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SUBCHRONIC TOXICITY OF *MANCOZEB* FUNGICIDE ON MALE WISTAR RATS: ULTRASTRUCTURAL ANALYSIS OF LIVER AND KIDNEY

[31]

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ABSTRACT

Dithiocarbamate mancozeb, an organometallic fungicide, was administered orally to male Wistar rats in two forms, pure technical 85% and formulated 80% WP, at sublethal doses of 125, 250 and 500 mg/kg b.w. for 28 days. Clinical chemical endpoints were measured after 2, 3, and 4 weeks of treatment initiation. Data disclosed that both compound formulations affected significantly serum enzyme activities particularly liver enzymes in a dose and time-dependent manner, compared with control. Such changes were accompanied with significant alterations in other parameters like; total protein, albumin, glucose, total lipids, triglycerides, cholestrol, urea, and creatinine. Furthermore, histological examination of liver and kidney showed several histopathogenic ubnormalities in the examined tissues of rats exposed to mancozeb either technical or formulated.

Keywords: Fungicides, Formulations, Toxicity, Liver, Kidney

INTRODUCTION

The increasing use of pesticides all over the world makes it necessary to reveal the toxic risk in populations of non-target organisms **(Hassal, 1990)**. Among these, Maneb, Zineb, Ziram, Mancozeb and Propineb are organometallic dithiocarbamate fungicides. Nowadays, mancozeb evokes controversial arguments in Egypt concerning its environmental hazardous effects and recently a ministerial decree (#719/2005) has banned mancozeb marketing. It has previously been reported that dithiocarbamates form metabolites called isothiocyanates which may disrupt protein synthesis and metabolism, by inactivating -SH groups in amino acids, proteins and enzymes **(Ware, 1983; Lukens, 1971)**. Ethylenethiourea, also one of the metabolites of ethylenebis-dithiocarbamate fungicides, has been reported to be carcinogenic, teratogenic and mutagenic in experimental animals **(Teramoto *et al* 1975; Larsson *et al* 1976)**.

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Mancozeb (Mn- and Zn-containing dithiocarbamate) a commonly used fungicide, have been shown to induce tumors in mouse skin and rats (**Subramoniam *et al* 1991**). Mancozeb was also found to cause an increase in glutathione transferase activity in the liver of new-born, weanling and adult rats (**Trivedi *et al* 1993**) and was found to alter the hematological parameters of aquatic organisms (rainbow trout) at sublethal concentrations for 3 weeks (**Atamanalp and Yanik, 2003**).

Moreover, agricultural exposure to the organomanganese fungicides is said to induce an extra-pyramidal syndrome resembling Parkinsonism (**Meco *et al* 1994**). **Soleo *et al* (1996)** suggested that ethylene-bis-dithiocarbamates rather than Mn may be primarily responsible for the cytotoxicity of organomanganese fungicides on neuronal systems.

Few studies have been carried out on the mechanisms of organometalic fungicide action or on the fate of fungicides in target organisms. However, many studies have been reported on the effects of heavy metals alone in a variety of organisms (**Kendrick *et al* 1992**). Such metals may also affect many key biological systems such as oxidative phosphorylation, membrane permeability and protein synthesis. Increases in trace metal levels in cells may result in chelation with biological components, particularly with enzymes, again causing cellular damage (**Lukens, 1971; Ware, 1983**). The current study aims to evaluate and compare the histopathological/biochemical effects of the organometallic fungicide mancozeb in two tested forms on different organs of male rats particularly liver and kidney.

MATERIAL AND METHODS

Chemicals: Mancozeb (manganese ethylene bis-dithiocarbamate; polymeric) was provided from EL-HELB Pesticides& Chemicals Co. (New Damietta, Egypt). The technical grade was 85% technical, while the formulated one was Anadol Gold (80% WP). Both physical and chemical characteristics of the tested formulation were firstly examined and confirmed by GC-FPD compared with the technical grade.

Animals and treatment: Male adult Wistar rats (70-80 days of age, 150±10 gm body weight) were housed and allowed to acclimatize for one week before initiation of treatment, feeding on a commercial diet and water ad libitum. Animals were divided into four groups for each of the tested compounds. Each group is composed of 10 rats and the four groups were treated with 0, 125, 250 and 500 mg/kg b.w. of technical and formulated mancozeb; respectively daily for 28 days. The doses administered represent a certain percentage of the oral LD_{50} of the tested compounds. All studies were conducted in accordance with Good Laboratory Practice Standards and Oral Toxicity Guidelines for Pesticide Testing (**Organization for Economic Cooperation and Development, OECD, 1995**).

Sample preparation: Blood was collected by orbital sinus technique (**Schalm, 1986**) from both treated and control rats at time intervals of 14, 21 and 28 days. Serum was separated via centrifugation at 3500 rpm for 10 minutes and kept frozen at -20°C for subsequent analysis. At the end of the study, all ani-

mals were killed, examined grossly and liver and kidney were removed and prepared for histological examination.

Biochemical measurements: Several toxicity biomarkers measuring liver and kidney functions were assayed including alanine and aspartate aminotransferases; ALT and AST, alkaline phosphatase; ALP, and acetyl cholinesterase activities; AChE, total protein, albumin, glucose, urea, creatinine. Furthermore, total lipids, cholesterol and triglyceride concentration were measured. All the examinations were performed using commercial diagnostic kits, Stanbio Co., Spain).

Histopathological examination: At the end of experimental period (28 days), animals were sacrificed (**OECD, 1995**). Liver and kidey were freshly dissected and placed in 10% formaldehyde and dehydrated in 70-100% ethanol series. They were then placed in paraffin baths at 58°C for paraffin inclusion. Sections of 4-6 µm were prepared from paraffin blocks using a rotary microtome. These sections were then stained with Hematoxylin-Eosin (H-E) and photographed using photomicroscope (**Carleton *et al* 1967**)

Statistical analysis: Data were expressed as mean ± S.E. and significant differences of values were analyzed using students T-test (**Snedecor and Cochran, 1989**).

RESULTS AND DISCUSSION

The potential biochemical end-points describing the dose-response relationship and concurrent histopathological impairments exerted in animals under mancozeb intoxication (technical or formulated) are highlightening in the following represented data obtained under the experimental conditions.

