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Biological effects of pyrimethinal on aquatic worms (*Tubifex tubifex*) under laboratory conditions

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ABSTRACT

Laboratory studies were conducted to determine the effects of different concentrations of pyrimethinal (5, 10, 15, 20 and 25 mg/l) on protein content, and some oxidative stress parameters in an aquatic worm, *Tubifex tubifex* after 2, 4, and 7 days of exposure. In addition, residues of the fungicide were quantified in water and in the worms. In water, pyrimethinal concentration decreased slowly (maximum – 6.4 ± 0.8% after 2 days for 25 mg/l). In the worms, it increased after 4 days and decreased thereafter, confirming that the worms were exposed to the fungicide and not to a degradation product. LC_{50} values of pyrimethinal were between 49.2 ± 0.58 and 39.5 ± 0.95 mg/l depending on exposure time. All the measured parameters appeared to be more sensitive than mortality. The activity of catalase (CAT) increased in response to the fungicide after 2 days of exposure to 25 mg/l of pyrimethinal (+ 84.95 %), demonstrating an oxidative stress in the worms. In contrast GST activity decreased, this decrease was significant for all fungicide concentrations after two days. The highest decrease of GST activity (- 29.7 %) was found after 7 days in the presence of 25 mg/l of pyrimethinal.

Keywords: Biomarker, pyrimethinal residues, oxidative stress, total protein, worms.

INTRODUCTION

Tubifex tubifex (Oligochaeta Tubificidae) is an aquatic oligochaete known to be very resistant to pollution; it is often the last to disappear from a contaminated site (Aston 1973, Lucan-Bouché *et al.*, 1999, Mosleh *et al.*, 2006 and 2007). Tubificid is a relevant potential biomonitor of aquatic

pollution because these worms are sedentary and widely distributed, frequently dominating the macrobiotic community in freshwater habitats. Furthermore, they dwell in the sediment, burrowing and ingesting large volumes of sediment for feeding and are thus exposed to contamination both through interstitial water and through contact with sediment particles. They also play a major role in bioturbation and in decomposition of organic matter. Although *T. tubifex* has been proposed as a test organism for ecotoxicological studies, very few data concerning the effects of different pollutants on this worm are available (Gillis *et al*., 2002, and 2004; Mosleh *et al.*, 2005 and Mosleh *et al.*, 2006).

In aquatic systems, certain fungicides are likely to be present at low but persistent concentrations. They may then cause lethal or sublethal effects in a wide range of non target organisms. Pyrimethanil is one of the major fungicides used against gray mold in grapes. Like other fungicides, it has been selected and synthesized for its biocidal properties and is applied to kill or control fungi. However, fungicides can be toxic to other forms of life. They may be introduced into natural aquatic systems by various means: incidentally during manufacturing, during their application (i.e., through aerial spray draft) and through surface water runoff, for example from vineyards, after application.

It is now well known that numerous pesticides can induce an increase in reactive oxygen species concentrations in cells and consequently a development of antioxidative defence systems such as catalase or glutathione-S- transferase (Davies *et al.* 1994; Cossu *et al.*, 1997; Davies and Cousins, 2000 and Mosleh *et al.*, 2007).

The aim of this research was to investigate the effect of chemical stress produced by the fungicide pyrimethinal on CAT and GST as antioxidant defense system in *T. tubifex*. In addition, total protein contents were measured to determine the toxic impact of the fungicide. In parallel the fate of the fungicide in the water and in the worms was quantified using HPLC.

MATERIALS AND METHODS

Origin of the worms and culture maintenance: *T. tubifex* worms were collected from a site near Cormicy sur Marne (Marne, France). This site is a retention basin that receives and confines runoff water from a vineyard. Sediment samples containing *T. tubifex* were collected and brought back to the laboratory. The worms were carefully extracted and washed several

times with tap water and then with a regional spring water (Source des Grands Bois, Fismes, France, Ca^{2+}: 124 mg/l; Mg^{2+}: 25 mg/l; Na^{+}: 6 mg/l; K^{+}: 2 mg/l; HCO_3^{-}: 399 mg/l, Cl^{-}: 22 mg/l; $SO4^{2-}$: 80; NO_3: 0.5 mg/l; hardness: 300 ± 10 mg/l $CaCO_3$; pH: 7 ± 0.1). *T. tubifex* cultures were accomplished with artificial sediment adapted from OECD Guideline 207 (OECD, 1984). Worms were cultured at 21 ± 1°C under a photoperiod of 12 h dark : 12 h light. The water was continuously aerated and dissolved oxygen keep at 60% saturation. *T. tubifex* were fed with TetraMin flakes (Tetra Werke, Melle, Germany) once a week.

