Toxicity, distribution, accumulation and cooking loss of malathion in tissues of tilapia and common carp fishes

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RESUMEN

Toxicidad, distribución, acumulación y pérdida en el cocinado de malatión en tejidos de pescados tilapia y carpa común.

La toxicidad aguda de malatión, pesticida organofosforado, para las dos especies de pescado analizadas, *Tilapia nilotica* (tilapia) y *Cyprinus carpio* (carpa común), fue seguida por estimación de la CL50 a intervalos desde las 24h hasta las 96h. Tilapia fue mucho más susceptible a la toxicidad del malatión cuando se comparó con carpa, y los datos disponibles proporcionaron que la CL50 del malatión para carpa común fue de 5-7 veces la CL50 para tilapia.

La acumulación del pesticida malatión en algunos órganos (músculos, agallas, intestino e hígado) de ambas especies de pescado fue estudiada después de la aplicación de concentraciones de 200, 300 y 400 ppb de malatión en agua durante 28 días. Se encontró una relación proporcional entre el tiempo de exposición y las concentraciones aplicadas en las dos especies de pescado. La velocidad de acumulación de malatión fue alta en hígado, seguido por intestino y agallas, mientras que la concentración más baja fue encontrada en músculos. El malatión acumulado en músculos de tilapia fue superior al acumulado en los de carpa, mientras que una tendencia reversible fue observada en la mayoría de los otros órganos.

El cocinado de algunas especies de pescado redujo eficazmente el contenido de malatión en sus músculos. La fritura de pescado en aceite condujo a un mayor porcentaje de pérdida de malatión que con otros métodos de cocinado.

PALABRAS-CLAVE: Carpa común - Cocinado - Malatión - Tilapia - Toxicidad.

SUMMARY

Toxicity, distribution, accumulation and cooking loss of malathion in tissues of tilapia and common carp fishes.

The acute toxicity of malathion as an organophosphorus pesticide to both of the tested fish species, i.e. Tilapia nilotica (tilapia) and cyprinus carpio (Common carp) was followed by estimating the LC50 at intervals from 24h up to 96h. Tilapia was much more susceptible to malathion toxicity when compared with carp and the available data proved that the LC50 of malathion to common carp fish was 5-7 times the LC50 for tilapia.

Accumulation of malathion pesticide in some organs (muscles, gills, intestine and liver) of both fish species was studied after application of 200, 300 and 400 ppb malathion in water for 28 days. A proportional relation was found in the two fish species between exposure time and the applied concentrations. The rate of malathion accumulation was higher in liver followed by intestine and gills whereas the lowest concentration was found in muscles. Accumulated malathion in tilapia muscles was higher than carp; while a reversible trend was observed in most other organs.

Cooking of the same fish species reduced effectively malathion content in their muscles. Frying of fish in oil lead to a higher loss percent of malathion than did the other methods of cooking.

KEY-WORDS: Common carp - Cooking – Malathion - Tilapia - Toxicity.

1. INTRODUCTION

Organophosphorus pesticides are commonly used in Egypt for either pest control and/or protection of crops. Data have begun to appear in the last few years concerning the presence of organophosphorus pesticides in bodies of water during farming operations. Malathion as one of the fangus organophosphorus pesticide were detected in most of the Egyptian lakes (Abou Donia, 1990). Intensive use of organophosphorus pesticides in agriculture lead to what is known as periodic contamination of natural water; a fact which assure that organophosphorus compounds are highly toxic to aquatic invertebrates and primarily to plankton.

Despite the widespread using of malathion in agriculture, its accumulation in fish exposed to a contaminated water still lacking. Pesticides can accumulate in fish tissues either as a result of feed intake or through a contact with pesticides in water, causing a hazard to public health (J.N. Luthin, American Society of Agronomy, Madison).

Organophosphorus pesticides on the other hand, are less persistent than organochlorines and malathion for instance can be degraded quickly in the environment by microorganisms. Organophosphorus may also appreciably reduce the standing crop of fish, both by decreasing the nutritive base and/or affecting the different stages of fish development and propagation.

