INTERNATIONAL JOURNAL OF
OCCUPATIONAL SAFETY AND ERGONOMICS 1995, VOL. 1, NO. 2, 167–178

Chlorfenvinphos Dermal Absorption in Rats: Histological and Ultrastructural Changes in the Skin and Internal Organs

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We studied the effect of chlorfenvinphos dermal absorption on the morphological picture of blood, on the histological and fine structure of tail skin, and on the histological structure of internal organs (lungs, heart, liver, spleen, and kidney) of rats. This study was conducted on 25 white Wistar rats, the tail skin of which was—or was not—hydrated before exposure. Rat tails were soaked in 0.5% or 0.05% chlorfenvinphos for 1 hr day for 3 months. Evident tendencies for a decrease in the absolute level of leukocytes and for an increase in the absolute level of erythrocytes in all experimental animals were observed. Histopathological changes in the internal organs were mildly manifested in only a few rats, mainly as liver and pulmonary hyperaemia. Rat tail skin at the direct exposure site showed hyperceratosis, intensive desquamation, and compensatory hyperplasia.

pesticide dermal absorption chlorfenvinphos

1. INTRODUCTION

The dermal penetration of a pesticide depends on the chemical structure of the active agent, its physical properties, molecule size, concentration, the size of the exposed skin surface and the resistance of skin. High skin permeability is typical of organophosphorus, chloroorganic and organomercuric compounds, nitrophenols, nitrocresols, and aniline oils.

Pesticides with a pH lower than 5 and higher than 8 have irritating properties. Alkaline substances cause the lysis of keratin, and acids induce protein precipitation. Both types of substances damage stratum corneum epidermidis.

Pesticides containing vaseline components, emulgents, surface-active substances, or organic solvents are much more dangerous than the same substances in the form of granulars. Dermal penetration depends on the concentration of a given substance and the duration of exposure to it.

Statement of the relevance of the reported research. Dermal absorption of chlorfenvinphos in rats cause changes in the absolute level of leukocytes and erythrocytes and histopathological changes as liver and pulmonary hyperaemia. Rat tail skin at the direct exposure site showed hyperceratosis, intensive desquamation and compensatory hyperplasia.

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Probably 65–85% of occupational intoxications with pesticides, both acute and chronic, are caused by dermal absorption. Experimental studies on animals show that cases of occupational intoxications with pesticides are caused by the acute dermal dose DL_{50} rather than by the acute oral dose DL_{50} (Bainova, 1982; Kundiev, 1975).

Exposure to pesticides as a result of their use in agriculture will vary according to the type of formulation, the method of application, and the protective measures used. By itself, the quantification of external exposure does not predict the amount absorbed, nor does it allow toxic hazard to be assessed: Information about skin penetration is also required (Carmichael, 1989)

Dermal absorption is investigated by methods in vitro, which enable us to establish the penetration mechanism, but do not allow us to investigate the role of blood during absorption.

Bucks et al. (1989) examined, in vitro, percutaneous absorption and stratum corneum epidermidis binding of alachlor and the effect of formulation dilution with water. Skin and human powdered stratum corneum epidermidis from these studies demonstrated a high capacity for alachlor. A soap-and-water solution effectively decontaminated powdered stratum corneum epidermidis. Using plasma as the receptor solution, penetration ranged from 0.5–4.0% of the applied dose for an 8-hr exposure period.

Methods in vivo are very important for the study of dermal absorption both by experimental animals and by people with occupational dermatitis. Investigations of toxokinetics of 12 C14-labeled pesticides have shown that all of them penetrate through the skin, with the lowest absorption level for dikwat and the highest absorption level for karbaryl (Feldman & Maiback, 1974).

Cholinesterase activity inhibition is often investigated in the evaluation of organophosphorus pesticide dermal penetration. The obtained results are interpreted on the basis of a strict dose–effect relationship (Fredriksson, 1969).

Dermal absorption was determined following topical application of C14-labeled lindane to the tail of rats. Trace levels of C14 activity were still detectable in blood and tail tissue on Day 72. The advantages of the rat tail model are highlighted (Moody, Grayhurst, & Ritter, 1989).

A study of occupational exposure to chlorfenvinphos by termite control workers engaged in spraying this organophosphorus insecticide indicates that urinary alkylphosphates were mainly derived from the dermal absorption of chlorfenvinphos. Sunaga, Yoshida, Ueda, Kasaka, and Hara (1989) suggested that biological monitoring using urinary alkylphosphates levels is a useful index for chlorfenvinphos exposure.

About 80% of occupational dermatitis is caused by irritating substances, and the majority of herbicides, fungicides, and selected insecticides have irritating properties, therefore, the World Health Organization (WHO) developed criteria for the classification of pesticide dermal toxicity, based on studies of acute dermal toxicity LD_{50} in rats (mg/kg cc). A different classification, designed by Kalojanowa, is used in Bulgaria. It is slightly different from the classification recommended by the WHO, both in relation to solid substances and liquids (Bainova, 1982). It is necessary to determine exactly the dermal toxicity of pesticides not only for legislative reasons, but primarily in order to improve their safety.

