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Activation of *In Vivo* Metabolism of Malathion in Male *Tilapia nilotica*

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Uptake and metabolism of organophosphorus insecticides are of major concern for monitoring their residues in fish and assessment of their metabolic fate. In addition activation of mixed function oxidases (MFO) may help in the study of metabolic pathways of malathion (Hassall 1990).

The present investigation aimed to follow the accumulation of malathion by the male *Tilapia nilotica* which is widely distributed in Nile River in Egpt. *In vivo* metabolism of the insecticide in exposed fish was also studied, both in the normal fish and those injected by phenolbarbital which leads to MFO activation.

MATERIALS AND METHODS

Adult *T. nilotica* were collected from River Nile, nearby Cairo, Egypt. Fishes weighing about 100-150 g were kept in glass aquaria (80 X 40 X 60 cm) containing river water. The general characteristics of river water are given in Table 1. The water samples were very low in heavy metals (concentration levels of Hg, Cd,Co, Cr and Pb were < 0.005 mg/L). The fish were fed twice daily a commercial diet (mixed protein and carbohydrate) and were kept at room temperature (25°C ± 2°C) for 48 hr before running the experiments. Male fishes were selected for this study to unify the specific xenobiotic enzymes liable to be involved *in vivo* degradation. either before or after injection with phenolbarbital.

Malathion (O,O-dimethyl-S-1,2-di(ethoxy carbonylethyl) phosphorodithioate)) together with four metabolites, namely, O,O-dimethyl phosphorodithioate (DPDT): O,O-dimethyl-phosphorothiolate (DPTL), O,O-dimethyl-phosphorothionate(DTN) and O,O-dimethylphosphate (DP), having a purity of 99 % were used (Sigma. USA) Phenolbarbital was used as an activator of MFO and was obtained from BDH, England. Ethyl alcohol was the solvent used for all chemicals used in this study.

A group of 60 male fishes were equally distributed into four aquaria containing river water (20 L). One aquarium served as control to which an appropriate volume of ethyl alcohol (1 ml/L) was added. The three others were fortified with malathion to yield final concentrations of 200, 500 and 1000 µg/L. Fishes of each group were sampled at time intervals of 1, 3, 5 and 7 -d of exposure. Each sample

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Table 1. General characteristics of raw Nile River water*

Parameter	Turbidity NTU	pH	TDS mg/L	Total bacterial count/ mL.22°C	D.O. mg 0_2/L	Total akalinity $CaC0_3$ mg/L	Total hardness $CaC0_3$ mg/L	COD mg 0_2/L
Concentration	15	7.8	270	3500	8.3	112	110	20

* Analysis was run according to APHA Standard Methods, 1989.

was represented by three fishes. Control fish samples were also collected at the same time intervals. Malathion solutions. of each concentration as well as the control were changed daily, and the aquaria were always areated by a gentle stream of air.

A second group of 30 fishes, was distributed in three aquaria containing malathion concentration as previously given. Fishes of this group were pre-injected with phenolbarbital (22.5 mg/150 g fish). Fish samples were collected at time intervals of 12 and 24 hr of exposure. Fish of all treatments were immediately killed. after the specific exposure period, and the tested organs (gills, intestine, muscle and liver) were removed and kept in aluminium foil at -40°C till analysis was run. Control fishes of the first group also served as the control for the second one.

Extraction and analysis of malathion residue as well as its metabolites were carried out according to the AOAC (1980) procedures. A GLC/mass spectroscopy unit (Hewlett Packard, model 5890) was used. The unit was equipped with Ni^{63} electron capture detector, mass selective detector and a capillary column (HP 101 25 m length. 0.2 mm I.D. and 0.2 µm film thickness). Helium was the carrier gas at a flow rate of 5 mL/min. The temperatures of the injector and detector were 210°C. respectively. Column temperature was programmed as follows : temperature raised to 160°C at a rate of 3°C/min and kept constant for 3 min. followed by an increase to 200°C at a rate of 3°C/min and maintained isothermal for 6 min. and finally increased to 225°C at a rate of 6°C/min and kept constant for 23 min. Recovery of malathion was 90, 92 and 95 % from fish tissues fortified by 2, 11 and 22 µg/g (wet weight), respectively. Detection limit according to this procedure was 0.01 µg/g (wet weight). Separation of malathion and its metabolites by the GLC procedure is presented in Figure 1.

