

DISTRIBUTION OF DERMALLY ABSORBED ^{14}C DDT IN THE ORGANS OF WISTAR RATS

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Abstract: The aim of study was evaluation of the concentration of dermally applied ^{14}C DDT in the organs of rats, and evaluation of histological and ultrastructural changes after the dermal application of unlabelled DDT standard in the organs of rats, in which the presence of this pesticide was determined by the radio-isotopic method. ^{14}C DDT of radiochemical purity of 97% was applied in the study. The activity of the preparation per 1 cm^2 of the tail skin was 22.2 KBq - ($175.38\text{ }\mu\text{g}$ DDT). The material for the study was taken directly after single exposure and 6 h, 8 h, 10 h, 12 h, 14 h, 18 h and 20 h after exposure. The following organs were taken for the determination of radioactivity of tissues: brain, heart, lung, liver, kidney, skin at the site of exposure. Unlabelled chemically pure, DDT (99.7%) was used to evaluate histological and ultrastructural changes after dermal application. Experimental animals received an oil emulsion of DDT ($10\text{ mg}/9\text{ cm}^2$), applied to the skin of the tail for 4 weeks. The time of exposure was 4h daily. The animals of the control group were dermally exposed to the emulsion at the same time and under the same conditions. 4 h after dermal exposure it was observed that the preparation applied on the surface of 9 cm^2 in the amount of $1.578\text{ }\mu\text{g}$ was present in the skin in the amount of $1\text{ }\mu\text{g}/9\text{ cm}^2$ ($0.11\text{ }\mu\text{g}/\text{cm}^2$), i.e. 0.063% of ^{14}C DDT applied dose. Directly after dermal application lasting 4 h the greatest amount of ^{14}C DDT was noted in the liver - $0.285\text{ }\mu\text{g}$, i.e. $0.033\text{ }\mu\text{g}/\text{g}$. Histopathological and ultrastructural changes were observed in the liver, kidney, lungs and heart.

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Key words: ^{14}C DDT - distribution in organs, DDT, dermal application, histopathology, ultrastructure.

INTRODUCTION

Rapid scientific and technical progress in recent years exerted an effect on chemization in agriculture and consequently, wide application of chemical plant protection. Due to the variety of chemical forms of biologically-active pesticides, their toxicity for the biocenosis and the whole ecosystem becomes a problem. Although officially chloroorganic pesticides have ceased to be applied there still appear reports indicating the presence of these compounds in human tissues and fluids. This results from their considerable prevalence in the environment and poor decomposition properties [7, 11].

Durable, toxic fluoroorganic compounds which are present in living organisms contaminate the natural environment and migrate to food products, as well as those which are directly applied during agrotechnical, zootechnical and hygienic treatments, currently constitute the most numerous and especially complicated group of food pollutants [4].

The discovery of the insecticidal properties of DDT (1,1,1-trichloro-2,2-bis-4-chloroethylene) originated the modern development of synthesis and application of chemical compounds in plant protection, as well as in other areas such as health protection where the control of the vectors of infectious diseases is applied (mosquitoes, lice, fleas) [3]. DDT - the compound which has been

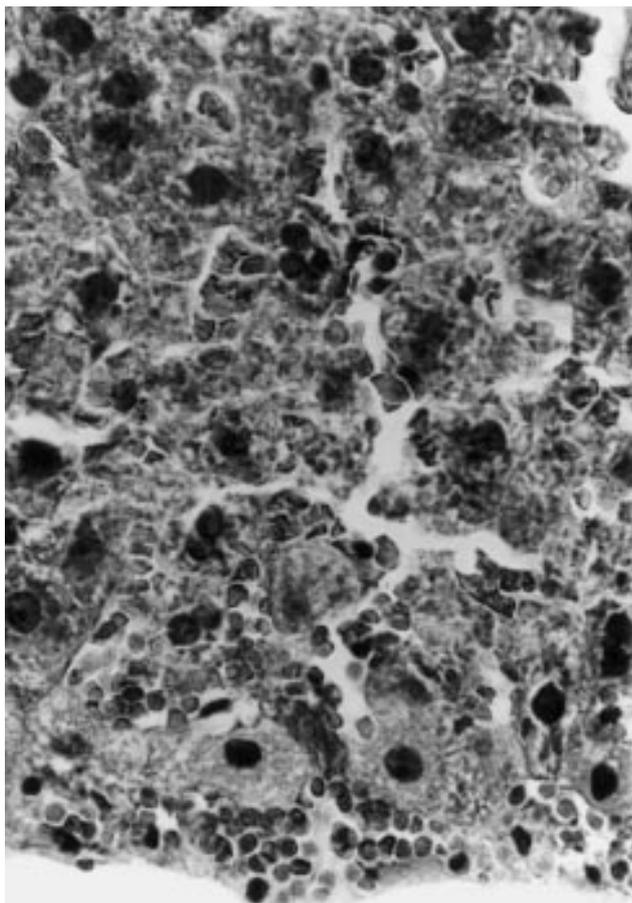


Figure 1. Liver of rat after dermal application DDT (10 mg/9 cm²). Subcapsular hyperaemia. H+E, × 160.

