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# Assessment of pesticide contaminated sediment using biological response of tropical chironomid, *Chironomus javanus* Kiffer as biomarker

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ABSTRACT

**Objective:** To investigate the use of a biomarker for assessment of the effects on the tropical chironomid, *Chironomus javanus (C. javanus)*, Kiffer of sediment contaminated with an insecticide (chlorpyrifos).

**Methods:** A wide range of biological responses to the tropical chironomid exposed were measured, including survival, growth rate and Acetylcholinesterase (AChE) activity.

**Results:** The measured median lethal concentration (96 h $LC_{50}$) of chlorpyrifos to *C. javanus* was 0.056 (95% *CI* 0.024–0.124) μg/kg. For sub-chronic levels of chlorpyrifos between 0.001 and 0.25 μg/kg administered for 10 days, the effects on the growth of *C. javanus* were reduced (larva size, head structure width and dry weight) at the significance level ($P < 0.01$) and the effects were concentration dependent. Following exposure to chlorpyrifos at the level of 0.001 μg/kg for 48 and 96 h, the AChE activity in *C. javanus* was inhibited compared with control samples ($P < 0.05$).

**Conclusions:** This study demonstrated that *C. javanus* was sensitive when exposed to chlorpyrifos. This species could serve as a potential biomarker for assessing pesticide contamination at low environmental persistence and provides limited effects data on the sensitivity of tropical biota to contaminants for ecological risk assessment of organophosphate pesticides in the tropical aquatic ecosystem.

## 1. Introduction

Contamination of the aquatic ecosystem is recognized as a significant issue when the sediment becomes a sink for pollutants such as pesticides [1]. Many pollutants can bind physically and chemically with sediments and persist for long periods of time to become bio-available depending under certain hydrological conditions and exert adverse effects on aquatic organisms [2]. Therefore, sediments may act both as a sink and as a source of pollution and sources of pollutants to the overlying water column and biota [3]. Consequently, sediment quality is crucial to the health of an aquatic ecosystem [4]. Herbicide and pesticide contamination of surface water is demonstrated worldwide as a major issue locally, regionally, nationally and globally [5,6].

In tropical countries having agro-economies, pesticides are used intensively. Growing concern has arisen from the effects of organophosphate (OP) and carbamate pesticides on aquatic organisms in fresh waters and sediments as a consequence of increased and continuous use [7–9]. This is particularly the case in Thailand being a principal global agricultural producer. Thailand is a rural country with a significant agricultural component of the economy. Thailand has increased agricultural exports by importing fertilizers and pesticides for intensive agricultural production. Pesticide use increases agricultural yield by protecting plant crops from pests, weeds and parasites [10]. Since importing pesticides began under the 'Green Revolution Policy' as part of Thailand's 1st National

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Economic and Social Development Plan in 1966, the total quantity has increased each year. More recently, pesticides use in Thailand has increased 3 times during 1995–2014, exceeding 100 thousand tons in 2015 [11]. Vegetable and fruit farming require the highest application of pesticides, in response to market demand for visually-perfect products. In 2015, organophosphates were the imported pesticides with the largest quantity followed by carbamates and organochlorines (OC); mostly herbicides were followed by insect control, disease control agents and plant growth regulators [11].

The widely used OP, chlorpyrifos whose primary application is for protection against pest and disease control in agriculture (paddy field, corn and cotton) in Thailand was the first imported high-volume insecticide [10]. Chlorpyrifos affects enzyme activity by inhibiting Acetylcholinesterase (AChE) activity and thereby affects neuromuscular functions of target species [12]. Acetylcholinesterase inhibitors cause toxic effects on organisms by chemical disruption of the normal nervous system function due to excessive accumulation of acetylcholine in the synapse area, leading to rapid muscular twitching and paralysis in the affected animals [13]. In particular, aquatic invertebrates in rivers and lakes can be exposed to concentrations of chlorpyrifos ranging from sub-lethal with several changes in behavioral and physiological patterns of aquatic organism to mortality when application rates are high (Pérez *et al.*, 2013). Besides, accidental exposure pesticide of edible aquatic species enters the food chain and can cause ecotoxicological effects, if not degraded [14,15].