El-Sarnagwy and Rizk (1978) traced the accumulation of malathion in fish tissues and they revealed that fatty tissues contained the highest amounts of pesticide residues followed by liver.

In the present work, the acute toxicity (LC50) of malathion pesticide in both Tilapia nilotica (tilapia) and Cyprinus carpio (common carp) was investigated over a 96-hr period. The accumulation of malathion in some organs of the selected fish species and the effects of some cooking methods on the disappearance of malathion from fish muscles were also studied.

2. MATERIALS AND METHODS

2.1. Materials

Malathion standard (57% active ingredient) was provided by R.B. Abel, New Jersey, Marine Science Consortium, Fort Hancock, NJ 07732.

Tilapia nilotica (tilapia) and Cyprinus carpio (common carp) fish were collected from Abbasa fish hatchery at El-Sharkia governorate. The alive fish samples of 5-7 cm in length and 25-30 g in weight were transported in polyethylene containers containing dechlorinated, aerated water and were transferred within 2 hours to a glass aquaria system available at the Laboratory of the National Research Center, Giza Governorate. Fish was acclimated in the glass aquaria (100 x 42 x 60 cm) for one week before bioassay tests and tap water was aerated with an air pump (9.000 cm^3/min) to minimize chlorine level before being used in the previous glass aquaria. The average water temperature in the aquaria during the experiment was 22 ± 1°C and the physicochemical properties of the water are shown in Table I.

2.2. Methods

2.2.1. Acute toxicity of organophosphorus pesticide to tilapia and common carp fish

Malathion was dissolved in acetone and diluted with water to prepare stock solution. A primary experiment was performed to determine the appropriate malathion concentrations to be applied; i.e. being 0.5, 1, 2, 4, 6 and 7.5 mg malathion/L water for tipalia and 14, 16, 18, 19, 20.5 and 22 mg malathion/L water for common carp.

Table I
Physicochemical properties of the water used in the aquaria

Tested properties	Values
Temperature	22±1°C
pH	7-8
Turbidity «NTU»	4.6
Total alkalinity as $CaCO_3$ mg/L	135.30
Total hardness (H) $CaCO_3$ mg/L	114.50
Ca .H as $CaCO_3$	68.08
Mg .H as $CaCO_3$	46.42
Dissolved oxygen (mg O_2/L)	5±0.5

Calculation of LC50

Regression analysis in its exponential form was estimated between concentration of the applied pesticide (log x) vs mortality % (log y).

2.2.2. Chronic toxicity and accumulation of malathion to tilapia and common carp fish

Living fish samples were divided into two sections in which on of them was treated with the tested pesticide for the sake of studying its accumulation in fish muscles, liver, intestine and gills. The other section was kept without treatment for the purpose of comparing results. The water of the aquaria was maintained at 22 ± 1°C and changed daily, and fish was fed three times a week. The tested pesticide was prepared in three different concentrations and added daily to the aquaria which contain the treated sections. The selected levels were around one tenth of LC50 values of 96 hr; i.e. 200, 300 and 400 µg malathion/L water.

Fish was sampled from each of the replicates of the aquaria for testing the accumulation rate of the investigated pesticide after 7, 14, 21 and 28 days. For estimating the accumulation rate of pesticides in gills, intestine and liver of the tested fish sample; these organs were collected from three fishes and minced together for obtaining a sufficient sample weight from which only 5-10 g were used. Results are calculated from an average of 3 replicates.

The AOAC (1980) of official method was applied as follows:

(a) **Extraction.**

Fifty g fresh ground fish flesh (5-10 g for other organs) was accurately weighed, transferred to a high speed blender and mixed for 2 min with 100 g anhydrous sodium sulfate in the presence of 150 mL petroleum ether. The extract was decanted through 500 mL Buchner funnel (containing two 12 cm Whatman Nº 1 filter papers) in a suction flask. The residue in the blender cup was reextracted with two 100 ml portions of petroleum ether, blended for 2 min for each portion, decanted through the Buchner funnel, and poaled with the first extract. The obtained extract was poured through a 40 x 25 mm column of anhydrous sodium sulfate, and the eluate was collected in a 500 mL flask and placed in a rotary evaporator, to obtain the fat.

