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Acute Toxicity of Organophosphorus Pesticides and Their Degradation By-products to *Daphnia magna*, *Lepidium sativum* and *Vibrio fischeri*

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1. Introduction

Organophosphorus pesticides (OPPs) attained the growing importance in pests control because of their rapid decomposition and less likely accumulation in environment. They are still of great concern however, for water sources contamination because of their high solubility in water and excessive usage. Their usage amounts were elevated after they were introduced as replacements for the highly persistent organochlorine pesticides.

They are classified into two main groups, organophosphates (P=O) and organothiophosphates (P=S) depending on whether oxygen or sulphur forms a double bond with the central phosphorous atom. They were found in environment with enough frequency (Ballesteros and Parrado, 2004) to constitute an ecotoxicological risk. Their concentration in water sources (Barcelo et al., 1990; Konstantinou et al., 2006), in air (Tuduri et al., 2006) and food (Bai et al., 2006; Darko and Akoto, 2008) can vary between a few ppb to ppm levels.

The presence of these pesticides can directly affect the health of aquatic and terresterial organisms and may present a threat to humans through contamination of drinking water supplies. OPPs always pose acute toxicity but not chronic toxicity on organisms because of their quick degradation (Ye et al., 2010).

OPPs are known to cause inhibition of acetylcholinesterase (AChE) in target tissues which leads to accumulation of acetylcholine. According to its key physiological role in nerve transmission, AChE is the target of various insecticides. AChE is an enzyme vital for normal nerve function and AChE inhibition leads to over stimulation of the central and peripheral nervous systems, resulting in neurotoxic effects in organisms. OPPs also produce oxidative stress in different tissues (Possamai et al., 2007) and shows genotoxic (Bolognesi, 2003; Cakir and Sarikaya, 2005, Arredondo et al., 2008) and immunotoxic (Yeh, et al., 2005; Day et al., 1995) effects. The majority of OPPs give rise to only slight inhibition of AChE by themselves, unless they undergo oxidative activation. This process involves the substitution of the sulfur atom in the P=S bond of the organophosphate pesticide with an oxygen atom resulting with formation of oxon derivatives (OPPs-oxons) (Fig. 1).

This substitution is a result of advanced oxidation processes such as O_3, O_3/UV, H_2O_2/UV, fenton, photo-fenton, TiO_2/UV, etc. in water treatment and natural oxidation processes such as UV radiation and microbial degradation. Combined oxidation systems decreases toxicity effects of by-products via enhancing mineralization. Kim et al. (2006) used *Vibrio fischeri* and