Determination of lethal concentrations: Tests were conducted to determine the lethal concentrations (LC_{50}, LC_{25} and LC_{10}) of pyrimethinal for worms under laboratory conditions. Different concentrations of this fungicide were prepared in 100 ml of distilled water three replicates per each. Then 40 worms were introduced into each container and placed into an incubation chamber (21 ± 1°C and 12:12 photoperiod). Three replicates without pyrimethinal were used as a control. After 1, 2, 4, and 7 days dead worms were counted and LC_{10}, LC_{25}, and LC_{50} values were determined graphically.

Sublethal effects of pyrimethinal: To assess the effects of the fungicide, the worms were randomly taken from the culture and exposed to various concentrations of pyrimethinal. The worms (approximately 1.5 g FW) were placed in crystallizing dishes (∅ = 8 cm) containing 100 ml spring water without sediment and fungicide was addea so that the final concentration was 5, 10, 15, 20 and 25 mg/l of active ingredient. The dishes were then incubated in the growth chambers as described above for 2, 4 and 7 days. Three dishes were prepared for each concentration and duration. After incubation, animals from each dish were weighed and the three replicates were pooled together and frozen (-80°C) for later analysis, which occurred approximately one week later.

Pyrimethinal extraction and quantification in worms and water: About 2 g of *T. tubifex* from each pooled and frozen sample was homogenized in 15 ml of dichloromethane (Chromanorm™ for HPLC, Prolabo) using a glass homogenizer immersed in ice. The homogenate was mechanically shaken for 120 min, filtered, and then washed twice with 10 ml of dichloromethane. The combined extracts were then centrifuged at 10, 000 *g* for 5 min at 4°C and quantitatively transferred to a volumetric flask.

The total extract was evaporated to dryness; the residue was dissolved in 1 ml acetonitrile. Then, the entire sample was transferred to a glass chromatographic column containing 5 g of activated Florisil. The column was eluted with 20 ml of 15% diethyl ether (80-60) /85% petroleum ether followed by 20 ml of 50 % diethyl ether/50 % petroleum ether. Both fractions were evaporated to dryness under a nitrogen stream; the residue was dissolved in 1 ml acetonitrile and injected into the high performance liquid chromatograph (HPLC).Water in which *T. tubifex* were incubated was analyzed directly with HPLC without pre-concentration.

T. tubifex extracts and water were analyzed with HPLC using a reversed phase column (Kromasil, C18 100 Å, 5 µm, 250 mm x 3 mm, CIL-Cluzeau). Elution of pyrimethinal was performed isocratically with 60% acetonitrile and 40% water (Millipore®). Pyrimethinal was detected and quantified by monitoring the UV absorbance (200 to 340 nm) with a diode array detector (Gynkotek UVD 340S). Standard solutions of technical pyrimethinal 99% of purity were prepared and injected to obtain a standard curve of peak area versus concentration (external standard method).

The rate of recovery was estimated by the addition of 1 mL methanol containing pure pyrimethinal (50 µg/ml) to 1 g of *T. tubifex*. The extraction procedure was carried out as described above. The average rate of pyrimethinal recovery, based on three replicates, was 89.9 ± 3.1%.

CAT, GST activities and total soluble protein content measurements: To evaluate enzyme activity, 1 g of frozen worms was homogenized at 4 °C in 2 ml of 2 mM Tris-HCl, pH 8, containing 20% glycerol, 2 mM mercaptoethanol, 6 µM leupeptin, and 0.5 mM phenyl methyl sulphonyl-fluoride (PMSF). The homogenate was then centrifuged at 18,000 g for 90 min at 4°C. The upper fatty layer was discarded and the final supernatant was subjected to protein, GST and CAT.The activity of GST was assayed as the increase of absorbance at 340 nm (Genesys 5 spectrophotometer, Spectronic instruments, Bioblock, Illkirch, France) due to the conjugation of glutathione (GSH) to 1-chloro-2,4-dinitrobenzene (CDNB) according to Regoli *et al.* (1997). Fresh solutions of GSH (40 mM) in sodium phosphate buffer (0.01 M, pH 7.6) and CDNB (40 mM) in 96% ethanol were prepared. The reaction mixture contained 250 µl of the GSH solution, 50 µl CDNB and 0.5 ml potassium phosphate buffer (0.1 M, pH 6.5). After mixing and incubating for 5 min at 25°C, the reaction was started by adding 50 µl of

