Chinese Journal of Oceanology and Limnology
Vol. 31 No. 1, P. 1-6, 2013
http://dx.doi.org/10.1007/s00343-013-2051-0

Acute toxicity and biodegradation of endosulfan by the polychaeta, *Perinereis aibuhitensis*, in an indoor culture*

KANG Kyoung Ho (康庆浩)[1, **], ZHANG Litao (张立涛)[2], ZHANG Zhifeng (张志峰)[2], SUI Zhenghong (隋正红)[2] , HUR Junwook (许准旭)[1]

[1] *Department of Aquaculture, Chonnam National University, Yeosu, Korea*

[2] *College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China*

Received Mar. 22, 2012; accepted in principle May 22, 2012; accepted for publication Jul. 24, 2012

Abstract The polychaete *Perinereis aibuhitensis*, a key species in estuarine ecosystems, can improve the culture condition of sediment. Endosulfan is an organochlorine pesticide used globally to control insects and mites; however, it is a source of pollution in aquaculture as a result of runoff or accidental release. In this study, we evaluated the toxicity of endosulfan to polychaeta and its ability to improve polluted sediment. Specifically, the effects of a series of endosulfan concentrations (0, 1.25, 2.5, 5, 10, 15, and 20 mg/L) were investigated, and the results indicated that the 24h median lethal concentration (24 h LC_{50}) was 55.57 mg/L, while the 48-h median lethal concentration (48h LC_{50}) was 15.56 mg/L, and the safe concentration was about 1.556 mg/L. In a 30-d exposure experiment, the animal specimen could decompose endosulfan effectively while improving endosulfan-polluted aquatic sediment.

Keyword: endosulfan; endosulfan sulfate; environment restoration; LC_{50}; *Perinereis aibuhitensis*

1 INTRODUCTION

The organochlorine pesticide endosulfan is used worldwide to control insects and mites. Endosulfan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxathiepin-3-oxide) is applied as a mixture that typically contains two diastereoisomers, known as α-endosulfan and β-endosulfan. Technical-grade endosulfan contains at least 94% α-endosulfan and β-endosulfan, with α- and β-isomers present at ratios ranging from 2:1 to 7:3 (Herrmann, 2002). Endosulfan enters aquatic environments via off-field movements through runoff of contaminated surface soil due to rain events, drift and overspray from aerial applications, and accidental releases of irrigation tail water (Pablo and Hyne, 2009). Upon entering the environment, endosulfan is changed to its diol form in water and its sulfate form in soil or sediment, after which it is further broken down to hydroxyl ether lactone, and alcohol forms (Martens, 1976; Miles and Moy, 1979; Stanley et al., 2009). Endosulfan sulfate, which is the sole metabolite of endosulfan, exerts toxicity to organisms similar to that of endosulfan (Berntssen et al., 2008). Endosulfan and endosulfan sulfate are persistent when absorbed by soil and sediment, with half-lives ranging from weeks to years (Peterson and Batley, 1993; Leonard et al., 2001).

Both endosulfan and endosulfan sulfate were reported as fish toxicants in the 1950s and are known to be highly toxic to non-target aquatic organisms (Maier-Bode, 1968; Rajeswari et al., 1988). In South Korea, endosulfan has been sold and is still widely used because of its high efficiency and low cost. Furthermore, human dietary intakes of endosulfan in South Korea increased significantly from 38.68 (1.3) ng/(kg bw·d) in 1994 to 92.17 (4.4) ng/(kg bw·d) in 2011 (Desalegn et al., 2011). Accordingly, it is important to reduce endosulfan pollution and its metabolite in seawater and aquatic sediments since seafood is the primary route of exposure.

To date, most studies related to endosulfan toxicity and its decomposition with respect to bioremediation

* Supported by Technology Development Program for Fisheries, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea
** Corresponding author: mobidic@chonnam.ac.kr

have been conducted in soil environments using microorganisms and earthworms (Kumar and Philip, 2006; Bhalerao and Puranik, 2007; Goswami et al., 2009; Liu et al., 2009), and there have been no investigations of benthic species in coastal regions or their role in endosulfan degradation. Considering its widespread use in agriculture, long distance transport and persistence in nature, it is important to assess the impact that endosulfan has on coastal benthos and its decay in the coastal sediment. *Perinereis aibuhitensis*, belonging to the class Polychaeta, is often used to improve coastal sediment. Indeed, this organism has been shown to have the potential to improve sediment conditions where scallop cultures exist. Because *P. aibuhitensis* is similar to the earthworm in its life habits, we selected *P. aibuhitensis* for this study.

