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Adverse Effect of Insecticides on Various Aspects of Fish's Biology and Physiology

Mahdi Banaee
Department of Aquaculture, Natural Resource and Environmental Faculty, Behbahan University of Technology, Iran

1. Introduction

Today, water quality management faces greater problems than at any time in its history. In addition to natural pollutants, varied contaminants exist in surface waters including multiple chemical compounds and different products of industrial and agricultural revolution. The insecticides constitute one group of these pollutants, both synthetic and natural, which contribute to the environmental problems. At present, it seems that the problem is more conspicuous in developing countries, where lately there has been an increase in the use of insecticides as a means of increasing agricultural productivity, without much concern to the consequences of indiscriminate application. There are many pathways by which insecticides leave their sites of application and distribute throughout the environment and enter the aquatic ecosystem. The major route of insecticides to water ecosystems in urban areas is through rainfall runoff and atmospheric deposition. Another source of water contamination by insecticides is from municipal and industrial dischargers. Most insecticides ultimately find their way into rivers, lakes and ponds (Tarahi Tabrizi, 2001; Honarpajouh, 2003; Bagheri, 2007; Shayeghi *et al*., 2007; Vryzas *et al*., 2009; Werimo *et al*., 2009; Arjmandi *et al*., 2010) and have been found to be highly toxic to non-target organisms that inhabit natural environments close to agricultural fields. The contamination of surface waters by insecticides is known to have ill effects on the growth, survival and reproduction of aquatic animals. In the past few years, the increase of mortality among the fish in various streams, lakes and ponds of around the world has drawn scholars' attention to the problems caused by insecticides and pesticides runoff associated with intense agricultural practices. Different concentrations of insecticides are present in many types of wastewater and numerous studies have found them to be toxic to aquatic organisms especially fish species (Talebi, 1998; Üner *et al*., 2006; Banaee *et al*., 2008). Fishes are particularly sensitive to the environmental contamination of water. Hence, pollutants such as insecticides may significantly damage certain physiological and biochemical processes when they enter into the organs of fishes (John, 2007; Banaee *et al*., 2011). Authors found out that different kinds of insecticides can cause serious impairment to physiological and health status of fishes (Begum, 2004; Monteiro *et al*., 2006; Siang *et al*., 2007; Banaee *et al*., 2009). Since fishes are important sources of proteins and lipids for humans and domestic animals, so health of fishes is very important for human beings. Recently, many studies have been conducted to determine the mechanisms of insecticides in fishes, with the ultimate goal of

monitoring, controlling and possibly intervening in xenobiotics exposure and its effects on the aquatic ecosystem. This chapter presents further information concerning the toxic effects of different concentrations of insecticides on various aspects of fish's biology and physiology. In other words, this chapter depicts the effects of insecticides on the survival chance, blood biochemical parameters, tissues and organs, reproduction, development and growth, nervous system, behavior, genetic and immune system of fish. The information given in this part facilitates the evaluation of potential toxic hazard resulting from exposure to different levels of these compounds.

2. Biokinetics and bioteransformation

After exposure to difference concentrations of insecticides in water, the fish absorbs them in its gills, skin or gastrointestinal tract. In the other words, Due to their lipophilicity, most insecticides easily permeate the biological membranes and it increases the sensitivity of fish to aqueous insecticides. Then, these compounds are rapidly metabolized and extracted, and may be bio-concentrated in various tissues of fish. In other words, bio-accumulation occurs if the insecticides is slowly metabolized or excreted from the body. As the amount of insecticide increases, it becomes more harmful to the consumer or animal. Accumulated insecticide can cause death or long-term damage. Ballesteros *et al.* (2011) showed that during the initial 24 h of exposure, insecticides may be transformed in various tissues of fish. However, some differences exist among tissues relating to metabolism rates, leading to different distribution models of the original compounds and their metabolites. For example, the low biodegradation and the high lipid solubility of some insecticides such as organochlorine insecticides have led to problems with the bio-concentrations of these compounds in different tissues of fish. In addition, since some fish are lower on the food chain, bioaccumulation of insecticides may increase in tissues of their predators and consumers, such as humans and thus affecting their health and survival. So, the bioaccumulation of these contaminants in fish and the potential biomagnification in humans are perceived as threats (Favari *et al*., 2002). Bioaccumulation rate of insecticides in fish depends on the species, life stages, the amount of fat reservation in different tissues and diet of fish, chemical and physical properties of insecticides and the rate of water pollution.

In order to facilitate the elimination and detoxification of toxic compounds, fishes have developed partly complex detoxification mechanisms including the release of several enzymes collectively termed xenobiotic metabolizing enzymes. Enzymatic biotransformation of insecticides can potentially alter their activity and toxicity. Enzymes participating in the biotransformation of insecticides are classified into phase I and phase II enzymes. The phase I enzymes, cytochrome P450 enzymes including CYP1A and CYP3A, are generally involved in the biotransformation of exogenous and endogenous compounds; thereby creating a more polar and water soluble compound. A great diversity of cytochrome P450 enzymes in fish has been recognized (Stegeman and Hahn, 1994), and CYP1A, CYP2B, CYP2E1, CYP2K1 and CYP3A have been recently identified in liver of some freshwater fish (Nabb *et al*., 2006) which play an important role in the detoxification of organophosphate and carbamate insecticides (Ferrari *et al*., 2007). The common pathways of biotransformation of different kinds of insecticides include three cytochrome P450 (CYP) mediated reactions: *O*-dealkylation, hydroxylation, and epoxidation of insecticides (Soldano *et al*., 1992; Keizer *et al*., 1995; Kitamura *et al*., 2000; Straus *et al*., 2000; Behrens & Segner, 2001; Nebbia, 2001). In phase II reactions, metabolites produced in phase I detoxification often conjugate with

