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Lethal concentration and toxicity stress of Carbosulfan, Glyphosate and Atrazine to freshwater air breathing fish *Channa punctatus* (Bloch)

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Abstract

The present study was undertaken to evaluate the lethal toxicity and stress of commercial formulations of carbosulfan (Aatank) insecticide, glyphosate (Roundup) and atrazine (Rasayanzine) herbicides toward freshwater air-breathing fish *Channa punctatus* (Bloch). The 96 h LC_{50} values, determined in a semi-static system by probit analysis as 0.268, 32.540 and 42.380 mg/l for carbosulfan, glyphosate and atrazine, respectively, indicated that the fish were more sensitive to carbosulfan than the other two herbicides. There were large variations in the safe levels estimated by different methods for the pesticides. In addition to dose and dose-time dependent increase in mortality rate, stress signs in the form of behavioral changes were observed in response to the test pesticides.

Keywords: *Channa punctatus*, Pesticides, LC_{50}, Safe level, Toxicity

Introduction

The use of pesticides has been recognized as part of agricultural practices throughout the world. Unfortunately the indiscriminate use of these pesticides to improve agricultural production and yield may have impacts on non-target organism, especially aquatic lives and environment. The world health organization (WHO 1992) reported that roughly 3 million cases of pesticides poisoning occur annually, resulting in 220,000 deaths worldwide. Many of these chemicals are mutagenic (Garaj-vrhovac and Zeljezic 2000; Kumar et al. 2009; Nwani et al. 2010), linked to the development of cancers (Leiss and Savitz 1995) or may lead to the developmental deficits (Arbuckel and Server 1998).

Carbosulfan [2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl [(dibutylamino)thio] methyl carbamate] belongs to the benzofuranyl methyl carbamate group of pesticides and has been widely used in agriculture for broad spectrum control of insect pests of crops such as caterpillars, green leaf hoppers, white-backed plant hopers, brown plant hoppers, gall midges, stem borers, leaf folder of paddy, white aphids of chilies (Giri et al. 2002). It has been

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reported to be effective against certain insect pests, which are not controlled by organo-chlorine or organo-phosphorous insecticides (Sahoo et al. 1990) and has been proposed for the control of pyrethroid resistant mosquitoes (Guillet et al. 2001).

Glyphosate (N-Phosphoromethyl glycine) is a broad spectrum, translocated herbicide, used primarily in agricultural applications for the control of a great variety of annual, biennial and perennial grasses, sedges, broad leaved weeds and woody shrubs, used in fruits orchards, vineyards, conifer plantations and many plantation crops (Ayoola 2008). It is also used for aquatic weed control in fish ponds, lakes, canals and slow running water (Tsui and Chu 2008). Glyphosate is perhaps the most important herbicide ever developed (WHO1994).

Atrazine (2-Chloro-4-ethylamino-6-Isopropylamino-s-triazine) is one of the most commonly used herbicides found in the rural environments. It is extensively used for corn, sorghum, sugar cane, pineapples, and to some extent in landscape vegetation. Rated as moderately toxic to aquatic species, atrazine is mobile in the environment and is among the most detected pesticides in streams, rivers, ponds, reservoirs and ground water (Battaglin et al. 2008).

The contamination of aquatic ecosystems by xenobiotics has gained increasing attention and several recent studies have demonstrated the toxicity and effects to fish under field and laboratory conditions (Abdul Farah et al. 2004; Pandey et al. 2005; Costa et al. 2008; Ayoola 2008; Langiano et al. 2009; Lushchak et al. 2009; Kumar et al. 2009; Nwani et al. 2010). Chronic exposure and accumulation of these xenobiotics by aquatic biota can result in biochemical and tissue burdens that produce adverse effects not only in the exposed organisms but also in organism including human beings (IARC 1993). Due to high water solubility, low persistence and extensive usage of these pesticides in the environment, exposure to non-target aquatic organisms is a source of concern. Therefore, it seems essential to study the lethal toxicity and stress of such environmental pollutants so as to formulate the strategies for safe guarding aquatic organisms.

