Ann Agric Environ Med 1999, 6, 151–159

STUDIES OF TOXICITY OF DERMALLY-ABSORBED NURELLE D 550 EC PREPARATIONS

Jadwiga Latuszyńska, Sabina Luty, Janina Halliop, Ewa Przylepa, Alina Tochman, Daniela Obuchowska, Elżbieta Korczak

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

Latuszyńska J, Luty S, Halliop J, Przylepa E, Tochman A, Obuchowska D, Korczak E: Studies of toxicity of dermally-absorbed Nurelle D 550 EC preparations. *Ann Agric Environ Med* 1999, **6**, 151–159.

Abstract: The aim of the study was the evaluation of the toxic effect of a two-component, preparation Nurelle D 550 EC (500 g of chlorpyrifos and 50 g cypermethrin per 1 l), administrated dermally. Toxicity was evaluated from histological and ultrastructural studies of the internal organs and immunotoxic effects (evaluation of phagocytical and bactericidal activity of neutrophils). The preparation for dermal application was applied in 2 concentrations (200 mg/kg/day of chlorpyrifos plus 20 mg/kg/day of cypermethrin or 1000 mg/kg/day of chlorpyrifos, plus 100 mg/kg/day of cypermethrin). The preparation was administrated on the tail skin of female Wistar rats for 4 hours daily for a period of 4 weeks. After 28 days of the experiment, the animals were anaesthetised and blood was taken from the heart to evaluate the granulocyte system. The following organs were taken for histological studies: liver, kidney, lung, heart, spleen, thymus and lymph nodes. Ultrastructural studies were carried out on the lung, liver, kidney and heart. The results of the study showed that dermal application of the pesticide Nurelle D 550 EC resulted in slight morphological and ultrastructural changes in the liver, kidney, lung and heart. The preparation examined slightly elevated the bactericidal activity of neutrophils. The differences, however, were not statistically significant. The phagocytic reaction in animals of both experimental groups did not differ from that observed in control group.

Address for correspondence: Jadwiga Latuszyńska, PhD, Department of Pathomorphology, Institute of Agricultural Medicine, P.O. Box 185, 20-950 Lublin, Poland.

Key words: dermal toxicity, chlorpyrifos, cypermethrin, histopathology, ultrastructure, neutrophil activity.

INTRODUCTION

The list of chemicals used in Poland in 1998 covers 742 pesticides, including approximately 172 pesticides composed of 2 or more active substances.

The use of pesticides in mixtures may result in decreased toxicity of mixture components, additive toxicity or synergistic effects. The components which have no synergistic effect in acute poisoning also do not show these properties in subacute and chronic intoxication [35].

In studies conducted on laboratory rats exposed to lithium chloride, used as a drug in psychiatry, almost a four-fold increase was observed in the sensitivity of animals to spasmatic effect of methomyl. The spasmatic state was not noted only after the administration of methomyl [30].

An interesting example of synergism in the toxic effect of 2 or more substances is the study conducted on hens exposed to one of the pyrethroids, permethrin and pyridostigmine (reversible cholinesterase inhibitors), for a period of 2 months. These preparations, when applied separately, did not exert a toxic effect. The exposure to two or three preparations led to the occurrence of serious neurological disorders in which 40–90% of animals died [11]. After intragastric administration of pyridostygmine

Received: 27 August 1999
Accepted: 6 October 1999

and subdermal administration of DEET (N,N-diethyl-m-toluamide) or chlorpyrifos, in cases of co-exposure, considerably greater neurophatologic changes were noted, compared to those observed after administration of each preparation separately [1].

The studies conducted *in vitro* on nerve cells exposed to pesticides (dimethoat, methyl azinofos, diazinon, methyl-pirimifos, benomyl) administrated separately and in mixtures showed that these pesticides, when administered separately, exerted a greater inhibitory effect on acetylcholinesterase activity than when applied in mixtures. Opposite results were obtained with respect to the synthesis of proteins [18].

Oritz *et al.* [21], in an acute experiment conducted on male rats, confirmed an interaction (LD_{50} and cerebral cholinesterase activity) between parathion and permethrin. Xylene, when used as a solvent, did not affect the inhibition of cerebral cholinesterase induced by permethrin. Also, no relationship was observed between mortality and the inhibition of cerebral cholinesterase activity in rats which received mixtures of both preparations, or these components separately.

Nurelle D 550 EC is an insecticide and its active substances are chlorpyrifos and cypermethrin. Chlorpyrifos (0,0-diethyl,0-3,5,6-trichloro-2-pirydyl-phosphorothioate) is an insecticide which, like other organophosphorus compounds, inhibits acetylcholine decomposition, and therefore increases the acetylcholine level in the synaptic space and stimulates specific receptors [19]. When administered orally in a dose of 1/10 LD_{50}, chlorpyrifos led to a considerable inhibition of lactate dehydrogenase, glutamic pyruvic transaminase and glutamyltransferase. The addition of 0.5% ascorbic acid to the diet had a protective effect on the enzymes examined [8]. The neurotoxic effect of chlorpyrifos has been examined by many researchers. Transient behavioural states were observed in newborn and 21-day-old rats after exposure to chlorpyrifos [4]. Chlorpyrifos may have an embriotoxic and fetotoxic effect [5]. However, no teratogenic or oncogenic effect was noted [9].

Cypermethrin [(*R*)-cyano-3-phenoxybenzyl (*1R*)-cis-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate] is an active pyrethroid intensively controlling a wide spectrum of pests in agriculture and animal breeding. It appears on the market in the form of a concentrated suspension or in a mixture with other insecticides (Nurelle D 550 EC). Administration of this preparation to rats in doses of 200 and 180 mg/kg body mass (b.m.) for a period of 5 and 13 weeks did not result in histopathologic and hematologic changes. A high dose led to the symptoms of poisoning associated with changes in the nervous system, as well as a decrease in the body mass, or increase in the mass of the liver and kidneys. Similar results were obtained based on studies conducted on dogs [33].