(b) **Cleanup by acetonitrile partitioning.**

The extracted fat was transferred to a 125 ml separatory funnel with the aid of 15 mL petroleum ether; 30 mL acetonitrile saturated with petroleum ether was added and the mixture was shaken vigorously for 1 min. The layers were allowed to separate and the acetonitrile layer was drained into a 1 L separatory funel containing 650 mL water, 40 mL saturated NaCl solution and 100 mL petroleum ether. Separation technique was repeated 3 times, beginning with the transfer of the extracted fat to the 125 mL separatory funnel.

All the extracts were collected in the 1 L separatory funnel and mixed thoroughly for 30-45 seconds. The layers were allowed to separate and the aqeous layer was drained into another 1 L separatory funnel containing 100 mL petroleum ether was added and the mixture was shaken vigorously for 15 S. After the layers separation, the petroleum ether layer was combined with the previous one and washed with two 100 mL portions of water. The petroleum ether layer was drawn off through a 50 x 25 mm column of

anhydrous sodium sulphate. The eluted petroleum ether extract was evaporated to 10 mL in a rotary evaporator after which it was transferred to a florisil column prepared as described below.

(c) **Cleanup by Florisil column.**

A glass column, 22 mm id, was filled with Florisil (60-100 mesh, P.R. grade; activated at 675°C for 3 h) to a height of 10 cm and topped with 1 cm anhydrous sodium sulfate. The column was prewetted with 40-50 mL petroleum ether; then the petroleum ether extract from the above (b) step was passed through the column at (5 mL/min). The column was eluted at the same rate using 200 mL eluting solvent (50% diethyl ether in petroleum ether). The eluate was concentrated to a dry film which was dissolved by 2 mL of n-hexane for determination.

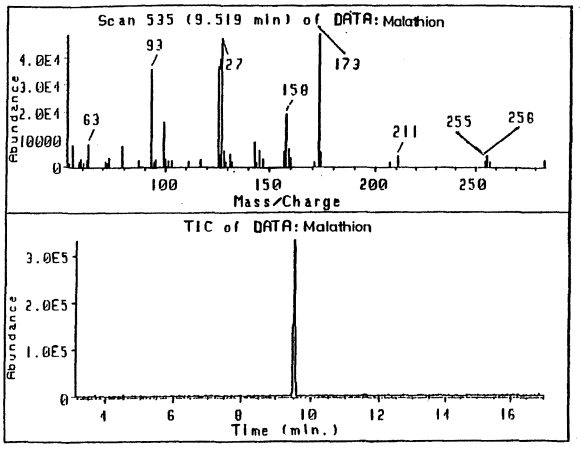

Fig. 1.
Separation of malathion by GC/MS.

(d) **Separation of malathion by GC/MS was performed under the following conditions:**

Apparatus.

Gas chromatograph-Hewlett-Packard Model 5890 A with HP 5970 series Mass selective detector and HP 3397 integrator was used. Interface temperature 280°C, oven temp.; the initial temp. was 100°C, increased to 225°C with a rate of 10°C/min; and helium was used as carrier gas.

Column stationary phase; capillary column HP 101 (methyl silicone fluid).

The extract of malathion was chromatographed by GC/MS as shown in Fig. 1 with its mass spectra.

Table II
Recovery and limit of detection of applied method

Pesticide	Spiked conc. µg/kg	Recovery %	Limit of detection µg/kg
	2	90	1.8
Malathion	11	92	10.12
	22	95	20.90

The sensitivity and recovery for the method were determined using samples spiked with 3 different concentrations (2, 11, 22 µg/kg) of the tested malathion. The results that given in Table II had been corrected for percent recovery of the malathion.