glutathione, uridyl-diphosphate glucose (UDPG), uridyl-diphosphate-glucuronic acid (UDPGA), amino acid derivatives and sulfate derivatives and can readily excrete from the fish body (Iannelli *et al*., 1994; Keizer *et al*., 1995; Kitamura *et al*., 2000; Straus *et al*., 2000; Behrens & Segner, 2001; Nebbia, 2001). In other words, final metabolites may also be excreted from the body of fish through the skin, gills, genital products, urine as sulphated and glucuronidated metabolites and stool as glutathione conjugated metabolites (Kitamura *et al*., 2000; Straus *et al*., 2000; Behrens & Segner, 2001; McKim & Lein, 2001; Nebbia, 2001).
Since metabolites produced during detoxification process may be more dangerous than parental compounds, these metabolites can cause serious damage in fish. Furthermore, the production of reactive oxygen species (ROS) during detoxification process can induce oxidative damage and may be a mechanism of toxicity for aquatic organisms living in environments receiving insecticides (Monteiro *et al*., 2009). ROS can indiscriminately attack and react with susceptible vital macromolecules -lipids, proteins and DNA- in living cells, inducing cytotoxicity and can result in serious disturbances in physiological cell processes (Dogan *et al*., 2011; Jin *et al*., 2011). Lipid peroxidation, the major contributor to the loss of cell function, DNA damage, enzyme inactivation, and hormone oxidation are bio-indicators of oxidative cell damage and examples of toxic mechanisms of insecticide induced ROS being involved in pathological processes and in the etiology of many fish diseases (Üner *et al*., 2006; Dogan *et al*., 2011).

3. Acute toxicity

In acute toxicity, sudden and intense mortality may be observed in a fish population exposed to toxic materials. The most apparent symptoms of insecticides' acute poisoning in fishes include lethargy, forward extension fins, pallor or blur parts of body, severe reaction to external stimuli and muscle spasms and sudden fast swimming in circles. The main clinical internal sings that can lead to death of fishes include neurological disorder and disruption of nerve functions, respiratory dysfunction and suffocation (Banaee *et al*., 2011).
Acute toxicity testing is widely used in order to identify the dose or exposure concentration and the time associated with death of 50 percent of the fish exposed to toxic materials which is expressed as LC_{50} in parts per million (ppm) or milligrams per liter (mg/L). In addition, we can use the LC_{50} value in the classification of insecticides based on potential toxicity for fishes. Furthermore, the relative acute toxicity of chemicals to fish can be categorized as follows:

Toxicity rating	**96 hr LC_{50}**
Slightly toxic	10-100 ppm
Moderately toxic	1-10 ppm
Highly toxic	0.1-1.0 ppm
Extremely toxic	Less than 0.1 ppm

Our literature reviews demonstrate that different fish species, even from the same family, show differences in the sensitivity to high concentrations of insecticides in water. Acute toxicity of different insecticides is influenced by the age, sex, genetic properties and body size of fish, water quality and its physicochemical parameters, and purity and formulation of insecticides.
The eight tables, which give relative acute toxicity of some insecticides to fishes, can be used to determine the potential toxicity to fish of using these compounds in farms around surface waters and to select products which less likely to cause problems. The data are derived from

laboratory studies and are given only as a guideline and not absolute data of the acute toxicity of the insecticides to different species of fish (Table 1-11).

Insecticide	species	Range of 96h LC_{50}	Reference
Akton	Channel catfish, Bluegill, Rainbow trout, Fathead minnow	0.17-1370 ppb	Jonson & Finley, 1980
Aldicarb	Fathead minnow	1.3-2.4 ppm	Pant *et al*., 1987
Aldrin	Chinook salmon, Rainbow trout, Fathead minnow, Black bullhead, Bluegill, Largemouth bass	2.3-53 ppb	Jonson & Finley, 1980
Allethrin	Rainbow trout, Bluegill	19-56 ppb	Jonson & Finley, 1980
Aminocrab	Cutthroat trout, Rainbow trout, Bluegill, Atlantic salmon, Fathead minnow, Channel catfish, Largemouth bass, Yellow perch, Brook trout	3.1-31 ppm	Jonson & Finley, 1980
Azinphos ethyl	Rainbow trout, Bluegill	1.1-20 ppb	Jonson & Finley, 1980
Azinphos methyl	Gilthead seabream, Coho salmon, Rainbow trout, Bluegill, Atlantic salmon, Brown trout, pike, Goldfish, Carp, Fathead minnow, Black bullhead, Channel catfish, Green sunfish, Largemouth bass, Black crappie, Yellow perch	2.1-4270 ppb	Arufe *et al*., 2007; Jonson & Finley, 1980
Azodrin	Rainbow trout, Bluegill, Channel catfish. Fathead minnow	4.9-50 ppm	Jonson & Finley, 1980