worm extract. Absorbance at 340 nm was followed during 4 min the activity was expressed as the consumption of nanomoles of CDNB per min per mg protein. CAT activity was determined as the decrease of absorbance at 240 nm due to H_2O_2 as described by Claiborne (1985). Briefly the assay mixture consisted of 1.95 ml of phosphate buffer (50 mM, pH 7.0), 1 ml of H_2O_2 (0.019 M), and 50 µl of worm extract. It was expressed as µmoles of H_2O_2 consumed per mg of protein per min. Protein concentrations were determined by dye binding method according to (Bradford, 1976).

Statistical analysis: In all cases, a minimum of two independent experiments were conducted. Data presented are means ± standard deviations. Data were analyzed with two way analyses of variance and pairwise multiple comparison procedure (Student-Newman-Keuls Method) was used to compare fungicide concentrations and duration of exposure at $p < 0.05$.

RESULTS AND DISCUSSION

Acute toxicity of pyrimethinal: No mortality was observed in the control group. The lethality was increased with increasing either pyrimethinal concentration or exposure time. The values of LC_{10} were 10.8 ± 0.09, 9.5 ± 0.08, 8.3 ± 0.08 and 8.05 ± 0.04 mg/l after 1, 2, 4, and 7 days, respectively (Table 1).

Table (1). Lethal concentrations (mg/l) of pyrimethinal in *Tubifex tubifex* after 2, 4, and 7 days of exposure.

Concentrations (mg/l)	Time (days)					
	2		4		7	
	Water	*T. tubifex*	Water	*T. tubifex*	Water	*T. tubifex*
Control	nd	nd	nd	nd	nd	nd
5	3.8 ± 0.72	1.5 ± 0.10	2.5 ± 0.62	3.8 ± 0.23	2.25 ± 0.23	3.1 ± 0.12
10	9.1 ± 1.10	3.5 ± 1.20	7.9 ± 1.01	7.5 ± 0.15	5.6 ± 0.25	6.2 ± 0.25
15	13.5 ± 1.82	5.6 ± 0.89	11.5 ± 1.05	10.5 ± 1.8	8.75 ± 0.89	9.8 ± 1.01
20	17.5 ± 1.32	8.5 ± 1.56	14.5 ± 1.25	12.6 ± 2.3	12.2 ± 1.25	11.5 ± 0.98
25	22.4 ± 0.98	10.9 ± 2.1	18.9 ± 2.01	16.1 ± 1.8	15.8 ± 2.5	13.5 ± 0.98

nd =Not detected.

Pyrimethinal residues:

The HPLC method allowed good and rapid separation and quantification of pyrimethinal. Residues of pyrimethinal were detected in all water and *T. tubifex* samples after exposure to the fungicide (Table 2). In water, the concentration was highest after 2 days and decreased thereafter. The relative

decrease was more rapid with low initial concentrations. Indeed, after 2 days 24 % of the fungicide was left with the lowest initial concentration (5 mg/l). Similarly, after 7 days the remaining fungicide was 55, 44, 41.7, 39 and 36.6 % of initial concentrations for 5, 10, 15, 20 and 25 mg/l, respectively. In the environment, decrease in the concentration of a pesticide in water may be due to adsorption (Widianarko and Van Straalen, 1996), translocation, and degradation. In our experiment, adsorption and translocation can be ruled out since no sediment was present and the experimental system was closed, leaving degradation (Anderson *et al.,* 1999), disappearance of the fungicide in our case is most probably due to biodegradation. The slower decrease with high pesticide concentrations may be attributed to a possible toxicity of the chemical to *T. tubifex* and the microorganisms that may be present in the medium as it was also proposed to explain lower degradation of higher concentration of isoproturon in soils (Mosleh *et al.,* 2003b).