The sensitivity of newborn rats to the toxic effect of cypermethrin is considerably greater than in adult rats [3]. Cypermethrin induces chromosome aberrations and the exchange of sister chromatides *in vivo* in the spleen of mice, in bone marrow, and *in vitro* in cell culture [2]. Supercypermethrin administered to rats intragastrically for 28 days (12.5, 8.75 and 4.38 mg/kg/day) had an inhibitory effect on phagocytic activity of granulocytes when administered in a higher dose, whereas lower doses had no influence on the parameters examined [29]. However, intragastric administration of alphacypermethrin for 28 days in a dose of 12 mg/kg/day had no toxic effect on the immunologic system [17].

In the literature available, no data were found concerning toxicity of dermally-applied pesticides mixtures composed of organophosphorus compounds and pyrethroids. Dermal absorption of pesticides is especially important in occupational poisonings. The speed of absorption depends on the solvent used and is usually slower than the uptake by other routes. There are, however, organophosphorus insecticides which are more toxic when administered dermally than orally. In the studies *in vitro*, the rat epidermis was 20 times more permeable to cypermethrin than human epidermis [23]. However, in an experiment conducted *in vivo* on rats, only 1% of dermally-applied cypermethrin marked with carbon was absorbed after 8 hours [24]. Studies carried out on humans showed that the dermal penetration of pyrethroids was low compared to other insecticides [10, 32].

The aim of the study was the investigation of the effect of a dermally-applied Nurelle D 550 EC preparation on the histological and ultrastructural appearance of selected organs, and on the phagocytic and bactericidal activity of peripheral blood neutrophils.

MATERIALS AND METHODS

Nurelle D 550 EC (500 g chlorpyrifos and 50 g cypermethrin) (Dow Elanco, USA) was used in the study. The application liquid was in the form of a water solution.

The study was conducted on 3 month old female Wistar rats, in good condition, with no macroscopic changes of the tail skin. The animals, fed with standard feed LSM [13], were separated into 3 groups (2 experimental and 1 control), of 10 rats each. Experimental groups received 200 mg/kg/day chlorpiryfos plus 20 mg/kg/day cypermethrin, or 1000 mg/kg/day chlorpiryfos plus 100 mg/kg/day cypermethrin dermally, for 4 weeks, except Saturdays and Sundays. The examined preparation was applied on the tail skin of rats with the use of an absorptive fabric FPP-15, and isolated from the environment with aluminium foil [27]. The time of exposure was 4 hours daily. The animals of the control group were exposed to the dermal absorption of the water at the same time and under the same conditions.

After 28 days of the experiment, the animals were anaesthetized and blood taken from the heart in order to evaluate the activity of granulocytic system. For evaluation of phagocytic properties of neutrophils, a phagocytic reaction with Bacto-Latex (Difco, USA) and

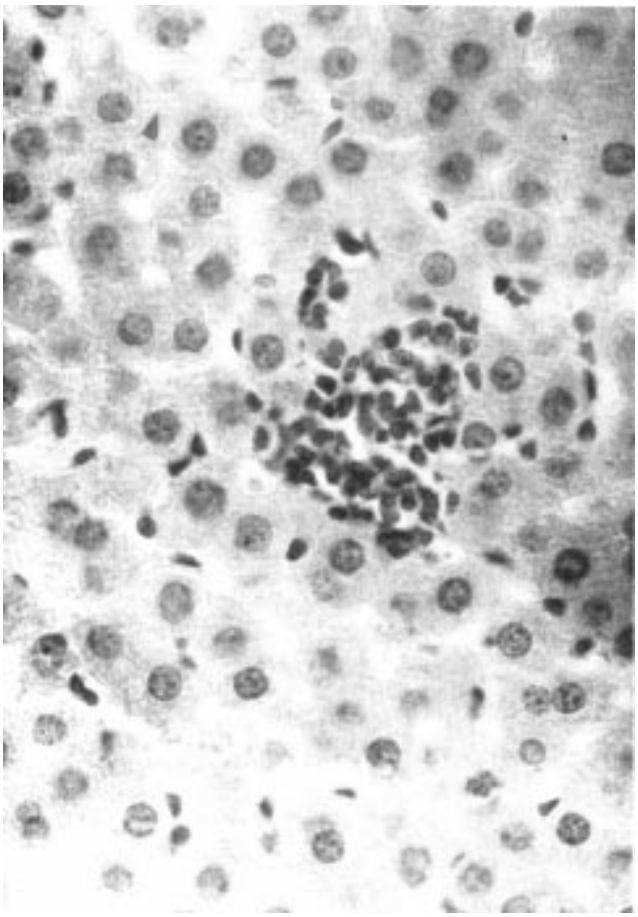

Figure 1. Liver of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). A few small infiltrations consisted of lymphocytes and histiocytes. In single hepatocytes signs of parenchyma degeneration. H-E, × 160.

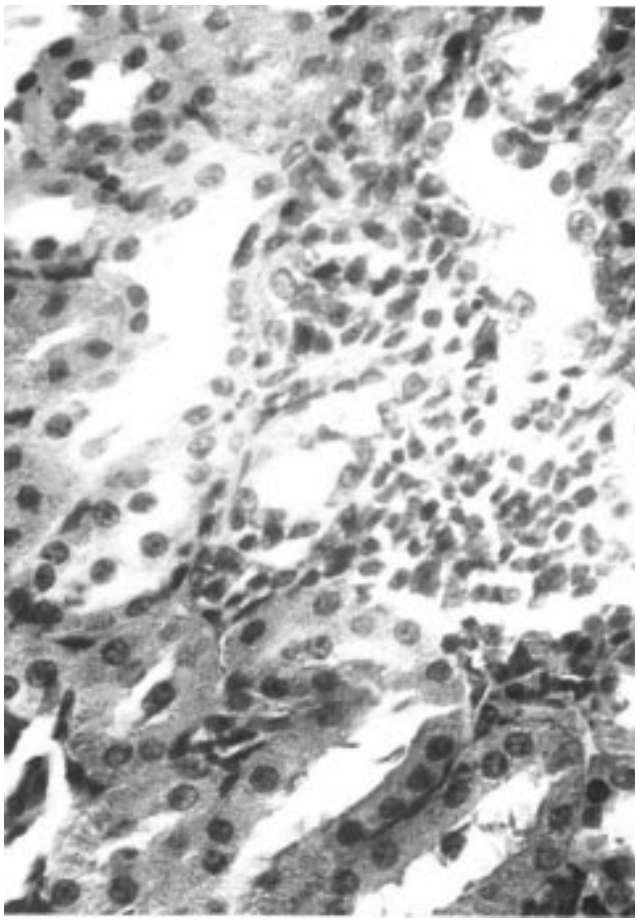

Figure 2. Kidney of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). A few infiltrations of mononuclear cells were noted between the proximal tubules or around blood vessels. H-E, × 160.

