Original Article
Toxicology International, *Jan-April 2016 / Vol-23 / Issue-1, 89-94*
ISSN (Print) : 0971-6580
ISSN (Online) : 0976-5131
DOI: 10.22506/ti/2016/v23/i1/146675

Toxic Effects of Quinalphos on Protein Content and Growth Rate in Freshwater Teleost, *Cyprinus carpio* (L.)

A. Padmanabha[1*], H. R. V. Reddy[1], N. B. Shridhar[2], Muttappa Khavi[1] and B. T. Naveen Kumar[3]

[1]Department of Aquatic Environment Management, College of Fisheries, Mangalore
[2]Department of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore
[3]Department of Aquaculture, College of Fisheries, Mangalore, India.
*padmanabha218@gmail.com

Abstract

Organophosphate based pesticides account for a major percentage of pesticides used in domestic, agricultural and industrial applications throughout the world. They are highly popular because they are effective, non persistent and relatively less expensive. Quinalphos is a synthetic organophosphate which has become a matter of concern because of its potentiality and hazardous effect. Hence, the present investigation was designed to assess the impact of quinalphos on total protein content in the selected tissues and growth rate of freshwater fish, *Cyprinus carpio*. The lethal toxicity of quinalphos to the fish exposed for 96 h was found to be 2.75 ppm. For sublethal toxicity study, the fish were exposed to two concentration viz., 1/5th of LC_{50} (0.550 ppm) and 1/10th of LC_{50} (0.275 ppm) along with control as reference for 4, 8, 16 and 32 days. Decrease in total protein concentration and growth rate were observed in 4th and 8th day of exposure, but on 16th and 32nd day of exposure all the values reached nearer to normal condition. The decline in total protein levels and relative growth rate at initial exposure periods may indicates the high energy demand associated with imposed quinalphos stress. Restoration of protein fractions to normal, implies that after 8 days of exposure there seems to exist an oscillatory phase in protein turnover towards a more synthetic phase leading to the establishment of recuperation and adaptation phenomena.

Keywords: Quinalphos, toxicity, freshwater teleost, total protein, growth

Received: 23-01-2016 **Revised:** 11-03-2016 **Accepted:** 02-04-2016

1. Introduction

Proteins are important organic substance required by organisms in tissue building. It constitutes the building block and the basic molecule for any biochemical reaction. They are intimately related with almost all physiological processes, which maintain a simple biochemical system in 'living condition'.[1] Proteins are mainly involved in the architecture of the cell; which is the chief source of nitrogenous metabolism. They are occupying a unique position in the metabolism of cell because of the proteinaceous nature of all the enzymes which mediate at various metabolic pathways.

The levels of total protein are considered to be major indices of the health status of teleosts. Both the protein degradation and synthesis are sensitive over a wide range of conditions and show changes to a variety of physical and chemical modulators. Detection of biochemical changes caused by pesticides in fish development therefore is essential to assess the adverse effects of pesticides on fish and to predict their impact on survival and growth.[2] Xenobiotics are potentially harmful to fish by inducing growth retardation.[3] The growth rate is an index associated with stress and is generally used as a sensitive and reliable end-point in chronic toxicity investigations.[4] Changes in growth rate of fish serve as an important biomarker in pesticide toxicity.

Quinalphos is a synthetic organophosphate, non-systemic, broad spectrum insecticide and acaricide, acting as a cholinesterase inhibitor with contact, stomach and respiratory action. The major use of quinalphos in farming is to protect corn, cotton and fruit trees against

* *Author for correspondence*

insects. It is a hard insecticide, which has become a matter of concern because of its potentiality and hazardous effect. Since quinalphos is extensively applied in agriculture for pest eradication, it is pertinent to study its hazardous effect on the aquatic system as it is assumed that the residue might affect the aquatic vertebrates.[5] Hence, the present study is an attempt to assess the impact of quinalphos on total protein content in the selected tissues (gills, liver and kidney) and growth rate of commercially valuable freshwater teleost, *Cyprinus carpio*, by exposing it to sublethal concentrations of the organophosphorous pesticide, quinalphos (25% EC: emulsifiable concentrate) for varying treatment durations. The gill was chosen as the major respiratory tissue and major site of uptake of xenobiotic chemicals.[6] The liver was chosen as the major site of accumulation, biotransformation and excretion of xenobiotic compounds,[7] whereas kidney was selected as the major route for elimination and rapid clearance of xenobiotic chemicals.[8]