In the present study, we evaluated the toxicity of endosulfan to polychaeta and its ability to improve a polluted sediment to determine whether the polychaete, *P. aibuhitensis*, could be introduced into endosulfan-polluted environments for ecological restoration purposes.

2 MATERIAL AND METHOD

2.1 Animal, sediment and seawater for indoor experiment

The test specimens, *P. aibuhitensis*, were collected from Mokpo, Jeonnam, Korea. Following collection, the specimens were immediately placed in an insulated box and transported to the laboratory. The specimens were then acclimated to the test conditions for 96 h before exposure to endosulfan. Sediment with a pH of 7 without infaunal organisms was purchased from a biotechnology company. The particle diameter of the sediment was less than 1 mm, and it was composed of 10% sand, 70% silt and 20% clay. The specimens burrowed into the sediment, and survived without showing any abnormal appearance or behavior.

2.2 Acute toxicity

Stock solutions of endosulfan (Fluka, Switzerland) were prepared by dissolving the crystalline solids into 15 mL acetonitrile at concentrations that varied depending on the experimental conditions. The primary tests for detection of acute toxicity were conducted using 5, 10, 20, and 40 mg/L for one day. None of the animals in the 5 mg/L group died; however, all in the 40 mg/L group did. Therefore, the acute toxicity bioassays were conducted at endosulfan concentrations of 0, 1.25, 2.5, 5, 10, 15, and 20 mg/L. According to the LC_{50}, the final concentration of endosulfan in the seawater for the long-term exposure experiment was set at 3 mg/L.

2.3 Endosulfan degradation experiment

The experiment was conducted in a 10 L tank with 5 cm of sediment on the bottom and 30 L of circulating seawater. Three replications were prepared for each group, and 30 polychaetes (length 16.0±1.3 mm; weight 0.4±0.1 g) were introduced to each tank. The average water conditions during the experiment were 22°C, pH 7.5, and a salinity of 30.6 g/L. Oxygenation and turbulence of water and sediment were produced by air pumps and air stones. In the acute toxicity test, the polychaetes were examined every 24 h and dead ones were removed. Over the 30-day long-term exposure experiment, seawater, sediment and polychaete samples were collected at days 0, 5, 10, 15, 20, 25, and 30 and stored at -20°C for further analysis. Nothing was supplied to the polychaete for the duration of the experiments.

2.4 Chemical analysis

The endosulfan and endosulfan sulfate concentrations of the sediment and specimens were measured at 0, 5, 15, 25, and 30 days by gas chromatography (Shimadzu 2010plus). To accomplish this, 50g sediments were extracted with 100 mL CH_3CN in a 300 r/min mixer for 30 min, then shaken for an additional 30 min after the addition of 20–30 g NaCl. For extraction of the polychaete tissue samples, 1–3 g of tissue were simply added to 100 mL of CH_3CN. The mixtures were then centrifuged at 3 000 r/min for 5 min, after which 10 mL of extract was decanted. The solvent was then removed, the residue extracted with 1mL of acetone/hexane (2/8 V/V) and the sample solution was loaded into a solid phase extraction cartridge (Florisil) and extracted with 5 mL of acetone/hexane (2/8 V/V). After removal of the solvent, the residue was dissolved in 1mL acetone and endosulfan was quantitatively analyzed using a gas chromatography (Shimadzu 2010 plus) equipped with an ECD and a 30 mm×0.32 mm×0.25 mm fused-silica DB-5 capillary column (Restok, RTx-5). The oven temperature program was as follows: 180°C for 1 min followed by an increase to 250°C at 3°C/min, which was held for 1 min, after which the temperature was increased to 300°C at 20°C/min,

where it was held for 3 min. Endosulfan and endosulfan sulfate concentrations were identified using a standard curve. Endosulfan diol, endosulfan ether, endosulfan hydroxyl ether and endosulfan lactone were also detected using this method.

The seawater samples were analyzed for total organic carbon (TOC) and total nitrogen (TN) at 0, 5, 10 and 15 days using a TOC/TN analyzer (Shimadzu, TOC-V/TNM-1) to monitor the eutrophication status.