Table 1. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Bensulide	Rainbow trout, Bluegill, Black bullhead, Common eel, Guppy, Sheephead minnow, Crucian carp	0.1-30 ppm	McAllister *et al*., 1986a, b;
Benzene hexachloride	Rainbow trout, Bluegill, Cutthroat trout, Goldfish, fathead minnow, Channel catfish, Largemouth bass	9-348 ppb	Jonson & Finley, 1980
Carbaryl	Coho salmon, Chinook salmon, Cutthroat trout, Atlantic salmon, Brown trout, Brook trout, Lake trout, Carp, Channel Catfish, Fathead Minnow, Rainbow Trout, Bluegill, Goldfish, Black bullhead, Green sunfish, Largemouth bass, Black crappie, Yellow perch	0.9-39 ppm	Jonson & Finley, 1980
Carbofuran	Walked catfish, *Poecilia reticulate*, Chubs	0.22-23 ppm	Dobšíková, 2003; Begum, 2004
Carbophenthion	Channel catfish, Bluegill, Green sunfish	0.02-6 ppm	Jonson & Finley, 1980
Carbosulfan	Bluegill, Cutthroat trout, Rainbow trout, Lake trout, Channel catfish	2.4-280 ppb	Yi *et al*., 2006; Jonson & Finley, 1980

Table 2. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Chlordane	Coho salmon, Cutthroat trout, Rainbow trout, Brown trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	3-115 ppb	Jonson & Finley, 1980
Chlorethoxyphos	Cutthroat trout, Rainbow trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	0.72-11.9 ppm	Jonson & Finley, 1980
Chlorfenapyr	Bluegill, Rainbow trout, channel catfish	6.5-14.7 ppb	Rand, 2004
Chlorpyrifos	Mosquito fish, Bluegill, Fathead minnow, Rainbow trout, Nile tilapia, Goldfish	0.57-3270 ppb	Davey *et al*., 1976; Holcombe *et al*., 1982; Bowman, 1988a, b; Gül, 2005; Wang *et al*., 2009
Coumaphos	Cutthroat trout, Rainbow trout, Lake trout, Channel catfish, Bluegill, Largemouth bass	0.34-1.2 ppm	Jonson & Finley, 1980
Cryolite	Rainbow trout, Bluegill	47-400 ppm	Jonson & Finley, 1980

Cypermethrin	Sheepshead minnow, Rainbow trout, Bluegill, Freshwater catfish	0.39-0.95 ppb	Jaber & Hawk, 1981; Sousa, 1998; Mishra et al., 2005
DDD	Rainbow trout, Fathead minnow, Channel catfish, Largemouth bass, Walleye	14-4400 ppb	Jonson & Finley, 1980
DDE	Rainbow trout, Atlantic salmon, Bluegill	32-240 ppb	Jonson & Finley, 1980

Table 3. Summary of acute toxicity.

Insecticide	**species**	**Range of 96h LC_{50}**	**Reference**
DDT	Coho samon, Rainbow trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass, Black bullhead, Yellow perch	1.5-21.5 ppb	Jonson & Finley, 1980
Deltamethrin	Guppies, Channa punctatus,	1.5-5.13 ppb	Viran et al., 2003; Sayeed et al., 2003
Diazinon	Cutthroat trout, Rainbow trout, Lake trout, Fathead minnow, Carp, Bluegill	0.9-2.6 ppm	Calmbacher, 1978a, b; Banaee *et al*., 2011; Banaee *et al*., 2008; Jonson & Finley, 1980
Dichlorvos	Lake Trout, Sheephead minnow	0.18-7.5 ppm	Mayer & Ellersieck 1986; Jones & Davis, 1994
Dicrotophos	Bluegill, Rainbow trout, Channel catfish	6.3-24.2 ppm	Jonson & Finley, 1980
Dieldrin	Cutthroat trout, Rainbow trout, Goldfish, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	1.2-19 ppb	Jonson & Finley, 1980
Diflubenzuron	Cutthroat trout, Rainbow trout, Brook trout, Fathead minnow, Channel catfish, Bluegill, Yellow perch	25-240 ppm	Jonson & Finley, 1980
Dimethoate	Rainbow trout, Bluegill	6-9.3 ppm	Jonson & Finley, 1980
Dimethrin	Fathead minnow, Channel catfish, Yellow perch, Bluegill,	28-1275 ppb	Jonson & Finley, 1980
Dinitrocresol	Rainbow trout, Bluegill	66-360 ppb	Jonson & Finley, 1980

Table 4. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Dioxathion	Cutthroat trout, Rainbow trout, Largemouth bass	22-110 ppb	Jonson & Finley, 1980
Disulfoton	Rainbow trout, Fatheah minnow, Channel catfish, Bluegill, Largemouth bass	60-4700 ppb	Jonson & Finley, 1980
d-Trans Allethrin	Coho salmon, Steelhead, Lake trout, pike, Fathead minnow, Channal catfish, Largemouth bass, Yellow perch	2.6-66 ppb	Jonson & Finley, 1980
Endosulfan	Striped bass, Bluegill, Rainbow trout, Fathead minnows, Asian swamp eel, Milk fish, Zebra fish	0.1-20 ppb	Mayer & Ellersieck, 1986; Siang *et al*., 2007; Capkin *et al*., 2006; Magesh & Kumaraguru, 2006, Velasco-Santamaría *et al*., 2011
Endrin	Rainbow trout, Goldfish, fathead minnow, black bullhead, Channel catfish, Mosquito fish, Bluegill, Largemouth bass, Yellow perch, carp	0.15-1.8 ppb	Jonson & Finley, 1980
EPN	Cutthroat trout, Rainbow trout, Channel catfish, Bluegill, Largemouth bass, Walleye	110-420 ppb	Jonson & Finley, 1980
Ethion	Cutthroat trout, Rainbow trout, Channel catfish, Bluegill, Largemouth bass, Fathead minnow	0.17-7.6 ppm	Jonson & Finley, 1980

