EFFECTS OF CHLORPYRIFOS (LORSBAN) ON REPRODUCTIVE PERFORMANCES OF GUPPY (*POECILIA RETICULATA*)

De Silva, P. M C. S
Department of Zoology, University of Ruhuna,
Matara, Sri Lanka.
Telephone: 0094 41 27025
Fax: 0094 4122683
Email: chathura@zoo.ruh.ac.lk

Samayawardhena, L. A,
Department of Zoology, University of Ruhuna,
Matara, Sri Lanka.
Telephone; 0094 41 27025
Fax: 0094 4122683
Email: samaya@zoo.ruh.ac.lk

Abstract

Toxicity of Chlorpyrifos on reproductive disorders including altered fertility, reduced viability of offspring, impaired hormone secretion and modified reproductive anatomy were little concerned. In the present study, we selected Guppy (*Poecilia reticulata*) to investigate the reproductive effects of Lorsban, a common insecticide in Sri Lanka. Male and female guppy were selected with proven fertility from our own colony and the groups of fish (n=12x6/group) were exposed to pre-determined 2µg / l, 0.002 µg /l Chlorpyrifos based on the 96 hrs LC50 for guppy. Mating behavior of pairs was recorded on the 2nd day of exposure. Offspring were counted and survival recorded on the 14th day. Gonopodial thrusts (4 /15 min, in 2 µg/l and 8/15 min in 0.002 µg/l) were significantly different from the control (11/15min, in the control). Similarly, live birth reduced significantly to 8/female in 2 µg/l compared to 27/female in the control group. Survival of offspring at the 14 days was reduced to 66% in 2 µg/l group. Our findings show that low soluble concentrations of Chlorpyrifos can impair reproductive behavior and capabilities of Guppy to a significant extent. F1 generation of treated fish showed reduced survival suggested importance of mating behavior. Pesticide exposure throughout embryonic development could result weak offspring and lesser their survival. Our study confirmed

Chlorpyrifos could potentially alter mating behavior, live birth and F1 survival of Guppy.

Introduction

Chlorpyrifos (O, O–diethyl–O (3,5,6–trichlor–2–pyridyl) phosphorothioate) is a broad-spectrum organophosphate insecticide with growing concern due to its aquatic toxicity (Foe, 1998; Bailey et al 1997). The toxicity effects may include neurological, behavioral and possibly reproductive effects (Mueller-Beilschmidt, 1990; Hill, 1995). Recent studies have revealed that Chloropyrifos together with Diazinon are responsible for most of the toxicity to aquatic organisms (de Vlaming et al., 1993; Moor et al., 1998; Foe et al., 1998). Especially it is very highly toxic to fresh water fish and aquatic invertebrates. The agricultural, residential and commercial use of Chloropyrifos on pest control leads to presence of Chloropyrifos in sufficient concentrations in agricultural runoff and as well as in urban storm water runoff resulting high toxic effects on *Ceridodaphnia* and *Mysidopis*, two zooplankton species (Corner et al., 1998; Larson et al., 1998). Similar toxicity assessment subjected by Lee and Jones-Lee (1997).

Toxicity of Chloropyrifos on zooplankton is well documented, but less work carried on fish. Johnson and Finley (1980), Odenkirchen et al, (1988) documented its acute toxicity. Reproductive disorders including altered fertility, reduced viability of offspring, impaired hormone secretion and modified reproductive anatomy were little concerned. Few early studies give some viable information on this aspect. Dursban exposure on fathead minnows for 200 days resulted a decline of offspring in first generation survival (USPHS, 1989). Growth of early life stage of California grunion (*Leuresthes tenuis*) was reduced 20% and 26% in two low concentrations of Chloropyrifos (Odenkirchen et al., 1988). But the validity of reproduction as a key parameter to evaluate the impacts of chemical pollutants Kime (1999) tends us to study the Chloropyrifos toxicity with some aspects of reproduction.

Guppy (*Poecilia reticulata*, Peters) is selected as the model organism, a livebearer fish species that was introduced to Sri Lanka as a biological tool in mosquito control. The experimental organism Guppy is a viviparous fish with a short reproductive period (Houde, 1997). Male guppies approach two methods of mating behaviour, sigmoid displays and gonopodial thrusts (Evens et al., 1999). They perform a sigmoid display in which the body is held in S- shape while fins are extended and quivered.