NBT test were used. In phagocytic test, cells containing 3 latex grains were considered as positive [26]. In the NBT test, cells containing large grains of formazan were also considered as positive [22]. In both tests, full peripheral blood was used. In each test, 100 cells were counted. The numbers of positive cells per 100 cells analysed were determined as indices of the tests applied.

For histological examination, the lung, liver, heart, kidney, thymus, spleen and lymphatic nodes were evaluated. Organs were fixed in 10% neutral buffered formalin, embedded in paraffin and stained with H+E. For ultrastructural studies, the heart, liver, kidney and lung were examined. The material was fixed in 4% glutaraldehyde buffered to pH 7.2-7.4 with 0.1 M sodium cocadylate and postfixed with 1% water solution of OsO_4. Dehydration was carried out with ethyl alcohol in a concentration up to absolute. The material was embedded in Epon 812. Ultrathin specimens were observed and photographs taken using a Tesla BS 500 electron microscope.

The results were presented as mean ($\bar{x}$) ± standard error (SEM). Statistical analysis was performed by the parametric Student's t-test.

RESULTS

Histologic and ultrastructural studies. In histological studies of the liver after administration of the higher dose of the mixture of chlorpyrifos and cypermethrin, in 40% of animals a few small infiltrations were observed consisting of lymphocytes and histiocytes located between hepatocytes. In single liver cells, signs of parenchyma degeneration were noted (Fig. 1).

On the level of an electron microscope, after administration of a lower dose of the mixture, slight empty cytoplasm spaces were observed in the vascular pole of single hepatocytes. These empty spaces were filled with a small amount of membranous and granular material (Fig. 4). When a higher dose of the pesticide was administered, the changes affected a greater number of cells. In some cells an increased number of peroxysomes were observed (Fig. 5).

In kidneys, after administration of the higher dose of the mixture, in 40% of animals a few infiltrations of mononuclear cells were noted between the proximal tubules or around blood vessels (Fig. 2).

In ultrastructural specimens from animals of the experimental group, which were administered chlorpyrifos

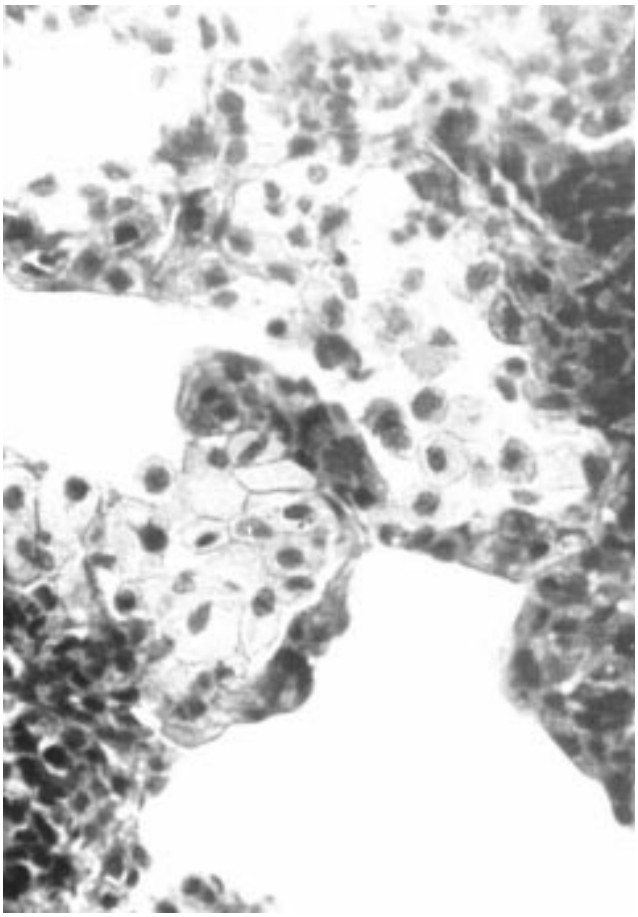

Figure 3. Lung of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). Foci of various sizes consisting of lymphatic tissue and foam cells. H-E, × 160.

and cypermethrin in a lower dose, degenerative changes of small intensity were observed in some cells of proximal tubules. These changes consisted of empty spaces and partial vacuolization of the cytoplasm. The above changes intensified after administration of a higher dose of the pesticide and were accompanied by an increase in the number of electron dense bodies (Fig. 6). The disorders also affected a few renal glomeruli and were manifested as vacuolization and lighting of basic cytoplasm of podocytes (Fig. 7).

In the lungs of a small number of animals, foci of various sizes were observed consisting of lymphatic tissue and foam cells, both beneath the capsule and in interalveolar septa (Fig. 3). Ultrastructural studies showed deviations in the structure of cells in a few alveoli, and only in short segments of the respiratory barrier after administration of a higher dose of the mixture. The changes were manifested by the swelling of both vascular endothelial cells and type I alveolar epithelial cells (Fig. 8).

In sub-microscopic studies, swelling of mitochondria was noted in some cardiomyocytes, as well as swelling of endothelium in capillary vessels of the heart muscle after administration of a higher dose of chlorpyrifos and cypermethrin (Fig. 9).

Table 1. Results of nitrobluetetrazolium test (NBT) and of the phagocytosis latex test (PLT) with whole blood neutrophils in rats exposed by dermal absorption to Nurelle D 550 EC (500 g chlorpyrifos and 50 g cypermethrin).