2.5 Statistical analysis

Statistical comparisons of the mean cumulative mortalities were examined by ANOVA at α=0.05. The concentration of endosulfan at which 50% mortality of the test organism occurred (LC_{50}) and its 95% confidence limit were estimated for 24 h and 48 h by Probit analysis (Finney, 1971). The LC_{50} value was multiplied by an application factor of 0.1 to estimate the safe concentration (Akinbulumo et al., 2005).

Repeated measures ANOVA followed by Duncan's multiple comparison was used to identify significant differences in TOC, TN in seawater, endosulfan and endosulfan sulfate concentrations after different elapsed times. Analyses were conducted using the SPSS statistical package (version 18.0).

3 RESULT

3.1 Acute toxicity bioassays

As shown in Table 1, none of the specimens died following exposure to low concentrations (1.25, 2.5, and 5 mg/L) of endosulfan during the course of the experiment, and at 24 h the mortalities at the concentrations of 10, 15, 20 mg/L were still low. There were also no significant differences (P>0.05) among concentrations at 24 h. However, at 48 h, the mortalities at concentrations of 10, 15, 20 mg/L increased to 26.7%, 48.3% and 63.3%, respectively. The mortalities in the presence of higher concentrations (10, 15, and 20 mg/L) were found to be significantly different (P<0.05) from those at lower concentrations (0, 1.25, 2.5, and 5 mg/L).

The probit of mortality was linearly dependent on the log concentration of endosulfan (Table 2). According to the regression equation, the 24 h median lethal concentration (24 h LC_{50}) of polychaete was 55.57 mg/L (Table 3), and its 95% confidence limits were 31.74–79.45. The 48-h LC_{50} of polychaete was 15.56 mg/L and its 95% confidence limits were 13.73–18.09.

Table 1 Toxicity of different concentrations of endosulfan to polychaeta, *Perinereis aibuhitensis*

Concentration(mg/L)	Cumulative mortality (%)		
	0 h	24 h	48 h
0	0^{c}	0^{c}	0^{c}
1.25	0^{c}	0^{c}	0^{c}
2.5	0^{c}	0^{c}	0^{c}
5	0^{c}	0^{c}	0^{c}
10	0^{c}	5.0^{bc}	26.7^{b}
15	0^{c}	6.7^{bc}	48.3^{a}
20	0^{c}	13.3^{bc}	63.3^{a}

Different letters indicate significant difference (P<0.05).

Table 2 Regression equation at different times

Time (h)	R^2	Regression equation
24	0.877	Y=-4.341+2.488X
48	0.999	Y=-4.414+3.476X

Table 3 The LC_{50} and safe concentration of endosulfan for the polychaeta, *Perinereis aibuhitensis*

	LC_{50} (mg/L)	95% confidence limit	Safe concentration (mg/L)
24 h	55.57	31.74–79.45	5.56
48 h	15.56	13.73–18.09	1.56

Probit analysis was carried out between the concentrations and mortalities in SPSS. When the R^2 at 24 h and 48 h was compared, the probity of mortality and log concentration at 48 h showed greater linearity. Thus, the concentration deemed safe was 1.56 mg/L.

3.2 Endosulfan and endosulfan sulfate for long-term exposure experiment

Endosulfan and endosulfan sulfate were not detected in seawater samples by HPLC analysis. The endosulfan concentration increased for the first five days in the control sediment with only endosulfan (Fig.1), then slowly declined and approached zero after 30 d. In the experimental group, the endosulfan concentration in the sediment at the beginning (t=0, 4.5 mg/L) was lower than that of the control sediment (t=0, 6.7 mg/L), indicating that endosulfan began to be consumed or decomposed by polychaeta. Furthermore, the endosulfan concentration in sediment of the experimental group decreased continuously, reaching 0.11 mg/L after 30 d. The maximum concentration of endosulfan in polychaeta

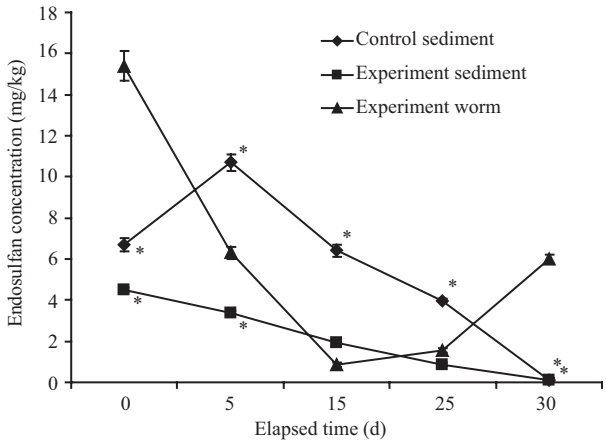

Fig.1 The concentration of endosulfan, in the sediment and in *P. aibuhitensis* at different elapsed times