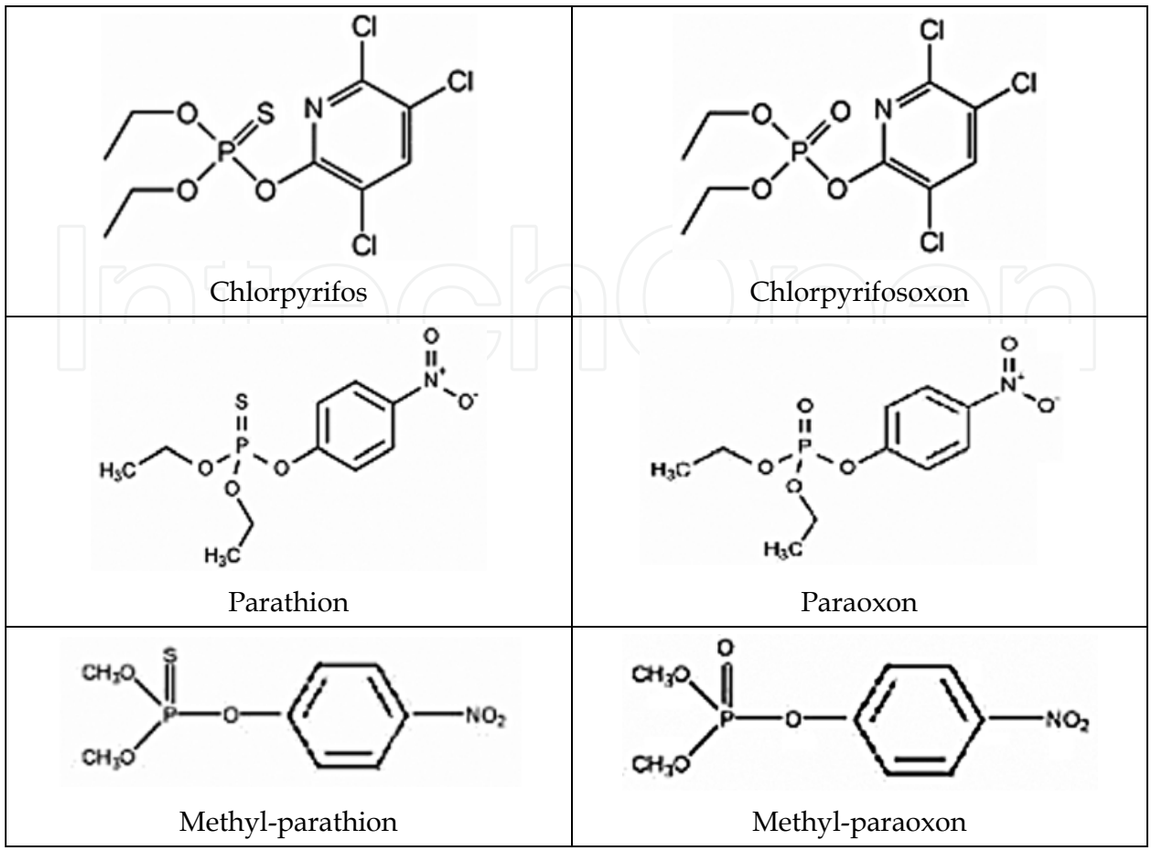

Fig. 1. Chemical structures of OPPs and OPPs-oxons

Daphnia magna bioassays to test acute toxicity of methyl parathion solutions treated by photocatalysis and photolysis. Test results showed that relative toxicity was reduced almost completely under photocatalysis for two of the tested organisms, whereas an 83% reduction for *Vibrio fischeri* and 65% reduction for *Daphnia magna* was achieved with photolysis alone.

There are some studies dealing with the ecotoxicity of OPPs (Burkepile et al., 1999; Zhang et al., 2008) but few provide data about the hazards of the degradation products (Kim et al., 2006; Kralj et al., 2007; Virag et al., 2007). According to Sparling and Fellers (2007) oxon analogs of chlorpyrifos, malathion, diazinon are 10 to 100 times more toxic for foothill yellow-legged frog (*Rana boylii*) than their parental forms. Kralj et al. (2007) studied degradation kinetics, toxicity, and degree of mineralization of malathion, malaoxon, isomalathion, and Radotion, during UV photolysis and TiO_2 photocatalysis. Formation of malaoxon, isomalathion or trimethyl phosphate esters correlated well with the induced toxicity (inhibition of acetylcholinesterase), which was observed in photocatalysis of malathion and Radotion, and in photolysis of malaoxon and Radotion.

Tsuda et al. (1997) reported that the 48 h LC_{50} values for killifish (*Uryzias latipes*) are 4.4 mg L^{-1} for diazinon and 1.8 mg L^{-1} for malathion while 0.22 mg L^{-1} for diazoxon and 0.28 mg L^{-1} for malaoxon.

Three pesticides, chlorpyrifos, parathion, methyl parathion, and their oxon derivatives; chlorpyrifosoxon, paraoxon, methyl paraoxon were involved in our study. Physical and chemical properties of parent compounds are given in Table 1. When compared with organochlorine pesticides their solubility in water is quite high.

Compound	Molecular Formula	Molecular Weight	Melting Point ^{0}C	Solubility in Water mg L^{-1}	Vapor Pressure	pKow
chlorpyrifos	$C_9H_{11}Cl_3NO_3PS$	350.59	41	1.4 (25 ^{0}C)	2.02X10^{-5}	4.96
parathion	$C_{10}H_{14}NO_5PS$	291.26	6.1	11 (20 ^{0}C)	6.68X10^{-6}	3.83
methyl parathion	$C_8H_{10}NO_5PS$	263.21	35	55 (20 ^{0}C)	1.72X10^{-5}	2.86

Table 1. Physical and chemical properties of OPPs (HSDB, 2010)

Parathion and its methyl analog are probably the most widely used organophosphorus insecticides in agriculture. Methyl parathion is a persistent pesticide commonly found in trace levels in the environment. According to a study about occurrence and temporal distribution of 49 pesticides and pesticide metabolites in air and rain samples conducted in Mississippi, the pesticide with the highest concentration in rain was methyl parathion (Coupe et al., 2000).

Methyl parathion is expected to have moderate to low mobility in soil. In moist soils, greater than 40% degrades to carbon dioxide or bound residues in 14 days, while in dry soils degradation is slower. Hydrolysis is expected to be an important process in moist soils, since methyl parathion hydrolyzes in natural water with half-lives ranging from 6.5 to 13 days at 40 ^{0}C and pH values less than 8. Half-lives in non-sterile sediment/water slurries range from 2.3 to 30 days. Aqueous photolysis half-lives range from 8 to 38 days; products include p-nitrophenol, O-methyl-O'-p-nitrophenylthiophosphoric acid, and methyl paraoxon (HSDB, 2010).

