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Effect of Various Levels of Dietary Malathion on Wistar Rats

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ABSTRACT

Malathion is a pesticide that is widely used in agriculture, residential landscaping, public recreation areas and in public health pest control programs such as mosquito eradication, it is the most commonly used organophosphate insecticide. Malathion were fed to 60 day-old Wistar rats at 50, 100, 500, 1000 and 2000 mg kg^{-1} of diet for 8 weeks. The 1000 and 2000 mg kg^{-1} of dietary Malathion were toxic but not lethal to rats and caused nephrohepatopathy, lymphocytic accumulation in vital organs, after 4 weeks macrocytic hypochromic anaemia and microcytic hypochromic anaemia after 8 weeks. Alterations of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities and cholesterol, globulin and urea concentrations.

Key words: Malathion, renal degenerative, lymphocytic accumulation, anaemia

INTRODUCTION

Malathion is S-1,2-bis(ethoxycarbonyl)ethyl O,O-dimethyl phosphorodithioate. It is likely that the results of earlier toxicological studies on Malathion have been substantially affected by impurities. Particular interest are isomalathion (S-1, 2-bis(ethoxycarbonyl) ethyl O,S-dimethyl phosphorodithioate) and various trialkyl phosphorothioates (Aldridge *et al*., 1985; Dinsdale, 1992). These compounds are notable for their pulmonary toxicity. Furthermore, isomalathion has a greater than additive effect when administered with malathion, probably due to carboxylesterase inhibition (Ryan and Fukuto, 1984). In fact, isomalathion appears to be the major impurity of Malathion and affects the LD_{50} of the commercial formulation. O,O,S-Trimethyl phosphorothioate and O,S,S-trimethyl phosphorothioate produce disorders of blood clotting (Keadtisuke *et al*., 1990) and O,O,S-trimethyl phosphorothioate produces an unusual neurotoxic syndrome with hypophagia, weight loss and hypothermia (Ohtaka *et al*., 1995). After an epidemic of Malathion poisoning among spraymen in Pakistan (Baker *et al*., 1978), WHO issued specifications for Malathion water-dispersible powders, which required that a 50% powder contain no more than 0.9% isomalathion after storage at 54°C for six days (Miles *et al*., 1979; WHO, 1985). Subsequently, major manufacturers, under the auspices of FAO, adopted a code of conduct which requires that, inter alia, the active ingredient and co-formulant of commercial formulation be the same as those tested toxicologically (FAO, 2005). Malathion was rapidly absorbed, biotransformed and excreted, predominantly in the urine but also in the faeces. After the low dose, 84% appeared in the urine of males and 88% in that of females within 72 h, mostly within 12 h: faecal elimination was 11 and

5.9% in males and females, respectively. Less than 1% of the administered dose was recovered in the tissues. At the high dose, urinary excretion was 76% for the males and 85% for the females; faecal elimination was 14 and 6.6%, respectively. Low concentrations were present in tissues at 72 h. After the repeated doses, 85 and 88% of the label was excreted in the urine within 72 h, mostly within the first 12 h and faecal elimination was 6.8% in males and 5.8% in females. Less than 1% of the dose was present in the tissues. The toxicokinetics of Malathion was studied by Garcia-Repetto *et al.* (1995) after oral administration of a dose of 467 mg kg^{-1} b.wt. (stated to be one-third of the LD_{50}) to male albino Wistar rats. A two-compartment model was discerned, the central compartment being blood, adipose tissue and muscle and the peripheral compartment, brain and liver. The half-life in blood was 1.4±0.25 days. In a fatal case of Malathion poisoning, Malathion and the mono-and dicarboxylic acids were found in cardiac blood and tissues, malaoxon being found additionally in most tissues (Morgade and Barquet, 1982).

MATERIALS AND METHODS

Experimental design: Thirty six 12 weeks-old male Wistar rats were housed within the premises of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum, with feed and water provided *ad libitum*.

The rats were allotted at random to six groups, each of 6 rats. Group 1 continued to be fed the normal diet and served as control. Groups Group 2-6 to be fed diets containing Malathion at 50, 100, 500, 1000 and 2000 mg kg^{-1} diet, respectively.

Average body weight and body weight gain for each group, were estimated weekly. Batches of 3 rats from each group were sacrificed at weeks 4 and 8 for pathological examination. Blood samples were collected from each of the killed rats. At necropsy, all rats were examined to identify gross lesions and specimens of the liver, kidneys, heart, spleen and intestines were fixed in 10% neutral buffered formalin and processed for histopathology.

Blood analyses: Serum samples were analyzed for the activity of aspartate aminotransferase (AST),alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and for concentrations of total protein, albumin, globulin, cholesterol and urea.

