REPRODUCTIVE TOXICITY EFFECTS OF CARBOFURAN EXPOSURE ON PREGNANT GUPPY *Poecilia reticulata*

Watiporn Yenchum[1, 2], Kingkeaw Wattanasirmkit[2, *], Jirarach Kitana[2]

[1]Biological Science Program, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand
[2]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

ABSTRACT: Carbofuran is one of the most toxic insecticides used in agriculture. Its toxic effects are not limited to animals but also on human. The main adverse health effects on human are neurological effects, irritation of skin and reproductive effects. However, the study of reproductive toxicity of carbofuran on human and wildlife is still limited. In the present study, guppy *Poecilia reticulata*, a live bearing fish, was selected as an aquatic animal model for an assessment of carbofuran effect during pregnancy on reproductive capability, viability of offspring and survival rate of offspring. One year old female guppies were exposed to 0.01 and 0.05 ppm of carbofuran until they produced their brood. Number of offspring from each pair was counted. The survival of offspring was monitored for 15 days after birth. The result shows that reproductive capability of female guppy exposed to 0.05 ppm carbofuran was significantly reduced to 9.0±0.3 individuals per female. Similarly, live birth indices significantly decreased to 92.46% and 77.58% in 0.01 ppm and 0.05 ppm treatment group, respectively compared with control groups. Moreover, survival of offspring at the 15th day after birth was significantly reduced to 90.68% and 68.18% in 0.01 ppm group and 0.05 ppm treatment group, respectively. Significant findings on morphological abnormality of the offspring revealed that 2% of the newborn in 0.05 ppm treatment had two heads in one body, indicating teratogenic effects of carbofuran firstly described in a non-mammalian species. The result from this study suggested that carbofuran can impair reproductive capability of female guppy and reduce live birth indices and the first generation (F_1) survival. The cautionary adverse effects found in this study suggested the need for further investigation of carbofuran effects on some other parameters significant in evaluating any risk to humans and animals in the environment.

Keywords: carbofuran, *Poecilia reticulata*, reproductive toxicity, fertility, fecundity, survival rate

INTRODUCTION

Carbofuran is one of the most important carbamate insecticides. Carbofuran is used to control a broad spectrum of insect pests in agricultural areas. It is normally sprayed directly onto soil and plants just after emergence to control pests [1]. Like other carbamate insecticides, carbofuran is acetylcholinesterase inhibitor. Carbofuran is classified in toxicity class 1b or highly toxic class [2]. Carbofuran is not only toxic to animal and human nervous system but also associates with reproductive toxicity [3]. Various insecticides widely used in agriculture can produce various types of reproductive and developmental disorders in animals and human. The reproductive toxicity of insecticide are expressed as alterations in reproductive ability, fertility, reproductive cycle, offspring viability, hormone secretion and structure of reproductive organs [4-7].

Reproduction is a key parameter used to investigate the effects of toxicants. In reproductive toxicity study, Results of exposure of the Charles River CD rats to carbofuran, at concentration of 0, 20 and 100 mg/kg, showed the reduction in body weight in the parental generation and the reduction in growth and survival of pup generations in 100 mg/kg treatment group [8]. Another similar study [9], 40 pregnant female Charles River CD rats were fed with 0, 20, 60 and 160 ppm carbofuran in the diet during gestation day 6th through 19th and result revealed that one-half of the fetus were found with visceral and skeletal abnormalities on gestation day 20th. In other report, carbofuran was administered to 10 to 12 CD rats by forced feeding from day 7th to 19th of gestation. The result showed that carbofuran was maternally toxic at the dose of 1, 3, and 5 mg/kg. Fetal toxicity was significant at 5 mg/kg exhibited as a reducing number of live fetuses per litter or increase in fetal mortality, and decrease in fetal body weight [10]. Reproductive toxicity of carbofuran in male rat is also reported. After carbofuran exposure, reduction in weight of epididymides, seminal vesicles, ventral prostate and coagulating glands were noted. Decrease sperm motility, reduced epididymal sperm count and increased morphological abnormalities in the head, neck, and tail regions of spermatozoa were also observed [11]. Testicular and spermatotoxic effects in rat were also noted at the carbofuran level higher than 0.2 mg/kg *in utero* or via lactation [12]. A study in rat reported from Sri Lanka concluded that carbofuran administered orally at the dose of 0.2, 0.4 and 0.8 mg/kg during early gestation is detrimental to