Parathion is 2-3 times more persistent than methyl parathion in natural water systems. Parathion is expected to have moderate to no mobility in soil. The primary oxidative pathway involves an initial hydrolysis to p-nitrophenol and diethylthiophosphoric acid; a second oxidative pathway involves oxidation to paraoxon (HSDB, 2010).

Chlorpyrifos is mainly used to control grain, cotton, fruit, and vegetable pests. Chlorpyrifos is acutely toxic to invertebrates and aquatic organisms (Pablo et al., 2008; Zhou et al., 2007; Gul, 2005). In soil, chlorpyrifos has half-lives of 33 to 56 days for soil-incorporated applications and 7-15 days for soil surface applications. Chlorpyrifos is expected to adsorb to suspended solids and sediment in aqueous media. The hydrolysis half-life of chlorpyrifos in distilled water at 25 ^{0}C was reported as 62 days (pH 4.7), 35 days (pH 6.9) and 22 days (pH 8.1) (HSDB, 2010). Microbial degradation contributes significantly to the dissipation of chlorpyrifos in freshwater, but is inhibited in seawater, leading to increased persistence (Bondarenko, 2004). Main oxidation by-products of chlorpyrifos are O,O-diethylphosphorothioate, TCP and chlorpyrifos-oxon (Kralj et al., 2007).

Ecotoxicological studies with a broader spectrum of aquatic organisms are needed to determine whether currently applied OPPs and their transformation products may constitute a potential risk to ecosystem. The potential utility of biomarkers for monitoring both environmental quality and the health of organisms inhabiting polluted ecosystems has received increasing attention during the recent years.

Three different biotests from different trophic levels were chosen in this study. For the trophic level, producers, the terrestrial plant *Lepidium sativum* was used. As representatives of the primary consumers the crustacea *Daphnia magna* was chosen. Representative for the decomposer was *Vibrio fischeri*. Since one simple bioassay never provides a safety estimation of the environmental hazard of a chemical, these three test organisms, which represent three different trophic levels, are incorporated.

For our knowledge no studies about OPPs-oxon's phtotoxicity have been reported. After agricultural applications of OPPs, natural effects, such as UV irradiation and microbial transformation, may cause decomposition of pesticides and formation of oxon derivatives. For phytotoxic evaluation of OPPs-oxon's *Lepidium sativum* test organism was selected. *Lepidium sativum*, known as garden cress, is a fast growing annual herb widely cultivated in temperate climates throughout the world for various culinary and medicinal uses (Moser et al., 2009).
Water flea *Daphnia magna* is a standardized test organism and has been widely used in toxicity tests (Jemec et al., 2007; Palma et al., 2008). *Daphnia magna* is very sensitive to OPPs (Barata et al., 2001). It is often inhabits in small water bodies around agricultural fields receiving OPPs treatments. In this study, acute effects of OPPs and their oxon derivatives on *Daphnia magna* was determined.
Luminescence bacteria test with *Vibrio fischeri,* commonly called Microtox test, is a convenient test to perform in a short time. The photo luminescent bioassay uses a suspension of *Vibrio fischeri* bacteria and measures the reduction in light output of its natural luminescence on exposure to the toxicant of interest (Kaiser, 1998). Bacterial bioluminescence is related with cell respiration, and any inhibition of cellular activity because of toxicant results in a decreased rate of respiration and a corresponding decrease in the rate of luminescence.

2. Aim of the study

The widespread use of OPPs for pest control poses a risk of contamination to aquatic and terrestrial environments. OPPs are transformed into degradation by-products both in biotic and abiotic processes. These compounds are toxic especially for the organisms in lower trophic levels. Toxicity assessments of both parent OPPs and their degradation by-products are necessary for safety consideration of OPPs applications.
The objective of this study is to investigate the toxicity of OPPs and their main degradation by-products; OPPs-oxons by using *Daphnia magna*, *Lepidium sativum* and *Vibrio fischeri*. For the trophic levels, the terrestrial plant *Lepidium sativum* was chosen as producer while representative for the consumers the crustacea *Daphnia magna* was selected. Representative for the decomposer was *Vibrio fischeri*. Conducted data in this study can be used for environmental risk assessment, and guide further use of pesticides correctly and appropriately.

3. Materials and methods

3.1 Chemicals

All chemicals used were of analytical grade. Chlorpyrifos, parathion, methyl parathion, and their oxon derivatives; chlorpyrifosoxon, paraoxon, methyl paraoxon were obtained from Accustandard Co. (USA). Stock solutions were prepared with dimethyl sulfoxide (DMSO) obtained from Merck (Palma et al., 2008). All solutions were stored in the dark at 4 ^{o}C. Working solutions were prepared by dilution of standard stock solution with distilled water.
Daphtoxkit was obtained from MicroBioTest Inc (SOP, 2009). The *Vibrio fischeri* bioassay used was LCK 480, obtained from Dr. Lange (ISO, 1998).