Chlorfenvinphos, used in our studies for dermal application, is an isomer of 2-chloro-1 (2,4-dichlorophenyl) vinyl diethyl phosphate. Chlorfenvinphos is the common name in general use. Trade names include Birlane, Enolofos, Dermaton, Sapecron, and Supona. Chlorfenvinphos was introduced in 1963 by Shell Development Company. It is an insecticide for use on foliage and soil. When sold under the name Dermaton, it is a miticide for veterinary use. Chlorfenvinphos shows an unusual degree of species variation in its acute toxicity. This applies to intravenous toxicity (LD_{50} in rats 6.6 mg/kg, in dogs 50 mg/kg), oral toxicity (LD_{50} in rats 1–15 mg/kg, in rabbits 300–1000 mg/kg, in dogs >5000–12000 mg/kg), as well as the dermal toxicity (LD_{50} in rats 30 mg/kg, in rabbits 400 mg/kg; Hayes and Laws, 1993).

Because of the unusually great difference in the toxicity of chlorfenvinphos in different species and because of the importance of dermal absorption associated with occupational exposure, its dermal toxicity was studied.

2. OBJECTIVE

The aim of this study was to investigate the effect of chlorfenvinphos dermal absorption on

1. the morphological picture of blood in rats;
2. the changes of body weight in animals during the 3-month experiment;
3. the histological and fine structure of the skin excised from the site of direct exposure, a site adjacent to the exposure zone, and a site 1.5–2.0 cm from the exposure zone; and
4. the histological structure of internal organs in rats exposed to chlorfenvinphos dermal absorption for 3 months.

3. MATERIALS AND METHODS

The study was conducted on 25 white Wistar strain rats with their initial weight ranging from 190–210 g. The animals were divided into five groups, 5 rats each. Among the experimental animals there were two groups with tail skin unhydrated before chlorfenvinphos exposure (the first and the third groups) and two groups with tails submerged in water for 1 hr directly before chlorfenvinphos exposure (the second and the fourth groups). In the first and the second groups rat tail skin was soaked in 0.5% chlorfenvinphos for 1 hr/day for 3 months. In the third and fourth groups rat tail skin was soaked in 0.05% chlorfenvinphos for 1 hr/day for 3 months. In the control group (the fifth group) rat tails were only hydrated, that is, soaked in water for 1 hr/day for 3 months.

The tails of all experimental rats were washed with soap and water prior to the application of chlorfenvinphos and then, after exposure, were thoroughly rinsed. Blood samples were taken from all rats prior to, and after completing, the experiment in order to determine the levels of erythrocytes, leukocytes, thrombocytes, and haemoglobin. Additionally, leukograms in all animals were studied. The animals in both the control and the experimental groups were weighed prior to the experiment as well as every week during the study. In order to investigate the differences in body weight between rats exposed to the dermal penetration of pesticide and rats in the control group, Student's t test was applied.

After 3 months all rats were anesthetized. Tail skin was excised for histological and ultrastructural studies from the following three sites: (a) the site of direct contact with the pesticide; (b) a site adjacent to the exposure zone; (c) a site 1.5–2.0 cm from the region of exposure. During the whole period of the study the site of exposure was limited by the application of collodium, which constituted a preventive border for the diffusion of liquids beyond the exposure site.

Additionally, the lungs, heart, liver, spleen and kidney were excised for histological studies. Histochemical examination by periodic acid–Schiff reaction (PAS) was applied to skin in order to detect polysaccharides.

4. RESULTS

After 6 weeks of dermal exposure to 0.05% chlorfenvinphos, 1 rat from Group 3 showed symptoms of spasticity and was anesthetized. The other animals did not show any clinical symptoms of intoxication.

Statistical analysis indicated that the body weight increase was lower only in the first and second experimental groups exposed to 0.5% chlorfenvinphos, in comparison with the control group.

In all experimental groups a decrease in the total number of leukocytes, compared to the initial values, was observed. After 3 months, absolute erythrocytes levels increased in all experimental animals (see Table 1).

The absolute level of neutrophils decreased during the course of the study in the experimental groups, except for the fourth group. Absolute eosinophils levels significantly increased

TABLE 1. Blood Morphology in Rats Pre- and Postexamination (Mean Values)

Examined Groups			Leucocytes G/l		Erythrocytes T/l		Hemoglobin g/l		Hematocrit		Thrombocytes G/l	
No.	Chlorfenvinphos concentration (%)	Skin condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1.	0.5	intact	7.90	6.00	7.32	7.73	12.50	10.80	0.48	0.54	848	770
2.	0.5	hydrated	10.83	9.65	6.80	8.40	12.90	14.60	0.47	0.51	870	870
3.	0.05	intact	7.85	6.40	7.40	8.70	13.90	14.50	0.48	0.49	872	900
4.	0.05	hydrated	6.80	5.70	7.90	8.70	13.00	13.20	0.41	0.51	891	892
5.		hydrated	10.20	10.00	7.73	7.71	12.20	12.80	0.46	0.49	830	870

in rats of the second and the third groups. The absolute lymphocyte level decreased in all experimental groups (see Table 2).