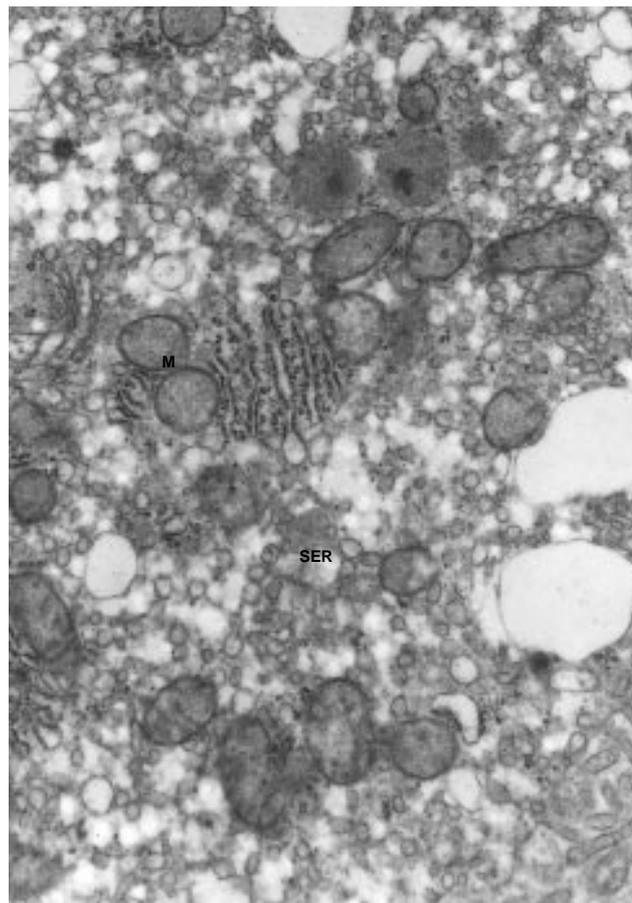


Figure 2. Liver of rat after dermal application DDT (10 mg/9 cm²). Proliferation of smooth endoplasmic reticulum (SER) and reduction of mitochondrial cristae (M). EM, × 12,000.

known for over 100 years (1974) and first applied as an insecticide in 1939, since currently withdrawn from production and turnover, or applied now to a very limited extent. In Poland, the application of DDT preparations was limited in 1973, and totally prohibited in 1976. In national food products, especially those of animal origin, DDT is still detected, although in constantly decreasing quantities [15]. In 1999, studies were conducted concerning the evaluation of the residues of chlorinated aromatic hydrocarbons in food products of animal origin. 2,700 tests were performed and low residues of chloroorganic pesticides were detected in 88% of the samples examined. DDT and its metabolites are still detected. The highest mean DDT concentrations were detected in hunted animals (in wild boars these concentrations being many times higher than in roe-deer and deer), as well as in carp, swine and cattle tissues, milk, and eggs - on the level of 0.05–0.10 mg/kg, while the lowest concentrations were observed in poultry tissues. In the material examined, in 8 samples (0.3%) the concentration of DDT exceeded the maximum allowable residues (MAR) - 1mg/kg [10].

DDT still remains on the list of biologically-active substances which create a special health risk for humans, animals and the environment [13]. Polychloric insecticides may penetrate into the bodies of mammals through the alimentary tract, airways and undamaged skin. In contrast

to other hydrocarbons, the property of poor penetration through the uninjured skin with the simultaneous ease of overcoming the chitinous covers of insects is a significant quality of DDT. DDT is easily soluble in fats, and therefore accumulates in fatty tissue and the skin, as well as in fruits, vegetables and cereal grain [6].

Studies of the absorption of ¹⁴C DDT from soil through the skin conducted by Wester *et al.* showed that DDT penetrates through the human skin in the amount of 1.0 ± 0.7%. The permeability of DDT into the skin increases when acetone is used as vehiculum (18.1 ± 13.4%) [19].

Polychloric insecticides are excreted with faeces, in only insignificant amounts, by the kidneys with urine, most often in a metabolised form. The daily value of DDT elimination is 1% of the amount accumulated.

Biotransformation of polychloric insecticides in homoiothermic organisms takes place according to various mechanisms with the domination of dechlorination, oxygenation and hydrolysis. DDT and its isomers create many metabolites in the organisms of mammals, of which, apart from the maternal substance (DDT), three metabolites - DDD, DDE, and DDA - are commonly detected in human and animal tissues and their excretions. DDD and DDE accumulate in the fatty tissue, and similar to DDT constitute contaminants of food products and tissue deposits in humans. DDA is the main metabolite

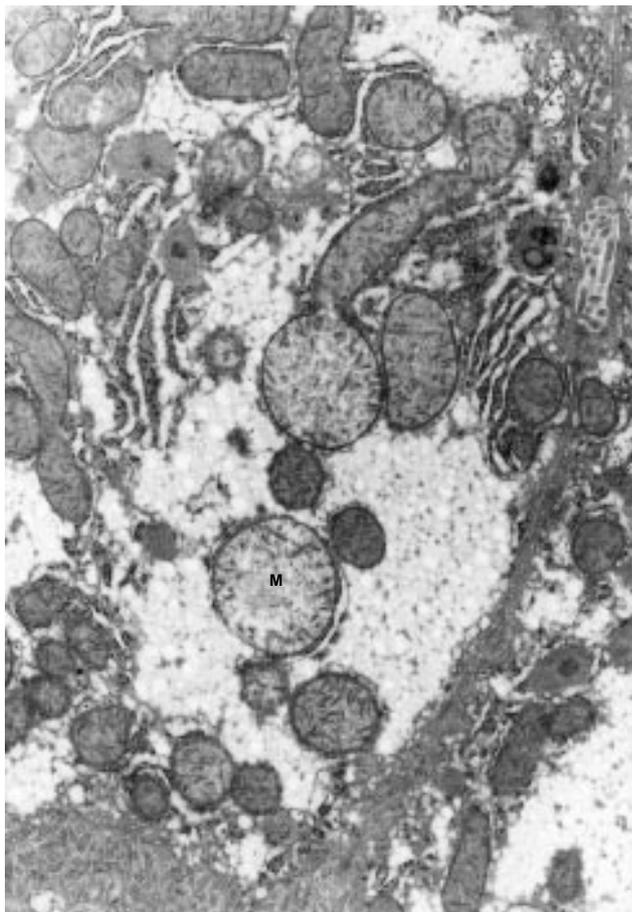


Figure 3. Liver of rat after dermal application DDT (10 mg/9 cm²). Spongy cytoplasm of hepatocytes and enlarged mitochondria (M). EM, $\times 12,000$.