3.2 Bioassay tests

To determination of the toxicity of OPPs and their main degradation by-products; OPPs-oxons by using *Daphnia magna*, *Lepidium sativum* and *Vibrio fischeri*, three different biotests

from different trophic levels were chosen in this study. For the trophic level of producers the terrestrial plant *Lepidium sativum* were used. The microtox test using luminescent bacteria was employed as representative for the decomposers. As representatives of the primary consumers the crustacea *Daphnia magna* was used. Properties of the selected tests protocols are described in Table 2.

Test	Trophic level	Group of organisms/plants	Type of test	Test duration	Test criterion	Test principles
Microtox* (*Vibrio fischeri*)	Decomposer	Bacteria	Acute	30 min	Inhibition of luminescence	Measure of luminescence reduction with luminometer
Daphtox* (*Daphnia magna*)	Primary consumer	Crustaceans	Acute	48 h	Immobility/ Mortality	Counting of dead and alive crustacean
*Lepidium sativum***	Producer	Garden cress	Chronic	3 day	Root length	Measurement of root length

*Aquatic test, **Terrestrial test

Table 2. Properties of the selected ecotoxicological tests

Water blank analyses without adding pesticides but including the solvents were carried out for controlling solvent effect to the toxicity. Quality control tests with potassium dichromate for all of the tests were performed. These tests should be repeated regularly to check the correct execution of the test procedure and the good physiological condition of the test organisms.

3.2.1 *Lepidium sativum* toxicity test

Garden cress test with *Lepidium sativum* was carried out according to Devare & Bahadir (1994). Phytotoxicity of OPPs were assayed by adding 25 seeds of *Lepidium sativum* onto 90 mm of two filter papers placed in a petri dish filled with 5 mL of sample. In the experiment, for the control six replicates and for the test samples three replicates were carried out. The dishes were covered and incubated in darkness for 72 hours. The lengths of the roots were measured after 72 h exposure duration and the inhibition of the root growth in the test solutions were calculated in comparison to the control. *Lepidium sativum* and test pictures are given in Fig. 2

3.2.2 *Daphnia magna* toxicity test

The toxicity tests on *Daphnia magna* bioassay were performed following the standard operational procedures of the respective Daphtoxkit F™ toxkits microbiotest (Fig. 3).
Standard freshwater solution for *Daphnia magna* toxicity tests was prepared from salt solutions provided in the test kit and aerated prior to use. For hatching of the *Daphnia magna* ephippia, they were transferred into a petri dish with 15 mL pre-aerated standard freshwater. *Daphnia magna* were hatched from eggs (ephippia) for 72 hours under continuous illumination (11000 lux) at 20-22 °C. 2 h prior to the test, the neonates were fed with a suspension of Spirulina micro-algal. Different concentrations and a control in five

Fig. 2. *Lepidium sativum* and test pictures

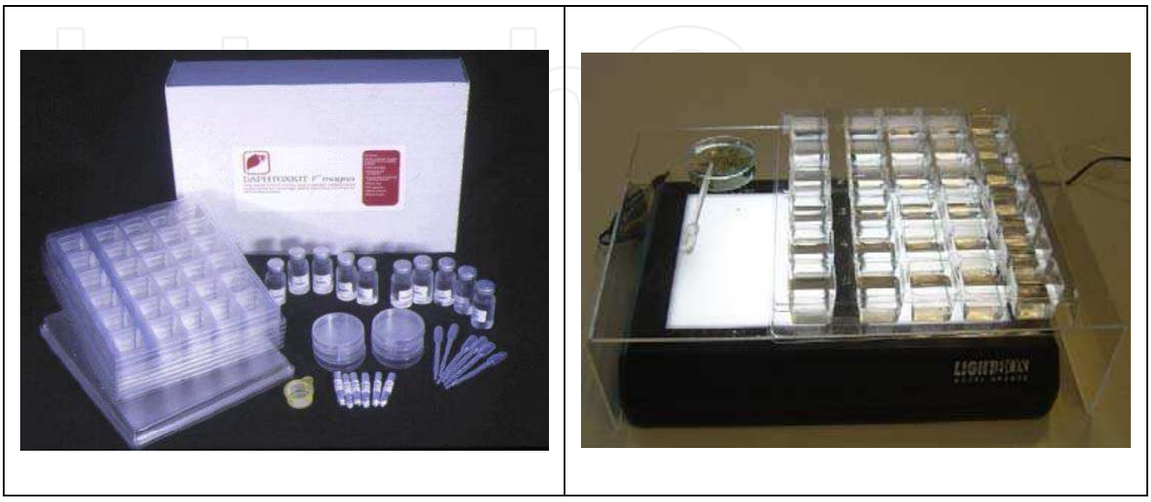

Fig. 3. Daphtoxkit pictures (www.microbiotests.be)

replicates were tested. Five *Daphnia magna* neonates were transferred into each cell Daphnids were exposed to samples of the tested agent for 48 h at a temperature of 20 °C in darkness. The immobile and dead daphnids were counted after 48 h of exposure. The percentage of dead and immobilized organisms and the EC_{50} values for samples were calculated.