Table 5. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Ethyl Parathion	Coho salmon, Cutthroat trout, Rainbow trout, Brown trout, Goldfish, Carp, Fathead minnow, Channel catfish, Bluegill, Black bullhead, Largemouth bass, Yellow perch	0.4-3.52 ppm	Jonson & Finley, 1980
Fenitrothion	Coho salmon, Cutthroat trout, Rainbow trout, Brown trout, Brook trout, Atlantic salmon, Goldfish, Bluegill, Channel catfish, Fathead minnow, Carp	1.7-12 ppm	Johnson & Finley, 1980; Woodward & Mauck, 1980; Jonson & Finley, 1980
Fenthion	Coho salmon, , Rainbow trout, Brown trout, Brook trout, Atlantic salmon, Goldfish, Yellow perch, Bluegill, Channel catfish, Green sunfish, Fathead minnow, Largemouth bass, Carp	1.1-3.4 ppm	Jonson & Finley, 1980

Fenvalerate	Zebra fish	3.5-193 ppb	Ma *et al*., 2009
Heptachlor	Rainbow trout, Northern pike, Fathead minnow, Black bullhead, Channel catfish, Redear sunfish, Bluegill, Largemouth bass,	5.3-63 ppb	Jonson & Finley, 1980
Isoprocarb	Goldfish	4.61 ppm	Wang *et al*., 2009

Table 6. Summary of acute toxicity.

Insecticide	**species**	**Range of 96h LC_{50}**	**Reference**
Kepone	Rainbow trout, Channel catfish, Bluegill, redear sunfish	30-225 ppb	Jonson & Finley, 1980
Leptophos	Rainbow trout, Lake trout, Fathead minnow, Bulegill	0.03-30 ppm	Jonson & Finley, 1980
Lindane	Eel, Tilapia, African Catfish, Coho salmon, Rainbow trout, Brown trout, Goldfish, Carp, Fathead minnow, Black bullhead, Green sunfish, Largemouth bass, Yellow perch	0.03-1.29 ppm	Ferrando *et al*., 1988, Feltz, 1971; Lawson *et al*., 2011; Jonson & Finley, 1980
Linuron	Bluegill, Rainbow trout	3-16.2 ppm	Wetzel, 1986; Mayer & Ellersieck,1986
Malathion	Coho salmon, Cutthroat trout, Rainbow trout, Brown trout, Lake trout, Goldfish, Carp, Fathead minnow, Black bullhead, Bluegill, Green sunfish, Largemouth bass, Yellow perch, Redear sunfish	4-12900 ppb	Mayer & Ellersieck,1986; Jonson & Finley, 1980
Methamidophos	Rainbow trout, Fathead minnow, Channel catfish, Bluegill	1.6-100 ppm	Jonson & Finley, 1980
Methomyl	Cutthroat trout, Rainbow trout, Atlantic salmon, Brook trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	0.3-6.8 ppm	Jonson & Finley, 1980; Yi et al., 2006

Table 7. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Methoxychlor	Rainbow trout, Atlantic salmon, Cutthroat trout, Brook trout, Lake trout, pike, Goldfish, Largemouth bass, Bluegill, Yellow perch, Fathead minnow, Channel catfish	15-64 ppb	Jonson & Finley, 1980
Methyl Parathion	Freshwater characid fish, Coho salmon, Cutthroat trout, Rainbow trout, Brown trout, Lake trout, Goldfish, Carp, Fathead minnow, Channel catfish, Bluegill, Black bullhead, Green sunfish, Largemouth bass, Yellow perch	0.25-9 ppm	Mayer & Ellersieck,1986; Monteiro et al., 2006; Jonson & Finley, 1980
Methyl Trithion	Cutthroat trout, Rainbow trout, Channel catfish, Bluegill, Largemouth bass	0.76-2.8 ppm	Jonson & Finley, 1980
Mexacarbate	Coho salmon, Cutthroat trout, Rainbow trout, Atlantic salmon, Lake trout, Carp, Fathead minnow, Channel catfish, Bluegill, Black bullhead, Largemouth bass, Yellow perch	0.32-23 ppm	Jonson & Finley, 1980
Mirex	Rainbow trout, Brown trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass, Yellow perch, Walleye	100 < ppm	Jonson & Finley, 1980

Table 8. Summary of acute toxicity.

