

## HISTOPATHOLOGICAL EFFECTS OF THE RESIDUAL MANEB AND ZINEB IN THE LETTUCES ON THE LIVER AND KIDNEY OF ALBINO MICE

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*SUMMARY: Suspensions of carbamate fungicides (maneb and zineb) were prepared at the concentrations of 200 g maneb/100 L and 300 g zineb/100 L respectively. These two doses were applied to the albino mice by way of lettuces during 13 weeks. Three applications were done weekly. The histopathological effects of the fungicides on mice were investigated. The test animals were observed to have larger livers with more heavily color than those of the controls. Another observation was the fat accumulation around the internal organs, particularly on liver, kidneys and pancreas of the test animals. Histological examination of the liver and kidney has shown mononuclear cell infiltration, tissue necrosis and congestion in mice fed with fungicide-applied lettuces. The changes obtained from tissues were discussed*

*Key words: Fungicides, mononuclear cell, infiltration.*

### INTRODUCTION

The most important class of fungicides for controlling the diseases of agricultural crops were known to be ethylenebisdithiocarbamate (EBDC) forms. The class includes nabam, maneb, mancozeb, metiram and zineb. EBDC's are unstable in the presence of moisture and oxygen as well as in biological systems. Ethylenethiourea, (ETU) a possible degradation product of the EBDC fungicides, has been reported to be goitrogenic, mutagenic and carcinogenic in rats (1,2). One of the studies on the subject has shown that cooking of feeds containing maneb can result in increased levels of ETU (3). Ethylenethiourea is a well-known and fairly stable compound. When administered two rats under various toxicological conditions, ETU has been shown to cause various pathological effects (4). Seifert reported that there was a retardation of growth, an apparent increase of thyroid weight and hyperplasia in

tissues of weanling rats given 1000 g ETU/kg for 8 days (5). The aim of the present study was to investigate and determine the histopathological effects of fungicides on male and female albino mice for three months.

### MATERIALS AND METHODS

#### Chemicals

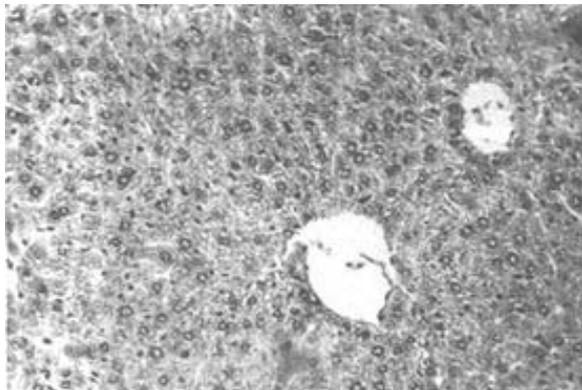
In this research, the following chemicals were used: Maneb (Dicotan M22) 80% pure, wettable powder (W.P), Zineb (Takineb-65) 65% pure, wettable powder. Maneb and zineb were obtained from Research Institute for Plant Protection Chemical and Equipment in Ankara, Turkey.

#### Lettuce treatment

The suspension of maneb was prepared at 200g/100L dose level while the suspension of zineb was prepared at the concentration of 300g/100L respectively. During the growing period, seedling plants of lettuce were treated with fungicides by spraying. Three applications were done on weekly basis.

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Figure 1: The control group of mice liver section. Hemtoxylen and eosin x175.



#### Treatment of mice

In this experiment, two month-old, 45 albino mice weighted 22-26 g were used. The animals were divided into three groups, each contained 15 mice. Five of the mice were housed in a cage during the period of 13 weeks. The laboratory conditions were kept at 22±2°C, 55-70% relative humidity and about 12-hr light/dark cycle. The fungicide treated lettuces were always kept under the natural conditions for two weeks in order to decompose these fungicides on plants before applications. Then, these lettuces were always washed twice and the animals were fed with these washed lettuces, for 13 weeks 19 µgr residual maneb/day and 10.92 µgr residual zineb/day were used (6).

