Effects of 2-phenoxyethanol anaesthesia on sheatfish (*Silurus glanis* L.)

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ABSTRACT: The aim of the study was to investigate the acute toxicity of 2-phenoxyethanol to sheatfish, and using the values of haematological and biochemical profiles of blood and histological tissue examinations to assess the effects of the fish exposure to that anaesthetic. The values of acute toxicity of 2-phenoxyethanol to sheatfish were found to be 10minLC50 0.77 ml/l, 10minLC0.1 0.42 ml/l, 10minLC99.9 1.90 ml/l, 96hLC50 0.29 ml/l, 96hLC0.1 0.20 ml/l, and 96hLC99.9 0.41 ml/l. The 10-min exposure to 2-phenoxyethanol at a concentration of 0.30 ml/l caused significantly higher values ($P < 0.05$) of packed cell volume (PCV), mean corpuscular volume (MCV), glucose (GLU) and albumins (ALB) immediately after anaesthesia. A significant decrease ($P < 0.05$) in the level of alanine aminotransferase (ALT) and a significant increase ($P < 0.05$) in the values of mean corpuscular haemoglobin concentration (MCHC) were found 24 h post anaesthesia. Histological examinations showed capillary ectasia of gill filaments immediately after 2-phenoxyethanol anaesthesia. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were demonstrated in other tissues (liver, spleen, cranial and caudal kidneys) following anaesthesia. The results of examinations suggest that the use of 2-phenoxyethanol at a concentration of 0.30 ml/l does not cause any irreversible damage in sheatfish.

Keywords: acute toxicity; haematological profile; biochemical profile of blood; histological examination of tissues

The use of anti-stress agents is a common practice in modern aquaculture. Such substances are used to induce anaesthesia during handling and sorting, tagging, artificial reproduction procedures or surgery, thus reducing stress-induced problems such as decreases in feeding and immune functions (Ross and Ross, 1984, 1999). The anaesthetics most commonly used in aquaculture are MS-222, benzocaine, quinaldine sulphate, methomidate, clove oil and 2-phenoxyethanol (Brown, 1988; Svoboda and Kolarova, 1999; Waterstrat, 1999), with anaesthesia being usually induced by immersing the fish in an anaesthetic solution.

Guilderhus and Marking (1987) defined three criteria that an anaesthetic applied in aquaculture must fulfil. It must be effective, safe and inexpensive. Their criteria for efficacy are as follows: fish must be sedated within 3 min after 15 min of exposure

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to anaesthetic, fish must regain normal swimming in 10 min, and all anaesthetized fish must survive. The effect of an anaesthetic on fish depends on a number of factors, including the concentration of an anaesthetic, water temperature, fish size and species (McFarland, 1960; Fereira et al., 1984; Soto and Burhanuddin, 1995).

The efficacy of 2-phenoxyethanol has been documented for many fish species: sockeye salmon (Sehdev et al., 1963); rainbow trout (Guilderhus and Marking, 1987; Iwama et al., 1988); platyfish (Guo et al., 1992); grass carp and silver carp (McCarter, 1992); guppy (Teo and Chen, 1993); black porgy (Hseu et al., 1996); goldfish (Weyl et al., 1996; Kaiser and Vine, 1998); perch (Hamackova et al., 2001); sea bream (Tort et al., 2002); tench (Myszkowski et al., 2003; Hamackova et al., 2004).

2-phenoxyethanol is used in the Czech Republic for short-term immobilization of fish before artificial spawning and whenever fish is handled outside water. The recommended concentration for anaesthetic purposes is 0.30 ml/l water bath (Svoboda and Kolarova, 1999; Hamackova et al., 2001). At present, effects of 2-phenoxyethanol on commercially produced fish are studied in a project regarding the application of principles of pharmacovigilance in aquaculture in the Czech Republic. In the first stage of the project, effects of 2-phenoxyethanol on common carp (Velisek and Svobodova, 2004a) and rainbow trout (Velisek and Svobodova, 2004b) were studied. In the second stage of the project, effects of clove oil on rainbow trout (Velisek et al., 2005a) and common carp (Velisek et al., 2005b) were studied. The aim of the present study was to investigate the acute toxicity of 2-phenoxyethanol to sheatfish, and on the basis of haematological indices, biochemical blood profile values and histological examinations to assess the changes in the organism of sheatfish induced by the anaesthetic.

