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Toxicological Assessment of Biodegraded Malathion in Albino Mice

N. E. Barlas

Department of Biology, Faculty of Science, Hacettepe University, Beytepe, 06532, Ankara, Turkey

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The disappearance of pesticide residues at a given location does not mean the end of the problem. Pesticides can be translocated, bioconcentrated or converted into more dangerous chemicals (Matsumura 1985). The organophosphorus compounds are widely used because of their rapid biodegradability and nonpersistent nature. It has been shown that these compounds acts on various enzyme systems (Nitiforo and Stein 1967; Abou-Donia 1978). Malathion is one of the most selective organophosphorus insecticides used in Turkey for the control of pests on vegetables, field crops, fruits and domestic animals.Malathion is known to have potent insecticidal activity and low mammalian toxicity (Martin and Worthing 1977). In B6531 mice fed malathion, however, statistically significant dose responsive increases in liver carcinomas and neoplastic nodules occured. In the F344 rat, there were increases in thyroid and benign mammary tumors in rats fed malaoxon, the more toxic metabolite/breakdown product of malathion (US-EPA 1988). This conversion is catalyzed by mixed-function oxidases in mammals and insects (Reuber 1985). Malathion is metabolized rapidly by the soil fungus *Trichoderma viride* and the bacterium Pseudomonas sp. (Matsumura 1966). These microorganisms are capable of utilizing malathion as a sole source of carbon.

In this study the effects of orally administered pesticide, both in the form of commercial and microorganism-degraded solutions, on albino mice were investigated.

MATERIALS AND METHODS

Malathion, technical purity 95 %, was obtained from the Research Institute for Plant Protection Chemical and Equipment in Ankara.

Malathion degrading microorganisms were isolated from soil samples by adding 1 g of soil to 99 mL of sterile

water and shaking the mixture vigorously for 3 minutes. From this stock, sterile dilutions of 10^{-2} and 10^{-3} were made in sterile petri dishes and mixed with soil extract agar of the type described by Allen (1951). Plates were incubated at 29°C for 7 days, then examined, and individual colonies were selected for identification and tested for their ability degrade malathion. Following further incubation, similar transfer was made to ensure culture purity. Malathion, dissolved in ethanol (0.5 % v/v), was added to 30 mL of mineral salts medium (0.5 g $(NH_4)_2HPO_4$; 0.2 g $MgSO_4.7H_2O$; 0.1 g K_2HPO_4;0.001 g $FeSO_4.7H_2O$; 0.01 g $Ca(NO_3)$ in 1 L distilled water, P^H,6.2) in a 150 mL incubation flask. The final concentration of this pesticide were kept at 6.7 ppm and that of ethanol was 0.5 % (v/v). Isolated microorganisms were inoculated into the mineral salt medium and the contents were incubated at 29 °C for 7 days (Rajagopal et al. 1983).

For determination of the malathion metabolite, the incubated solutions containing mix culture microorganisms were filtered through a Whatman No 1 Milipore filters into a sterile flask. These filtrates and controls (contain malathion but not contain microorganisms) were extracted 3 times with 1:3 n-hexane in 125 mL separatory funnels and analyzed to determine the amount of biodegradation by-products. Norris and Kuckar (1959) reported that n-hexane is the best solvent for malathion in GC experiments. All extracts were evaporated and the residues were redissolved in 2 mL absolute methanol. Malathion residues in microorganisms growth medium were analyzed with a Finnigan Mat 4615 model GC/MS using 30M DB 5,33 MM ID, 25U FT conditions. The column temperatures were programmed at 10°C min^{-1} from 80°C to 270°C. Helium at a flow rate of 10 ml min^{-1} was used as a carrier gas. Duplicate injections(0.8 µg)of each sample were analyzed.