2.2.3. Cooking Methods

Samples of tilapia and common carp fish which contain maximun concentration of accumulated malathion in their muscles (2.63 ppm for the former and 1.70 ppm for the latter; Tables V and VI) were taken from the aquaria for cooking operations. Head, skin, fins and viscera were removed. Pieces of muscular tissues of each contaminated fish were ground in a mixer for 3 min and about 100 g of the previous samples were weighed and formed in a berger style shapes (7 cm diameter and 1 cm thickness). The aforementioned fish styles were cooked by the following different methods:

a) Boiling in water at 100°C for 30 min.
b) Frying in maize oil at 180°C for 15 min.
c) Roasting at 260°C for 20 min.

Malathion residue in cooked fish samples was determined as previously mentioned.

3. RESULTS AND DISCUSSION

3.1. Acute toxicity

Table III summarizes mortality patterns in relation to malathion dosage. No mortality was taken place in control samples. The LC50 of the selected fish species (tilapia and common carp) were calculated by exponential regression analysis using the applied malathion concentrations (log x) against mortality % (log y). From Table IV it could be noticed that the sensitivity to malathion poisoning varied among the two tested species. The LC50 of tilapia during the test periods of exposure (24, 48, 72, 96 h) were 3.75, 2.59, 2.25 and 1.98 mg/L. The corresponding LC50 of malathion with common carp were 19.86, 18.65, 17.37 and 16.60 mg/L, respectively. In 24 h up to 96 h acute tests with malathion, tilapia died faster than carp. Tilapia was much more susceptible to malathion toxicity when compared with carp. The LC50 shown in Table IV. For the two selected fish species were within the range reported by Verma et al (1982). Differences between species in the rate of malathion uptake, detoxification, and elimination, or differential sensitivity of their electron transport systems to inhibition by malathion could contribute to the differential response to the toxicant among species.

3.2. Chronic toxicity and accumulation of malathion by tilapia and carp

Data presented in Table V and Fig. 2 show the level of malathion accumulation in the different organs of tilapia and carp exposed to 200, 300 and 400 µg malathion/L for several intervals up to 28 days.

Table III
Mortality percent of the selected fish samples exposed to malathion

Nº of aquarium	Fish samples	Concentration used (mg/L)	Initial count of fish	Count of dead fish after				%
				24 h	48 h	72 h	96 h	Mortality
1	*Tilapia nilotica*	0.0	10	–	–	–	–	00
2		0.5	10	1	1	–	–	20
3		1.0	10	2	1	–	1	40
4		2.0	10	3	1	1	–	50
5		4.0	10	5	1	1	1	80
6		6.0	10	7	1	1	–	90
7		7.5	10	9	1	–	–	100
1	*Cyprinus carpio*	0.0	10	–	–	–	–	00
2		14.0	10	–	1	1	2	40
3		16.0	10	1	1	1	2	50
4		18.0	10	2	3	3	–	80
5		19.0	10	4	2	2	1	90
6		20.0	10	8	1	1	–	100
7		20.5	10	9	1	–	–	100

Table IV
LC_{50} values at different periods (hr) from treatment with malathion as calculated by regression analysis

	24 hr	48 hr	72 hr	96 hr
		Tilapia nilotica		
A	0.0861	0.5578	0.4923	0.6757
B	0.7735	0.5718	0.5985	0.5668
r	0.9970	0.9949	0.9984	0.9849
LC_{50}	3.7500	2.5900	2.2500	1.9800
		Cyprinus carpio		
A	9.669^{-09}	6.842^{-06}	7.1678^{-04}	7.9165^{-03}
B	7.4830	5.4018	3.9588	3.1147
r	0.9811	0.9877	0.9525	0.9021
LC_{50}	19.8600	18.6500	17.3700	16.6000

Results (Table V and Fig. 2) indicate a proportional relation between the time of exposure and the accumulated concentrations within the applied doses. Exposure of tilapia and carp fish samples to different concentrations of malathion, produce descending accumulation pattern in different organs and arranged as follows: liver, intestine, gills and muscles. The highest accumulation ratio of malathion was found after 28 days from exposure of tilapia to a concentration of 200 ppb. The accumulated amounts in muscle, gills, intestine and liver were 1.40, 2.43, 3.27 and 9.04 ppm respectively. The corresponding accumulation ratios in the aforementioned organs were 7.00, 12.15, 16.35 and 45.20 (Table V). From the same table accumulation ratios resulted from using 300 ppb malathion concentration after 28 days were 7.83, 10.00, 11.87 and 45.33, respectively. Mean while, the corresponding values with the highest applied concentration of malathion (400 ppb) were 6.58, 10.55, 15.00 and 37.00 in muscle, gills, intestine and liver, respectively.

