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The comparison of anesthetic potency and toxicity of 2-phenoxyethanol and 1-phenoxy-2-propanol for juvenile common carp

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Abstract: The aim of this study was to investigate anesthetic potency and acute toxicity of 2-phenoxyethanol (2PE) and 1-phenoxy-2-propanol (PP) for common carp. During efficacy tests, individual phases of anesthesia and recovery, as well as behavior and survival rates, were determined. As effective time for anesthesia and recovery, 3 and 10 min respectively were chosen. Determined efficient concentrations were 600 mg L^{-1} for 2PE and 415 and 460 mg L^{-1} for PP. Acute toxicity tests were performed according to OECD Norm Acute Toxicity Tests for Fish No. 203. To establish the 96-h LC_{50} of PP and 2PE, juvenile carps were exposed to six different concentrations in three replicates. Control fish were exposed to substance-free water. Mortality of fish and water characteristics were measured every 8 h. Determined 96-h LC_{50} values of 2PE and PP were 327.9 mg L^{-1} and 304.2 mg L^{-1}, respectively. Results obtained during experiments proved that both 2PE and PP have good efficacy and can be classified as relatively harmless for fish. This suggests that both 2PE and PP can be used as safe anesthetics for common carp. However, similar anesthesia time together with extended recovery time suggest PP as a potentially better anesthetic for aquatic organisms.

Key words: 2-Phenoxyethanol, 1-phenoxy-2-propanol, efficacy, toxicity, common carp

1. Introduction

In aquaculture anesthetics are used for various purposes ranging from mild sedation during transport to general sedation in order to reduce the stress caused by various manipulations such as controlled propagation, sampling, and other physiological examinations (1–4). Although different preparations are available on the market, not all of them meet the standards for fish anesthetics (5). Therefore, research looking for new safe anesthetics for fish is being carried out.

The anesthetics most commonly used in aquaculture are tricaine methane sulfonate (MS 222), benzocaine, quinaldine sulfate, metomidate, clove oil, and 2-phenoxyethanol (6). 2-Phenoxyethanol (2PE) is a moderately water-soluble, colorless, and aromatic liquid used as an effective and safe fish anesthetic. 2PE seems to be suitable for use in aquaculture due to its easy preparation, low cost of anesthesia, rapid induction, and bactericidal and fungicidal properties (1,7). However, the major disadvantage of 2PE is rapid recovery time; fish often fully recover during manipulations conducted out of anesthetic solution. The relatively high anesthetic concentration needed for fish is another drawback of 2PE anesthesia (1). The recommended concentration of 2PE for fish anesthetic baths varies from 167 mg dm^{-3} to 442 mg L^{-1} depending on fish species (2,3,8). In some cases, even higher concentrations up to 660 mg L^{-1} (personal observations) are needed to obtain general anesthesia.

1-Phenoxy-2-propanol (PP) is a 2PE derivative. PP forms a clear and colorless liquid with a slight odor at room temperature. Although more hydrophobic than would be expected from its molecular weight, it can be dissolved in water (solubility is 11,700 mg L^{-1}). However, it seems to be an effective anesthetic for gastropods, pulmonates, bivalves, and nudibranchs due to its ability to reversible eliminate neural activity and reduction of muscle contraction force (9). PP is rapidly absorbed, distributed throughout the body, metabolized, and eliminated from invertebrate and probably fish organisms (9–12).

The aim of this study was to determine and compare the anesthetic potency and toxicity of 2PE and PP for common carp (*Cyprinus carpio*).

2. Materials and methods

2.1. Fish

The experiments were carried out on young carps (n = 330) of 107 ± 20.9 g in weight and 190 ± 11.9 cm in total length. Fish were supplied by a local fish farm in Ostróda, Poland.

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Fish were acclimated for 2 weeks before the experiment in a 0.3 m^3 tanks. Fish were fed ad libitum with commercial pellet feed. The water temperature during acclimation and the experiment was 19.5 ± 0.5 °C. Water pH was 7.4 ± 0.5 and the oxygen saturation was maintained above 80%.

2.2. Anesthetics

For the experiments 2PE and PP were used. Both 2PE and PP were supplied by Sigma Aldrich (USA). Before the experiment, an alcohol solution of 2PE and PP (respectively 330.6 and 50 mg mL^{-1}) was prepared.

