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Using Zooplankton, *Moina Micrura* Kurz to Evaluate the Ecotoxicology of Pesticides Used in Paddy Fields of Thailand

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1. Introduction

Thailand is an agricultural country where agriculture is a very important part of the economy. Thailand expanded exports of agricultural products and also imports fertilizers and pesticides intensively. Pesticides are used widely in agriculture and trade of agricultural products to increase agricultural yield and to protect plant from diseases, weeds and insect damage (Department of Agricultural, 2010). Since pesticides were first imported into Thailand under the "Green Revolution Policy" as part of the 1st National Economic and Social Development Plan in 1966, the total amount of imported pesticides has dramatically increased year by year. Most pesticides used in the country are imported (Department of Pollution Control, 2005), and the quantities of imported agricultural pesticides have increased 3 times from 1994 to 2005, reaching more than 80 thousand tonnes in 2004. Pesticides are applied in the highest quantity in vegetable and fruit farming, where market pressure for appearance is higher. In 2000, organophosphates contributed the majority of imported pesticides followed by carbonates and organochlorines; most were herbicides, followed by insecticides, disease control agents and plant growth regulators (Department of Pollution Control, 2002).
The result from increasing pesticides uses has resulted in significant increased crop contamination and human health hazard (Office of Epidemiological, 2009). The risk of pesticide contamination in fruits and vegetables in Thai market often occurs.
Rice is the major crop and food source for most Asian countries including Thailand. Rice production from paddy fields faces variety of pests that require a range of pesticides and herbicides to manage the presence of insects and weeds, as well as fungal and bacterial pathogens. Indeed, losses of the total world rice crop due to insects have been estimated to occur at a rate of 28% per annum, which is four times greater than the average for other world cereal crops. More than 90% of the global end-user market in pesticides for rice production is applied in Asia (Abdullah, 1995). In Thailand, pesticides play an important part and widely use on rice production because its benefits in pest control and increased rice production. Therefore, pesticide contamination in draining water from paddy field has been one of non-point source pollution in aquatic ecosystem (Sanchez et al., 2006).

This is attributed to be relatively large amounts pesticides applied in paddy field, in addition to common practice of draining the paddy water in draining canals (Tejada, 1995). Around 95 % of freshwater in Thailand is withdrawn to irrigate the more than 5 million hectares of irrigated agriculture. Waste water from this activity may pose significant environmental hazards for aquatic ecosystem in particularly aquatic biota. Furthermore, this contaminate affect wildlife species ether by direct exposure or through bioaccumulation in food web.

Pesticide contamination sites associated with paddy field activities may pose significant environmental hazards for terrestrial and aquatic ecosystems. They are important sources of agro-sourced pollution and may result in ecotoxicological effects, particularly following transfer of irrigation waters following use. Ecotoxicological effects occur at all trophic levels, from the molecular to the ecosystem level and effects may be observed via biomonitoring with both individual organisms and the ecosystem function and structure.

Pesticide monitoring is traditionally based on evaluations of individual pesticides identified through chemical analyses. A variety of techniques may permit an examination of actual pesticides, herbicides and their metabolites that are present (Iwai et al., 2007). These techniques are based on sampling approaches that use concentration following collection or during collection. Although these techniques still are not able to show the direct response that ecotoxicity gives, they do give an indication of what is inducing the response of the organism. However, chemical analyses obviously do not reveal complex interaction phenomena and polar degradation products are often missed. In contrast to the use of chemical analyses, the ecotoxicity bioassay approach integrates the biological effects of all compounds present and factors such as bioavailability, synergism, or antagonism are reflected directly in the bioassay results.

Ecotoxicological assessment of pesticides in paddy field are therefore expected to give a more comprehensive indication of environmental effects. The use of ecotoxicological assessment to evaluate the impact of pesticide residues in the paddy field is strongly recommended in order to have a more direct and integrated estimate of environmental impact. In fact, biological response to a complex mixture of chemicals integrates different factors such as pH and solubility, antagonism or synergism, and the bioavailability of substances.

Pesticides contamination associated with paddy field has been increased a big concern in Thailand. For risk assessment study on the impact of pesticides on aquatic environments that surrounding area, information about effect of pesticides on local species were limited, especially the ecotoxicological data on aquatic organism in Thailand, and it unknown, whether ecological effects test guideline developed elsewhere in the world (US. EPA, ATSM etc) may be use in Thailand. Countries located in the tropical zone rely, mostly, on data from temperate countries about ecotoxicity data. However, this data may be not suitable for tropical countries. Due to the difference organisms species, temperature, rainfall, and agriculture practices that might greatly influence pesticides behavior (Abdullah et al., 1997) and toxicity of pesticides on organisms. Considering the climate adaption of tropical species, assessment of effects of pesticide use on local ecosystem should be performed with local species since their sensitive to toxicants may differ considerably form temperate organism (Domingues et al., 2007). Differential response of organism representing diverse physiological capabilities and niches in aquatic system can help focus field studies where nontarget effect due to off - site movement of pesticides are suspected.

Therefore, Thailand need ecological effects test guideline, this guideline typically derived data on toxicological response of local organism to environmental contaminant. The toxicity test is procedure that involves the exposure of organism to complex environmental sample under controled condition to determine if adverse effects have occurred (Edmondson, 1959).

The objective of this study selected the fresh water cladoceran *Moina micrura* Kurz order *Cladocera*, family *Moinidea*. In Thailand, this zooplankton is very common in pond, muddy pool and paddy field and it can be mass culture by some local fish farmer as a high quality fish food. *M. micrura* is an ideal animal for ecological relevance, wide occurrence, short life cycle, genetic uniformity, relative ease of culture in the laboratory and more sensitive to toxicants (Wang, 1994; Wongrat, 2001). The present study was determine the acute and chronic toxicity of pesticides on *M. micrura*. The result would be useful as an input to developing a biomonitoring tool and using local species test for evaluation pesticide contamination in Thailand aquatic ecosystem.