3.2.3 *Vibrio fischeri* toxicity test

Luminescence bacteria test with *Vibrio fischeri* was carried out according to DIN/EN/ISO 11348-2 (Luminescent bacteria test LCK 482) measured with Dr. Lange LUMIStox 300 Luminometer (Fig. 4). The pH of the sample was adjusted to pH 7. Since *Vibrio fischeri* is a marine organism, an adjustment of the osmotic pressure of the samples was applied to obtain samples with 2% salinity, using a concentrated salt solution.

A dilution series of the sample was prepared directly in the glass cells according to DIN/EN/ISO 11348-2 and including the so called "G1" dilution of 80 %. Bioluminescence bacteria *Vibrio fischeri* were in freezedried form and activated prior to use by a reconstitution solution. For that, 12 mL reactivation solution for the luminescence bacteria was added into the reactivation tube and tempered to 15 °C for 30 min. Frozen luminescence bacteria were thawed in a water bath at room temperature for 2 min. 0.5 mL reactivation solution were added into the luminescence bacteria tube and tempered to 15 °C for 15 min.

Toxicity was assessed by measuring the inhibition of the luminescence bacteria after 30 min of incubation at 15 °C and the EC_{50} values and validation data were calculated according to DIN/EN/ISO 11348-2.

Fig. 4. Dr. Lange LUMIStox 300 Luminometer

3.3 EC_{50} and toxic unit

In order to assess the acute toxicity, test results are expressed in EC_{50}. EC_{50} values are the concentration responsible for the inhibition/mortality in 50% of the tested population in the different volumes of sample. The data expressed as EC_{50} were transformed into Toxic Units (TU) to reveal the direct relationship between toxic effects and the test system used. TU was calculated according to equation (1).

$$TU = \left[{}^{1}\!/_{EC_{50}} \right] x100 \quad (1)$$

4. Discussion

Toxicity of the reference compound, potassium dichromate was observed 0.9 mg L^{-1} for Daphtox with *Daphnia magna*, 4.1 mg L^{-1} for Microtox with *Vibrio fischeri*, 15 mg L^{-1} (root length) for *Lepidium sativum*, which is within the limits accepted by the ISO methods. According to their responses for reference tests, order of the trophic levels is the primary consumer, the decomposer and the producer, respectively.

The 72 h EC_{50} values of the OPPs and OPPs-oxons obtained for *Lepidium sativum, Daphnia magna* and *Vibrio fischeri* in our research were given in Table 3 and Table 4 respectively.

Lepidium sativum test results show that chlorpyrifosoxon, paraoxon and methyl paraoxon inhibit root growth. Negative inhibition effect was observed up to 50 mg L^{-1} concentration for parent compounds, so upper concentrations were not investigated. Straw et al. (1996) reported negative inhibition effect of chlorpyrifos on 2–4 year old Sitka spruce. Trees treated with chlorpyrifos showed a 25% increase in height growth and a 13% increase in side shoot extension growth after 2 years compared with control trees.

		Chlorpyrifos	Parathion	Methyl Parathion	Ref.
Lepidium sativum	EC_{50} (µg L^{-1})	n.d	n.d	n.d	In our study, 2010
	TU (µg L^{-1})	n.d	n.d	n.d	In our study, 2010
Daphnia magna	EC_{50} (µg L^{-1})	0.37	6.35	1.14	In our study, 2010
		0.74	-	-	Palma et al. (2008)
		-	2.2	-	Guilhermino et al. (1996)
	TU (µg L^{-1})	270	15	87	In our study, 2010
Vibrio fischeri	EC_{50} (µg L^{-1})	23190	12650	1187	In our study, 2010
		2840	-	-	Palma et al. (2008)
	TU (µg L^{-1})	0.004	0.008	0.08	In our study, 2010
WHO Class.	LD_{50} (mg kg^{-1})	Moderately hazardous 135	Extremely Hazadous 13	Extremely Hazadous 14	WHO (2005)

n.d. not determined

Table 3. EC_{50}, TU and LD_{50} values of the OPPs investigated against three tested organisms

According to toxic effects on root growth, toxicity order of the investigated compounds were paraoxon (EC_{50}: 0.634 mg L^{-1}), methyl paraoxon (EC_{50}: 1.599 mg L^{-1}) and chlorpyrifosoxon (EC_{50}: 2.048 mg L^{-1}) respectively. The toxicity of pesticides is investigated during their registration process, but the toxicity of their degradation products to the plant is unexplored. However, pesticides sprayed on the plant and soil surface are exposed to effect of microbial degradation and UV photons resulting with decomposition of the molecule, so inhibition effects of the transformation products should be noted.

