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Effects of Short and Long Exposure to the Anesthetic 2-Phenoxyethanol Mixed with Ethyl Alcohol on Common Carp (*Cyprinus carpio* L., 1758) Fingerlings

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Key words: common carp, anesthesia, ethyl alcohol, 2-phenoxyethanol

Abstract

Common carp fingerlings (*Cyprinus carpio* L., 1758; 7.70±0.4 g; 8.38±0.8 cm) were exposed to different concentrations of 2-phenoxyethanol mixed with pure ethyl alcohol (1:1) in an open system for a short (until induction of anesthesia) or long (48 h) duration, then transferred to clean water and maintained for 96 h. The effects of the anesthetic on induction time, anesthetic stage, opercular rate, recovery time, recovery stage, and mortality were observed. For short exposure, the most suitable concentrations were 0.8, 1, and 1.2 ml/l. For long exposure, transportation, and deep sedation and partial loss of equilibrium (anesthetic stages 2 and 3), the preferable concentrations were 0. 4 and 0.6 ml/l. No mortality was observed at any concentration.

Introduction

Transport for short or long durations can be stressful to fish but stress can be reduced if fish are partially or fully anesthetized. Several anesthetics have been used in this respect. One is 2-phenoxyethanol.

The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. It is an inexpensive, colorless, oily, aromatic liquid anesthetic, slightly soluble in water (26.7 g/l) but readily soluble in ethyl alcohol (Weyl et al., 1996; Velisek and Svobodova, 2004). Induction and recovery times with 2-phenoxyethanol are short and manipulation of fish is easy (Mohamed, 1999).

The anesthetic efficacy of 2-phenoxyethanol has been documented for many fish species including rainbow trout (Gilderhus and Marking, 1987), cod (Mattson and Riple, 1989), and tench (Ross and Ross, 1999; Myszkowski et al., 2003; Hamackova et al, 2004). Guo et al. (1995) suggested that 2-phenoxyethanol was more suitable than either quinate or MS-222 to sedate non-food (ornamental) fishes during live transport. The effect

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of 2-phenoxyethanol on hematological parameters of carp fry was studied by Adamek et al. (1993). Kaiser and Vine (1998) studied the anesthetic effects of three concentrations of 2-phenoxyethanol (0, 0.25, and 0.35 ml/l) on goldfish (3.99±1.99 g) in three packing densities (25, 50, and 75 fish/500 ml) in plastic bags but did not recommend its use for transport.

In these studies, 2-phenoxyethanol was used as a pure substance and was not dissolved in ethyl alcohol. Nor have its long duration effects been sufficiently studied. The present study was designed to test the effects of short and long exposure to different combinations of 2-phenoxyethanol and ethyl alcohol on common carp (*Cyprinus carpio*) fingerlings in an open system. Common carp of 5-10 cm are generally preferred for transplantation into lakes or dam lakes (Salehi, 2004; Sagat, Fisheries Department, Regional Directorate of the State Hydraulic Works, Adana, Turkey, pers. comm.).

Materials and Methods

The experiment was carried out in 5-l glass aquaria in the laboratory of the Fisheries Faculty of the University of Cukurova. Common carp were obtained from the Fisheries Department of the Regional Directorate of the State Hydraulic Works in Adana. The fish were starved 24 h prior to the experiment (Weyl et al., 1996). The mean weight and total length of the fish were 7.70±0.4 g and 8.38±0.8 cm. Ten fish were stocked into each aquarium. Aquaria were aerated continuously. Water temperature and dissolved oxygen were maintained at 22.4-23ºC and 7.8-8mg/l. The anesthetic solution was prepared by mixing 2-phenoxyethanol and pure ethyl alcohol (96%; Merck Chemicals) at the rate of 1:1.

The study included two experiments with triplicates of each treatment. In the first experiment, five concentrations (0.6, 0.8, 1, 1.2, 1.4 ml/l) of the anesthetic solution were tested for a short duration (induction time). In the second experiment, four concentrations (0.2, 0.4, 0.6, 0.8 ml/l) were tested for a longer duration (48 h). After application of the anesthetic, the opercular rate (beats/min) was determined. Fish mortality was monitored during anesthesia and 1, 10, 20, 48, and 96 h after transfer to fresh water. Induction and recovery times were recorded in minutes. Anesthesia and recovery stages are presented in Tables 1 and 2.

Table 1. Stages of anesthesia in fish (Keene et al., 1998, modified from McFarland, 1959; Jolly et al. 1972).

