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Formalin Toxicity to Caspian roach, Rutilus rutilus caspicus Fry

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ABSTRACT Formalin is a common disinfectant and therapeutic agent in fisheries-related activities. Considering large variation in susceptibility of different species to formalin toxicity, it is necessary to determine its toxicity and safety margins in different species, separately. Therefore, to investigate formalin acute toxicity and safety margins on Caspian roach, an experiment was conducted in 1g Caspian roachs *Rutilus rutilus caspicus*. Fish were exposed to 0, 30, 40, 45, 50, 55, 60, 70, 100, 150, 200, 250, 300 and 400 ppm formalin and mortality was recorded after 0.5, 1, 12, 24, 48, 72 and 96h. 0.5-96 h LC50 values were 49-246 ppm. The lowest observed effect concentrations (LOEC) were found to be 40-150 ppm at different periods. No observed effect concentrations (NOEC) were 30-100 ppm at different periods. According to regression model for NOEC versus time, concentrations of 88.4, 80.3, 51.2 and 40.1 ppm are suggested as the maximum concentrations for 0.5, 1, 12 and 24 h bath treatment, respectively. Results showed the recommendations for formalin safety in the literatures are not applicable and valid for Caspian roach. Such differences could be due to species or experimental differences which should be taken into account before treating.

Key words: *Caspian roach, Disinfectant, LOEC, NOEC, Toxicity*

1 INTRODUCTION

Increased fish production in fisheries is often accompanied by increased incidence of fish diseases of parasites and thus requires the use of therapeutic or prophylactic drugs. Varieties of chemical disinfectant and prophylactic agents are commonly used in all aquaculture and fisheries activities. Short- and long-term bath are two main methods for disinfection as well as disease prevention and treatment. Formalin is a widely-used therapeutic for ecto-parasite control, gill and skin bacterial disease (Post, 1987; Speare and Ferguson, 1989; Smith *et al.*, 1993; Thorburn and Moccia, 1993; Rach *et al.*, 1997; Klinger and Floyd, 1998). Concentration of 150-250ppm over 30-60 min, and 15-25 ppm over the longer period (depending on species and life stage) have been suggested for short- and long-term formalin treatment, respectively (Stoskopf, 1988; Luzzana and Valfre, 1993; Powel *et al.*, 1996; Klinger and Floyd, 1998).

Beside the therapeutic and disinfecting effect of formalin, it can cause toxicity in fish,

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too. Some previous studies reported the toxicity (48h- median lethal concentration (LC50)) of formalin in certain fish species such as *Trachinotu carolinus* (Birdsong and Avault, 1971), *Clarias macrocephalus* (Kanchanaburangkul, 1988), *Chanos chanos* (Cruz and Pitogo, 1989) and *Sphoeroides annulatus* (Fajer-Ávila *et al*., 2003). The reported values in these studies were 74-260 ppm, suggesting involvement of some factors in formalin toxicity.

Species and treatment condition affect chemical performance and toxicity (Hoffman and Meyer 1974; Kabata, 1985), therefore, the toxicity of the chemical to fish should be determined before applying it for disease control. On the other hand, therapeutic materials might introduce to the water as a result of poor management which in turn, can cause water contamination as well as acute or chronic toxicity. Water contamination with formalin might occur as a result of aquaculture- and fisheries-related practices. Also, active ingredient of the formalin is formaldehyde, which is introduced to aquatic ecosystem via varieties of sources. For instance, formaldehyde is present in rain water at the level of 0.11-0.17ppm (Howard, 1990). It is used in dental drug such as mouthwash as well as in resins as sterilant (Hohreiter and Rigg, 2001). Thus, water contamination with formaldehyde is not unlikely and study on its toxicity in certain fish species would be of interest.