MATERIAL AND METHODS

2-phenoxyethanol characteristics

The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. Its summary formula is $C_8H_{10}O_2$, molar weight 138.17 g/l, density 1.107–1.108, peroxide content less than 0.005% and the boiling temperature is 245°C. The anaesthetic is slightly soluble in water (26.7 g/l) but readily soluble in ethanol. The anaesthetic affects fish through the skin and gills.

The anaesthetic is marketed by MERCK-Schucherd (Hohenbrunn, Germany) in 2.5- and 1-litre packages or in other volumes by request.

Acute toxicity of 2-phenoxyethanol

Acute toxicity of 2-phenoxyethanol was determined by the OECD 203 "Fish, Acute Toxicity Test". For 96-h and 10-min LC50 trials, sheatfish of 4.31 ± 1.11 (mean ± SD) average weight and 78 ± 29 mm average body length were used.

96-h LC50 test. Experimental fish were exposed to concentrations of 0.10, 0.15, 0.20, 0.25, 0.30 and 0.40 ml/l 2-phenoxyethanol dissolved in diluting water (pH 7.51; acid neutralization capacity – $ANA_{4.5}$ 1.29 mmol/l; total ammonia 0.03 mg/l; NO_3^- 7.45 mg/l; NO_2^- 0.003 mg/l; PO_4^{3-} 0.02 mg/l; chemical oxygen demand – COD_{Mn} 1.5 mg/l), and controls were placed in diluting water with no tested substance added. Ten sheatfishes were used for each concentration and for the control group. The fish and their behaviour, water temperature, pH and oxygen saturation were monitored throughout the tests at individual concentrations and in the control aquarium. Mean lethal concentration (96hLC50) and also 96hLC0.1 and 96hLC99.9 were calculated from mortality rates over the period of 96 hours.

10-min LC50 test. For 10 min, the fish were exposed to concentrations of 0.30, 0.50, 0.60, 0.80, 0.90 and 1.10 ml/l of 2-phenoxyethanol dissolved in diluting water. Ten sheatfishes were used for each concentration and for the control group. Diluting water of the same parameters as in previous trials was used. During the 10-min test period, changes in physiological parameters of fish and fish mortality figures were recorded, and after the sheatfish had been moved to clean water, the time of their recovery from anaesthesia was determined. Mean lethal concentrations (10minLC50) and also 10minLC0.1 and 10minC99.9 were calculated from mortality rates over the period of 10 min.

Within the tests, the onsets of individual phases of anaesthesia and recovery rates were studied. Evaluations were made in four consecutive phases (Theinpoint and Niemegeers, 1965; Yoshikawa et al., 1988):

(1) Acceleration and subsequent deceleration of opercular movements, a partial loss of reactivity to external stimuli.

(2) Loss of equilibrium, opercular movements very slow, fish still reactive to strong stimuli.
(3) Total loss of reactivity, fish are lying on the tank bottom and do not respond to handling.
(4) Complete cessation of opercular movements, fish die if left in the bath for too long.

Lethal concentration levels (LC50, LC0.1 and LC99.9) were determined by the probit analysis using EKO-TOX 5.1 software.

Haematological blood profile after exposure to 2-phenoxyethanol

For the haematological blood profile tests, sheatfish of 94.90 ± 55.23 g average weight and 253.0 ± 74.60 mm average body length were used. A total of 40 fishes divided into four groups were examined: Control I (before the anaesthetic administration), Experiment I (immediately after 10-min anaesthesia at the concentration of 0.30 ml/l), Experiment II (24 h after 10-min anaesthesia) and Control II (controls examined in parallel with Experiment II). The fish were anaesthetized for 10 min by 2-phenoxyethanol at a concentration of 0.30 ml/l. Heparinized injection needles were used to take samples of blood from the hearts of fish stunned by a blow with a blunt object over the head. To stabilize blood samples, an aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodova et al., 1986).