Alternatively they may attempt sneaky mating, in which the female is approached sideways or from behind and the modified anal fin the gonopodium is thrust toward the genital pore. Successful mating produced a litter size range from 12 – 46 in monthly intervals depending on circumstances (Hutchins, 1996). In this study we observed their mating behaviour representing an organism level parameter, brood size and the survival of the offspring as a population level parameter with two more or less similar concentrations of Chloropyrifos.

2 Materials and Methods

2.1 Study population and their maintenance

We collected wild guppies (*Poecilia reticulata*) from the urban cannel systems around the Nilwala river basin in the southern region of Sri Lanka. Fishes were returned to the laboratory and stocked in (200 l) tanks. This stock aquarium received fully aerated water from a header tank. The water temperature was kept at 26 ± 2 C^0.These fishes were fed with special aquaria food purchased through local market. By maintaining this colony we select female guppy that belongs to the highest length class (3.5±1.0 cm) was selected as the test female animals assuming that they were well suited to give birth to offspring. They were separated into (18 l) tanks and monitored for 4 – 6 weeks, until they had given birth to offspring due to early fertilization in stock aquaria. Adult males representing the length class (2.0 ± 1.0 cm) were selected based on their early sexual behaviour.

2.2 Stress exposure

Pre determined 2 µg / l, 0.002 µg / l were used as the exposure concentrations based on the 96 Hrs LC 50 for guppy (our own study). Each pair of guppy was transferred to the tanks (23x23x35cm), that contained 10 l of Lorsban® EC 40% (Chloropyrifos) solutions for consecutive three days together with controls. Chlorinated free tap water was used in the process of dilution of the pesticide. Test solutions were changed every 24 hours followed by the addition of fresh Chloropyrifos solution. After three day exposure time male guppies were removed and the females were kept in the test solutions until they produced their brood.

2.3 Mating behaviour

Sigmoid displays and gonopodial thrusts characterize male mating behaviour of guppy. In sigmoid displays the cooperation of the female is necessary hence we observed the number of gonopodial thrusts as our observation because it is an alternative mating tactic, which does not require female reception. When the modified anal fin, gonopodium made contact with the female genital region it was referred as a successful attempt of mating. A day after exposure to the concentrations the male mating behaviour was observed for consecutive two days. The number of gonopodial thrusts performed by each male in the tanks was calculated over 15 minutes using a counting deviser.

2.4 Reproductive rate and Offspring survival

The reproductive capabilities of the pair were estimated by counting the total number of offspring born to the female. The reproductive rate was calculated as the average number of offspring born per female for each concentration and the control. The number of offspring born to each pair was separated into glass tanks (23 x 23 x 35 mm) and monitored for consecutive two weeks. The mortality in each set of offspring was observed in every 24 hrs and the total number of dead offspring was determined after a period of two weeks.

Statistical analysis

The 96 hrs LC50 was determined through probit analysis, using the software SPSS for windows 98.The relationship between the observations in exposed concentrations and control experiments were investigated by ANOVA modeling and comparisons by Student-Newman-Keuls test. All analysis was subjected at < 0.001 probability level.

3.Results

During the overall study period General appearance was prime in condition and no pathological effects were observed. Also there were no changes in feed intake in both control and treatment groups. The 96 hrs LC 50 was 7.17 μg / l. Mating behavior observed as number of gonopodial thrusts performed by male in 8.00 hr, 12.00 hr, and 6.00 hr respectively, with control and two treatments as given in Figure 1.

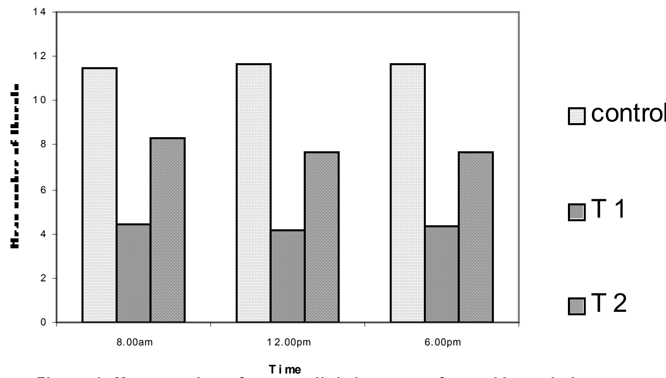

Figure 1: Mean number of gonopodial thrusts performed by male in different periods of the day.