Insecticide	species	Range of 96h LC_{50}	Reference
Monocrotophos	Tilapia, Mosquito fish	11.5-20.5 ppm	Rao, 2006; Kavitha & Rao, 2007
Naled	Cutthroat trout, Rainbow trout, Lake trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	0.13-3.3 ppm	Jonson & Finley, 1980
Oxydemeton-methyl	Rainbow trout, Channel catfish, Bulegill, Largemouth bass, Walleye	13-31.5 ppm	Jonson & Finley, 1980
Permethrin	Brook trout	1.4-7.9 ppb	Jonson & Finley, 1980
Phorate	Cutthroat trout, Rainbow trout, Northern pike, Largemouth bass, Channel catfish, Bluegill	2-110 ppb	Jonson & Finley, 1980

Phosmet	Coho salmon, Rainbow trout, Fathead minnow, Channel catfish, bluegill, Smallmouth bass, Largemouth bass	0.15-10.6 ppm	Jonson & Finley, 1980
Phosphamidon	Rainbow trout, Fathead minnow, Channel catfish, Bluegill	3.4-100 ppm	Jonson & Finley, 1980
Phoxim	Coho salmon, Atlantic salmon, Rainbow trout, Brown trout, Brook trout, Northern pike, Fathead minnow, Channel catfish, bluegill	0.11-2.9 ppm	Jonson & Finley, 1980
Propoxur	Goldfish, Rainbow trout, Fathead minnow, Bluegill	4.8-36.2 ppm	Wang *et al*., 2009; Jonson & Finley, 1980

Table 9. Summary of acute toxicity.

Insecticide	**species**	**Range of 96h LC_{50}**	**Reference**
Pyrethrum	Coho salmon, Atlantic salmon, Brown trout, Lake trout, Channel catfish, bluegill	13-65 ppb	Jonson & Finley, 1980
Resmethrin	Cho salmon, Lake trout, Fathead minnow, Channel catfish, bluegill	1.7-9.9 ppb	Jonson & Finley, 1980
Ronnel	Rainbow trout, Channel catfish, bluegill, Cutthroat trout, Lake trout	0.6-1.6 ppm	Jonson & Finley, 1980
Rotenone	Rainbow trout, Channel catfish, bluegill	2.6-36 ppb	Jonson & Finley, 1980
RU-1169	Coho salmon, Atlantic salmon, Lake trout, fathead minnow, White sucker, Bluegill	0.3-28 ppb	Jonson & Finley, 1980
S-Bioallethrin	Fathead minnow, Channel catfish, Bluegill, Yellow perch	7.8-90 ppb	Jonson & Finley, 1980
SD-17250	Coho salmon, Rainbow trout, bluegill	1.5-5.7 ppm	Jonson & Finley, 1980
Strobane	Bluegill, Rainbow trout	8.7-12 ppb	Jonson & Finley, 1980
TEPP	Rainbow trout, Fathead minnow, Bluegill	240-980 ppb	Jonson & Finley, 1980

Table 10. Summary of acute toxicity.

Insecticide	species	Range of 96h LC50	Reference
Temephos	Cutthroat trout, Rainbow trout, Atlantic trout, Brook trout, Lake trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	1.44-34 ppm	Jonson & Finley, 1980
Thanite	Rainbow trout, Channel catfish, Bluegill	1.2-3.7 ppm	Jonson & Finley, 1980
Thiodicarb	Bluegill, Rainbow trout	1.4-3.3 ppm	Yi *et al*., 2006
Toxaphene	Coho salmon, Rainbow trout, Brown trout, Goldfish, Carp, Fathead minnow, Black bullhead, Channel catfish, Bluegill, Largemouth bass, Yellow perch	2-18 ppb	Jonson & Finley, 1980
Trichlorfon	Eel, Rainbow trout, Cutthroat trout, Atlantic salmon, Brown trout, Brook trout, Lake trout, Fathead minnow, Channel catfish, Bluegill, Largemouth bass	0.36-9.2 ppm	Lopes *et al*., 2006; Jonson & Finley, 1980

Table 11. Summary of acute toxicity.

4. Sub-lethal toxicity

Sub-lethal toxicity testing was planned based on one tenth or more of LC_{50} dose in moderate periods. In sub-lethal toxicity, the organs or biological systems which may be affected at such exposure can be respiratory, hepatic, haematopoietic, nervous, cardiovascular, and reproductive and immune systems. Different biomarkers of fish exposed to insecticides are usually evaluated in these experiments. Insecticides may lead to changes in the blood biochemical parameters and haematological profile of fish which can be investigated as biomarker in pollution monitoring (Mushigeri & David, 2005; Banaee *et al*., 2008; Kavitha & Rao, 2009). In fact, these compounds may induce alterations in the activities or levels of a number of different enzyme systems, including those necessary for biochemical reactions in cells (Banaee *et al*., 2011). Decreased rate of growth, reproductive disorder, spinal deformities, histopathological changes (Benli & Özkul, 2010) in gills, liver, haematopoietic tissue such as spleen, head of kidney, and renal tubules, endocrine tissues as well as brain, neurological and behavioral disorder and genetic defects are other biological indicators of exposure to insecticides which are described in details in the following sections.

5. Chronic toxicity

Chronic toxicity tests commonly include the measurement of long term effects of low concentrations of insecticides on the survival, growth, reproduction, nervous system and other biological and physiological aspects of fishes. Type of injury to fish in chronic toxicity is similar to sub-lethal toxicity damage, but the frequency and intensity injury and lesion resulting from chronic toxicity may be more or even less than damage of sub-lethal toxicity. Therefore, this experiment is important in insecticides toxicology.