Table V
Concentrations of malathion (mg/kg wet weight) accumulated by *Tilapia nilotica* fish

Applied concentration (µg/L)	Time of exposure (days)	Concentration in the tested specimens*			
		Muscle	Gills	Intestine	Liver
	7	0.21	0.80	0.91	2.30
200	14	0.42	1.12	1.19	4.82
	21	0.73	1.23	2.29	7.64
	28	1.40	2.43	3.27	9.04
Accumulation ratio		7.00	12.15	16.35	45.20
	7	0.42	1.20	1.28	4.10
300	14	0.73	1.65	1.88	5.10
	21	1.27	1.78	2.94	8.50
	28	2.35	3.00	3.56	13.60
Accumulation ratio		7.83	10.00	11.87	45.33
	7	0.75	1.59	2.46	4.50
400	14	1.50	2.29	3.00	5.84
	21	1.64	3.72	3.92	10.90
	28	2.63	4.22	6.00	14.80
Accumulation ratio		6.58	10.55	15.00	37.00

* Concentration expressed as mg/kg wet weight (ppm).

Malathion accumulation by carp after exposure to 200 ppb of malathion for 28 days (Table VI and Fig. 3), was 1.32, 4.20, 9.40 and 12.10 ppm in muscle, gills, intestine and liver, respectively. The corresponding calculated ratios of accumulation were 6.60, 21.00, 47.00 and 60.50 as seen in the same table. Exposing the same fish species to 300