2.3. Testing of anesthetic efficacy

The anesthetic efficacy was tested according to the methodology proposed by Siwicki (13). While testing, specified phases of anesthesia were measured (Table 1). The time to reach each stage of anesthesia as well as stages during recovery time (return of operculum and body movement, equilibrium recovery, and ability to active swimming) were recorded using an electronic stopwatch. Three criteria proposed by Marking and Meyer (5) were used in determining the effectiveness of the test substances: anesthesia induction within 3 min, recovery occurrence within 10 min, and no deaths after 48 h from exposure. Fulfillment of these criteria made it possible to determine the usefulness of the tested compounds as anesthetics.

To test the efficacy of the compounds, five different concentrations for both anesthetics were selected during preliminary tests. For 2PE the following test concentrations were selected: 400, 500, 600, 700, and 800 mg L^{-1}. Selected concentrations for PP were 290, 375, 415, 460, and 540 mg L^{-1}.

Seven juvenile fish were individually exposed to each anesthetic concentration. Each fish was randomly caught from the tank and transferred to a 15-L experimental tank. Each anesthetic tank was filled with 12 L of mechanically aerated tap water containing different concentrations of anesthetic. Time to reach stage III of anesthesia was measured. The maximum exposure time to each anesthetic concentration was 10 min. Following application of the anesthesia, fish were immediately removed from the anesthetic tank and placed in a tank containing anesthetic-free water to determine the time needed for full recovery of fish. After termination of the experiment, fish were moved to a stock tank and monitored for 48 h.

2.4. Testing of anesthetic toxicity

Acute toxicity tests of 2PE and PP were conducted according to OECD No. 203 for fishes. Semistatic tests were conducted in 15-L tanks filled with tap water. For maintaining a constant concentration of anesthetic during the assays, the test medium was replaced in both control and experimental tanks every 8 h.

To establish the 96-h LC_{50} of PP and 2PE, juvenile carps were exposed to six different concentrations (270, 284, 298, 313, 328, and 345 mg L^{-1} for PP and 294, 309, 324, 340, 357, and 375 mg L^{-1} for 2PE) in three replicates. Control fish were exposed to substance-free water.

Seven juvenile carp were used for each chosen concentration and for a control group. A total number of 294 juvenile carp were used. Test animals were not fed from 2 days prior to the experiments. Mortality was recorded every 8 h and the 96-h median lethal concentration (96-h LC_{50}) with 95% confidence limit of PP and 2PE was estimated by probit analysis (14). Measurements of temperature, oxygen content, and pH value were made every 8 h.

3. Results

3.1. Anesthetic efficacy of 2PE and PP

No mortality was observed during anesthesia and the following 48 h in both anesthetic treatments. The induction and recovery times (mean ± SD) of fish exposed to 2PE and PP are shown in Table 2.

No excitation stage was observed in any group of both used anesthetics. All chosen concentrations of 2PE and PP induced surgical anesthesia in fish within 10 min. However, only one concentration of 2PE (600 mg L^{-1}) and two concentrations of PP (415 and 460 mg L^{-1}) fulfilled the established criteria and were considered as effective. It was observable that anesthesia induction times decreased with increasing concentrations of PP and 2PE (Table 2).

Table 1. The stages of anesthesia.

Stage	Level of anesthesia	Description
I	Excitation	Anxiety, increased physical activity, rapid gill ventilation rate
II	Light anesthesia	Loss of equilibrium, decreased gill ventilation rate, decreased muscle tone, loss of pain perception
IIa	Loss of balance	
IIb	Myorelaxation	
IIc	Loss of pain perception	
III	Deep anesthesia	No physical activity, rare gill movement
IV	Overdose	No physical activity, no gill movement, death

3.2. Toxicity of PP

The temperature of the experimental bath during the LC_{50} tests of 96-h exposure to PP and 2PE LC_{50} was 20 ± 0.3 °C, the dissolved oxygen concentrations did not drop below 70% (80%–94%), and the pH ranged between 7.97 and 8.33. Based on the results of the tests, determined lethal concentrations of PP and 2PE in common carp were 307.1 mg L^{-1} for PP and 327.9 mg L^{-1} for 2PE. Particular lethal concentrations of PP and 2PE with confidence intervals as well as mean and standard deviations are shown in Table 3. No deaths were noticed at the lowest used concentrations in both anesthetics (270 and 284 mg L^{-1} for PP and 294 and 309 mg L^{-1} for 2PE) during the 96-h trial.

4. Discussion

The definition of anesthetic efficacy can be subjective and varies between authors (5,15–17), but all of them suggest that ideal anesthetics should meet several criteria such

Table 2. Times to reach stages II and III and recovery times for juvenile common carp anesthetized with various concentrations of PP and 2PE.