In the worms the concentration of pyrimethinal was maximum after 4 days for the lowest 2 initial concentrations (5 and 10 mg/l) and decreased thereafter (Table 2). Decreasing fungicide availability in the water may not solely be invoked to explain the concentration decrease in the animals; indeed concentration decrease was not as rapid in the worms as in the water. This increase in body concentration followed by a decrease that was observed in worms exposed to the fungicide corresponds to what was observed in the case of isoproturon accumulation in *Lumbricus terrestris* (Mosleh *et al.*, 2003b) and it fits the model established by Widianarko and Van Straalen (1996). In this model, 2 exponentials describe the 2 parameters that explain pesticide concentration in the organisms. These parameters are availability and regular metabolism and excretion of the pesticide. The data obtained with the lowest 2 concentrations apparently did not follow the model, however, it is possible that the increase of fungicide concentration in the worms within the first two days, that is before the first sampling time point.

Table (2). Residues of pyrimethinal in *Tubifex tubifex* (µg/l) and water (mg/l) after 2, 4, and 7 days of exposure to the fungicide.

Toxicity	Time (days)			
	1	2	4	7
LC_{10}	10.8 ± 0.09	9.5 ± 0.08	8.3 ± 0.08	8.05 ± 0.04
LC_{25}	25.6 ± 0.15	22.3 ± 0.08	20.2 ± 0.09	19.3 ± 0.03
LC_{50}	49.2 ± 0.58	45.6 ± 0.98	42.3 ± 0.08	39.5 ± 0.95

Antioxidative enzyme activity: During the 7 days of the experiment, GST activity of the control worms did not show any significant variation at time intervals, it was approximately 225 ± 10.1 nmol CDNB/min/mg protein (Table 3). In the presence of pyrimethinal, the activity of GST was lower than in the controls, this decrease was significant for all fungicide concentrations after two days. The highest decrease of GST activity (- 29.7 %) was found after 7 days in the presence of 25 mg/l of pyrimethinal.

Table (3). Effects of various concentrations of pyrimethinal on glutathione-S- transferase (nmoles CDNB/min/mg protein) of *Tubifex tubifex* after 2, 4, and 7 days of exposure.

Concentrations (mg/l)	Time (days)		
	2	4	7
Control	225.5 ± 10.1	230.2 ± 9.1	228.6 ± 6.8
5	210.2 ± 15.3	203.1 ± 8.5*	200.2 ±7.8*
10	205.5 ± 5.60*	198.5 ± 5.3*	193.3 ± 5.6*
15	195.8 ± 3.30*	189.6 ± 2.8*	180.2 ± 4.8*
20	182.4 ± 5.50*	178.3 ± 4.5*	175.2 ± 2.9*
25	175.5 ± 3.50*	169.3 ± 6.0*	160.5 ± 3.9*

Data presented are mean ± standard deviation.
*Significantly different from control at $p < 0.05$ in a Student-Newman—Keuls test.

GST plays a crucial role in cellular protection against toxic compounds such as pesticides and heavy metals (Anderson, 1997; Anderson and Luo 1998, Mosleh *et al* 2005). This group of enzymes catalyzes the nucleophilic addition of the thiol moiety of reduced glutathione (GSH) electrophilic xenobiotics and thus converted to a wide variety of electrophilic compounds, such as pesticides and heavy metals, forgein compounds are thus converted to N-acetyl cystine S-conjugates (mercapturic acid), which are then excreted in the urine and faces (Almar *et al.*, 1987). In the present study, the level of GST in worms was found to be lower than in controls (Table 3). It contradicts most studies in which an increase of GST activity is observed in response to pollutants. It is the case in *T. tubifex* exposed to copper (Mosleh *et al.*, 2004). The decrease of GST activity in those studies might arise from the decontamination of the pesticides by the formation of glutathione conjugates, as described for example in *Macrobarchium malcolmsonii* exposed to endosulfan (Saravana-Bhavan and Geraldine, 2000). The lower level of GST in pyrimethinal treated worms seems to indicate that GST is not involved in the detoxification of pyrimethinal in

this organism as observed in vertebrates where the fungicide is metabolized via a metabolic addition of glucuronic acid with or without prior hydroxylation of the fungicide (Anderson *et al.,* 1999). To explain the lower activity of GST, it may be hypothesized that the enzyme is no longer in the alarm phase when the activity increases but in the exhaustion phase, as observed for high concentration of fungicide with catalase.