As regards to the toxic effects on liver as an organ with several complex activities, liver injury due to pesticide intoxication results in release of liver enzymes (AST, ALT, ALP) into blood stream as index of cellular degeneration and lysosomal activity linked to pesticide elimination (**Verplanke *et al* 2000**). Accordingly, data in Table (1) showed that technical mancozeb caused a significant elevation in ALT activity at all the tested dose regimens during the whole experimental period. While, AST activity was significantly decreased at 125 and 250 mg/kg throughout the test period, but the higher dose (500 mg/kg) caused elevation in AST activity. Furthermore, all doses caused significant increase in ALP activity after 28 days of treatment. The lower dose inhibited ALP activity while the higher stimulated the enzyme activity after 14 and 21 days of treatment; respectively. Comparatively, ALT and ALP activities were decreased significantly in a dose-dependent manner after treatment with Anadol Gold at all the tested doses and allover the experimental period while, AST activity was significantly increased also in a dose-dependent manner after all time intervals (Table, 2).

Since mancozeb is an organometalic fungicide, the released metals (Mn and Zn) may affect many key biological systems such as oxidative phosphorylation, membrane permeability and protein synthesis. Increases in trace metal levels in cells may result in chelation with biological components, particularly with enzymes, again causing cellular damage (**Lukens, 1971; Ware, 1983**).

The obtained data are matching with the previous literatures studying the toxicity of mancozeb. **Kackar *et al* (1997& 1999)** reported that mancozeb has produced significant enzymatic changes in the activities of AST, ALT and ALP after oral administration of 500, 1000, 1500 mg/kg to male rats for 30, 90, 180 and 360 days. Also, **Szepvolgyi *et al* (1989)** showed that Dithan M-45 (80% mancozeb) decreased the detoxication capacity of the rat liver at 253 and 379 mg/kg for 12 week. Furthermore, the present findings run parellel in part with **Hashizame *et al* (1992)** who stated that a significant increase in each of serum AST and ALP after administration of bithional sulfoxid and thiobendazole fungicides to rats and mice.

Interstingly, AChE activity shown in Tables (1) and (2) indicates that both forms of mancozeb caused significant increase in AChE activity.The technical one caused significant increase of the enzyme activity in a dose and time-dependent manner whereas the intermediate dose of the formulated mancozeb (250 mg/kg) caused significant elevation after all tested time intervals and the higher dose (500 mg/kg) caused a lesser degree of enzymatic activation. These data agree with **Kackar *et al* (1999)** who stated that mancozeb altered significantly the activity of rat AChE throughout the study period (360 days) in a dose dependent manner. On the other hand, **Siddiqui *et al* (1990)** showed that AChE activity was unaffected in rats orally administrated with mancozeb at 250 mg/kg for 30 days. Also, **El-Halwagy *et al* (2003)** reported that administration of sulfur containing fungicide (Panadol 8%) at 1/5 or 1/10 LD_{50} for 30 days showed non-significant changes in serum AChE activity throughout the experimental period.

Leakage of enzymes into the blood stream was accompanied with disturbance in protein content due to affection of membrane permability and protein biosynthesis. The current data showed an increase in serum total protein and albumin particularly after 28 day of treatment with the technical mancozeb but during the other tested periods, the lower dose caused significant elevation in protein content and albumin while the higher dose exhibited a decreasing trend (Table, 1). On the other hand, treatment with Anadol Gold decreased the total protein content and albumin non-significantly during the whole experimental period, compared with the corresonding control.

Similarly, **Dacosto *et al* (1994)** recorded that rabbits serum total protein and albumin levels were apparently altered by triphenytin acetate fungicide treatment at 15, 75 and 150 ppm for 70 days. Also, **Mahadevaswami *et al* (2000)** showed that the level of total protein was elevated in the liver of rats treated with 700 and 800 mg/kg b.w. /day of mancozeb for 15 consecutive days. Also, rats treated by 1/10 LD_{50} of Panadol 8% sc fungicide showed a significant decrease in serum total protein and albumin concentration **(El-Halwagy *et al* 2003).**

Blood glucose concentration was significantly increased after treatment with technical mancozeb for 21 days, while, it was noticed that all the tested concentrations showed a decreasing pattern of glucose level in a dose-dependent manner after 14 and 28 days of exposure. Treatment with Anadol Gold caused significant increase in blood glucose level after all times of treatment of all dose regimens in a dose and time-dependent manner

Table 1. Effect of technical mancozeb on indicators of liver function in male Wistar rats (Data expressed as mean ± SE)

Indicator	Experimental period (day)											
	14				21				28			
	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg
ALT	35.62	84.66	46.08	52.86	31.20	59.28	54.96	46.38	43.68	40.56	54.02	50.10
(U/L)	± 2.85	±11.79**	±1.35*	± 2.67**	±1.60	±7.64*	±4.44**	±7.75	±7.64	±4.51	±8.17	±4.19
AST	90.86	74.21	82.11	95.26	96.75	72.63	95.26	105.00	100.72	68.55	54.15	123.29
(U/L)	±6.27	±4.94**	±1.77*	±5.85	±2.13	±7.62*	±7.05	±10.90	±6.67	±7.83*	±4.04***	±11.19
ALP	213.73	192.59	272.66	266.03	204.22	85.54	164.96	282.37	194.92	206.70	277.81	207.47
(U/L)	±31.21	±19.82	±30.62	±32.74	±7.79	±4.52**	±14.84**	±42.64*	±1.24	±22.83	±5.02***	±4.54
AChE	123.92	112.42	175.87	180.56	121.3	140.52	168.63	183.11	128.29	127.75	200.57	235.91
(U/L)	±6.32	±16.28	±12.12*	±19.12*	±10.06	±12.55	±16.48	±8.35**	±4.71	±6.32	±12.64**	15.27***
T. protein	5.07	7.83	3.39	3.18	4.02	10.36	2.96	3.30	6.25	7.43	8.27	7.33
(gm/dl)	±0.46	±0.53*	±0.85*	±0.24*	±0.46	±0.80**	±0.22	±0.44	±0.75	±0.83	±0.69	±0.062
Albumin	2.18	5.61	3.31	3.09	2.35	7.55	2.79	2.99	2.18	7 35	6.29	5.16
(gm/dl)	±0.18	±0.61**	±0.45	±0.14	±0.18	±0.52**	±0.45	±0.47	±0.18	±0.71***	±0.51***	±0.79*
Glucose	119.07	106.23	100.75	94.98	107.68	132.20	147.06	131.14	115.37	108.53	100.65	84.14
(mg/dl)	±6.78	±8.95	±9.74	±3.44**	±4.55	±11.84	±8.14**	±13.85	±10.16	±5.76	±9.55*	±3.51*

