ECOTOXICITY ANALYSIS OF TRICLOSAN AGAINST *MOINA MACROCOPA* A ZOOPLANKTON

Abstract

Several artificial chemicals are categorized as potential environmental endocrine-disrupting chemicals (EDCs), they affect the health of livestock, wildlife and human beings. In recent years, human exposure to environmental EDCs has received an increased awareness due to their association with altered human health as documented by several epidemiological and experimental studies. As a new and ubiquitous trace organic pollutant, endocrine-disrupting compounds (EDCs) can cause endocrine-disrupting effects on organisms even at low levels. The current study is attempted to estimate the acute and chronic effect of triclosan on *Moina macrocopa*, which was collected and cultured from the local water body in Tiruchirappalli district. The acute toxicity was estimated as LD50 (55ug/ml). In the case of chronic exposure study 20 % of viability was observed for 15 days. A docking study revealed that RRM Protein has a binding affinity for triclosan. The amino acid lysine at 140th position forms a hydrogen bond with triclosan.

Authors

M Rajathi D Modilal
PG and Research
Department of Zoology
Holy Cross College (Autonomous)
Tiruchirapalli.

Anusiya T
PG and Research
Department of Zoology
Holy Cross College (Autonomous)
Tiruchirapalli.

I. INTRODUCTION

Endocrine Disrupting Chemicals (EDC) are a wide category of organic and inorganic substances that have a population-level impact at a very low concentration (nanogram levels). Organisms may be exposed to endocrine disruptors through the consumption of food and beverages, the use of pesticides, the usage of plastic bottles, detergent and flame retardants in food and cosmetic(Martinez-Jeronimo and Gutierrez-Valdivia., 1991; Park *et al*., 2017). In essence, when one comes in contact with these substances through our diet, air or water, endocrine disrupting chemicals may be dangerous in even modest concentration. Slight fluctuations in hormone levels can have a large impact on biological processes and development. Wildlife is negatively affected by endocrine disrupting substances. Endocrine disruptor are chemicals that mimic or alter the body's hormones. These chemicals have been linked to developmental, reproductive, brain, and other tissues. Endocrine disruptors include both natural and man-made chemicals; certain endocrine disruptor takes a while to degrade in the environment. They can eventually be dangerous due to such trait. An exogenous chemical or mixture known as a "endocrine disruptor" alters the way the endocrine system functions and consequently has a negative impact on the health of an intact organism, as well as its offspring and subpopulations. Attention has been drawn to the endocrine effects of many compounds, including metals like arsenic compound and parabens, which are commonly used in cosmetics and toiletries and are components of UV-screen(Jia *et al*., 2018). This endocrine disruption is closely related to the dysregulation of reproduction and development, which is used to manage the population of organisms exposed to pollutant(Silva *et al*., 2015) Some of the EDC are Bisphenol A (BPA), Bisphenol F (BPF), Bisphenol S (BPS), Dioxins, Perchlorates, Perfluoroalkyl and Polyfluoroalkyl substances (PFAS), Phthalates, Phytoestrogens, Polybrominated diphenyl ethers (PBDE), Polychlorinated biphenyls (PCB), Triclosan, Lead, Arsenic, Mercury, Atrazine, glycol ethers etc(Wong *et al*., 1995). Zooplankton population address the environmental fluctuation condition(Wuerz *et al*., 2019) ranging from seasonal and predictable to unusual(Razak *et al.*, 2022) and unpredictable occurrence(Syberg *et al*., 2017).

Zooplankton is an important community in the aquatic ecosystem for energy transfer from primary producers to fishes. Zooplankton species distribution and abundance are influenced by environmental factors such as water transparency, climate change and nutritional food content. The abundance and diversity of zooplankton and phytoplankton are affected by available diets in the environment. Therefore, water salinity shifts also can alter the original taxa composition and ecological processes, such as primary productivity, decomposition, nutrients cycles and food web function; salinity increase in freshwater can also reduce zooplankton richness, especially in the cladoceran community and thus, change the adaptation of the species to a more salt-tolerant species. Cladoceran are an important group in zooplankton community with most of the species living in freshwater environments with salinities less than one. The exposure to EDC can lead to long term effects on reproduction and development which can become evident later, even at sexual maturity and or adulthood. The identification and characterization of this 'early exposure –late effects' pattern of EDCs still represent a risk assessor(Kato *et al*., 2011; Ignoto *et al*., 2022). The Moinidae family of crustaceans, also known as water fleas, includes *Moina sp*., which can survive in both brackish and marine environments and inhibits freshwater. *Moina* sp. has an asexual and sexual phase in its reproductive cycle. Most of the population, which reproduces asexually, is usually made up of females. *Moina sp*. reproduces when it is only 4 to 7 days

old under ideal circumstances, with a brood size of 4 to 22 per female. Every 1.5 to 2.0 days, broods are produced, with many females having 2 to 6 broods in their lifetimes. The generation of males and sexual reproduction under unfavourable environmental conditions leads to the production of resting eggs (ephippia).

When brine shrimp eggs are produced, this situation is comparable. In populations of *Moina sp*., a sudden decrease in the food supply and a considerable shift in the habitat serve as the catalysts for the transition from asexual to sexual reproduction. The pharmaceutical and personal care products (PPCPs) have been widely used, resulting in great concern in recent years as emerging threats to aquatic environments and human health(Jung *et al*., 2020). Triclosan (TCS) is an antimicrobial agents used in several personal care products including toothpastes, mouth rinses and other frequently used products ,such as plastics, shoes, textiles, and food packaging materials(Verdu *et al*.,2021).