Because of potential pollution problems, ecological risk assessment and environmental impact monitoring have become important tools for pollution and contamination management to assess effects in aquatic ecosystems by establishing effective risk-reducing measures [16,17]. A biomarker response is an efficient method for assessing the effect of a contaminant in an ecosystem using biological responses of an organism. A biomarker can be used to assess the effects of small quantities of pollutants on organisms subjected to long-term exposure [18]. Biomarker responses are measured by observing physical, biological and/or biochemical changes such as in growth, reproduction, and enzyme activity in organisms [19,20]. Biomarkers enable discrete effects of exposure from pollutants to be detected, and identify the incidence of exposure to, and the effects caused by pollutants thus providing an indication of potential effects to higher trophic levels of species [20,21]. However, limited studies using species sensitivity distributions have shown that tropical aquatic species do not differ in sensitivity to contaminants for similar temperate species and that it may be difficult to predict sensitivity between different climatic regions [22]. However, tropical aquatic ecosystems differ ecologically from habitats of temperate localities in both physicochemical and biological structures. The diversity of the subtropics and tropics is usually higher than that found in temperate zones and thus the number of species potentially affected by exposure to particular pollutants can be greater [23].

In this study, the tropical chironomid species, (*Chironomus javanus*, Kiffer) (*C. javanus*) were selected as the test bio-indicator organism, because they are commonly found benthic organism and adapted to both temperate and tropical aquatic ecosystems and well suited to test the toxicity of sediments, both in situ and in the laboratory [24]. Their discrete life stages are easily identified a short life cycle under laboratory conditions and food source for juvenile and adult fish and aquatic birds [25]. Chironomids are demonstrated as indicators of acute and chronic toxicity in sediments and water contaminated with a variety of pollutants [26,27]. The aim of this study was to evaluate the acute and chronic effects of chlorpyrifos toxicity on *C. javanus*, by using biological responses of *C. javanus* (survival, growth rate and AChE activity as a biomarker).

## 2. Materials and methods

### 2.1. Test organism

The test species employed was the tropical chironomid *C. javanus,* Kiffer which was isolated from an upstream part of the Nam Phong River catchment, above the Ubolratana dam (16°46′13″N 102°37′16″E) in North East Thailand. It was cultured under controlled laboratory conditions following the OECD 218 method [28]. The chironomid was held in the Ecotoxicology and Environmental Sciences Laboratory at Khon Kaen University, 50 km south of Ubolratana dam. The chironomid was held at a temperature of (25 ± 2) °C under a light sequence of 16 h: 8 h light and dark photoperiod in plastic aquaria of size 30 × 60 × 35 $cm^3$. A pure culture of *C. javanus* was obtained from egg masses and placed in 500 mL beakers containing 250 mL of laboratory water with shredded tissue paper prepared following Batac-Catalan and White [29]. The top of each beaker was covered with a net to trap emerging chironomids. Continuous gentle aeration to maintain optimum oxygen level was provided by an aquarium pump and the test water was replaced weekly. Following hatching, the cultured larvae grew to the second larval stage (instar 2) before being used in survival and growth experiments, and the fourth larval stage (instar 4) before being used in enzyme activity experiment.

### 2.2. Test chemical

The OP chlorpyrifos (40% W/V) preparation ('the chemical') was purchased from the local market of Khon Kaen province, Thailand, under the trade name Lorsban, supplied by Sotus International Chemical Supplies Limited, Bang Kong, Thailand. Gas chromatography (GC) analysis was used to quantify 40% W/V chlorpyrifos stock solution. Calibration standard were prepared with 99.5% purity chlorpyrifos (Chem Service, Inc., city, USA.) Stock solutions were prepared by dissolving 'the chemical' in a salt solution ($NaHCO_3$: 48 mg/L; $CaCl_2 \bullet 2H_2O$: 30 mg/L; $MgSO_4 \bullet H_2O$: 30 mg/L; KCl: 2 mg/L) in 50 mL deionized water according to OECD 218 [27] and immediately before use. Further diluting of stock solutions was made in deionized water. Gas chromatography (GC) analysis was used to check the chlorpyrifos concentrations in each stock solution.

### 2.3. Experiment design

The study investigated the use of biological responses for evaluation of effects of chlorpyrifos on *C. javanus*: (i) the acute toxicity (96 h-$LC_{50}$) for survival; (ii) the sub-chronic effect on growth after 10 days exposure; and (iii) the effect on AChE activity after 48 and 96 h exposure.