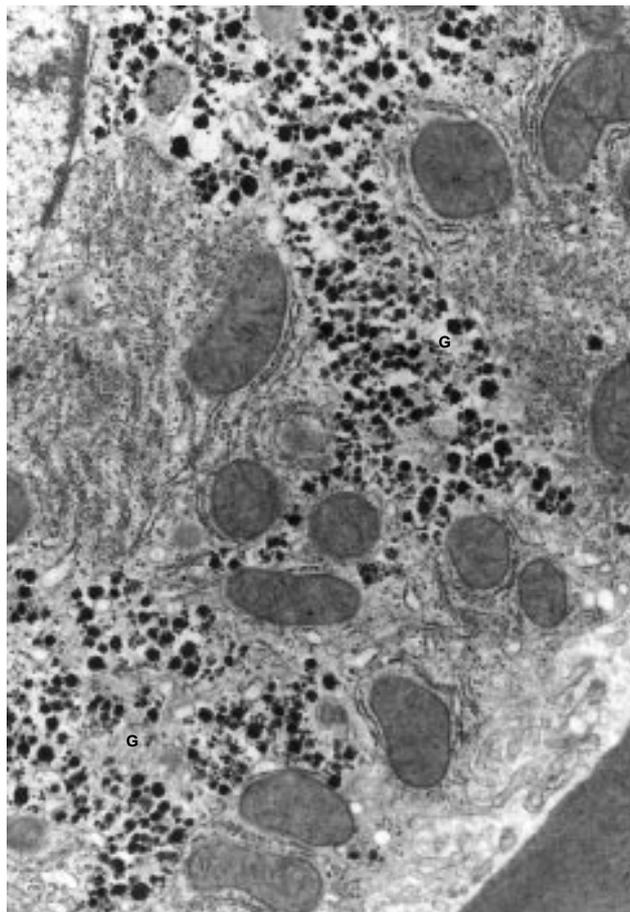


Figure 4. Liver of rat – control group. Granules of glycogen (G).

excreted with urine and serves as a basis for the analytical evaluation of occupational and population exposure to DDT.

A special case is excretion with milk in breast feeding women. Studies of the level of DDT and its metabolites - DDE and DDD - in milk of women from the Poznań Region conducted in 1997 showed that in this area the levels of DDT and its metabolites were 7.7 times lower, compared to the results of the studies carried out in 1971 [14].

The studies conducted independently by Snedeker *et al.*, Zheng *et al.*, and Romieu *et al.* indicated that there is no relationship between the level of DDE and DDT in tissues and serum, and risk of breast cancer [12, 17, 21].

Long-term experiments on animals showed that polychloric insecticides have the property of causing the majority of the known distant effects. Mutagenic, cancerogenic and immunotoxic effect of DDT was confirmed [3].

OBJECTIVE

The aim of the study was:

- evaluation of the concentration of dermally applied ^{14}C DDT in the organs of rats,
- evaluation of histological and ultrastructural changes after the dermal application of unlabelled DDT standard in the organs of rats, in which the presence of this pesticide was determined by the radio-isotopic method.

MATERIAL AND METHODS

Studies in which ^{14}C DDT was applied were conducted on male Wistar rats with a body mass of 200–300 g, fed with standard LSM fodder and watered *ad libitum* [9]. The animals were divided into 8 experimental groups, 2 rats in each group ($n = 16$) and 1 control group ($n = 4$).

DDT of radiochemical purity of 97% labelled with ^{14}C of activity of 45 MBq/ml was applied in the study. The application liquid was a solution of ^{14}C DDT in ethyl alcohol. 1 ml of the preparation of the activity of 200 KBq/ml, containing 1578.42 μg DDT was applied on the tail skin according to the Massmann's method in own modification [18].

The activity of the preparation per 1 cm² of the tail skin was 22.2 KBq - (175.38 μg DDT). The material for the study was taken directly after a single exposure and 6 h, 8 h, 10 h, 12 h, 14 h, 18 h and 20h after exposure. The following organs were taken for determination of radioactivity of tissues: brain, heart, lungs, liver, kidney, skin at the site of exposure.

Homogenates were prepared in the manual homogenizer. 5 ml of PBS (buffered solution of physiological salt with calcium chloride and magnesium chloride; BIOMED) was added to the fragments of organs.

For the determinations of radioactivity, 0.3 g of the homogenate of organs was taken, and NSC Amersham

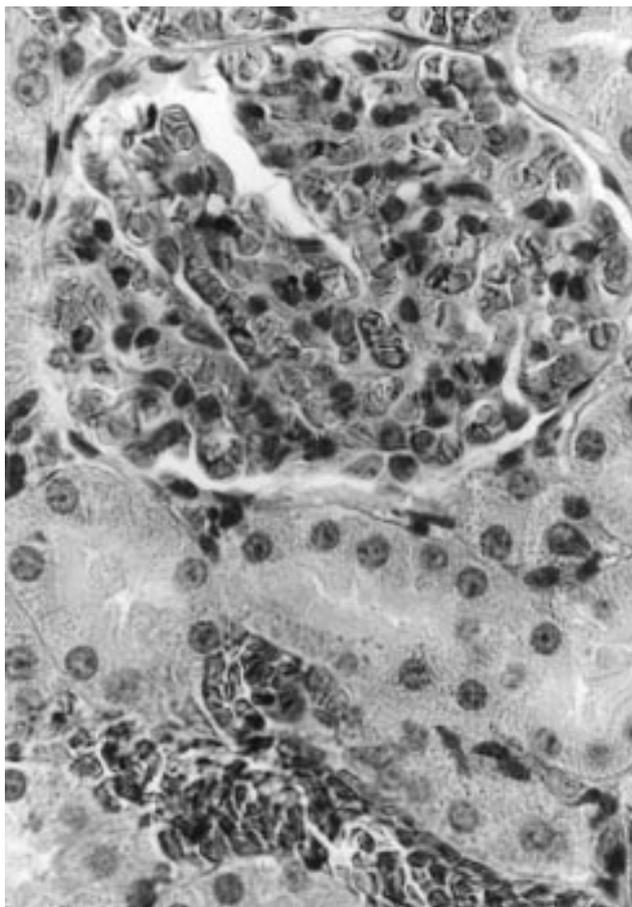


Figure 5. Kidney of rat after dermal application DDT (10 mg/9 cm²). Hyperaemia of renal glomerules. H + E, × 160.