**Correspondence to: Kingkeaw Wattanasirmkit*
E-mail: oum_bio@hotmail.com
Tel. +66 (0) 2218 5251, Fax. +66 (0) 2218 5256

pregnancy (enhanced preimplantation losses) and possibly harmful to neonatal development [13]. Similarly, post-implantation losses were noted after exposure to carbofuran during mid-gestation [14]. Carbofuran is not only toxic to animals but can also affect on human. The main adverse health effects after exposed to carbofuran are difficulty in breathing, headaches, neurological or phychological effects and irritation of skin and mucous membrane [15]. Moreover, effects on immune, cancer and reproductive effects were reported [15, 16]. The manifestation of these effects depends on the concentration and duration of exposure. Most reports on adverse effects of carbofuran in human were retrospect in nature and usually deal with high and acute dose. Carbofuran and its major metabolites can cross the placental barrier and produce serious effects on the maternal-placental-fetal unit [17, 18]. Moreover, a case of carbamate pesticide poisoning of a pregnant woman by carbofuran ingestion is presented. The mother recovered from the poisoning in the hospital but necrosis of the fetus was found. Toxicological findings of the liver, brain, and kidney of the fetus revealed carbofuran in concentrations comparable with the mother's blood [19]. In addition, women who reported to have agricultural exposure during pregnancy showed risk of gestational diabetes mellitus (GDM) in association with ever-use of carbofuran [20].

In the environment, carbofuran is degraded by photolysis, hydrolysis and microbial decomposition. Hydrolysis half-life of carbofuran in paddy water at 25°C and pH 7 is 240 hours [21]. Although carbofuran is rapidly biodegradable in the environment, overspray and runoff of this insecticide from agricultural fields may easily contaminate bodies of water. Contaminated carbofuran is highly toxic to wildlife including aquatic animals like fish. Because of their habitat, fish are susceptible to the toxic effects of insecticide residues in water and sediment of aquatic environment that receive runoff from agricultural areas. Insecticides and their residues, washed into the water systems, can cause acute and chronic toxicity to fish. Disruptions of fish reproductive process with alterations in gonad structure by insecticide are well documented [22-26]. Whereas studies on monitoring health status of fish including biochemical, physiological and histopathological biomarkers are undergoing, parallel investigation of ecological endpoints such as abundance, growth of fry, litter size and survival ability should be monitored as well.

Guppy *Poecilia reticulata* Peters is viviparous fish belonging to the family Poeciliidae. It is an omnivorous fish. Guppy is selected as a biological model because it has a short reproductive cycle [27, 28]. A readily availability and a small body size make it easily to handle. Guppy is used in variety of studies including behaviour, life history evolution, water quality, genetic and ecotoxicology. Guppy is very useful for studying environmental pollution problems [29, 30].

Even though the reproductive toxicity of carbofuran on small mammals is well documented, few studies were carried out on fish which is more susceptible to the insecticide effects due to exposure via aquatic environment. Reports about reproductive disorders of fish including altered fertility, viability and survival of offspring are rare. Report on similar issues in a viviparous fish, an important model for pregnancy, is even rarer. Therefore, the objectives of this study were to investigate the effects of carbofuran exposure on reproductive capability, live birth index and offspring survival of pregnant guppy *Poecilia reticulata*.

