Poll Res. 40 (3) : 1086-1090 (2021)

ISSN 0257–8050

ASSESSMENT OF ACUTE TOXICITY AND HISTOPATHOLOGY OF THE ENDOSULFAN IN *ERIOCHEIR HEPUENSIS* DAI, 1991 (CRUSTACEA: BRACHYURA: GRAPSOIDEA: VARUNIDAE)

AMAAL GH. YASSER[A]*, NADA M. AL- BAGHDADI[A] AND MURTADA D. NASER[A]

[a] *Department of Marine Biology, Marine Science Centre, University of Basrah, Basrah, Iraq*

(Received 10 May, 2021; Accepted 20 June, 2021)

ABSTRACT

Endosulfan is widely used in agricultural activities and overfishing in the south of Iraq, impacting negatively on the ecology and species that live in the region's waters. The acute toxicity of endosulfan, an organochlorine insecticide, to the edible crab *Eriocheir hepuensis* (DAI) was investigated in this work. 96h LC_{50} value of endosulfan on crab was 55.15 µg/L. With increasing toxicant concentration and exposure time, the exposed crab displayed restlessness, escape behavior, increased scaphognathite activity. The sublethal exposure studies were done for up one week and two weeks at 1/8 of LC_{50} 96 h for histopathological analysis. The most histopathological alterations were hyperplasia (HY), lamellar collapse (CL), and after two weeks exposure was rupture of pilaster cells (RCP).

KEY WORDS : Overfishing, Organochlorine, 96h LC_{50}

INTRODUCTION

Endosulfan is a non-systemic organochlorine insecticide that is widely used on a variety of agricultural crops (Sarma *et al.,* 2012). Endosulfan, an organochlorine pesticide, is one of the most hazardous pesticides for aquatic organisms, particularly fish, and has been designated by the US Environmental Protection Agency as a "priority contaminant" (Cengiz and Unlu, 2002). It can reach water bodies through surface runoff and air drift from surrounding agricultural fields after application, causing harm to non-target aquatic creatures (Miglioranza *et al*., 2002). Despite its limited water persistence, it can bind to organic matter in both suspended and bottom particles, allowing it to stay in sediments for years (Weber *et al.,* 2010).

In the south of Iraq, endosulfan is commonly used in agricultural activities and overfishing, wreaking havoc on the ecology and animals that inhabit the region's waters (Yasser *et al*., 2008; Yasser *et al.,* 2010; Yasser and Naser, 2011; Yasser, 2012, Al-Gheezy *et al*., 2018).

Pesticide exposure has been linked to a variety of histopathological consequences in animals such as prawns and crabs. The gills are the first organ to come into touch with pollutants in the environment. They are extremely sensitive to harmful compounds because to their huge surface area, which allows for easier contact and absorption. As a result of the quick absorption through the gills, the toxic response in the gills is likewise rapid (Negro, 2015; Jesus *et al.,* 2020).

Eriocheir hepuensis is an alien species that has lately been recorded from several locations in Iraq's south (Naser *et al.,* 2012). The goal of this study was to assess endosulfan's acute toxicity in the hairy crab *E. hepuensis* and impact of the sublethal concentration of endosulfan on the crab gills.

MATERIALS AND METHODS

Crab

Healthy crabs with initial body carapace width of 35 ± 0.25 mm (mean ±SE) were collected from the Shatt Al-Basrah canal during November 2011. They were transported to the laboratory in aerated plastic bags.

The crabs were acclimatized for 15 days in glass aquaria 100 l. filled with dechlorinated tap water of the same quality as used in the test under laboratory conditions. During this time, no deaths were reported. An electric air pump was used to maintain continual aeration in each aquarium.

Analysis of water chemical parameters

The following were the physicochemical properties of clorine-free tap water used in both acclimation and experiments: Total hardness 137 mg/l as $CaCO_3$, nitriate 1.7 mg/l, nitrite 0.006 mg/l, and salinity 0.7 PSU. pH 7.5, dissolved oxygen 7.0 mg/l, total hardness 137 mg/l as $CaCO_3$, nitriate 1.7 mg/l, nitrite 0.006 mg/l, and salinity 0.7 PSU. To avoid fungal and bacterial infection, the aquaria were changed on a daily basis.