In the anesthetized rat, which had showed growing symptoms of nervous system disorders, no histopathological changes were observed in the examined organs. The control group animals showed only renal congestion without histopathological changes in other organs.

In rats exposed to 0.05% chlorfenvinphos penetration through intact skin, there were no histopathological changes in the heart, kidney, and spleen. Occasionally, subcapsular liver hyperaemia and focal congestion in the lungs were observed. Similarly, no histopathological changes in the heart, kidney, and spleen were found in animals exposed to 0.05% chlorfenvinphos through hydrated skin. It was only in 1 rat that subcapsular liver hyperaemia occurred, as well as extravasation in pulmonary alveoli and perivascular infiltrations in the lungs.

In rats exposed to 0.5% chlorfenvinphos penetration through hydrated and intact skin, slides of the heart and spleen did not show any pathological changes. No histopathological changes were observed in the kidneys of animals with the skin intact prior to exposure, either. However, renal congestion occurred in rats with tails hydrated before exposure. Evident liver congestion was observed in rats with intact skin. Pulmonary histopathological changes were similar in both experimental groups with intact and hydrated skin exposed to 0.5% chlorfenvinphos penetration. They manifested themselves as perivascular infiltrations and wider interalveolar septa.

In the control group with hydrated tail skin, epidermis did not show any deviations from the standard apart from slight condensation of the nuclei in the stratum basale. No histopathological changes were found in the dermis or in the subcutaneous layer.

No histopathological changes were observed in the skin adjacent to the site of exposure and 1.5–2.0 cm from the exposed zone. However, ultrastructural examination of hydrated skin showed changes in the membrane basale of the epidermis and in the cells of all its layers. The structure of the membrane basale was different. Some segments did not indicate any deviations from normal, but occasionally we observed sites of amorphous material of a higher electron

TABLE 2. Types of Leucocytes Pre- and Postexamination (Mean Values)

Examined Groups			Neutrophils G/l		Eosinophils G/l		Basophils G/l		Lymphocytes G/l		Monocytes G/l	
No.	Chlorfenvinphos concentration (%)	Skin condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1.	0.5	intact	3.21	2.99	0.53	0.37	—	—	4.08	2.63	0.14	0.34
2.	0.5	hydrated	4.48	3.88	0.29	1.36	—	—	5.75	4.39	0.30	0.43
3.	0.05	intact	2.89	2.47	0.38	0.93	—	—	4.34	1.95	0.26	0.41
4.	0.05	hydrated	2.36	2.55	0.38	0.27	—	—	3.71	2.62	0.25	0.21
5.		hydrated	3.63	3.67	0.38	0.38	—	—	5.80	5.65	0.27	0.31

density that formed the membrane. Then, the bright space, usually dividing it from the cell membrane of the stratum basale, was not present (see Figure 1). In the cells of the stratum basale, the stratum spinosum epidermidis and the stratum granulosum epidermidis, and the mitochondria were swollen and their matrix was electron bright with a few short fragments of cristae. The stratum corneum epidermidis usually adhered to the cells of the stratum granulosum epidermidis. The contours of the corneous plates were irregular and their position was looser than in normal skin. The internal structure of corneous plates did not significantly differ from normal.

In the hydrated skin in the zone adjacent to the exposed site, the membrane basale had a similar structure. In both the stratum basale and the stratum spinosum epidermidis, a considerable accumulation of cells was frequently observed, with a great number of ribosomes and nuclei with evenly distributed chromatin, that is, cells with features typical of young, weakly differentiated cells (see Figure 2). The mitochondria were swollen in the cells of all epidermal layers. The structure of the stratum corneum epidermidis was normal.

Two centimeters from the hydrated site, the membrane basale (between the epidermis and the dermis) did not show any deviation from the norm. Also, the structure of cells of the stratum basale was unchanged, with swollen mitochondria rarely found. The mitochondria of the higher layers were more swollen, with the normal structure of other cytoplasmic organellae. The stratum corneum epidermidis adhered to the lower stratum granulosum epidermidis and did not show any changes.

In rats with hydrated skin exposed to 0.5% chlorfenvinphos, the stratum corneum epidermis was significantly thicker. Its lower parts of compact consistency adhered to the other epidermis layers with the upper layers stratified and unstuck from the epidermis. The stratum spinosum epidermidis showed hyperplasia, especially in the perifollicular area. The stratum granulasum epidermidis became focally thicker. In the stratum basale no histopathological changes were observed. The slides of the dermis and the subcutaneous layer did not differ from the slides of the control group.