In this investigation, a total of 45 Swiss albino mice (10 wks-old, 30 females and 15 males) weighing 18.2-32.6 g each were used. The animals were obtained from the Ankara Poisons Center, Refik Saydam Hygiene Center. The mice were divided at random into three treatment groups, each having 10 females and 5 males.The mice in group 1 as control, received pellet food and water ad libitum; each mouse in group 2 received 100 µg commercial malathion daily while the ones in group 3 administered filtrated malathion that contained a mixture of 25.6 µg malathion, 14.17 µg malathion monocarboxylic acid and 7.56 µg malaoxon/mouse/d for 15 wks. The females and males were housed in separete glass cages with metal covers during the study period of 15 wks. The mice were allowed to acclimatize to laboratory conditions of 19-24 °C and 60-70 % relative humidity for a minimum of 1 wk prior to

dosing. The dose for the maximum growth of microorganisms was used as treatment quantitate. The incubation solutions containing microorganisms were filtered through a Whatman No.1 filter paper and kept at 4°C for 1 wk. Unincubated commercial malathion and filtrated solutions were given daily as drinking water in a glass bottle for 15 wks. The bottles were cleaned daily. Food consumption was about 3 g/mouse/d. Water and food consumption of mice was recorded daily and the animals were weighed weekly throughout the study.

After 15 weeks, all surviving mice were sacrificed by cervical dislocation, and selected organs (kidneys, liver, spleen, ovaries and testes) were excised and weighed. Blood samples, for serum analysis, were collected by syringe from the heart into non-heparinized tubes and were centrifugated at 1500 rpm for 15 min. The serum was transferred to another tube and analyzed for alkaline phosphatase (AP),alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities (Vural et al.l986).The levels of creatinine, urea, total protein and albumin were measured automatically by a DACOS Discrete Analyzer with Continuous Optical Scanning Instrument (Coulter Electro,Inc).

A Student t-test was used for statistical analysis of organ weights, and a repeated measurement variation of analysis method was employed for the analysis of biochemical results (Sümbüllüoglu and Sümbüllüoglu 1987). The 0.05 level of significance for probability was used as the criterion of statistical significance.

RESULTS AND DISCUSSION

Six species of microorganisms having the ability to degrade malathion were isolated from soil samples except for three of them which did not grow in mineral salt medium (Barlas 1992). The results are shown in Table 1.

The degradation of commercial malathion (100 µg) in mineral salt medium proceeded slowly for the first 6 days and very rapidly thereafter. We suggest that microbial action was responsible for biodegradation of malathion (Figure 1). The amount of malathion residues and degradation products in mineral salt medium reached to 25.6 µg malathion, 14.17 µg malathion monocarboxylic acid and 7.56 µg malaoxon after 10 days when 100 µg of malathion was applied initially.

Some of the pesticides are degraded quite rapidly in the environment, thus, they are unlikely to accumulate even under conditions of repeated annual applications.

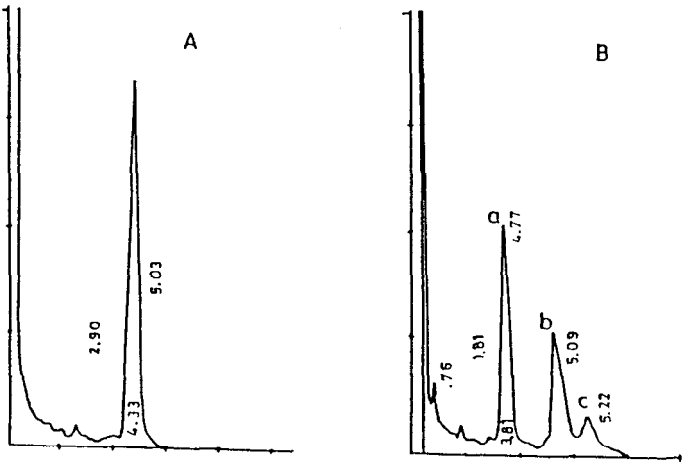

Figure l.GC/MS of control malathion (A) and after 1 week incubation treatment with microorganisms (B) a: malathion, b: malathion monocarboxylic acid c: malaoxon