Feeding

During the acclimation stage, the crabs were fed Andontasp once a day, at least one hour before the tank water was replaced.

Test chemical and acute toxicity

Static acute bioassays were conducted using commercial grade Thiodan-35 EC® (35% endosulfan and 64% naphtha; made in Iran). The nominal concentrations used were 10, 25, 35, 50 and 70 µg/l. Every three hours for 96 hours, each crab was gently prodded with a needle. Crabs that did not respond within one minute were recorded as dead and were removed from the experiment.

Sublethal experiment

The sublethal exposure studies were done for up one week at 1/8 of LC_{50} 96 h for histopathological analysis.

Histopathological examination

The specimens of *E. hepuensis* were promptly dissected in the laboratory. Using sterile scissors, samples of the hepatopancreas and gills were extracted. The samples were then preserved in Davidson's solution for 24 hours in vials that were properly identified and sealed (Arockia-Vasanthi *et al.*, 2014). The samples were cleaned and kept in 70% ethanol after 24 hours. The gills were then dehydrated in increasing of ethanol concentrations, diaphanized in xylol, and impregnated with paraffin. Hematoxylin and eosin were used to stain cross-sections of roughly 5 m thickness. The alterations were photographed with compound microscope equipped with digital camera.

Statistical analysis

96h LC_{50} values were determined using Finney's (1971) probit analysis LC_{50} determination method and version 1.00 of the software developed by EPA (1999).

RESULTS

The present finding revealed that the organochlorine endosulfan is toxic pesticide to *E. hepuensis*. In the acute toxicity testing, the toxicity of this compound on the tested crabs increased with increasing concentration and/or exposure time. Determined 96 hLC50 value of endosulfan for *E. hepuensis* was found as 55.15 µg/L (Table 1). (Fig. 1) shows the concentration -response plot.

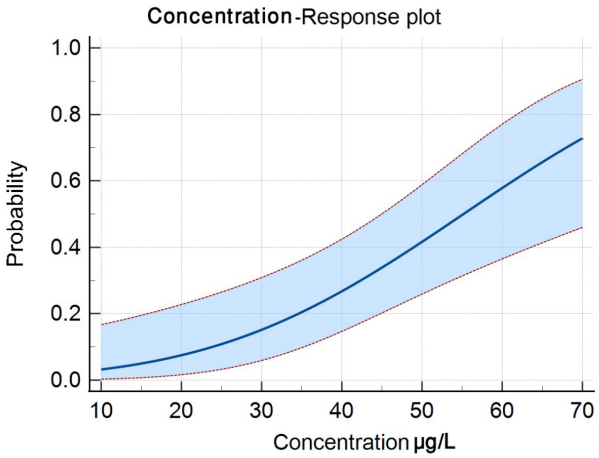

Fig. 1. Concentration response plot

In a concentration-dependent way, crabs exposed to varying concentrations of endosulfan showed altered behavioural responses including as hyperactivity, escape behavior, scaphognathite pounding. They revealed enhanced scaphognathite activity during the first hours of exposure, as well as the generation of air bubbles and coughing, at low concentrations.

The most frequently seen histological lesion in gills is the collapse of pillar cells was caused by the disturbance of pillar cells and hyperplasia of the lamellae was detected in the first week, while rupture of pilaster cells can be seen after the second week (Fig. 2).

DISCUSSION

Endosulfan's acute toxicity to aquatic crustacea

Table 1. Concentration-Response table

Probability	Concentration µg/L	95% lower confidence interval	95% upper confidence interval
0.01	-1.74789	-60.18554	16.66479
0.02	4.91974	-45.70449	21.28218
0.025	7.21367	-40.74564	22.89396
0.05	14.92111	-24.21156	28.43668
0.1	23.80729	-5.55244	35.23071
0.2	34.56776	15.72181	44.77823
0.25	38.65572	23.03355	49.17582
0.5	55.15339	44.94147	74.52303
0.75	71.65107	58.79147	107.92817
0.8	75.73902	61.80921	116.61976
0.9	86.49949	69.43938	139.81135
0.95	95.38567	75.53939	159.16449
0.975	103.09311	80.7458	176.0349
0.98	105.38705	82.28458	181.06674
0.99	112.05468	86.73573	195.71402