6. Side effect of insecticides on various aspects of fish's biology and physiology

6.1 Alterations in blood biochemical parameters

Insecticides can cause serious impairment to physiological and health status of fish. Therefore, biochemical tests are routine laboratory tests useful in recognizing acute or chronic toxicity of insecticides (Banaee *et al*., 2008; Al-Kahtani, 2011) and can be a practical tool to diagnose toxicity effects in target organs and to determine the physiological status in fish. Blood biochemistry test gives indicates what is happening in the body of fish exposed to insecticides. When different tissues are injured, the damaged cells release specific enzymes into plasma and we can recognize their abnormality levels in blood. Then it helps locate the lesions. Also, if certain organs are not eliminating certain waste products or not synthesizing certain important materials, this can tell us they are not functioning properly. In some cases due to the severity of the damage to tissues, particularly liver, synthesis of many biochemical parameters may reduce significantly in cells, which can decrease some biochemical factors in blood of fish exposed to insecticides. These changes were observed in *Channa punctatus* (Agrahari *et al*., 2007), *Oreochromis niloticus* (Velisek *et al*., 2004), *O.mossambicus* (Arockia and Mitton, 2006; Matos *et al*., 2007), *Heteropneustes fossilis* (Saha & Kaviraj, 2009), *Cirrhinus mrigala* (Prashanth & Neelagund, 2008) *Clarias batrachus* (Begum, 2005; Ptnaik, 2010), *Cyprinus carpio* (Banaee *et al*., 2008), *Oncorhynchus mykiss* (Banaee *et al*., 2011), *Colisa fasciatus* (Singh *et al*., 2004) which were exposed to monocrotophos, bifenthrin, carbaryl, dimethoate, cypermethrin, sevin, diazinon, and malathion respectively.

6.2 Tissue and organ damage

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. In histopathology, we can provide information about the health and functionality of organs. Tissues injuries and damages in organs can result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents. Frequency and intensity of tissue lesions depend on the concentrations of insecticides and the length of the period fish are exposed to toxins. Nevertheless, many insecticides cause specific or non-specific histopathological damage (Fanta *et al*., 2003). For example, histopathological lesions in the liver tissue of freshwater fish (*Cirrhinus mrigala*) (Velmurugan *et al*., 2009) and common carp carp (*Cyprinus carpio*) (Banaee *et al*., In press) were observed after 10 and 30 days exposure to sublethal concentrations of dichlorvos and diazinon insecticides, respectively. Other researchers reported the same histopathological alterations in different tissues of fish treated with diazinon (Duttaa & Meijer, 2003; Banaee *et al*., 2011), deltamethrin (Cengiz, 2006; Cengiz & Unlu, 2006), fenitrothion (Benli & Özkul, 2010).

6.3 Reproductive dysfunction

Reproduction guarantees the survival of fish population. Any changes in environmental parameters or physiological conditions of fish can affect its reproductive success. Since fish may be exposed to environmental pollutants, including insecticides, herbicides, heavy metals and other xenobiotics, disorders may occur in their natural reproductive process. Recent researches showed the dysfunction in the reproductive systems of fishes exposed to insecticides. Insecticides' effects on reproductive biology of fishes are numerous, and include decreased fecundity, testicular and ovarian histological damage (Duttaa & Meijer,

2003; Banaee *et al*., 2009), vitellogenesis process impairment (Haider and Upadhyaya, 1985;), and disruption in steroidogenesis process (Zaheer Khan & Law, 2005), delay in gonads maturation (Skandhan *et al*., 2008), alter in reproductive and parental behavior (Jaensson *et al*., 2007), impairment in olfactory response and disorder in reproductive migrations (Scholz *et al*., 2000), as well as disruption in coordinating courtship behavior of male and female fish and time of spawning (Jaensson *et al*., 2007).

Some insecticides are known as endocrine disrupting chemicals (EDC) which can interfere with the normal functioning of endocrine system in fish. Adverse effects of insecticides on the hypothalamus-pituitary-gonads axis can also play a significant role in causing reproductive failures in fish. In fishes, chronic toxicity of insecticides can change sex steroid hormone levels in plasma. While the mechanism is not exactly known, it is possible that insecticides and their metabolites disrupt reproductive systems through activation or inhibition of key enzymes which participated in the steroid hormone biosynthesis in fishes. For example, DDT, endosulfan, methoxychlor and some other insecticides possess estrogenic properties and are probably capable of disrupting functions of endocrine system and causing disorder in the reproductive system of fish (Arukwe, 2001). These compounds may directly or indirectly interact with natural hormones, changing the hormone functions and thus altering physiological cellular response or mutate the natural patterns of hormone synthesis and metabolism. Impact of organophosphate insecticides such as malathion, diazinon and fenitrothion on the hypothalamus-pituitary-gonads axis and also disturbance in hormones associated with reproductive systems were studied by Kapur & Toor, (1978); Singh and Singh, (1987); Maxwell & Dutta, (2005); Skandhan *et al*. (2008).

Insecticides can also cause adverse effects on gonad histology, morphology and its growth. In addition, there are significant relationships between blood sex steroid hormone concentrations, sperm or oocytes quality, rate of fecundity and histopathological alterations in ovary and testis of fish exposed to different insecticides (Duttaa & Meijer, 2003; Maxwell & Dutta, 2005). Banaee *et al*., (2008) reported that diazinon inhibits steroidogenesis in testis of male carp by histopatological alterations. Research results showed that direct toxic effects of insecticides on seminiferous tubules or Leydig cells may be the most important parameter for the low quality of sperms in fish (Fadakar Masouleh *et al*., 2011). Similar results were reported in walking catfish (*Clarias batrachus*), freshwater eel (*Monopterus albus*), and Atlantic salmon (*Salmo salar*) that were exposed to different insecticides (Singh & Singh, 1987; Singh, 1989; Moore & Waring, 1996; 2001).