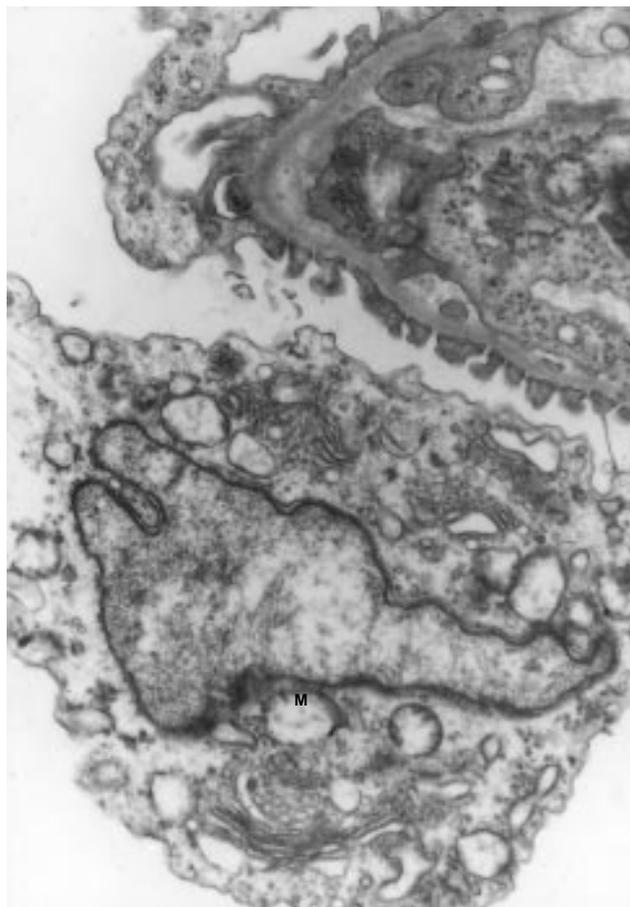


Figure 6. Kidney of rat after dermal application DDT (10 mg/9 cm²). Renal glomerule. Swollen mitochondria (M) in podocyte. EM, × 12,000.

solubiliser (2 ml) added. The samples were then shaken at a temperature of 50°C for a period of 2 h (350 shakes per min). 10 ml of Sigma scintillation liquid was added to the each sample and then shaken for 5 min. at room temperature.

β radiation coming from ¹⁴C was measured by the Wallac scintillation meter (LSC) model 1409, computer controlled, Multi Calc software. Measurements lasting for 5 min. were performed for each sample, and for each sample the measurement was repeated 3 times.

In general, β radiation measurements were performed for 360 samples. The quantities of ¹⁴C DDT absorbed were evaluated with the use of mean values for 3 results obtained for the same material, as well as mean values obtained in 2 rats in each group examined.

Control groups, in which the number of animals was twice as numerous as experimental groups, were evaluated in a similar way.

The amount of radioactivity in 0.3 g of examined tissues was calculated per whole organ and $\mu\text{g/g}$ tissue (Tab. 2).

Chemically pure DDT (99.7%), Prochem Co. Ltd., Warsaw, was used to evaluate histologic and ultrastructural changes after dermal application.

DDT was suspended in an emulsion of arabic gum, olive oil and water in the proportion 1 : 2; 1.5. The study was carried out on Wistar rats. Body mass of the animals of the experimental group at the beginning of the

experiment ranged within 200–230 g, while in the control group – 220–230 g. The experimental group covered 10 rats, the control group - 5 animals.

Experimental animals received for 4 weeks (except Saturdays and Sundays) an oil emulsion of DDT (10 mg/9 cm²), applied to the skin of the tail according to Massmann's method in own modification [18]. The time of exposure was 4 h daily. The animals of the control group were dermally exposed to the emulsion at the same time and under the same conditions.

After the experiment, the animals were anaesthetized and organs taken for histologic and ultrastructural examinations.

The following organs were taken for histologic examinations: brain, liver, lung, heart, kidney

The organs for histological examinations were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with H+E. The brain for histological examinations was perfused with a solution of methanol, formalin and glacial acetic acid, embedded in paraffin and stained by the Nissel method [20].

The following organs were taken for ultrastructural studies: liver, kidney, lung and heart. For ultrastructural studies, organs were fixed in 4% glutaraldehyde buffered to a pH of 7.2–7.4 with 0.1 m sodium cacodylate, and post-fixed with 1% solution of OsO₄ in water. Dehydration was carried out by ethyl alcohol in graded concentrations up to

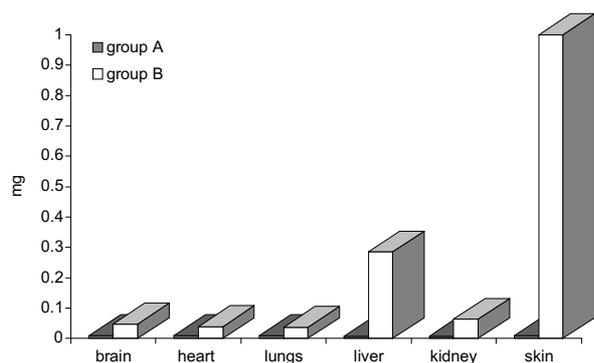


Figure 7. Results of studies of radioactivity in tissues of rats, expressed in µg per whole organ.

