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