The present study is thus aimed at examining the toxicity and effects of carbosulfan insecticide, glyphosate and atrazine herbicides on fresh water fish *Channa punctatus* by determining the LC_{50} values and analyzing behavioral changes due to toxic stress. The study further focused on estimation of the safe level as well as strengthened the base line data that could be used to estimate comparative sensitivity to these pesticides.

Materials and methods

Experimental fish specimen and chemicals

Freshwater air-breathing fish *Channa punctatus* (Bloch; Family; Channidae, Order: Perciformes) were procured from local sources. This species was selected for bioassays because of some ecotoxicological characteristics such as wide distribution, availability throughout the year, easy maintenance under laboratory conditions and commercial importance.

Table 1. Specifications of the test pesticides

Pesticide	CAS. eg. Number	Supplier	Grade	Chemical Name	Alternative Name
Carbosulfan	55285-14-8	Northern Minerals Ltd, India	Commercial formulation (25 % EC)	[2,3-dihydro-2,2-dimethyl-7-benzofuranyl [(dibutylamimo)thio] methyl carbamate]	Aatank
Glyphosate	1071-83-6	Monsato India Ltd	Commercial formulation (41% SL)	N-Phosphorom Ethyl glycine	Roundup
Atrazine	1912-24-9	Krishi Rasayan, India Exports Pvt India	Commercial formulation (50% WP)	2-chloro-4-ethylamino-6-isopropylamino-s-atrazine	Rasayanzine

The specimens had an average (± SD) weight and length of 13.38 + 0.21 g and 11.15 + 0.14 cm, respectively. Fish specimens were subjected to prophylactic treatment by batting twice in 0.05% potassium permanganate ($KMnO_4$) for two min to avoid any dermal infections. The fishes were then acclimatized for two weeks under laboratory conditions in semi static systems. They were fed boiled eggs, minced goat liver and poultry waste materials during acclimatization. The fecal matter and other waste materials were siphoned off daily to reduce ammonia content in water. The test pesticides used in the present study, commercial formulations of carbosulfan (Aatank), glyphosate (Roundup) and atrazine (rasayanzine) had specifications listed in Table 1.

Determination of sub-lethal concentrations

Acute toxicity assay to determine the 96 h LC_{50} value of carbosulfan, glyphosate and atrazine was conducted with definitive test in semi-static system in laboratory as per standard methods (APHA, AWWA, WPCE, 2005). The range finding test was carried out prior to the definitive test to determine the concentration of the test solution. The experiment was conducted in glass aquaria (60×30×30 cm) containing 40l of dechlorinated and gentle aerated water. The water with the pesticide was changed after every 48h to counterbalance decreasing pesticides concentrations.

A set of 10 fish specimen were randomly exposed to each of the carbosulfan (0.1, 0.2, 0.3, 0.4, 0.5 mg/l), glyphosate (22.0, 26.0, 30.0, 34.0, 38.0 mg/l) and atrazine (25.0, 32.0, 39.0, 46.0, 53.0 mg/l) concentrations. Another set of 10 fish were also simultaneously maintained in tap water (0.00 mg/l) as the control. The experiment was set in triplicate and fish were not fed during the experimentation as recommended by Ward and Parrish (1982) and Reish and Oshida (1987). Exposure time was 96 h after which mean mortality from a particular dose and its replicate was calculated. A fish was considered dead when it did not respond after gentle prodding with a glass rod; dead fish were removed from the tank immediately. Fish behavior was observed during the exposure. The median lethal concentration (LC_{50}) of the test pesticides was calculated from the data obtained in acute toxicity bioassays following the probit analysis method as described by Finney (1971). The 95% confidence limits of the LC_{50} values obtained by Finney (1971) method were calculated with the formula of Mohapatra and Rengarajan (1995). The safe level estimation after 96 h exposure for all pesticides was based on Hart et al. (1948), Sprague (1971), Committee on Water Quality Criteria (CWQC 1972), National Academy of Sciences/National Academy of Engineering (NAS/NAE 1973), and International Joint Commission (IJC 1977).