Triclosan has been used in almost every part of the world including in European Union, where approximately 350 tons of triclosan are produced annually(Caserta *et al*., 2008). Triclosan's has been detected in wastewater treatment effluents(Kadiene *et al*., 2020) at concentration between 0.01 and 2.7µg/L after entering wastewater treatment, it is not completely removed and consequently it is released into the environment. In Portugal, triclosan was detected in urban wastewater samples at low level of concentration:124.1 ng/L. Other concern is that even after triclosan prohibition in some countries, it stills remains a problem as it aggregates in wastewater sludge and it might be transferred to water environment, persisting for months and years.TCS has been widely used in household personal care products(Saaristo *et al*.,2009) and must be detected at a high frequency in sewage treatment plan (STP). The effects of TCS on life cycle parameters (e.g., survival and reproduction). These zooplankton are used as a laboratory model species due to its small size (less than 1mm), sexual dimorphism, high fecundity and great tolerance against stress (temperature, pH, salinity)(Falfushynska *et al*., 2017; Yuslan *et al*., 2021) Among these unique features that have demonstrated utility in Acute(Brenda Karen Gonzalez-Perez *et al*.,2018) and Chronic ecotoxicity(Nandini and Sarma., 2019), also their behavioral changes of exposing TCS with treated and control were compared also with their growth aspects response to the triclosan. The present investigation was conducted to study the effect of triclosan on *Moina macrocopa*, its toxicity and lethal dose.

II. MATERIALS AND METHODS

1. **Sample Collection:** The sample was collected from (Uyyankondan river) sewage water at three different regions and they were filtered to remove the debris, it was maintained in a dechlorinated freshwater. To identify type of zooplankton, they were observed under the light microscope, after that zooplankton were maintained under laboratory conditions at suitable temperature.

2. **Culture Maintenance:** *Moina macrocopa* was cultured under a laboratory condition with ambient temperature of 27℃ and pH 7. In this study, the filtered sewage water organisms were transferred and maintained in a dechlorinated freshwater. The feed of this zooplankton was spirulina fed once a day. And water was replenished once in two days on dechlorinated freshwater. The process is carried frequently to maintain the culture.

3. **Reagents**: In this study of exposure chemicals, Triclosan with molecular formula of $C_{12}H_7Cl_3O_2$ and molecular weight (MW=289.54, purity (>98.0%), Lot.JL400 TE) was taken, Dimethyl sulfoxide (DMSO) an organosulfur compound as a penetrating vehicle was used as a solvent to enhance the solubility of the tested solution. To prepare the concentration stock solution of (500 mg/ml). Triclosan was dissolved in dimethyl sulfoxide (DMSO).

4. **Acute Toxicity of TCS in *Moina* (nauplii) and Ovigerous Female**: Ten *nauplii* were collected shortly after hatching (<12 hours), along with food it has been treated to various concentration of triclosan (TCS), (control, vehicle control,10, 50 and 100 µg/L) for 48 hours in order to assess the variation in mortality of nauplii. **Acute toxicity** for ten ovigerous females were given 96 hours of exposure to various concentration of triclosan (control, vehicle control,100, 500, and 800µg/L). Every experiment was carried out in biological duplicate. Under the light microscope with low magnified power, the number of dead nauplii and ovigerous females was counted separately to determine the mortality rate. Every 24 hours throughout the experiment, each beaker represented a unique treatment condition, was replenished with half of the water to the initial volume. Once daily, ovigerous females were fed spirulina.

5. **Chronic Toxicity:** In Chronic test, ten nauplii were exposed beyond providing food and various triclosan (TCS) were used during the treatment (control, vehicle control, 10, 50, and 100µg/L). This study was carried out by the duration of 15 days. Each plate in the experiment, which represented a different treatment condition, along with dechlorinated water, chemicals also be refilled by every 24 hours with half of its initial volume. At the end of the day, total number of *Moina macrocopa* were recorded together with nauplii, adult and ovigerous female were also observed under the microscope. Throughout the experiment spirulina were fed daily.

6. **Immobilization Test:** In these tests, organisms with less than 24 hours were incubated. *Moina macrocopa* is checked for immobilization 24 and 48 hours after the test's start, number of immobilized and mobilized organisms was noted. Immobile organisms were those that did not move after the test beakers were gently stirred. Moreover, the mortality of nauplii can be noted for periods of 24 and 48 hours. Five nauplii were used in the experiment for each treatment and control. During the 48-hour experiment, nauplii were individually exposed to the test solution of triclosan in 50 ml dechlorinated freshwater without any feeding. Although, during the experiment water and chemicals was refilled with half of its initial volume by 24 hours. To conduct an experiment using biological duplicates. In these studies, concentration of triclosan (control, vehicle control, 100, 500, and 1000 μg/L) was implemented. (Ana Rita R. silva, *et al*.)

7. **Fecundity Test:** Single ovigerous female were subjected to TCS, (control, vehicle control,10, 50, and 100µg/L in 50 ml of dechlorinated freshwater to assess the effects of TCS on fecundity. Using a light microscope, the number of nauplii in each plate was counted. Also, the male and female were observed in an adult stage of *Moina macrocopa*. Once every 24 hours, 50% of the test solution was replaced during the TCS exposure period. Ovigerous female were fed spirulina once daily.

8. **DNA Isolation:** The cultures were inoculated in to 100 ml of fresh water with aerator and spirulina feed for every 8 hrs once, until it reached the number of individuals for molecular work.

