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Histopathological Investigations of the Effects of Malathion on Dwarf Lizards (*Lacerta parva*, Boulenger 1887)

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Toxic pesticide chemicals and their degradation products are common on the foods of vertebrate and invertebrate animals living around agricultural fields (Sauter and Stell 1972). The deleterious effects of pesticides on the entire food chain are not known. In addition, we do not have information on impacts of decomposition products and how they will affect animals.

Malathion is very widely used in agricultural pest control because of its high insecticidal activity but low mammalian toxicity. Although there are many studies reporting the effects of malathion on fish, frogs, birds and mammals (Dutta and Marcelino 1990, Kumar and Ansari 1986, Ramalingam 1988, Moitra and Lal 1989, Rosenbaum *et al.* 1988, Pascual *et al.* 1991, Takahashi *et al.* 1987), studies of its toxicity to reptiles are quite rare (Littrel 1983).

In the present study, three dose levels of malathion were applied to the lizard (*Lacerta parva*, Boulenger 1887) for 16 weeks by oral ingestion and possible toxicological effects were investigated anatomically and histopathologically.

MATERIALS AND METHODS

Lizards were collected by hand from localities near Tokathan and Harmandalı villages in Eskişehir. The lizards were divided at random into five treatment groups, three of which were treated with malathion (group 1: 1 mg/kg, group 2: 2 mg/kg and group 3: 3 mg/kg) and two of which were control groups (control group and fat-control group), each containing 50 animals. Lizards were fed daily with grasshoppers, young cockroaches, ants, small flies and flour-moth larva. The lizards in the control group were given only the food mentioned above while the ones in the fat-control group were given sunflower oil in addition to

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their daily food. Mean wet mass of the animals was 2.82 ± 0.1 g prior to the experiment. All animals were weighed weekly throughout the study.

Temperature was kept at 27°C in day time and 18°C at night. Relative humidity was about 60 %. During the experiment lasting 16 weeks, the photoperiod was 12 hours of light, 12 hours of dark.Fluorescent lamps (Philips TLD 36 W/33) were used as a light source.

Malathion, technical purity 96%, was obtained from the Research Institute for Plant Protection Chemical and Equipment in Ankara. The median lethal dose (LD_{50}) of malathion on dwarf lizards was calculated according to the Probit Analysis Method (Finney 1952) and determined as 169.8 mg/kg (Özelmas 1993).

Technical malathion was dissolved in sunflower oil and then doses of 1 mg/kg, 2 mg/kg and 3 mg/kg were prepared. The dosages were applied into the mouth of the animals by using automatic pipettes everyday. Lizards in the fat-control group received sunflower oil of an amount equal to that in which technical malathion was dissolved for the malathion-dosed groups. Gilson pipetman P 20 and P 100 models were used to inject the doses to the lizards orally.

After 16 weeks, all surviving lizards were killed by decapitation. Sacrificed animals were autopsied and gross findings were recorded. Selected organs were dissected out and fixed in Bouin's solution. Fixed tissue portions from liver, kidney and small intestine were embedded in paraffin, sectioned and stained by hematoxylin-eosin for routine microscopic examination.

RESULTS AND DISCUSSION

Anatomical and histopathological investigation was studied with surviving 103 lizards out of 250 since the rest of animals died during the experiment lasting 16 weeks. 32 animals in control group, 16 animals in fat-control group, 32 animals in 1 mg/kg dose group, 30 animals in 2 mg/kg dose group and 36 animals in 3 mg/kg dose group died during the experiment.

Anatomical examinations of internal organs showed that there were heavy lipid accumulation around kidney, liver and intestines of lizards in dosed groups. Similar lipid accumulation in the same places was also determined in lizards in fat-control group but not in control animals. Pale-colored livers and degenerated kidneys were observed in malathion-treated lizards.

The fatty degeneration observed in the liver of lizards treated with malathion was very widespread in all examined areas whereas it was determined only in some fields in the liver of fat-control animals. Mononuclear cell infiltration and congestion in venous sinusoids also occured to some extent in dosed animals when compared with controls (Figure 1-3). There was edema around vena centralis of liver in lizards given 2 mg malathion/kg. Necrosis was also seen at highest dose in a small area near fatty degenerative fields (Figure 4). There was a decrease in the number of Kupffer cells in the liver of animals in all malathion-treated groups.