MATERIALS AND METHODS

Experimental animal

Guppy *Poecilia reticulata* used in the experiment was obtained from the laboratory stock of Department of Biology, Faculty of Science, Chulalongkorn University. One year old guppies were used in this experiment. The mean length of the females was 2.8±1.0 cm. The fish were acclimatized in 12-L glass aquarium in dechlorinated tap water with aeration prior to the experiment. During the experiment, they were maintained in 27-30°C water, on 12h light: 12h dark photoperiod, and were fed twice daily with commercial guppy food (See-All Aquariums Co., Ltd.).

Carbofuran

The commercial grade of carbofuran, 2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, or Furadan 3G (P.T. Bina Gunakimia Ltd., Indonesia) was used in this experiment. Carbofuran was dissolved in dimethylsulphoxide (DMSO) before diluting into water to obtain the final concentration at 0.01 and 0.05 ppm. The maximum concentration of DMSO used to dissolve the pesticide was 5 ppb. Therefore, solvent control group was contained DMSO at a concentration of 5 ppb.

Experimental and Control Groups

In this study, the experimental groups were consisted of two groups, the treatment and the solvent control group. The treatment group was the group of guppy that exposed to carbofuran at concentration of 0.01 and 0.05 ppm. While, the solvent control group was the group of guppy that exposed to DMSO solution at a concentration of 5 ppb. This solvent control group provided a baseline data for interpreting the test results along with the (actual) control group, which was the group of guppy that exposed to filtered tap water. During the experiment, all groups were maintained by the same procedures and conditions (i.e., 12h light: 12h dark photoperiod and water temperature was 27-30°C). The experimental protocols were conducted according to a guideline

Table 1 Mean number of offspring per female in different concentrations (female guppy=20/group)

	Control (Mean ± SE)	Solvent control (Mean ± SE)	Treatment (Mean ± SE)	
			0.01 ppm	0.05 ppm
Reproductive capability	10.0±0.1[a]	10.0±0.1[a]	10.0±0.1[a]	9.0±0.3[b]

Mean with different letters (a, b) within a row are significantly different ($P < 0.05$)

Table 2 Mean live birth index of offspring in different concentrations

	Control (Mean ± SE)	Solvent control (Mean ± SE)	Treatment (Mean ± SE)	
			0.01 ppm	0.05 ppm
Live birth index (%)	99.22±0.33[a]	99.69±0.21[a]	92.46±0.66[b]	77.58±2.54[c]

Mean with different letters (a, b, c) within a row are significantly different ($P < 0.05$)

for animal care and use, which have been approved by Chulalongkorn University Animal Care and Use Committee (CU-ACUC Protocol Review No. 0923011).

Reproductive indices

Female guppies were isolated into 2-L glass aquarium and monitored for 6 to 8 weeks until they were given birth to offspring due to earlier fertilization in stock aquarium. The final concentrations of carbofuran were prepared at 0.01 and 0.05 ppm. These concentrations were sublethal concentration that calculated from the LC_{50} at 96 hours for guppy (unpublished data). A pair of male and female guppy was transferred to a glass aquarium for breeding. After five-day, male guppy was removed and the female was kept in the test solutions until they produced their brood. Twenty female guppies were used in each concentration. The static renewal system was used throughout the experiment. After offspring was born, total number of offspring, number of death offspring and number of viable offspring were observed and recorded. Then the offspring of each female was separated into glass aquarium contained the same concentration of carbofuran as mother tank for survival study. After offspring was born, the morphology of newborn in each individual was observed. The survival of offspring in each aquarium was monitored every 24 hours for 15 days.

Data Analysis

The reproductive capability (the litter size of guppy), live birth index and survival percentage [31] were calculated. The reproductive capability was done by determining average number of offspring per female for each concentration and the control, the live birth index by multiplying number of viable offspring per a total number of offspring by 100, and the survival percentage by multiply number of viable offspring on a given day per a total number of offspring by 100, respectively. The data were statistically analyzed with Kruskal-Wallis One Way Analysis of Variance on Ranks followed by Dunn's Method. $P < 0.05$ was considered to be significant.