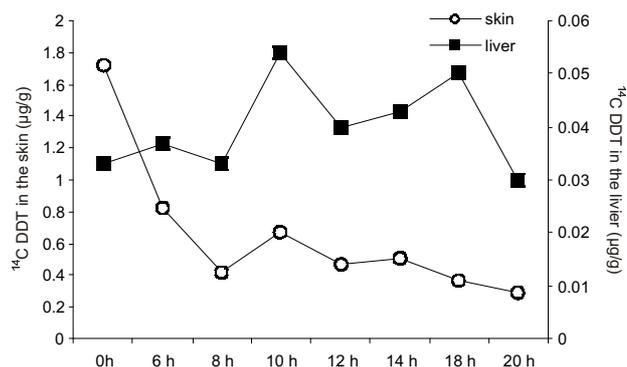


Figure 8. ¹⁴C DDT in the skin and in the liver at the site of application - during 20 h after dermal application.

absolute. 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

Dermal application of ¹⁴C DDT

4 h after dermal exposure it was observed that the preparation applied on the surface of 9 cm² in the amount of 1,578 µg was present in the skin in the amount of 1 µg/cm² (0.11 µg/cm²), which constituted 0.063% of ¹⁴C DDT applied to the skin (Tab. 1).

Table 2 and Figures 7, 8 presents the distribution of ¹⁴C DDT in organs from 4–20 h after the dermal application.

Immediately after 4h exposure, the total amount of ¹⁴C DDT in all examined organs was 1.473 µg, the highest in skin (1.00 µg/g whole organ) and liver (0.285 µg/g whole organ).

Table 1. Amount of ¹⁴C DDT absorbed through the tail skin of Wistar rats during 4 h exposure.

Absorption surface in cm ²	¹⁴ C DDT applied in µg	¹⁴ CDDT absorbed	
		Amount in µg	% of applied dose
9	1,578.42	1.0	0.063

Table 2. Level of ¹⁴C DDT in the organs of Wistar rats.

	Immediately		6 h		8 h		10 h		12 h		14 h		18 h		20 h	
	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g	µg	µg/g
Skin	1.00	1.724	0.48	0.827	0.237	0.419	0.392	0.676	0.27	0.465	0.292	0.503	0.21	0.362	0.168	0.290
Brain	0.048	0.03	0.059	0.037	0.043	0.027	0.064	0.039	0.059	0.037	0.048	0.030	0.54	0.034	0.048	0.030
Heart	0.039	0.047	0.033	0.040	0.017	0.020	0.028	0.034	0.019	0.023	0.028	0.034	0.028	0.034	0.022	0.026
Lungs	0.037	0.033	0.037	0.033	0.030	0.027	0.037	0.033	0.037	0.033	0.037	0.033	0.045	0.040	0.034	0.030
Liver	0.285	0.033	0.313	0.037	0.285	0.033	0.465	0.054	0.342	0.040	0.370	0.043	0.427	0.050	0.285	0.033
Kidney	0.064	0.036	0.070	0.040	0.047	0.027	0.047	0.027	0.047	0.027	0.058	0.033	0.047	0.027	0.052	0.030

The amount of ¹⁴C DDT expressed in µg per g of examined tissues was the highest in skin (1.724 µg/g) and heart (0.047 µg/g).

Amount of ¹⁴C DDT in the organs of animals of the experimental group 4 h after dermal application: it was observed that during this time the greatest amount of the preparation reached the liver (0.285 µg), followed by the kidney - 0.064 µg ¹⁴C DDT, the brain - 0.048 µg, the heart - 0.039 µg and the lungs - 0.037 µg ¹⁴C DDT.

The amount of ¹⁴C DDT in the skin at the site of application decreased within 6 h by approximately 50%. 20 h after dermal application about 15% of the ¹⁴C DDT dose absorbed was still present in the skin.

In the liver, the greatest amount of ¹⁴C DDT was noted 10 h after dermal exposure of (0.054 µg/g). After the subsequent 2 h this amount decreased to 0.040 µg/g. After 14 h it increased and after 18 h reached the next peak - 0.050 µg/g.

Results of histologic and ultrastructural studies

Liver. In histologic specimens of the liver parenchymatous degeneration was observed in hepatocytes, as well as fine infiltrations of mononuclear cells and subcapsular hyperaemia in 30% of animals (Fig. 1).

Liver cells showed ultrastructural changes of various degrees of intensity. Hepatocytes with less advanced changes were filled with a great amount of fine alveoli of smooth endoplasmic reticulum of indistinct and irregular

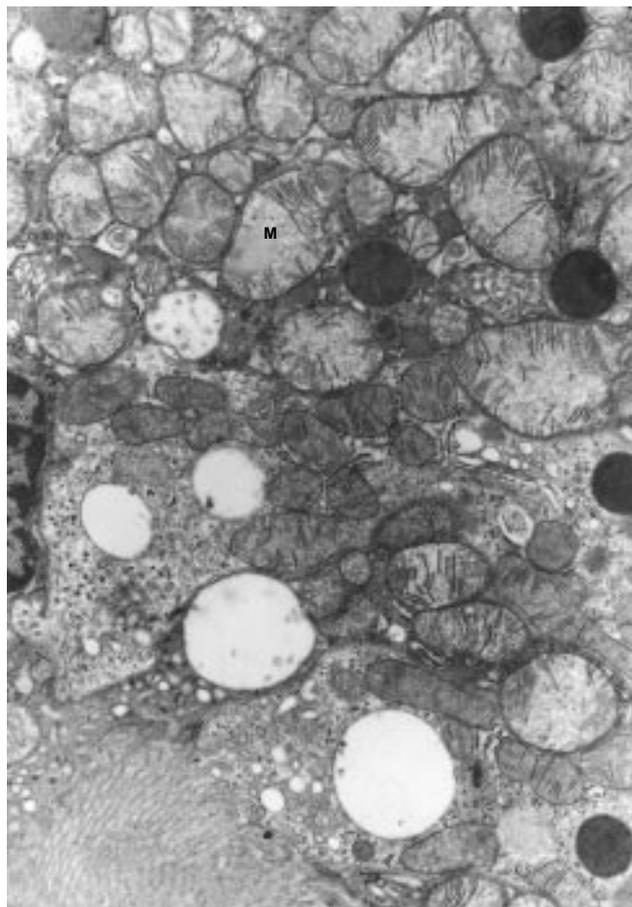


Figure 9. Kidney of rat after dermal application DDT (10 mg/9 cm²). Large mitochondria (M) in proximal tubule. EM, × 15 000.