Caspian roach *Rutilus rutilus caspicus*, is a valuable species of Caspian sea. It is a very toothsome for the local people occupied the Caspian basin. There is always a huge demand for this fish in the local markets. This species was so scant in the market, during the previous years, however, it is relatively abundant in the market compared to the previous which is believed to be related to stock rebuilding performed by fisheries organization as well as capture managements. At present, it is artificially propagated and reared by Iranian fisheries organization to release into the sea for stock rebuilding. In the involved farms, formalin is used as disinfectant of culture facilities (tanks, nets, etc) as well as therapeutic agent, as well as some other chemicals. According to Khara *et al.* (2011) Caspian roach might be the host for the ecto-parasites diplostomum, *Diplostomum spathaceum* as well as anchor worm, *Lernaea cyprinacea*. Formalin can be used to eliminate these parasites, if occur (Al-Hamed and Hermiz, 1973; Larsen *et al*., 2005). However, there are no information about its toxicity and safety in Caspian roach. Such information can provide and basic knowledge necessary for therapeutic treatments, too. Thus, the aim of the present work was to determine 0.5-96 h LC50 of formalin to evaluate its toxicity and marginal safety in Caspian roach.

2 MATERIALS AND METHODS

2.1 Fish and maintenance condition

A total of 3500 Caspian roach fingerlings (0.9 ± 0.012g) were provided from Bony Fish Propagation and Rearing Center of Sijeval (Bandar Torkaman, Iran). Fish were packed in 10 plastic bags (100 L in volume) filled with water and oxygen in portion of 1:2. Fish were transferred to Aquaculture Research Center of Gorgan University of Agricultural Sciences and Natural Resources, within 20 min. At arrival, fish were stocked in a fiberglass tank (2000 l). Fish were maintained under continually-aerated condition in the tank for 7 days, during which they were fed (~1% of body weight, once a day) by trout commercial pellet (Biomar, France; 0.8 mm in diameter). Water exchange was about 50% daily (dechlorinated tap water of city Gorgan). Photoperiod was 14:10 light: dark (natural). Temperature was almost maintained constant (23±1ºC) using central temperature control system. Water quality parameters were: dissolved oxygen (DO) > 6 mg/l, pH = 7.89-8.01, salinity = 0.2 ppt, total hardness = 180 mg/l ($CaCO_3$), alkalinity = 168 mg/l ($CaCO_3$),

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magnesium = 1 mg/l, calcium = 75 mg/l, iron = 0.01 mg/l, potassium = 9 mg/l and sulphate = 7 mg/l. DO, pH, temperature and salinity were measured using portable multiparameter meter (sensION 156, USA). Water total hardness, alkalinity, magnesium, calcium, iron, potassium and sulphate levels were determined using portable photometers with commercial kits provided by the manufacturer (Wagtecch Portable Photometer 7100, Berkshire, UK). No mortality was observed during this period.

2.2 Toxicity test

The static non-renewal test (Weber, 1993) was performed in order to evaluate the acute toxicity. Based on preliminary tests and pervious results, fish were exposed to concentrations of 0 (control), 30, 40, 45, 50, 55, 60, 70, 100, 150, 200, 250, 300 and 400 ppm formalin (Merck, Darmstadt, Germany) and mortalities were recorded at 0.5, 1, 12, 24, 48, 72 and 96 h, thereafter. Three 25 l, white and cylindrical tanks were used for each concentration. Total of 25 fish were stocked in 12 l water. Fish were allowed to adapt to these tanks for 10 days under aerated condition, during which they were fed (~1% of body weight, once a day) by trout commercial pellet (Biomare, France; 0.8 mm in diameter). Water exchange was about 80% every other day. Photoperiod was 14:10 light: dark (natural). Temperature was maintained constant (23±1ºC). DO was 7.4 -7.7 mg/l. pH range was 7.93-8.01. Water total hardness and alkalinity were 171-179 mg/l, 160-170 mg/l, respectively. No mortality was observed during this period. Feeding was stopped 24 h before dosing. Formalin was inoculated to each tank in order to achieve desired concentrations. Feeding was ceased at the dosing point and thereafter. pH, DO, total hardness and alkalinity were 8.01, 8.13 mg/l, 170 mg/l ($CaCO_3$) and 160 mg/l ($CaCO_3$) before dosing, respectively, and were checked twice a day after dosing. Other water chemistry was measured only before dosing: magnesium = 1.1 mg/l, calcium = 70 mg/l, iron = 0.01 mg/l, potassium = 9 mg/l and sulphate = 7.3 mg/l. Mortality and behavioral changes were recorded during the test period.