*,**,*** Significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$; respectively

Table 2. Effect of formulated mancozeb (Anadol Gold, 80% WP) on indicators of liver function in male Wistar rats (Data expressed as mean ± SE)

Indicator	Experimental period (day)											
	14				21				28			
	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg
ALT	42.34	27.44	27.01	28.74	30.11	29.79	26.28	21.44	26.82	20.20	23.29	36.81
(U/L)	±2.72	±3.85*	±4.86*	±3.08*	±6.09	±2.42	±3.93	±3.35	±5.95	±2.11	±2.11	±2.36
AST	89.05	71.41	123.27	148.88	99.51	97.67	117.81	113.20	100.66	103.23	110.61	124.71
(U/L)	±5.68	±8.36	±21.06	±5.31***	±3.69	±10.26	±4.73	±7.64	±2.85	±7.58	±5.26	±13.42
ALP	258.47	249.91	214.43	159.24	273.14	226.59	199.06	196.85	271.05	149.14	123.29	123.91
(U/L)	±18.45	±25.87	±15.39	±6.18***	±16.54	±22.45	±18.82*	±13.65**	±11.77	±24.60**	±8.35	±5.64***
AChE	116.25	131.89	187.03	169.94	147.23	144.36	227.50	144.87	126.47	144.36	195.79	162.24
(U/L)	±7.41	±4.42	±12.42**	±8.25*	±11.73	±12.73	±15.27**	±6.57	±13.98	±5.46	±15.35*	±8.51
T. protein	7.60	5.542	5.89	6.74	7.51	6.47	8.18	6.43	7.82	6.55	7.45±	6.33
(gm/dl)	±0.88	±0.65	±0.75	±0.61	±0.19	±0.61	±0.59	±0.54	±0.92	±0.47	0.65	±0.21
Albumin	5.71	3.32	5.38	4.13	5.45	4.49	4.91	5.25	5.15	3.72	4.61	4.62
(gm/dl)	±0.46	±0.41**	±0.38	±0.23*	±0.48	±0.21	±0.39	±0.43	±0.66	±0.61	±0.43	±0.13
Glucose	96.41	124.39	130.75	122.17	98.88	115.31	112.79	98.41	96.53	127.08	153.61	151.91
(mg/dl)	±9.64	±9.34	±3.85	±9.59	±6.60	±3.69	±3.55	±5.85	±1.38	±6.02**	±9.07***	±7.41***

*, ** and *** Significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$; respectively.

(Tables, 1, 2). Alteration of blood sugar level was previously documented after exposure to similar fungicides (**Rizk Alla *et al* 2004**).

Furthermore, lipid profile was found to be affected due to mancozeb intoxication reflecting disturbance in lipid metabolism possibly due to peroxidation. Abnormal serum lipids levels also may be secondary to a number of liver disease, biliary cirrhosis and nephritic syndrome (**Kelly, 1993**). Data represented in Table (3) exhibited that technical mancozeb has led to significant increase in total lipids at the doses 125 and 500 mg/kg only after 21 day and at 250 mg/kg after 14 days of exposure while the general trend was decreasing in the level of total lipids throughout the whole exposure period. Furthermore, level of triglycerides was fluctuating, it was decreased significantly after treatment for 14 and 28 days but increased after 21 day of treatment. Serum cholesterol level was significantly increased after all doses regimens at all different times of exposure.

Some pesticides including fungicides are already known to disrupt lipid metabolism (**Timbrell, 1991**). **Abu El-Zahab *et al* (1991)** have indicated a link between serum cholesterol levels and the metabolism of certain metals. A high degree of fatty change occurred in most organs of the females administered with 400 ppm propineb fungicide where Zn is known to affect lipid peroxidation in biological membranes (**Prasadad, 1979**). **Terada *et al* (1998)** reported a significant increase in total lipids, cholesterol and triglycerides in rats treated with Mepanipyrin fungicide.

On the other hand, formulated mancozeb caused a significant decrease in levels of total lipids and total cholesterol with all doses after different time intervals compared with the control. Also, triglycerides showed the same decreasing pattern except at the doses of 250 and 500 mg/kg after 14 day of exposure.

The obtained results agree with that reported by **Kackar *et al* (1997)** who stated that serum cholesterol increased in rats treated with 500 mg/kg mancozeb for 90 days. Also **Szepvolgyi *et al* (1989)** reported that serum cholesterol level was increased significantly in rats given Dithane M-45 (80% mancozeb) at 75 mg/kg for 12 week. It was also found that doses of 113,169 and 253 mg/kg caused elevation of triglyceride content. Rats treated with 600,700 and 800 mg/kg/day mancozeb showed a signifigant decrease in the level of total lipids, phospholipid and neutral lipid in liver (**Mahadevaswami *et al* 2000**) but **Baligar and Kaliwal, (2001)** reported that exposure to 500 mg/kg/day mancozeb for 30 days caused a significant increase in total lipids in the liver of treated rats.

Exposure to fungicides-containing metals results in metal accumulation in certain tissues and organs of the exposed organisms. It is also known that metals may cause extensive damage to the organs in which they accumulate causing biochemical and histopathological changes (**Goyer, 1986; Wlostowski, 1992**).

Histopathological examination of the liver disclosed that both of technical mancozeb and Anadol Gold caused an obviously histopathological alterations. Both doses of technical mancozeb (500, 125 mg/Kg) caused hyperplasia of Von Kupffer cells accompanied with dilation and congestion of portal vein with red blood cells and the sinusoidal spaces were engarged with large focal area of

Table 3. Effect of mancozeb (technical and formulated) on lipid profile in plasma of male Wistar rats (Data expressed as mean ± SE)