Ultrastructural examinations indicated that the structure of the membrane basale was normal only in some short segments. Most of it contained irregularly distributed electron-

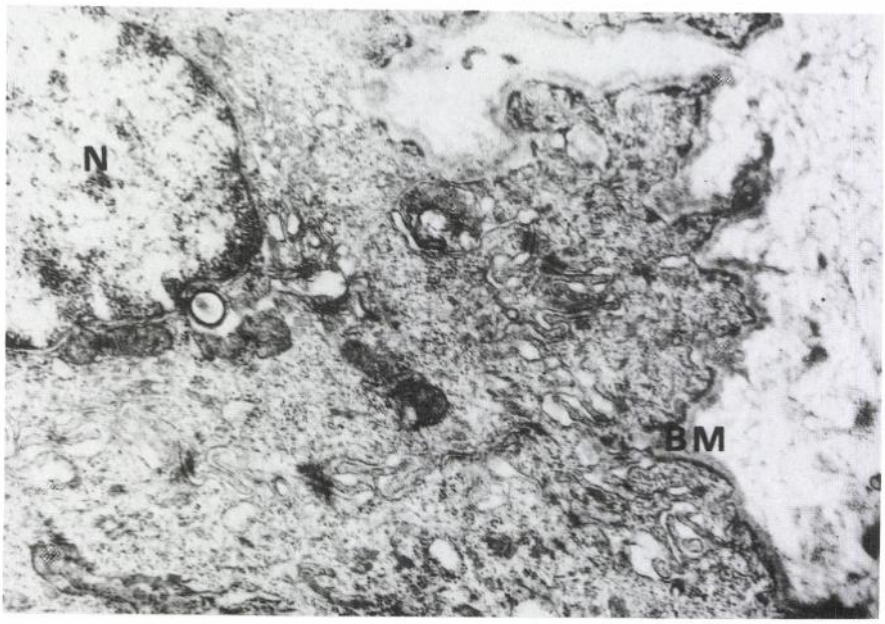

Figure 1. Amorphous material of high electron density formed the membrane basale. Bright space dividing it from the cell membrane of the stratum basale was not present. Mag. ca 25 000x. *Note.* BM = basement membrane; N = nucleus of the cell.

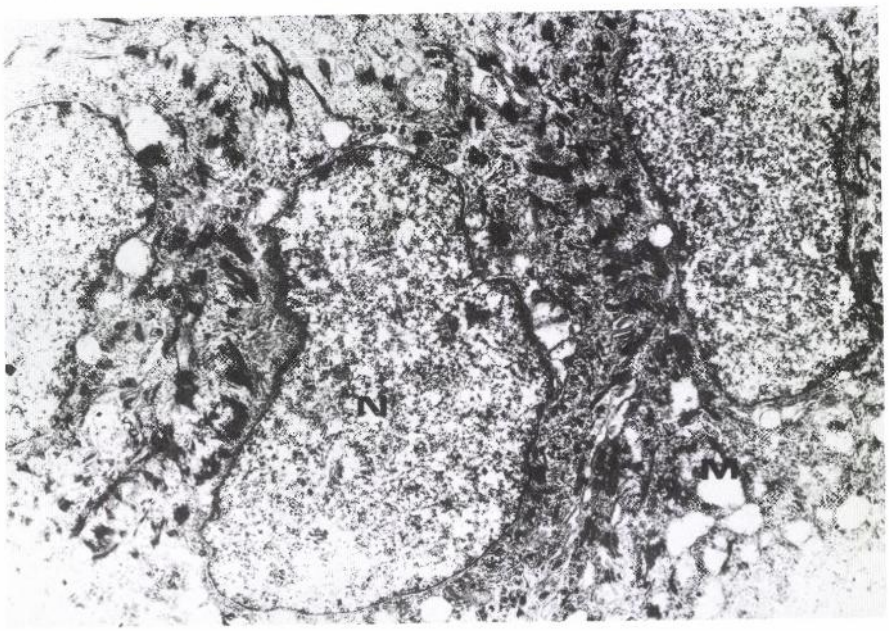

Figure 2. A great number of ribosomes and evenly distributed chromatin in the nucleus of the stratum basale cell. Mag. ca 14 500x. *Note.* N = nucleus of the cell.

dense material. In some places its accumulation was so great that the membrane basale became significantly thicker. However, the continuity of the membrane was always uninterrupted (see Figure 3). Similarly, swollen mitochondria were observed in the cells of all layers.

The stratum corneum epidermidis was formed out of plates surrounded by the pleated cell membrane, which frequently showed an irregular internal structure (see Figure 4).

In rats exposed to 0.05% chlorfenvinphos through hydrated skin, the hyperplasia of the stratum spinosum epidermidis was greater. The structure of the remaining epidermal layers, the dermis, and the subcutaneous layer was normal.