outlines, compared to the control group. Between them, single, enlarged vacuoles and a few lipid drops were noted. A reduced number of cristae, or their lack, was observed in mitochondria. In other cells, which were the most numerous, membranes of the smooth endoplasmic reticulum were not visible, therefore cytoplasm at these sites had a spongy appearance. In such hepatocytes mitochondria were considerably enlarged, swollen, possessed a light matrix and a reduced number of cristae (Figs. 2 and 3). This type of mitochondria were specific for parenchymatous degeneration. Ergastoplasm's canals in the liver cells were unchanged or slightly widened to a degree compared to that noted for the control group (Fig. 4). Generally, no glycogen granules were observed in hepatocytes.

Kidney. Hyperaemia of renal glomerules was noted in 30% of the animals (Fig. 5). Only in 1 rat were inflammatory infiltration in the medulla of the kidney, the presence of granular - lymphatic infiltration in the renal pelvis, and parenchymatous degeneration of single cells of proximal tubules observed.

Ultrastructural changes were observed both in the renal glomerules and in all sections of the nephron. Endothelium of capillaries of the renal glomerules was partly swollen and had partly lost the character of a fenestrated membrane.

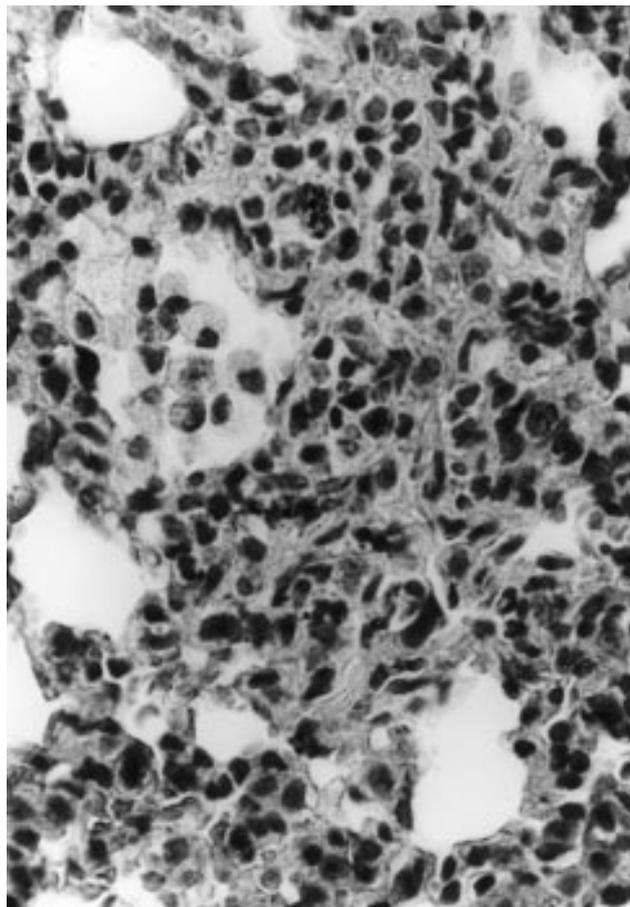


Figure 10. Lung of rat after dermal application DDT (10 mg/9 cm²). Widened interalveolar septa and pulmonary macrophages in lumen of alveoli. H + E, × 160.

Swollen mitochondria and widened ergastoplasm's canals were present in the podocytes (Fig. 6). In proximal tubules, large, swollen mitochondria were noted, similar to those described above in the liver (Fig. 9). A considerable part of cells of all sections of the nephron showed vacuolization of the cytoplasm.

Lungs. No changes were observed in histologic specimens of the lungs. Slight widening of interalveolar septa occurred in both groups - experimental and control (Fig. 10).

Ultrastructural changes in the lungs were noted in the blood-air barrier. The endothelium of the capillary vessels was partly swollen and contained few micropinocytosis alveoli (Fig. 11). In type II pneumocytes considerably widened ergastoplasm's canals were observed and numerous lamellar granules, as well as irregular microvilli on the free surface. Lumen of pulmonary alveoli was filled with fragments of decomposed cellular structures (Fig. 12).

Heart. Inflammatory infiltrations in the heart muscle were noted in 40% of rats (Fig. 13).

Swollen mitochondria were present in cardiomyocytes to the degree comparable with the control, and vacuoles were filled with a small amount of electron-dense material (Fig. 14).