Treatment	Parameter	Experimental period (day)											
		14				21				28			
		0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg
Mancozeb (technical, 85%)	T. lipids (mg/dl)	60.30 ±2.90	51.30 ±2.90	83.70 ±5.30**	47.50 ±1.80**	50.90 ±4.80	65.00 ±3.30*	43.20 ±1.80	66.80 ±2.30	49.30 ±4.10	44.60 ±2.40	44.20 ±0.70	42.60 ±3.70
	T. cholestrol (mg/dl)	73.77 ±8.08	128.70 ±12.29*	116.36 ±8.67*	132.01 ±15.78*	69.01 ±5.03	47.79 ±3.38*	129.41 ±5.59***	159.10 ±12.47***	57.72 ±4.88	86.58 ±1.00**	70.48 ±1.29	117.70 ±3.01***
	Triglyceride (mg/dl)	69.26 ±1.67	47.99 ±7.13*	52.66 ±6.90*	53.05 ±5.13*	71.62 ±7.93	59.51 ±7.72	86.58 ±11.29	86.34 ±8.97	64.54 ±4.99	44.67 ±3.62*	71.81 ±14.01	47.10 ±6.04
Anadol gold (80%, WP)	T. lipids (mg/dl)	65.75 ±6.12	19.95 ±2.00***	14.55 ±2.20***	16.45 ±1.95***	77.95 ±6.35	10.35 ±1.40***	17.20 ±1.90***	11.45 ±1.55***	79.95 ±4.65	28.05 ±2.35***	42.65 ±7.15**	32.30 ±3.10***
	T. cholestrol (mg/dl)	49.19 ±1.24	21.97 ±2.38***	38.93 ±1.84**	33.63 ±1.31***	68.94 ±4.94	38.33 ±4.50**	63.25 ±4.16	58.85 ±5.71	50.05 ±2.53	51.74 ±2.22	43.12 ±1.64	67.13 ±4.98*
	Triglyceride (mg/dl)	52.58 ±6.33	36.31 ±5.73	58.18 ±7.47	71.26 ±3.92*	72.44 ±10.63	50.24 ±4.99	38.09 ±5.74*	50.88 ±3.37	75.68 ±5.43	46.35 ±10.24	65.13 ±5.05	79.53 ±10.04

*, ** and *** Significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$; respectively.

inflammatory cells (Fig. 2 a,b). Also severe necrosis and hemosiderin pigments were seen in case of treatment with the lower dose (Fig. 3 a,b). Necrosis may be due either to severe degeneration or to metabolic disturbance and inhibition of protein synthesis in the liver cells or to direct effect of haemorrhage. Similarly, formulated mancozeb exhibited a similar pattern accompanied with hydropic and fatty degeneration of hepatocytes and absence of the regular arrangement of hepatic strands and architecture (Figs. 4 a,b and 5 a,b).

These results agree with **Belpoggi *et al* (2002)** who reported that mancozeb at the concentrations of 10, 100, 500 and 1000 ppm fed for 104 weeks caused, an increase in hepatocarcinomas in rat liver. On the other hand, **Jeffrey *et al* (2000)** showed that there is no histological alterations in the liver of male rats treated with metiram (EBDC) fungicide at 84 mg/kg/day for 13 week.

Concerning renal involvement upon exposure to mancozeb, determination of blood urea and creatinine levels indicated a worsening of kidney function. Table (4) showed that technical mancozeb caused a significant decrease in blood urea level particularly after 21 and 28 days of exposure accompanied with a decreasing pattern in creatinine concentration except at the higher dose which caused a significant elevtion in creatinine concentration after 14 day of treatment. In case of treatment with Anadol Gold (80% WP), a significant increase in both urea and creatinine concentrations at all doses after 28 days of treatment was recorded. The highest concentrations of urea and creatinine were recorded after 14 and 28 days of exposure; respectively. Elevation in serum creatinine may be due to increase in the protein catabolic rate.

A comparative study of **Dacosto *et al* (1994)** reported that triphenyltine acetate fungicide increased blood urea nitrogen levels in rabbit treated by 15, 75 and 150 ppm for 70 days and suggesting a possible renal involvement. While, **Jeffrey *et al* (2000)** reported that rats fed on diet containing metiram (EBDC) fungicide at 960 ppm for 13 weeks showed a significant decrease in blood urea concentration. **Hasegawa *et al* (1993)** showed that propineb causes cancer in the thyroid, kidney and urinary bladder of rats.

Similar to the liver tissues, kidney tissues have been seriously affected by mancozeb intoxication and such impairments become more prominent with the higher dose of the fungicide rather than the lower one. The type of renal damage observed under the influence of technical mancozeb included deposition of hyaline casts in the renal tubular vacuoles (Fig. 6) and congestion of blood vessels between the convoluted tubules (Fig. 7). Compatatively, formulated mancozeb caused greater changes than the technical one summarizing in appearance of focal congestion in glomeruli, interstatial haemorrhage and inflammatroy cells between the renal tubules in the corticomedullary junction (Fig. 8 a,b). Such haemorrhage may be due to an increase of intravascular tension or venous congestion or due to a depolarization of mucopolysaccharides which permits blood to leave the vascular system. The lower dose of formulated mancozeb caused similar changes in addition to dilation of collecting tubules accompanied with marked glomerular degeneration and lysis (Fig. 9 a,b).

Similar findings to the recorded histopathological changes were obtained when propineb (Zn-dithiocarbamate) administered orally to female Wistar rats

Figures (1-9) describing the histopathological changes of liver and kidney in treated rats

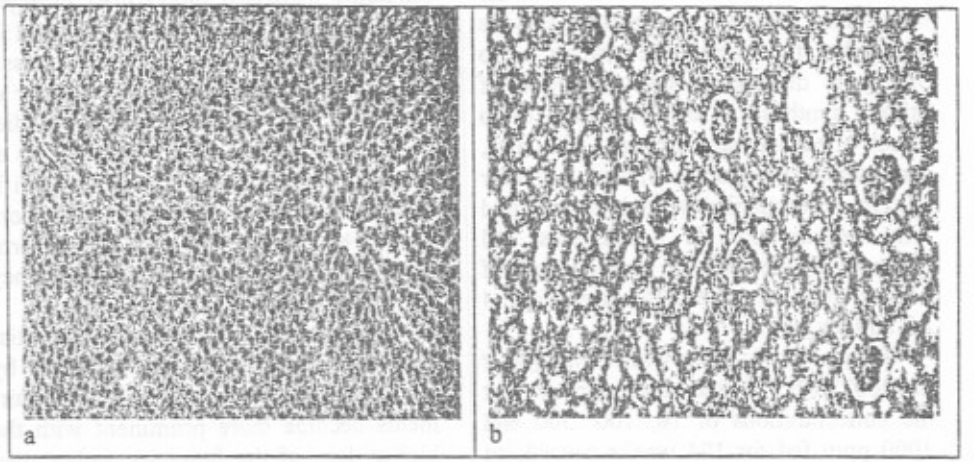

Fig. (1a,b). Section in control liver and kidney of albino rat, showing normal lobular architecture of hepatocytes and renal cells; respectively. (X200)