		Chlorpyrifosoxon	Paraoxon	Methyl Paraoxon	Ref.
Lepidium sativum	EC_{50} (μg L^{-1})	2050	630	1600	In our study, 2010
	TU (μg L^{-1})	0.048	0.158	0.062	In our study, 2010
Daphnia magna	EC50 (μg L^{-1})	0.31	0.76	0.28	In our study, 2010
		-	0.2	-	Guilhermino et al. (1996)
	TU (μg L^{-1})	322	131	357	In our study, 2010
Vibrio fischeri	EC50 (μg L^{-1})	4380	4140	4404	In our study, 2010
	TU (μg L^{-1})	0.022	0.024	0.022	In our study, 2010

n.d. not determined

Table 4. EC_{50} and TU values of the OPPs-oxons investigated against three tested organisms

By comparing the results reached with the toxicological response of test organism *Daphnia magna*, toxicity order of the tested pesticides were chlorpyrifos (EC_{50}: 0.37 mg L^{-1}), methyl parathion (EC_{50}: 1.14 mg L^{-1}) and parathion (EC_{50}: 6.35 mg L^{-1}), from most to least. This order changes when oxon derivatives are investigated. Toxicity order of the tested OPPs-oxons is methyl paraoxon (EC_{50}: 0.28 mg L^{-1}), chlorpyrifosoxon (EC_{50}: 0.31 mg L^{-1}) and paraoxon (EC_{50}: 0.76 mg L^{-1}).

According to the acute toxicity classification system reported by Persoone et al. (2003), one can consider as class I (no acute toxicity, TU<0.4), class II (slightly acute toxicity, 0.4<TU<1), class III (acute toxicity, 1<TU<10), class IV (high acute toxicity, 10<TU<100), class V (very high acute toxicity, TU ≥100). Considering the toxic unit classification, chlorpyrifos is in class V (very high acute toxicity) for *Daphnia magna* and class I (no acute toxicity) for *Vibrio fischeri*. Parathion is in class IV (high acute toxicity) for *Daphnia magna and* class I (no acute toxicity) for *Vibrio fischeri*. Methyl parathion is similarly in class IV (high acute toxicity) for *Daphnia magna and* class I (no acute toxicity) for *Vibrio fischeri. Chlorpyrifosoxon, paraoxon and methl paraoxon are in class V* (very high acute toxicity) for *Daphnia magna* and in class I (no acute toxicity) for *Lepidium sativum* and *Vibrio fischeri.*

OPPs impairs the nerves function and consequently the normal mobility of organisms, which is the most frequent observed endpoint of the acute toxicity test with water fleas (Tisler et al., 2009). Neurotoxic effects in organism result with mortality.

In this study, mortality/immobility was the evaluated endpoint of the acute toxicity. Among parent OPPs chlorpyrifos was the most toxic insecticide to Daphnia magna. Guzella et. al. (1997) reported similar results in a study on acute toxicity of 11 OPPs to two marine invertebrates *Artemia sp.* and *Brachionus plicatilis*. Chlorpyrifos was the most toxic insecticide to both species while parathion and methyl parathion showed lower toxic responses. Palma et. al. (2009) reported developmental abnormality for *Daphnia magna* embryos exposed to a concentration of 0.01 µg L^{-1} chlorpyrifos. Besides, chlorpyrifos possesses an inhibitory effect on soil bacteria during the initial periods after its treatment (Xiaoqiang et al., 2008).

Evaluating the calculated EC_{50} values, acute effects of chlorpyrifos and chlorpyrifosoxon on survival parameters of *Daphnia magna* are very close to each other. On the other hand, paraoxon is nearly eight times and methyl paraoxon is nearly four times more toxic than their parental forms. Dzyadevych and Chovelon (2002) used Lumistox test for toxicity determination of methyl parathion and methyl paraoxon. The inhibition levels for methyl parathion and methyl paraoxon were roughly 2% and 25%, respectively.