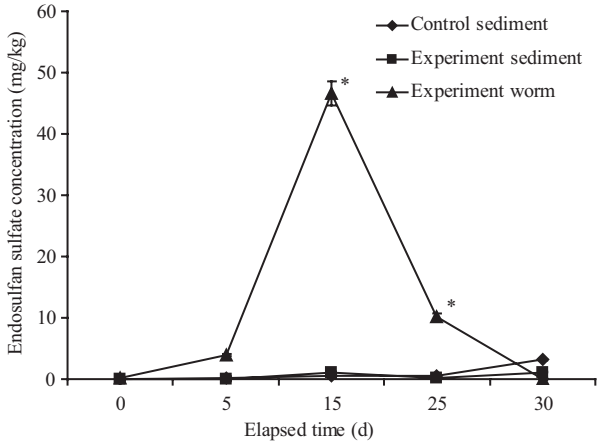

Fig.2 The concentration of endosulfan sulfate, in the sediment and in *P. aibuhitensis* at different elapsed times

* indicates significantly different from the time 0 (P<0.05).

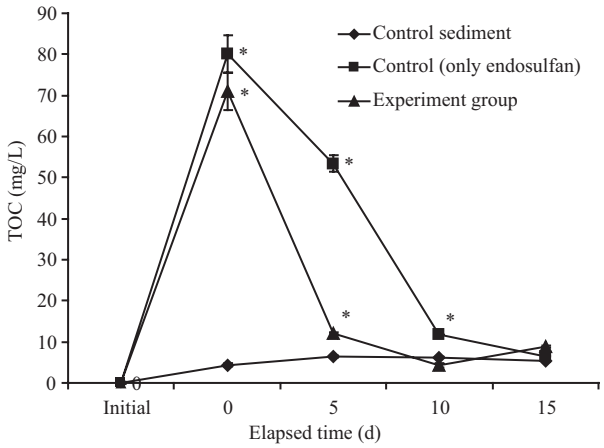

Fig.3 The total organic carbon (TOC) of seawater at different elapsed times

* indicates significantly different from initial TOC (P<0.05). Initial TOC means the TOC of the sediment in the natural condition.

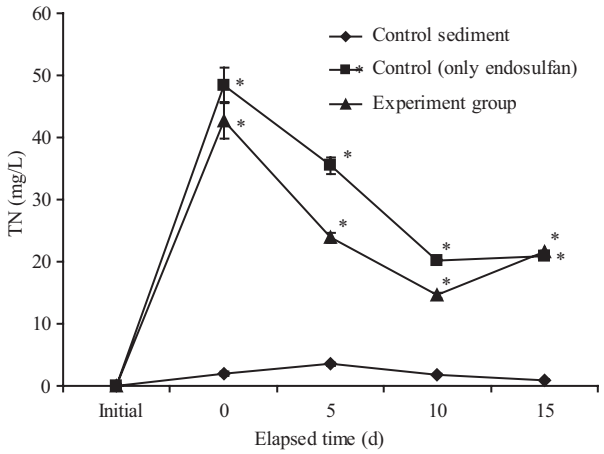

Fig.4 The total nitrogen (TN) of seawater at different elapsed times

* indicates significantly different from initial TN (P<0.05). Initial TN means the TN of the seawater in the natural condition.

was observed at time 0, after which it decreased; however, one-way ANOVA revealed no significant differences among groups.

Endosulfan sulfate, a well-known primary metabolite of endosulfan, was also detected in worms (Fig.2). Specifically, the maximum concentration was observed at 15 d, after which it decayed completely. The formation of endosulfan sulfate occurred simultaneously with the decomposition of endosulfan. In the sediment for both the control and experimental group, the concentrations of endosulfan sulfate were less than 3.15 mg/kg.

3.3 Seawater improvement during long-term exposure experiment

For seawater (Fig.3 and Fig.4), the total organic carbon (TOC) and total nitrogen (TN) increased significantly (P<0.05) upon introduction of endosulfan dissolved in acetonitrile, after which they decreased with time.