Control worms showed stable CAT activities during the 7 days of the experiment (Table 4). CAT activities in worms exposed to pyrimethinal were found to be higher than in control worms (Table 4). The stimulation of CAT activity was clearly concentration and time-dependent, it increased in response to the fungicide concentrations but the increase was most pronounced in worms exposed to the highest concentration of pyrimethinal (25 mg/l).CAT activity was significantly increased after 2 days of exposure to 25 mg/l of pyrimethinal (+84.95%), it increased further with exposure time and after 4 and 7days it was respectively 94 and 104 % higher than in control worms respectively (Table 4).

CAT is an enzyme that is known to detoxify reactive oxygen species in the cells. The increase CAT activity in worms in pyrimethinal most probably occurred in response to an excess of reactive oxygen species in the *T. tubifex*, indicating that an oxidative stress in the animal in response to the fungicide (Hazarika and Sarkar, 2001).

Table (4). Effects of various concentrations of pyrimethinal on CAT (nmoles H_2O_2/min/mg protein) of *Tubifex tubifex* after 2, 4, and 7 days of exposure.

Concentrations (mg/l)	Time (days)		
	2	4	7
Control	29.9 ± 2.3	30.2 ± 5.1	29.5 ± 4.6
5	35.6 ± 1.5*	38.3 ± 2.8	42.4 ± 2.5*
10	41.5 ± 1.1*	44.2 ± 3.5*	46.3 ± 1.3*
15	45.5 ± 2.6*	49.3 ± 4.1*	51.2 ± 4.3*
20	51.8 ± 3.2*	52.6 ± 2.9*	52.1 ± 3.9*
25	55.3 ± 2.4*	58.5 ± 2.4*	60.2 ± 3.5*

Data presented are mean ± standard deviation.
*Significantly different from control at $p < 0.05$ in a Student-Newman—Keuls test.

In control, *T. tubifex* concentration of soluble protein remained stable throughout the experiments and ranged between 1300.9 ± 11.3 and 1390.8 ± 15.3 µg/g FW after 2 and 7 days of exposure, respectively (Table 5) which indicate that they remained stable during the experiment. In all pyrimethinal treated worms, a clear reduction of total protein content was observed either the duration of exposure or the concentrations of fungicide.

Table (5). Effects of various concentrations of pyrimethinal on total soluble protein content (µg/g FW) of *Tubifex tubifex* after 2, 4, and 7 days of exposure.

Concentrations (mg/l)	Time (days)		
	2	4	7
Control	1300.9 ± 11.3	1368.9 ± 18.5	1390.8 ± 15.3
5	1250.2 ± 12.5*	1238.5 ± 13.6	1183.2 ± 23.2*
10	1220.5 ± 14.5*	1155.4 ± 11.9*	1101.5 ± 21.9*
15	1150.3 ± 12.9*	1051.3 ± 14.9*	975.42 ± 11.1*
20	1105.2 ± 11.8*	950.36 ± 13.1*	920.52 ± 22.3*
25	1082.2 ± 21.3*	920.23 ± 12.8*	869.33 ± 20.5*

Data presented are mean ± standard deviation.
*Significantly different from control at $p < 0.05$ in a Student-Newman—Keuls test.

Lower concentrations of soluble protein in treated worms suggest that physiological compensatory mechanisms be activated to provide intermediates for deriving energy. Since it appears that the lower growth rate of worms may be due to less food intake, the reduction of protein content may be ascribed to a catabolism of protein in response to worm energy demand as suggested for an isopod in response to parathion (Ribeiro *et al.*, 2001). Several authors have shown that the reduction of worm protein content was one of the primary toxic effects of various pesticides; this decrease of protein content appeared to be an early defense reaction to the pesticides stress in animals. In earlier work, it was found that total protein of *T. tubifex* exposed to 10 mgl of isoproturon was reduced by 13% after 2 days (Mosleh *et al.*, 2004). Also, Mosleh *et al* (2003a) found that the reduction of total protein of earthworms (*Apporectodea caliginosa*) might be the primary effect of chlorfluazuron, while it comes as a secondary effect for other pesticides (cypermethrin, aldicarb, profenofos, atrazine and metalaxyl). To overcome the stress situation, animals require high energy and this energy demand may have led to protein catabolism. Furthermore, this decrease in protein content might be due to a mechanical lipoprotein