#### Morphological examination

Unfed mice were weighed weekly during the experiment. After the feeding period of 13 weeks, the mice were killed by cervical dislocation and kidneys, liver, spleen, ovaries, testes, thyroid and parathyroid glands were removed from all the ani-

Figure 3: Congestion in the liver of mice given maneb. Hemtoxylen and eosin x115.

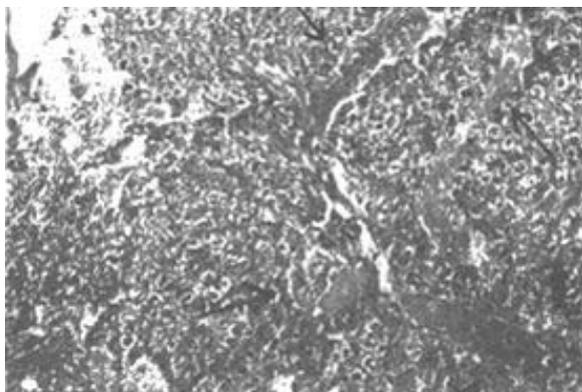
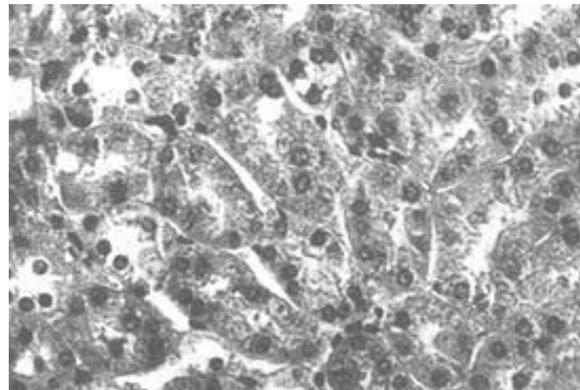


Figure 2: The control group of mice kidney section. Hemtoxylen and eosin x230.



mals and evaluated in the terms of weight and histopathological view. Tissue samples from those organs were fixed in 10% formaldehyde and they were embedded in the paraffin blocks. After staining procedure with hematoxyline-eosin, the sections were examined under light microscope. Student's t test was employed for statistical analyses of internal organ weights.

#### RESULTS

At the end of the tenth week, 2 male mice which administered maneb died. At the ninth and tenth week, 4 male mice given zineb died. None of the mice from control group died. During the course of the study two of the mice from test group showed no difference when compared to controls. Pathological changes were found in 22 mice (6 male and 6 female mice given maneb and 4 male and 6 female mice given zineb). Macroscopically and microscopically the organs of the control group were observed normal (Figures 1, 2). Internal organs (liver and kidneys) of the experimental mice were found to be heavier and darker in color than those of the controls. Fat accumulation and congestion was observed in liver and kidneys of mice treated with residual fungicides. Light microscopical examinations revealed congestion in all sections of liver (Figure 3). Parenchymal necrosis and mononuclear inflammatory cell infiltration were determined in the liver tissues of three mice given zineb. Mononuclear cell infiltration were observed in liver of all tested animals (Figure 4). Minimal congestion, and focal mononuclear inflammatory cell infiltration was observed in the kidneys of all tested mice (Figure 5). Other organs of tested animals showed no histopathological changes.

Figure 4: Focal necrosis and necrobiotic changes in the liver of mice given zineb. Hemtoxylen and eosin x175.