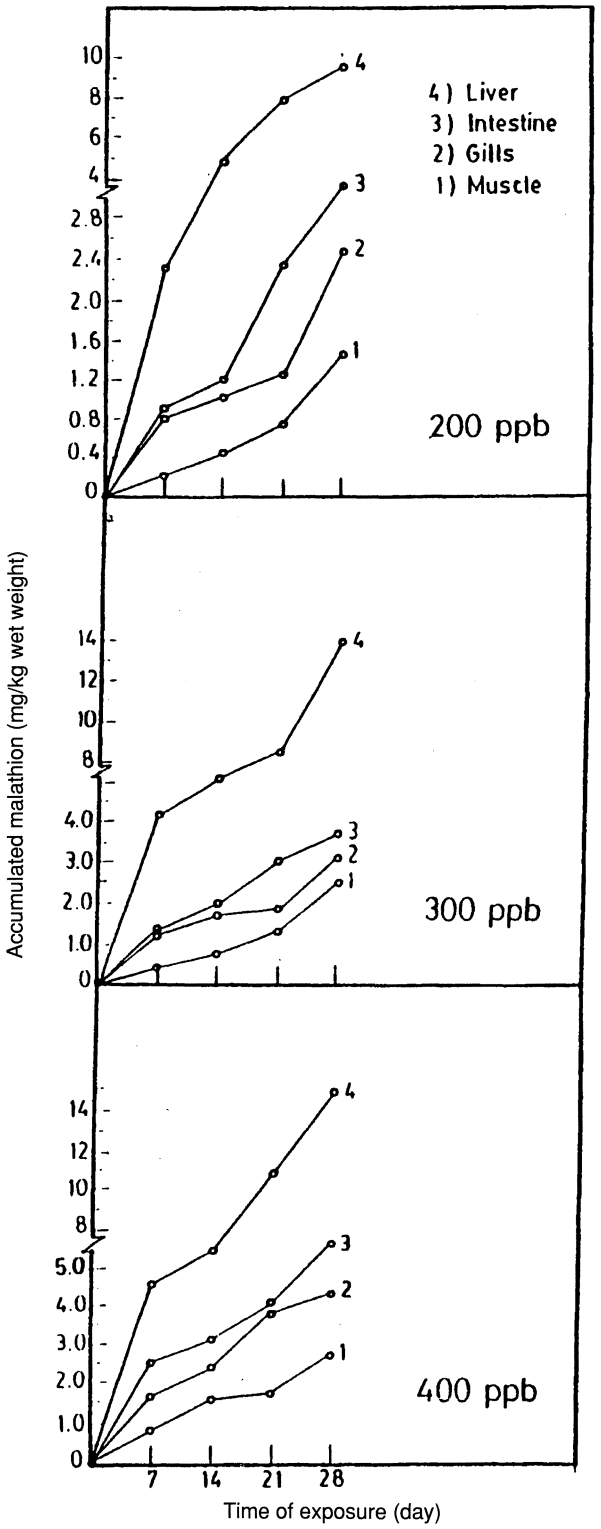

Fig.2
Accumulation of malathion in organs of *T. nilotica* exposed to different malathion concentrations for 28 days.

ppb resulted in a higher accumulation but the accumulation ratio was lower (Table VI).

Tilapia also accumulated malathion in their muscles; but with higher level than carp in all the 3 applied concentrations whereas a reversible trend had been observed in most of the other organs (gills, intestine and liver). Variation in tendency of the tested fish organs towards the accumulation of malathion may be explained as follows:

1. The presence of natural defence system in fish muscle which retard the penetration of malathion and control its diffusion toward the inner parts of the organs. This pattern was more pronounced in carp fish.

2. The physiological activity of individual fish species which permit or limit the accumulation of malathion.

Such a conclusion is justified by Rach and Gingerich (1986) who studied the toxic effects of accumulated rotenone in fish.

Table VI
Concentrations of malathion (mg/kg wet weight) accumulated by *Cyprinus carpio* fish

Applied concentration (µg/L)	Time of exposure (days)	Concentration in the tested specimens*			
		Muscle	Gills	Intestine	Liver
	7	0.60	0.74	1.70	2.68
200	14	0.90	1.89	2.80	3.92
	21	1.03	3.10	5.20	8.40
	28	1.32	4.20	9.40	12.10
Accumulation ratio		6.60	21.00	47.00	60.50
	7	0.87	1.90	2.10	3.20
300	14	1.20	2.90	4.10	5.80
	21	1.30	4.92	7.30	10.20
	28	1.40	6.10	10.50	13.41
Accumulation ratio		4.67	20.33	35.00	44.70
	7	1.01	2.70	3.00	4.32
400	14	1.39	3.28	5.86	9.93
	21	1.45	4.69	9.80	13.29
	28	1.70	7.64	12.47	14.80
Accumulation ratio		4.25	19.10	31.18	37.00

* Concentration expressed as mg/kg wet weight (ppm).

3.3. Effect of technological processes on malathion residues in tilapia and carp

Samples of tilapia and carp which contain the maximum concentration of accumulated malathion in their muscles, i.e. 2.63 ppm for the former and 1.7 ppm for the latter were taken for cooking process.

In case of boiling in water for 30 min (Table VII) malathion loss reached about 35 and 39% in carp and tilapia, respectively.

Table VII shows that the disappearance of malathion by roasting of fish at 260°C for 20 mins was high being 60% in case of carp fish and 65% in case of tilapia.

Frying of the contaminated muscles in maize oil at 180°C for 15 mins reduced effectively malathion content by about 83 and 94% of the original content. The rate of