Further for species identification, Universal COI gene sequence of the zooplankton was amplified with the extracted DNA using universal primers LCO1490 F 5'- GGT CAA CAA ATC ATA AAG ATA TTG G and HCO 2196 R 5'- TAA ACT TCA GGG TGA CCA AAA AAT CA (Edwards *et al*. 1989). Amplification was carried out in a thermal cycler (Gene Amp 2700, Applied Biosystems, USA) using following temperature program: initial denaturation at 94ºC for 5 min, 30 cycles of 94ºC for 30 s, 55ºC for 30 s and 72ºC for 1.5 min, and final extension of 72ºC for 15 min.

Amplified PCR products were purified and prepared for Cycle sequencing using the Big Dye® Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA). Denatured products were subjected for sequencing in forward and reverse direction individually using Genetic Analyzer 3500 (Life Technologies Corporation, Applied Biosystems®, California 94404, USA) as per manufacture's instruction. Sequences were aligned and edited using Mega software version 11 (Tamura *et al*. 2020) to confirm the species NCBI BLAST analysis were performed. From the BLAST analysis evolutionary history was inferred using the Neighbor-Joining method.

9. **RNA Extraction and Gene Expression:** From the species of *Moina macrocopa* RNA was isolated using TRI reagent. Further it was converted into cDNA for gene expression studies. cDNA conversion was carried out R 2D 1st strand cDNA synthesis kit by GCC BIOTECH. Primer for dsx like genes were designed and procured from GCC biotech.

Forward and reverse primer for DSX gene.

Table 1: Primer Design

Primer	**Sequence**
DSXF	ATCCTCCAACAACTCGAGCG
DSXR	TCGCGTAGCATGGACACATT

Amplification cycles were 94℃ for 7 mins and 30x (94℃ for 30 sec, 52℃ for 30 sec, 72℃ for 30 sec). After the PCR, amplification results were visualized by performing 1.5% agarose gel electrophoresis and ethidium bromide staining.

III. DOCKING

The structure of RRM1 was not known when this study was designed, only the sequence obtained from whole genome sequence by short gun method was collected. The popular sequence alignment approach BLAST (Basic Local Alignment Search Tool) was used to detect homolog protein sequences to RRM1 of *Moina macrocopa* in database. The BLAST algorithm is very efficient due to its heuristic approximation of the Smith-Waterman algorithm. The resulting sequences was used to construct structure. Windows platform package of EMBL Expasy. The modelled structures were further subjected to energy minimization and validation by 200 steps of energy minimization using the Newton Raphson

algorithm and the CHARMm version 22 force field (Brooks, Bruccoleri *et al*. 1983). The resulting energy minimized protein structures were further analysed with the PROCHECK and the QMEAN score as provided by the SWISS-MODEL server (Laskowski, Chistyakov *et al*. 2005; Benkert, Tosatto *et al*. 2008) and selected the best structure. The ligand structure was collected from the drug database. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein-ligand interaction energies (Accelrys Software Inc, 2008)

IV. RESULTS

The water sample was collected from the river water, the city sewage water and the site at which they join in the Uyyankondan river. The water was rich in zooplanktons.

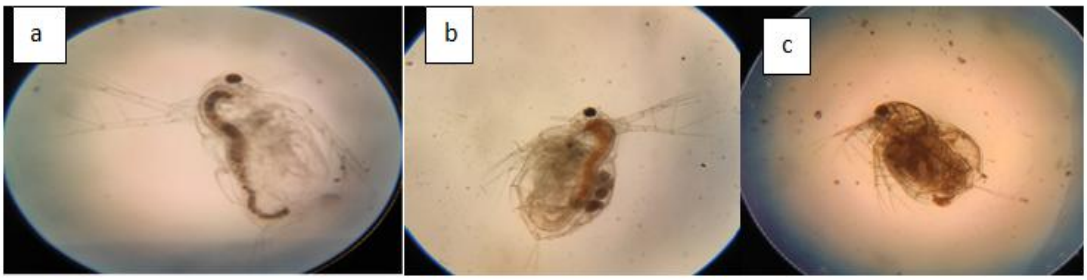

Figure 1: Photograph of a. *Moina macrocopa* b. Ovigerous Female of *Moina macrocopa* c.Nauplii of *Moina macrocopa*

Based on the morphological characters, the zooplanktons were preliminarily screened and it was found that the sample was rich in *cyclopoid copepods* and *Moina* species, among the isolated organisms, *Moina* species was predominant. Both were cultured, between the two *Moina* grew fast, hence it was selected for the current study. The *Moina* species isolated was subjected to species identification and it was found out that the species belonged to *Moina macrocopa*(Fig 1). The acute toxicity of *Moina macrocopa* of nauplii is depicted in figure 2, the results show that there is no significant mortality in control and vehicle control groups at 24 and 48 h of exposure to different concentrations of Triclosan. LD50 was calculated from the linear regression equation and it was found that the LD50 of 24 h was 44 ug/L and for 48 h exposure it was 32 ug/L (Figure 2).