Examined groups	N	Index of NBT ($\bar{x}$ ± SEM)	Index of PLT ($\bar{x}$ ± SEM)
Rats exposed to: Chlorpyrifos 200 mg/kg Cypermethrin 20 mg/kg	10	7.5 ± 1.68	93.0 ± 0.74
Rats exposed to: Chlorpyrifos 1000 mg/kg Cypermethrin 100 mg/kg	10	9.1 ± 2.17	93.0 ± 1.04
Control group	10	5.4 ± 1.29	89.6 ± 2.01

No changes were observed in the histologic structure of spleen, thymus and lymph nodes.

Immunologic studies. Chlorpyrifos plus cypermethrin applied to the tail skin in doses of 1000 mg/kg/day plus 100 mg/kg/day or 200 mg/kg/day plus 20 mg/kg/day, respectively, caused a slightly elevated bactericidal activity of neutrophils compared to the control group. The differences, however, were not statistically significant. The slightly elevated, phagocytic reaction in animals of both experimental groups did not differ statistically from that observed in the control group (Tab. 1).

DISCUSSION

Based on our previous studies concerning the effect of chlorfenvinfos, carbaryl, and the mixture of both preparations administered intragastrically for a period of 3 months in doses of 1/10, 1/50 and 1/100 LD_{50}, no clear destructive changes were observed in the internal organs of rats [14]. A single dermal application of 5% chlorpyrifos also did not cause changes in the internal organs [27]. No changes were observed in the liver after oral administration of chlorpyrifos in the dose 50 mg/kg [34]. Alphacypermethrin dermally-applied in rats in the dose 250 mg/kg/day for a period of 28 days led to slight changes in the liver, kidneys, lungs and heart, discernable by light and electron microscopy [15].

The studies by Łukowicz-Ratajczak and Krechniak [16] on the influence of cypermethrin on the renal function and metabolism showed that this preparation has no nephrotoxic effect. Toukhy and Girgis [28], described an inhibitory effect of cypermethrin on the activity of total ATP in the liver of rats, which may cause disorders in the active transport of Na+, K+ and Ca++ ions, and lead to pathologic changes in the liver cells.

Perger and Szadkowski [23], reported a slight growth of nodules in the lungs of female rats after administration of cypermethrin. In other subacute and acute studies of the toxicity of pyrethroids on laboratory animals,

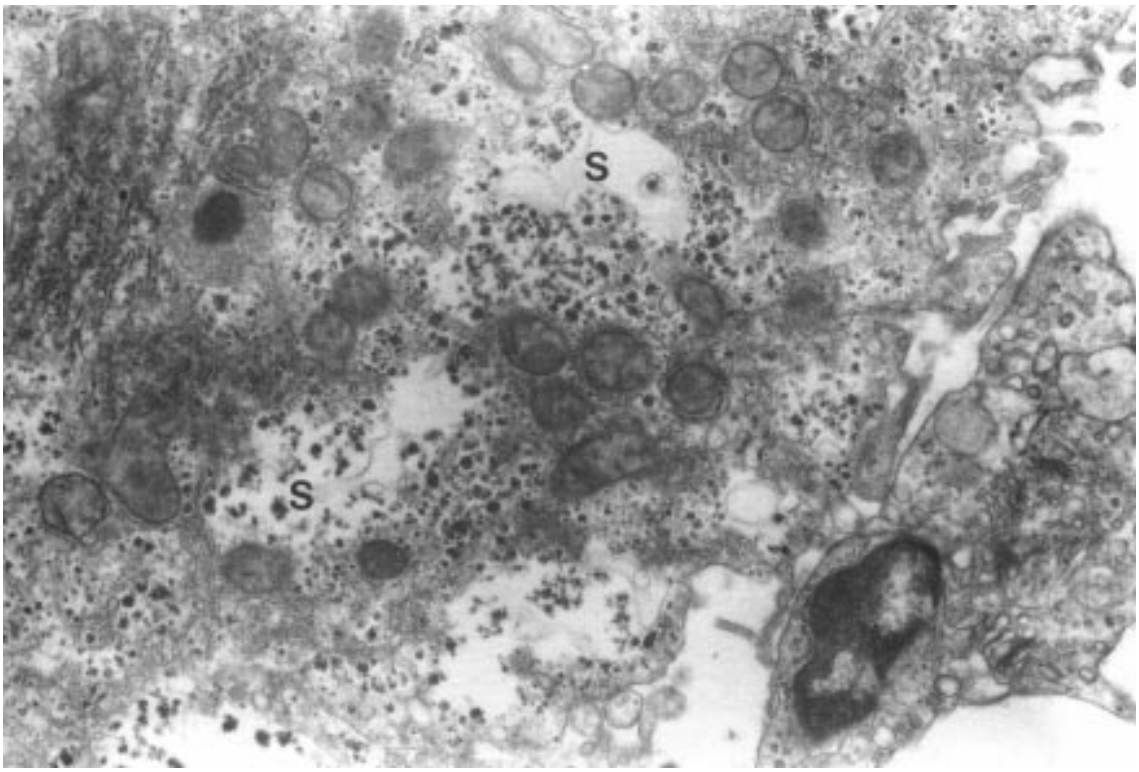

Figure 4. Liver of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (200 mg/kg chlorpyrifos and 20 mg/kg cypermethrin). Empty spaces within a hepatocyte (S) with a small amount of membranous and granular material. EM, × 15 000.