Table 1. Degradation of malathion by mixed culture of soil microorganisms

Microorganisms	Malathion
Spedenium sp.	+
Rhizopus sp.	−
Aspergillus niger	+
Myceliasterilla sp.	+
Gliocladium sp.	−
Fusarium sp.	−
Bacillus sp.	+
Pseudomonas sp.	+
Arthrobacter sp.	+
Micrococcus sp.	−

+: degradable
-: non degradable

Artrobacter and *Aspergillus* sp are well adapted to soil niche because they are very resistant to desication and nutrient deprivation. These genus are usually flexible nutritionally and can even degrade some herbicides and pesticides; it is probably important in the mineralization of complex organic molecules (Harley and Klein 1993). These experiments indicate that pesticides can be converted to water soluble or toxic compounds by soil microorganisms. In the present study, it was shown

that malathion is biodegraded by mixed culture soil microorganisms. Isolated microorganisms from the soil samples don't have endotoxin and didn't release exotoxin in the growth medium (Harley and Klein 1993). They could release organic acids and enzymes. These products don't have a toxic effects on animals. This is very important because these insecticide-degrading microorganisms may have a positive effect in minimizing soil pollution or preventing ground water from contamination by persistent pesticides. Malathion was found to be metabolized quickly by a soil fungus, *Trichoderma viride,* and a bacterium, *Pseudomonas* sp. (Matsumura and Boush 1966; Singh and Seth 1989). In aqueous-soil-free systems (200ml:5ppm) inoculated with a soil extract, a log phase occured in 7 days followed by a rapid malathion loss, likely due to microbial degradation (Konrad et a1.1969). These findings were consistent with our results.

Anatomical and biochemical investigation was studied with 42 animals. 3 female animals given filtrated malathion died during the experiment. The initial and final body weights and absolute organ weights (liver, spleen, kidneys, ovarium and testes) in each group are given in Table 2. An increase in the mean body weights was found to be significantly lower in tested animals than control ones. Body weight loss of the animals exposed to the oral application of pesticides may be attributed to the taste and odor of these chemicals that cause unwillingness to the foods (Akay 1984). Statistically significant decreases in spleen weights in male mice were found in animals dosed with filtrated malathion. Other organs did not show any change in weight. According to Durham et a1.(1965),a decrease in body weights may be due to decreased food consumption. Vos et al.(1989) reported that environmental pollutants such as pesticides and metals caused changes in the weights of lymphoid organs such as thymus, spleen and lymph nodes in mice and rats. In our study, statistically significant decreases in spleen weights of male mice were found in animals dosed with filtrated malathion.

The effects of the commercial and filtrated solutions of malathion on serum enzyme activities (AP, ALT, AST and levels of creatinine, urea, albumin and total protein) are shown in Table 3. At the end of the experiment, serum alkaline phosphatase activity showed statistically significant increases in female mice but decreases in males in filtrated malathion group. Aspartate aminotransferase activity increased slightly in females in the filtrated malathion group also. Alanine aminotransferase activity decreased in all male mice but, it decreased only in females dosed with commercial malathion. Nitiforo and Stein (1967) and Abou-Donia (1978) found an increase in acid and alkaline phosphatase activity in the chicken plasma and suggested the

Table 2. The mean body and organ weights of control mice and mice dosed with commercial and filtrated solution of malathion for 15 weeks