#### 2.3.1. Acute toxicity test

The test sediment was OECD artificial sediment [28]. The sediment comprised 75% quartz sand, 20% kaolin clay, 5% sphagnum peat and calcium carbonate to adjust the pH to 6.5–

7.5. The sediment was prepared by adding different concentrations of chlorpyrifos (dry weight basis) and de-ionized water to give the final moisture in the range of 30–50% and chlorpyrifos at 0, 0.001, 0.01, 0.1, 1 and 10 μg/kg dry sediment. The desired amount of chlorpyrifos was thoroughly mixed into sediment as an aqueous solution to give the working concentration of chlorpyrifos, and sediment was placed into glass jars. All concentrations in this study were the stated nominal concentrations based on the measured 'chemical' concentration. Acute toxicity tests were performed to measure the chlorpyrifos $LC_{50}$ data of second instar of chironomid larvae in test sediments. The static bioassay procedure was used in all experiments and performed according to OECD 218 [27]. Five test concentrations of chlorpyrifos in sediment and a control with six replications of each concentration were used. The day before the addition of second instar larvae, 500 mL glass beakers were filled with 100 g sediment and 400 mL reconstituted commercial mineral water. Ten chironomid larvae were placed into each container test for 96 h. Following completion of the test, the sediments were passed through a 425 μm stainless steel sieve and mortality of the larvae was observed. The test water quality (temperature, pH, conductivity, dissolved oxygen (DO) concentration and hardness) were measured according to the APHA 2013 test procedures [30].

#### 2.3.2. Sub-chronic effect of chlorpyrifos on growth rate of chironomids

This evaluation was performed to determine the temperature influence on chlorpyrifos toxicity and the growth of chironomid test organisms (head capsule width, body length, and dry weight). Second instar *C. javanus* were exposed for 10 days to non-lethal concentrations of chlorpyrifos (0, 0.001, 0.01, 0.05 and 0.25 μg/kg dry sediment) based on the method used for the acute test ($LC_{10}$) described above. Ten *C. javanus* were added to each of six replication beakers for different exposures treatments. Chironomids were removed from the containers after 10 days of exposure and then were passed through a 425 μm stainless steel sieve and rinsed with distilled water. For measuring dry weight, chironomids were transferred to aluminum weighing boats, dried for 24 h at 60 °C in an oven, and weighed using an analytical balance. Chironomid samples were preserved in 70% ethanol before measurement of width of head capsule as the distance between most distant lateral sides of head capsule margins. Body length was measured as distance between posterior points of head capsule and posterior prologs with the use of an OLYMPUS SZX7 binocular microscope (Olympus Corp., Tokyo, Japan) with a calibrated eye-piece micrometer.

#### 2.3.3. Effect of chlorpyrifos on Acetylcholinesterase (AChE) activity of in chironomid

The assays of enzyme, was performed to determine the AChE activity in chironomid after exposure to chlorpyrifos for 48 and 96 h. Sediment was spiked with chlorpyrifos to give test concentrations of 0.001, 0.01, 0.10 and 0.25 μg/kg dry sediment and four replications of fourth instar larvae per treatment and control were used. Surviving test chironomids from the tests were snap-frozen individually in micro centrifuge tubes and then stored frozen at −20 °C for a maximum of one week. Whole snap-frozen chironomid were homogenized in ice-cooled buffer 325 μL (0.02 M phosphate buffer pH 8.0, containing 1% Triton X-100) using a tissue homogenizer (Scilogex D160, Berlin CT06037, USA). The homogenate was centrifuged at 14 000×*g* at 4 °C for 15 min. The supernatant of each sample was stored at −20 °C for no more than 3 days until enzymatic activity assays were completed.

The AChE activity assay was based on the method described by Ellaman [30] and was adapted to a microplate reader. In brief, the homogenate samples were mixed with 8 mM 5,5-di-thio-nitrobenzene acid (DTNB) in phosphate buffer containing 0.75 mg/L$NaHCO_3$ and 50 μL of 16 mM acetylthiocholine iodide. After incubation at 30 °C for 5 min, AChE level was determined for rAChE activity at 412 nm. Results were expressed as nmol/mg protein. Total protein content in the homogenate was measured using the Bradford method [31] at 595 nm, using bovine serum albumin as a standard protein.

### 2.4. Statistical analysis

The concentration at the 96 h-$LC_{50}$ at 95% confidence level were calculated using Probit Analysis by the statistical package SPSS [32]. The data obtained from various experiments were analyzed using one-way analysis of variance (ANOVA) to test for variations between treatment group and control and a significance level of ($P < 0.05$) with Statistica 8 software 9 (Version 8, USA).