2. Materials and methods

2.1 Test organism culture

The Moina micrura obtained from Fisheries Research Institute, Khon Khaen (Khon Khaen, Thailand) and have been maintained in cultured under control laboratory conditions in Ecotoxicology and Environmental Sciences Laboratory, Faculty of Agriculture, Khon Kaen University, Thailand. The culture was incubated at 25± 2 ^{0}C with 16:8 h light:dark photoperiod. *M. Micrura* were cultured using moderately hardwater and fed on single-celled green alga, *Chlorella vulagaris* from axenic culture. The medium, used for zooplankton, as well as for experiments, was tap water at the Faculty of Agriculture, Khon Kaen University, Thailand. Water was filtered by using 0.45μm polymembrane filter. Dissolved oxygen concentration was between 5-7 mg/L and pH was 7-8. The culturing period for one generation was 2 weeks before testing.

2.2 Test chemicals

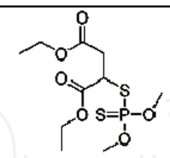
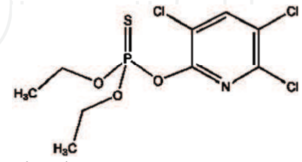
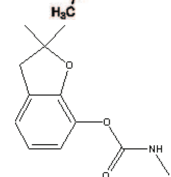
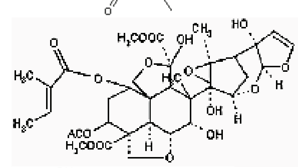
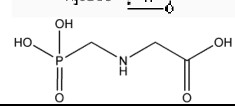
The pesticides test were selected from the common pesticides used in paddy filed of Thailand. Five selected pesticides were malathion 58% w/v (CAS: 121-75-5), chlorpyrifos 40% w/v (CAS:2921-88-2), carbofuran 3 % GR (CAS:1563-66-2), neem extract 40% w/v (CAS:1141-17-6) and glyphosate 36% w/v (CAS:1071-83-6) (Table 1.). Stock solutions were prepared by dissolving the pesticide directly in distill water immediately before to each experiment. Stock solution were added to each of three replication test beakers (50 ml total volume) to obtain nominal exposure concentration. Rang in nominal aqueous exposure concentration of chlorpyrifos,malathaion, glyphosate, carbofuran and neem extract on *M. micrura*, were arranged in geometic series between 0.5 -0.0005, 1-50, 500-2500, 3-15 and 50-250μg/L respectively.

2.3 Experiment design

2.3.1 Acute toxicity test

Preliminary acute toxicity tests were conducted in order to calculate malathion, chlorpyrifos, carbofuran, neem extract and gyphosate LC_{50} data. All experiments were performed according to the US.EPA document OPPTS 850.1010 (1996) for determining 48 h LC_{50} values for *M. micrura*. Three replication of 10 neonates (<24 h) per treatment and control laboratory well – wate were used. The neonates were exposed in a 150 ml glass beaker containing 50 ml for each test concentration and control were static bioassay under laboratory. Test organisms were not fed during the testing period. Observation motality was made at 24 and

48 h, and results recorded. For water quality, temperature, pH, conductivity and dissolved oxygen were measured according to APHA (1992).

Pesticide	Group of pesticide	Chemical name and number (Chemical Abstract Service)	Structure
Malathion	Insecticide (organophosphate)	O,O-dimethyl phosphorodithioate of diethyl mercapto- succinate ; CAS no. 121-75-5	
Chlorpyrifos	Insecticide (organophosphate)	Phosphorodithioic acid, O,O-diethyl O- (3,5,6-trichloro-2-pyridyl) ester; CAS no:2921-88-2	
Carbofuran	Insecticide (carbamate)	3,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate CAS no. 1563-66-2	
Neem extract	Insecticide (biopesticide)	Azadirachin; CAS no. 1141-17-6	
Glyphosate	Herbicide (amine)	N - (Phosphonomethyl) glycine; CAS no. 1071-83-6	

Source: Extoxnet (1996); Chemical Book (2007); Compendium of Pesticide common name (2008 a, 2008 b, 2008 c)

Table 1. Chemical formulation of pesticides tested with *M. micrura* .

2.3.2 Chronic toxicity test

Chronic toxicity of pesticides to *M. micrura* followed the procedure recommend by US.EPA document 6004-91/002 (1994). Based on acute toxicity result, *M. micrura* were exposure to control and concentration test malathion concentration of 0.05, 0.25 and 0.50 µg/L, chlorpyrifos concentration of 0.00005, 0.00025 and 0.00045 µg/L, carbofuran concentration of 0.25,1.00 and 2.50 µg/L, neem extract concentration of 15, 40 and 65 µg/L and glyphosate concentration of 50, 250 and 325 µg/L. In the chronic tests, three replication of 10 neonates <(24 h)per treatment and control laboratory well – water were used. The neonates were exposed in a 50 ml glass beaker containing 30 ml for each test concentration and control. Test organism were fed with a concentrated suspension of the green algae, *Chlorella* sp. Test solution and food were renewed completely every day. The measurement of water quality at the beginning and end of the test on control and treatments. The number of offspring was noted each day used to evaluate the effect of pesticide on reproduction of test organism.

2.4 Statistic analysis

The values of lethal concentration 24 and 48 h LC_{50} and 95 % confidence limit were caculated by appropriate statistical method intervals by probit analysis.Data from chronic test were analyzed using ANOVA with SPSS version 12 statistical software to detected variation significances ($P<0.05$) between treatment group and control.

3. Results and discussion

Acute toxicity

Table 2. show the estimated 48-h LC_{50} for pesticides, with were calculated from standard toxicity test with *M. micrura*.