2. Materials and Methods

2.1 Test animal collection and maintenance

Common carp (2-3 cm) fry procured from government fish farm were transported to the college fish farm in well oxygenated polythene bags containing clean pond water. The fry were reared to fingerlings (6.5-7.5 cm) in a pond (5×5×1) using formulated fish feed. The fingerlings were transferred to 1000 L fiber reinforced plastic tank in the laboratory after proper acclimation. Vigorous aeration was provided in the tanks with a photoperiod of a 12:12 h light and dark cycle. The walls of the holding tank were thoroughly cleaned and excreta were siphoned off on daily basis to prevent the building of ammonia in the medium. Fish were conditioned in holding tank for 10 days and starved for 24 h before utilizing them for the experiments.

2.2 Quinalphos

Quinalphos, which is a trade name of Ekalux EC 25 [O,O-diethyl-O-(2-quinoxalinyl)- phosphorothiate] supplied by Chethana Agro Chemicals, Mangalore, India was used throughout the study. For each experiment the required concentrations were prepared from fresh stock solutions.

2.3 Lethal toxicity study

Lethal toxicity study was carried out by following the standard guidelines of APHA[9] to determine the lethal (LC_{50}) level of quinolphos using static renewal system. The experiments were conducted in triplicate with appropriate controls. The animals were not fed during the experiment and water was never aerated. The test solution was replenished every 24 h. The percentage mortality of fish for each concentration was taken into account for 96 h. The 96 h LC_{50} value of quinalphos was calculated by probit analysis.[10]

2.4 Sublethal toxicity study

Individuals measuring 7.0±0.5 cm in total length and weighing 10.0±1.0 g were selected for the present study. The fish were divided into 3 groups with the first group was held in tap water serving as control and the others as experimental groups. The experimental groups were subjected to the assessment of sublethal toxicity by exposing them to two selected sublethal quinalphos (25% EC) concentrations of 0.275 ppm ($1/10^{th}$ of LC_{50} / 96 h) and 0.55 ppm ($1/5^{th}$ of LC_{50} / 96 h). The 96 h LC_{50} of quinalphos for fingerlings *C. carpio* was previously determined as 2.75 ppm.

Ten healthy animals were stocked in 50 L of freshwater in a glass aquarium. Triplicates were run for each concentration. Renewal assay for static test was applied.[9] The water with insecticide was replaced every 24 h to maintain constant quinalphos (25% EC) nominal concentrations. The control fish were maintained in the same manner. There were no fish mortalities during the experiment. Control and experimental fish were fed once daily with floating feed, at a rate of approximately 3% of their body weight. The specimens were sampled after 4, 8, 16 and 32 days of exposure for estimation of total protein content and relative growth rate.

2.5 Estimation of total protein content

The gills, liver and kidney tissues were isolated and homogenized in ice-cold phosphate buffer and centrifuged at 3000 rpm for 15 minutes. The supernatant was collected for the estimation of total protein. Total protein content in the tissues was estimated by the method of Lowry *et al.*[11] using bovine serum albumin as standard. Samples were treated with folin-phenol reagent and the absorbance was measured at 750 nm using a Thermo Fisher Scientific (Evolution 300) UV-Visible spectrophotometer. Protein concentration was expressed as mg protein/g wet weight of the tissue.

2.6 Estimation of relative growth rate

The total initial weights of the fish were estimated in each tank before the beginning of trial and the final weights of the fish were measured after the trial ended. Fish growth was determined by the total initial weight and the final weight of 10 fish per tank. The following equation was applied to measure the relative growth rate (RGR):

$$RGR = ((m_t - m_0)/m_0) \times 100\%$$

Where, m_0 is the initial weight at the start of the trial
m_t is the final weight at the end of the trial.

3. Statistical analysis

The data were subjected to statistical analysis employing ANOVA to compare variables among controls and treatments and Duncan's multiple range test at $P < 0.05$[12,13] to determine which individual groups were significantly differen-t from control. All the data are expressed as mean ± standard deviation (SD) of the mean of six observations.