## 3. Results

### 3.1. Acute toxicity test

Survival of *C. javanus* in controlled samples exceeded 90% after the 96 h test period (Table 1). The significant chlorpyrifos toxicity effect on mean survival of *C. javanus* was found at the chlorpyrifos concentration above 0.001 μg/kg compared with control ($P < 0.05$) (Table 1). The 96 h-$LC_{50}$ value of chlorpyrifos on *C. javanus* in this study was 0.056 μg/kg with a 95% confidence interval of 0.024–0.124 μg/kg.

### 3.2. Sub-chronic effect of chlorpyrifos on growth rate of chironomids

For sub-chronic effect of chlorpyrifos on *C. javanus*, the mean ± SD of head capsule width, body length and dry weight were (0.28 ± 0.02) mm, (8.27 ± 0.22) and (0.44 ± 0.01) mg, respectively (Table 2). After 10 days exposure, *C. javanus* growth characteristics of larvae in test species had declined with

**Table 1**
Acute toxicity of chlorpyrifos contaminated sediment on survival (%) of *C. javanus* 96 h.

| Chlorpyrifos (μg/kg) | Survival |
|---|---|
| 0 | $90.00 \pm 0.87^{a}$ |
| 0.001 | $75.56 \pm 3.53^{b}$* |
| 0.01 | $54.44 \pm 1.13^{c}$ |
| 0.1 | $48.89 \pm 0.93^{c}$ |
| 1 | $36.67 \pm 0.71^{d}$ |
| 10 | $18.89 \pm 1.36^{e}$ |
| CV (%) | 10.83 |

Note: Values are expressed as mean ± SD. *Indicate lowest observed effect concentration; LOEC) superscript indicate significant difference (LSD, $P < 0.05$) from control at 96 h.

**Table 2**
Subchronic effects of chlorpyrifos contaminated sediment on head capsule width, body length and dry weight of *C. javanus* at 10 days.

| Chlorpyrifos (μg/kg) | Head capsule width (mm) | Body length (mm) | Dry weight (mg) |
|---|---|---|---|
| 0 | 0.28 ± 0.02$^{ab}$ | 8.27 ± 0.22$^{a}$ | 0.44 ± 0.01$^{ab}$ |
| 0.001 | 0.27 ± 0.01$^{b}$ | 8.00 ± 0.32$^{a}$ | 0.39 ± 0.02$^{c*}$ |
| 0.01 | 0.27 ± 0.06$^{b}$ | 6.30 ± 0.44$^{b*}$ | 0.38 ± 0.30$^{c}$ |
| 0.05 | 0.23 ± 0.02$^{c*}$ | 6.18 ± 0.38$^{b}$ | 0.38 ± 0.51$^{cd}$ |
| 0.25 | 0.20 ± 0.01$^{cd}$ | 5.89 ± 1.89$^{c}$ | 0.20 ± 0.80$^{d}$ |

Note: Results are expressed as mean ± SD; *$P$ < 0.01 compared with control; different letters in superscript indicate significant difference from control as each experiment at 10 days.

**Table 3**
Effect of chlorpyrifos contaminated sediment on AChE activity in C. javanusat 48 and 96 h.

| Chlorpyrifos (μg/kg) | AchE activity (nmol/min/mg protein) | |
|---|---|---|
| | 48 h | 96 h |
| 0 | 33.68 ± 1.01$^{a}$ (0) | 39.70 ± 1.55$^{a}$ (0) |
| 0.001 | 12.05 ± 0.96$^{b}$* (64.22) | 5.60 ± 0.92$^{b}$* (85.89) |
| 0.01 | 9.81 ± 1.16$^{b}$* (70.87) | 5.60 ± 1.22$^{b}$* (85.89) |
| 0.05 | 9.50 ± 1.82$^{b}$* (71.79) | 5.13 ± 0.97$^{b}$* (87.08) |
| 0.25 | 7.90 ± 1.44$^{b}$* (76.54) | 3.54 ± 1.26$^{b}$* (91.08) |

Note: Values are expressed as mean ± SD; *$P$ < 0.05 compared with control; different letters in superscript indicate significant difference among different value as each exposure. Value in parentheses indicates value of % AChE inhibition relative to control. Under tropical condition with the temperature of (25 ± 2) °C.

increasing concentration of chlorpyrifos. Growth was significantly affected at ≥0.05 μg/kg for all test species when compared with the control ($P$ < 0.01).