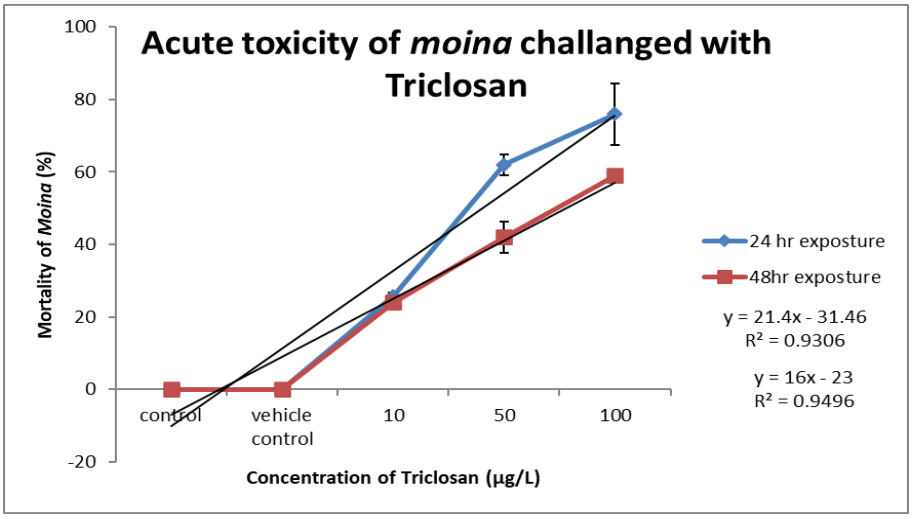

Figure 2: Mortality of M. macrocopa exposure of TCS

Table 2: Acute toxicity of Triclosan on survival and mortality of nauplii of *M. macrocopa* at 24 h and 48 h of exposure

Group	No of Survival at 24 h	No of mortality at 24 h	No of survival at 48 h	No of mortality at 48 h
Control	**10**	**0**	10	0
vehicle control	**9.5**	**0.5**	8.5	1.5
10µg/L	**7.5**	**2.5**	7.5	2.5
50µg/L	**6**	**6**	5.5	4.5
100µg/L	**3**	**7**	4	6

From table 2, it is observed that morality of the nauplii increased as the concentration of triclosan dose and time of exposure to triclosan increased. Mortality of nauplii was observed at the highest concentration of triclosan 100 ug/L for both 24 h and 48 h study. The acute toxicity of ovigerous female of *Moina macrocopa* against different concentrations of triclosan is given in figure 3, able 4 and 5, the results shows that there was no significant mortality in control and vehicle control groups. The study revealed that LD 50 value of adult *Moina macrocopa* range from 230 to 360 ug/L concentration for 24 to 76 h exposure.

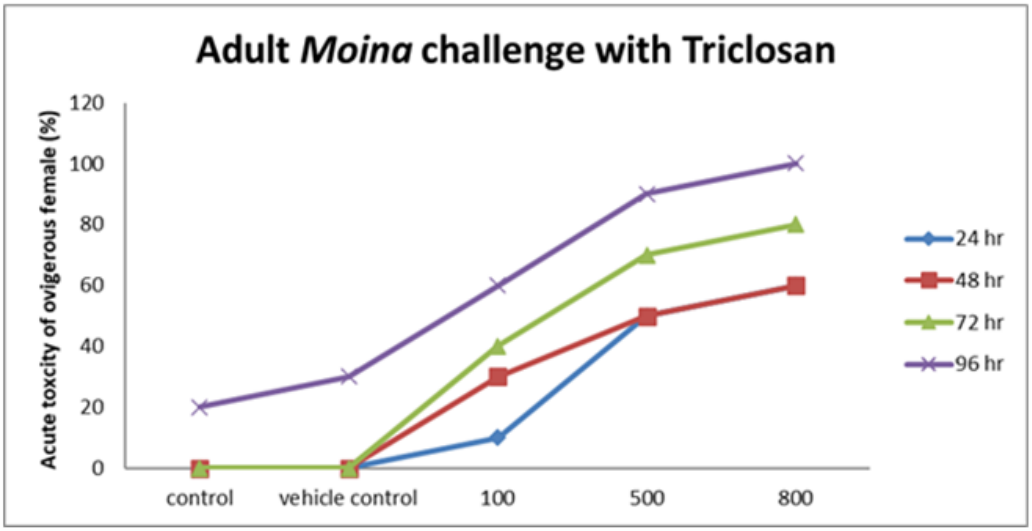

Figure 3: Mortality of ovigerous female to different concentrations of triclosan for a period of 96 h

Table 3: Tabulation for Survival of ovigerous female

Concentration	24 h	48 h	72 h	96 h
Control	10	10	10	8.5
vehicle control	10	10	9.5	7.5
100µg/L	8.5	6.5	6	4.5
500µg/L	6	5	3.5	1.5
800µg/L	4	5.5	2	0

Table 4: Tabulation for mortality of ovigerous female

concentration	24 h	48 h	72 h	96 h
Control	0	0	0	1.5
vehicle control	0	0	0.5	2.5
100µg/L	1.5	3.5	4	5.5
500µg/L	4	5	6.5	8.5
800µg/L	6	6.5	8	10

Whereas for 96 h exposure it was around 80ug/L. Ovigerous female of *Moina macrocopa* showed a low survival and high morality as the concentration of triclosan increased from100 ug/L to 800 ug/L(Table 3 and 4).

The chronic exposure study results are shown in figure 4 and in table 5, study was initiated with 10 nauplii in each group and allowed for two life cycles of *Moina macrocopa* which is equal to 15 days of time period. At the end of the study period control group shows 30 individuals with 5 Ovigerous females and 10 nauplii's and sexually undisguisable adults. Whereas in vehicle control it was 3, 8 and 8 individuals, respectively. In both the groups

mortality was observed which was 6 and 11 individuals, respectively. Increasing concentration of triclosan shows increasing mortality rate as evident from the table 5.

Table 5: Observed values of Chronic test.