formation, which will be used to repair damaged cells, tissues, and organs (Ribeiro *et al.*, 2001; Saravana-Bhavan and Geraldine, 2001; Mosleh *et al.*, 2003a). The significant decrease of protein content observed in *T. Tubifex* exposed to pyrimethinal could be related to the development of defense mechanism induced to overcome the stress situation. Such hypothesis has been advanced to explain changes of protein content observed in the crab *Barytelphusa guerini* and *Macrobarchium malcolmsonii* exposed to endosulfan (Saravana-Bhavan and Geraldine, 2000).

CONCLUSION

At the tested concentrations, degradation in water was relatively high since 50% of the initial concentration persisted after 7 days. Accumulation of pyrimethinal in *T. tubifex* occurred during the first 4 days of exposure; the decrease of concentrations towards the end of the experiment may be related to the development of degradation processes in *T. tubifex*. Sublethal toxic effect of this fungicide is clearly shown by significant reduction of GST, total soluble protein content and increased in CAT.

REFERENCES

Almar, M.M., Mayans, J.D. and Rmero, F.J. (1987). Glutathione content and GSH S-transferase activity in mid gut gland of *Procambarus clarkii,* sex difference, the effect of fasting and their implications in cadmium toxicity. Comparative Biochemistry and Physiology 87C: 433-440.

Anderson, C., Brumhard, B., Ditgens, K. and Reiner, H. (1999). Metabolism of fenhexamid (KBR 2738) in plants, animals, and the environment. Pflanzenschutz-Nachrichten Bayer 52: 227-251.

Anderson, M.E. (1997). Glutathione and glutathione delivery compounds. Advances in Pharmacology 38: 65-78.

Anderson, M.E. and Luo, JL (1998). Glutathione therapy: From pro drugs to genes. Seminars in Liver Disease 41: 1309-1319.

Aston, R.J. (1973). Tubificids and water quality: a review. Environ. Pollut. 5: 1–10.

Bradford, M.N. (1976). A rapid and sensitive method for the quantitation of micrograms of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Claiborne, A. (1985). Catalase activity, In Greenwald, R.A., Handbook of Methods in Oxygen Radical Research. CRC Press Boca-Raton, pp. 283-284.

Cossu, C., Doyotte, A., Jacquin, M.C. and Vasseur, P. (1997). Biomarqueurs du stress oxydant chez les animaux aquatiques. In : Lagadic, L., Caquet, T., Amiard, J.C., Ramade F., (Ed.) Biomarqueurs en Ecotoxicologie: Aspects Fondamentaux. Masson, Paris, pp. 149-161.

Davies, J.M., Lowry, C.V. and Davies, K.J.A. (1994). Free radicals. In: Nohl, H., Esterbauer, H., Rice-Evans C., (Ed.) Environment Medicine and Toxicology. Richelieu Press, London, pp. 563-578.

Davies, S.R. and Cousins, R. (2000). Metallothionein expression in animal: a physiological perspective and function. Recent Advances in Nutrition Sciences, 1085-1088.

Gillis, B.L., Diener, L.C., Reynoldson, T.R. and Dixon, D. G. (2002). Cadmium-induced production of a metallothionein like protein in *Tubifex tubifex* (Oligochaeta) and *Chironomus riparius* (Diptera): correlation with reproduction and growth. Environmental Toxicology and Chemistry 21:1836-1844.

Gillis, P.L., Reynoldson, T.B. and Dixon, D.G. (2004). Natural variation in a metallothionein-like protein in *Tubifex tubifex* in the absence of metal exposure. Ecotoxicol. Environ. Safe., 58: 22–28.

Hazarika, A. and Sarkar, S.N. (2001). Effect of isoproturon pretreatment on the biochemical toxicodynamics of anilofos in male rats. Toxicology 165: 87–95.

Lucan-Bouché, M.L., Biagianti-Risbourg, S., Arsac, F. and Vernet, G. (1999). An original decontamination process developed by the

aquatic oligochaete *Tubifex tub\ifex* exposed to copper and lead. Aquatic Toxicology 45: 9-17.