### 3.3. Effect of chlorpyrifos on Acetylcholinesterase (AChE) activity of in chironomid

The results of the effects of chlorpyrifos on AChE activity in *C. javanus* are shown in Table 3. *C. javanus* after exposure to chlorpyrifos at concentrations above 0.001 μg/kg showed significantly increased percentage of inhibition of AChE activity compared to the controls ($P$ < 0.05), at the respective exposure time of 48 or 96 h. After exposure to chlorpyrifos for 48 h at the highest chlorpyrifos concentration (0.25 μg/kg), the AChE activity in *C. javanus* was inhibited up to 76.5% and when exposed for 96 h inhibition reached 91.08% (Table 3).

## 4. Discussion

In this study, the 96 h-$LC_{50}$ value of chlorpyrifos on *C. javanus* was 0.056 μg/kg. This indicated that the $LC_{50}$ value of chlorpyrifos had a high toxicity on chironomid larvae (0.1 < $LC_{50}$ mg/L) based on 96 h-$LC_{50}$ of an aquatic organism [33]. Chlorpyrifos is an OP that binds with acethycholinesterase, and breaks down the neurotransmitted acetylcholine so that subsequent impulses can be transmitted across the synapse. The inhibiting acethycholinesterase results in repeated, uncontrolled firing of neurons leading to mortality usually by asphyxiation as respiratory control is lost [33,34]. Chlorpyrifos is relatively more hydrophobic than most other insecticides, with a log $K_{ow}$ (log octanol–water partition coefficient) that is 5.0 [14]. The $K_{ow}$ value of chlorpyrifos indicates its preferential partitioning into the organic matter rather than in water resulting in its strong binding with sediment; and therefore it has higher toxicity to the sediment dwelling chironomid. Due to this, the sediment provides a habitat and food source for larval stages of chironomid, which exposed to sediment-associated chemicals directly [35]. Ingestion of sediment particles has been proposed as the major route for accumulation of elevated pesticide contamination in detritus-feeding animals [36,37]. Thus toxicity, bioaccumulation levels, and trophic-transfer of pesticide contaminants in aquatic environment and into food chain can cause ecotoxicity problems and subsequently effect on human health if transferred through the food chain [38]. The outcome of this study indicated that the 96 h-$LC_{50}$ of chlorpyrifos for *C. javanus* was more sensitive than found for other standard chironomids [*Chironomus riparius (C. riparius)* and *Chironomus tentans (C. tentans)*], which was 0.09–0.47 μg/kg [39,40].

The sub-chronic toxicity of chlorpyrifos on *C. javanus* growth was observed at a concentration of ≥0.05 μg/kg. This is in agreement with the results obtain by Hasenbein *et al.* [36] who reported that chlorpyrifos had effects of reducing the growth of *Chironomus dilutus.* Also, the findings of Faria *et al.* [41] reported that chlorpyrifos caused a reduction of development time, reproduction, and molting of an aquatic organism. Moreover, the present study shows low concentration of chlorpyrifos (≥0.05 μg/kg) in sediment, affects the growth of *C. javanus.* Therefore, assessing the environmental impacts growth parameters are expected to be significant indicators of effects in an ecological system as growth change influences the biomass, reproduction, food quantity and diversity or companion of aquatic organisms according to Du *et al.* [42] and Hasenbein *et al.* [36] who also reported that effects of sediment toxicity on invertebrate organisms include reduction emergence, case-abandonment and reduced growth are the most important factors affecting reproductive output of invertebrate organisms. Effect-based endpoints, designed to assess sublethal impairments are often more sensitive and better predictors of deleterious effects associated with contaminated sediment [43,44]. This study supported the idea of using the biological response of tropical chironomid, *C. javanus* as biomarker for assessing pesticide contaminated sediment, which could predict possible environment impacts for the organophosphate pollutant chlorpyrifos.

The results for the enzyme activity study indicated that chlorpyrifos concentration above 0.001 μg/kg caused inhibition of AChE activity in *C. javanus* with dose in a time-dependence manner. The mechanism of the inhibition effects has been proposed as chlorpyrifos-inhibited AChE activity by binding to active site serine, resulting in irreversible inhibition of enzymes and thereby increasing both the level and duration of action of the neurotransmitter acetylcholine. Accumulation of acetylcholine will result in prolonged muscle contraction and prolonged electrical activity at nerve endings causing uncontrolled movement [45,46]. These results are consistent with Pérez *et al.* [34] and Kheir *et al.* [47] who reported that chlorpyrifos has a direct inhibition effect of AChE activity in *C. riparius*. Moreover, previous studies reported that chlorpyrifos has an ability to inhibit AChE in *C. tentans* and *C. riparius* in a dose and time-dependent manner [48,49]. These studies also indicate that *C. javanus* was sensitive to low concentrations of chlorpyrifos (0.001 μg/kg) and inhibited AChE activity to over 50% from

24 h exposure. Thus, AChE activity in *C. javanus* can be used as a biomarker for assessing the contamination of sediments with chlorpyrifos representing the OP group of pesticides that have low persistence in the environment [50].