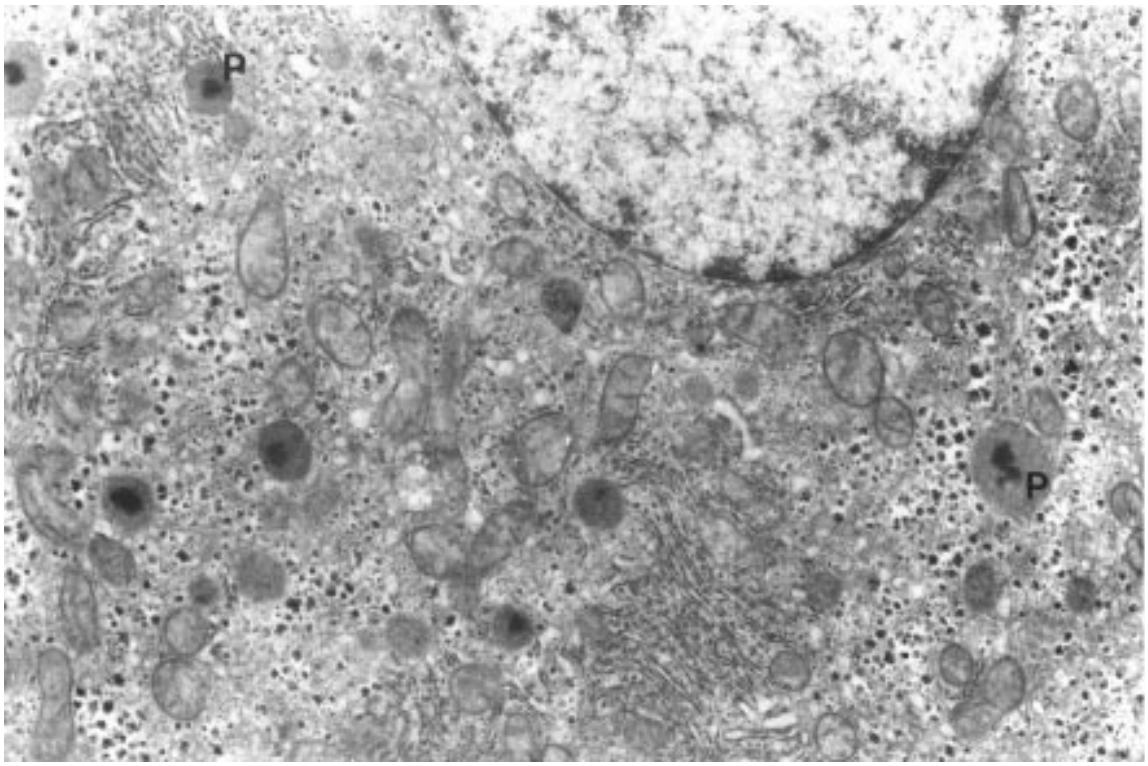

Figure 5. Liver of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). Increased number of peroxysomes (P) in some cells. EM, × 10 000.

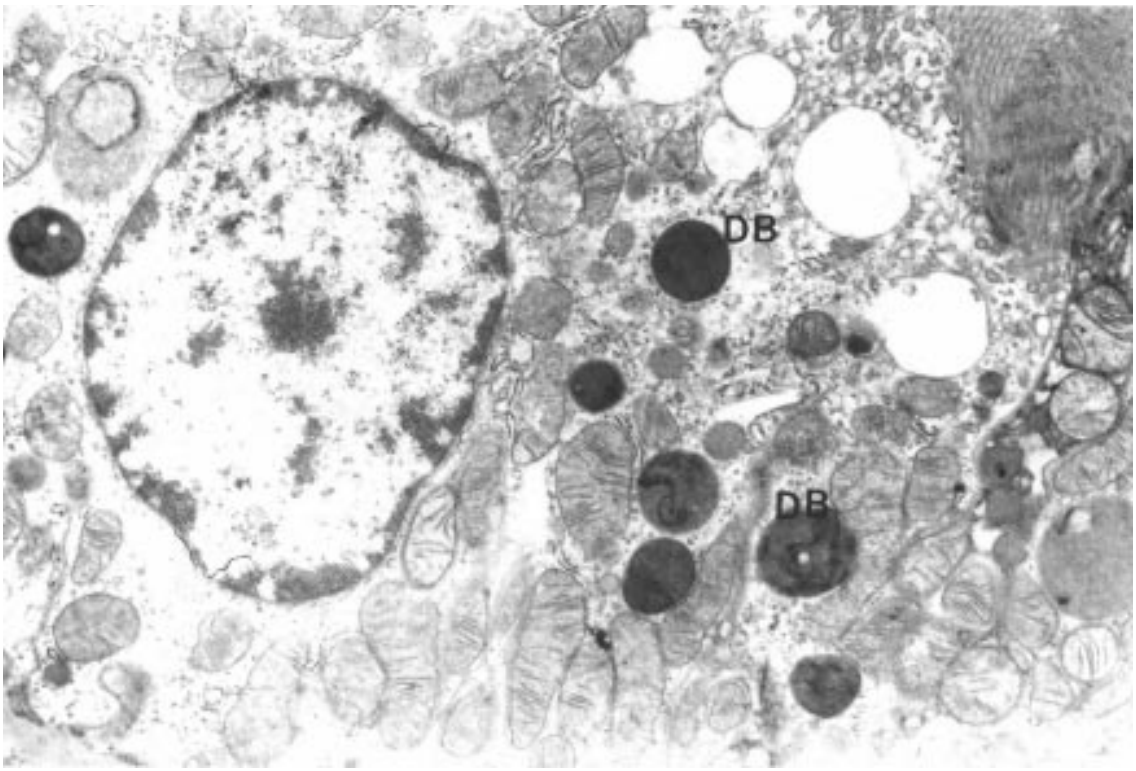

Figure 6. Kidney of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). An increase in the number of electron dense bodies (DB). EM, × 10 000.