	Control	vehicle control	10µg/L	50µg/L	100µg/L
No of ovigerous female	**5**	**3**	**1**	**0**	**0**
No of nauplii	**10**	**8**	**2**	**0**	**0**
No of adult	**9**	**8**	**2**	**2**	**0**
Dead	**6**	**11**	**16**	**19**	**23**

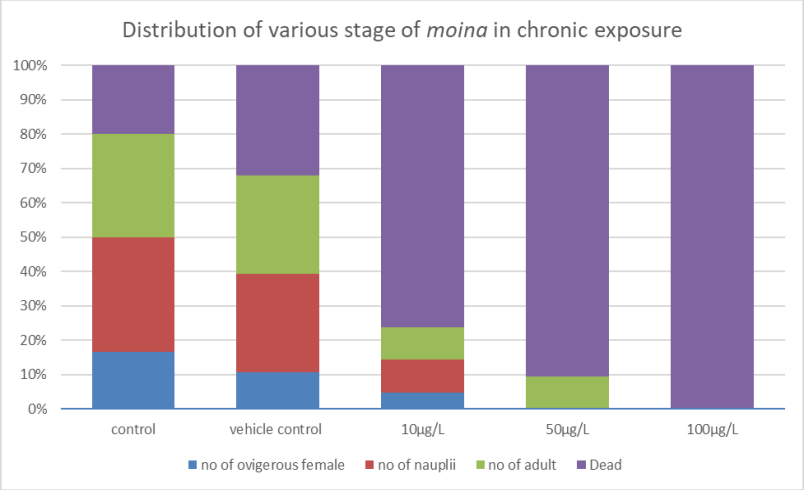

Figure 4: Different stages of M. macrocopa in chronic exposure

Immobilization tests were carried to identify if the species were able to be mobilize or immobilize at different concentrations of triclosan(Table 6 and Figure 5). In this test,5 nauplii were introduced in each plate for a period of 24 h and 48 h respectively. Results shows that nauplii were immobilized by 20% and mortality of nauplii is 90 % (Figure 5).

Table 6: Rate of Immobilization of nauplii at 24 h and 48 h of triclosan exposure**.**

	Immobilized at 24 h	Immobilized at 48 h
Control	**0.5**	**0**
vehicle control	**1.5**	**0.5**
100µg/L	**1**	**1**
500µg/L	**0**	**1**
1000µg/L	**0**	**1**

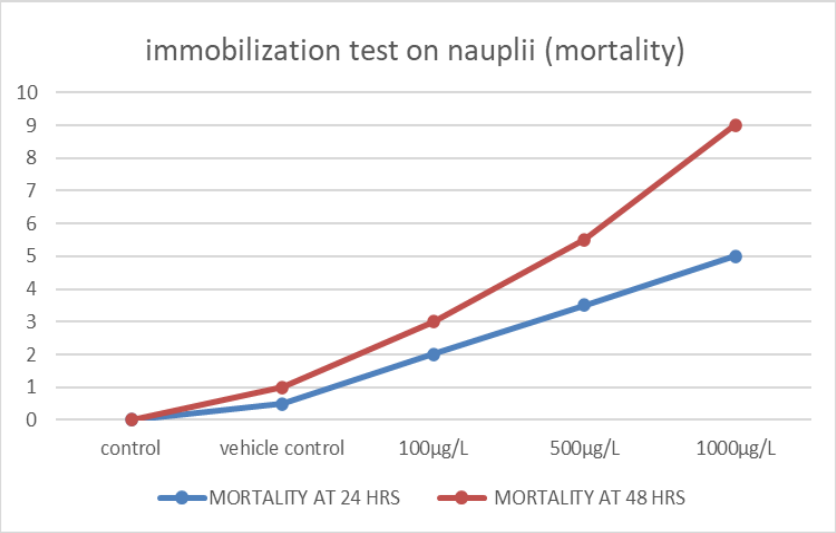

Figure 5: Mortality of Immobilization Test

V. FECUNDITY TEST

The fecundity analysis was studied in duplicates with 6 young ones for each group and observed for 15 days for male female maturation from the sexually undistinguishable young ones. The study revealed that in control and vehicle control the young ones turn to 2.5: 3.5 female to male ration and without any mortality. Triclosan challenge gradually increased the male dominance and the female adults was gradually reduced along with increasing mortality. The results are shown in Figure 6 and table 7. To identify the male and female *Moina macrocopa*, a slight difference is observed, like male are smaller than female and female body structure are slandering also its antenna are larger than male, along with its characteristic features we also identified the sex of the *Moina*.

Table 7: Tabulation for Fecundity Test

	no of nauplii	no of male	no of female	no of mortality	no of survival
Control	**6**	**2.5**	**3.5**	**0**	**6**
Vehicle control	**6**	**3.5**	**2.5**	**0**	**6**
10µg/L	**6**	**2.5**	**2.5**	**1**	**5**
50µg/L	**6**	**2**	**1**	**2.5**	**3.5**
100µg/L	**4**	**0**	**0**	**4**	**0**

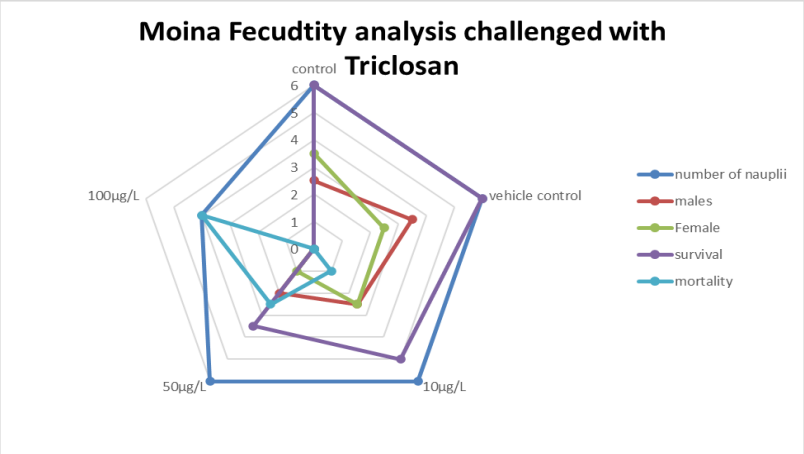

Figure 6: Fecundity Test

VI. DNA ISOLATION

The FASTA sequence is provided below after alignment and editing of COI gene submitted to NCBI. **Accession number provided by NCBI is** OQ726603.