The indices used to evaluate the haematological profile included erythrocyte count (Er), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (Leuko) and differential leukocyte count (Leukogram). The procedures were based on Unified Methods for Haematological Examination of Fish (Svobodova et al., 1986).

Results of haematological examinations were tested by the analysis of variance using the Statistica 6.0 (ANOVA – Tukey's test) software.

Biochemical blood plasma profile after exposure to 2-phenoxyethanol

For the biochemical profile of blood plasma tests, sheatfish of 94.90 ± 55.23 g average weight and 253.0 ± 74.60 mm average body length were used.

Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4°C, 837 × g). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), calcium (Ca^{2+}) and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories Inc., U.S.A.) manufactured by Medisoft was used.

Results of biochemical examination were tested by the analysis of variance using the Statistica 6.0 (ANOVA – Tukey's test) software.

Histological examination of tissues

For the histological examination of tissues, sheatfish of 94.90 ± 55.23 g average weight and 253.0 ± 74.60 mm average body length were used.

After blood sampling, samples of gills, liver, cranial and caudal kidney and spleen were taken for histological examinations. The samples were immediately fixed in 10% formaldehyde, drained and embedded in paraffin. Sections were made of the paraffin blocks and stained with haematoxylin-eosin.

RESULTS

Acute toxicity of 2-phenoxyethanol

During the 96-hour LC50 tests, the mean water temperature was 19.7–20.4°C, pH was 7.41–7.66 and water oxygen levels were at 96–103% saturation. On the basis of tests of acute toxicity to sheatfish, the 96-hour lethal concentrations of 2-phenoxyethanol were determined (96hLC50 0.29 ml/l, 96hLC0.1 0.20 ml/l, and 96hLC99.9 0.41 ml/l).

The autopsy performed after the acute toxicity test revealed increased amounts of watery mucus on body surfaces, and the gills were matt dark in colour. The body cavity contained excess moisture, and an increased injection of visceral vessels was also observed.

During the 10-min LC50 tests, water temperature was 19.7°C, pH was 7.69 and water oxygen level was at 96% saturation. On the basis of tests of acute toxicity to sheatfish, the 10-min lethal concentrations of 2-phenoxyethanol were determined (10minLC50