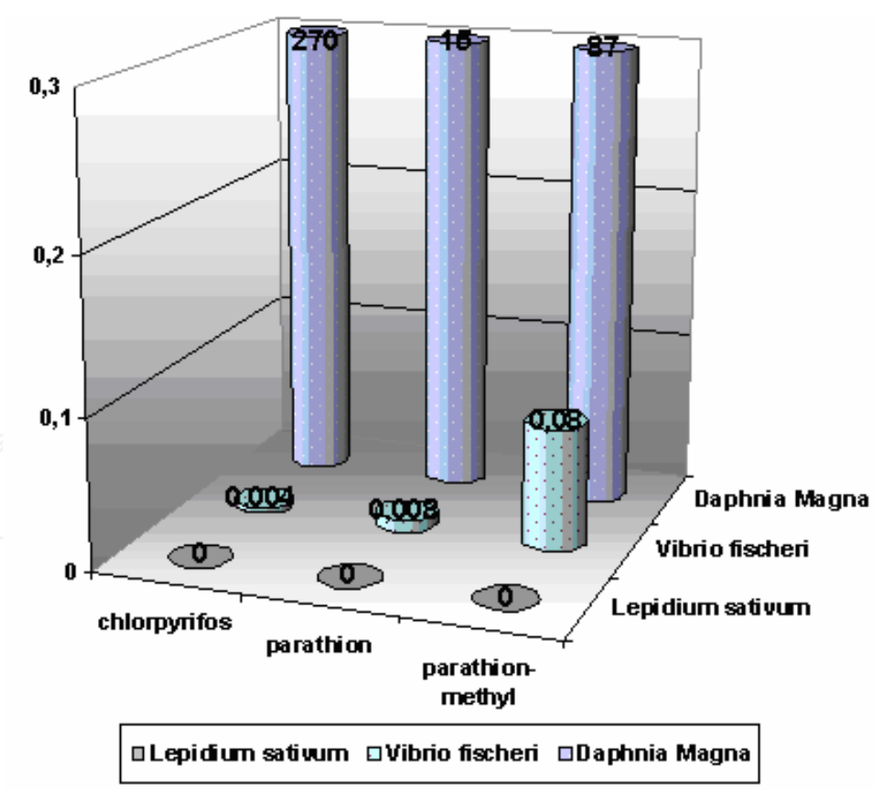

Fig. 5. TU values for test organisms; *Daphnia Magna, Lepidium sativum* and *Vibrio fischeri* in case of OPPs treatment

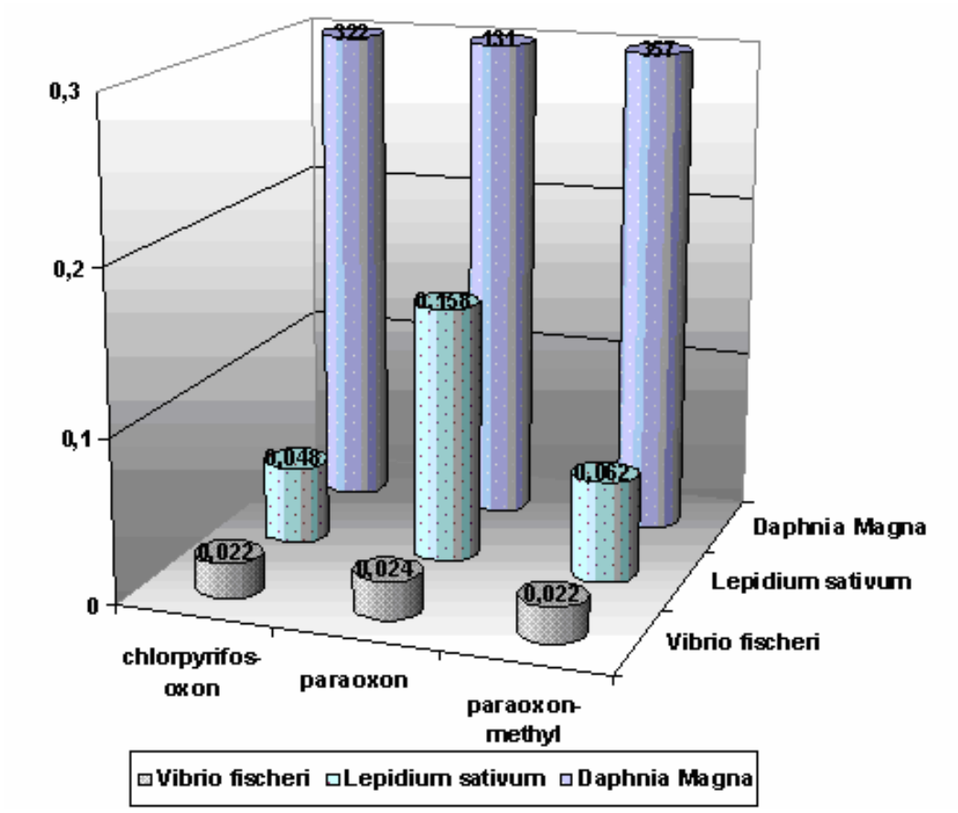

Fig. 6. TU values for test organisms; *Daphnia Magna, Lepidium sativum* and *Vibrio fischeri* in case of OPPs-oxons treatment

EC_{50} values are in ppm levels for *Daphnia magna* and OPPs can be detected in these levels in aquatic environments. TU values clearly demonstrate that *Daphnia magna* test organism is much more sensitive to OPPs and OPPs-oxons than *Vibrio fischeri* and *Lepidium sativum* and this difference is shown in Figure 5 and Figure 6. TU values for test organisms; *Daphnia Magna, Lepidium sativum* and *Vibrio fischeri* in case of OPPs treatment is given in Figure 5 and in case of OPPs-oxons treatment is given in Figure 6.