Malathion.: Our 48-h *M. Micrura* LC50 of 10.44 µg/L was compareable to the 48-h LC_{50} to other Cladocera species. This results shown 48-h- LC_{50} *M. Micrura* were nealy repoted the 5-10 µg/L for *M. marcocopa* by Wang et al. (1994) and the 8 and 13 µg/L that reported by Khan et al. (1993) and Siefirt (1987) for *D. Magna.* Differnce in LC_{50} value were observed wih *Cerodahnia dubia* have been reported between 1.14 -3.35 µg/L(Hernadez et al.,2004; Maul et al., 2006; Nelson et al.,1997,1998; Ankley et al., 1991).

Chlorpyrifos: Of the five pesticides tested in this study, chlorpyrifos was the most toxic to *M. Micrura*. The 48-h LC_{50} value for *M. micrura* was 0.08 µg/L. Other values in literature were higher between 0.13-3.7 µg/L using *D. ambigue, D. Magnaand and D. Duplex* (Caceres et al.,2007; Van Wijngaarden et al., 1993; Barata et al.,2004; Kersting et al.,1997; Van der Hoeven and Gerrisen, 1997).

Carbofuran: The 48-h LC_{50} for *M. micrura* obtain in this study 6.96 µg/L is compareable to the 48-h LC50Of 2.69 µg/L obtain with *C. Dubia* (Nerberg et al.,1997) for carbofuran in *D. magna* were higher than concentration tested (6.96 µg/L). In comparison, Poirer (1990); DBR (2000) and Dopsikova (2003) found an acute 48-h LC_{50} were 86.1, 38.6 and 18.7 µg/L respectively.

Neem extract: The present study found that the 48-h LC_{50} for neem extract in *M. micrura* was 196.3 µg/L. The acute toxicity data for *D.magna* with 48-h LC_{50} were 570- 1,250 µg/L (John , 2001; Stark ,2001; Scott and Kaushik, 2001), and were <6000 – 380,000 µg/L for *D. Duplex* (Goktepe and Plhak, 2002,2003)**.**

Glyphosate: Glyphosate was the lowest toxic (LC50 was 3042 µg/L) to *M. micrura*. Other values in reports were higher between 1150 – 107,000 µg/L and 30000 µg/L for *C. Dubia and D. Magnaa,* respectively. The LC50 value of pesticides showed that toxicity of chlorpyrifos > carbofuran > malathion > neem extract > glyphosate. *M micrura* were susceptible to pesticides from µg/L to mg/L, with chlorpyrifos was the most toxic (LC50 = 0.08 µg/L) and glyphosate was the lowest toxic (LC50 = 3042 µg/L) to *M. micrura*.

Pesticides	48 h-L50 (µg/L)
Malathion	10.44 (9.10 -11.85)
Chlorpyrifos	0.08 (0.03 - 0.20)
Carbofuran	6.96 (5.97 – 7.63)
Neem extract	196.3 (161.5 -263.9)
Glyphosate	3043 (1974 – 1778)

Table 2. Acute toxicity (Medium lethal concentration [LC_{50}]) of pesticides on *M. micrura* at 48 h.

In this studied were founded that toxicity of the insecticide group (chlorpyrifos, carbofuran, malathion and neem extract) were more toxic to *M. micrura* than the herbicide group (glyphosate), because insecticide had mode of action that affect on organism directly but herbicide acted in indirect way. US. EPA (1998) reported chlorpyrifos had very high toxicity to freshwater fish and aquatic invertebrates, carbofuran and neem extract had higher toxicity but glyphosate had less toxicity on zooplankton (Henry et al.,1994; ENTOXNET, 1996; PMRA, 2002; Dopsikova, 2003; Saglam and Saler, 2005). On the basis LC_{50} value, *M. micrura* of this study were sensitive to pesticides nearly Ceriodapnia species but were more sensitive to pesticides than Dahpnia species indicated by other studies in Table 3. Due to Daphnia species were bigger than Moina species and Ceriodapnia species thus its tolerant than M. Micrura and Ceriodapnia species. The result were found similar to Scott and Kaushik (1998); Liane (2002) and Grant and Schmutter (1987) reported were size, age, species, life-cycle of zooplankton and environment such as temparature, pH and harness have influent to chemical toxicity on zooplankton.

The observation of *M micrura*, after treated with pesticides especially in high concentration, the swimming activity of *M. micrura* was changed. They moved faster then normal conditions, after a time later, the movement on antenna and limbs become slowly and death after that. Concentration of pesticides had disrupt respiratory membrane of *M. micrura*, their swimming behavior changes in high concentration and *M. micrura* were loosed their original colored. The similar results were reported in Rassolzadegan (2000); Saglam and Saler (2005)John et al. (2007)

Chronic toxicity

Effect of sublethal pesticides concentration on the number of offspring per female of *M. micrura* is shown in (Table 4). Number of offspring per female of *M. micrura* was significant reduced ($P<0.05$) at malathion concentration 0.50 µg/L, chlorpyrifos concentation greater than 0.00025 µg/L, at carbofuran concentration at 2.50 µg/L and at glyphasate concentration 325 µg/L. For neem extract concentration had no effect on the number of offspring per female significantly ($P>0.05$). Sublethal effects for each pesticide, were founded similar to other reports (Wong et al, 1995; Alberdi et al,1996; US EPA 2006). An estimate of no observed effect concentration (NOEC) and lowest observed concentration (LOEC) were 0.25 and 0.50 µg/L for malathion, 0.00005 and 0.00025 µg/L for chlorpyrifos, 1.00 and 2.50 µg/L for carbofuran, 250 and 325 for glyphosate and LOEC 65 µg/L for neem extract. Cladocerans contribute an important component of aquatic ecosystem especially, for fish food source. If the number of clardocerans were down, it may affect fish and another organisms.

The number of offspring per female is one endpoint used to determine the maximum acceptable - toxicant concentration (MATC). The 16 % reproduction impairment have been used as the endpoint for many aquatic ecotoxicology (Biesinger and Chistensen, 1972). Therefore, this studies used 16 % reproduction impairment estimate the chronic values MATCs for pesticides (Table 4). According to the obtained results the calculated values of MATCs and 48-h LC_{50} were for estimate application factor (AF) of pesticides on *M. micrura* (Table 5).