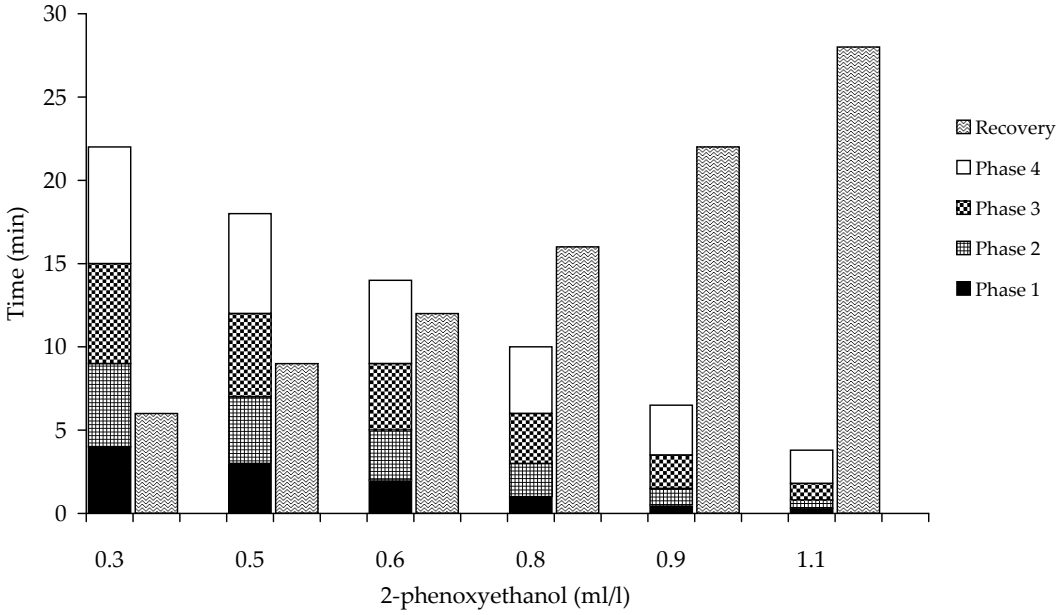

Figure 1. Effects of 2-phenoxyethanol concentrations on the onset of individual phases of anaesthesia and recovery in sheatfish

0.77 ml/l, 10minLC0.1 0.42 ml/l and 10minLC99.9 1.90 ml/l).

Effects of 2-phenoxyethanol concentrations on the time of the onset of anaesthesia, duration of its individual stages and on the course of recovery are shown in Figure 1.

Haematological blood profile after exposure to 2-phenoxyethanol

Effects of 2-phenoxyethanol on the haematological profile of sheatfish are shown in Table 1 and 2. The 10-min exposure to 2-phenoxyethanol at a concentration of 0.30 ml/l caused a significant ($P < 0.05$) increase in PVC and MCV immediately after anaesthesia. A significant ($P < 0.05$) increase in the value of MCHC 24 h post anaesthesia was found. The remaining indices (Er, Hb, MCH, Leuko and Leukogram) were at comparable levels in all groups.

Biochemical blood plasma profile after exposure to 2-phenoxyethanol

Effects of 2-phenoxyethanol on the blood plasma biochemical profile of sheatfish are given in Table 3. The 10-min exposure to 2-phenoxyethanolu at a

Table 1. Effects of 2-phenoxyethanol anaesthesia on haematological indices in sheatfish

Indices	Control I (before anaesthesia) (n = 10)	Experimental I (immediately after anaesthesia) (n = 10)	Experimental II (24 h after anaesthesia) (n = 10)	Control II (after 24 h) (n = 10)
Er (T/l)	0.82 ± 0.23[a]	0.56 ± 0.21[a]	0.50 ± 0.24[a]	0.76 ± 0.25[a]
Hb (g/l)	38.54 ± 6.84[a]	43.69 ± 9.22[a]	43.32 ± 9.27[a]	39.51 ± 5.89[a]
PCV (l/l)	0.21 ± 0.04[a]	0.27 ± 0.04[b]	0.19 ± 0.03[a]	0.20 ± 0.03[a]
MCV (fl)	266.94 ± 138.52[a]	440.39 ± 549.73[b]	392.55 ± 220.85[a]	229.19 ± 160.49[a]
MCH (pg)	53.0 ± 24.38[a]	68.93 ± 115.55[a]	84.43 ± 59.62[a]	50.04 ± 46.35[a]
MCHC (g/l)	177.56 ± 31.42[a]	161.29 ± 27.50[a]	226.19 ± 28.53[b]	171.67 ± 37.66[a]
Leuko (G/l)	20.40 ± 4.04[a]	13.60 ± 5.55[a]	14.20 ± 5.78[a]	14.0 ± 7.50[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Table 2. Effects of 2-phenoxyethanol anaesthesia on differential leukocyte counts in sheatfish