RESULTS AND DISCUSSION

Uptake and accumulation levels of malathion in the various tested organs of male *T. nilotica* are giving in Table 2. There is a general trend for malathion residues to increase as its concentration was increased and fish exposure period was extended from 1 to 7 -d. Total malathion residues. after 7 -d, in case of 1000 µg/L, 500 µg/L and 200 µg/L treatments amounted to 3.14, 1.88 and 1.45 µg/g (wet weight)

Table 2. Concentration of malathion residues (µg/g wet weigh) in different organs of normal and induced MFO activated male of *T. nilotica.*

Exposure time	Malathion treatment											
	1000 µg/L				500 µg/L				200 µg/L			
	Liver	Muscle	Gills	Intest.	Liver	Muscle	Gills	Intest.	Liver	Muscle	Gills	Intest..
Normal male fish												
in days												
1	0.46	0.30	0.30	0.72	0.25	0.09	0.15	0.25	0.20	0.01	0.12	0.20
3	0.60	0.38	0.38	0.85	0.35	0.17	0.19	0.30	0.28	0.10	0.15	0.25
5	0.68	0.50	0.52	1.10	0.40	0.28	0.25	0.38	0.30	0.20	0.20	0.30
7	0.77	0.62	0.55	1.20	0.48	0.70	0.30	0.40	0.38	0.50	0.25	0.32
Induced MFO activated fish												
in hr.												
12	0.68	0.55	0.45	1.00	0.21	0.40	0.15	0.35	0.30	0.05	0.20	0.30
24	0.30	0.30	0.25	0.50	0.15	0.52	0.08	0.25	0.41	0.55	0.35	0.45

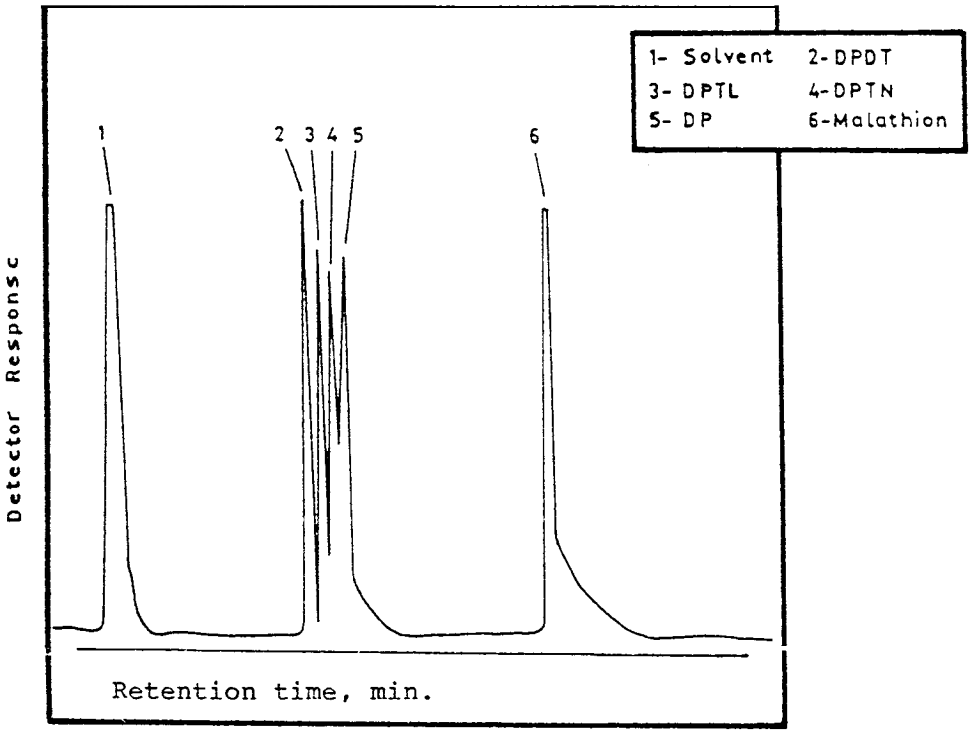

Figure 1. Separation of malathion and its metabolities by GLC ·

respectively. In addition. residue levels of the insecticide were subject to considerable variation in the tested organs. After 7 -d of exposure. malathion residues in the tested organs showed the following order :