1. The possible organism based on the BLAST: *Moina macrocopa*.

> OQ726603

CAACAAATCATAAAGATATTGGTACACTCTATTTCATATTTGGAATCTGATCAGG
TATAGTAGGAACAGCGCTTAGTATACTTATCCGATTTGAACTAGCTCAAGCAGGA
AATTTTATTGGAGATGATCAAGTTTATAATGTAATCGTAACTGCCCATGCGTTTAT
TATAATTTTCTTCATAGTTATACCAATTTTAATTGGAGGGTTTGGTAATTGACTAG
TACCCTTAATATTAGGAGCCCCTGATATAGCTTTCCCCCGACTAAATAATTTAAG
TTTTTGACTTTTACCCCCCGCTCTTACACTACTACTAGTAGGAGGGGCTGTAGAA
AGAGGAGCAGGAACAGGATGAACAGTCTATCCTCCATTATCAGCAGGAATTGCT
CACGCGGGAGCATCTGTTGATTTAAGAATTTTTTCTCTTCATCTAGCAGGAATTTC
TTCTATTTTAGGAGCTGTAAATTTCATTACAACAATTATTAATATACGAACACAA
GGAATAACTCTTGATCGAATC

2. **Phylogenetic Analysis:** To uncover the variations and their functional role, the corresponding amino acid sequences from the available COI genes of M. macrocopa strains were aligned by ClustalW multiple sequence alignment as shown in figure 7

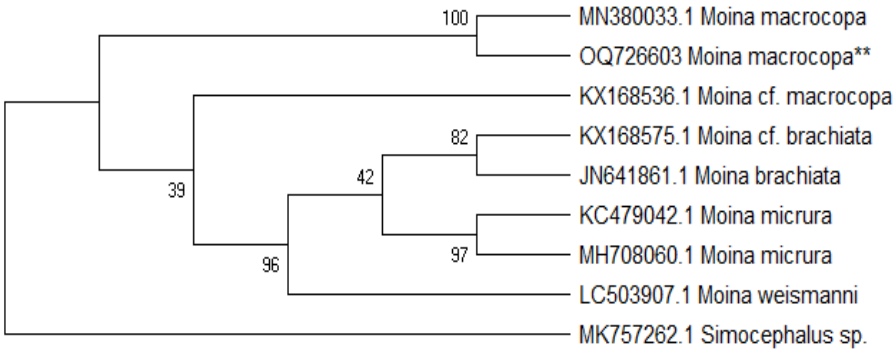

Figure 7: Phylogeneictreeof*Moina*macrocoppa

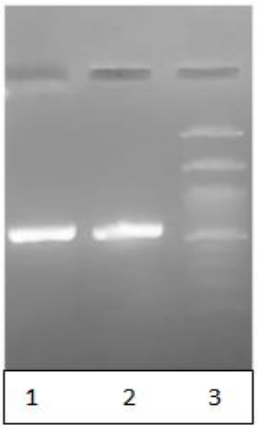

Figure 8: Lane 1: Positive control; Lane 2:sample; Lane 3:100bp DNA ladder

Gel image amplification COI gene for species identification shows the presence of 500 bp band, which confirms the presence of the gene.

VII. GENE EXPRESSION

The current study also studied the modulation of DSX gene of *Moina* on triclosan challenge. Since the gene sequence of *Moina macrocopa* is not yet annotated, possible gene sequence was extrapolated from the short gun whole genome sequence using phylogenetic analysis with various taxon, it was found the conserved sequence is present in all taxa, based on phylogenetic analysis, the primer was designed (Table 1).

Since Triclosan is an endocrine disruptor, it alters the gene expression by binding to RRM1 motif of DNA binding protein. Since the structure of RRM1 is not elucidated so far homology modeling was done using EMBL Expasy homology tool using PDB ID of 6r5k. The docking was conducted with EMBL EXPASY tool.

VIII. DOCKING

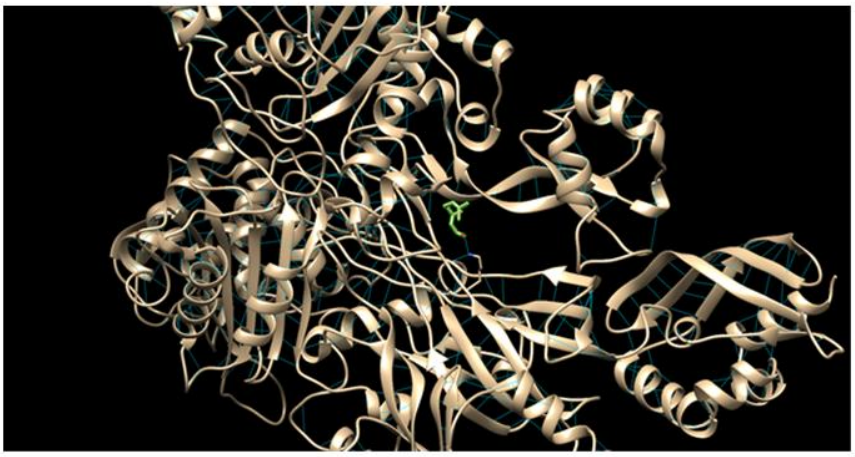

Figure 9: Interaction of RRM1 protein with the triclosan has been studied with the help of molecular docking work. The golden colour back bone is representing the RRM1 protein and green colour molecule is the ligand Triclosan. Blue colour line indicating the Hydrogen bonds triclosan interacting with 148th amino acid lysine molecule.