This value was used to predict the safe concentration (SC)applies for pollutant prevention in aquatic ecosystem. However, the application factor will vary with type of pesticide and organism (Mounth and Stephan, 1967).

Pesticide	Species	48-h LC_{50}	References
Malathion	*M. micrura*	10.44	This studies
	M. marcocapa	5-10	Wang et al. (1994)
	C. dubia	3.18	Hernadez et al. (2004)
		3.35	Maul et al. (2006)
		1.14	Nelson et al. (1997, 1998)
		2.12	Ankley et al. (1991)
	D. magna	0.90	Ren et al. (2007)
		8.0	Khan et al. (1993)
		13	Siefirt. 1987)
Clorpyrifos	*M. micrura*	0.08	This studies
	C. dubia	0.117	Bailey. (1997)
		0.056	Harmon et al. (2003)
	D. ambigue	0.050	El- Merhibi et al. (2004)
		0.035	Harmon et al. (2003)
	D. magna	0.30 - 0.80	Caceres et al. (2007)
		1.28	Van Wijngaarden et al. (1993)
	D. duplex	1.0 - 3.7	Barata et al. (2004)
		0.13	Kersting et al. (1997)
		> 1.6	Van der Hoeven and Gerrisen. (1997)
		0.17-0.49	Hooftmant et al. (1993)
Carbofuran	*M. micrura*	6.96	This studies
	C. dubia	2.69	Nerberg et al. (1997)
		> 20	Poirer (1990)
		> 162	DBR (2000)
	D. magna	86.1	Dopsikova (2003)
		38.6	
		18.7	
Neem extract	*M. micrura*	196.3	This studies
	D. magna	1250	John. (2001)
	D. duplex	570 - 680	Stark. (2001)
		570	Scott and Kaushik. (2001)
		<6000 - 243000	Goktepe and Plhak. (2002)
		30000 - 380000	Goktepe and Plhak. (2003)
Glyphosate	*M. micrura*	3043	This studies
	C. dubia	1150	Hensen et al. (1994)
		5890 - 107000	Tsui et al. (2004)
	D. spinulata	30000	Lutufu et al. (2001)
	D. magna	20000 - 21880	Al –Omar et al. (2000)
	D. duplex	218000	Henry et al. (1994)
		7900	Office of pesticide program. (2000)

Table 3. Comparison of 48-h LC_{50} (µg/L) Value of *Moina micrura* and another clardocerans species.

Aquatic ecosystems in tropical regions differ from those in temperate regions. The biodiversity in tropical zones is higher than that in temperate zones, which means that in tropic regions there are potentially more species that can be exposed to certain pollutants. However, many countries in the tropics are developing countries, in which pollution control is not carried out due to a lack of funds and other resources. Furthermore environmental quality criteria for some pollutants are often obtained by extrapolating toxicity data derived for a reduced number of species mainly distributed in temperate regions (e.g. Europe or the US) (Kim *et al.*, 2001 in Kwok *et al.*, 2007). Kwok *et al.* (2007) investigated to which extent the sensitivity distributions of temperate species to toxic substances were similar to those of tropical species. They found that the temperate species seemed to be more sensitive to metals than the tropical species (Kwok *et al.*, 2007). However, it should be noted that these differences might be due to the different species composition included in the species sensitivity distributions (SSD). Kwok *et* al (2007) used mainly fish species, which could be less sensitive to pollutants than the invertebrate species that are predominantly used in the temperate species sensitivity distributions. A better comparison can be made when using similar taxonomic groups for the distribution.

In Thailand ecotoxicological research is quite new and has many limitations. Although ecotoxicological issues arise in this country and there is a need for water quality management and ecological risk assessment tools, there is a lack of ecotoxicological data on aquatic organisms from Thailand. Until now, like other developing countries, they have relied on over sea data to develop ecotoxicological test guidelines. However, these guidelines may be unsuitable for Thailand. The Thai indigenous aquatic organisms might be more or less sensitive to contaminants than their temperate surrogate species (Iwai, 2004; Iwai and Noller, 2010; Somparn et al., 2010). Moreover, there are differences in physicochemical and biological characteristics of aquatic habitats between tropical and temperate regions (Kwok et al., 2007). The characteristic of the sediment and water in Thai rivers may differ from those in other countries (Iwai and Noller, 2010; Somparn et al., 2010), influencing the concentration, availability and accumulation of pollutants and therefore their toxicity. An example of this is given by Jeon et al. (2010). They found that clay and food content in the water influence the toxicity of pollutants on aquatic biota.

Tirado et al. (2008) report that the main rivers in Thailand were monitored from 1993 to 1999 for the presence of pesticide residues; most water samples contained insecticide and herbicide residues in levels above advisable limits, whereas less contamination was observed in sediment samples. In river water, organochlorine pesticides were detected in 40.62% of the samples (in concentration ranging from 0.01 to 1.21 µg/L), organophosphate pesticides were detected in 20.62% of samples (in concentration ranging from 0.01 to 5.74 µg/L). The safety limit established by the European Union is 0.1 µg/L for any single pesticide and 0.5 µg/l for the sum of all pesticides detected. Both organochlorine and organophosphate pesticide residues were found above those safety limits. Additional compounds, like carbamate pesticides were detected in 12.39% of samples (in concentration ranging from 0.01 to 13.67 µg/l), triazines were detected in 20.0% of samples (in concentration ranging from 0.01 to 6.63 µg/L), and paraquat was detected in 21.36% of samples (in concentration ranging from 0.14 to 87.0 µg/L) (Chulintorn et al., 2002). An earlier study has also found residues of the pesticides DDT and dieldrin in five Thai rivers (Upper Ping, Lower Ping, Wang, Yom, Nan, Chee), in concentrations above acceptable standard levels (Sombatsiri, 1997). The Division of Agricultural Toxic Substances in the Department of Agriculture (Ministry of Agriculture and Cooperatives) has also monitored the presence of pesticide residues in rivers and canals around agricultural areas in the country. The contamination of pesticides in water and sediments was generally low in water resources used for domestic consumption like ponds and reservoirs that have no connection to agricultural plantations. However, the water resources in certain agricultural areas, like orchid and ornamental plantations, were contaminated with organophosphate and carbamate insecticides. From 1999 to 2001, a survey of three major rivers along paddy field areas (Thachin river in Suphanburi and Nakornpathom, the Chao Phraya river in Pathumthani and Nonthaburi, and the Bangpakong river in Chachengsao), found the highest residues of the insecticide endosulfan in the Thachin River, followed by the Chao Phraya and Bangpakong Rivers. In all cases, the levels of pesticide residues were above the safety limit set by the European Union (0.1 µg/L) (Chatsantiprapha, et. al., 2002).