4. Results

In the present studies no fish died during the acclimation period before exposure, after exposure during sublethal toxicity tests and in control group during lethal toxicity tests. Acute toxicity (LC_{50}) of quinalphos for the freshwater fish, *Cyprinus carpio* was found to be 2.75 ppm at 96 h [Figure 1]. Fish mortality elevated with increasing the concentration of quinalphos [Table 1]. Increased mucus secretion, colour changes, behavioural changes such as rapid swimming, increased opercular rate, hyperactivity and loss of balance were important changes found in the individuals exposed to quinalphos. Sublethal concentrations of quinalphos were determined according to 96 h LC_{50} i.e. 1/5th of LC_{50} (0.550 ppm) and 1/10th of LC_{50} (0.275 ppm).

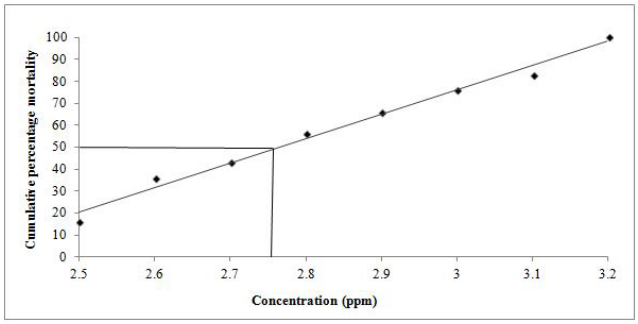


Figure 1. Graphical derivation of 96 h LC_{50} for quinalphos in *Cyprinus carpio*.

The levels of total proteins declined in both at low and high sublethal concentrations of quinalphos relative to controls in all the selected tissues (gills, liver and kidney) of fish after 4th day exposure and continued its declination up to 8th day exposure periods. From 8th day onwards their levels gradually elevated and came nearer to control at 32nd day exposure period [Table 2]. The growth rate of test fishes exposed to sublethal concentrations of quinalphos was found to lower than that in control. Decrease in body weight and relative growth rate were observed in 4th and 8th day of exposure, but on 16th and 32nd day of exposure all the values reached nearer to normal condition. [Table 3 and Figure 2]. The growth rate was very low in fish exposed to 1/5th of LC_{50} than that of the 1/10th of LC_{50} of quinalphos. Statistical analysis indicated significant ($P < 0.05$) variation in total protein content, body weight and relative growth rate in both the treated groups compared to control.

Table 1. Mortality of *Cyprinus carpio* in different concentrations of quinalphos (25% EC) at 96 h exposure periods

Concentration (ppm)	No. of fishes used for each trial	No. of fishes died			Mean % mortality
		Trial 1	Trial 2	Trial 3	
2.5	10	2	1	2	16
2.6	10	4	3	4	36
2.7	10	4	4	5	43
2.8	10	6	5	6	56
2.9	10	7	6	7	66
3.0	10	8	7	8	76
3.1	10	9	8	8	83
3.2	10	10	10	10	100

Table 2. Impact of sublethal concentrations of quinalphos on total protein content of *Cyprinus carpio*

Tissue	Treatment	Exposure days			
		4th day	8th day	16th day	32nd day
Gills	Control	16.17 ± 0.36	16.98 ± 0.31	16.56 ± 0.45	17.68 ± 0.73
	0.275 ppm	14.87 ± 0.24	12.99 ± 0.18*	13.61 ± 0.28	15.20 ± 0.25
	0.550 ppm	11.38 ± 0.16*	10.82 ± 0.23*	12.52 ± 0.15*	14.14 ± 0.29
Liver	Control	31.78 ± 0.52	31.91 ± 0.56	32.62 ± 0.34	34.23 ± 0.94
	0.275 ppm	27.37 ± 0.34*	26.76 ± 0.26*	28.42 ± 0.57*	31.03 ± 0.52
	0.550 ppm	24.18 ± 0.21*	22.62 ± 0.46*	25.29 ± 0.76*	27.94 ± 0.25*
Kidney	Control	20.34 ± 0.84	20.26 ± 0.47	21.89 ± 0.55	22.76 ± 0.63
	0.275 ppm	19.07 ± 0.14	19.30 ± 0.28	19.99 ± 0.38	20.61 ± 0.35
	0.550 ppm	17.38 ± 0.46	16.80 ± 0.36*	18.52 ± 0.65	19.94 ± 0.46

All values are expressed as mean±SD of six individual samples. *indicates significant difference from control at $P < 0.05$.