Concentrations [mg dm^{-3}]	**Time to reach stage IIc [min:s]**	**Time to reach stage III [min:s]**	**Recovery time [min:s]**
2-Phenoxyethanol			
400	07:37	-	03:47
500	04:40	08:23	04:37
600	02:55	06:27	03:16
700	01:53	04:08	04:01
800	01:45	03:31	04:59
1-Phenoxy-2-propanol			
290	07:57	-	03:55
375	05:33	07:58	05:27
415	03:38	08:50	06:57
460	03:52	07:47	05:27
540	02:21	05:06	06:51

All data are presented as mean values ± SD.

Table 3. Calculated 96-h LC_{50} values (mg dm^{-3}) of PP and 2PE with 95% confidence intervals for common carp.

2-Phenoxyethanol		
Test series	LC_{50} (mg dm^{-3})	95% Confidence interval
1	337.2	325.9–346.5
2	324.6	311.9–335.1
3	322.1	310.1–331.4
Mean LC [mg dm^{-3}]	327,9	
Standard deviation	9,8	
1-Phenoxy-2-propanol		
Test series	LC50 (mg dm^{-3})	95% Confidence interval
1	313.3	301.2–324.5
2	310.8	299.6–322.2
3	297.1	287.3–306.6
Mean LC [mg dm^{-3}]	307.1	
Standard deviation	10.4	

as simple administration, rapid induction of anesthesia, maintenance of the anesthesia state and rapid recovery, low tissue residues, and effectiveness at low concentrations (5). However, anesthetic effectiveness can be influenced by various factors such as health and the physical condition of fish, the oxygen concentration in the experimental medium, or water temperature (18,19).

Induction and recovery times of anesthesia as well as rate of operculum movements can be influenced by the concentration of the used substance (20). Results presented in Table 2 clearly indicate that the onset of particular anesthesia phases depends on the used concentration of 2PE and PP. During PP anesthesia, induction time decreased proportionally to used concentration and ranged between 2.5 and 8 min for achieving stage IIc of anesthesia (Figure). Recovery time ranged from 4 min at the lowest concentration to about 7 min at the highest concentration of PP. The same tendency is observable in fish anesthetized with 2PE. However, both anesthesia and recovery times in the case of 2PE are shorter when compared to fish anesthetized with PP. Longer recovery time during PP anesthesia indicates that it can be more accurate for use during general anesthesia than 2PE, which is known to cause early awakening of fish from the anesthesia state. Lower doses of PP being needed to anesthetize fish (Table 2) can also act in favor of PP, suggesting its usefulness during longer transportation of fish.

2PE and PP have similar physicochemical properties such as density, water solubility, and partition coefficient (LogP). Partition coefficients of biologically active compounds are widely used for the study of structure–activity relationships. It is known that substances