In 2001, groundwater in the lower Central and the lower Northeastern region of Thailand was contaminated with pesticides residues, in many cases in concentration above the safety limit set by the EU (0.1 µg/l). In the lower Central region during the rainy season in 2001, 68% of 15 GRL-TN-03-2008 the total groundwater samples were contaminated with endosulfan and other insecticides, in concentration ranging from 0.02 to 3.2 µg/l, and paraquat, 2,4-D, butachlor, atrazine and metribuzin herbicide residues ranging from 0.02 to 18.9 µg/l. In lower Northeastern region during the dry season in 2001, 71.2% of the total groundwater samples were contaminated with endosulfan and other insecticides, in concentrations from 0.01 to 0.33 µg/l, and atrazine and paraquat herbicide residues at the level of 0.5-4.0 µg/l (Sakultiangtrong, et.al., 2002). In 1993, the Department of Agriculture investigated shallow groundwater wells from Rayong Province. From 160 samples collected from wells, 67% were contaminated with organochlorine and organophosphate pesticides, but in concentration below the safety limits (Pollution Control Department, 2004).

Pesticide	Concentration (µg/L)	Number of offspring per female	% Reproductive impairment
Malathion	0.00	55.13±0.45a	0.00
	0.05	53.13±0.50a	3.68
	0.25	49.03±0.70a	11.06
	0.50	36.50±0.46b	33.79
Chlorpyrifos	0.00	53.37±0.35a	0.00
	0.00005	53.37±0.35a	6.741
	0.00025	49.77±0.41b	9.43
	0.00045	43.00±0.52c	28.23
Carbofuran	0.00	53.37±0.35a	0.00
	0.00005	53.37±0.35a	6.74
	0.00025	49.77±0.41b	19.43
	0.00045	43.00±0.52c	28.23
Neem extrat	0.00	56.33±3.15a	0.00
	15.00	55.10±2.12a	2.18
	40.00	53.76±1.72a	4.56
	65.00	52.33±1.99a	7.10
Glyphosate	0.00	56.06±1.62a	0.00
	50.00	55.17±0.95a	1.59
	250.00	47.66±2.12a	14.19
	325.00	42.43±3.74b	24.31

*Note:Value are maen + standard deviation. Mean with the same letter in the column are not significantly different (P>0.05).

Table 4. Chronic toxicity of malathion, chlorpyrifos, carbofuran, neem extract and gyphosate on the number of offspring per female and % reproductive impairment of M. micrura.

Pesticides	48 h-LC_{50} (μg/L)	MATC	AF
Malathion	10.44	0.36	0.03
Chlorpyrifos	0.08	0.0001	0.001
Carbofuran	6.96	2.41	0.35
Neem extract	196.3	281.89	0.09
Glyphosate	3043	172.04	0.88

Table 5. The maximum acceptable - toxicant concentration (MATC) and Application factor (AF) for each pesticide.

4. Conclusion

The aim of this study was using zooplankton, *Moina micrura* Kurz. which is an important species in aquatic ecosystem of Thailand to evaluated ecotoxicity of main pesticide used in paddy field (malathion, chlorpyrifos, carbofuran, neem extract (azadirachtin) and glyphosate). The acute toxicity (48-h LC_{50}) of malathion, chlorpyrifos, carbofuran, neem extract and glyphosate on *M. micrura* were 10.44, 0.08, 6.96, 196.3 and 3043 μg/L, respectively. Chlorpyrifos had highest toxicity followed by carbofuran, malathion, neem extract and glyphosate, respectively. Chronic toxicity test, the effect of pesticides to *M. micrura* on reproduction was studies by observing the number of offspring per female. Reproduction have significant reduced ($P<0.05$), with concentration of malathion at 0.50 μg/L, chlorpyrifos greater than 0.0025 μg/L, carbofuran at 2.50 μg/L and the concentration of glyphosate at 325 μg/L affected on reducing the number of offspring per female significantly ($P<0.05$). The neem extract had no significantly ($P>0.05$) effect on the number of offspring per female. The maximum acceptable - toxicant concentration (MATCs) of malathion, chlorpyrifos, carbofuran, neem extract and glyphosate were 0.36, 0.0001, 2.41, 172 and 281.9 μg/L, respectively. The result would be useful as an input to developing a biomonitoring tool for evaluation pesticide contamination in Thailand aquatic ecosystem.

Effect of experimental condition including duration test organism and end point on observed toxicity of pesticide to *M. micrura* were evaluated. Relative sensitivities of test varies with pesticide type. Among five pesticides toxicity test, chlorpyrifos had highest acute toxicity on *M. micrura* followed by carbofuran, malathion, neem extract and gyphosate, respectively. The significant reducing effect on number of offspring per female of *M. micrura* were observed in the present of malathion, chlorpyrifos carbofuran and glyphosate. For neem extract had no effect on the number of offspring per female. The results indicate that reproductivity parameters are very important interm of pesticide impact on aqutic population such as *M. micrura*. However, in the natural environment aquatic organism are often exposure to multiple pesticides simultaneously. Therefore under natural condition, there is the potential of pesticides may act in additive or synergistic manner, although the sensitivity of aquatic biota to multiple pesticides cannot be predicted by the individual pesticide sensitivities generate in this study.