Chlorpyrifos had a very toxic effect on the chironomid *C. javanus* after short- and long-term exposure (survival, growth and AChE activity). We have shown the following:

That *C. javanus* was a more sensitive organism to chlorpyrifos than temperate chironomid test species used for standard testing (*C. riparius and C. tentans*); Biological response of *C. javanus*, especially inhibitor of AChE activity is sensitive to chlorpyrifos (0.001 μg/kg) after a short term (48 h); The integration of sublethal endpoints in sediment quality monitoring and pesticide regulation efforts could improve identification of low-level pesticide concentrations that may eventually cause negative effects on food webs and community structure in aquatic environments; *C. javanus* can be used as a test organism for assessing chlorpyrifos contamination and possibly other pesticides from the OP group that have low persistence in the environment; and the measurement of the biological responses of *C. javanus* could be a promising biomarker for sediment contaminated with short persistence pesticides and to provide exposure data for developing environmental risk-assessment tools for pesticide contamination in tropical freshwater aquatic ecosystems.

The finding of this work provides useful data for the ecological risk assessment of chlorpyrifos application in the tropical aquatic ecosystem.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Guilherme S, Santos MA, Gaivão I, Pacheco M. Are DNA-damaging effects induced by herbicide formulations (Roundup® and Garlon®) in fish transient and reversible up on cessation of exposure. *Aquat Toxicol* 2014; **155**: 213-21.

[2] Zoppini A, Ademollo N, Amalfitano S, Casella P, Patrolecco L, Polesello S. Organic priority substances and microbial processes in river sediments subject to contrasting hydrological conditions. *Sci Total Environ* 2014; **484**: 74-83.

[3] Dimuthu WM, Wijeyaratne N. Application of pollution indices to quantify the pollution status of shallow sediments of the Bolgoda Lake, Sri Lanka. *Nat Sci Found Sri Lanka* 2016; **44**(3): 279-89.

[4] Cerejeira MJ, Viana P, Batista S, Pereira T, Silva E, Valério MJ, et al. Pesticides in Portuguese surface and ground waters. *Water Res* 2003; **37**: 1055-63.

[5] Konstantinou IK, Hela DG, Albanis TA. The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environ Pollut* 2006; **141**: 555-70.

[6] Castillo LE, De La Cruz E, Ruepert C. Ecotoxicology and pesticides in tropical aquatic ecosystems of Central America. *Environ Toxicol Chem* 1997; **16**: 41-51.

[7] Tam NT, Berg H, Tuyen PT, Van Cong N. Effect of chlorpyrifos ethyl on acetylcholinesterase activity in climbing perch (*Anabas testudineus*, Bloch, 1972). *Arch Environ Contam Toxicol* 2015; **69**(4): 515-24.

[8] López-Doval JC, Montagner CC, Alburquerque AF, Moschini-Carlos V, Umbuzeiro G, Pompêo M. Nutrients, emerging pollutants and pesticides in a tropical urban reservoir: spatial distributions and risk assessment. *Sci Total Environ* 2017; **575**: 1307-24.

[9] Thailand Pesticide Alert Network (Thai-PAN); Pesticides used in Thailand. Available from: http://www.thaipan.org/node/826 [Access on 12th March, 2015]

[10] Department of Agricultural. Office of agricultural economics. Thailand; Importing Pesticide in Thailand. Available from: http://www.oae.go.th/ewt_news.php [Access on 25th January, 2015]

[11] Coelho S, Oliveira R, Pereira S, Musso C, Domingues I, Bhujel RC, et al. Assessing lethal and sub-lethal effects of trichlorfon on different trophic levels. *Aquat Toxicol* 2011; **103**(3–4): 191-8.

[12] Giesy JP, Solomon KR, Christopher CG, Giddings JM, Mackay D, Dwayne RJ, et al. Ecological risk assessment for chlorpyrifos in terrestrial and aquatic systems in the United States. *Rev Environ Contam Toxicol* 2014; **231**: 1-11.

[13] Moore DRJ, Teed RS, Greer C, Solomon KR, Giesy JP. Refined avian risk assessment for chlorpyrifos in the United States. *Rev Environ Contam Toxicol* 2014; **231**: 163-217.