4 DISCUSSION

Endosulfan, like other cyclodiene insecticides, has been shown to cause neurotoxicity through GABA-gated chloride channel inhibition (Naqvi and Vaishnavi, 1993; Jia and Misra, 2007). Endosulfan causes decreased adenylate energy charge, oxygen consumption, hemolymph amino acids, succinate dehydrogenase, heart-rate (mussel) and altered osmoregulation (Naqvi and Vaishnavi, 1993). The present results indicated that the endosulfan is slightly toxic (10–100 mg/L) to *P. aibuhitensis* (Kamrin,

1997). The LC_{50} of endosulfan at 48h for fish has been reported to be less than 0.1 mg/L (Gill et al., 1991; Desalegn et al., 2011), while it was shown to be below 1 mg/L for crustaceans (Gill et al., 1991). When compared to other aquatic organisms, polychaete can endure high concentrations of endosulfan.

The accumulation of endosulfan by the polychaeta was highest at time 0 (Fig.1), indicating that their accumulation rate of endosulfan is very high. Freshwater animals have been found to be able to accumulate endosulfan to some extent, but then lose the compound rapidly during depuration (Naqvi and Newton, 1990). Pablo and Hyne (2009) pointed out that endosulfan had a tendency to move preferentially toward or remain bound onto solid gravel and sediment substrate. This may be the main reason for the increase of endosulfan that was observed in the control sediment at Day 5. In the chronic toxicity experiment, endosulfan was not detected in the seawater, which was likely due to the hydrophobic nature of endosulfan (1.32 mg/L) resulting in its adsorption to soil particles (Bhalerao and Puranik, 2007; Pablo and Hyne, 2009). The fact that the endosulfan sulfate concentration in polychaete eventually decreased and only trace amounts were detected in the sediment of both the control and experimental group suggests that endosulfan sulfate was not extruded from the *P. aibuhitensis*, and that it decomposed further inside the organisms. Other possible metabolites, such as endosulfan diol, endosulfan ether, endosulfan hydroxyl ether and endosulfan lactone, were not detected at any time. Microbes have commonly been used to decompose endosulfan, but animals have rarely been applied for its remediation. We previously found that endosulfan decomposition by polychaete was in accordance with that by *Aspergillus niger*, the concentration of endosulfan decreased while endosulfan sulfate increased, and the endosulfan and endosulfan sulfate completely disappeared in the presence of *A. niger* while the endosulfan increased in the presence of polychaete (Bhalerao and Puranik, 2007).

Endosulfan pollution is often also accompanied by organic pollution. Therefore, we measured the TOC and TN of seawater. The TOC and TN were elevated at the beginning of the experiment, possibly because of the CH_3CN solvent that was used to dissolve the endosulfan. Accordingly, the subsequent decrease in TOC and TN could have been caused by loss of this solvent by the aeration of seawater during circulation through the system, sorption to the sediment (Leonard et al., 2001) and decomposition by polychaete in the experimental group. However, it should be noted that the decrease of TOC and TN in the experimental group was more dramatic than that in the control group, indicating that the polychaete influenced the reduction. The movement of benthic invertebrates through sediment can accelerate solvent volatilization and create conditions favorable for their aerobic decomposition. This finding and interpretation can be applied to the removal of other volatile organic pollutants that contaminate coastal sediments.

Overall, the results of this study indicated that polychaeta could improve environments that have been polluted by endosulfan. Indeed, the organic and endosulfan concentrations decreased significantly ($P<0.05$) under experimental conditions. Accordingly, *P. aibuhitensis* are a good candidate for the remediation of endosulfan contaminated coastal environments.

References

Akinbulumo M O, Fagbenro O A, Fasakin E A. 2005. Acute toxicity of ethanolic extract of Derris elliptica roots to African catfish (*Clarias gariepinus*, Burchell, 1822) fingerlings. *Biol. Environ. Sci. J. Tropics*, **2**: 126-131.

Berntssen M H, Glover C N, Robb D H, Jakobsen J V, Petri D. 2008. Accumulation and elimination kinetics of dietary endosulfan in Atlantic salmon (*Salmo salar*). *Aqua. Toxicol.*, **86**(1): 104-111.

Bhalerao T S, Puranik P R. 2007. Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. *International Biodeterioration and Biodegradation*, **59**: 315-319.