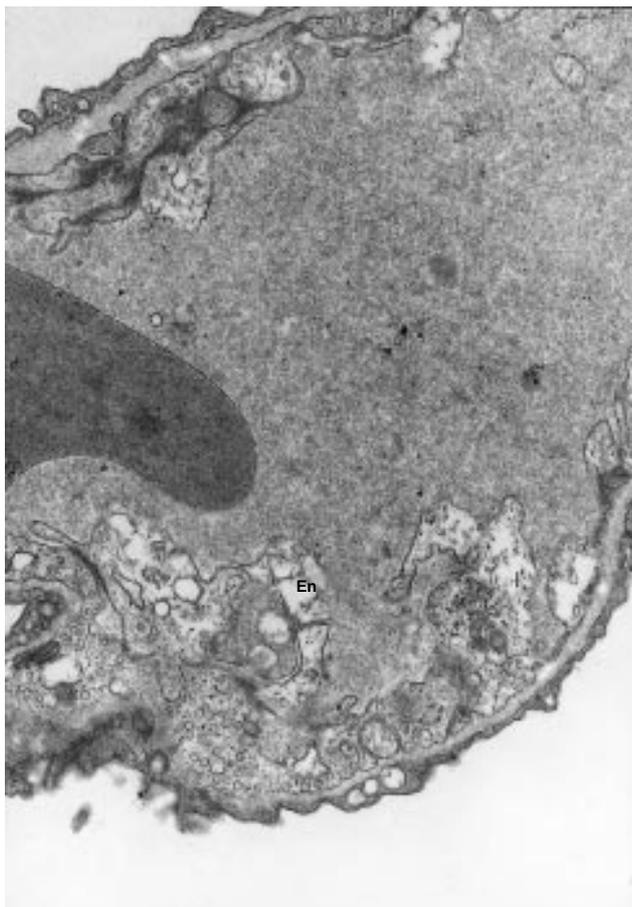


Figure 11. Lung of rat after dermal application DDT ($10 \text{ mg}/9 \text{ cm}^2$). Swollen endothelium (En) in the capillary vessels. EM, $\times 10,000$.

Adrenal glands. No changes were observed in the adrenal glands.

DISCUSSION

Shah *et al.* applied ^{14}C DDT to the rat's skin in the amount of $4 \mu\text{g}/\text{cm}^2$. This preparation, similar to parathion and carbaryl, penetrated through the skin in the amount of $>85\%$ during 5 days. After intraperitoneal administration of ^{14}C DDT it was excreted with urine during 5 days in an amount less than 5%, whereas parathion and carbaryl - in about 80% [16].

In own studies, a significantly higher dose of DDT was used for dermal application ($175.38 \mu\text{g}/\text{cm}^2$). After a single 4 h dermal application of $1.578 \mu\text{g}/9 \text{ cm}^2$ ^{14}C DDT was present in the skin in the amount of $1.0 \mu\text{g}$ (0.063% of the dose applied). The lowest level of ^{14}C DDT in the skin was noted 20 h after dermal application - 8.8% of the dose absorbed.

In the case of administration of the unlabelled DDT standard, 10 mg of DDT/ 9 cm^2 was used, this dose constituting approximately 1/50 LD50 of the dermal dose. On the surface of 1 cm^2 there was 1.11 mg of the preparation, which constituted a 10 times greater amount, compared to ^{14}C DDT. Considering the same method of dermal application of both forms of DDT it may be

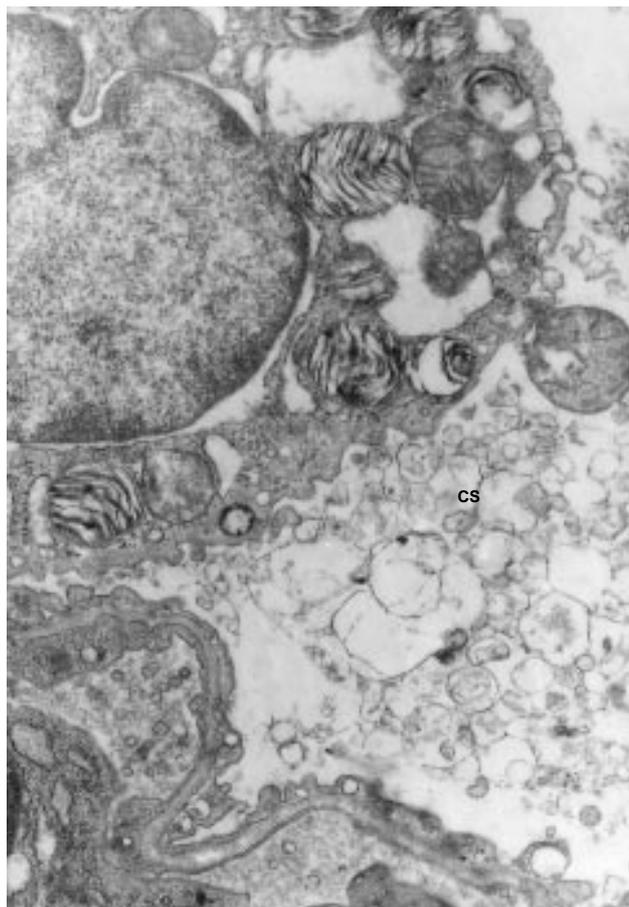


Figure 12. Lung of rat after dermal application DDT ($10 \text{ mg}/9 \text{ cm}^2$). Lumen of pulmonary alveoli are filled with fragments of cellular structures (CS). EM, $\times 12,000$.

presumed that in the liver, DDT administered in the form of a standard occurred 4 h after exposure in the amount of about $3.0 \mu\text{g}$. Then, 10 h after the dermal application this amount increased to about $5 \mu\text{g}$ and remained on a similar level for the next 14 h. Histologic and ultrastructural changes were most clearly visible in the liver. Approximately 30% of the DDT dose applied reached this organ within 4 h after the dermal exposure.

Ben Rhouma *et al.* also described histologic changes in the liver in the form of vacuolization of the cytoplasm and necrotic foci in Wistar rats poisoned with DDT for 10 days (50 and 100 mg/kg) [2].