Indices		Control I (before anaesthesia) (n = 10)	Experimental I (immediately after anaesthesia) (n = 10)	Experimental II (24 h after anaesthesia) (n = 10)	Control II (after 24 h) (n = 10)
Lymphocytes	%	66.20 ± 10.27[a]	65.25 ± 8.92[a]	67.60 ± 19.63[a]	78.70 ± 7.06[a]
	g/l	13.50 ± 2.09[a]	8.87 ± 1.21[a]	9.81 ± 1.71[a]	11.04 ± 1.00[a]
Monocytes	%	0.90 ± 1.22[a]	1.60 ± 1.14[a]	1.15 ± 0.98[a]	0.45 ± 0.79[a]
	g/l	0.18 ± 0.25[a]	0.22 ± 0.16[a]	0.10 ± 0.08[a]	0.06 ± 0.11[a]
Neutrophile granulocyte segments	%	17.0 ± 6.54[a]	25.25 ± 10.44[a]	11.25 ± 6.24[a]	9.35 ± 2.34 [a]
	g/l	3.47 ± 1.33[a]	3.43 ± 1.42[a]	1.01 ± 0.56[a]	0.75 ± 0.33[a]
Neutrophile granulocyte rods	%	10.95 ± 8.64 [a]	6.95 ± 2.75[a]	12.00 ± 15.72[a]	13.65 ± 6.57[a]
	g/l	2.23 ± 1.76[a]	0.95 ± 0.37[a]	1.70 ± 1.41 [a]	1.91 ± 0.92[a]
Developmental phases – myeloid sequence	%	1.40 ± 1.24[a]	0.70 ± 0.75[a]	2.10 ± 1.91[a]	0.90 ± 0.58[a]
	g/l	0.27 ± 0.23[a]	0.10 ± 0.15[a]	0.19 ± 0.16[a]	0.13 ± 0.10[a]
Eosinophiles	%	2.80 ± 2.71[a]	0.75 ± 0.51[a]	0.90 ± 0.70[a]	0.85 ± 0.95[a]
	g/l	0.57 ± 0.47[a]	0.06 ± 0.07[a]	0.08 ± 0.06[a]	0.11 ± 0.21[a]
Basophiles	%	0.75 ± 1.15[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.10 ± 0.30[a]
	g/l	0.15 ± 0.23[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.01 ± 0.04[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

concentration of 0.30 ml/l caused a significant ($P < 0.05$) increase in the concentration of glucose and albumins immediately after anaesthesia. Their values returned back to the normal within 24 hours. The level of alanine aminotransferase was found to decrease 24 h post anaesthesia. The remaining indices (TP, GLOB, NH_3, TRIG, AST, LDH, CK, Ca^{2+} and PHOS) were at comparable levels in all groups.

Table 3. Effects of 2-phenoxyethanol anaesthesia on biochemical indices of blood plasma in sheatfish

Indices	Control I (before anaesthesia) (n = 10)	Experiment I (immediately after anaesthesia) (n = 10)	Experiment II (24 h after anaesthesia) (n = 10)	Control II (after 24 h) (n = 10)
GLU (mmol/l)	7.24 ± 2.63[a]	10.52 ± 3.43 [b]	6.52 ± 2.26 [a]	6.91 ± 1.90 [a]
TP (g/l)	35.30 ± 4.0[a]	36.40 ± 2.37[a]	34.20 ± 1.17[a]	32.50 ± 2.70[a]
ALB (g/l)	3.80 ± 1.83[a]	6.50 ± 0.92[b]	3.20 ± 0.98[a]	2.70 ± 1.27[a]
GLOB (g/l)	31.60 ± 2.11[a]	32.80 ± 1.40[a]	31.30 ± 0.90[a]	29.60 ± 1.69[a]
NH_3 (µmol/l)	931.60 ± 68.09[a]	946.80 ± 66.33[a]	939.40 ± 70.03[a]	936.79 ± 76.11[a]
TAG (mmol/l)	1.68 ± 0.39[a]	1.83 ± 0.50[a]	1.27 ± 0.11[a]	1.07 ± 0.25[a]
AST (µkat/l)	7.43 ± 0.60[a]	7.21 ± 0.86[a]	7.38 ± 0.79[a]	7.53 ± 0.71[a]
ALT (µkat/l)	0.19 ± 0.09[a]	0.18 ± 0.11[a]	0.09 ± 0.09[b]	0.18 ± 0.10[a]
LDH (µkat/l)	8.96 ± 4.18[a]	8.78 ± 5.66[a]	8.67 ± 5.12[a]	8.89 ± 6.65[a]
CK (µkat/l)	44.82 ± 2.89[a]	43.11 ± 3.26[a]	44.64 ± 4.01[a]	44.08 ± 4.34[a]
Ca^{2+} (mmol/l)	2.30 ± 0.21[a]	2.31 ± 0.14[a]	2.13 ± 0.23[a]	2.08 ± 0.08[a]
PHOS (mmol/l)	1.16 ± 0.14[a]	1.35 ± 0.23[a]	1.28 ± 0.22[a]	1.37 ± 0.18[a]