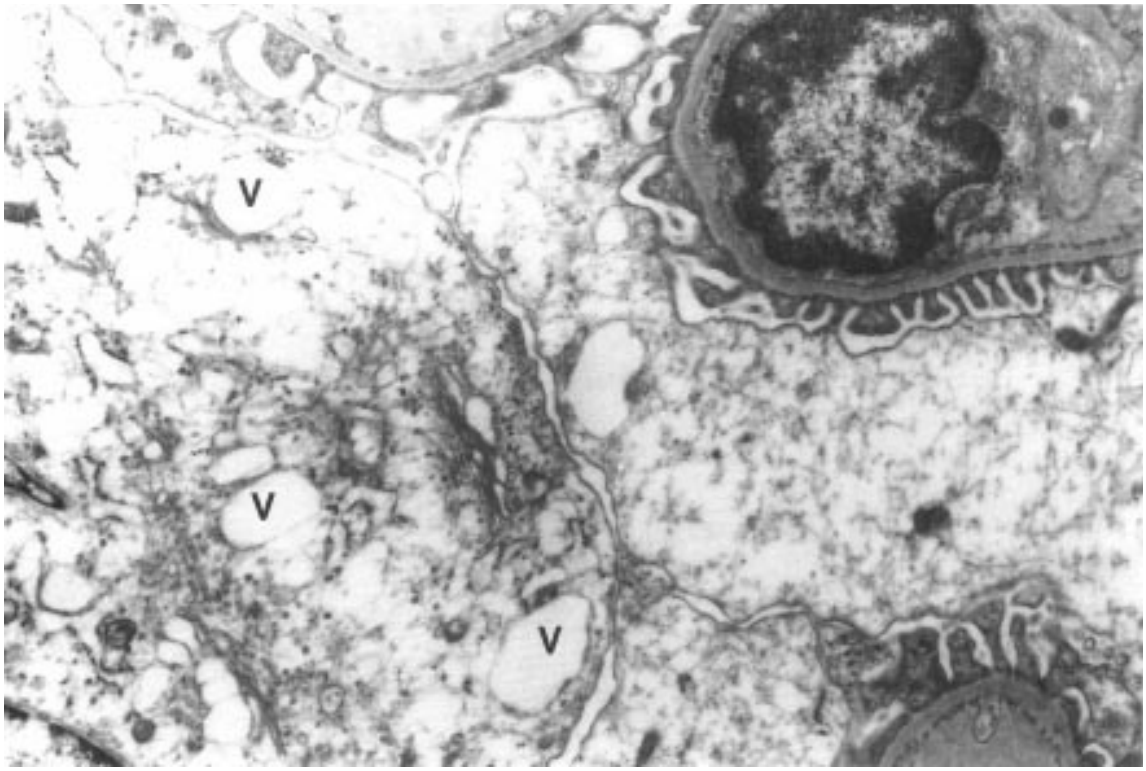

Figure 7. Kidney of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). Vacuolization (V) and lighting of basic cytoplasm of podocytes . EM, × 10 000.

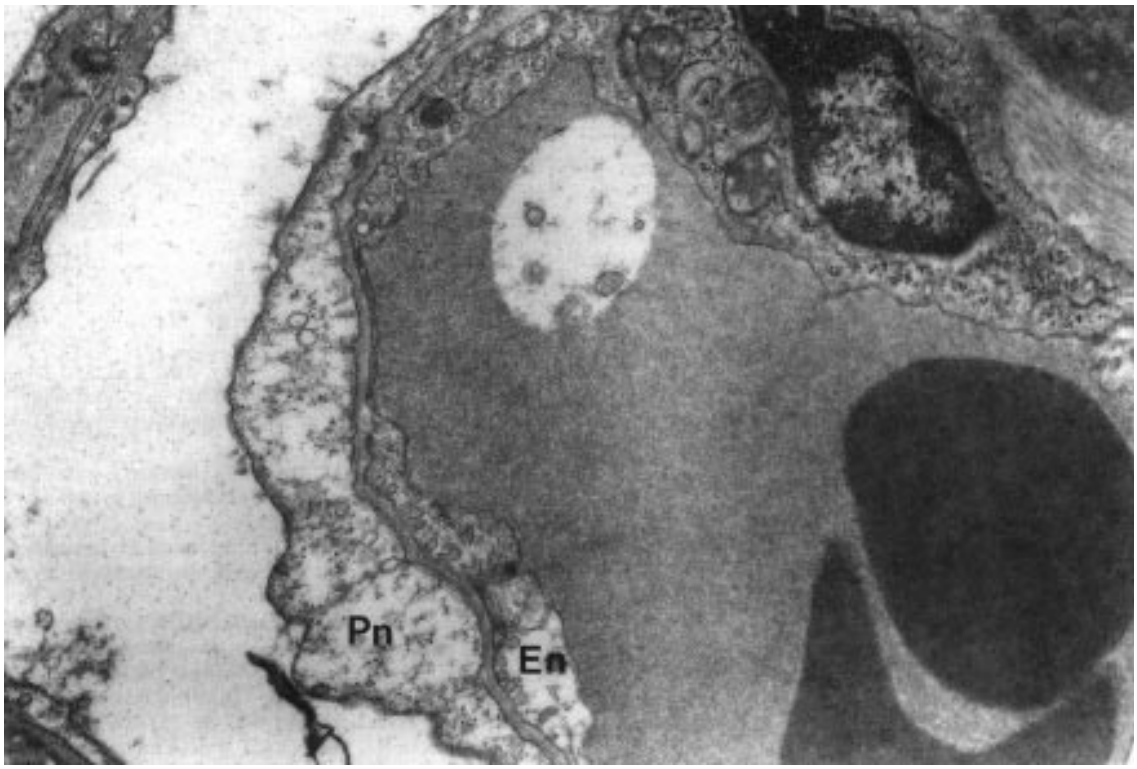

Figure 8. Lung of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). The swelling of vascular endothelial cells (En) and type I alveolar epithelial cells (Pn). EM, × 20 000.