Our histologic studies of the liver conducted after the administration of unlabelled DDT standard showed the presence of fine infiltrations and parenchymatous degeneration in hepatocytes. On the ultrastructural level, changes were observed in the mitochondria of the liver cells (enlargement, swelling, diminished electron density of the matrix, reduction in mitochondrial cristae) were specific of this type of degeneration and suggested disorders in the process of cellular respiration. Moreover, in the cytoplasm of hepatocytes an overgrowth of smooth endoplasmic reticulum was observed. This is a structural component of the microsomal fraction. Microsomal fraction is associated with biotransformation of DDT to the reactive intermediary compound DDT [1]. In these

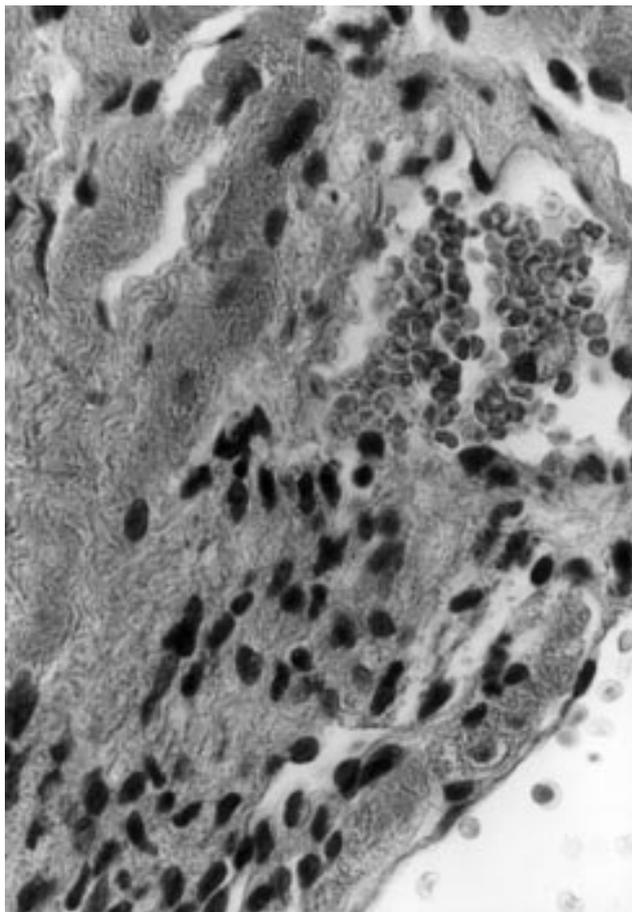


Figure 13. Heart of rat after dermal application DDT (10 mg/9 cm²). Inflammatory infiltration between cardiomyocytes. H + E, × 160.

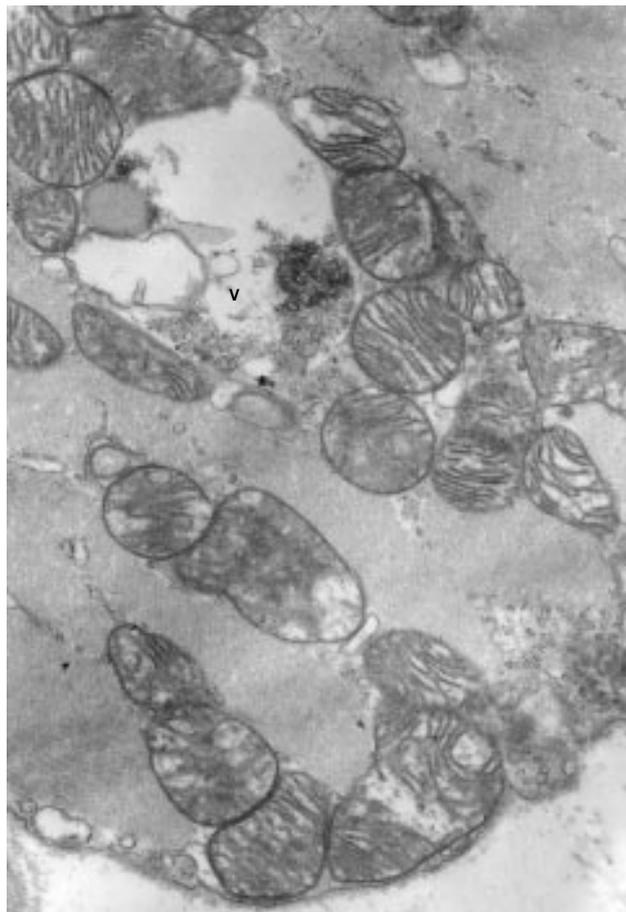


Figure 14. Heart of rat after dermal application DDT (10 mg/9 cm²). Vacuoles (V) filled with small amount of electron dense material. EM, × 15,000.

processes participate enzymes of the system of monooxygenases, the final oxidase of which is the cytochrome P-450. Kostka *et al.* described a clear, induction effect of the pesticide examined on the cytochrome P-450 of phenobarbital type [8]. Usually, an overgrowth of smooth endoplasmic reticulum is accompanied by glucogen loss. This was also true in the case of our studies.

Based on the results of own studies obtained with the use of ¹⁴C DDT, it may be expected that DDT standard in the kidney occurred in the amount of 0.6 µg directly after dermal exposure.

In the kidneys, changes of the type of parenchymatous degeneration were also observed in histologic studies, which was clearly confirmed by the ultrastructure of the cells of renal tubuli. Other changes noted in the sub-microscopic structure of the kidney (changes in the glomerules) may suggest that DDT exerts an effect of the permeability of cellular membranes due to its affinity to their lipid component.

The smallest amounts of DDT reached the lungs (0.3 µg), therefore the lack of histologic changes in this organ may not be surprising.

The destabilising effect of DDT on cellular membranes may be confirmed by ultrastructural changes occurring in the lungs (the presence of the remains of decomposed cellular organelles in the lumen of pulmonary alveoli).

Studies of the metabolism and excretion of labelled ¹⁴C DDT, DDD, DDE and DDMU administered intraperitoneally to male rats and Japanese quails, conducted by Fawcett *et al.*, showed that the greatest accumulation of the preparations in both species were noted in the skin and fatty tissue [5].

Studies carried out by B. Komorowski *et al.* indicated that the contents of chloroorganic pesticides in the tissues of humans not exposed constantly to the means of plant protection (mean age 57) was much too small to induce symptoms of acute poisoning. The results obtained showed a statistically significantly higher level of DDE in the tissues of the adrenal glands, heart and liver. The contents of DDT and its metabolites in the adrenal glands was higher than in the liver, on the level of statistical significance of 95% [7].

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