IX. DISCUSSION

The collected wastewater was rich in *Moina* like organism, they were collected and cultured (Rottmann *et al*.2003) in-fish tanks with aerator, temperature control and feed. The well grown cultures were periodically collected and subjected to analysis. The species of *Moina macrocopa* was identified by molecular technique COI gene sequencing. The fresh water and city sewage water mingling junction is known for diversified zooplanktons. the current study also supports the earlier report.

Among them *Moina macrocopa* was cultured and used in current study. With the help of COI sequencing, the species was identified as *Moina macrocopa* and the sequence was submitted into NCBI database with the accession number OQ726603. The current study of acute toxicity of the *moina macrocopa* was studied with nauplii and adult and both showed different LD 50 value, this observation was supported by earlier studies. *Moina* is an important part of freshwater food chain and food web. The endocrine disruptors are group of molecules which are highly heterogeneous in nature, that includes numerous chemicals and are a significant concern to public health. The current study is first of its kind as per the literature survey, there are very few reports on *Moina* species challenged with pollutants. With respect to the life cycle study, fecundity analysis also there is no direct literature for *Moina macrocopa* fecundity analysis with respect to pollutant such as triclosan.

DSX gene has been reported for sex differentiation for various organisms, it has been reported that environmental endocrine disruptor's modulate its expression. However, the gene is not yet studied in zooplanktons which are prime consumers in food chain. This current study attempted to explore DSX gene with the help of ortholog gene information. In the current study we were not able to amplify DSX gene from transcript level as well as genomic level. From the docking study, the current work has proved that Triclosan has strong affinity

towards RRM1 which is multi gene regulating element of the genome. This interaction may lead to beneficial or adverse effect to the exposed organism, which requires further study.This study is first, kind addressing toxicity (acute, Chronic, recovery study and fecundity) effect of a triclosan on *Moina macrocopa*. This study further open avenue for further molecular characterization including ecotoxicity, biomagnification and environmental health issues.

X. SUMMARY AND CONCLUSION

We conclude that the ecotoxicity study both acute and chronic at different concenrations of triclosan was done on *Moina macrocopa*, this is the first study conducted in addition immobilization test and fecundity experiment was also carried out. The reproduction of *Moina* is affected, if proper measures are no taken against triclosan it will decrease the rate of population among aquatic organisms. The female *Moina macrocopa* was converted into male after it released its brood and some *Moina macrocopa* were parthenogenetic female. The FDA recently restricted the use of TCS to specific soap formulations.

XI. ACKNOWLEDGEMENT

Authors wish to acknowledge the help received from Dr. R. Muhil and team, Par Life Science and Research Private Limited, Woraiyur, Tiruchirappalli 620 003 in execution of the work.

REFFERENCE

[1] Martinez-Jeronimo, F., & Gutierrez-Valdivia, A. (1991). Fecundity, reproduction, and growth of *Moina macrocopa* fed different algae. Hydrobiologia, 222, 49-55.
[2] Park, J. C., Han, J., Lee, M. C., Seo, J. S., & Lee, J. S. (2017). Effects of triclosan (TCS) on fecundity, the antioxidant system, and oxidative stress-mediated gene expression in the copepod *Tigriopus japonicus*. Aquatic Toxicology, 189, 16-24.
[3] Jia, J., Liu, X., Li, L., Lei, C., Dong, Y., Wu, G., & Hu, G. (2018). Transcriptional and translational relationship in environmental stress: RNAseq and ITRAQ proteomic analysis between sexually reproducing and parthenogenetic females in *Moina micrura*. Frontiers in Physiology, 9, 812.
[4] Silva, A. R. R., Cardoso, D. N., Cruz, A., Lourenço, J., Mendo, S., Soares, A. M., & Loureiro, S. (2015). Ecotoxicity and genotoxicity of a binary combination of triclosan and carbendazim to *Daphnia magna*. Ecotoxicology and environmental safety, 115, 279-290.
[5] Wong, C. K., Chu, K. H., & Shum, F. F. (1995). Acute and chronic toxicity of malathion to the freshwater cladoceran *Moina macrocopa*. Water, Air, and Soil Pollution, 84, 399-405.
[6] Wuerz, M., Whyard, S., Loadman, N. L., Wiegand, M. D., & Huebner, J. D. (2019). Sex determination and gene expression in *Daphnia magna* exposed to juvenile hormone. Journal of Plankton Research, 41(4), 393-406.
[7] Razak, M. R., Aris, A. Z., Yusoff, F. M., Yusof, Z. N. B., Abidin, A. A. Z., Kim, S. D., & Kim, K. W. (2022). Risk assessment of bisphenol analogues towards mortality, heart rate and stress-mediated gene expression in cladocerans *Moina micrura*. Environmental Geochemistry and Health, 1-17.
[8] Syberg, K., Nielsen, A., Khan, F. R., Banta, G. T., Palmqvist, A., & Jepsen, P. M. (2017). Microplastic potentiates triclosan toxicity to the marine copepod *Acartia tonsa* (Dana). Journal of Toxicology and Environmental Health, Part A, 80(23-24), 1369-1371.
[9] Kato, Y., Kobayashi, K., Watanabe, H., & Iguchi, T. (2011). Environmental sex determination in the branchiopod crustacean *Daphnia magna*: deep conservation of a Doublesex gene in the sex-determining pathway. PLoS Genetics, 7(3), e1001345.
[10] Ignoto, S., Pecoraro, R., Scalisi, E. M., Buttigè, S. E., Contino, M., Ferruggia, G., ... & Brundo, M. V. (2022). Acute Toxicity of a Marine Emerging Pollutant (Promethazine Hydrochloride) on *Artemia* sp. ACS omega, 7(44), 39619-39623.