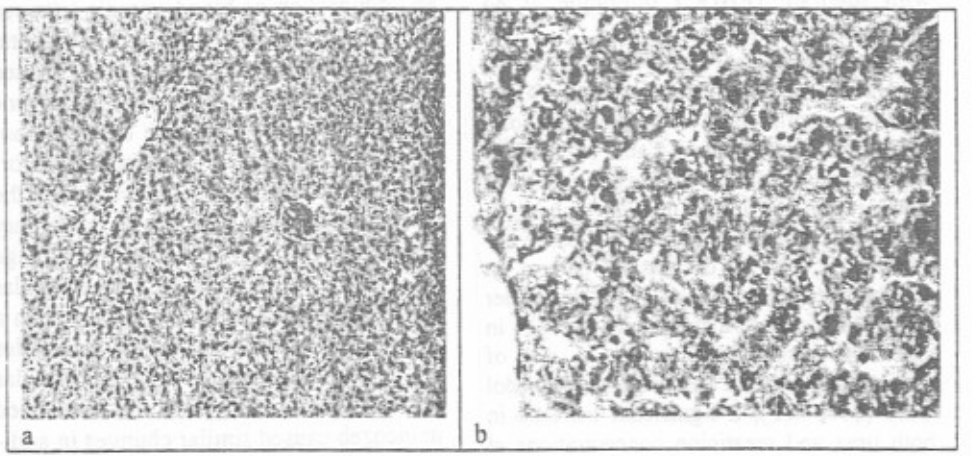

Fig. (2a, b). Histopathological effects of technical mancozeb on liver of rats treated with 500 mg/Kg, showing hyperplasia of Von Kupffer cells, most of hepatocytes appear as coagulative masses of degenerated cytoplasm, dilation and congestion of the portal vein with red blood cells and the sinusoidal spaces engarged with large focal area of mononuclear inflammatory cells (X 200, 400).

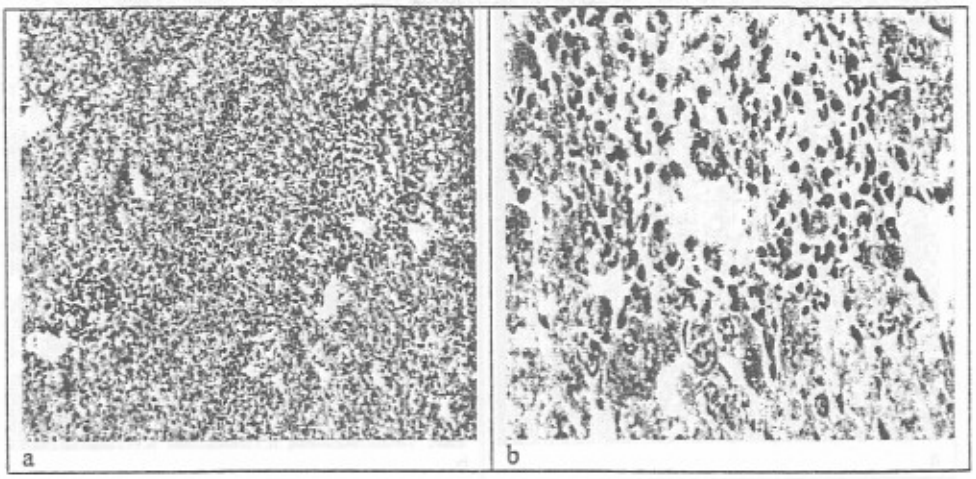

Fig. (3a, b). Photomicrograph of section in liver of rats received 125 mg/Kg of technical mancozeb showing severe necrosis, hyperplasia of Kupffer cells, congestion of sinusoids between hepatocytes and hemosiderin pigments are seen (X200, 400).

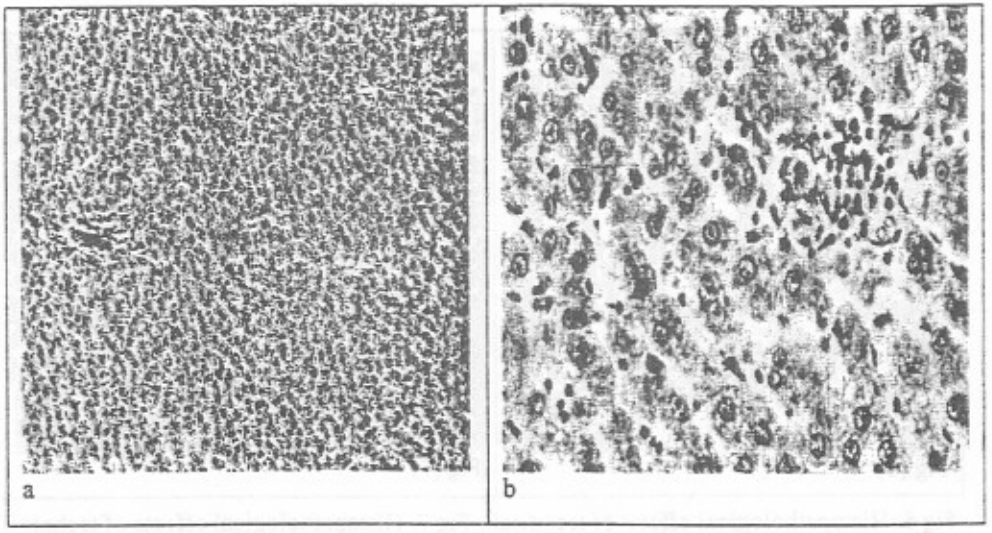

Fig (4a,b). Histopathological effects of formulated mancozeb (Anadol gold) on liver of rats treated with 500 mg/Kg showing focal areas of inflammatory cells in the whole hepatic lobular architecture, ill-defined cell boundaries, hydropic degeneration of hepatocytes and hemosiderin pigments are highly distributed among Kupffer cells (X200, 400).