The epidermis of rats exposed to 0.05% or to 0.5% chlorfenvinphos through intact skin showed hyperkeratosis and the stratum corneum epidermidis separated from other layers. The stratum granulosum epidermidis and the stratum spinosum epidermidis became slightly thicker. No pathological changes were observed in the stratum germinativum epidermidis.

The penetration of 0.5% and 0.05% chlorfenvinphos through hydrated skin caused an evident hyperplasia of the stratum basale, which was more intense than at the site of direct exposure. In addition, hyperplasia of the stratum spinosum epidermidis and of the stratum granulosum epidermidis was observed. Similar changes, but of smaller intensity, were found in the epidermis of animals exposed to 0.5% or 0.05% chlorfenvinphos through intact skin.

Investigations of the fine structure were performed only on rats exposed to 0.5% chlorfenvinphos through hydrated skin. Dense, irregularly distributed material was found in the membrane basale. Young and occasionally even dividing cells were quite frequently found in the stratum basale and the stratum spinosum epidermidis (see Figure 5). Swollen mitochondria were observed in cells of all layers. The fine structure of the stratum corneum epidermidis was varied. Apart from squamae of the structure slightly different from normal, we observed others with irregular outlines and with clearly heterogenous internal structure (see Figure 6).

In rats of all groups, the epidermis at the sites distant from the exposure zone did not show any pathological changes. It is only in the cells of the dermis that infiltrations were occasionally found (see Figures 7 and 8). The fine structure of the membrane basale was close to normal. Sporadically, it became slightly thicker. In the cells of all layers a few swollen mitochondria

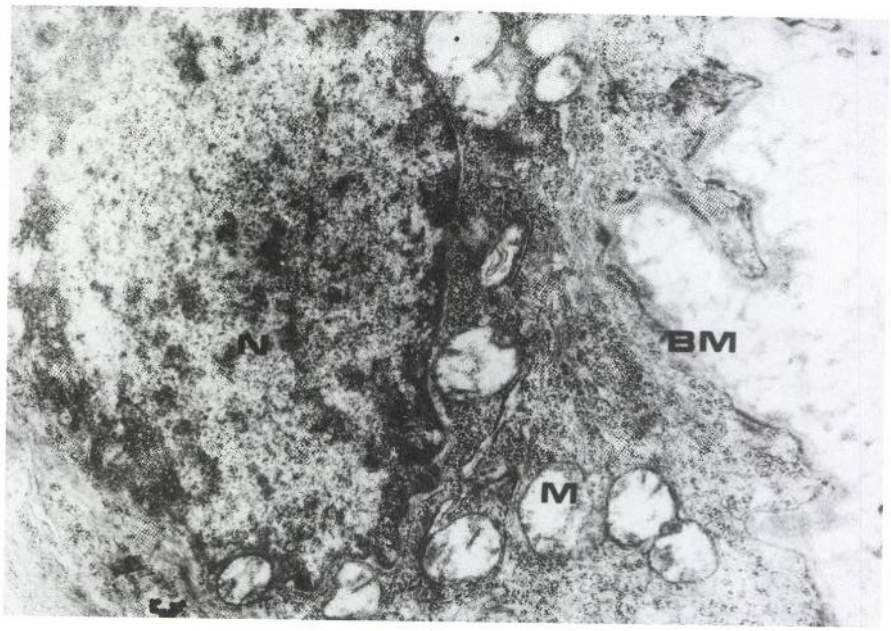

Figure 3. Continuity of the membrane basale uninterrupted but at some place it is significantly thicker because of electron-dense material. Mag. ca 25 000x. *Note.* BM = basement membrane; M = mitochondria; N = nucleus of the cell.