Table 3. Effect of quinalphos exposure on body weight of *Cyprinus carpio*

Treatment	Weight gain/loss (g/10 fish)			
	4th day	8th day	16th day	32nd day
Control	2.34 ± 0.20	6.22 ± 0.59	13.05 ± 1.46	25.71 ± 2.35
0.275 ppm	-4.11 ± 0.38*	-0.44 ± 0.27*	3.37 ± 0.25*	14.03 ± 1.29*
0.550 ppm	-7.82 ± 0.60*	-5.44 ± 0.42*	0.53 ± 0.18*	11.15 ± 1.68*

All values are expressed as mean±SD of six individual samples. *indicates significant difference from control at $P < 0.05$.

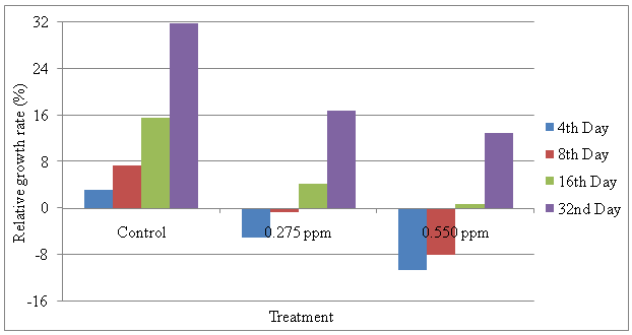


Figure 2. Relative growth rate of *Cyprinus carpio* exposed to sublethal concentrations of quinalphos.

5. Discussion

The quantity of protein is dependent on the rate of protein synthesis or on rate of its degradation.[14] During stress, fish need more energy to detoxify toxicants and to overcome stress the protein is used to meet the increased energy demand.[15] Thus the depletion of protein fraction in gill, liver and kidney tissues may have been due to their degradation and possible utilization for metabolic purposes. The decrement in the protein content suggests the existence of a high proteolytic activity and impairment in the protein biosynthesis.

The effects of pesticides on protein content of fish have been observed by a number of investigators. Magar and Shaik[16] observed decline in protein content in tissues of fish *Channa punctatus* on exposure to sublethal concentration of malathion. Singh and Singh[17] reported decline in total proteins in the fish *Trichogaster fasciatus* on exposure to pesticide dipterex. Fahmy[18] noticed significant decrease in total proteins in various tissues of *Oreochromis niloticus* on exposure to malathion. Vidhya and Nair[19] observed protein content decreased in tissues of *Etroplus suratensis* on exposure to sublethal concentration of λ- cyhalothrin. All these studies correlate with the decline in total proteins. In later half of exposure the total proteins gradually elevated and came nearer to control at 32nd day exposure period. Giridhar and Neeraja[20] reported decrease in total proteins in different organs of fish, *Labeo rohita* at initial exposure period and elevation in protein content in later exposure periods also coincides with the present study. Dixon and Sprague[21] and Kito *et al.*[22] reported an increase in protein content may also help to fortify the organs for developing resistance to the imposed toxic stress and synthesis of enzymes necessary for detoxification. In the present study the shifts in protein metabolism might to compensate with situation shown by the animal for its survival. The survival ability of animals

exposed to stress majorly depends on their protein synthetic potentials. The proteins are the major source of energy during chronic conditions besides carbohydrates.[23] Young[24] reported protein budget of a cell can be taken as an important diagnostic tool in the evaluation of its physiological standards. Pesticides are known to interfere in protein synthesis and degradation there by altering the dynamic equilibrium.[25]

Before changes in growth occur, changes in biochemical composition should become apparent. The use of energy stores (glycogen, fat) might be initiated and protein synthesis might decrease. Protein is a major component of an organism's body mass and RNA is necessary for the synthesis of protein. Consequently, a positive relationship between the concentration of RNA and the rate of protein synthesis has been suggested. Typically, maximum RNA:DNA ratios should occur during peaks of protein production. Based on these observations, protein, RNA and DNA relationships have been implied as a promising biomarker of reduced growth.[26]