It is quite evident that the time and the number of attempts were not significantly different. But number of gonopodial thrusts by male was considerably reducing with increased concentration of LORSBAN. In control mean number of gonopodial thrusts were recorded as 11 while in males in 2 µg / l (T 1) it was 4 and 8 in the lowest concentration 0.002 µg / l (T 2) (Figure 2).

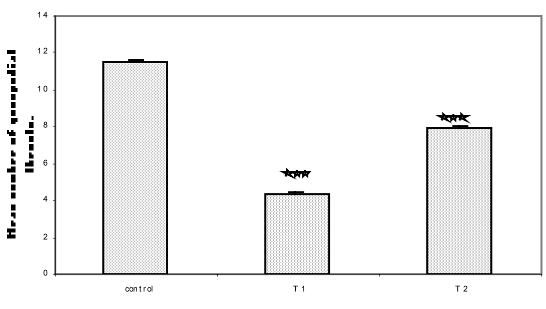

Figure 2 : Mean number of gonopodial thrusts performed by male in exposed concentrations (***P< 0.001).

Each of these values were significantly different at P<0.001. The litter size calculated as number of offspring per female varies to minimum number of 18 to maximum of 36 in the control, which made it to average of 27 offspring per female. Female guppy in exposed concentration 2 µg / l recorded least number of offspring ranging 5 – 11 and mean of 8 offspring per female. But in the lowest concentration the mean number of offspring per female has risen to 24.

Although the mean number of attempts were reduced at 0.002µg / l, still exposed females were able to produce a closer number of offspring as the control (Figure 3).

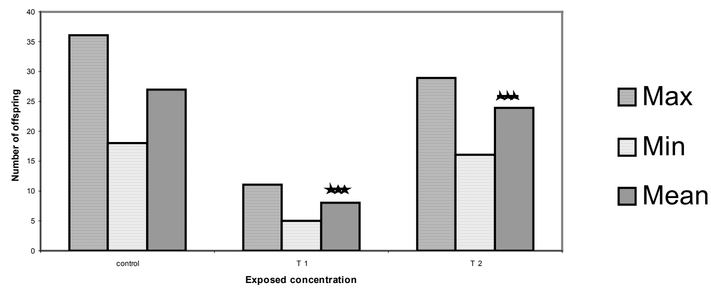

Figure 3: Mean,maximum,and minimum number of offspring per female with different concentration (Mean,***P< 0.001).

Produced offspring in control for additional 14 days were recorded a highest percentage survival, over 86% while in the concentration of 2µg / l it was less than 66. Quite contrast to the observations related to number of offspring in the control and exposed concentration, 0.002µg / l, the percentage survival of this concentration significantly lesser than control (Figure 4).

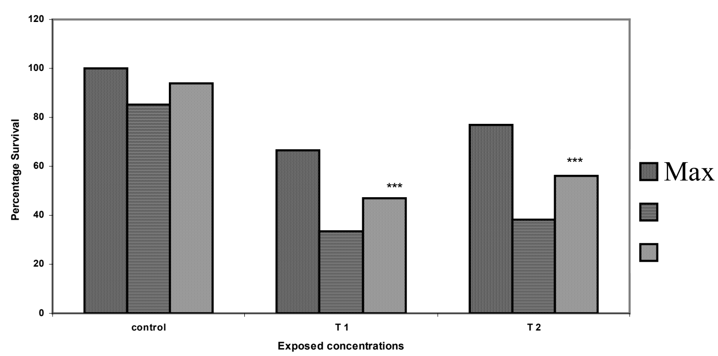

Figure 4: Recorded maximum,minimum and mean percentage survival with exposed concentrations(***P< 0.001)

4.Discussion

Fish exposed to xenobiotic pollutants have manifested a range of reproductive defects including behavioral, anatomical, physiological levels from reduced fertility to alternation of sexual behaviour and one of the key parameters to evaluate the impact of xenobiotics (Jones et al, 1997; Kime, 1999). Reproductive behavior it self is a key phase of the reproduction cycle of Guppy, which ensures the successful mating. Endler (1987) and Houde (1997) have illustrated the two approaches as sigmoid displays and alternative tactic gonopodial thrusts difference in change of temperature, in presence of predators. But the effects of pesticides the sexual behaviour was either poorly highlighted or neglected. Although the degradation of Chloropyrifos is rapid still it can cause serious consequences on fish as it coincides with this key phase of their reproduction cycle, mating behaviour. Our study suggests that even low concentrations of Chloropyrifos well below LC50 value heavily ceased male mating behaviour. Similarly Matthiessen and Logan (1984) suggested that 0.002 – 0.0015 mg/l exposure of Endosulfan inhibited male reproductive behavior of *Sarotherodon mossambicus.*