[14] Mackay D, Giesy JP, Solomon KR. Fate in the environment and long-range atmospheric transport of the organophosphorus insecticide, chlorpyrifos and its oxon. *Rev Environ Contam Toxicol* 2014; **231**: 35-76.

[15] Connon RE, Geist J, Werner I. Effect-based tools for monitoring and predicting the ecotoxicological effects of chemicals in the aquatic environment. *Sensors* 2012; **12**(9): 12741-71.

[16] Kim HJ, Koedrith P, Seo YR. Ecotoxicogenomic approaches for understanding molecular mechanisms of environmental chemical toxicity using aquatic invertebrate, Daphnia model organism. *Int J Mol Sci* 2015; **16**(6): 12261-87.

[17] Chaffai AH. Usefulness of bioindicators and biomarkers in pollution biomonitoring. *J Biotechnol Wellness* 2014; **3**: 19-26.

[18] Sanchez W, Burgeot T, Porcher JM. A novel 'Integrated Biomarker Response' calculation based on reference deviation concept. *Environ Sci Pollut Res Int* 2013; **20**(5): 2721-5.

[19] Bae MJ, Park YS. Biological early warning system based on the responses of aquatic organisms to disturbances: a review. *Sci Total Environ* 2014; **466–467**: 635-49.

[20] Kaviraj A, Unlu E, Gupta A, El Nemr A. Biomarkers of environmental pollutants. *Biomed Res Int* 2014; **2014**: 1-2.

[21] Gissi F, Stauber JL, Bine TM, Golding LA, Adams MS, Schlekat CE, et al. A review of nickel toxicity to marine and estuarine tropical biota with particular reference to the South East Asian and Melanesian region. *Environ Pollut* 2016; **218**: 1308-23.

[22] Somparn A, Iwai CB, Noller B. Development of the indigenous chironomid species ecotoxicology test: tool for water quality management in Thailand. *IJERD* 2010; **1**(1): 32-6.

[23] Rebechi D, Richardi Vi S, Vicentini M, Guiloski IC, Silva de HCA, Navarro-Silva1 MA. Low malathion concentrations influence metabolism in *Chironomus sancticaroli* (Diptera, Chironomidae) in acute and chronic toxicity tests. *Rev Bras Entomol* 2014; **58**(3): 296-301.

[24] Adakole JA, Dauda MZ, Muhammad AA. Toxicity assessment of bindare stream sediment on chironomid nutrient composition and growth using sediment contact bioassay: Zaria, Nigeria. *J Water Resour Hydraul Eng* 2015; **2**(4): 198-204.

[25] Ebau W, Rawi CSM, Din Z, Al-Shami SA. Toxicity of cadmium and lead on tropical midge larvae, *Chironomus kiiensis* Tokunaga and *Chironomus javanus* Kieffer (Diptera: Chironomidae). *Asian Pac J Trop Biomed* 2012; **2**: 631-4.

[26] Choung CB, Hyne R, Stevens MM, Hose GC. The ecological effects of a herbicide-insecticide mixture on an experimental freshwater ecosystem. *Environ Pollut* 2013; **172**: 264-74.

[27] OECD (The Organisation for Economic Cooperation and Development). Guidelines for the testing of chemicals, sediment-water

chironomid toxicity test using spiked sediment. OECD 218; 2004. Available from: http://www.oecd-ilibrary.org/environment/test-no-218-sediment-water-chironomid-toxicity-using-spiked-sediment_9789264070264-en.

[28] Batac-Catalan Z, White DS. Chironomustentas. *Entomol News* 1982; **93**: 54.

[29] APHA (American Public Health Association). *American water works association and federal water pollution control administration. Standard method for the examination of water and wastewater*. Washington DC: American Public Health Association; 2013.

[30] Ellman G, Courtney K, Andres JV, Featherstone R. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88-95.

[31] Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-54.

[32] Statistical Package for the Social Sciences. *SPSS statistical software, version 11.5 for windows*. Chicago, Illinois, United States: SPSS, SPSS Inc.; 2003.

[33] Katagi1 T, Tanaka H. Metabolism, bioaccumulation, and toxicity of pesticides in aquatic insect larvae. *J Pestic Sci* 2016; **41**(2): 25-37.

[34] Pérez J, Monteiro MS, Quintaneiro C, Amadeu MVM, Loureiro S. Characterization of cholinesterases in *Chironomus riparius* and the effects of three herbicides on chlorpyrifos toxicity. *Aquat Toxicol* 2013; **15**(144–145): 296-302.