Data analysis

The data obtained were statistically analyzed by statistical package SPSS (version 16). The data were subjected to one way ANOVA and Duncan’s multiple range test was used to determine the significance difference at 5 % probability level.

Results

Physicochemical characteristics of the test water

The physicochemical characteristics of the test water are presented in Table 2. The water temperature varied from 24.20 to 25.40 ºC, pH 7.4 to 7.90 while salinity ranged from 0.09 to 0.17 mg/l. The dissolved oxygen concentration ranged from 6.70 to 7.80 mg/l, conductivity values varied from 260 to 300 µM/cm, while total hardness varied from 200 to 230 mg/l $CaCO_3$ during the experimental period.

Toxicity stress and poisoning symptoms in fish

Fish exposed to different concentrations of carbosulfan, glyphosate and atrazine displayed uncoordinated behavior. At the initial exposure, fish were alert, stopped swimming and remained static in position in response to sudden changes in the surrounding environment. After sometime they tried to avoid the toxic water with fast swimming and jumping. Faster opercula activity was observed as surfacing and gulping of air. In tanks with higher concentrations of test pesticide especially carbosulfan, swimming of the fish was very erratic with vigorous jerks of the body. Their fins became hard and stretched due to stretching of body muscles.

They secreted copious amounts of mucus from whole body continuously and soon thick layer of mucus was found deposited in the buccal cavity and gills. Body pigmentation was increased. Ultimately fish lost their balance and consciousness, engaged in rolling movement and became exhausted and lethargic. Lastly they remained in vertical position for a few minutes with anterior side or terminal mouth up near the surface of the water trying to gulp air and tail in a downward direction. Soon they settled at the bottom of the tank, and after some time their bellies turned upward and the fish died, while the opercula remained wide open exposing the gills.

Table 2. Physicochemical properties of the test water

S. N.	Characteristics	Unit	Mean	Range
1	Air temperature	°C	26-30	25.60-26.60
2	Water temperature	°C	24.40	24.20-25.40
3	pH	-	7.60	7.40-7.90
4	Salinity	mg/l	0.12	0.09-0.17
5	Dissolved oxygen	mg/l	6.80	6.70-7.80
6	Conductivity	µM/cm	282	260.30.0
7	Total hardness	mg/l	220	200-30

Table 3. Lethal concentrations of carbosulfan, glyphosate and atrazine (mg/l) (95% confidence intervals) depending on exposure time for *C. punctatus*)*

Pesticide	Lethal Concentration	Exposure time (h)			
		24	48	72	96
Carbosulfan	LC_{10}	0.215^{a}	0.178^{b}	0.162^{b}	0.160^{b}
		(0.136-20.266)	(0.122-0.187)	(0.128-0.189)	(0.134-0.191)
	LC_{50}	0.629^{a}	0.295^{b}	0.280^{b}	0.268^{b}
		(0.502-1.017)	(0.264-0.325)	(0.252-0.307)	(0.243-0.293)
	LC_{90}	1.846^{a}	0.548^{b}	0.482^{b}	0.435^{b}
		(1.105-6.540)	(0.0477-0.677)	(0.429-0.573)	(0.392-0.505)
Glyphosate	LC_{10}	36.250^{a}	34.709^{b}	27.991^{c}	27.179^{c}
		(33.540-37.802)	(32.409-35.871)	(26.160-29.350)	(22.324-29.395)
	LC_{50}	40.788^{a}	37.330^{b}	33.991^{c}	32.540^{c}
		(39.500-41.947)	(36.215-38.230)	(32.210-34.59)	(30.990-34.112)
	LC_{90}	45.890^{a}	40.140^{b}	37.520^{c}	35.054^{c}
		(44.267-49.177)	(39.007-42.111)	(36.140-39.371)	(38.054-43.960)
Atrazine	LC_{10}	43.042^{a}	37.383^{b}	34.664^{c}	33.192^{c}
		(36.661-46.872)	(33.42-40.111)	(31.322-37061)	(29.920-35.542)
	LC_{50}	64.053^{a}	49.100^{b}	44.412^{c}	42.380^{c}
		(57.806-79.920)	(46.623-51.914)	(42.252-46.623)	(40.218-44.406).
	LC_{90}	95.321^{a}	64.483^{b}	56.902^{c}	54.113^{c}
		(77.411-160.716)	(59.511-73.515)	(53.322-62.722)	(50.804-59.332)