The observed reduction in the growth rate of test fishes is due to an indirect effect of toxicant on macromolecular syntheses which are secondary effects induced by physiological stress.[27] Similar reductions in growth rate of fish have been reported by a number of investigators. Moraes *et al.*[28] observed the acute toxicity of pyrethroid-based insecticides in the neotropical freshwater fish, *Brycon amazonicus* and exposure to synthetic pyrethroid pesticides is known to decrease growth and impair swimming performance. Kim and Kang[29] reported reduced growth rate of Rockfish (*Sebartes schlegeli*) due to Cu stress and there was an inverse relationship between growth and Cu exposure. Hayat *et al.*[30] exposed the fingerlings of three major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala,* to sublethal concentrations of Manganese for 30 days. During this exposure period, all the fish species showed negative growth.

Growth can be considered a complex indicator of metabolic activity. If water pollution occurs in an environment, then growth may be inhibited.[31] According to Brocksen's[32] research, animals fed higher concentrations of pesticides have decreased feeding and growth rates. It is clear that the body weight of the fish changed after 4 days of exposure to quinalphos (Table 2). The decline in relative growth rate at initial exposure periods may indicates the high energy demand associated with imposed quinalphos stress. Sublethal levels of a wide variety of toxicants have been found to slow the growth of fish larvae or juveniles.[33] This can be due to increased metabolic expenditure for detoxification and maintenance of the normal body functions. Thus it is inferred that exposure to quinalphos in sublethal doses affects protein content and normal growth rate of the fish at initial periods. But recovery in later periods may be revitalization phenomenon as every organism strives to overcome the stress to prove its existence.

6. Acknowledgments

The authors acknowledge the help and support rendered by Dr. Shrikrishna Isloor, Associate Professor, Dept. of Veterinary Microbiology and Mr. Avinash Bhat, Research Associate, Dept. of Veterinary Pharmacology and Toxicology, Veterinary College, Bangalore, during the research work.

7. Conflict of Interest

None Declared

8. References

1. Joshi PP, Kulkarni GK. Cypermethrin and fenvalerate induced protein alterations in freshwater crab, *Barytelphusa cunicularis* (Westwood). Recent Res Sci Tech 2011; 3(12):7-10.
2. Varo I, Serrano R, Pitarch E, Amat F, Lopez FJ, Navarro JC. Bioaccumulation of chlorpyrifos through an experimental food chain: study of protein HSP70 as biomarker of sublethal stress in fish. Arch Environ Contam Toxicol 2002; 42:229–235.
3. Gad NS, Saad AS. Effect of environmental pollution by phenol on some physiological parameters of *Oreochromis niloticus.* Global Veterinaria 2008; 2(6):312-319.
4. Huang DJ, Chen HC. Oxygen consumption, ammonia-N excretion and growth rate in juvenile green neon shrimp (*Neocaridina denticulata*) exposed to chlordane and lindane. Acta Zool Taiwan 2004; 14(2):65-76.
5. Das BK, Mukherjee SC. Sublethal effects of quinalphos on selected blood parameters of *Labeo rohita* (Ham) fingerlings. Asian Fish Sci 2000; 13:225-233.
6. Cengiz EI, Unlu E. Histopathology of gills in mosquito fish, *Gambusia affinis* after long-term exposure to sublethal concentrations of malathion. J Environ Sci Health 2003; 38(B):581-589.
7. Triebskorn R, Kohler HR, Honnen W, Schramm M, Adams SM. Induction of heat shock proteins, changes in liver ultrastructure and alterations of fish behavior: Are these biomarkers related and are they useful to reflect the state