[35] Diepens NJ, Beltman WH, Koelmans AA, Van den Brink PJ, Baveco JM. Dynamics and recovery of a sediment-exposed *Chironomus riparius* population: a modelling approach. *Environ Pollut* 2016; **213**: 741-50.

[36] Hasenbein S, Lawler SP. The use of growth and behavioral endpoints to assess the effects of pesticide mixtures upon aquatic organisms. *Ecotoxicology* 2015; **24**: 746-59.

[37] Ingersoll CG, Kunz JL, Hughes JP, Wang N, Ireland DS, Mount DR. Relative sensitivity of an amphipod *Hyalella azteca*, a midge *Chironomus dilutus*, and a unionid mussel *Lampsilis siliquoidea* to a toxic sediment. *Environ Toxicol Chem* 2015; **34**(5): 1134-44.

[38] Walls LD, Li LY, Hall KJ. Trace metal contamination due to acid rock drainage and its impacts on the fish-bearing Pennask Creek watershed, British Columbia. *Water Air Soil Pollut* 2013; **224**: 1675-95.

[39] Lydy MJ, Austin KR. Toxicity assessment of pesticide mixtures typical of the Sacramento-San Joaquim delta using *Chironomustentans*. *Arch Environ Contam Toxicol* 2004; **48**: 49-55.

[40] Harwood AD, You J, Lydy MJ. Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: toxicokenetic confirmation. *Environ Toxicol Chem* 2009; **28**: 1051-8.

[41] Faria MS, Re A, Malcato J, Silva PCLD, Pestana J, Agra AR, et al. Biological and functional responsesof in situ bioassays with *Chironomus riparius* larvae to assess river water quality and contamination. *Sci Total Environ* 2006; **371**: 125-37.

[42] Du J, Pang J, You J. Bioavailability-based chronic toxicity measurements of permethrin to *Chironomus dilutus*. *Environ Toxicol Chem* 2013; **32**(6): 1403-11.

[43] Deanovic LA, Markiewicz D, Stillway M, Fong S, Werner I. Comparing the effectiveness of chronic water column tests with the crustaceans *Hyalella azteca* (order: Amphipoda) and C*eriodaphnia dubia* (order: Cladocera) in detecting toxicity of current use insecticides. *Environ Toxicol Chem* 2013; **32**(3): 707-12.

[44] Rasmussen JJ, Wiberg-Larsen P, Kristensen EA, Cedergreen N, Friberg N. Pyrethroid effects on freshwater invertebrates: a meta-analysis of pulse exposures. *Environ Pollut* 2013; **182**: 479-85.

[45] Rajini A, Revathy K. Effect of combination pesticide on acetylholine esterase activity in freshwater fish *Danio Rerio*. *Int J Pharm Bio Sci* 2015; **6**(1): 1305-10.

[46] Rodríguez-Fuentes G, Rubio-Escalante FJ, Noreña-Barroso E, Escalante-Herrera KS, Schlenk D. Impacts of oxidative stress on acetylcholinesterase transcription, and activity in embryos of zebrafish (*Danio rerio*) following chlorpyrifos exposure. *Comp Biochem Physiol C Toxicol Pharmacol* 2015; **172–173**: 19-25.

[47] Kheir RH, Ibrahim J, Lewis A, Callaghan A, Crane M. Comparison of acetylcholinesterase and glutathione-*S*-transferase activity in *Chironomus riparius*, Meigen exposed to chemical-spiked sediments. *Environ Contam Toxicol* 2001; **66**: 603-10.

[48] Callaghan A, Hirtthe G, Fisher T, Crane M. Effect of acute exposure to chlorpyrifoson developmental parametersand biochemical biomarkers in *Chironomus riparius* Meigen. *Ecotoxicol Environ Saf* 2001; **50**: 19-24.

[49] Domingues A, Guilhermino BC, Amadeu MVM, Soares A, Antonio JA, Nogueira A. Assessing dimethoate contamination in temperate and tropical climates: Potential use of biomarkers in bioassays with two chironomid species. *Chemosphere* 2007; **69**: 145-54.

[50] Park SY, Nair PMG, Choi J. Characterization and expression of superoxide dismutase genes in *Chironomus riparius* (Diptera, Chironomidae) larvae as a potential biomarker of ecotoxicity. *Comp Biochem Physiol C Pharmacol* 2012; **156**: 187-94.