*Values with different alphabet superscript differ significantly ($P < 0.01$) between exposure time within lethal concentrations.

Median lethal concentration and application factor

Median lethal concentration (LC_{50}) is the most widely accepted basis for acute toxicity test and it is the concentration of a test chemical, which kills 50 % of the test organism in a particular length of exposure, usually 96 h. Generally in toxicity test, death is a decisive criterion because it is easy to determine and have obvious biological and ecological significance. The LC_{50} values (with 95% confidence limits) of different concentrations of carbosulfan were 0.629 (0.50-1.1017), 0.295 (0.264-0.325), 0.280 (0.252-0.307), and 0.268 (0.243-0.293) mg/l for 24, 48, 72 and 96 h, respectively, while that of glyphosate and atrazine were 40.788 (39.500-41.947), 37.330

(36.215-38.230), 33.991 (32.210-34.540) and 32.540 (30.990-34.112) and 64.053 (57.806-79.920), 49.100 (46.623-51.914), 44.412 (42.252-46.623) and 42.380 (40.218-44.406) mg/l for 24, 48, 72 and 96 h respectively (Table 3). A dose dependent increase and time dependent decrease were observed in mortality rate as the exposure time increased from 24 to 96 h; the median concentration was reduced. There were significant differences ($P < 0.05$) in LC_{10-90} values obtained for different times of exposure. No mortality was however observed in the control during the experimental period. There were variations in safe levels estimated by different methods at 96 h of fish as presented in Table 4.

Table 4. Estimate of safe levels of carbosulfan, glyphosate and atrazine at 96 h exposure time

Chemical	96h LC_{50} (mg/l)	Method	AF	Safe level
Carbosulfan	0.268	Hart et al. (1948)*	-	1.950×10^{-3}
		Sprague(1971)	0.1	2.680×10^{-2}
		CWQC(1972) NAS/NAE	0.01	2.680×10^{-3}
		(1973)	0.1-0.00001	2.680×10^{-2} to 2.68×10^{-6}
		CCREM (1991)	0.05	1.340×10^{-2}
		IJC (1977)	5% of 96 h LC_{50}	1.340×10^{-2}
Glyphosate	32.540	Hart et al. (1948)	-	9.381×10^{-1}
		Sprague (1971)	0.1	3.254
		CWQC (1972)	0.01	3.254×10^{-1}
		NAS/NAE (1973)	0.1-0.00001	3.254 to 03.254×10^{-4}
		CCREM (1991)	0.05	1.627
		IJC (1977)	5% of 96 h LC_{50}	1.627
Atrazine	42.380	Hart et al. (1948)	-	8.657
		Sprague (1971)	0.1	4.240
		CWQC (1972)	0.01	4.24×10^{-1}
		NAS/NAE (1973)	0.1-0.00001	$4.24-04.24 \times 10^{-4}$
		CCREM (1991)	0.05	2.129
		IJC (1977)	5% of 96 h LC_{50}	2.129

*$C = 48\ h\ LC_{50} \times 0.03/S^2$ where C is the presumable harmless concentration and $S = 24h\ LC_{50}/48\ h\ LC_{50}$.