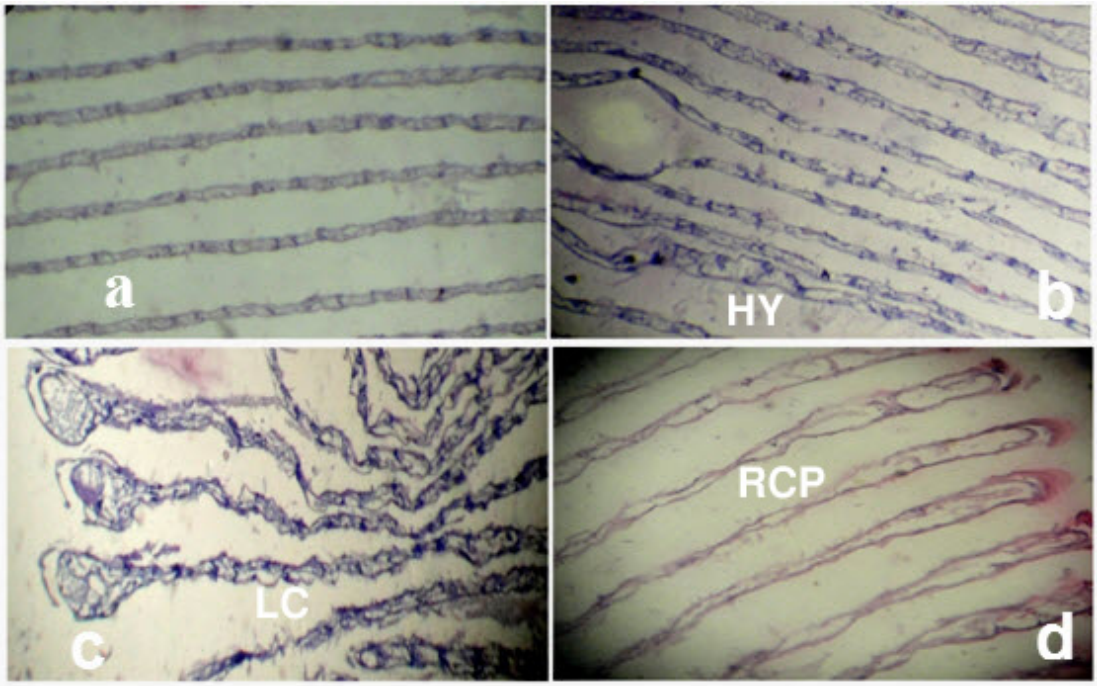

Fig. 2. Cross-sections of gill lamellae of *E. hepuensis* after 1 week. a- Control showing normal lamellae with uniform interlamellar spaces. b–d -exposed crab showing hyperplasia (HY) (b), lamellar collapse (CL)(c), after two weeks rupture of pilaster cells (RCP) (d) H&E stain, 10x

species has been studied in various ways. Li *et al.* (2006) estimated that endosulfan's 96-h LC_{50} value for *Eriocheir sinensis* was 142.2 µg/L, while the same insecticide's LC50 value for the atyid shrimp *Cardina laevis* was 1.02 µg/L Sucahyo *et al.* (2008). The other prawns endosulfan's 96-h LC_{50}s were 0.2, 17.1 and 0.32 for species *Macrobrachium rosenbergii*, *Palaemon macrodactylus* and *Palaemonetes pugio*, respectively, (Lombardi *et al.*, 2001; Wirth *et al.*, 2001 and Key *et al.*, 2003). When comparing the estimated 96-h LC_{50} value for endosulan with other insecticides toxicity, it appears that acute toxicity of endosulfan in *E. hepuensis* is more more than twice sensitive than chlorpyrifos's toxicity for species *E. sinensis*. On the contrary, when we compare with other prawns species toxicity, the species *E. hepuensis* reveals

higher tolerances to the insecticides endosulfan.

In control group, The crabs were able to remain calm and walk regularly from one side of the container to the other, while in the exposed groups to endosulfan, the crabs tend to be suffering hyperactivity in the beginning then slowly motionless. This behaviour has been noted by Deyashi *et al.,* 2019, in their experiments on the species *Varuna litterata* exposed to mahua oil cake aqueous extract. Dyer and Uglow(1978) and Vardhanan and Radhakrishna (2002) employed scaphognathite activity as an indicator to determine metal toxicity in crabs. The same behavior that noticed by Srivastava *et al.,* 2013 in freshwater crab *Barytelphus aguerini* exposed to chlorpyrifos.