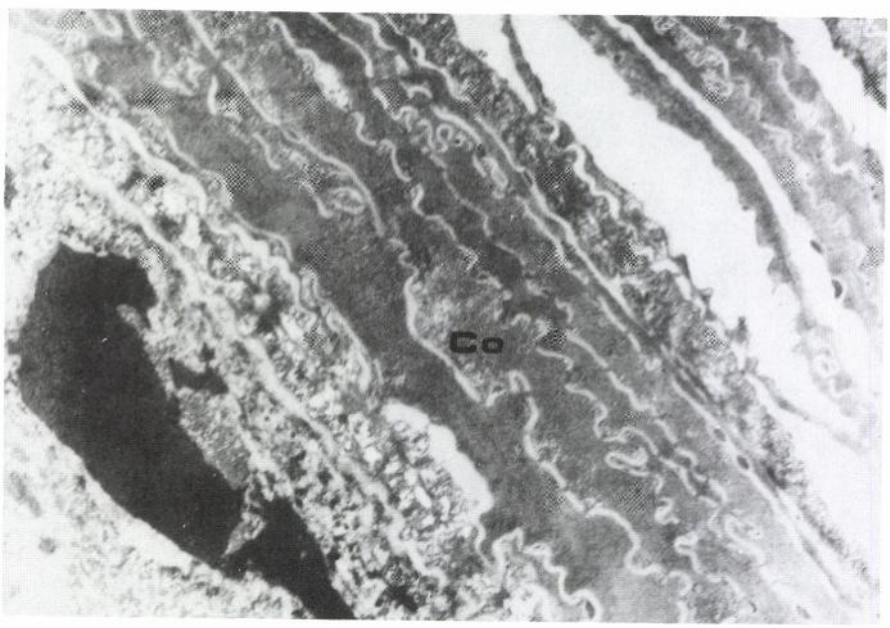

Figure 4. Irregular internal structure of stratum corneum epidermidis. Mag ca 25 000x. *Note.* Co = corneus plates.

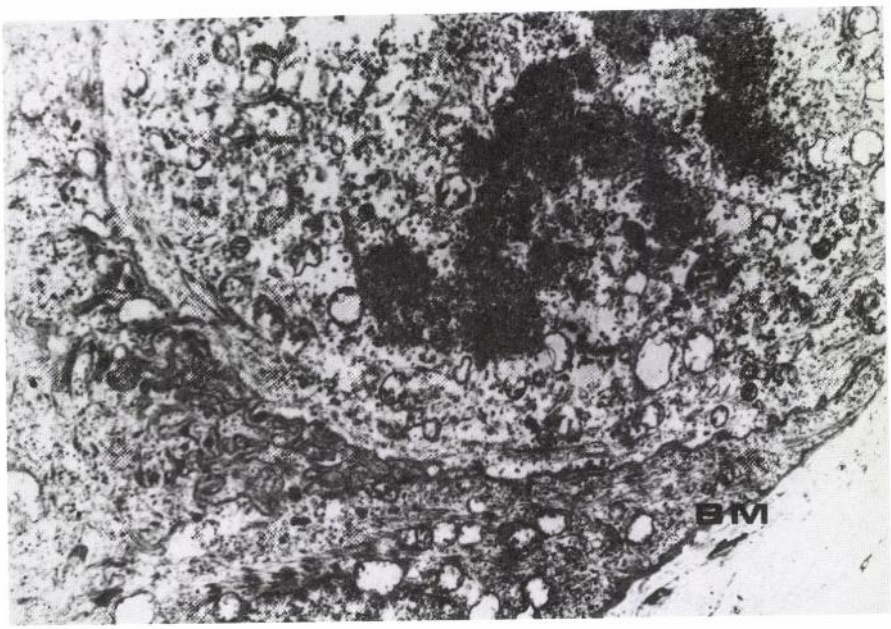

Figure 5. A dividing cell in the stratum basale. Mag. ca 14 000x. *Note.* BM = basement membrane.

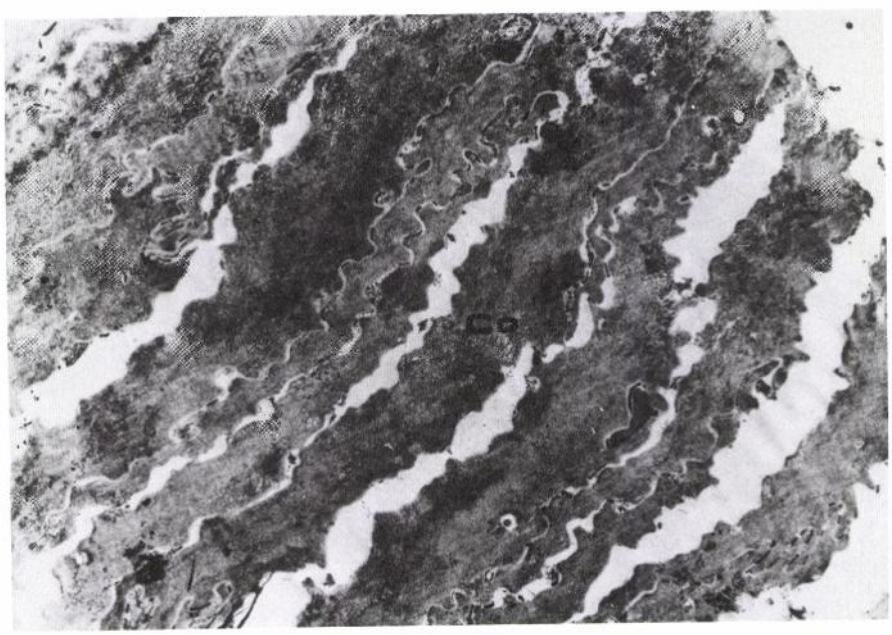

Figure 6. Squames with irregular outlines and heterogenous internternal structure. Mag. ca 14 000x. *Note.* Co = corneus plates.