2.3 Statistical analysis

LC50 values and confidence intervals were calculated using EPA Probit Analysis Program V. 1.5 for each group, separately. All data were accepted if calculated chi-square for heterogeneity was lower than the tabular value at the 0.05 level. The lowest observed effect concentration (LOEC) determined as the minimum concentration which caused mortalities at each time point (0.5-96 h) (Rand, 1995). No observed effect concentration (NOEC) determined as maximum concentration at which no mortality was occurred. (Rand, 1995).

3 RESULTS

No mortality was observed in the control group during the trial. Fish showed behavioral changes after formalin exposure, including: avoidance behavior (fish gathered in a corner of the tanks), increase in opercular rate, severe reaction to stimuli (a mild blow on the tank made fish very excited). While exposure period was progressing, these symptoms were accompanied by imbalanced or upside down swimming, coming to surface or going to bottom of the chamber in the form of upside down or laying on one side and finally death, in higher concentrations.

Mortality rate of the treatments are shown in Table 1. 0.5-96 h LC50 values were estimated to be 49-246ppm (Table 2). LC50 values for formalin decreased logarithmically ($P<0.0001$; $R^2 = 0.917$) along with time (Figure 1). LOEC values decreased logarithmically ($P < 0.0001$; $R^2 = 0.878$) along with time (Figure 1). NOEC values were logarithmically correlated with time ($P < 0.0001$; $R^2 = 0.917$) (Figure 1).

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Table 1 Mean observed mortality following formalin exposure over 0.5-96h.

	Time (h)						
Concentration (ppm)	0.5	1	12	24	48	72	96
400	25	25	NT	NT	NT	NT	NT
300	15	19	NT	NT	NT	NT	NT
250	10	13	NT	NT	NT	NT	NT
200	8	9	NT	NT	NT	NT	NT
150	4	6	NT	NT	NT	NT	NT
100	0	1	25	25	25	NT	NT
70	0	0	25	25	25	25	NT
60	NT	NT	10	20	20	22	22
55	NT	NT	5	13	13	17	19
50	NT	NT	3	7	7	10	16
45	NT	NT	0	3	3	3	5
40	NT	NT	0	0	0	1	3
30	NT	NT	0	0	0	0	0

NT = not tested.

Table 2 0.5-96 h LC50 values of formalin for Caspian roach.

		95% confidence limit					
Time	Concentration (ppm)	Lower	Upper	Slope ± S.E	Intercept ± S.E	Calculated Chi-square	Tubular Chi-square
0.5	246	223	271	6.2 ± 0.91	-9.7 ± 2.2	6.9	9.5
1	219	198	242	5.7 ± 0.87	-8.3 ± 1.8	4.4	11.1
12	63	60	67	12.8 ± 2.1	-18 ± 3.8	0.66	9.5
24	53	51	55	18.4 ± 2.6	-26.8 ± 4.5	1.6	11.1
48	53	51	55	18.4 ± 2.6	-26.8 ± 4.5	1.6	11.1
72	51	49	53	18.1 ± 2.5	-26 ± 4.3	0.66	11.1
96	49	47	50	15.1 ± 2.1	-20.6 ± 3.6	2.5	11.1