Moore et al., (1997) focused on interesting phenomenon stating as successful mating behavior sometimes depend on the secretion of chemicals by female fish to elicit a response that both triggers production of sperm and male mating behaviour. Pesticides such as carbofuran and diazinon both could disrupt male fish to detect such chemicals. Similar mechanism could be suggested for Chloropyrifos since its effects are more similar to diazinon. Further research on this aspect might be able to confirm this mechanism. In our early study using guppy juveniles and Chloropyrifos suggested that exposed animals had signs of paralysis even in low concentrations of this pesticide. These signs of paralysis which caused them, incapable of moving might lead to the reduction of their mating behaviour. Male guppy reduces their mating tactics in the presence of predators (Endler, 1987). In natural environment impacts of Chloropyrifos alone with predators might cause the situation worst.

Guppy, which can produce the brood to the external as fries and its short reproductive cycle, provides an excellent model to examine effects of Chloropyrifos on female fertility. The sperm storage of female guppy ensures that female could fertilized new embryos even if she was unable to remate (Constantz, 1989). Since we used females, which confirmed as no early mating, prior to exposure of Chloropyrifos we strongly suggest that reduction of average number of offspring per female associated with pesticide exposure. It is further highlighted as the highest concentration was used have been recorded the least number of offspring.

It is true that male vertebrates produce more sperm than eggs. But studies on fish and mammals confirmed that small change of sperm quality and quantity could reduce female fertility which might resulted fewer offspring (Kime, 1999). Change of mating behaviour in exposed males could lead to change of sperm quality and quantity resulting few offspring in exposed groups. The validity of this observation needs further research on Chloropyrifos effects on sperm quality and quantity. Decrease production of yolk protein resulting from inhibition of ovarian or liver function may lead to small number of eggs (Tyler et al, 1990). In this study we did not focus on this aspect but this relationship should be revealed with this experiment as well. Several authors suggested decreasing number of offspring with various pesticides. Yasuno (1980) used the same species Guppy as model organism to evaluate the effects of Fenitrothion and he suggested that reduced number of juveniles in pesticide contaminated female population.

Exposure of Dursban on Fathead minnows for 200 days resulted the reduction of first generation of offspring. Hose et al, (1989) and Thomas et al, (1989) suggested similar results on fish as the test species. Another aspect that could have a possible relationship was percentage survival of the juveniles born. Although females in the lowest concentration were able to produce quite similar number of offspring with control their survival was significantly lesser than control. Xenobiotics could pass on from mother to offspring when it was developing. Pesticide exposure throughout this embryonic development could result week offspring that makes them struggle to survival. Less than 66 % of survival in exposed concentrations of this study revealed that fries affected by Chloropyrifos, although they were not entirely exposed. Contaminated yolk passed on from mothers who have accumulated high pesticide burden and liver alterations itself might lead to the nutrition content and quality of the eggs, which were the possible causes for weak offspring production. Also we suggest that Chloropyrifos could decrease the responses to stresses and further decrease in growth and metabolism could possibly affect these juveniles, ability to survival. In wild they might highly struggling to survival since they are vulnerable to combination of stress conditions including effects of pesticides.

There is increasing evidence that some of the problems found in fish now being applied to human population as well (Colborn, et al 1996). So suggested effects of chlorpyrifos on guppy might insight into a much more general view as well. We can conclude that LORSBAN (Chlorpyrifos) could potentially impair mating behavior and the effects could be extended to survival of F1 up to 14 days after birth.

References

Bailey, H. C., Miller, J.L., Miller, M.J., Wiborg, L.C., Deanovic, L., Shed, T. 1997. Joint acute toxicity of diazinon and chlorpyrifos to *Ceriodaphnia dubia*. Environmental Toxicology and Chemistry.16 (11). 2304-2308.

Colborn.T, Dumasanoski and Myres, J.P. 1996. Our Stolen future. Little brown. London.

Connor, V., 1995. Status of urban storm runoff projects. Central Valley Regional Water Quality Control Board, Sacramento, CA.