of pollution in the field? J. Aquat Ecosyst Stress Recovery 1997; 6:57-73.
8. Pereira P, De-Pablo H, Pacheco M, Vale C. The relevance of temporal and organ specific factors on metals accumulation and biochemical effects in feral fish (*Liza aurata*) under a moderate contamination scenario. Ecotoxicol Environ Saf 2010; 73:805-816.
9. APHA, AWWA, WPCF. Standard methods for the examination of water and wastewater. Washington, DC: American Public Health Association 1981; pp 1134.
10. Finney DJ. Probit Analysis, 3rd Ed. Cambridge University Press, London 1971; 330 pp.
11. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193:265-275.
12. Duncan DB. Multiple range and multiple *F* – tests. Biometrics 1955; 11:1-42.
13. Snedecor GW, Cochran GW. Statistical Methods. Oxford and IBH Publishing Company, Calcutta, India 1968.
14. Ogueji EO, Auta J. Investigation of biochemical effects of acute concentration of lambda-cyhalothrin on African catfish, *Clarias gariepinus* (Teguels). J Fish Intern 2007; 2(1):86-90.
15. Chebbi SG, David M. Modulation in the protein metabolism under sublethal concentration of quinalphos intoxication in the freshwater common carp, *Cyprinus carpio* (Linnaeus, 1758). Int J Pharm Biol Arch 2011; 2(4):1183-1189.
16. Magar RS, Shaikh A. Biochemical changes in proteins and amino acids in *Channa punctatus* in responses to sublethal treatment with the insecticide malathion. Trends in Life Sci 2012; 1(3):2319–4731.
17. Singh SP, Singh S. Metabolic effects of an organophosphorus dipterex in *Trichogaster fasciatus*. Ind J Biol Res 2011; 1(1):61-66.
18. Fahmy GH. Malathion toxicity: Effect of some metabolic activities in *Oreochromis niloticus*, the Tilapia fish. Int J Bio Biochem Bioinf 2012; 2(1):52-55.
19. Vidhya V, Nair CR. Lambda-cyhalothrin induced protein alternations in *Etroplus suratensis* (Bloch). Global J Pharmacol 2013; 7(2):166-171.
20. Neeraja SRK, Giridhar P. Effect of deltamethrin on some aspects of protein metabolism in fresh water fish *Labeo rohita* (Hamilton). Int J Pharm Pharm Sci 2014; 6(7):494-497.
21. Dixon DG, Sprague JB. Copper bioaccumulation and hepato protein synthesis during acclimation to copper by juvenile rainbow trout. Aquatic Toxicol 1981; 1:69-81.
22. Kito H, Osc Y, Sato T, Ishikawa T. Formation of metalloprotein in fish. Comp Biochem Physiol 1982; 73C (1):129-34.
23. Umminger BL. Physiological studies on super cooled kill fish, *Fundus heteroclitus*. III. Carbohydrate metabolism and survival at subzero temperature. J Exp Zool 1970; 173:159-74.
24. Young UR. In: Mammalia protein metabolism (H.N. Mumo. Ed), Academic Press. New York 1970; 4:485.
25. Rekha P, John J. Study on the changes in the levels of protein metabolism in λ cyhalothrin induced hepatotoxicity in fresh water Tilapia (*Oreochromis mossambicus*). Adv Appl Sci Res 2011; 2(3):57-62.
26. Heath AG. Use of physiological and biochemical measures in pollution biology. In: Water pollution and fish physiology. Lewis Publishers, CRC Press, Baco Raton, FL 1995; pp 325-342.
27. Miliou H, Zaboukas N, Moraitou-Apostolopoulou M. Biochemical composition, growth and survival of the guppy, *Poecilia reticulata* during chronic sublethal exposure to cadmium. Arch Environ Contam Toxicol 1998; 35:5863.
28. Moraes FD, De FP, Venturini LRX, Cortella PA, Rossi, Moraes G. Acute toxicity of pyrethroid-based insecticides in the Neotropical freshwater fish, *Brycon amazonicus*. *Ecotoxicol Environ Contam 2013; 8(2):59-64.*
29. Kim SG, Kang JC. Effect of dietary copper exposure on accumulation, growth and hematological parameters of the juvenile rockfish, (*Sebstes schlegeli)*. Mar Environ Res 2004; 58:65-82.
30. Hayat S, Javed M, Razzaq S. Growth performance of metal stressed major carps viz. *Catla catla, Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. Pakistan Vet J 2007; 27:8-12.
31. Cleveland L, Hamilton SJ. Toxicity of the organophosphorus defoliant to rainbow trout, *Salmo gairdneri* and channel catfish *Ictalurus punctatus*. Aquat Toxicol 1983; 4:341-355.
32. Brocksen RW. Influence of competition on the food consumption and production of animals in laboratory stream communities. MS thesis, Oregon State University, Corvallis, OR 1966; 82 pp.
33. Woltering DM. The growth response in fish chronic and early life stage toxicity tests: a critical review. Aquat Toxicol 1984; 5:1–21.