Oxon derivatives of chlorpyrifos, parathion and methyl parathion increase inhibition effect on *Daphnia magna* and *Vibrio fischeri* test organisms. For *Lepidium sativum* test organism, parent compounds did not show any inhibition effect up to 50 mg L^{-1} concentration, so TU values are accepted as zero. On the other hand OPPs-oxons inhibition effect on *Lepidium sativum* test organism is bigger than *Vibrio fischeri*.

EC_{50} values for *Daphnia magna* can also be used to understand toxic effects of analytes on other aquatic organisms. Daphnids are more sensitive to toxicants than fish; therefore, the EC_{50} values for *Daphnia magna* can be used as an upper threshold for fish tests (Zvinavashe et al., 2009).

There are studies using acetylcholinesterase (AChE) bioassays that rely on inhibiting AChE activity (Kralj et al., 2007). Oxon derivatives have higher AChE inhibition capacity, however AChE inhibition does not explain all the symptoms of OPPs intoxication. Dzyadevych and Chovelon (2002) reported that effect of methyl parathion and methyl paraoxon on the luminescent bacteria is not related to cholinesterase activity. Xuereb et al.(2009) investigated relations between whole-body acetylcholinesterase (AChE) inhibition and changes in locomotor behaviours in adult male *Gammarus fossarum* exposed to chlorpyriphos and reported that significant mortality was observed from 50% AChE inhibition This result suggests that, for chlorpyriphos, the observed mortality was not directly related to AChE inhibition but that an additional toxic mode of action occurred.

Biotransformation of OPPs to oxon derivatives in the body of the organism may also occur. Metabolic activation occurs by monooxygenase enzymes to form the active oxon analogues

This metabolic activation occurs especially in crustacean species compared to fish and mollusc species (Takimoto et al., 1987). Some species have an elimination capacity. According to the results of the study conducted by Ashauer et al. (2006) accumulated chlorpyrifos in *Gammarus pulex* is rapidly eliminated, suggesting a biotransformation capacity in this species. This capacity of species may be used for bioremediation of contaminated environments. Cycon et al. (2009) used three bacterial strains *Serratia liquefaciens, Serratia marcescens and Pseudomonas sp* to determine their capacity to use in bioremediation of contaminated soil and reported that isolated bacterial strains may have potential for use in bioremediation of diazinon-contaminated soils.

Additional studies are required to test combined toxicity of OPPs and OPPs-oxons, since the compounds usually applied simultaneously or one after another for crop protection. According to the results of the study conducted by Xiaoqiang et al. (2008) the inhibitory effect of chlorpyrifos on soil microorganisms was increased by its combination with chlorothalonil.

Since biological techniques are inefficient for the treatment of pesticide containing wastewaters chemical water treatment processes are preferred. A number of different Advanced Oxidation Processes are widely used for water treatment for sanitation and oxidation purposes. In many cases total mineralization is not possible. OPPs transformation to more toxic products during the application of these processes make it essential to use more convenient treatment methods and measurement of toxicity. According to the results conducted in our study, *Daphnia magna* appears to be the most sensitive method.

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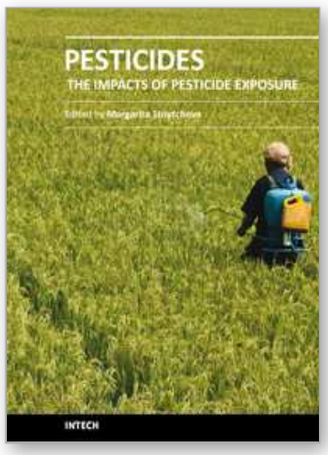

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Pesticides are supposed to complete their intended function without â€œany unreasonable risk to man or the environmentâ€. Pesticides approval and registration are performed â€œtaking into account the economic, social and environmental costs and benefits of the use of any pesticideâ€. The present book documents the various adverse impacts of pesticides usage: pollution, dietary intake and health effects such as birth defects, neurological disorders, cancer and hormone disruption. Risk assessment methods and the involvement of molecular modeling to the knowledge of pesticides are highlighted, too. The volume summarizes the expertise of leading specialists from all over the world.

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