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)

Histological examination of tissues

All specimens of sheatfish showed capillary ectasia of gill filaments immediately after clove oil anaesthesia. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were demonstrated in other tissues (liver, spleen, cranial and caudal kidneys) following anaesthesia.

DISCUSSION

Acute toxicity of 2-phenoxyethanol to fish is investigated from the aspect of 2-phenoxyethanol use as an anaesthetic, and of the risk of water contamination with anaesthetizing baths. The 10minLC50 (LC0.1; LC99.9) values characterize 2-phenoxyethanol toxicity in the case of a 10-min exposure to the anaesthetic.

Woynarowich and Horvat (1980) reported 0.30 ml/l 2-phenoxyethanol as a safe concentration for the anaesthesia of channel catfish (*Ictalurus punctatus*), adding that exposures longer than 15 min prolonged recovery times and increased mortality.

Sensitivity to anaesthetics is generally affected by the health and physical condition of the fish. It may also be affected by oxygen concentrations: an oxygen deficiency increases the effectiveness of anaesthetics (Svobodova et al., 1987).

The most important factor affecting the efficiency of 2-phenoxyethanol in fish is, however, water temperature: the higher the temperature, the higher the efficiency of the anaesthetic in fish. Repeated use of 2-phenoxyethanol increases the fish tolerance to the anaesthetic (Weyl et al., 1996). Because young fish are more sensitive to 2-phenoxyethanol than old fish, lower concentrations of the anaesthetic should be used for the former, as they provide much wider safety anaesthesia margins (Barton and Helfrich, 1981).

It follows from Figure 1 that the onset of individual phases of anaesthesia and recovery times depended on 2-phenoxyethanol concentrations used.

To evaluate haematological and biochemical profiles of blood and histopathological changes in sheatfish tissues the 2-phenoxyethanol concentration of 0.30 ml/l was used in the present study. Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). The values determined in the present study suggest that internal organs and tissues of sheatfish are not altered by 2-phenoxyethanol anaesthesia. This conclusion was also confirmed by the result of histological examinations of parenchymatous organs.

In our experiments with sheatfish, a significant increase ($P < 0.05$) in blood plasma glucose and albumins was observed immediately after the 10-min 2-phenoxyethanol anaesthesia. Increased glucose and albumins levels returned to the normal 24 h after anaesthesia. The level of alanine aminotransferase was found to decrease 24 h post anaesthesia.

2-phenoxyethanol, a common fish anaesthetic, is widely applied in closed transport systems of fish (Guo et al., 1992; Teo and Chen 1993; Kaiser and Vine, 1998). Compared with quinate, MS-222, and methomidate, 2-phenoxyethanol is the most effective in decreasing the metabolic activity and mortality of transported fish (Guo et al., 1992).

A disadvantage of 2-phenoxyethanol is its relatively low therapeutic index, i.e. the ratio of therapeutic to toxic concentrations. The generally reported optimum ratio is 1:4 or higher (Svobodova and Vykusova, 1991). A comparison between the concentration used in 10-min anaesthesia of fish (0.30 ml/l) and the 10minLC50 values (0.77 ml/l) suggests that the 2-phenoxyethanol therapeutic index is 1:2.6.

When 2-phenoxyethanol is used, labour safety regulations should be strictly observed because the anaesthetic is toxic and harmful to humans. It may cause fatigue and drowsiness of the staff in poorly ventilated rooms (Svoboda and Kolarova, 1999).

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