The results showed *M. micrura* to be sensitive test organism, Thus its a good bioindicator and useful to developing a biomonitoring tool for evaluation pesticide contamination in Thailand aquatic ecosystem. However, in order to obtain more precise and conclusive toxicology data on application of these pesticide in paddy field and evaluation toxicity of pesticides on organism, similar study using another local freshwater in Thailand.

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6. References

Abdullah, A.R., Bajet, C.M, Matin, M.A., Nhan, D.D. & Sulaiman, A.H. (2007). Ecotoxicology of pesticides in the tropical paddy field system. *Environmental Toxicology and Chemistry*, Vol.26, No. 1, pp.59-70, ISSN 0730-7268

Ankly, G.T., Call, D.J., Cox, J.S., M.D., Kahl, M.D., Hoke, R.A. & Kosian, P.A. (1994) Organic carbon patitioning as a basis for predicting the toxicity of chlorpyrifos in sediment. *Environmental Toxicology and Chemistry*, Vol.13, No.4, pp.621- 626, ISSN 0730-7268

American Public Health Association. (1992). American Water Works Association and Federal Water Pollution Control Administration. *Standard method for the Examinationof Water and Wastewater*. American Public Health Association, ISBN 0-875-53217-9,Washington, D.C.

Alberdi J.L., Sáenz M.E., Di Marzio W. D. and Tortorelli M. C. (1996). Comparative Acute Toxicity of Two Herbicides, Paraquat and Glyphosate to *Daphnia magna* and *D. spinulata*. *Journal of Earth and Environmental Science*, (March 1996),Vol.57, No.2, pp. 229-235, ISSN 0007-4861

Al-Omar, M.A & Hassan, N.A. (2000). Simple and Rapid Method for the Detection of Early Signs of Toxicity in *Daphnia magna* Straus. *Bulletin of Environmental Toxicology and Chemistry*, Vol.65, No.5, pp.553- 559, ISSN 0007-4861

Bailey, H.C., DiGiorgio, C., Kroll, K., J., Miller, L., Hinton, D.E.& Starrett. G. (1996) . Development of Procedures for Identifying Pesticide Toxicity in Ambient Waters: Carbofuran, Diazinon, Chlorpyrifos. *Environmental Toxicology and Chemistry*, (June 1996), Vol.15, No.6, pp. 837-845, ISSN 0730-7268

Barata, C., Solayan A.& Porte,C. (2004). Role of B-Esterases in Assessing Toxicity of Organophosphorus (Chlorpyrifos, Malathion) and Carbamate (Carbofuran) Pesticides to *Daphnia magna*. Aquatic Toxicology, (July 2003),Vol.66, No.2, pp. 125-139, ISSN 0166-445x

Bailey, H.C., Miller, J.L., Miller, M.J., Wiborg, L.C., Deanovic, L. & Shed. T. (1997). Joint Acute Toxicity of Diazinon and Chlorpyrifos to Ceriodaphnia dubia. *Environmental Toxicology and Chemistry*, Vol.11, No.6, pp. 2304-2308, ISSN 0730-7268

Biesinger. K.E. & G.M. Chistensen. (1972). Effects of Various Metals on Survival, Growth, Reproduction, Metabolism of *D. magna*. *Journal of the Fisheries Research Board of Canada,* Vol. 29, pp. 1691-1700

Caceres, T., He, W. Naidu, R. & Megharaj, M. (2007). Toxicity of Chlorpyrifos and TCP Alone and in Combination to Daphnia carinata: The Influence of Microbial Degradation in Natural Water. *Water Resevation*, (June 2007), Vol.41, No.19, pp. 4497-4503, ISSN 0043-1354

Dobsikova, R. (2003). Acute toxicology of carbofuran to selected species of aquatic and terrestrial organisms. *Journal of Plant Protection Science*, (September 2003), Vol 39, No.3, pp103-108, ISSN 1212-2580

Domingues, I., Guilhermino, L., Soares, A.M.V.M.& Nogueira, A.J.A. (2007). Assessing dimethoate contamination in temperate and tropical climate: Potential use of biomarkers in bioassays with two chironomid species. Zhemospher, Vol.69, pp. 145-154, ISSN 0045-6535

DPR. (2002). Ground Water Database. In: *Environmental Monitoring Branch*. California Department of Pesticide Regulation

Edmondson, W.T. (1959). *Freshwater Biology*, In: John Wiley & Son, 2nd,(Ed),.pp. 1248, ISBN 978-0471232988, New York.

Extension Toxicology Network. (1996). Glyphosate. Pesticide Information Profiles. Extension Toxicology. In: Entox, 12.12.2010, Avaliable form http://www.ace.orst.edu/info/extoxnet/

Goktepe, I. & L.C. Plhak. (2002). Comparative Toxicity of Two Azadirachtin-Based Neem Pesticides to *Daphnia pulex*. *Environmental Contamination and Toxicology*, (June 200), Vol. 21, No.1, pp. 31-36, ISSN 0730-7268

________.(2003). Acute Toxicity Assessment of Azadirachtin-Based Pesticides Using Murine Hybridoma and Oyster Cells. *Journal of Environmental Science Health Part B,* (December), Vol. 38, No.2, pp.169-180, ISSN 1532-4109

Harmon, S.M., Specht, W.L. & Chandler, G.T. (2003). A Comparison of the Daphnids Ceriodaphnia dubia and Daphnia ambigua for Their Utilization in Routine Toxicity Testing in the Southeastern United States. *Achive of Environmental Contamination and Toxicology*, (January 2003), Vol. 45, No., pp. 79-85