Stage	*Description*	*Behavior*
0	Normal	Reacts to external stimuli; opercular rate and muscle tone normal
1	Light sedation	Slight loss of reactivity to external visual and tactile stimuli; opercular rate slightly decreased; equilibrium normal
2	Deep sedation	Total loss of reactivity to external stimuli except very strong pressure; slight decrease in opercular rate; equilibrium normal
3	Partial loss of equilibrium	Partial loss of muscle tone; increased opercular rate; reacts only to strong tactile and vibrational stimuli
4	Total loss of equilibrium	Total loss of muscle tone and equilibrium; slow but regular opercular rate; loss of spinal reflexes
5	Loss of reflex reactivity	Total loss of reactivity; opercular movements slow and irregular; heart rate very slow; loss of all reflexes
6	Medullary collapse	Opercular movements cease; cardiac arrest usually follows quickly

Results

Results are given in Table 3.

Discussion

Low doses of ethyl alcohol have no known anesthetic properties on fishes (Anderson et al., 1997; Munday and Wilson, 1997) and have been used to dilute clove oil (Anderson et al., 1997; Bressler and Ron, 2004). In most fishes, the effective anesthetic dose of 2-phenoxyethanol ranges 0.2-0.6 ml/l (Gilderhus and Marking 1987; Mattson and Riple, 1989;

Table 2. Stages of recovery from anesthesia in fish (Keene et al., 1998 modified from Hikasa et al., 1986).

Stage	*Behavior*
1	Decreased opercular movement
2	Partial recovery of equilibrium; partial recovery of swimming motion
3	Total recovery of equilibrium
4	Reappearance of avoidance swimming motion; reaction to external stimuli; behavioral response still stolid
5	Swimming, rarely striking head firmly to sides or against bank of the tank
6	Total behavioral recovery; normal swimming

Table 3. Short and long duration anesthetic effects of 2-phenoxyethanol dissolved in ethyl alcohol (1:1) on common carp fingerlings.

Anesthetic concentration (ml/l)	*Induction time (min)*	*Anesthethic stage*	*Opercular rate (per min)*	*Recovery time (min)*	*Recovery stage*
Short duration					
0	-	-	72-73	-	-
0.6	5-6	4	11-12	2	5-6
0.8	2-3	4	17	6	5
1	1	4	19	8-9	4-5
1.2	0.5-1	4	19	8-9	4-5
1.4	0.25-1	4	28.06	13-15	4-5
Long duration (48 h)					
0	-	-	72-73	-	-
0.2	-	-	78	-	-
0.4	13	2	30	1	3
0.6	5-6	3-4	38	2	3
0.8	2-3	4	48	6	3

Hseu et al, 1994, 1997, 1998; Hseu and Ting, 1995; Weyl et al., 1996). This study shows that lower quantities of 2-phenoxyethanol may be more effective for common carp fingerlings when diluted with ethyl alcohol.

In a study of common carp fingerlings (5.15±0.12 g) exposed to concentrations of 0.4, 0.5, 0.6, and 0.7 ml 2-phenoxyethanol/l at 27-29°C and 6-7 ppm dissolved oxygen, induction time was 9.63±0.19, 3.95±0.17, 2.15±0.03, and 0.95±0.11 min, respectively (Mohamed, 1999). Except for the low concentrations, recovery time was 0.48±0.02, 0.56±0.04, and 0.55±0.03 min. The optimum effective concentration suggested by Mohamed (1999) was 0.6 ml/l. In our study with added ethyl alcohol, induction time in all concentrations in both experiments was shorter than in Mohamed (1999). This, and the better recovery time, indicates that concentrations of 0.6 and 0.8 ml/l are more suitable for common carp fingerlings.

Opercular beats are an indicator of stress. They increase and decrease according to the type and concentration of the anesthetic. For example, enhancing concentrations of quinaldine sulfate and diazapem from 2.5 to 20 mg/l increased the opercular rate in sea bream (*Sparus aurata*) while enhancing concentrations of clove oil decreased it in sockeye salmon (*Oncorhynchus nerka*; Kumlu and Yanar, 1999; Woody et al., 2002). In our short duration experiment, the opercular rate of the fish decreased sharply in 0.6 ml/l and was slightly higher in the other concentrations. In the long duration experiment, the opercular rate slightly increased in the 0.2 ml/l concentration (probably because fish were not anesthetized at this concentration) and dropped in higher concentrations. Mortality in the study was nil, as in Mohamed (1999).

In conclusion, our results show that concentrations of 0.8, 1, and 1.2 ml/l of 2-phenoxyethanol diluted in ethyl alcohol (1:1) in water temperatures of 22-23ºC are more suitable for short-term handling and transportation of common carp fingerlings while 0.4 and 0.6 ml/l are preferable for deep sedation or the partial loss of equilibrium required for long-term transportation.

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