Hemoglobin (Hb) concentration, Red Blood Cell (RBC) counts, Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and White Blood Cell (WBC) counts were determined by standard methods (Schalm *et al.*, 1975).

Statistical analysis: The significance of differences between means was compared at each time point using Duncan's multiple range test after ANOVA for one-way classified data (Snedecor and Cochran, 1989).

RESULTS

Changes in growth: The effects of treatment with diets containing 50-2000 mg kg^{-1} of Malathion on growth of the rats are shown in Table 1, none of the animals died during the course of the experiment.

Pathological changes: No pathological changes were detected in the vital organs of the rats fed the 50 mg kg^{-1} Malathion (group 2) compared with rats on the control diet (group 1). Mild

Table 1: Changes in mean body weights and mean body weight gains in rats fed diets containing various levels of malathion 4 and 8 weeks

Treatment groups	Initial body weight (g)	Body weight (g)	Body weight gain (g)
4 weeks			
Control (normal diet)	77	97.5±1.3	20.5±1.2
Malathion (50 mg kg^{-1})	76.2	94.5±1.2^{NS}	18.3±1.2^{NS}
Malathion (100 mg kg^{-1})	85.7	98.0±2.1^{NS}	21.3±2.1^{NS}
Malathion (500 mg kg^{-1})	76.7	97.5±1.9^{NS}	20.8±1.8^{NS}
Malathion (1000 mg kg^{-1})	76.5	99.2±2.4^{NS}	22.7±2.3^{NS}
Malathion (2000 mg kg^{-1})	76.5	96.0±1.1^{NS}	19.5±0.8^{NS}
8 weeks			
Control (normal diet)	101.4	124.7±0.9	23.3±0.9
Malathion (50 mg kg^{-1})	92.3	118.3±1.2^{NS}	26.0±1.2^{NS}
Malathion (100 mg kg^{-1})	94.3	120.3±1.3^{NS}	26.0±1.5^{NS}
Malathion (500 mg kg^{-1})	98	123.0±2.3^{NS}	25.0±3.1^{NS}
Malathion (1000 mg kg^{-1})	104.3	125.0±1.5^{NS}	20.7±1.2^{NS}
Malathion (2000 mg kg^{-1})	96.7	125.0±1.7^{NS}	28.3±1.9^{NS}

Values are Means+SE, NS: Not significant

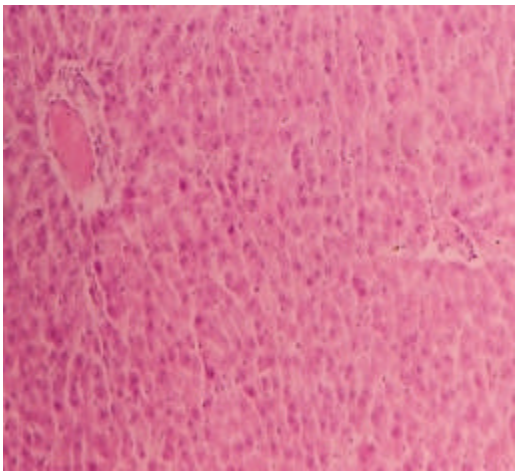

Fig. 1: Generalized necrosis and congestion in a liver of a rat fed a diet containing 2000 mg kg^{-1} Malathion for 8 weeks. H and E x100

degenerative changes were, however, observed in the liver generalized necrosis and congestion of the rats fed a diet containing 2000 mg kg^{-1} Malathion (group 6) (Fig. 1). There were, in addition, to renal degenerative changes, necrotic foci with lymphocytes. No significant lesions were observed in the heart and spleen.

Hematological changes are summarized in Table 2. After 4 weeks of treatment, the values of Hb, MCH and MCHC were lower ($p<0.05$) in all groups than control. The values of RBC and MCV were higher ($p<0.05$) in groups 2-6 than control (group 1). The value of PCV was higher ($p<0.05$) in group 2 than control and other groups. After 8 weeks of treatment, the values of Hb, MCV, MCH and MCHC were lower ($p<0.05$) in groups 2-6 than control (group 1). The value of RBC were higher ($p<0.05$) in groups 2-6 and PCV in group 3 was higher ($p<0.05$) than control and other groups.