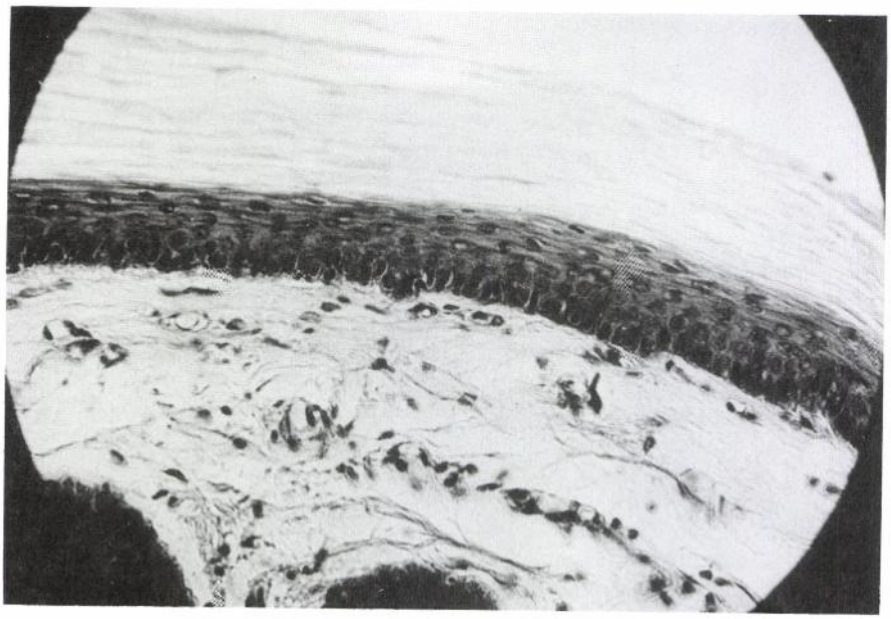

Figure 7. Dermis cells infiltrations and hyperaemia. Mag. ca 200x, h+e.

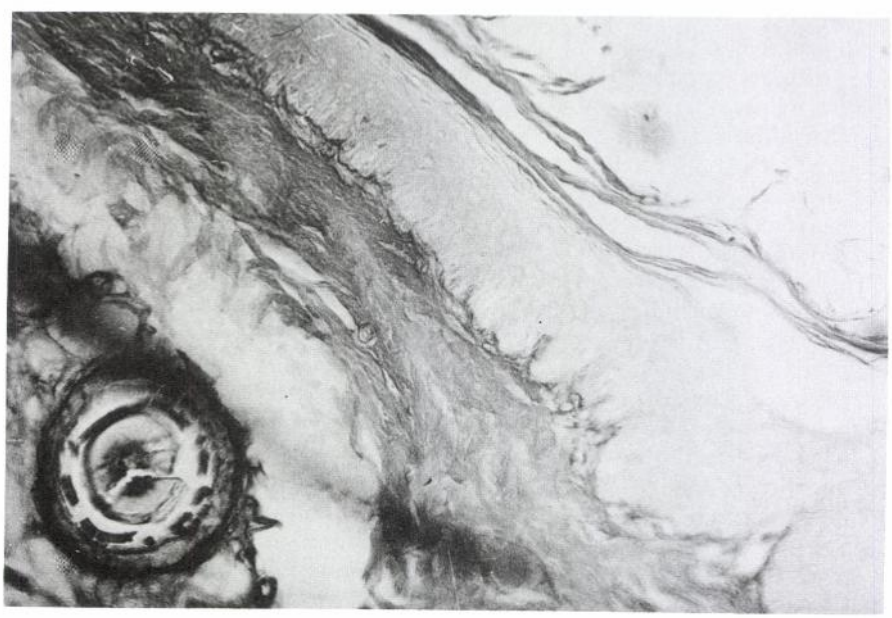

Figure 8. The basement membrane dyed by the PAS method (Schiff). Mag. ca 400x.

were found. The structure of the stratum granulosum epidermidis and the stratum corneum epidermidis was normal.

5. DISCUSSION

A lot of studies were performed to explain whether skin from various animals and their various body sites have different permeability properties. In Scott, Gorrigan, Smith, and Mason (1991), differences in permeability can be related to interspecies differences in skin structure, but only with the relatively slowly absorbed penetrants. Many research workers have evaluated the rat tail model to estimate the dermal absorption of pesticides as a highlighted model to predict skin resorption toxicity in man. Based on previous studies of skin structure in rabbits, guinea pigs, and rats from various body sites, Luty, Halliop, Tochman, and Latalski (1993a, 1993b) arrived at the same conclusions. Using the rat tail skin model they investigated the function of the skin barrier in the penetration of chlorfenvinphos. Rat tail skin was hydrated, it had the surface lipid membrane removed or the stratum corneum epidermidis damaged. The greatest penetration of pesticide was noted through hydrated skin (Luty et al., 1993a, 1993b). Therefore, the skin hydrated before topical application of chlorfenvinphos was used in the investigations described here.