Sex	Exposure	n	Initial body weight	Final body weight	Mean Weights (g) Liver	Kidney	Spleen	Ovarium	Testis
Male	Control	5	27.14±1.51	38.70±2.02	1.61±0.005	0.30±0.045	0.22±0.45	_____	0.11±0.015
	Com. Malath.	5	25.96±1.45	35.50±1.13*	1.70±0.12	0.24±0.013	0.21±0.31	_____	0.09±0.003
	Filt.Malath.	5	26.80±1.79	31.89±2.40*	1.45±0.11	0.27±0.013	0.11±0.008*	_____	0.11±0.004
Female	Control	10	23.90±0.99	35.50±1.12	1.61±0.072	0.21±0.012	0.22±0.012	0.52±0.10	_____
	Com.Malath.	10	22.02±0.38	30.96±1.12*	1.47±0.041	0.20±0.006	0.17±0.018	0.41±0.085	_____
	Filt.Malath.	7	24.23±0.89	28.14±1.32*	1.61±0.2	0.19±0.009	0.21±0.034	0.69±0.13	_____

Values ± S.D.
*:Statistically significant at 0.05 level
n: Number of animals

Table 3.Serum enzyme activities of control mice and mice dosed with commercial and filtrated solutions of malathion for 15 weeks

GROUPS	n	UREA (mg/dl)	CREATININE (mg/dl)	ALKALINE PHOSPHATASE (AP) U/L	ALANINE AMINOTRANSFERASE ALT (GPT) U/L	ASPARTATE AMINOTRANSFERASE AST (GOT) U/L	TOTAL PROTEIN (g/dl)	ALBUMIN (g/dl)
Female								
Control	10	26.60±1.44	0.65±0.02	72.6±7.13	80.00±6.51	509±58.94	6.8±0.1	3.0±0.05
Com. Malath.	10	23.70±2.98	0.50±0.01	89.00±6.30	53.20±3.42*	352.5±22.3	5.8±0.21	2.56±0.08
Filt. Malath.	7	28.60±2.72	0.46±0.02	94.30±12.1*	82.30±12.08	622±160.9	7.03±0.88	2.9±0.05
Male								
Control	5	34.00±0.56	0.90±0.03	115±2.48	138±3.10	505±15.9	6.7±0.03	2.8±0.0
Com. Malath.	5	37.00±1.66	0.61±0.02	50.00±2.25*	80.00±4.43*	480±1.80	6.9±0.02	3.00±0.03
Filt. Malath.	5	37.00±2.16	0.50±1.63*	52.00±3.65*	78.00±2.30*	463±14.8	6.8±0.02	3.00±0.03

Values ± S.D.
*:Statistically significant at 0.05 level
n: Number of animals

possibility of lysosomal membrane releasing the enzyme from the liver damage following the treatment of an organophosphorus pesticide. Husain et a1.(1987) reported that the increases in the activities of AP, AST, and ALT along with other enzymes suggested that malathion may have an effect on tissue cells in female rats given 137.5 µg/kg of malathion for 32 days. When rats were dosed with increasing oral doses of malathion for 36 days, the activity of aspartate aminotransferase and alanine aminotransferase in the serum was reduced by malathion (Sadek et a1.1989). Zayed et a1.(1993) reported that malathion residues caused increasing of the activity of serum esterases AST, ALT and AP. Some nonspecific responses are related to hepatic changes induced by pesticides, including induction of serum aminotransferases, lactic dehydrogenase and alkaline phosphatase. Damage to liver cells leads to changes in their enzyme levels. The inhibition of aminotransferases might explain in part the observed body weight loss in chronic toxicity studies of the insecticides (Sadek et a1.1989). Increasing levels of AST and ALT are usually due to leakage of damaged membranes (Götz 1981). These reports support our findings. We found a statistically significant decrease in creatinine level in male mice dosed with filtered malathion, but such a decrease was not observed in female mice. The importance of increases and decreases shown in some of the enzyme activities depending on the sex of mice could not be explained in the present study. For urea, total protein and albumen, no statistically significant difference was observed between control and test groups .

On the basis of the results obtained, it may be concluded that commercial malathion and it's degradation products together have detrimental effects on mice over a period of 15 wks of treatment. The degradation products of malathion formed by mixed cultures of microorganisms apparently have more impact on changes in body weight and some enzyme levels than malathion itself.

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