Intestine > Liver > Muscles > Gills. for the 1000 µg/L treatment.
Muscles > Liver > Intestine > Gills. for the 500 µgL treatment. and
Muscles > Liver > Intestine > Gills. for the 200 µg/L treatment

Binder et al. (1984) previously reported that residue levels of malathion in carp (*Cyprinus carrpio*) exposed to 5 mg/L of the insecticide for 4 -d. were subject to variation in the different organs and exhibited the following order. Liver > muscle > blood > gills > brain. Low residue levels attained by the present study may be due to slow uptake rate of malathion. and/or its rapid conversion to metabolites. Available results are in agreement with that reported by Cook and Moore (1976) in case of carp fish fed with malathion.

The distribution of total metabolite residues in the tested fish organs. after 24 hr of exposure. is given in Table 3 High residue levels of metabolites were found in the intestine and gills. and amunted to 42.8 % and 37.4 % of the total value. Such high levels of metabolites indicate that the two organs contain active xenobiotic enzymatic systems which act as defensive mechanism against toxic chemicals. Also. results may explain the relatively low malathion residue levels in the intestine and gills especially in case of the medium and low malathion treatments

Table 3. Total concentration of malathion metabolites (µg/g wet weight) in normal male *T. nilotica* and after MFO activation (24 hr exposure).

Malathion treatment µg/L	Tested organs of control fishs					Tested organs of MFO activated fish				
	Liver	Muscle	Gills	Intest.	Total treatment µg/L	Liver	Muscle	Gills	Intest.	Total treatment µg/L
1000	0.10	0.09	0.40	0.58	1.17	0.30	0.46	0.55	0.79	2.10
500	0.19	0.07	0.36	0.34	0.96	0.32	0.33	0.38	0.46	1.49
200	0.03	0.04	0.22	0.20	0.49	0.28	0.33	0.20	0.38	1.19
Total metabolites per organ	0.30	0.20	0.98	1.12	2.62	0.90	1.12	1.13	1.63	4.78
% of total	12.20	7.60	37.4	42.80	100	18.83	32.43	12.64	34.10	100

Table 4. Concentrations of malathion metabolites (µg/g wet weight) in various organs of male *T. nilotica* with activated MFO (24-hr exposure)*.

Fish organ	Malathion treatments											
	1000 µg/L				500 µg/L				200 µg/L			
	DPDT	DPTL	DPTN	DP	DPDT	DPTL	DPTN	DP	DPDT	DPTL	DPTN	DP
Liver	0.20	0.10	ND	ND	0.10	0.10	0.10	0.02	0.05	0.05	0.10	0.08
Muscle	0.08	0.22	0.16	ND	0.10	0.09	0.10	0.04	0.08	0.05	0.10	0.10
Gills	0.10	0.18	0.12	0.15	0.09	0.12	0.05	0.12	0.01	0.09	0.04	0.06
Intestine	0.20	0.23	0.20	0.11	0.10	0.12	0.18	0.06	0.10	0.10	0.10	0.08

*ND = not detected (Detection limit 0.01 µg/g).