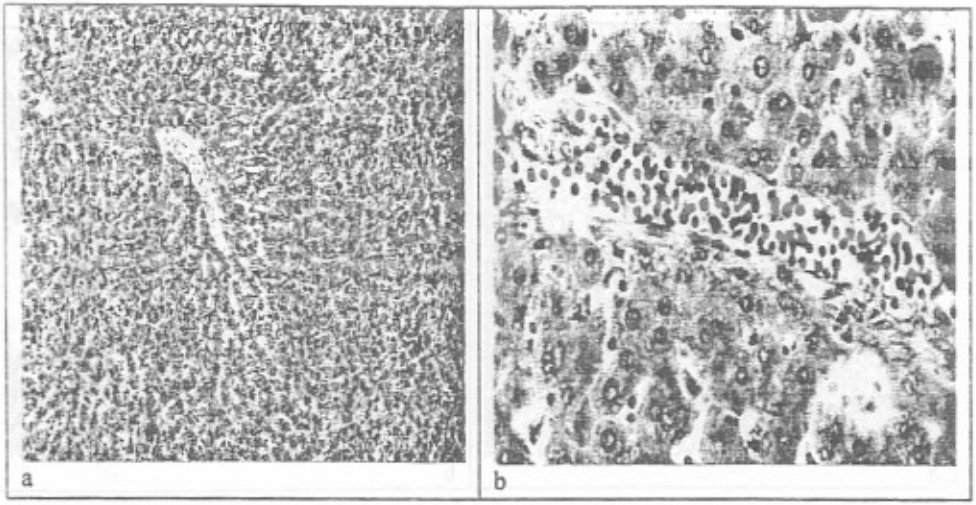

Fig. (5a,b). Histopathological effects of formulated mancozeb (Anadol gold) on liver of rats treated with 125 mg/Kg showing the same pattern exhibited by the high dose shown in Fig 4, in addition to hydropic and fatty degeneration of lysed hepatocytes, widened and dilated C.V., and absence of the regular arrangement of hepatic strands and architecture (X200, 400).

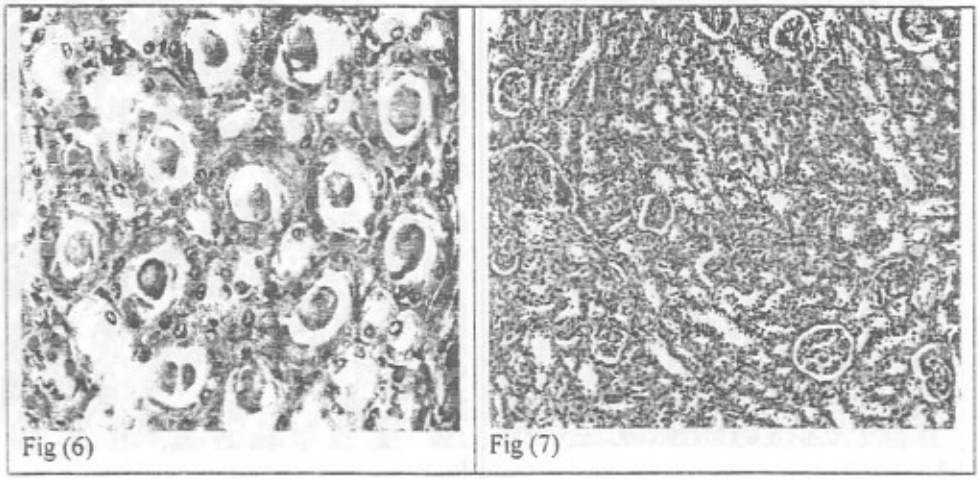

Fig 6. Histopathological effects of technical mancozeb on kidney of rats treated with 500 mg/Kg showing deposition of pink hyaline casts in the renal tubular vacuoles (X200).

Fig 7. Histopathological effects of technical mancozeb on kidney of rats treated with 125 mg/Kg showing congested blood vessels between the convoluted tubules (X200).

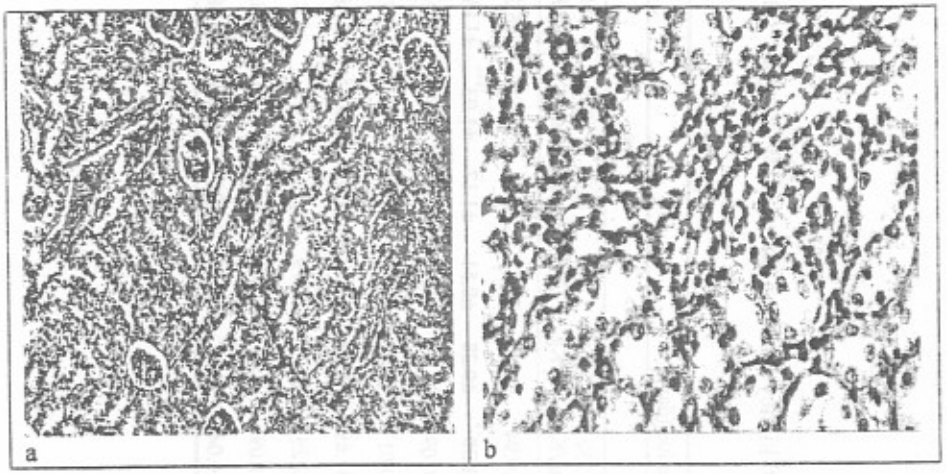

Fig. (8a,b). Histopathological effects of Anadol gold on kidney of rats treated with 500 mg/Kg showing focal congestion in glomeruli, interstitial haemorrhage and inflammatory cells between the renal tubules in the corticomedullary junction (X200, 400).

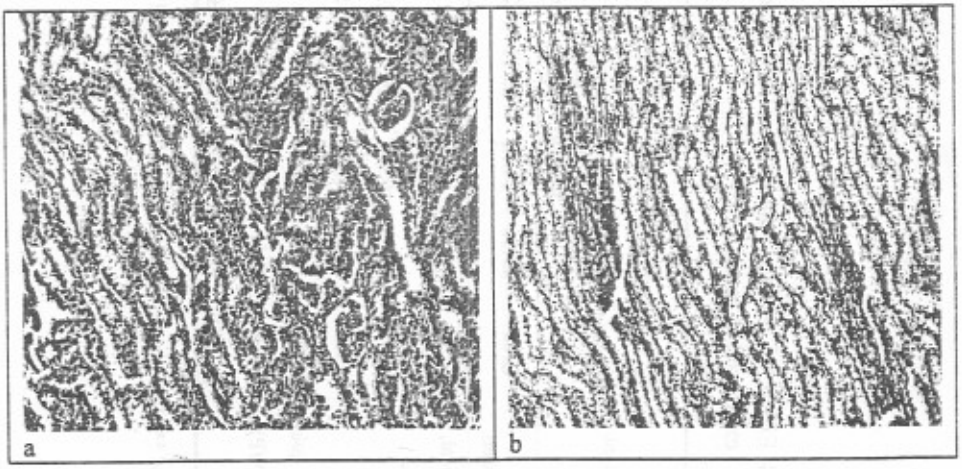

Fig. (9a,b). Histopathological effects of Anadol gold on kidney of rats treated with 125 mg/Kg showing the same effect exerted above in addition to dilation, lysis of collecting tubules and marked glomerular degeneration (X200, 400).