In most experimental animals exposed to 0.05% or 0.5% chlorfenvinphos for 3 months, no symptoms of poisoning were observed. After 6 weeks only 1 rat showed evident symptoms of intoxication. The toxicity of the formulation dermally absorbed through hydrated and intact skin expressed itself as a statistically lower body weight increase only in rats exposed to 0.5% chlorfenvinphos water emulsion.

Histopathological changes in the internal organs of rats exposed to chlorfenvinphos dermal penetration for 3 months were insignificant. They were observed in a few rats only, and they revealed themselves as liver hyperaemia, pulmonary congestion, focal perivascular inflammation, and wider interalveolar septa.

Pelletier, Ritter, and Caron (1990), who examined the effects both of skin preapplication treatments and postapplication cleaning agents on the dermal absorption of 2,4-dichlorophenoxyacetic acid dimethylamine, found that the rat tails retained more than 75% of the material, thus preventing its absorption into the blood stream.

We may, therefore, assume that the insignificant histopathological changes in some of the animals, which were noted in our studies, were induced by a small amount of pesticide that had penetrated into the blood stream.

The histological examination of the epidermis in the zone of direct exposure to chlorfenvinphos and in the adjacent zone showed hyperkeratosis and a clear desquamation of the stratum corneum epidermidis. Simultaneously, compensatory hyperplasia of the stratum granulosum epidermidis and especially of the stratum spinosum epidermidis was observed. In the zone adjacent to the exposure site, the keratinization of the epidermis was significantly smaller, whereas in the remaining epidermis layers evident signs of greater germinative activity were noted. The stratum basale consisted of densely located young cells. The stratum spinosum epidermidis and focally the stratum granulosum epidermidis got thicker.

Electron microscopic studies confirm the histopathological results. In the epidermis at the site of exposure and in the adjacent zone, we observed a great concentration of young undifferentiated cells, occasionally during the process of division. Moreover, the position of the internal structure of the corneous plates was disturbed. However, the disorders were not greater. In fact, sometimes they were even of smaller intensity than the changes observed after a 1-month exposure to chlorfenvinphos (Luty et al., 1993a, 1993b). The changes in epidermis are most probably caused by the following mechanisms: (a) the stimulation of the stratum germinativum epidermidis, due to water and chlorfenvinphos, caused an intensification of the differentiation process; (b) the triggering of the adaptation mechanisms by the 3-month exposure. In addition, the pictures of fine structure showed the abnormal structure of the membrane basale with its continuity preserved.

Hardy, Swenny, and Bellows (1978) observed the damaged membrane basale in the epidermis of mice grown in vitro with an excess of vitamin A. It is assumed that the cells of the

deepest epidermis layers participate in the formation of the stratum basale. The long-lasting disorders of the respiration process in cells, confirmed in our studies by the evidently swollen mitochondria, may exert an influence on the metabolism of the cells and, consequently, lead to disorders in the normal synthesis of the membrane basale forming material.

A comparison of the pictures of skin at the direct exposure site and in an adjacent zone allows us to suppose that the investigated formulation penetrated through the epidermal barrier in the area of the stratum corneum epidermidis. Moreover, at the direct exposure site, it not only penetrated through the intracellular spaces to the dermis, but also easily spread in all directions.

The histological picture of the skin in sites adjacent to the exposure zone suggests that chlorfenvinphos penetrated into that area omitting the stratum corneum epidermidis.

6. CONCLUSIONS

1. Statistically significant lower body weight increases during the experiment, compared with the control group, were found only in rats exposed to higher chlorfenvinphos doses.
2. Evident downward tendencies in the absolute level of leukocytes and an increase in the absolute level of erythrocytes in all experimental animals dermally exposed to chlorfenvinphos were observed.
3. Histopathological changes in the internal organs were mildly manifested and were observed only in a few rats, mainly as liver and pulmonary hyperaemia.
4. The histological structure of rat tail skin varied according to the topical application of chlorfenvinphos:
   a) At the direct exposure site the dermis showed hyperkeratosis with intensive desquamation and compensatory hyperplasia of the stratum granulosum epidermidis and the stratum spinosum epidermidis;
   b) At a site adjacent to the exposed area the degree of keratosis, and the growth of the stratum granulosum epidermidis and the stratum spinosum epidermidis, was smaller compared with the direct exposure site. However, the stratum basale of the epidermis showed features of intensive growth;
   c) At a site 1.5–2.0 cm from the direct exposure site no significant histopathological changes were observed in the structure of epidermis or in the remaining skin layers.
5. The results of histological studies suggest that chlorfenvinphos penetrated through the epidermis barrier at the site of direct exposure, and then diffused vertically into the epidermis through intracellular spaces. Simultaneously, it diffused horizontally to areas up to about 1 cm from the direct exposure site.

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