Table 2: Haematological changes in rats fed diets containing various levels of malathion 4 and 8 weeks

Parameters	Treatment groups: Control (normal diets)	Malathion (50 mg kg^{-1})	Malathion (100 mg kg^{-1})	Malathion (500 mg kg^{-1})	Malathion (1000 mg kg^{-1})	Malathion (2000 mg kg^{-1})
4 weeks						
Hb (g dL^{-1})	16.7±0.4	14.4±0.2*	12.5±0.6*	12.6±0.9*	12.1±0.5*	12.8±0.2*
PCV (%)	35.3±1.2	40.8±1.0*	35.5±1.5^{NS}	37.8±2.9^{NS}	35.4±2.2^{NS}	36.6±0.3^{NS}
RBC (10^{6} mm^{3})	5.4±0.5	6.9±0.3*	6.0±0.2*	6.1±0.5*	5.8±0.3*	5.9±0.2*
MCV (m^{3})	39.9±4.0	59.5±1.3*	59.6±1.6*	62.1±0.6*	60.8±0.9*	62.2±1.3*
MCH (pg)	45.2±4.6	21.1±0.7*	21.0±0.7*	20.7±0.4*	20.9±0.4*	21.8±0.8*
MCHC (%)	48.6±0.3	35.4±0.4*	35.3±0.2*	33.3±0.3*	34.3±0.8*	35.0±0.6*
8 weeks						
Hb (g dL^{-1})	17.1±0.4	12.4±0.6*	13.7±1.0*	12.5±0.5*	11.9±0.1*	12.5±0.6*
PCV (%)	33.4±1.2	35.7±1.7^{NS}	40.5±3.4*	36.0±0.6^{NS}	34.8±0.3^{NS}	35.5±1.5^{NS}
RBC (10^{6} mm^{3})	5.8±0.4	6.3±0.3*	6.7±0.5*	5.6±0.1*	5.5±0.05*	6.0±0.2*
MCV (m^{3})	68.5±2.6	57.0±0.4*	60.7±1.0*	61.3±2.3*	62.8±0.2*	59.6±1.6*
MCH (pg)	42.4±1.1	19.8±0.07*	20.6±0.2*	22.3±0.4*	21.4±0.3*	21.0±0.7*
MCHC (%)	48.9±2.5	34.8±0.09*	34.0±0.4*	34.7±0.9*	34.1±0.5*	35.3±0.2*

Values are Means+SE, NS: Not significant, *p<0.05

Table 3: Serobiochemical changes in rats fed diets containing various levels of malathion 4 and 8 weeks

Parameters	Treatment groups: Control (normal diets)	Malathion (50 mg kg^{-1})	Malathion (100 mg kg^{-1})	Malathion (500 mg kg^{-1})	Malathion (1000 mg kg^{-1})	Malathion (2000 mg kg^{-1})
4 weeks						
AST (iu)	24.7±2.4	85.0±17.5*	90.3±6.9*	101.3±2.0*	122.7±8.2*	95.7±19.5*
ALT (iu)	15.3±0.3	69.0±19.1*	80.0±7.0*	92.7±16.8*	48.0±9.1*	52.3±9.4*
ALP (iu)	127.7±2.4	116.0±0.6*	81.3±0.2*	125.0±0.1*	129.7±0.2	128.0±0.2
T. Protein (g dL^{-1})	7.9±0.1	3.3±0.4*	3.1±0.1*	3.4±0.2*	4.0±0.1*	4.6±0.03*
Albumin (g dL^{-1})	4.1±0.1	1.9±0.4*	2.1±0.1*	1.5±0.2*	3.5±0.3*	3.7±0.2*
Globulin (g dL^{-1})	3.8±0.1	1.4±0.03*	1.0±0.0*	1.9±0.0*	0.5±0.0*	0.9±0.0*
Cholesterol (mg dL^{-1})	80.0±2.5	138.3±19.6*	162.7±14.5*	143.7±24.8*	142.7±3.7*	140.7±7.4*
Urea (mg dL^{-1})	50.0±7.2	116.0±18.2*	81.3±12.1*	125.0±5.3	129.7±10.3	128.0±3.8
8 weeks						
AST (iu)	25.0±2.1	88.3±11.8*	97.3±4.6*	105.3±8.8*	120.0±5.7*	92.0±14.2*
ALT (iu)	16.7±0.7	61.3±12.2*	78.0±4.9*	78.3±5.2*	58.7±2.3*	52.7±10.0*
ALP (iu)	135.0±6.4	107.7±0.4*	118.0±0.5*	125.3±0.4*	130.0±0.2*	127.7±0.3*
T. Protein (g dL^{-1})	8.1±0.08	3.1±0.3*	2.8±0.2*	3.3±0.3*	3.5±0.3*	4.3±0.2*
Albumin (g dL^{-1})	4.1±0.2	2.6±0.3*	2.3±0.4*	1.7±0.2*	3.4±0.5*	3.4±0.4*
Globulin (g dL^{-1})	4.0±0.2	0.5±0.03*	0.5±0.0*	1.6±0.0*	0.1±0.0*	0.9±0.03*
Cholesterol (mg dL^{-1})	83.7±2.7	132.7±10.8*	157.0±13.4*	164.3±13.9*	139.7±3.9*	133.7±12.2*
Urea (mg dL^{-1})	56.7±4.4	107.7±6.1*	118.0±5.5*	125.3±8.6*	130.0±5.9*	127.7±5.9*