Table 4. Effect of technical and formulated mancozeb on indicators of kidney function in male Wistar rats (Data expressed as mean ± SE)

Treatment	Indicator	Experimental period (day)											
		14				21				28			
		0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg	0	125 mg/Kg	250 mg/Kg	500 mg/Kg
Mancozeb (technical, 85%)	Urea (mg/dl)	25.36 ±0.87	24.92 ±1.33	23.91 ±0.49	21.08 ±0.32**	27.14 ±0.81	22.54 ±0.93	10.94 ±1.38**	25.30 ±0.28	26.41 ±0.51	25.46 ±1.17	13.21 ±1.03***	16.22 ±1.09***
	Creatinine (mg/dl)	0.24 ±0.03	0.08 ±0.00***	0.19 ±0.02	0.41 ±0.03**	0.26 ±0.02	0.034 ±0.02	0.09 ±0.01***	0.19 ±0.02	0.26 ±0.02	0.18 ±0.02	0.29 ±0.02	0.24 ±0.03
Anadol gold (80%, WP)	Urea (mg/dl)	26.72 ±1.16	41.17 ±2.90***	45.49 ±1.02***	54.96 ±1.49***	28.06 ±1.05	34.21 ±2.99	37.69 ±2.29**	35.47 ±1.95*	21.19 ±2.79	31.97 ±1.72***	43.85 ±1.72***	36.19 ±0.85**
	Creatinine (mg/dl)	0.61 ±0.00	0.61 ±0.05	1.43 ±0.06***	0.88 ±0.09*	0.95 ±0.04	1.02 ±0.06	1.09 ±0.04*	1.16 ±0.07*	0.77 ±0.07	1.43 ±0.06***	1.09 ±0.07*	1.35 ±0.08***

*, ** and *** Significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$; respectively.

(Guven *et al* 1999) where histological examination of the liver and kidney of the fetuses and treated pregnant females showed a variety of histopathological changes primarily in the liver and kidney and adenocarcinomas of the uterus.

From the above observations, the histopathological findings confirm the biochemical results and showing that liver and kidney represent sensitive target organs under subchronic mancozeb intoxication. In most cases, the formulated form of mancozeb seems to be more hazardous than the technical one may be due to the synergistic effects of the additives coupled to the technical material. Furthermore, the formulation may carry some unknown impurities that may potentiate the toxic effects of the original compound. Since technical mancozeb is an organometallic compound can enter cells much more readily than the inorganic metal ion (Guven *et al* 1999), at least part of their toxicity may remit from a rapid rise in intracellular metal concentrations. However, the metabolic fate of this compound (metal release, excretion etc) and the toxicity of the organic moieties also require further study.

REFERENCES

Abu El-Zahab, H.; W. Abdel Aal; R. Awadallah; T. Mikhail and K. Zakaria (1991). The correlation between serum cholesterol and some trace elements in serum, liver and heart of rats fed with high cholesterol diet. *Nahrung, 35: 827-834.*

Atamanalp, M. and T. Yanik (2003). Alterations in hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to mancozeb. *Turk. J. Vet. Anim. Sci., 27: 1213-1217.*

Baligar, P. and B. Kaliwal (2001). Induction of gonadal toxicity to female rats after chronic exposure to mancozeb. *Ind. J. Health, 39(3): 235-243.*

Belpoggi, F.; M. Soffritti; M. Guarino; L. Lambertini; D. Cevolani and C. Maltoni (2002). Results of long-term experimental studies on the carcinogenicity of ethylene-bis-dithiocarbamet (Mancozeb) in rats. *Ann. NY. Acad. Sci., 982: 123-136.*

Carleton, H.; R. Druy; E. Willingaton and S. Coneron (1967). *Histological Techniques, 4th Ed.*, pp. 125-137. Oxford Univ Press, N.Y., USA.

Dacosto, M.; O. Abate and C. Nebbia (1994). Subacute toxicity of triphenyltin acetate (TPTA) in the rabbit. Clinical observations and hematologic, hematochemical and enzymatic changes. *Schweiz Arch. Tiechnol., 136(3): 111-115.*

El-Halwagy, M.; G. Abd El-Raheim and M. Rizk Alla (2003). Subacute effect of sulfur containing fungicide on some hematological and biochemical changes in rat. *Egypt J. Appl. Sci., 18(8B): 681-694.*

Goyer, R. (1986). Toxic effects of metals. In: *Casarett and Doul's Toxicology, The Basic Science of Poisons*, edited by Mary Amdur, O. John Doull and Curtis D. Klaassen), pp. 623-680. Pergamon Press, N.Y., USA.

Guven, K.; E. Deveci and D. de Pomerai (1999). The accumulation and histological effects of the organometallic fungicide Propineb on the organs of the fetuses and female rats during pregnancy. *Tr. J. Biol., 22: 413-422.*

Hasegawa, R.; R. Cabral; T. Hoshiya; K. Hakoi; T. Ogiso; P. Bonyaphiphat; T. Shirai and N. Ito (1993). Carcinogenic potential of some pesticides in a

medium-term multi-organ bioassay in rats. *Int. J. Cancer, 54: 489-493.*

Hashizame, K.; T. Yasui and H. Nagano (1992). Studies on the fate of thiabendazol in mice. *Japanese J. Toxicol. Environ. Health, 38(1): 69-77.*

Hassal, K. (1990). *The Biochemistry and Use of Pesticides:* Structure, metabolism, mode of action and uses in crop protection. **pp. 263-361,** VCH Publishers Inc., N.Y., USA.