The gills are important for the species that rely on them for respiration, osmoregulation, ion excretion, and other functions; however, when the gills perform activities other than those already performed, such as heavy metal detoxification or pesticide detoxification, their structure may be damaged (Negro, 2015). Furthermore, because the gills are the first organs to come into touch with the aquatic environment, they are susceptible to stress from toxins in the water. As a result, gills have been crucial in the research of biomonitoring since they are an effective biomarker of pollution (Jesus *et al.,* 2020). Because it is sensitive, and when in direct contact with water and toxins, the gills respond with diverse structural alterations, the gills are considered the best organ to study biomarkers (or biological responses altered due to the presence of xenobiotics) (Carvalho-Neta *et al.,* 2019). In most aquatic contamination investigations, the gill responses are the most visible in acute exposure because the gills are immediately exposed to xenobiotics in the environment, responding fast and dramatically to the impacts of xenobiotics (Jesus *et al.,* 2020).

In conclusion,Endosulfan is widely used in agricultural activities and overfishing in south of Iraq, wreaking havoc on the ecology and creatures that live in the region's waters. In the present study,endosulanshowed a high lethal toxicity for *E. hepuensis*, the 96-h LC50 value was 55.15µg/L. Histopathological alterations in the gills of the crabs were severely impacted.

REFERENCES

Al-Gheezy, K., Yasser, A. G., Naser, M. and Rigby, M. 2018. Comparative acute toxicity of chlorofet and thiodan to the amphipod *Parhyale basrensis* (Salman, 1986) from Iraq. *Journal of Biological Studies*. 1(1): 1-8.

Arockia-Vasanthi, L., Muruganandam, A., Revathi, P., Baskar, B., Jayapriyan, K., Baburajendran, R. and Munuswam, N. 2014. The application of histo-cytopathological biomarkers in the mud crab (Forskal) to assess heavy metal toxicity in Pulicat Lake, Chennai. *Marine Pollution Bulletin*. 81: 85-93. doi: 10.1016/j.marpolbul.2014.02.016

Carvalho-Neta, R.N.F., Mota Andrade, T.S.O., Oliveira, S.R.S., Torres, A.R., Cardoso, W.S., Santos, D.M.S., Batista, W.S., Serra, I.M.R. and Brito, N.M. 2019. Biochemical and morphological responses in *Ucides cordatus* (Crustacea, Decapoda) as indicators of contamination status in mangroves and port areas from northern Brazil. *Environmental Science and Pollution Research*. 26(16): 15884-15893. doi: 10.1007/s11356-019-04849-0

Cengiz, E.I. and Unlu, E. 2002. Histological changes in the gill of mosquito sh, *Gambusia afnis* exposed to endosulfan. *Bull Environ Contam Toxicol.* 68 : 290-296

Dyer, M.F. and Uglow, R.F. 1978. Gill chamber ventilation scaphognathite movement in *Crangon crangon* (L.). *J. Exp. Mar. Biol. Ecol.* 32 : 209-218.

Finney, D.J. 1971. *Probit Analysis,* 3rd edn. Cambridge University Press, New York, p 668

Jesus, W. B. D., Oliveira, S. R. S. D., Andrade, T. D. S. D. O. M., Sousa, J. B. M., Pinheiro-Sousa, D. B., Santos, D. M. S. and Carvalho-Neta, R. N. F. 2020. Biological responses in gills and hepatopancreas of *Ucidescordatus* (Crustacea, Decapoda, Ocypodidae) as indicative of environmental contamination in mangrove areas in Maranhão State, Brazil. *Latin American Journal of Aquatic Research*. 48(2): 226-236.

Key, P.B., Chung, K.W., Opatkiewicz, A.D., Wirth, E.F. and Fulton, M.H. 2003. Toxicity of the insecticides fipronil and endosulfan to selected life stages of the grass shrimp (*Palaemonetes pugio*). *Bull Environ Contam Toxicol.* 70 : 533-540.

Li, K., Chen, L. Q., Li, E. C. and Zhou, Z. K. 2006. Acute toxicity of the pesticides chlorpyrifos and atrazine to the Chinese mitten-handed crab, *Eriocheir sinensis*. *Bulletin of Environmental Contamination & Toxicology.* 77(6).