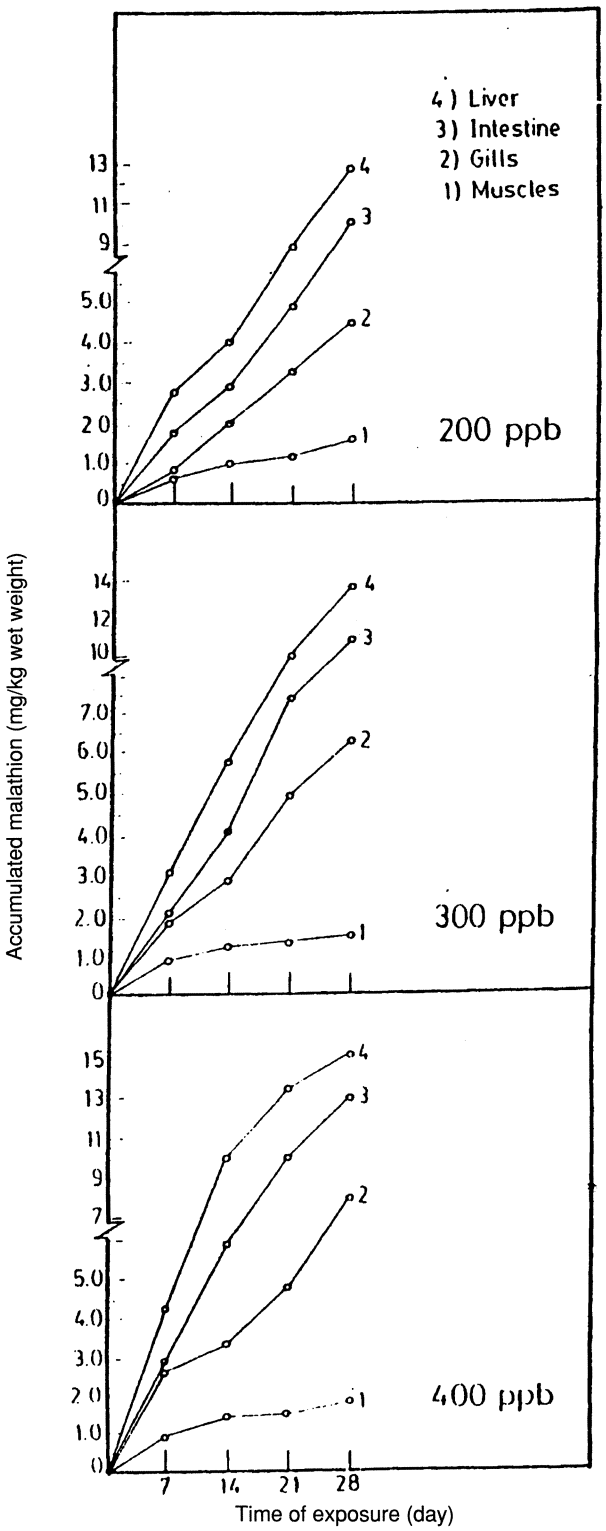

Fig. 3
Accumulation of malathion in organs of *C. carpio* exposed to different malathion concentrations for 28 days.

disappearance of malathion residue by frying in maize oil was greater than by the other previous methods.

From the previous presentation, it is clear that, the pattern of getting rid of higher levels of malathion was as follows: frying > roasting > boiling. In addition, all of the applied technological treatments reduced malathion content in tilapia to an extent which is greater than that in carp.

Table VII
Disappearance of malathion in the tested fish samples by different technological processes

Cooking method	Concentration as µg malathion/ 100 gm wet samples		% Disappearance
	Raw fish	Cooked fish	
	T. nilotica		
Boiling (100°C)	263.0	160.82	38.85
Roasting (260°C)	263.0	92.26	64.92
Frying (160°C)	263.0	16.20	93.85
	C. Carpio		
Boiling (100°C)	170.0	111.0	34.71
Roasting (260°C)	170.0	68.0	59.71
Frying (160°C)	170.0	29.0	82.94

It could also be concluded that both cooking temperature and heating media (water or oil) affect on the disappearance of malathion from both tilapia and carp meat. It seems likely that cooking in oil was much more effective in reducing malathion content in burger shaped fish than did heating temperatures up to 260°C.

However, loss of malathion might be caused by degradation, hydrolysis, and chemical as well as physical factors such as solubility in water or oil, volatibility thermostability and chemical reactions as indicated by Liska et al. (1967) and Lee and Lee (1985).

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