Jeffrey, M.; L. Charles; A. Tobia and B. Ravenz Waay (2000). Subchronic and chronic toxicological investigations on metiram: The lack of a carcinogenic response in rodents. *Toxicol. Sci., 54: 481-492.*

Kackar, R.; M. Srivastava and R. Raizada (1997). Induction of gonadal toxicity to male rats after chronic exposure to mancozeb. *Ind. Health, 35(1): 104-111.*

Kackar, R.; M. Srivastava and R. Raizada (1999). Assessment of toxicological effects of mancozeb in male rats after chronic exposure. *Ind. J. Exp. Biol., 37(6): 553-559.*

Kelly, W. (1993). The liver and biliary system. In: *Pathology of Domestic Animals*, Jubb, K.V. and N., Plameer (eds.). **Vol. 2, 4th Ed., pp. 319-406.** Academic press, London.

Kendrick, M.; M. May; M. Plishka and K. Robinson (1992). *Metals in Biological Systems.* **p. 179.** E. Horwood, N.Y., USA.

Larsson, K.; C. Arnander; E. Cekanova and M. Kjellberg (1976). Studies of teratogenic effects of the dithiocarbamates maneb, mancozeb and propineb. *Teratology, 14: 171-184.*

Lukens, R. (1971). *Chemistry of Fungicidal Action.* **p. 125.** Springer-Verlag, N.Y., USA.

Mahadevaswami, M.; U. Jadaram Kunti; M. Hiremath and B. Kaliwal (2000). Effect of mancozeb on ovarian compensatory hypertrophy and biochemical constituents in hemicastrated albino rat. *Reprod. Toxicol., 14(2): 127-134.*

-Meco, G.; V. Bonifati; N. Vanacore and E. Fabrizio (1994). Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). *Scand. J. Work. Environ. Health, 20: 301-305.*

Organization for Economic Cooperation and Development (OECD): *Guidelines for Testing Chemicals*, **406 (Adopted in 1995)**

Prasadad, A. (1979). Clinical, biochemical and pharmacological role of zinc. *Ann. Rev. Pharmacol. Toxicol., 20: 393-426.*

Rizk Alla, M.; S. Abo-Seada and M. Talha (2004). Biochemical effects of crunch on male albino rats. *Egypt J. Appl. Sci., 19(1): 212-223.*

Schalm, O. (1986). *Veterinary Hematology.* **4th Ed., pp. 21-36.** Lea and Febiger, Philadelphia, USA.

Snedecor, G.W. and W.G. Cochran. (1989). *Statistical Methods.* **8th Ed.,** 503pp. Ames, Iowa State Univ Press, USA.

Siddiqui, A.; S. Srivastava and B. Ali (1990). Effect of mancozeb on hydrolytic metabolism of xenobiotics. *Res. Commun. Chem. Pathol. Pharmacol., 70(2): 249-252.*

Soleo, L.; G. Defazio; R. Scarcelli; R. Zefferino; P. Livrea and V. Foa (1996). Toxicity of fungicides containing ethylene-bis-dithiocarbamate in serumless dissociated mesencephalic-striatal primary coculture. *Arch. Toxicol., 70: 678-682.*

Subramoniam, A.; D. Agrawal; S. Srivastava and P. Seth (1991). Influence of mancozeb on mitogenically responsive lipids in rat cerebrum and liver. ***Ind. J. Exp. Biol., 29: 943-5.***

Szepvolgyi, J.; K. Nagy; K. Sajgone Vukan; A. Regoly Merel; K. Soos; K. Toth; A. Pinter and M. Antal (1989). Subacute toxicological examination of Dithane M-45. ***Food Chem. Toxicol., 27(8): 531-538.***

Terada, M.; F. Mizuhashi; T. Tanita; H. Inueand and K. Murata (1998). Mepanipyrn induces fatty liver in rats but not in mice and dogs. ***J. Toxicol. Sci., 23(3): 223-234.***

Teramoto, S.; M. Kaneda and Y. Shirasu (1975). Teratogenicity of ethylenethiourea in rats. Relationship of dosage and time of administration to types of malformation. ***Teratology, pp. 215-216.***

Timbrell, J. (1991). ***Principles of Biochemical Toxicology.*** **p. 415.** Taylor and Francis, London.

Trivedi, N.; R. Kakkar; M. Srivastava; A. Mithal and R. Raizada (1993). Effect of oral administration of fungicide-mancozeb on thyroid gland of rat. ***Ind. J. Exp. Biol., 31: 564-566.***

Verplanke, A.; L. Bloemen; E. Brouwer; N. Van Sittert and P. Boogaard (2000). Occupational exposure to cis-1,3-dichloropronene: biochemical effect monitoring of kidney and liver function. ***Occup. Environ. Med., 57: 745-751.***

Ware, G. (1983). ***Pesticides: Theory and Application.*** **p. 293.** W.H. Freeman and Company, N.Y., USA.

Wlostowski, T. (1992). On metallothionein, cadmium, copper and zinc relationships in the liver and kidney of adult rats. ***Comp. Biochem. Physiol., 103C: 35-41.***

مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ،١٤(١) ، ٤٥٧-٤٧٤ ، ٢٠٠٦

السمية تحت-مزمنة للمبيد الفطرى مانكوزيب على ذكور فئران وستر التحليل البنائى الدقيق لأنسجة الكبد و الكلى

[٣١]

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ينتمى المانكوزيب لمجموعة دايثيوكرباميت ويعد مبيد فطرى معدنى-عضوى وقد أعطى عن طريق الفم لذكور فئران وستر فى صورتين أحدهما نقية ٨٥% والأخرى مجهزة ٨٠% عند جرعات تحت مميتة وهى ١٢٥و ٢٥٠و ٥٠٠ مجم/كجم من وزن الجسم و لمدة ٢٨ يوم.

وقد تم قياس بعض المحددات الكيميائية/الطبية بعد مروم ٢و٣و٤ أسابيع من بدء المعاملة. وقد أظهرت النتائج أن كلا المركبين قد أثر بصورة معنوية على نشاط أنزيمات السيرم وخاصة أنزيمات الكبد وذلك فى نمط يعتمد على الجرعة و زمن المعاملة مقارنة مع الكنترول . وقد تلازمت هذه التغيرات مع تغيرات مصاحبة معنوية فى دلائل أخرى مثل كمية البروتين الكلى والزلال والجلوكوز والدهون الكلية وثلاثى الجلسريد والكوليسترول واليوريا والكرياتينين.

وفضلا عن ذلك فقد أظهر فحص أنسجة الكبد والكلى عدة تغيرات تشريحية فى أنسجة الفئران المعاملة سواء بالصورة النقية أو المجهزة للمركب.

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