In fat-control group, there was parenchymal degeneration in some of the tubules in the cortex of kidney. Slight fatty changes were also noted in some of the interstitial areas of cortex. In 1 mg malathion/kg group, there were subcapsular hemorrhages (or congestions), fatty changes followed by the degeneration of interstitial tissues in inner cortex. In addition, there was vacuolar degeneration in the tubules of outer cortex. In 2 mg malathion/kg group, the degree of histopathological changes was much higher than 1 mg/kg dose group. The cells and the fibrils of connective tissue were mostly detected instead of parenchymal tissue both in cortex and medulla. In 3 mg malathion/kg group, there was an apparent structural changes in both proximal and distal tubules when compared with controls (Figure 5-6). Pyknosis and karyorrhexis were observed in such degenerated tubule cells. The degree of the degeneration was highest in this dose group.

There was a slight increase in the number of goblet cells in small intestine of lizards in 1 mg malathion/kg group compared to animals in fat-control group. Hypersecretion of goblet cell and perinuclear vacuolization were also noted in this dose group. In 2 mg malathion/kg group, there was excessive mucus accumulation in the apical cytoplasm, perinuclear vacuolization, degeneration and desquamation of epithelial cells, atrophic changes in duedonum mucosa and degeneration of the villi's structure in comparison with controls.The histopathologic changes determined in 3 mg malathion/kg dose group were similar to those in 2 mg malathion/kg dose group but the degree of degeneration was found to be heavier (Figure 7-8).

Various animals were used to determine the possible toxic effects of pesticide chemicals on human body by many researchers (Richmonds and Dutta 1988, Ramalingam, 1988, Pascual *et al.* 1991). However, there was no information published in the literature about the effects of any organophosphate pesticide on lizards. This condition may be attributed to that they are small-sized cold-blooded animals, their remote relationship with humans,

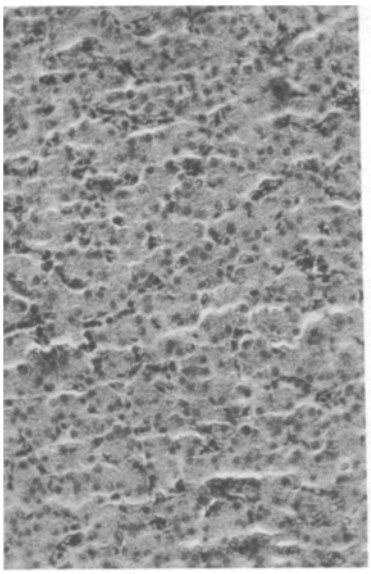

Figure 1:Liver section of lizard in control group.H-E.X 300

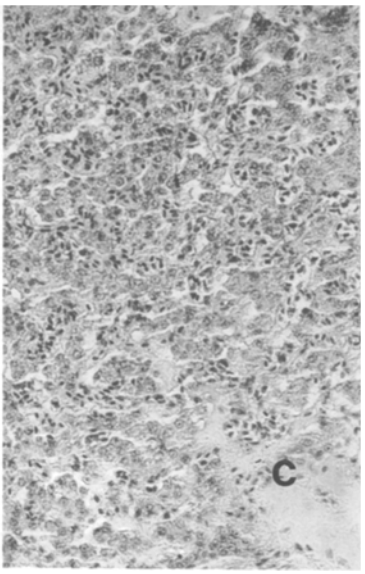

Figure 2:Congestion(c) in the liver of lizard in 1 mg/kg dose group. H-E.X300

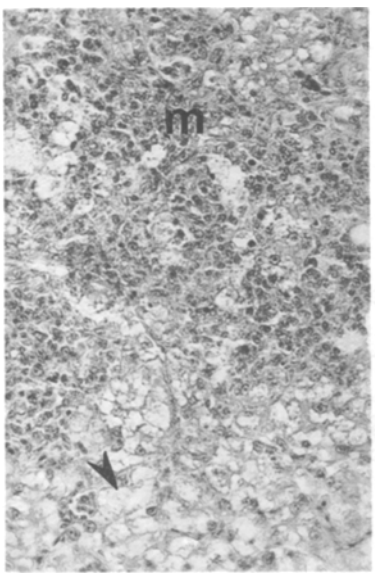

Figure 3:Mononuclear cell infiltration(m) and slightly degenerated cells (arrow) in the liver of lizard dosed with 2 mg/kg. H-E. X 300