RESULTS

Reproductive Capability

The result of mean reproductive capability is shown in Table 1. The maximum value of reproductive capability was found in control group (13 individuals/female) whereas the lowest litter size was found in the treatment group exposed to 0.05 ppm carbofuran (6 individuals/female). The mean litter size was not different (10.0±0.1 individuals/female) between both control groups and the 0.01 ppm treatment group. On the other hand, the reproductive capability was significantly decreased to 9.0±0.3 individuals per female in the 0.05 ppm treatment group ($P < 0.05$).

Live Birth Indices

The live birth indices in control and solvent control groups were found at the highest percentage. The mean live birth indices in control, solvent control, 0.01 ppm and 0.05 ppm treatment groups were 99.22±0.33%, 99.69±0.21%, 92.46±0.66% and 77.58±2.54%, respectively. The mean live birth indices in both treatment groups were significantly different from both control groups ($P < 0.05$). The result is shown in Table 2.

Survival Percentage

The survivals of offspring in control and solvent control groups were recorded for 15 days post partum. The survival percentages were high at 99.22±0.33% and 99.69±0.21%, respectively. The survival of offspring in treatment group at concentration of 0.01 ppm was reduced from 91.68±0.83% to 90.68±0.83% during 15 days. In 0.05 ppm treatment group, the survival of offspring was decreased from 73.32±2.48% to 68.18 ±2.42% during 15 days. The survival percentages of offspring in treatment groups at concentration of 0.01 ppm and 0.05 ppm carbofuran were significantly lower than control and solvent control groups ($P < 0.05$). The result of mean survival index of offspring is shown in Table 3.

Morphological Abnormality of Offspring

The morphology of offspring in control, solvent control and 0.01 ppm treatment group showed normal character. On the other hands, the morphological

Table 3 Mean survival index of offspring in different concentrations at 1st to 15th day

Day	Survival index (%) (Mean ± SE)			
	Control	Solvent control	Treatmant	
			0.01 ppm	0.05 ppm
1st	99.22±0.33[a]	99.69±0.21[a]	91.80±0.72[b]	73.32±2.48[c]
2nd	99.22±0.33[a]	99.69±0.21[a]	91.80±0.72[b]	73.32±2.48[c]
3th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	69.31±2.45[c]
4th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	69.31±2.45[c]
5th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
6th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
7th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
8st	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
9th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
10th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
11th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
12th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
13th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
14th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]
15th	99.22±0.33[a]	99.69±0.21[a]	90.68±0.83[b]	68.18±2.42[c]

Mean with different letters (a, b, c) within a row are significantly different ($P < 0.05$)

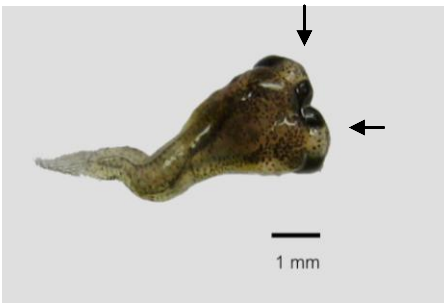

Figure 1 The morphological abnormality of newborn guppy in treatment group at 0.05 ppm of carbofuran. Two percent of newborn guppy in this treatment group showed two heads (arrows) in one body

abnormality of offspring was found only in treatment group at 0.05 ppm of carbofuran. It is of importance to note that the newborn guppy had two heads in one body. Ten individuals of offspring or approximately 2% of total offspring in 0.05 ppm treatment group were found with this morphological abnormality (Figure 1).

DISCUSSION

Although the degradation of carbofuran is rapid in environment, its extensive use probably causes adverse effects on fish reproduction [4]. The viviparity of the guppy and its short reproductive cycle make this fish to be an excellent model for the assessment of effects of carbofuran on female fertility and viability of offspring [27, 28]. Results from our study suggested that even at a low concentration, carbofuran can cause reproductive disorders in exposed guppies including reduced viability of offspring, litter size and survival of offspring, respectively. The reproductive capability, determined by the litter size, was significantly decreased. It explained that the changes of eggs quality and quantity could reduce female fertility which might result in decrease of offspring number [32]. Many studies [33-37] reported that carbofuran reduced the quality and quantity of eggs resulting in low number of offspring or reduced reproductive capability of female by disrupting egg development or by reducing vitellogenin production.