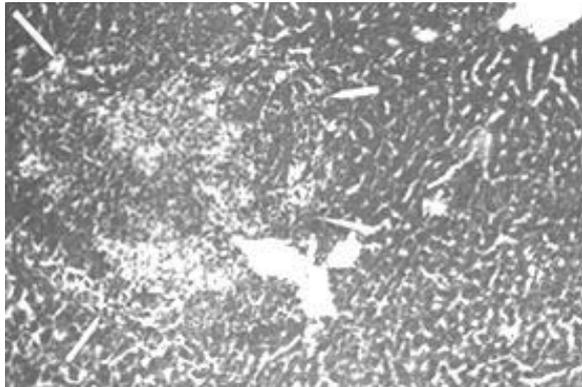
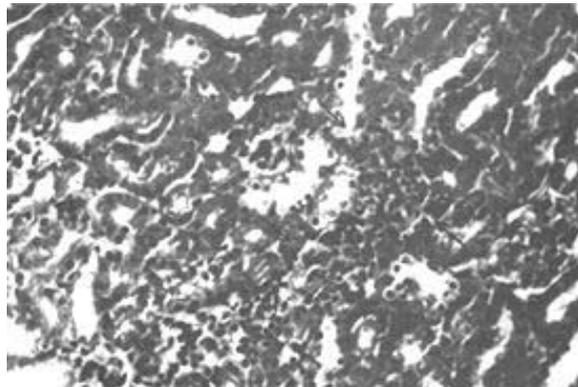


Figure 5: Lymphocytic infiltration in the kidney of mice given menab. Hemtoxylen and eosin x230.



## DISCUSSION

In this study, the toxicological effects in mice of the residues of maneb and zineb on lettuces were examined histopathologically.

We determined an increase in the liver weight of mice given fungicide residues. A subacute toxicological study on the effects of the EBDC-containing fungicide Dithane M-45 was carried out in male Wistar rats. 169 mg Dithane/kg body weight caused a decrease in the growth of the rats. Relative weights of the liver were significantly increased in rats given 75 mg Dithane/kg body weight (7). Darker color observed in liver tested of tested animals may be attributed to the functional and structural disorders in this organ. Emir *et al.* (8) reported that acid phosphatase activity showed an apparent decrease in liver.

In histopathological observation, mononuclear cell infiltration and necrosis in liver may cause a decrease in the detoxification capacity of this organ. Szepvolgyi *et al.* (7) reported that Dithane M-45 (80% Mancozeb, manganaseethylnebisdithiocarbamate-zinc ion complex) decreased the detoxicating capacity of the liver. The same effects of the residual maneb and zineb were determined histopathologically in kidney of animals. The histopathologic changes in kidney may be caused during the course of excretion of these residues by way of urine. At the relatively high dose levels tested, ETU (Ethylenthiourea) and EDA (ethylene diamine) were readily absorbed in the intestinal tract and almost exclusively excreted in urine (9,10). After a single oral dose of about 5 mg/kg of ( $^{14}\text{C}$ ) maneb to female rats about

half of the  $^{14}\text{C}$ -activity was excreted in urine and excretion was completed within 3 days (11). Therefore most of the administered maneb must be converted into degradation products in the intestinal tract. The fat accumulation around the intestinal organs of mice given fungicide residues were more than those of the controls. This may be attributed to the normal detoxification mechanism of fungicides in the organism. It is commonly known that when a xenobiotic enters into the organism, there are many ways to destroy it. One of them is the accumulation of these toxic materials in the fat tissue. Therefore, xenobiotic-exposed organisms have always increased amount of lipids in their bodies(7).

No obvious thyroid changes were detected by histological examination in mice administered maneb and zineb residues. Although other investigators have found detrimental effects of maneb and zineb in thyroid, could not find any sign of histopatological change in thyroid glands of maneb and zineb given mice (2,2, 13).

The differences in thyroid tissues from our experiment and other studies might depend on the level of dosage, treatment period, and the fungicides, while the other researchers treated the animals directly with the original compound, we applied indirectly, by way lettuces.

The histopathological results of the present study indicated that the residual maneb and zineb have detrimental effects on mice during 13 weeks. The data obtained from this research forced us to offer the limited use and then giving up these fungicides in near future.

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