Values are Means+SE, NS: Not significant, *p<0.05

Serobiochemical changes these data are presented in Table 3. After 4 weeks of treatment, the activity of serum AST and ALT in groups 2,3,4,5 and 6 were higher ($p<0.05$) than control (group1). The activity of ALP were lower ($p<0.05$) in groups 2, 3, 4, 5 and 6 than control. Total protein,

albumin and globulin concentrations were lower ($p<0.05$) in groups 2-6 than control and other groups. Cholesterol and Urea concentrations were higher ($p<0.05$) in group 2,3,4,5 and 6 than other groups. After 8 weeks of treatment, the activity of serum AST and ALT in groups 2-6 were higher ($p<0.05$) than control (group1). The activity of ALP were lower ($p<0.05$) in groups 4, 5 and 6 and higher ($p<0.05$) in group 2 than other groups. Total protein, albumin and globulin concentrations were lower ($p<0.05$) in groups 2-6 than control and other groups. Cholesterol and Urea concentrations were higher ($p<0.05$) in group 2,3,4,5 and 6 than other groups.

DISCUSSION

There were no differences in mean body weight gains between the groups of rats for the 8 week full period. This may be explained by the feeding of identical diets to each group and the useful randomized assignment for examination. The results of the present study indicate that feeding rats Malathion at 50, 100, 500, 1000 and 2000 mg kg^{-1} of the normal diet for 60 days is not toxic as evidenced by the absence of mortality, of clinical changes, of growth impairment and of lesions in the vital organs. The damage to these organs probably contributed to the elevated serum AST and ALT activities and cholesterol and urea concentrations and the decreased albumin concentration and ALP activity. The increase in MCV without significant effects on MCHC indicates macrocytic normochromic anemia. In previous similar research work, differences were seen between treated groups in total protein and albumin concentrations and a significant decrease in alkaline phosphatase activity was seen in animals at the two highest doses. Animals at 20 000 ppm had a significant decrease in weight gain in comparison with the control group. Haemoglobin count and haematocrit were decreased in males at the high dose, while the mean corpuscular volume and mean cell haemoglobin were decreased in males at doses >5000 ppm. In females, the erythrocyte count was marginally increased at doses >500 ppm, while the mean corpuscular volume was decreased at 10 000 and 20 000 ppm and the mean cell haemoglobin was decreased at doses >5000 ppm (Daly, 1993b). Decreases in mean haemoglobin concentration, haematocrit, mean corpuscular volume and mean cell haemoglobin were seen in animals of each sex at the two highest doses at 6, 12 and 18 months, although all parameters were not affected at all the time intervals and there was a tendency for improvement during the study. The mean cell haemoglobin concentration was decreased in males at the two highest doses only at 12 months, accompanied by an increase in platelet count. Alkaline phosphatase activity was reduced in comparison with concurrent controls in animals of each sex at the two highest doses at 6 and 12 months and at the highest dose at 18 months. Aspartate aminotransferase activity was reduced in females at doses >500 ppm at 12 months and at the highest dose at 18 months. Alanine aminotransferase activity was also decreased in females at the three highest doses at 12 months. gamma-Glutamyl transpeptidase activity was increased consistently in males at the two highest doses from 12 months and at most intervals in females. Cholesterol content was increased in animals of each sex at the two highest doses at 6, 12, 18 and 24 months (Daly, 1993a). The mean body weight of the animals at the high dose was decreased in comparison with the controls. Decreased weight gain at the next highest dose and the latter on the absence of fetal toxicity at any dose (Siglin, 1985). The present results and the studies of Mohamed and Adam (1990a, b) and Ahmed and Adam (1979) suggested that serum urea determination is of value in the assessment of renal toxicity in goat. A decrease in serum Albumin concentration due to the failure of hepatic synthesis may occur in

association with an increase of the globulins specially gamma globulins such effect have been demonstrated in young ruminants intoxicated with seneciojacoboea (Ford *et al*., 1968).

CONCLUSION

It is concluded that Dietary levels of 50 mg kg^{-1} of malathion are not lethal to Wistar rats but the higher level is capable of producing hepatonephropathy.

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