The live birth indices and the survival of offspring in treatment group at concentration of 0.01 ppm and 0.05 ppm carbofuran were significantly reduced compared with the control groups. Although the female guppies exposed to the low concentration of carbofuran (0.01 ppm) were capable of producing quite similar number of offspring with the controls (10 individuals per female), their survival was significantly lower. The decrease of percentage live birth index may be a result of the possibility that carbofuran could pass from mother to offspring during the embryonic development. Carbofuran exposure throughout this embryonic development could result in weaker offspring making them struggle to survive. Contaminated yolk from mothers who exposed to high carbofuran burden could result in liver alterations and this might affect to the nutrition content and quality of the eggs, which were the possible causes for weak offspring production. In other studies, the guppy was exposed to chlorpyrifos (insecticide) concentrations (0.002 mg/L and 2 mg/L) for 3 days. The chlorpyrifos significantly reduced live birth index and survival of offspring of guppy [38]. The hatching percentage of fresh water fish *Labio rohita* were reduced after exposed to sublethal concentration of carbofuran at 0.06, 0.15 and 0.30 ppm [37].

In the present study, the survival of offspring in 0.05 ppm treatment group at day 15 was less than 68.18 %. This may imply that carbofuran can pass from mother during pregnancy and result in infirmity of offspring and affected its survival. Furthermore, the low survival percentage in the treatment groups may be a result of carbofuran acute toxic effect after newborn guppies were directly exposed to carbofuran after birth.

The morphological abnormality of offspring was found in 0.05 ppm treatment group with approximately 2% of the newborn guppies found with two heads in one body. The abnormality of morphology of offspring may be involved with vertical transfer of carbofuran from mother to offspring during the embryonic development. These results indicate potential teratogenic effects of carbofuran firstly described in a non-mammalian species.

Of important to this study, few studies reported on the adverse reproductive effects of carbofuran. Forty-five percent of urban African-American women who live and work on farm showed detectable levels of carbofuran in maternal plasma and umbilical cord blood [39]. In male worker exposed to carbofuran, seminal analysis revealed a total concentration of 42 million spermatozoa/mL with 17% motility and 20% normal shape. The second patient presented a total concentration of 5 million spermatozoa/mL with 6% motility and 2% normal shape. The patients presented a similar percentage of binucleated spermatozoa (28 and 26%) and of multinucleated spermatids (10 and 6%) [40].

The results from this study suggest that carbofuran could decrease responses to stress conditions and further decrease growth and metabolism, and could ultimately affect the survival of offspring. In the environment, newborn guppies will be highly struggle to survive since they are vulnerable to a variety of stress conditions including effects from insecticide residues. The adverse effects may cause the decrease in population size of the newborn fish by reducing its survival. In conclusion, the present study exhibited that the low concentration of carbofuran could potentially impair reproductive capabilities of female guppy, reduce the live birth index, reduce survival of F_1 generation of guppy and disruption the embryo development. This suggested that maternal carbofuran exposure throughout the embryonic development could result in weak offspring as well as their survival and abnormality.

Overall, these experiments show adverse effects on small fish in organism level (litter size, live birth index and survival percentage). The cautionary adverse effects of this compound suggests the need for further investigation of carbofuran effects on some other parameters significant in evaluating the risk to humans and animals in the environment.

ACKNOWLEDGEMENT

This study was supported by Department of Biology, Faculty of Science, Chulalongkorn University. The authors wish to thank the Development and Promotion of Science and Technology Talents Project (DPST) and Graduate School, Chulalongkorn University who supports research fund.

