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Original Article

Determination of lethal concentrations of indoxacarb in fingerling *Cyprinus carpio* at two temperatures

Ali Taheri Mirghaed, Melika Ghelichpour*

Department of Aquatic Animal Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Abstract: Acute (24-96 hrs) toxicity of indoxacarb, a new insecticide generation, was determined in *Cyprinus carpio* under the semi-static condition. Fish (~5 g) were exposed to increasing concentrations of indoxacarb over 24-96 hrs at 17ºC, and over 24 hrs at 22ºC, and mortality was recorded every 24 hrs. Indoxacarb-LC_{50} values at 17ºC were found to be 37.55, 20.92, 18.77 and 16.85 ppm after 24, 48, 72 and 96 hrs, respectively. LC_{50} after 24 hrs at 22ºC was 21.55 ppm, which was significantly lower than that obtained at 17ºC. The lowest observed effect concentration (LOEC) values at 17ºC were 14 ppm for 24, 48, 72 hrs, and 11 ppm for 96 hrs. No observed effect concentration (NOEC) values at 17ºC were 11 ppm for 24, 48, 72 hrs, and 8 ppm for 96 hrs. NOEC and LOEC values after 24 hrs at 22ºC were 8 and 11 ppm, respectively. The results indicated that indoxacarb is classified as "harmful" substance in common carp and, the higher temperature, the more toxicity of indoxacarb.

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Introduction

Chemical pesticides are considered as an economic access to control pests; however, these chemicals may be highly toxic to non-target species in the environment. Nowadays, the indiscriminate use of pesticides and their excretion to the aquatic ecosystem threaten the health of organisms (Rao, 2006).

Indoxacarb is an oxadiazin pesticide used to control sucking pests of crops, especially acts against lepidopteran larvae. The chemical name of indoxacarb is (S)-methyl 7- chloro-2, 5-dihydro-2-[[(methoxycarbonyl) [4-(trifluoromethoxy) phenyl] amino] carbonyl] indeno[1,2-e][1,3,4] oxadiazine-4a(3H)-carboxylate (C22H17ClF3N3O7). It is marketed under the names Indoxacarb Technical Insecticide, Steward Insecticide and Avaunt Insecticide. Avant is more common name for this substance in Iran. This relatively new insecticide was registered in California, January 2001 (Monkada, 2003). As soon as indoxacarb is absorbed or ingested by insects, feeding cessation occurs. It kills pests by binding to a site on sodium channels and blocking the flow of sodium ions into nerve cells. The result of exposure is impaired nerve function, feeding cessation, paralysis and death of pests. It may take days for insects to die (Brugger, 1997).

Indoxacarb is used on a range of crops, which include fruits (apples, pears and tomatoes), vegetables (broccoli, Brussels sprouts, cabbage, cauliflower, eggplant, potato, and lettuce), soybeans, alfalfa and peanuts. It is used to control or suppress many insects, including Beet armyworm, Cabbage looper, Corn earworm, Diamondback moth, Fall armyworm, Imported cabbageworm, Southern armyworm, Tomato pinworm and Tomato fruitworm (Dupont, 2002).

This pesticide is somehow a hydrophobic pesticide and its penetration to aquatic system is slow, so it is considered as a safe insecticide in environmental standpoint. It provides a much safer alternative to some pesticides such as organophosphates and

* Corresponding author: Melika Ghelichpour
E-mail address: ml.ghelichpour@gmail.com

pyrethroids. The relatively low mammalian toxicity of this insecticide provides improved safety to workers, as well as terrestrial mammals and birds when compared to competitive organophosphates and carbamates. Lack of cross resistance to existing insect control products, environmental suitability and its safety to non-target organisms makes indoxacarb an excellent candidate for controlling some pests (Wing et al., 2000).

Since pH elevation and sunlight speed up indoxacarb hydrolysis and photolysis, it seems that this insecticide has low persistence in aquatic system. However, it is classified as moderately to very highly toxic to freshwater and estuarine/marine fish on an acute basis (Moncada, 2003).