Discussion

Acute toxicity data has been used to derive water quality guidelines for regulatory measures (Sunderam et al. 1994). The results of the LC_{50} (median lethal concentration) of the present study at 96 h were 0.268, 32.540 and 42.380 mg/l for carbosulfan, glyphosate and atrazine respectively. The results showed that carbosulfan was more toxic to the fish than glyphosate and atrazine and toxicity of the pesticides was both time and concentration dependent, thus accounting for differences in LC_{10-90} values obtained at different concentrations and times of exposure. The test result of the 96 h LC_{50} of *C. punctatus* exposed to carbosulfan obtained in the present study was slightly higher than the 96 h LC_{50} value of 0.231 and 0.122 mg/l estimated by Boran et al. (2007) for rainbow trout (*Oncorhincus mykiss*) and guppy (*Poecillia reticulata*) for the same pesticide, respectively. The 96 h LC_{50} of 32.540 mg/l reported for *C. punctatus* exposed to glyphosate in the present study was higher than 13.69 mg/l recorded by Langiano et al. (2009) for *Prochilodus lineatus* but was lower than 620 mg/l recorded by Nesković (1993) for *Cyprinus carpio*.

Our value however was within the 2-55 mg/l range reported by Hildebrand et al. (1982); Jiraungkoorskul et al. (2003) and Glusczak et al. (2007) for other fish species exposed to glyphosate. The 96 h LC_{50} of 42.380 mg/l reported for *C. punctatus* exposed to atrazine in the present study differed from the report of Bathe et al. (1973), Neskovic et al. (1993) and Hussein et al. (1996) who reported LC_{50} of 16.0, 18.8 and 9.37 mg l-1 for *Lepomis macrochirus* (Bluegill sunfish), *Cyprinus carpio* and *Oreochromis niloticus* respectively exposed to atrazine. Toxicity of chemicals to aquatic organisms has been shown to be affected by age, size and health of the species (Abdul-Farah et al. 2004). Physiological parameters like quality, temperature, pH, dissolved oxygen and turbidity of water, amount and kind of aquatic vegetation, concentration and formulation of chemical and its exposure also greatly influence such studies (Gupta et al.1981).

The estimated safe levels obtained in the present study showed variations among the various pesticides. However, the large variation in safe levels determined by various methods has resulted in controversy over its acceptability (Buikema et al. 1982; Pandey et al. 2005). Mount and Stephan (1967) underscored the fact that extrapolation of laboratory data to the field is not always meaningful, and hence it is difficult to decide on an acceptable concentration based on the laboratory experiments that may be considered "safe" in the field. Kennega (1979) emphasized that the major weakness in calculation of accumulation factor (AF) is its dependence on LC_{50} value.

Behavioral changes as a result of stress are further accepted as the most sensitive indication of potential toxic effects. The various behavioral changes like restlessness, abnormal swimming behavior, vigorous jerks of body, loss of balance, myotonia and anorexia observed in *C. punctatus* are similar to the observations of Hussein et al. (1996), Pandey et al. (2005) and Chandra (2008) in fishes exposed to various pesticides.

Conclusion

The result of the present study showed that carbosulfan insecticide was more toxic to *C. punctatus* than glyphosate and atrazine herbicides. All the pesticides induced toxic stress inform of behavioral changes in the fish. Acute toxicity studies have been recognized as the very first step in determining the water quality requirements of fish and reveal toxicant concentrations (LC_{50}) that cause fish mortality even at short exposure. However, chemical determination of any persistent toxicant concentration in water as well in sediment may not provide information on the severity of contamination, especially in the case of sublethal levels.

Biological monitoring using a series of assays having different endpoints could allow a sensitive approach to predict the potential risk of pesticides which is helpful in formulating the "safe levels" of such bioaccumulative chemicals. Therefore, studies demonstrating the sensitivity of genotoxic effects of pesticides in aquatic organisms particularly in fish are needed in assessment of possible risk to similar species in natural environment.

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