REFERENCES

1. U.S. EPA. Carbofuran. Washington, D.C.: Office of Water, U.S. Environmental Protection Agency; 1995.
2. World Health Organization. The Recommended Classification of Pesticides by Hazards and Guidelines to classification. Geneva: World Health Organization; 1999.
3. Public Health Goal. Carbofuran. California: Office of Environmental Health Hazard Assessment, California Environmental Protection Agency Pesticide and Environmental Toxicology; 2000.
4. Colborn T, vom Saal FS, Soto AM. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect. 1993 Oct; 101(5): 378-84.
5. Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet. 1993 May 29; 341(8857): 1392-5.
6. Kelce WR, Monosson E, Gamcsik MP, Laws SC, Gray LE, Jr. Environmental hormone disruptors: evidence that vinclozolin developmental toxicity is mediated by antiandrogenic metabolites. Toxicol Appl *Pharmacol.* 1994 Jun; 126(2): 276-85.
7. Kumar S. Occupational exposure associated with reproductive dysfunction. J Occup Health. 2004 Jan; 46(1): 1-19.
8. World Health Organization. Carbofuran in Drinking-water. Geneva: World Health Organization; 2004.
9. IRDC. Teratology and post natal study in the rat with carbofuran dietary administration. (Study No. FMC A80-44/IrDC 167-154). IRDC conducted for FMC Corporation; 1981.
10. Courtney KD, Andrews JE, Springer J, Dalley L. Teratogenic evaluation of the pesticides baygon, carbofuran, dimethoate and EPN. J Environ Sci Health B. 1985 Aug; 20(4): 373-406.
11. Pant N, Prasad AK, Srivastava SC, Shankar R, Srivastava SP. Effect of oral administration of carbofuran on male reproductive system of rat. Hum Exp Toxicol. 1995 Nov; 14(11): 889-94.
12. Pant N, Shankar R, Srivastava SP. *In utero* and lactational exposure of carbofuran to rats: effect on testes and sperm. Hum Exp Toxicol. 1997 May; 16(5): 267-72.
13. Jayatunga YNA, Dangalle CD, Ratnasooriya WD. Hazardous effects of carbofuran on pregnancy outcome of rats. Med Sci Res. 1998; 26: 33-7.
14. Jayatunga YNA, Dangalle CD, Ratnasooriya WD. Effects of midterm exposure to carbofuran on pregnancy outcome of rats. Med Sci Res. 1998; 26: 679-83.
15. Environmental Protection Agent (EPA). Interim Reregistration Eligibility Decision (IRED) Document for Carbofuran. Office of Environmental Health Hazard Assessment; 2006.
16. Bacigalupo MA, Meroni G, Longhi R. Determination of carbofuran in water by homogeneous immunoassay using selectively conjugate mastoparan and terbium/dipicolinic acid fluorescent complex. Talanta. 2006 Jul 15; 69(5): 1106-11.
17. Gupta RC. Carbofuran toxicity. J Toxicol Environ Health. 1994 Dec; 43(4): 383-418.