Constanz, G.D. 1989. Reproductive biology of poeciliid fishes. Ecology and evolution of livebearing fishes (Poeciliiadae)(Ed.by G.Kmeffe & F.F Snelson, Jr) pp33-50. EnglewoodCliffs, New Jersy, Prentice Hall.

deVlaming, V. DiGiorgio, C. and Deonovic, L., "Insecticide-Caused Toxicity in the Alamo River," Presented at the NorCal SETAC annual meeting, Reno., NV, June. (1998).

Endler, J. A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). Anim.Behav.35. 1376-1385.

Evens.J.P, Magurran.A.E. 1999. Geographic variation in sperm production by Trinidadian guppies. Proc.R.Soc.Lond. B266, 2083-2087.

Foe, C., Deanovic, L., Hinton, D. 1998. Toxicity Identification Evaluations of Orchard Dormant Spray Storm Runoff. California Regional Water Quality Control Board, Central Valley Region, Sacramento, CA.

Hill, E.F. 1995. Organophosphorus and Carbamate Pesticides. Pp. 243-274 *in* D. J. Hoffman, B.A. Rattner, G. A. Burton, Jr., and J. Cairns, Jr. (eds.). Handbook of Ecotoxicology. Lewis Publishers, Boca Raton, FL.

Hose, J.E., Cross., Smith, S.G ., Diehl.D.1989. Reproductive impairment in a fish inhabiting a contaminated coastal environment off southern California. Env. Poll. 57, 139-148.

Houde, A. E. 1997.Sex, colour, and mate choice in Guppies. Princeton University Press. New Jercy.USA.

Hutchins,L.1996.Freshwaterresources.OnlinePublication.http://www.Aqualink.com/Fresh/Z1-Guppy.

Jones, J .C., Reynolds J.D. 1997.Effects of pollution on reproductive behaviour of fishes. Rev. Fish.Biol. & Fisheries 7. 463-491.

Johnson, W.W., Finley, M.T. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates. U.S.Fish.Wild.Serv.Resour.Pub.137, 98-99.

Kime.D.E. 1999. Endocrine disrupting chemicals.,ed R.F Hester and R.M Harrison, Issues in Environ.Sci.and Tech.12. 27-48.

Landis, W.G., Yu, M.H. 1995. Introduction to Environmental Toxicology: Impacts of Chemicals Upon Ecological Systems. Lewis Publishers, Boca Raton, FL.

Larsen, K. L, Connor, V.M and Hinton, D.E."Sacramento River watershed Program Toxicity Monitoring Results 1996 - 1997,SETEC annual meeting Reno, NV.June.(1998).

Lee, G.F., Jones-Lee.1998. A Development of a regulatory approach for OP pesticide toxicity to aquatic life in receiving waters for urban storm water runoff. SETAC Meeting, Reno, NV.

Matthiessen, P.Logazn, J.W.M.1984.The effects of low concentrations of endosulfan insecticide on reproductive behavior in the tropical cichild fish, *Sarotherdon mossambicus*. Bull. Contam. Toxicol.33.575 - 583.

Mueller-Beilschmidt, D. 1990. Toxicology and environmental fate of synthetic pyrethroids. J. Pest. Reform .10(3). 33-34.

Moore, M. T., Huggett, D.B., Gillespie, W.B., Rodgers, J.H.J., Cooper, C.M. 1998. Comparative toxicity of chlordane, chlorpyrifos, and aldicarb to four aquatic testing organisms. Archives Environmental Contamination and Toxicology. 34. 152-157.

Odenkirchen, E.W., Eisler, R. 1988.Chlorpyrifos hazards to fish, wild life, and invertebrates synoptic review. U.S fish wild. Serv Biol. Rep.85, 9-11.

Thomas, P. 1990.Teleost model for studying the effects of chemicals on female reproductive endocrine function. J. Exp. Zool.Suppl.4. 126-128.

Tyler.C.R, Sumpter.J.P.1996.Oocyte growth and development in teleosts. Rev.Fish.Biol.Fisheries.6, 287.

U.S. Environmental Protection Agency. Registration Standard (Second Round Review) for the Registration of Pesticide Products Containing Chlorpyrifos. Washington, DC, 1989.5-44.

Yasuno.M, Hatakeyama.S, Miyashita.M., 1990. Effects on reproduction in the guppy (*Poeciliia reticulata*) under chronic exposure to Temphos and Fenitrothion. Bull. Env.Contam.Toxicol. 25, 29-33.

Acknowledgements

Authors acknowledge University of Ruhuna research grant (RU/SF/RP/99/05) for providing financial assistance.