Henry C.J., Higgins K.F. & Buhl K.J. (1994). Acute Toxicity and Hazard Assessment of Rodeo, X-77 Spreader, and Chem-Trol to Aquatic Invertebrates. *Environmental Contamination and Toxicology*, (April 1994), Vol. 27, No.3, pp. 392-399

Hooftman, R.N., K. Van de Guchte & Roghair, C.J. (1993). Development of Ecotoxicological Test Systems to Assess Contaminated Sediments. *In:Project.B6/8995*, pp.41, The Netherlands Integrated Program on Soil Research (PCB), Netherlands

Iwai, C. (2004). Ecotoxicology of heavy metals on Thailand aquatic ecosystem. (oral presentation), *The conference INTERACT 2004 - Science-a smart state to be in Analytical chemistry, Ecotoxicology, Environmental chemistry and Electrochemistry,* Conrad Jupiters, Gold Coast, Queensland, Australia, July 2-9, 2004

Iwai, C.B. & Noller, B. (2010). Ecotoxicological assessment of diffuse pollution using biomonitoring tool for sustainable land use in Thailand. *Journal of Environmental Sciences*, (January 2010), Vol.22, No.6, pp.1-6 ISSN 1001-0742

Iwai, C., Somparn, A & Noller, B. (2010). The use of local midge species Chironomus striatipennis to develop sediment toxicity test in Thailand. (oral presentation) *The first International Conference on Environmental Pollution, Restoration, and Management (SETAC Asia/Pacific Joint Conference)* Ho Chi Minh City, Viet Nam, March1-5, 2010

Iwai, C.B., Sujira, H., Somparn, A., Komarova, T., Mueller, J. & Noller, B.N. (2007) Monitoring pesticides in the paddy field ecosystem in Northeast Thailand for environmental and health risks. Chapter 12 in Rational Environmental Management of Agrochemicals: Risk Assessment, Monitoring and Remedial Action. First Edition Eds. I.R. Kennedy, K.R. Solomon, S.J. Gee, A.N. Crossan, S. Wang and F. Sanchez-Bayo ACS Symposium Series 966, American Chemical Society, Washington, DC. OXFORD UNIVESITY PRESS, USA, pp 259-273 ISBN 0841274207

Jeon, J., Ra, J.S., Lee, S.H., Lee, M.J., Yu, S.H & Kim, S.D. (2010). Role of food and clay particles in toxicity of copper and diazinon using *Daphnia magna*. *Ecotoxicology and Environmental Safety*, (March 2010), Vol.73, pp. 400–406 ISSN 1090-2414

John M., Brausch B.B. & Philip N.S. (2007). Acute and Sub-Lethal Toxicity of Three POEA Surfactant Formulations to *Daphnia magna*. *Bulletin Environmental Toxicology and Chemistry*, (May2007), Vol.18, pp. 510-514

Kwok, K.W.H., Leung, K.M.Y., Chu, V.K.H., Lam, P.K.S., Morritt, D., Maltby, L., Brock, T.C.M., Brink, P.J. van den, Warne, M.St.J.& Crane, M. (2007). Comparison of Tropical and Temperate Freshwater Animal Species' Acute Sensitivities to Chemicals: Implications for Deriving Safe Extrapolation Factors. *Integrated Environmental Assessment and Management*, (April,2006), Vol.3, No.1, pp. 49-67, ISSN 1551-3777

Khan, A.A., Haque, N. & Khan, R.R. (1993). Effect of Malathion on Daphnia magna Under Laboratory Conditions. *Indian Journal Environmental Health*, Vol.35, No.4, pp.333-337 ISSN 0367-827X

Kim, Y.H., Cowan, C.E., Sanchez-Osuna M. &Kimerle, R. (2001). Environmental risk assessment and management of chemicals in Asia/Pacific countries. In: *Proceedings of the UNIDO/SETAC Regional Symposium and Workshop*,held at Seoul, Republic of Korea, April 20–24, 1998; United Nations Industrial Development Organization; Seoul (KR): Hanrimwon

Maul, J.D., Farris, J.L. & Lydy, M.J. (2006). Interaction of Chemical Cues from Fish Tissues and Organophosphorous Pesticides on *Ceriodaphnia dubia* Survival. *Environmental Pollution*, (August 2006), Vol.141, No.1, pp.90-97, ISSN 0269-7491

Norberg-King, T.J., Durhan, E.J.. Ankley, G.T & Robert, E. (1991). Application of Toxicity Identification Evaluation Procedures to the Ambient Waters of the Colusa Basin Drain, California, *Environmental Contamination and Toxicology*, Vol. 10, pp. 891-900 , ISSN 0730-7268

Nelson, S.M & Roline, R.A. (1997). *Comparison of Rapid Toxicity Tests with a Standard Acute Test. Report BR-EE010*, Technical Service Center, Bureau of Reclamation, Denver, CO, pp.12, NTIS PB97-158919

________. (1998). Evaluation of the Sensitivity of Rapid Toxicity Tests Relative to Daphnid Acute Lethality Tests. Bulletin of *Environmental Contamination and Toxicology*, (Norvember 1997), Vol. 60, pp. 292-299, ISSN 0007-4861

National Research Council. (1992). Neem: A tree for solving lobal problems, National Academy Press, ISBN 0-309-04686-6, Washington, DC.

Office of Pesticide Programs.(2000). *Ecotoxicity Database (Formerly: Environmental Effects Database (EEDB): Environmental Fate and Effects Division*. US. Environmental Protection Agency. Washington, DC.

Poirier, S. (1990). *Memorandum*: Memo to R Spehar, Aug.29, US Environmental Protection Agency, National Water QualityLaboratory, Duluth. MN

PMRA. (2000). *Neemix 45 Regulatory Note REG 2000-1*. Pest Management Regulation Agency Health Canada Ottawa, Ontario.