There are very limited studies about the effect of this insecticide on fish. Veeraiah et al. (2013) investigated the biochemical parameters of Indian major carp *Labeo rohita* exposed to lethal and sub-lethal concentrations of indoxacarb showing that indoxacarb is highly toxic to fish with 96 hrs LC_{50} of 0.053 ppm. Also, indoxacarb exposure resulted in decrease in protein, glycogen and nucleic acids content in different organs. As, there is no information available about the toxicity of this pesticide to common carp, *Cyprinus carpio*, hence, this study aimed to determine the lethal concentrations of indoxacarb in common carp at two different temperatures.

Materials and methods

Fish and maintenance conditions: In summer 2015, a total of 700 fingerlings were obtained from Fishery Research Station of Gharahsoo (Bandar Turkman, Iran) and brought to the laboratory. Fish were stocked in a 2000 L fiberglass tank for adaptation to the laboratory conditions. The fish were maintained under continuously-aerated condition in the tank for seven days. They were fed commercial pellets (Energy Co., 4EF3000) at 2% body weight per day, twice a day. Water exchange was about fifty percent daily (ground water of Gharahsoo). The fish total length and weight were 5.83 ± 0.87 cm and 5.64 ± 0.74 g, respectively.

Water quality parameters were as following: temperature = 17ºC, pH = 8.23 ± 0.02, dissolved oxygen = 8.68 ± 0.5 ppm, oxygen saturation = 97.05 ± 2.3 %, electro conductivity = 4.8 ± 0.15 µs m^{-1}, salinity = 2.8 ± 0.08 ppt. These parameters were measured by Hach HQ40d portable pH, conductivity, dissolve oxygen and salinity meter (Loveland, Colorado, USA). Total hardness 300 ± 17 ppm (as $CaCO_3$), alkalinity 350.75 ± 20 ppm (as $CaCO_3$), and calcium 110.8 ± 11 ppm were measured by a Portable photometer (Wagtech 7100, Berkshire, UK). No mortality was observed during adaption period.

Toxicity test: Lethal concentration was determined at two temperatures, 17 ± 1 and 22 ± 1°C, according to OECD (1992). Based on the preliminary tests, the fish were exposed to concentrations of 0 (control), 8, 11, 14, 17, 20, 23, 26, 35, 45 and 60 ppm indoxacarb (Hefiran Co., 15%, Tehran, Iran). At 22°C, mortality was recorded after 24 hrs exposure, whereas, at 17°C mortality was recorded at 24, 48, 72 and 96 hrs. Eleven tanks were assigned for each temperature. A 300 L, white and cylindrical tank was used for each concentration. Each tank stocked with 30 fish (150 g biomass) and 160 L water. The fish were allowed to adapt to these conditions for seven days under aerated condition, during which they were fed (2% of body weight, twice a day) with commercial pellets (Energy Co., 4EF3000). Tanks' water was exchanged fifty percent daily. Water quality parameters were monitored during experiment and they were similar to those of adaptation period. No mortality was observed during this period. Feeding was ceased 24 hrs before dosing. Indoxacarb solution was added to each tank to set concentrations. Seventy five percent of the exposed solution was renewed each day to maintain water quality and the appropriate concentration of indoxacarb. During the experiment, the fish behavior and the number of dead fish were recorded at 24 hrs intervals.

Statistical analysis: LC_{50} values were calculated using probit regression in SPSS v.22. The lowest observed effect concentration (LOEC) was

Table 1. LC_5 (ppm) of indoxacarb after 24, 48, 72 and 96 hrs at 17 and 22ºC.

Time	Temperature	LC_5	95% Confidence Limits Lower	95% Confidence Limits Upper	Slope ± S.E	Intercept ± S.E
24 hrs	22ºC	14.98	7.86	17.83	10.42 ± 1.25	-13.89 ± 1.65
24 hrs	17ºC	19.57	13.76	23.61	5.81 ± 0.68	-9.15 ± 0.96
48 hrs	17ºC	14.80	11.41	16.70	10.94 ± 1.37	-14.45 ± 1.80
72 hrs	17ºC	13.70	12.17	14.80	12.03 ± 1.50	-15.31 ± 1.93
96 hrs	17ºC	12.10	10.67	13.15	11.44 ± 1.39	-14.03 ± 1.73

Table 2. LC_{50} (ppm) of indoxacarb after 24, 48, 72 and 96 hrs at 17 and 22ºC. Asterisk in front of 24 hrs LC_{50} at 22ºC means significant difference compared to 24 hrs LC_{50} at 17ºC.