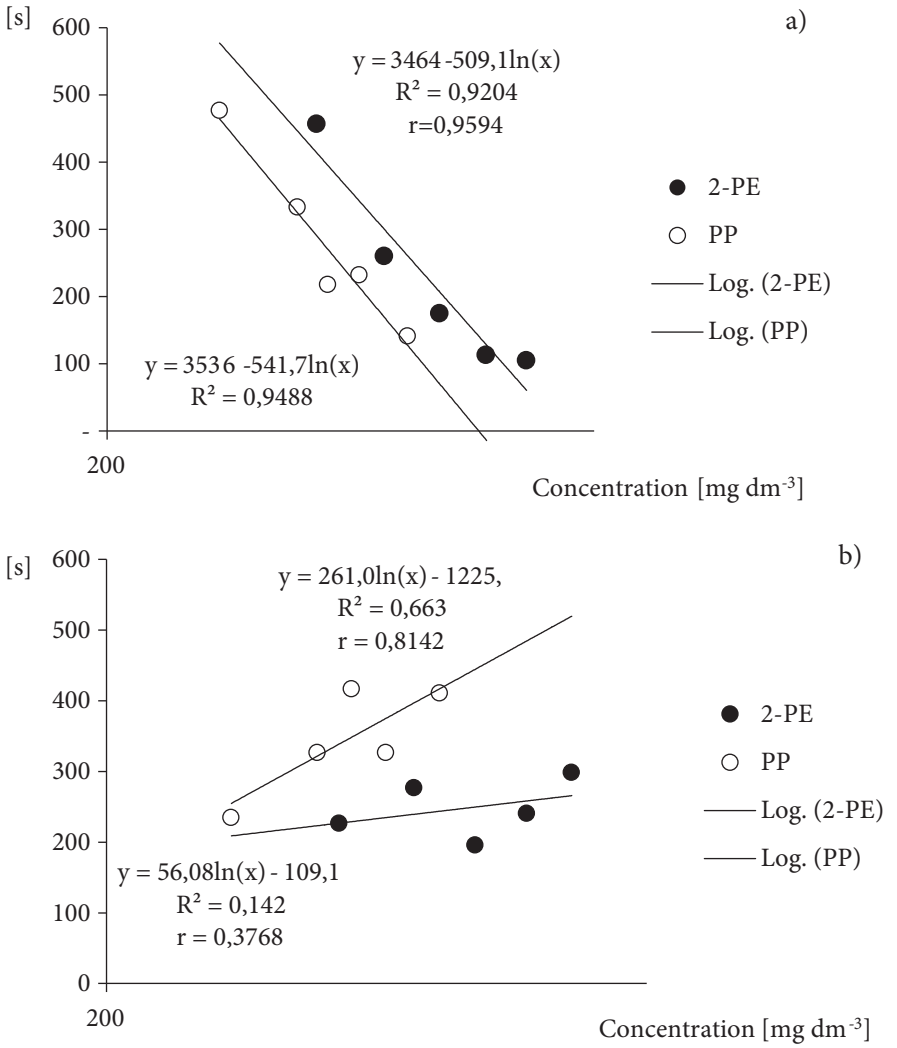

Figure. Correlation between anesthetic concentration of PP and 2PE and anesthesia (a) and recovery (b) times in juvenile common carp.

with high LogP values tend to adsorb more readily to organic matter, and chemicals with LogP values above 4.5 may have the potential to bioconcentrate in living organisms. Both 2PE and PP have relatively low partition coefficients (respectively 1.15 and 1.5), which, with low bioconcentration factors (respectively 0.35 and 0.77), indicates a very limited potential for bioaccumulation in living organisms of these compounds.

The use of acute toxicity tests for assessing the potential hazard of chemical contaminants to aquatic organisms is well documented (21–23). According to Oksama and Kristoffersson (24), after achieving the median lethal concentration point, the toxicity curve becomes parallel to the time axis. Any prolongation of the exposure time will no longer increase fish mortality. Acute toxicity tests usually provide estimates of the exposure concentration of a stressor, pollutant, or poisonous substance causing 50% mortality (LC_{50}) to test organisms during a specified period of time. Among the most frequently performed toxicity tests, the 10-min LC_{50} and 96-h LC_{50} can be included.

The determination of a substance's acute toxicity is important not only for its usage in fish anesthesia and the appropriate treatment concentration for anesthetic baths, but also for possible contamination of the water environment by such anesthetics. Factors affecting substance pharmacology and toxicology involve the systems that control absorption, distribution, metabolism, and excretion. To the main factors, personal and population features can also be included, such as nutritional and hormonal status, sex, health, genetics, and age of the organism (25). The EU criteria for toxicity classify substances as very toxic (≤1 mg L^{-1}), toxic (≤10 mg L^{-1}), harmful (≤100 mg L^{-1}), and not classified (>100 mg L^{-1}). Estimated values of the 96-h LC_{50} values for 2PE and PP in our tests were 327.9 and 307.1 mg L^{-1}, respectively (Table 3). That ranks both anesthetics among relatively harmless substances. Results obtained during biochemical tests of PP on common carp seem to confirm that fact (26).

Literature data about 96-h LC50 values of 2PE for juvenile fish range from 188.7 mg L^{-1} (common carp) to 338 mg L^{-1} (zebrafish) (2,28). Even higher values of LC_{50} for 2PE were obtained for zebrafish embryos at 486 mg L^{-1} during 168-h LC_{50} tests (27). However, according to Barton and Helfrich (8), younger fish are more sensitive to anesthetic concentrations, which seems to be confirmed when we compare the results obtained in our work with results obtained by Velišek and Svobodová (2). The 96-h LC_{50} value of 2PE for common carp calculated by them (188.7 mg L^{-1}) is much lower than the LC_{50} obtained in our work (327.9 mg L^{-1}).

There are no published data concerning PP effectiveness and toxicity for juvenile fish available. However, considering similarities between 2PE and PP we can assume that also in the case of PP juvenile fish can be more sensitive to anesthetic concentrations, so lower doses of anesthetics should be used.

Results obtained during these experiments suggest that both 2PE and PP can be considered as effective anesthetics for juvenile common carp. Our studies showed that PP has relatively longer recovery times than 2PE. Similar anesthesia time together with extended recovery time makes PP a potentially better anesthetic for aquatic organisms. Conducted acute toxicity tests show that both of the used substances can be classified as relatively harmless for fish, suggesting that both 2PE and PP can be used as safe anesthetics for common carp.

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