18. Barr DB, Ananth CV, Yan X, Lashley S, Smulian JC, Ledoux TA, et al. Pesticide concentrations in maternal and umbilical cord sera and their relation to birth outcomes in a population of pregnant women and newborns in New Jersey. Sci Total Environ. 2010 Jan 15; 408(4): 790-5.
19. Klys M, Kosun J, Pach J, Kamenczak A. Carbofuran poisoning of pregnant woman and fetus per ingestion. J Forensic Sci. 1989 Nov; 34(6): 1413-6.
20. Saldana TM, Basso O, Hoppin JA, Baird DD, Knott C, Blair A, et al. Pesticide exposure and self-reported gestational diabetes mellitus in the Agricultural Health Study. Diabetes Care. 2007 Mar; 30(3): 529-34.
21. Environmental Protection Agent (EPA). Public Health Goal for carbofuran In Drinking Water. California: Office of Environmental Health Hazard Assessment; 2002.
22. Mlambo SS, van Vuren JHJ, Barnhoorn IEJ, Bornman MS. Histopathological changes in the reproductive system (ovaries and testes) of Oreochromis mossambicus following exposure to DDT. Environmental Toxicology and Pharmacology. 2009; 28(1): 133-9.
23. Hinck JE, Blazer VS, Denslow ND, Echols KR, Gross TS, May TW, et al. Chemical contaminants, health indicators, and reproductive biomarker responses in fish from the Colorado River and its tributaries. Sci Total Environ. 2007 Jun 1; 378(3): 376-402.
24. Dutta HM, Meijer HJ. Sublethal effects of diazinon on the structure of the testis of bluegill, Lepomis macrochirus: a microscopic analysis. Environ Pollut. 2003; 125(3): 355-60.
25. Chatterjee S, Kumar Dasmahapatra A, Ghosh R. Disruption of pituitary-ovarian axis by carbofuran in catfish, Heteropneustes fossilis (Bloch). Comp Biochem Physiol C Toxicol Pharmacol. 2001 Jul; 129(3): 265-73.
26. Houde AE. Sex, colour and mate choice in guppies. New Jersey: Princeton University Press; 1997.
27. Castro BB, Sobral O, Guilhermino L, Ribeiro R. An in situ bioassay integrating individual and biochemical responses using small fish species. Ecotoxicology. 2004 Oct; 13(7): 667-81.
28. Constanz GD. Reproductive biology of poeciliid fishes. New Jersey: Prentice Hall; 1989.
29. Camilo BG, Wualberto T, Socorro S, Laura P. Contribution to vital statistic of guppy *Poecilia reticulata* Peters (Pisces: Cyprinodontifomes: Poecillidae) pond population Santa Marta, Colombia. Panam JAS. 2008; 3: 335-9.
30. Wester PW, van der Ven LTM, Vethaak AD, Grinwis GCM, Vos JG. Aquatic toxicology: opportunities for enhancement through histopathology. Environ Toxicol Pharmacol. 2002; 11(3-4): 289-95.
31. Donald JE. The basic of toxicity testing. London: CRC Press; 1992.
32. Kime DE. Endocrine disrupting chemicals. In: Hester RF, Harrison RM, editors. Environmental Science and Technology. Cambridge: The Royal Society of Chemistry; 1999.
33. Chatterjee S, Ghosh R. Toxicity of carbamate pesticide, carbofuran technical 75DB to the fertilization of eggs of catfish *Heteropneustes fossilis* (Bloch). Bull Environ Contam Toxicol. 1995; 55: 111-5.
34. Chatterjee S. Studies on the effect of pesticide on reproduction of female catfish, Heteropneustes fossilis (Bloch). Doctoral dissertation, Calcutta University, Calcutta, West Bengal, India; 1996.
35. Chatterjee S, Dutta AB, Ghosh R. Impact of carbofuran technical in the oocyte maturation of *Heteropneustes fossilis* (Bloch). Arch Environ Contam Toxicol. 1997; 32: 426-30.
36. Adhikari S, Sarkar B, Chattopadhyay A, Chattopadhyay DN, Sarkar SK, Ayyappan S. Carbofuran induced changes in breeding of a freshwater fish, *Labeo rohita* (Hamilton). Toxicol Environ Chem. 2008; 90(3): 457 - 65.
37. Tyler CR, Sumpter JP. Oocyte growth and development in teleosts. Rev Fish Biol Fish. 1996; 6(3): 287-318.
38. De Silva PM, Samayawardhena LA. Effects of chlorpyrifos on reproductive performances of guppy (*Poecilia reticulata*). Chemosphere. 2005 Mar; 58(9): 1293-9.
39. Whyatt RM, Barr DB, Camann DE, Kinney PL, Barr JR, Andrews HF, et al. Contemporary-use pesticides in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and newborns. Environ Health Perspect. 2003 May; 111(5): 749-56.
40. Gallegos-Avila G, Ancer-Rodriguez J, Niderhauser-Garcia A, Ortega-Martinez M, Jaramillo-Rangel G. Multinucleation of spermatozoa and spermatids in infertile men chronically exposed to carbofuran. Reprod Toxicol. 2010 Jul; 29(4): 458-60.