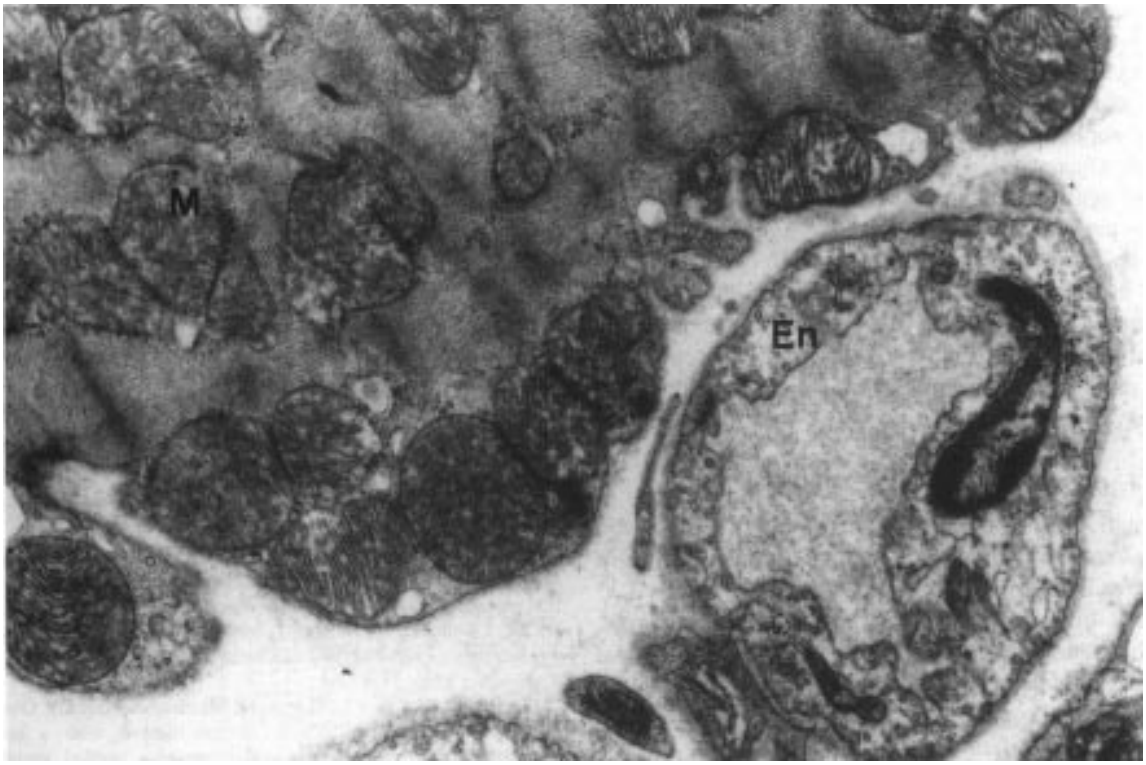

Figure 9. Myocardium of a rat exposed by dermal absorption to chlorpyrifos and cypermethrin (1000 mg/kg chlorpyrifos and 100 mg/kg cypermethrin). Swelling of mitochondria (M) in cardiomyocytes and swelling of endothelium (En) in capillary vessels. EM, × 10 000.

administered in high doses, a decrease in the body weight was observed, as well as hypertrophy in the liver and kidneys [23]. After administration of fenvalerate, a great number of micro-granulomas in the liver and kidneys of mice and rats was reported [20].

In our studies, after administration of the mixture of chlorpyrifos and cypermethrin for 28 days (4 hour/day), histologic changes in the liver, kidneys, lungs and heart were observed by light and electron microscopy, especially after administration of a higher dose of the mixture. The changes after administration of the mixture of chlorpyrifos and cypermethrin were not greater than those noted after administration of alphacypermethrin alone.

The studies by Tulińska *et at.* [29] showed that supercypermethrin administered orally resulted in an elevated humoral and cellular response in Wistar rats, in a subacute experiment (28 days) after administration of 1/40 LD_{50} of this preparation, whereas higher doses (1/20 and 1/14 LD_{50}) had a suppressive effect. Desi *et al.* [6, 7] reported suppression of humoral and cellular immunological response after administration of cypermethrin. Cypermethrin (suspended in oil) administered orally to male rats in doses of 5, 10, 20 and 40 mg/kg daily for 90 days caused a humoral response and leucopenia, but only after administration of the highest dose [31]. Based on the studies on humans (males and females) participating in the production of liquid pesticides, a significant decrease was noted in the number of neutrophils, while in NBT test an increase in oxidant production by neutrophils was noted [12]. Similar studies conducted on humans producing dusty pesticides and exposed to inhalation of dust containing 28-65% SiO_2 showed an increase in oxidant production in the NBT test and in phagocytic test in females and males, compared to the control group [25].

In the studies of the immunotoxicity of alphacypermethrin dermally applied for 4 weeks, the preparation examined did not significantly elevate the bactericidal and phagocytic activity of neutrophils in the dose 50 mg/kg/day [15].

In the present study, chlorpyrifos and cypermethrin applied to the tail skin of rats in both doses, slightly elevated the oxidant production by neutrophils, compared to the control group.These differences, however, were not statistically significant. The phagocytic reaction in animals of both experimental groups did not differ from that observed in the control group.

CONCLUSIONS

1. Small histopathologic changes were observed in the liver, kidney and lung after dermal application of a chlorpyrifos and cypermethrin mixture.
2. Ultrastructural changes were observed in the liver, kidney, lung and heart.
3. The pesticide examined slightly elevated the oxidant prodution by neutrophils; the differences, however, were not statistically significant.
4. Phagocytic reaction in animals exposed to the examined pesticide did not differ from that observed in the control group.