Exposure of fish eggs and milt to insecticides also reduced the level of fertilization, hatching rate and the larval survivability. Further studies on bluegills (*Lepomis macrochirus*), atlantic salmon (*Salmo salar* L.) demonstrated that the gametes and fertilized eggs were sensitive to the insecticides (Tanner & Knuth, 1996; Moore & Waring, 2001) suggesting a further toxic impact of these toxicants on the fish reproduction. In addition, the waste of energy in the fish exposed to insecticides reduces their reproductive ability.

6.4 Development disorders

Study of development disorders caused by insecticides is to emphasize the links between the concentrations of toxins and dysfunction in normal development from embryonic to puberty periods. So, impairment in the normal development and the growth may reduce the fish's survival chance.

Embryos and larvae may be directly exposed to insecticides, through the yolk or via parental transfer in viviparous fish (Viant *et al*., 2006). Spinal deformities, mostly scoliosis and lordosis, and morphological abnormalities were among the more adverse effects registered for insecticides toxicity. Other alterations in the embryo of fish exposed to insecticides also consist of yolk sac edema, pericardial edema and crooked body of larvae (Xu *et al*., 2010). Teratogenic effects of carbaryl insecticides on the embryo of fish have been proved (Todd and Leeuwen, 2002). Similar results were reported in silversides *Menidia beryllina* exposed to tebufos during embryogenesis (Middaugh *et al*., 1990; Hemmer *et al*., 1990).

The most important factors decreasing fish growth consist of disorder in feeding behaviors, decrease in feeding rate, dysfunction in metabolism process and waste of energy to overcome the stress caused by insecticide exposure (Tripathi *et al*., 2003). For example, disorder in the metabolism of carbohydrates, proteins and lipids in various tissues, particularly liver of fish exposed to insecticides, may reduce their growth rates. Begum (2004) found out that protein and carbohydrate metabolism in the liver and muscle tissue is disrupted on the exposure to a carbofuran insecticide. In addition, exposure during embryonic or larval stage can result in behavioral abnormalities, such as decreased ability to capture prey after hatching, functional deficiencies or slowing of growth and finally death (Kuster, 2005; Viant *et al*., 2006; Arufe *et al*., 2007). These changes were observed in larvae and embryo of zebra fish (*Danio rerio*) in contact with endosulfan (Velasco-Santamaria *et al*., 2011), beta-cyprmethrin (Xu *et al*., 2010); paraoxon-methyl (Küster, 2005) and sevin (carbaryl insecticide) (Todd and Leeuwen, 2002).

6.5 Neurotoxicity

The primary mechanism of organophosphate and carbamate insecticides toxicity is well known – they function as inhibitors of acetycholinestrase enzyme (AChE) or and butyrylcholinesterase (BChE), as well as disturbing the metabolism of other neurotransmitters such as γ-aminobutyrate (GABA). The synthetic pyrethroids change normal neuronal function by interfering in the function of ion channels in the nerve cell membrane, alterations in intracellular calcium ion concentrations and possibly by blocking GABA receptors. Organochlorine insecticides act primarily by changing the transport of ions across the nerve cell membranes, thus altering the ability of nerve to stimulate.

Fish exposure to these insecticides is frequently assessed by determining the alterations in AChE in brain, muscle, plasma and other tissues or probably GABA activity in brain (Banaee, 2010). AChE is an enzyme responsible for inactivating the neurotransmitter acetylcholine (Fulton & Key, 2001). AChE inactivation results in the accumulation of the neurotransmitter acetylcholine in cholinergic synapses space, leading to synaptic blockage and disruption of signal transmission (Ferrari *et al*., 2004; 2007a, b). Inhibition of AChE induces alteration in the swimming behavior, shaking palsy, spasms and other undesirable effects (Sharbide *et al*., 2011). Disturbances in AChE activity can also impair feeding, identification and avoidance and escaping from predators, spatial orientation of the species, and reproductive behavior (Bretaud *et al*., 2000). Thus, AChE inhibition is considered to be a specific biomarker of exposure to organophosphorus and carbamate insecticides like diazinon, chlorpyrifos, propoxur, isoprocarb, (Üner *et al*., 2006; Cong *et al*., 2008; 2009; Wang

et al., 2009; Banaee *et al*., 2011;). Similar results have been observed for pyrethroids insecticide toxicity (Koprucu *et al*., 2006). Disorder in γ-aminobutyrate (GABA) system in brain of rainbow trout exposed to sub-lethal lindane was reported by Aldegunde *et al*., (1999). GABA receptors inhibit the transmission of nerve impulses; thus disturbances in this receptor would also lead to an over stimulation of the nerves.

In addition, nervous tissue has weaker antioxidant defense system than other kinds of tissue. On the other hand, the brain as center of the nervous system in fish contains low levels of enzymatic and non-enzymatic antioxidant and higher levels of oxidizable unsaturated lipids and catecholamines. So, nerve tissue is very sensitive to oxidative stress damage induced insecticide toxicity compared with other tissues (Üner *et al*., 2006; Li *et al*., 2010). These results have been reported by other scientists (Senger *et al*., 2005).