Time	Temperatu	LC_{50}	95% Confidence Limits Lower	95% Confidence Limits Upper	Slope±S.E	Intercept±S.E
24 hrs	22ºC	21.55*	18.25	26.70	10.42±1.25	-13.89±1.65
24 hrs	17ºC	37.55	32.26	46.44	5.81±0.68	-9.15±0.96
48 hrs	17ºC	20.92	19.12	23.08	10.94±1.37	-14.45±1.80
72 hrs	17ºC	18.77	17.87	19.70	12.03±1.50	-15.31±1.93
96 hrs	17ºC	16.85	15.97	17.73	11.44±1.39	-14.03±1.73

Table 3. LC_{95} (ppm) of indoxacarb after 24, 48, 72 and 96 hrs at 17 and 22ºC.

Time	Temperature	LC_{95}	95% Confidence Limits Lower	95% Confidence Limits Upper	Slope ± S.E	Intercept ± S.E
24 hrs	22ºC	31.00	55.33	124.9	10.42 ± 1.25	-13.89 ± 1.65
24 hrs	17ºC	72.05	25.54	66.26	5.81 ± 0.68	-9.15 ± 0.96
48 hrs	17ºC	29.57	25.95	39.38	10.94 ± 1.37	-14.45 ± 1.80
72 hrs	17ºC	25.72	23.90	28.87	12.03 ± 1.50	-15.31 ± 1.93
96 hrs	17ºC	23.48	21.79	26.22	11.44 ± 1.39	-14.03 ± 1.73

determined as the minimum concentration which caused mortalities at each time point (Rand, 1995). No observed effect concentration (NOEC) was determined as maximum concentration at which no mortality was occurred (Rand, 1995). Difference between 24 hrs-LC_{50} of the temperatures was considered significance if LC_{50} of one temperature was outside the confidence interval of the other temperature (Marking and Bills, 1975; Hoseini and Jafar Nodeh, 2011). Significant difference between mortality percentages was investigated using t-test.

Results

No mortality was observed in the control group during the exposure. The control and 8 ppm indoxacarb treatment showed normal swimming and natural body coloration during the experiment. Abnormal behavioral changes such as dark coloration, unilateral or bilateral exophthalmia, loss of equilibrium, lateral swimming near the surface, motionless on the bottom of tank, hyper excitement and lethargy were observed in the fish exposed to higher indoxacarb concentrations (20-60 ppm) during 24 hrs of exposure period. These behaviors were observed in 11 ppm of indoxacarb 72 hrs after the start of the experiment, and in 14 ppm indoxacarb 48 hrs after exposure.

LC_{5-95}, NOEC and LOEC of indoxacarb are presented in Tables 1-4. LC_{50} after 24, 48, 72 and 96 hrs at 17ºC were 37.55 (32.26-42.44), 20.92 (19.12-23.08), 18.77 (17.87-19.70) and 16.85 (15.97-17.73) ppm, respectively. LC_{50} after 24 hrs at 22ºC was 21.55 (18.25-26.70). There was a significant difference between 24 hrs-LC_{50} at the tested

Table 4. NOEC and LOEC of indoxacarb after 24, 48, 72 and 96 hrs at 17 and 22ºC.