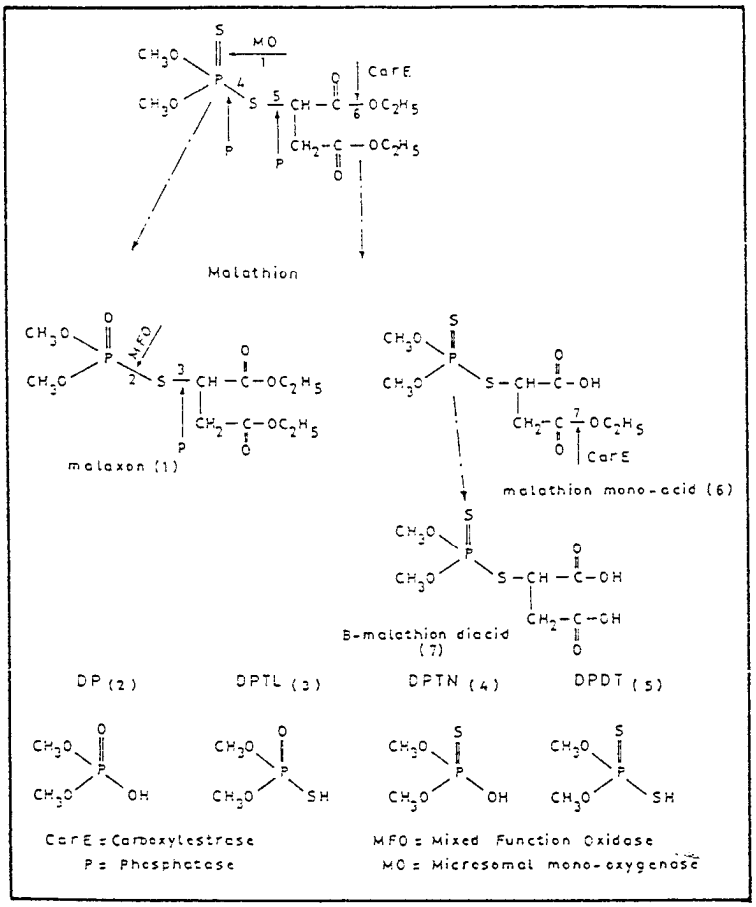

Figure 2. Metabolic pathways of malathion by xenobiotic enzyme systems (Modified after Hassall 1990).

Injection of male fish with MFO activator, resulted in active uptake of malathion in case of the 200 µg/L treatment compared with the normal fish (Table 2). Fish with activated MFO, attained high residues of total metabolites compared with the normal fish, and their concentrations increased as malathion concentration was increased (Table 3). The intestine and gills of induced MFO activated fish accumlated relatively high levels of malathion metabolites. These results still confirm that the enzymatic systems of the two organs play an important role in malathion metabolism. The relative distribution of total metabolites in the tested organs revealed that the liver attained the lowest level of the studied metabolites (18.8%). The concentration levels of DPDT, DPTL, DPTN and DP in the tested organs of induced MFO activated fish of the various treatments are given in Table 4. The residues of each metaboiite were subject to variation according to the tested organs and malathion treatment. Such results tend to reflect the possible variation in the enzymatic systems and their activities in the different organs investigated.

Percent distribution of each of the metabolites in normal and of fish with induced

Table 5. Percent distribution of malathion metabolites in the tested organs of control and activated MFO fish µg/g wet weight (24 hr post-exposure).

Metabolite	Total metabolites in tested organs µg/g		% of each metabolite	
	Normal fish	MFO activated fish	Normal fish	MFO activated fish
DPDT	0.04	1.26	1.5	26.4
DPTL	0.68	1.54	26.0	30.3
DPTN	1.25	1.25	47.7	26.2
DP	0.65	0.82	24.7	17.1

MFO activation is given in Table 5. In normal exposed fish. DPDT was represented by the lowest ratio of 1.5 % DPTN amounted to 47.7 % of the total metabolites whereas DPTL and DP showed intermediate values of 26 % and 24.8%, respectively. In fish with activated MFO the relative ratios of metabolites were changed in favour for DPDT (26.4 %) and DPTL (30.3 %), whereas the ratios of DPTN and DP decreased. The present study was mainly concerned with the metabolites retained by the different tested organs rather than the mono- and dicarboxylic acid derivatives of malathion which are excreted to the surrounding water (Huston and Roberts 1985, Muan and Share 1989). Several enzymatic systems are likely to be involved in malathion metabolism. Figure 2 (Hayes 1982. Hassall 1990). According to metabolite ratios given in Table 5, malathion metabolism in normal fish could proceed directly via the cleavage of P-S linkage to yield the major metabolite DPTN or via the hydrolysis of malaxon at the S-C linkage to yield DPTL together with the attack of the P-S linkage to yield DP. Malaxon, has to be first produced by the oxidation of the sulphur of malathion by MFO. Hence the yields of DPTL and DP are both controlled by rate of metabolic conversion of malathion to malaxon, and their concentrations approach each other.

In the case of fish with activated MFO, the ratio of DPDT was considerably increased indicating that direct attack of malathion at the S-C linkage was enhanced (Table 5). The ratio of DPTL increased which indicates the active conversion of malathion to malaxon and the subsequent cleavage of the S-C linkage by phosphatases (Figure 2).

The present investigation revealed some of the possible metabolic pathways of malathion in *T. nilotica* with special emphasis on the relative distribution of malathion residue and its metabolites in the gills, intestine. muscles and the liver.

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