6.6 Behavioral alterations

Behavioral changes are the most sensitive indicators of potential toxic effects. The behavioral and the swimming patterns of the fish exposed to different insecticides include changes in swimming behavior, feeding activities, predation, competition, reproduction and species-species social interactions such as aggression (Cong *et al*., 2008; 2009; Werner and Oram, 2008). Banaee *et al*. (2008; 2011) reported similar behavioral responses in common carp and rainbow trout exposed to sub-lethal levels of diazinon. In fact, most insecticides influence the behavior patterns of fish by interfering with the nervous systems and sensory receptors (Keizer *et al*., 1995; Pan & Dutta, 1998; Cong *et al*., 2008; 2009); and this incident may impair the identification of situation and development of appropriate response by the fish exposed to insecticide. The effect of certain insecticides on the activity of acetylcholinestrase may lead to a decreased mobility in fish (Bretaud *et al*., 2000). Researchers have reported the same alterations in *Oryzias latipes*, *Cyprinus carpio*, *Labeo rohita*, *Oncorhynchus tshawytscha*, *O.latipes*, *Cirrhinus mrigala*, *Oreochromis niloticus*, *Clarias gariepinus* treated with chlorpyrifos (Rice *et al*., 1997; Halappa & David, 2009), malathion (Patil & David, 2008), diazinon (Scholz *et al*., 2000), endosulfan (Gormley & Teather, 2003), Fenvalerate (Mushigeri & David, 2005), fenitrothion (Benli & Özkul, 2010), dimethoate (Auta *et al*., 2002), respectively.

6.7 Genotoxicity

In genetic-toxicology any heritable damage or DNA inactivation resulting from the animal's exposure to xenobiotics is studied. Genotoxic chemicals such as insecticides have common chemicals and physical properties that enable them interact with genetic materials (Campana *et al*., 1999; Çava & Ergene-Gözükara, 2003; Candioti *et al*., 2010; Dogan et al., 2011). The mutation that may result from an interaction between a chemical and the genetic material is a heritable change in the cell genotype, and thus the error may be transferred to the daughter cell or the next generation. Carcinogenesis and the formation of some tumors in different tissues of fish exposed to insecticides may also be caused by genotoxic properties of these xenobiotics. One of the ill effects of insecticides' arrival into surface waters may be an induction of chromosomal damage in eggs and larvae of fishes in different stages of development.

Some insecticides that behave as endocrine active compounds can change the expression of vital genes resulting in unusual concentrations of plasma steroid hormones and reproductive dysfunction or immuosuppression (Jin *et al*., 2010).

6.8 Immunosuppression

The immune system of fish is important for defense against a variety of pathogens. The system is very sensitive to homeostatic adjustments via endocrine regulation and is influenced by the biochemical status of the nervous system. Thus, any impairment in the nervous system and disturbance in the biochemical homeostasis can weaken the immune system of fish. On the other hand, insecticides may alter the function of the immune system and result in immunodepression, uncontrolled cell proliferation, and alterations of the host defense mechanisms including innate immunity and acquire immunity against pathogens.
Different insecticides at sub-lethal levels have been recognized as stressors causing immune-suppression in fish (Werner and Oram, 2008). In addition, some insecticides may exert immunotoxic effects by altering the transcription of important mediators of the fish's immune system (Eder *et al.*, 2009). Effects of insecticides like P,P'-DDE, lindane, cypermethrin, chlorpyrifos, diazinon on the immune factors of fish such as Interleukin-1β (IL-1β), IL-1β receptor (IL-1R1), Interferon gamma (IFN-γ2b), TNFα, MHCIα, MHCIIα, Mx, TLR9, IγML and C- reactive protein (CRP), TCRα in head- kidney leucocytes, Lysozyme activity, chemiluminuscence (CL) response and immunocompetent cells population size, IgM levels, value of white blood cells (WBC) and respiratory burst activity, head kidney phagocytes and peripheral blood leucocytes, etc., have been reported by scholars (Betoulle *et al*., 2000; Khoshbavar-Rostami *et al*., 2006; Banaee *et al*., 2008; Cuesta *et al*., 2008; Girón-Pérez *et al*., 2009; Shelley *et al*., 2009; Ahmadi *et al*., 2011; Jin *et al*., 2011, Wang *et al*., 2011). The exposure to sub-lethal concentrations of insecticides is what probably makes fish vulnerable to infectious diseases because of their immune-depressive effects (Zelikoff *et al*., 2000). For example, the susceptibility of juvenile chinoock salmon (*O.tshawytscha*) to infectious hematopoietic necrosis virus was significantly increased in fish exposed to sub-lethal concentrations of esfenvalerate (Clifford *et al*., 2005). Similar results were reported in goldfish and common carp that were exposed to carbaryl and lindane respectively (Shea, 1983; Shea & Berry, 1984; Cossarini-dunier & Hattenberger, 1988).

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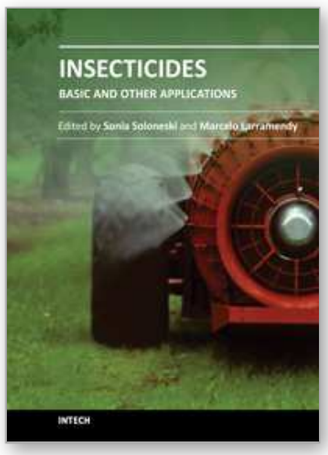

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It is our hope that this book will be of interest and use not only to scientists, but also to the food-producing industry, governments, politicians and consumers as well. If we are able to stimulate this interest, albeit in a small way, we have achieved our goal.

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Phone: +385 (51) 770 447
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Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
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