Time	Temperature	NOEC	LOE
24 hrs	22ºC	8	11
24 hrs	17ºC	11	14
48 hrs	17ºC	11	14
72 hrs	17ºC	11	14
96 hrs	17ºC	8	11

temperatures. NOEC after 24, 48, 72 and 96 hrs at 17ºC were 11, 11, 11 and 8 ppm, respectively. LOEC after 24, 48, 72 and 96 hrs at 17ºC were 14, 14, 14 and 11 ppm, respectively. NOEC and LOEC after 24 hrs at 22ºC were 8 and 11 ppm, respectively. Mortality of fish exposed to different concentrations of indoxacarb, at 22ºC was significantly higher than 17ºC (Fig. 1).

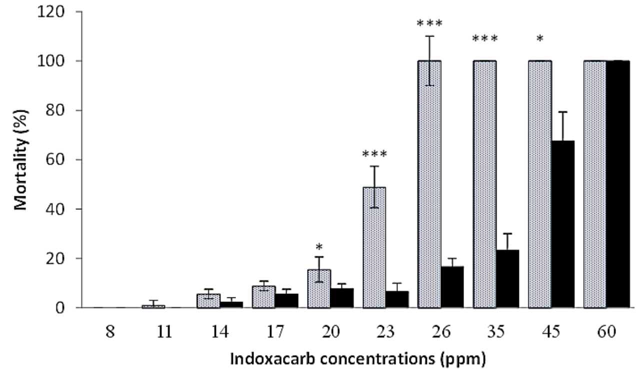

Figure 1. Mortality percentages at the final time point of observation (17 and 22ºC: 24 hrs after exposure). Columns represent means and standard deviation. Gray bars show mortalities at 22ºC and black bars show mortalities at 17ºC. Asterisks above the bars show significant difference in mortality percentages between the temperatures. * $P<0.05$; *** $P<0.001$.

Discussion

The results revealed that the severity of the behavioral responses was dependent to the indoxacarb concentration and exposure time. There were no studies about fish behavioral alterations which are exposed to indoxacarb. Environmental pollutants such as metals, pesticides, and other organic pollutants cause serious risks to aquatic organisms. Accordingly, there is a great deal of researches about physiological mechanisms in animals exposed to contaminants. Behavioral signs of toxicity appear to be ideal tools to assess the effects of aquatic pollutants on fish, because behavioral indicators reflex the physiological states. Toxicant exposure may completely alter behaviors that are essential for fish survival in natural ecosystems, particularly in toxicant concentrations lower than lethal concentration. Behavioral changes are mainly related to cholinesterase inhibition, alternation of brain neurotransmitter levels, sensory deficiency, and impaired gonadal or thyroid hormone levels. Scott and Sloman (2004) investigated interrelationships between behavioral and physiological indicators of toxicity in fish. Also, a positive correlation was observed between brain acetyl cholinesterase activity and swimming speed of mosquito fish, *Gambusia affinis* after lethal exposure of an organophosphate pesticide, monocrotophos (Kavitha and Rao, 2007).

According to 96 hrs LC_{50}, indoxacarb is classified as "harmful" substance in common carp (Commission Directive, 2001). The results showed that LC_{50} of indoxacarb in the present study was higher than other studies which reported 96 hrs LC_{50} of 0.024-0.053 in different fish species (Veeraiah et al., 2013; Hoke, 1997). Such differences may be species specific. Also, water physico-chemical properties are the most important factors involved in the different results of indoxacarb toxicity. Hydrolysis rates of indoxacarb elevate with increasing pH. The half-life at pH 5, 7 and 9 were considered to be approximately 500, 38 and 1 day, respectively (Ferraro and McEuen, 1996). In this study, pH was 8.23 ± 0.02; therefore, it can cause faster hydrolysis of indoxacarb and continuously lower toxicity in comparison with other studies; although, those studies did not mention water pH. Other water physic-chemical factors may affect toxicity of pesticide. For instance, increasing total alkalinity from 19 to 90-120 ppm is resulted in decreasing mortality of *Oncorhynchus mykiss* exposed to methiocarb and endosulfan (Altinok et al., 2006; Capkin et al., 2006). Water alkalinity in the present study was relatively high (~350 ppm) and this may be another reason for higher LC_{50} compared to the previous studies that needs a further study.