REFERENCES

1. Abou-Donia MB, Wilmarth KR, Abdel-Rahman AA, Jensen KF, Oehme FW, Kurt TL: Increased neurotoxicity following concurrent exposure to pyridostigmine bromide, DEET and chlorpyrifos. *Fundam Appl Toxicol* 1996, **34**, 201-222.
2. Amer SM, Ibrahim AA, El-Sherbeny KM: Induction of chromosomal aberations and sister chromatid exchange in vivo and in vitro by the insecticide cypermethrin. *J Appl Toxicol* 1993, **13**, 341-345.
3. Cantalamessa F: Acute toxicity of two pyrethroids, permethrin and cypermethrin in neonatal and adult rats. *Arch Toxicol* 1993, **67**, 510-513.
4. Chanda SM, Pope CN: Neurochemical and neurobehavioral effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. *Pharmacol Biochem Behav* 1996, **4**, 771-776.
5. Deacon MM, Murray JS, Pilny MK, Rao KS, Dittenber DA, Hanley TR, John JA: Embryotoxicity and fetotoxicity of orally administered chlorpyrifos in mice. *Toxicol Appl Pharmacol* 1980, **54**, 31-40.
6. Desi J, Dobronyi I, Varga L: Immuno-, neuro- and general toxicologic animals studies on a systhemic pyrethroid. *Ecotoxicol Environ Safety* 1986, **12**, 220-223.
7. Desi J, Varga L, Dobronyi I, Szklenarik G: Immunotoxicological investigation of the effect of a pesticide: cypermethrin. *Arch.Toxicol* 1985, **8**, 305-309.
8. Enann EE, El Sebae AH, Enann OH, El Fiki S: In-vivo interaction of some organophosphorus insecticides with different biochemical targets in white rats. *J Environ Sci Health* B 1982, **17,** 549-570
9. FAO and WHO working groups: Chlorpyrifos. FAO Plant Production and Protection Paper 1982, **49**, 153-156.
10. Feldmann RJ, Maibach HJ: Percutaneous penetration of some pesticides and herbicides in man. *Toxicol Appl Pharmacol* 1974, **28**, 126-132.
11. Jensen K, Oehme FW, Kurt TL: Neurotoxicity resulting from coexposure to pyridostygmine bromide, DEET, and permethrin: Implications of Gulf War chemical exposures.. *J Toxicol Environ Health* 1996, **48**, 35-56.
12. Kluciński P, Hrycek A, Stasiura-Zielińska H, Kossmann S, Tustanowski J, Friedek D, Kamińska-Kołodziej B: Humoral and cellular immunity rates in chemical plant workers employed in the production of liquid pesticides. *Int J Occup Med Environ Health* 1996, **9**, 103-110.
13. Królikowska-Prasał I, Kifer-Wysocka E, Matysiak W, Romanowska-Sarlej J: Morfologische Beurteilung und Analyse von Superelementen in der Leber von Ratten, die mit Kraftwerk-Aschen enthaltendem Futter gefuttert wurden. *Gegenbaurs Morphol Jahrb* Leipzig 1990, **136**, 565-574.
14. Latuszyńska J, Tość-Luty S, Przylepa E: Wpływ chlorfenwinfosu, karbarylu i mieszaniny obu preparatów na narządy wewnętrzne szczurów. *Bromat Chem Toksykol* 1973, **6**, 359-365.
15. Luty S, Latuszyńska J, Halliop J, Tochman A, Obuchowska D, Przylepa E, Korczak B: Toxicity of dermally applied alpha-cypermethrin in rats. *Ann Agric Environ Med*. 1998, **5**, 109-115.
16. Łukowicz-Ratajczak J, Krechniak J: Wpływ dekametryny i cypermetryny na funkcję i metabolizm nerek. *Bromat Chem Toksykol* 1991, **24**, 133-137.
17. Madsen C, Claesson MH, Ropke C: Immunotoxicity of the pyrethroid insecticides deltamethrin and alpha-cypermethrin. *Toxicol* 1996, **3**, 219-227.
18. Marinovich M, Ghilardi FM, Galli CL: Effect of pesticide mixtures on in vitro nervous cells; comparison with single pesticides. *Toxicol* 1996, **3**, 219-227.
19. Nostrandt AC, Padilla S, Moser VC: The relationship of oral chlorpyrifos effects on behavior, cholinesterase inhibition and muscarinic receptor density in rat. *Pharmacol Biochem Behav* 1997, **1**, 15-23.
20. Okuno Y., Ito S, Seki T, Murakami M, Kadota T, Miyamoto J: Fenvalerate – induced granulomatous changes in rats and mice. *J Toxicol Sci* 1986, **11**, 53-66.
21. Ortiz D, Yanez L, Gomez H, Martinez-Salazar JA, Diaz-Barriga F: Acute toxicological effects in rats treated with a mixture of commercially formulated products containing methyl parathion and permethrin. *Ecotoxicol Environ Saf* 1995, **32**, 154-158.
22. Park BH, Fikrig SM, Smithwick EM: Infection and nitroblue-tetrazolium reduction by neutrophils. *Lancet* 1986, **2**, 532.

23. Perger G, Szadkowski D: Toxicology of pyrethroids and their relevance to human health. *Ann Agric Environ Med* 1994, **1**, 11-17.

24. Scott RC, Ramsey JD: Comparison of the in vivo and in vitro percutaneous absorption of a lipophilic molecule (cypermethrin, a pyrethroid insecticide). *J Invest Dermatol* 1987, **9,** 142-146.

25. Śliwiński Z, Hermanowicz A, Kossmann S, Hrycek A: Neutrophil function in chemical plant workers employed in the production of dust pesticides. *Pol J Occup Med* 1991,**4-3,** 1991.

26. Ślopek S: *Immunology in Practice*. PZWL Warszawa, 1979.

27. Tość-Luty S, Latuszyńska J, Halliop J., Tochman A, Przylepa E, Bychawski E, Obuchowska D: Skin penetration of selected pesticides. *Ann Agric Environ Med* 1994, **1**, 57-67.

28. Toukhy MA, Girgis RS: In vivo and in vitro studies on the effect of larvin and cypermethrin on adenosine triphosphate activity of male rats. *J Environ Sci Health* B 1993, **28**, 599-619.

29. Tulińska J, Kubova J, Janota S, Nyulossy S: Investigation of immunotoxicity of supercypermethrin forte in the Wistar rat. *Hum Exp Toxicol* 1995, **14**, 399-403.

30. Turski WA: Charakterystyka toksykologiczna pestycydów - wybrane problemy. **In:** Pomorska K (Ed): *Zagrożenia Chemiczne w Rolnictwie*, 31–41. Institute of Agriculture Medicine, Lublin 1997.

31. Varshneya C, Singh T, Sharma LD, Bahga HS, Garg SK: Immunotoxic response of cypermethrin, a synthetic pyrethroid. *Indian J Physiol Pharmacol* 1992, **36**, 123-126.

32. Wester RC, Maibach HJ: In vivo percutaneous absorption and decontamination of pesticides in humans. *J Toxicol Environ Health* 1985, **16,** 25-37.

33. WHO-working group: Alpha-cypermethrin. *Environmental Health Criteria* 1992, **142**, 112.

34. Vodela JK, Dalvi RR: Comparative toxicological studies of chlorpyrifos in rats and chickens. *Vet Hum Toxicol* 1995, **37**, 1-3.

35. Wysocka-Paruszewska B, Osicka A, Brzeziński J, Gradowska J: Ocena skojarzonego działania nowych insektycydów fosforoorganicznych w mieszaninach dwuskładnikowych. *Bromat Chem Toksykol* 1974, **7**, 195-200.