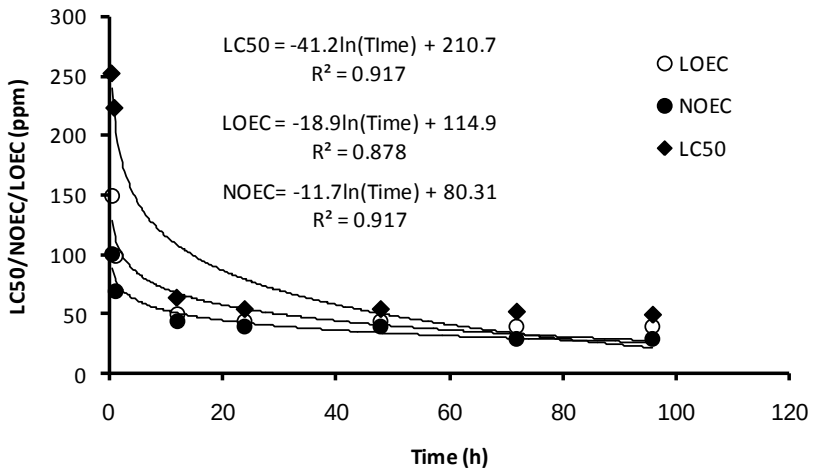

Figure 1 Correlation between formalin LC50, NOEC and LOEC values and time.

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4 DISCUSSION

Data on the toxic and safe margin of the chemicals and drugs are useful in both aquaculture and natural resources management. This was the first work on toxicity and safety of formalin in Caspian roach fry. Since the previous studies were conducted under different experimental conditions, precise comparison between the results is impossible. In an early study, 24-96 h formalin LC50 was found to be 69-84 ppm in *T. carolinus* (Birdsong and Avault, 1971). This low levels of toxicity compared to present study might be as a result of both species difference as well as water salinity, as it has been established that the lower formalin toxicity occurred at intermediate (isosmotic) salinities (Reardon and Harrell, 1990). 96h LC50 values of formalin were found to be 4.9-15.4 in *M. saxatilis* (1.8g), depending on salinity, suggesting this species has low tolerance to formalin (Reardon and Harrell, 1990). Importance of fish weight in formalin toxicity was demonstrated in *O. niloticus* (Dureza, 1995). Higher formalin LC50 values have been reported for *S. annulatus* (79-1095 ppm over 0.5-72h) (Fajer-Ávila *et al*., 2003). These higher values might be as a result of higher water salinity (34 ppt) as well as higher fish weight (~15g) along with species difference. Likewise, high values in 6g *C. chanos* (a cyprinid species) seem to be due to same reasons (Cruz and Pitogo, 1989). Other study on *C. macrocephalus* demonstrated that this species is more tolerant to formalin than Caspian roach, since 12-96 h LC50 values reported to be 82-95 ppm (Kanchanaburangkul, 1988). Generally, Caspian roach seems to be more susceptible to formalin than the other tested species, with the exception of *M. saxatilis*. Observed behavioral changes suggest that fish experienced stress as a result of formalin exposure. Avoidance, high opercular rate and excitability are behavioral signs of stress (Wendelaar Bonga, 1997). Previous studies showed pathological effects of formalin on (Cruz and Pitogo, 1989). Formalin-induced gill damages, including epithelial rise or separation along with gill tissue protein denaturation and hardening, disrupt normal functions such as osmoregluation and gas exchange, which are stressful for fish (Wendelaar Bonga, 1997). As the proportion of the gill surface to whole body surface negatively correlated to fish size, small fish are more susceptible to formalin toxicity than large ones. This may explain lower tolerance of Caspian roach to formalin compared to the other tested species.