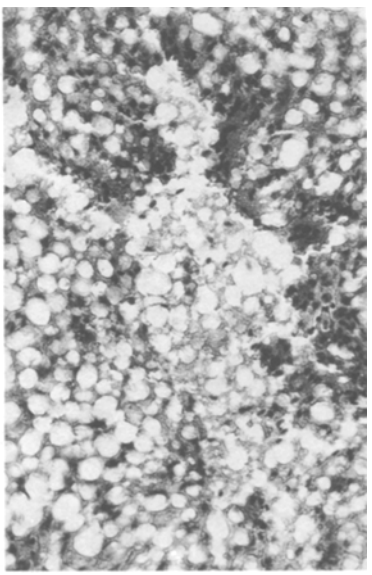

Figure 4:Necrosis around heavy fatty degeneration in the liver of lizard treated with 3 mg/kg. H-E. X 300

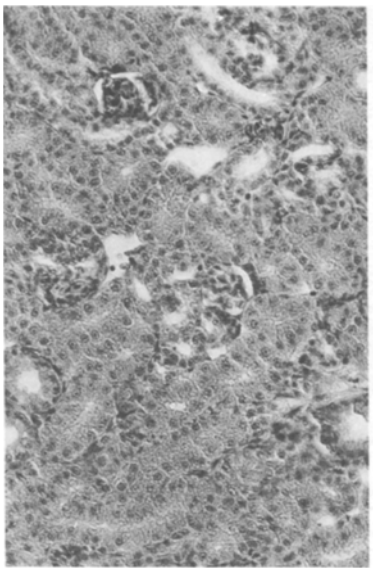

Figure 5:Kidney section of control lizard. H-E. X 300

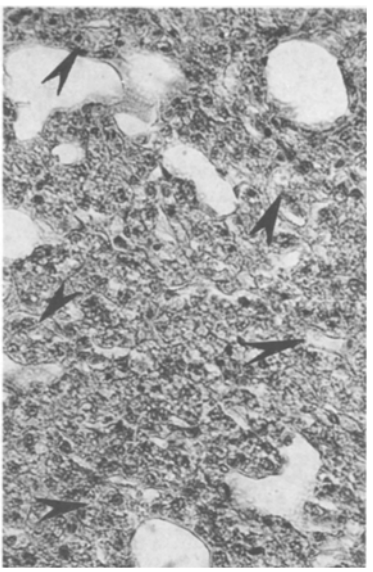

Figure 6:Degenerated tubules (arrows) in the kidney of lizard given 3 mg/kg.H-E. X 480

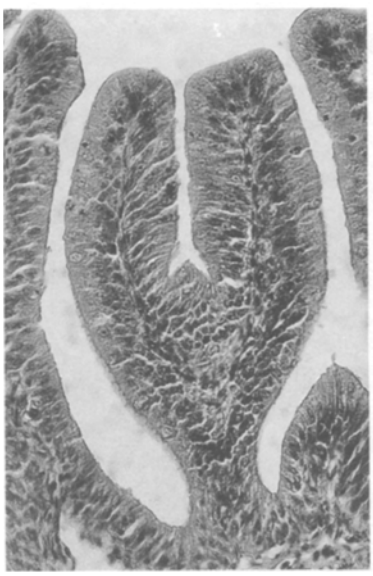

Figure 7:Small intestine section of control lizard.H-E. X 300

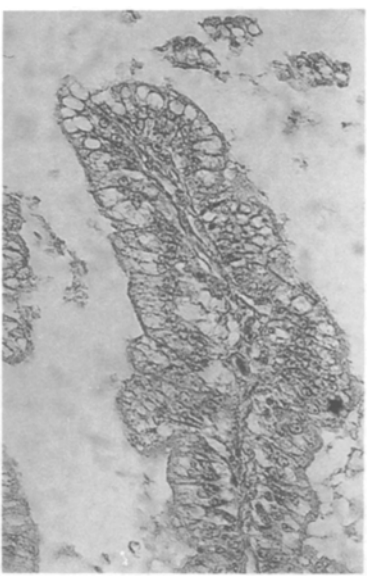

Figure 8:Changes in the structure of villi caused by atrophy and heavy degenerations in epithelial cells in the intestine of lizard treated with 3 mg/kg. H-E.X 300

difficulties in catching them in nature, and finally, difficulties in working with them in laboratory conditions. However, dwarf lizards live around meadows and empty arid fields; habitats in which they live are surrounded by sown fields and water sources. So, they are likely to be exposed to pesticides or to their metabolites used near those places.