Cyprinus carpio is an ectothermic organism, hence, temperature is a fundamental factor influencing all its physiological processes. Many authors affirmed the assumption that temperature elevation increases toxicity of harmful substances in certain species (Fisher and Wadleigh, 1985; Persoone et al., 1989; Song et al., 1997; Van Wezel and Jonker, 1998; Heugens et al., 2001). Exposure to a toxic substance would increase metabolism and oxygen demand of fish as an ectothermic organism; with elevated temperature, the oxygen solubility in water is decreased, therefore, temperature elevation may worsen the toxic effects of the toxic substance (Osterauer and Kohler, 2008). In addition, bioavailability of toxic materials like any substances is dependent on temperature. At high temperatures, the absorption of substances by aquatic animals is elevated due to better solubility of the substance and an intensified distribution or active uptake rate of the substance by gill or skin (Heugens et al., 2001; Osterauer and Kohler, 2008). According to this, toxic effect of a substance on an aquatic organism may be potentiated with increasing temperature. Similarly, Altinok et al. (2006) and Capkin et al. (2006) reported increase in methiocarb and endosulfan toxicity in *O. mykiss* as a result of temperature elevation.

As conclusion, the results suggest that indoxacarb is categorized as "harmful" pesticide in common carp with 96 hrs LC_{50} value of 16.85 ppm. Also, the pesticide toxicity increases along with temperature elevation.

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چکیده فارسی

تعیین غلظت‌های کشندۀ سم ایندوکساکارب بر کپورماهیان انگشت‌قد در دو دمای مختلف

علی طاهری میرقائد، ملیکا قلیچ پور*
گروه بهداشت آبزیان، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران.

چکیده:
سمیت حاد حشره‌کش جدید ایندوکساکارب (۲۴- ۹۶ ساعت) در ماهی کپورمعمولی (*Cyprinus carpio*) در شرایط نیمه ایستا تعیین شد. ماهی‌های حدود ۵ گرم در دمای ۱۷ درجۀ سانتی‌گراد به مدت ۲۴ تا ۹۶ ساعت و در درجه‌حرارت ۲۲ درجۀ سانتی‌گراد به مدت ۲۴ ساعت در معرض غلظت‌های مختلف ایندوکساکارب قرار گرفتند و درصد مرگ‌ومیر هر ۲۴ ساعت گزارش شد. در دمای ۱۷ درجۀ سانتی‌گراد مقادیر LC_{50} برای سم ایندوکساکارب پس از ۲۴، ۴۸، ۷۲ و ۹۶ ساعت به‌ترتیب ۲۰/۳۷،۹۲/۵۵، ۱۸/۷۷ و ۱۶/۸۵ ppm به‌دست آمد. در درجه حرارت ۲۲ درجۀ سانتی‌گراد، مقدار LC_{50} پس از ۲۴ ساعت ۲۱/۵۵ ppm محاسبه شد که به‌طور معنی‌داری کمتر از مقدار معادل آن در دمای ۱۷ درجۀ سانتی‌گراد بود. مقدار کمترین غلظت مؤثر (LOEC) در درجه‌حرارت ۱۷ درجۀ سانتی‌گراد طی زمان‌های ۲۴، ۴۸ و ۷۲ ساعت ۱۴ ppm و پس از ۹۶ ساعت ۱۱ppm محاسبه شد. غلظت بی‌اثر (NOEC) این سم در دمای ۱۷ درجۀ سانتی‌گراد پس از ۲۴، ۴۸ و ۷۲ ساعت ۱۱ ppm و پس از گذشت ۹۶ ساعت ۸ ppm بود. غلظت بی‌اثر و کمترین غلظت مؤثر سم ایندوکساکارب در دمای ۲۲ درجۀ سانتی‌گراد و پس از ۲۴ ساعت به‌ترتیب ۸ و ۱۱ ppm بود. نتایج نشان داد که سم ایندوکساکارب در دستۀ مواد مضر قرار داشته و افزایش دما سبب افزایش سمیت این حشره‌کش می‌گردد.

کلمات کلیدی: کپورمعمولی، حشره‌کش، سمیت، LC_{50}.