Lombardi, J.V., Machado-Neto, J.G.,Brossi-Garcia, A.L., Marques, H.L.A. and Kubo, E. 2001. Acute toxicity of the pesticides endosulfan and ametryne to the freshwater prawn *Macrobrachium rosenbergii* (De Man). *Bull. Environ. Contam. Toxicol.* 67: 665-671.

Miglioranza, K.S.B., Gonza´ lezSagrario, M.A., Aizpu´ n de M oreno, J.E., Moreno, V.J Escalante, A.H. and Osterrieth, M.L. 2002. Agricultural soil as a potential source of organochlorine pesticides into nearby pond. *Environ. Sci. Pollut. Res. Int.* 9 : 250-256.

Naser, M. D., Page, T. J., Ng, N.K., Apel, M., Yasser, A. G., Bishop, J. M., Ng, P. K.L. and Clark, P. F. 2012). Invasive records of *Eriocheir sinensis* Dai, 1991 (Crustacea: Brachyura: Grapsoidea: Varunidae): Implications and taxonomic considerations. *BioInvasions Records.* 1(1) : 71-86.

Negro, C.L. 2015. Histopathological effects of endosulfan to hepatopancreas, gills, and ovary of the freshwater crab *Zilchiopsiscollastinensis* (Decapoda: Trichodactylidae). *Ecotoxicology and Environmental Safety*. 113: 87-94. doi: 10.1016/ j.ecoenv.2014.11.025

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

Sarma, K., Dalvi, R. S., Pal, A. K., Sahu, N. P., Chatterjee, N., Mukherjee, S. C. and Baruah, K. 2012. Acute and chronic effects of endosulfan on the haemato-immunological and histopathological responses of a threatened freshwater sh, spotted murrel. *Channa punctatus*. *Fish Physiol Biochem*. 38 : 499-509.

Srivastava, J. Y. O. T. I. M. A., Das, S. H. O. B. H. A., Pandey, R. K. and Das, V. K. 2013. Acute toxicity and behavioural responses of freshwater crab *Barytelphusa guerini* to chlorpyrifos exposure. *J. Appl.Biosci*. 39(1) : 56-59.

Sucahyo, D., van Straalen, N.M., Krave, A. and van Gestel, C.A.M. 2008. Acute toxicity of pesticides to the tropical freshwater shrimp *Caridina laevis*. *Ecotox Environ Saf.* 69 (3) : 421-427.

Vardhanan, S.Y. and RadhaKrishnan,T. T. 2002. Acute toxicity evaluation of Copper, Arsenic and HCH to paddy field crab, *Paratelphusa hydrodromous* (Herb.). *J. Environ. Biol.* 23(4): 387-392.

Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson, M Hung, H. and Bidleman, T. 2010. Endosulfan, a global pesticide: a review of its fate in the environment and occurrence in the Arctic. *Sci. Total Environ.* 408: 2966-2984.

Wirth, E.F., Lund, S.A., Fulton, M.H. and Scott, G.I. 2001. Determination ofacute mortality in adults and sublethal embryo responses of *Palaemonetes pugio* to endosulfan and methoprene exposure. *Aquat Toxicol*. 53 : 9-18.

Yasser, A. G. 2012. Haematological and micronuclei alterations in common carp *Cyprinus carpio* exposed to Cypermethrin. *J. Thi-Qar Sci.* 3(3): 37-50.

Yasser, A. G. and Naser, M. D. 2011. Impact of pollutants on fish collected from different parts of Shatt Al-Arab River: a histopathological study. *Environmental Monitoring and Assessment*. 181(1): 175-182.

Yasser, A. G., Naser, M. D. and Al-Gheezy, K. W. 2010. The effect of chlorpyriphos pesticides on the biochemical contents in the tissues of fresh water snail *Bellamya bengalensis* (LAMARCK). *Marsh Bulletin*. 5(1) : 96-102.

Yasser, A.G., Naser, M.D. and Aziz, N.M. 2008. Acute toxicity of endosulfan to immature and adult gastropods *Lymnaea radix cor* (Annandale and Prashad, 1919). *J. Thi-Qar Sci.* 1 : 13-18.