The increased rate of fat tissue accumulation around internal organs of warm-blooded animals exposed to pesticide chemicals was reported by researchers (Akay 1980, Barlas 1992). In the present study, the obvious fatty accumulation around internal organs may be attributed to the known function of fat tissue in the sequestration of pesticides. Sequestration of pesticides is similar in warm-blooded animals and lizards.

Histological studies carried out on dwarf lizards in this experiment is similar to studies of different animals. In the study of Kumar and Ansari (1986) it was concluded that 0.9 mg/L concentration of malathion to zebra fish exposed for 4 months caused degeneration in liver. Ramalingam (1988) reported that malathion caused congestion in venous sinusoids in the liver of fish *Sarotherodon mossambicus* . Hepatotoxicity of malathion was observed as necrosis formation in the liver of blue gill sunfish (Richmonds and Dutta 1988). Malathion having high insecticidal activity and low mammalian toxicity caused a significant effect in the liver of lizards in our study when administrated in very low doses as 1-3 mg/kg. This effect may be attributed to the difference in the forms of degradation by malathion in warm-blooded animals and cold-blooded ones. Another point which supports this explanation is the differences in the number of Kupffer cells in warm-blooded and cold-blooded animals exposed to malathion. In the present experiment, there was a decrease in the number of Kupffer cells in all malathion- treated lizards whereas commercial malathion caused an increase in Kupffer cells of mice at 4.16 mg/kg dose orally for 15 weeks (Barlas 1992). Regarding the decrease in Kupffer cells it can be concluded that malathion may weaken the immunological defense mechanism in liver. Therefore, mononuclear cells may inflitrate the tissue and maintain immunological defense of liver instead of Kupffer cells. In our study, we observed mononuclear cell inflitration in the liver of all treated animals.

The damages in the kidney of lizards given malathion seemed to be dose-dependent since 1 mg/kg dose caused congestion, fatty changes and degeneration of interstitial tissues in cortex while the most remarkable injuries including heavy fatty degeneration and fibrosis were observed in 2-3 mg/kg dose group. It is likely that slight

fatty changes that also occured in the tissues of fat-control lizards may be associated with sunflower oil given to those animals, but heavy degeneration in the kidney (that was metanephrose type as in mammals) of lizards in 2 and 3 mg/kg dose group were thought due to the deleterious effect of malathion. Although malathion shows low mammalian toxicity, commercial malathion at 4.16 mg/kg dose caused histopathological changes such as mononuclear cell inflitration, degeneration in proximal and distal tubules in the kidney of mice after 15 weeks (Barlas 1992). These findings were consistent with our results.

Three doses of malathion showed different degrees of degenerative changes in small intestines of animals in our experiment. Excessive increase in the secretion of goblet cells was predominant in all treated animals. Richmonds and Dutta (1989) reported that after malathion exposure, an excessive amount of mucus was found over the gills of live bluegill fish. Khangarot (1982) reported that the gills of living animals treated with zinc were covered by a film of coagulated mucus. Mucus secretion increased on the skin of *Puntius sarana* fish exposed to malathion (Moitra and Lal 1989). A similar observation was reported in fish by Dutta and Marcelino (1990). The observed increase of mucus secretion is thought to be the defense response of tissue induced by the action of malathion. The organism tried to diminish the absorbtion of the toxicant from gastrointestinal tract in this way. Desquamation of epithelial cells of intestine in treated animals was a consistent finding with the reports of Areechon (1988) in catfish given malathion.

The results obtained from this study demonstrated that malathion widely used in Turkey have deleterious effects upon the lizards. Histopathological investigation showed that animals were affected even by very low doses. This means, uncontrolled use of malathion or related compounds will certainly endanger not only the lives of lizards but also affect food chain and ecological balance of nature negatively.

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