Mosleh Y.Y., Ismail, SM., Ahmed, M.T. and Ahems, Y.M. (2003a). Comparative toxicity and biochemical responses of certain pesticides on the mature earthworms *Aporrectodea caliginosa* under laboratory conditions. Environmental Toxicology 18: 338-346.

Mosleh, Y.Y., Paris-Palacios, S., Couderchet, M. and Vernet, G. (2003b). Effects of the herbicide isoproturon on survival, growth rate, and protein content of mature earthworms (*Lumbricus terrestris* L.) and its fate in the soil. Applied Soil Ecology 23: 69-77.

Mosleh, Y.Y., Paris-Palacios, S., Arnoult, F., Couderchet, M., Biagianti-Risbourg, S. and Vernet, G. (2004). Metallothionein induction in the aquatic Oligocheate *Tubifex tubifex* exposed to the herbicide isoproturon. Environmental Toxicology 19: 88-93.

Mosleh, Y.Y., Paris-Palacios, S., Couderchet, M., Biagianti-Risbourg, S. and Vernet, G. (2005). Effects of the herbicide isoproturon on metallothioneins, growth and antioxidative defences in the aquatic worm *Tubifex tubifex* (Oligochaeta, Tubificidae). Ecotoxicology 14 (5): 559-57.

Mosleh, Y.Y., Paris-Palacios, S. and Biagianti-Risbourg, S. (2006). Metallothioneins induction and antioxidative response in aquatic worms *Tubifex tubifex* (Oligochaeta, Tubificidae) exposed to copper. Chemosphere 64: 121–128.

Mosleh Y. Y., Paris-Palacios, S., Mohamed T. Ahmed, F.M. Mahmoud, M.A. Osman and Sylvie Biagianti-Risbourg (2007). Effects of chitosan on oxidative stress and metallothioneins in aquatic worm *Tubifex tubifex* (Oligochaeta, Tubifcidae). Chemosphere 67: 167–175.

OECD (1984). Guidelines for Testing of Chemicals. Test 207:Earthworms Acute Toxicity Tests. Paris: Organization for Economic Co-operation and Development (OECD).

Regoli. F. Nigro, M. Bertoli. E., Principato, J. and Orlando, E. (1997). Defences against oxidative stress in the Antartic scallop *Adamussium colbecki* and effects of acute exposure to metals. Hydrobiologia 355: 139-144.

Ribeiro, S., Sousa,. J.P., Noguerira, A.J.A. and Soares, A.M.V.M. (2001). Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus.* Ecotoxicol. Environ. Safe., 49:131-138.

Saravana Bhavan, P. and Geraldine, P. (2000). Aberration in various parameters of bioenergetic in the *Macrobarchium malcomsonii* following exposure to endosulfan. Aquaculture 1: 141-150.

Saravana Bhavan, P. and Geraldine, P. (2001). Biochemical stress responses in tissues of the prawn *Macrobarchium malcomsonii* on exposure to endosulfan. Pesticide Biochemistry and Physiology 70: 27-41.

Widianarko, B. and Van Straalen, N. (1996). Toxicokinetics-based survival analysis in bioassays using non-persistent chemicals. Environmental Toxicology and Chemistry 15: 402-406.

التأثيرات البيولوجية للمبيد الفطرى بيريميثينال على ديدان المياة العذبة *Tubifex tubifex* معمليا

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أجريت تجربة معملية لتقدير متبقيات المبيد الفطري بيريميثينال داخل المياه وديدان المياه العذبة وأوضحت النتائج المتحصل عليها إن تركيز المبيد الفطري يقل ببط داخل المياه في حين يحدث تراكم للمبيد داخل الديدان بعد 4 أيام ويحدث له تكسير بعد ذلك . وتم أيضا تقدير التركيز اللازم لقتل 50% من الديدان وكانت بين 39.5 و 49.2 ملى جرام /لتر. وتم تقدير فاعلية الكتلاز ولوخط زيادة الكتلاز عند إضافة المبيد إلى المياه بعد 2 يوم من المعاملة. وتم تقدير اوكسيديف استريز ولوحظ ان ج –اس- تى يقل ايضا نتيجة وجود المبيد داخل البيئة (المياة).