Desalegn B, Takasuga T, Harada K H, Hitomi T, Fujii Y, Yang H R, Wang P, Senevirathna S, Koizumi A. 2011. Historical trends in human dietary intakes of endosulfan and toxaphene in China, Korea and Japan. *Chemosphere*, **83**: 1 398-1 405.

Finney D J. 1971. Probit analysis (3rd edition). Cambridge University Press, Cambridge, UK. 318p.

Gill T S, Pande J, Tewari H. 1991. Individual and combined toxicity of common pesticides to teleost *Puntius conchonius* Hamilton. *Indian J. Exp. Biol.*, **29**(2): 145-148.

Goswami S, Vig K, Singh D K. 2009. Biodegradation of α and β endosulfan by *Aspergillus sydoni*. *Chemosphere*, **75**: 883-888.

Herrmann M. 2002. Preliminary risk profile of endosulfan. Umweltbundesamt, Berlin. Germany.

Jia Z, Misra H P. 2007. Developmental exposure to pesticides zineb and/or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals later in life. *Neurotoxicology*, **28**(4): 727-735.

Kamrin M A. 1997. Pesticide profiles: toxicity, environmental

impact, and fate. CRC/Lewis Publishers, Boca Raton, FL. 676p

Kang K H, Zhang Z F, Bao Z M, Zhou B, Kim Y H, Webster C D. 2010. Effects of clamworm, Perinereis aibuhiteusis, density on acid volatile sulfide, chemical oxygen demand, and loss of ignition in benthic sediment, and on survival of scallop, *Chlamys farreri*, spat. *J*. *World Aqua*. *Soc*., **41**: 131-138.

Kumar M, Philip L. 2006. Bioremediation of endosulfan contaminated soil and water optimization of operating conditions in laboratory scale reactors. *J*. *Hazard*. *Mater*., **136B**: 354-364.

Leonard A W, Hyne R V, Lim R P, Leigh K A, Le J, Beckett R. 2001. Fate and toxicity of endosulfan in Namoi River water and bottom sediment. *J*. *Environ*. *Qual*., **30**(3): 750-759.

Liu W, Zhu L S, Wang J, Wang J H, Xie H, Song Y. 2009. Assessment of the genotoxicity of endosulfan in earthworm and white clover plants using the comet assay. *Arch*. *Environ*. *Contam*. *Toxicol*., **56**(4): 742-746.

Maier-Bode H. 1968. Properties, effects, residues and analytics of insecticide endosulfan. *Residue Rev*., **22**: 1-44.

Martens R. 1976. Degradation of [8, 9,-14C] endosulfan by soil microorganisms. *Appl*. *Environ*. *Microbiol*., **31**(6): 853-858.

Miles J R W, Moy P. 1979. Degradation of endosulfan and its metabolites by a mixed culture of soil microorganisms. *Bulletin of Environ*. *Contam*. *Toxicol*., **23**(1): 13-19.

Naqvi S M, Newton D J. 1990. Bioaccumulation of endosulfan (Thiodan insecticide) in the tissues of Louisiana crayfish, *Procambarus clarkia*. *J*. *Environ*. *Sci*. *Health B*, **25**(4): 511-526.

Naqvi S M, Vaishnavi C. 1993. Bioaccumulative potential and toxicity of endosulfan insecticide to non-target animals. *Comp*. *Biochem*. *Physiol*. *C*, **105C**(3): 347-361.

Pablo F, Hyne R. 2009. Endosulfan application to a stream Mesocosm: studies on fate, uptake into passive samplers and caged toxicity test with the fish *M*. *ambigua*. *Arch*. *Environ*. *Contam*. *Toxicol*, **56**(3): 525-535.

Peterson S M, Batley G E. 1993. The fate of endosulfan in aquatic ecosystems. *Environ*. *Pollution*, **82**(2): 143-152.

Rajeswari K, Kalarani V, Reddy D C, Ramamurthi R. 1988. Acute toxicity of endosulfan to crabs: effect on hydromineral balance. *Bulletin of Environ*. *Contam*. *Toxicol*., **40**: 212-218.

Stanley K A, Curtis L R, Simonich S L, Tanguay R L. 2009. Endosulfan I and endosulfan sulfate disrupts zebrafish embryonic development. *Aquat*. *Toxicol*., **95**(4): 355-361.