Regression models for LC50 and LOEC suggest importance of concentration on formalin toxicity is more pronounced over short (0.5-1h) compared to long (12-96h) periods. It suggests the need for crucial care when high formalin concentration is used over short period for fish treatment (short-term bath). Partially similar models were detected in other fish species (Cruz and Pitogo, 1989; Fajer-Ávila *et al*., 2003). Using these models in combination with the data on effectiveness of formalin against certain pathogen, safe formalin concentration can be estimated for short- and long-term bath, in Caspian roach. Previous authors suggested 150-250 ppm formalin could be applied over 0.5-1 h as short-term bath for fish (Stoskopf, 1988; Luzzana and Valfre, 1993; Powel *et al.*, 1996; Klinger and Floyd, 1998). According to Table 2 and Figure 1, 150 and 250 ppm formalin causes 10-17% and 52-62% mortality in Caspian roach (~ 1g) over 0.5-1 h. According to Figure 2, LOEC of formalin over 0.5-1 h are calculated to be 88.4 and 80.3, respectively, which can be used for short-term bath and are about the one third of the recommendations (Stoskopf, 1988; Luzzana and Valfre, 1993; Powel *et al.*, 1996; Klinger and Floyd, 1998). This suggests the need for reevaluating the formalin effective and therapeutic doses for treatment in each fish

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species before application. Likewise, formalin concentrations for long-term (12-24h) treatment in Caspian roach are calculated to be maximum 51.2 and 40.1, respectively.

5 CONCLUSIONS

It is concluded that Caspian roach is more susceptible to formalin than other tested species and recommended doses in the literatures are not suitable for treatment in this species. Maximum formalin concentrations of 88.4, 80.3, 51.2 and 40.1ppm are suggested for 0.5, 1, 12 and 24 h treatment in Caspian roach under the present conditions.

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مسمومیت (LC50) فرمالین در بچه ماهیان نورس کلمه خزر

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چکیده فرمالین یک مادهٔ ضدعفونی کننده و درمانی در فعالیت‌های شیلاتی می‌باشد. با توجه به تفاوت‌های زیاد در میزان حساسیت گونه‌های مختلف به فرمالین، ضروری است تا سمیت آن برای گونه‌های مختلف به طور مجزا ارزیابی گردد. بنابراین جهت بررسی سمیت حاد و غلظت بی‌خطر فرمالین در ماهی کلمهٔ دریای خزر، آزمایشی روی ماهیان یک گرمی انجام شد. ماهیان در معرض غلظتهای غلظت‌های ۰، ۳۰، ۴۰، ۴۵، ۵۰، ۵۵، ۶۰، ۷۰، ۱۰۰، ۱۵۰، ۲۰۰، ۲۵۰، ۳۰۰ و ۴۰۰ ppm فرمالین قرار گرفتند و تلفات آنها پس از ۰/۵، ۱، ۱۲، ۲۴، ۴۸، ۷۲ و ۹۶ ساعت ثبت گردید. مقادیر LC50 پس از ۰/۵-۹۶ ساعت، ۲۴۶-۴۹ ppm برآورد گردید. همچنین میزان حداقل غلظت مؤثر (LOEC) فرمالین در زمان‌های۹۶-۰/۵ساعت، ۴۰-۱۵۰ ppm به‌دست آمد. حداکثر غلظت مجاز (NOEC) فرمالین در زمان‌های۹۶-۰/۵ساعت، ۳۰-۱۰۰ ppm به‌دست آمد. بر اساس مدل رگرسیونیِ رابطهٔ زمان و NOEC، غلظت‌های پایین‌تر از ۸۸/۴، ۸۰/۳، ۵۱/۲ و ۴۰/۱ ppm به ترتیب به عنوان حداکثر غلظت حمام‌های بلند مدت ۰/۵، ۱، ۱۲ و ۲۴ ساعته پیشنهاد شدند. نتایج نشان داد که غلظت‌های بی‌خطر پیشنهاد شده در منابع علمی، برای ماهی کلمه دریای خزر قابل کاربرد و معتبر نیستند. این تفاوت‌ها می‌تواند به دلیل تفاوت‌های گونه‌ای یا تفاوت در شرایط آزمایشی باشد.

کلمات کلیدی: LOEC، NOEC، ضدعفونی کننده، کلمه دریای خزر، مسمومیت

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