[11] Jung, J. W., Kang, J. S., Choi, J., & Park, J. W. (2020). Chronic toxicity of endocrine disrupting chemicals used in plastic products in Korean resident species: Implications for aquatic ecological risk assessment. Ecotoxicology and Environmental Safety, 192, 110309.
[12] Verdú, I., González-Pleiter, M., Leganés, F., Rosal, R., & Fernández-Piñas, F. (2021). Microplastics can act as vector of the biocide triclosan exerting damage to freshwater microalgae. Chemosphere, 266, 129193.
[13] Caserta, D., Maranghi, L., Mantovani, A., Marci, R., Maranghi, F., & Moscarini, M. (2008). Impact of endocrine disruptor chemicals in gynaecology. Human reproduction update, 14(1), 59-72.
[14] Kadiene, E. U., Ouddane, B., Gong, H. Y., Kim, M. S., Lee, J. S., Pan, Y. J., ... & Souissi, S. (2020). Differential gene expression profile of male and female copepods in response to cadmium exposure. Ecotoxicology and Environmental Safety, 204, 111048.
[15] Saaristo, M., Craft, J. A., Lehtonen, K. K., & Lindström, K. (2009). Sand goby (Pomatoschistus minutus)males exposed to an endocrine disrupting chemical fail in nest and mate competition. Hormones and Behavior, 56(3), 315-321.
[16] Falfushynska, H. I., Gnatyshyna, L. L., Horyn, O., & Stoliar, O. B. (2017). Vulnerability of marsh frog *Pelophylax ridibundus* to the typical wastewater effluents ibuprofen, triclosan and estrone, detected by multi-biomarker approach. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 202, 26-38.
[17] Yuslan, A., Najuwa, S., Hagiwara, A., Ghaffar, M. A., Suhaimi, H., & Rasdi, N. W. (2021). Production performance of *Moina macrocopa* (Straus 1820) (Crustacea, cladocera) cultured in different salinities: the effect on growth, survival, reproduction, and fatty acid composition of the neonates. Diversity, 13(3), 105.
[18] Brenda Karen Gonzalez-Perez, S.S.S. Sarmab, M.E. Castellanos-Paez, S. Nandini,2018. Multigenerational effects of triclosan on the demography of *Plationus patulus* and *Brachionus havanaensis* (ROTIFERA). Vol.147, pg. no 275-282.
[19] Nandini, S., & Sarma, S. S. S. (2019). Reproductive strategies of *Moina* (Cladocera) in relation to their habitat. Limnetica, 38(1), 137-145.
[20] Xu, S. L., Zhou, W., Chen, P., Zhou, J. K., Zou, X., Wang, C. L., ... & Zhao, Y. L. (2014). Identification and expression analysis of a doublesex1 gene in *Daphnia pulex* during different reproductive stages. Development genes and evolution, 224, 147-157.
[21] Kabery, K., Anisuzzaman, M., Jeong, U. C., & Kang, S. J. (2019). Culture of *Moina macrocopa* using different types of organic wastes. Asian Journal of Fisheries and Aquatic Research, 4(1), 1-13.
[22] David R. Orvos, Donald J. Versteeg, Josef Inauen, Marie Capdevielle, Arthur Rothenstein, Virginia Cunningham (2009). Aquatic toxicity of Triclosan, Volume 21 Pages:1323-1532.
[23] Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.
[24] Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.
[25] Nei M. and Kumar S. (2000). Molecular Evolution and Phylogenetics. Oxford University Press, New York.
[26] Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.
[27] Weatherly, L. M., & Gosse, J. A. (2017). Triclosan exposure, transformation, and human health effects. Journal of toxicology and environmental health. Part B, Critical reviews, 20(8), 447–469.
[28] Peng, Y., Luo, Y., Nie, X. P., Liao, W., Yang, Y. F., & Ying, G. G. (2013). Toxic effects of triclosan on the detoxification system and breeding of *Daphnia magna*. Ecotoxicology, 22, 1384-1394.
[29] Annamalai, J., & Namasivayam, V. (2015). Endocrine disrupting chemicals in the atmosphere: Their effects on humans and wildlife. Environment international, 76, 78-97.
[30] Navis, S., Waterkeyn, A., De Meester, L., & Brendonck, L. (2018). Acute and chronic effects of exposure to the juvenile hormone analog fenoxycarb during sexual reproduction in *Daphnia magna*. Ecotoxicology, 27, 627-634.
[31] Lin, D., Li, Y., Zhou, Q., Xu, Y., & Wang, D. (2014). Effect of triclosan on reproduction, DNA damage and heat shock protein gene expression of the earthworm *Eisenia fetida*. Ecotoxicology, 23, 1826-1832