Rassoulzadegan, M & Akyurtakli N. (2002) An Investigation on the Toxic Effects of Malathion (Organophosphate Insecticide) on the *Daphnia magna* Straus, 1820 (Crustacea, Cladocera). *Turky zoology*, (April,2001), Vol.26, pp. 349-355

Ren, Z., J. Zha, M. Ma, Z. Wang & Gerhardt, A. (2007). The Early Warning of Aquatic Organophosphorus Pesticide Contamination by On-Line Monitoring Behavioral Changes of *Daphnia magna*. *Environmental Monitoring and Assessment*, (August 2006), Vol.134, No.1-3, pp.373-383, ISSN 1573-2959

Sanchez P., Kubitza K., Dohment G.D & Tarzona, J.V. (2006). Aquatic risk assessment of new rice herbicide proxydrin. *Environmental Pollutant*, (August 2005), Vol.142,pp.181-189, ISSN 0269-7491

Saler, S. & Saglam, N. (2005). Acute toxicity of Malathaion on *Daphnia magna* Straus, 1820. *Journal of Biological Sciences,* Vol.5, No3, pp 297-299, ISSN 1727-3048

Siefert, R.E. (1987). Effects of Dursban (Chlorpyrifos) on Aquatic Organisms in Enclosures in a Natural Pond. *In:* Review *of environmental Contamination and Toxicology Vol. 16*, Spinger verlag, ISBN 0-387-98627-8, New York

Stark, J.D. (2001). Population-Level Effects of the Neem Insecticide, Neemix, on *Daphnia pulex*. *Environmental Science Health Part B*, (June 2001), Vol.36, No.4, pp.457-465 , ISSN 0360-1234

Somparn, A., Iwai, C.B. and Noller, B. (2010) Development of the Indigenous Chironomid Species as Ecotoxicology Test: Tool for Water Quality Management in Thailand. *International Journal of Environmental and Rural Development*, (March 2010), Vol.1, No.1, pp. 31-36, ISSN 2185-159X

Tsui, M.T.K. & Chu,L.M. (2004). Comparative Toxicity of Glyphosate-Based Herbicides: Aqueous and Sediment Porewater Exposures. *Archvieof Environmental Contamination and Toxicology*, (August 2003), Vol. 46, No.3, pp. 316-323, ISSN 0090-4343

Tirado, R. Englande A. J., Promakasikorn, L & Novotny, V. (2008). Use of agrochemicals in Thailand and itsconsequences for the environment, In: Technical Note 03/2008.Greenpeace Research Laboratories.

Tejada, A.W. (1995) Pesticides residues in foods and the environment as consequence of crop production. *Philippine Agriculture*, Vol.78,pp. 63-79

Wong, C. K., Chu K. H. & Shum F. F. (1995). Acute and Chronic Toxicity of Malathion to the Freshwater Cladoceran *Moina macrocopa*. *Water Air Soil Pollutio*n, (November 1994), Vol.84, No.3/4, pp. 399-405, ISSN 0049-6979

Wongrat, L. (2001). *Zooplankton*. In*: Faculty of Fisheries of Kasetsart University,* (Ed.),pp. 787, *Thailand*, ISBN 9-745538412-4

US. Environmental Protection Agency. (1993). Environmental fate and effects division. Pesticide environmental fate one line summary: Carbofuran. Washington, DC.

________. (1996). Ecological Effects Test Guidelines. In: *OPPTS 850.1010 Aquatic Invertebrate Acute Toxicology Test Freshwater Daphnia*. Department of Pesticide Regulation US Environmental

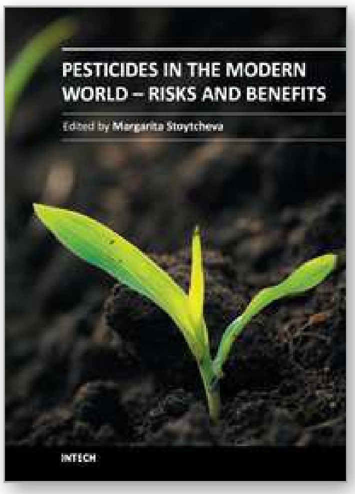
________. (1998). *Registration Standard (Second Round Review) for the Reregistration of Pesticide Products Containing Chlorpyrifos*. Office of Pesticide Programs, US EPA, Washington, DC

US. Environmental Protection Agency. (2002). Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. In :*EPA-821-R-02-013*, 4th edn, pp. 230,Cincinnati, OH

________. (2006). Interim Reregistration Eligibility Decision (IRED) Document for Carbofuran. In: *EPA-738-R-06-031*. Department of Pesticide Regulation US Environmental

Van der Hoeven, N& Gerritsen, A.A.M. (1997). Effects of Chlorpyrifos on Individuals and Populations of Daphnia pulex in the Laboratory and Field. *Environmental Contamination and Toxicology*, (December 1997), Vol. 16, No.12, pp. 2438-2447, ISSN 0730-7268

Van Wijngaarden, R., P. Leeuwangh, W.G.H. Lucassen, K. Romijn, R. Ronday &. Van der Veld, R. (1993). Acute Toxicity of Chlorpyrifos to Fish, a Newt, and Aquatic Invertebrates. *Bulletin of Environmental Contamination and Toxicology*,Vol. 51, No.5, pp. 716-723, ISSN 0007-4861



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This book is a compilation of 29 chapters focused on: pesticides and food production, environmental effects of pesticides, and pesticides mobility, transport and fate. The first book section addresses the benefits of the pest control for crop protection and food supply increasing, and the associated risks of food contamination. The second book section is dedicated to the effects of pesticides on the non-target organisms and the environment such as: effects involving pollinators, effects on nutrient cycling in ecosystems, effects on soil erosion, structure and fertility, effects on water quality, and pesticides resistance development. The third book section furnishes numerous data contributing to the better understanding of the pesticides mobility, transport and fate. The addressed in this book issues should attract the public concern to support rational decisions to pesticides use.