Effects of 2-Phenoxyethanol Anaesthesia on Haematological Profile on Common Carp (*Cyprinus carpio*) and Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

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The aim of this study was to assess changes in haematological profile of common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) after the exposure to anaesthetic 2-phenoxyethanol. The haematological profile was assessed before, immediately after 10 min of anaesthesia and 24 h after the anaesthesia with recommended concentration of 0.30 ml·l^{-1} 2-phenoxyethanol. The 10-min exposure to 2-phenoxyethanol of common carp caused the significant increase ($p < 0.01$) in the haematocrit value and relative and actual count of monocytes immediately after the anaesthesia. These values returned back to normal within 24 hours. In rainbow trout, 2-phenoxyethanol anaesthesia had no effect on the haematological profile. Results of the examinations suggest that the use of 2-phenoxyethanol at the concentration of 0.30 ml·l^{-1} does not cause irreversible damage of the blood in common carp and rainbow trout.

Anaesthetic, erythrocyte profile, leukocyte profile, fish

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 and surgery procedures, in order to reduce stress-induced problems such as decreases in food intake and immunity (Roubach et al. 2005).

A number of chemicals have proved effective in anaesthetisation of fish (Velíšek et al. 2006). Each of them has its own advantages and drawbacks. The anaesthetics most commonly used in aquaculture are MS-222, benzocaine, quinaldine sulphate, clove oil and 2-phenoxyethanol (Svoboda and Kolářová 1999). Anaesthesia is usually induced by immersing the fish in an anaesthetic solution.

At this time, only MS-222 (tricaine methanesulfonate) is registered for use on food fish in the U. S. and the United Kingdom. However, many compounds have been evaluated experimentally and some of them are being used on non-food fish and in research (Coyle et al. 2004).

Anaesthesia is achieved by placing the fish into an anaesthetic solution that is absorbed through the gills and enters the arterial blood, from where it acts on the central nervous system (Ross and Ross 1999). Some anaesthetics reduce or block the activation of the hypothalamic-pituitary-interrenal (HPI) axis associated with stressors and thus decrease or prevent the release of the stress hormone cortisol to the bloodstream of fish (Hoskonen and Pirhonen 2006). After return of the anaesthetised fish to the fresh water, the anaesthetics or their metabolites are excreted via the gills (Ross and Ross 1999). The efficacy and safety of any anaesthetic agent varies among species, life stages and environmental conditions.

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In the Czech Republic, 2-phenoxyethanol is used for short-term immobilisation of fish before artificial spawning and whenever fish is handled outside water. The recommended concentration for anaesthetic purposes is 0.30 ml·l^{-1} water bath (Svoboda and Kolářová 1999). At present, the effects on 2-phenoxyethanol on commercially produced fish are studied in a project regarding the application of principles of pharmacovigilancy in aquaculture in the Czech Republic. In the first stage of the project, the effects of 2-phenoxyethanol on biochemical profile of common carp (Velíšek and Svobodová 2004a) and rainbow trout (Velíšek and Svobodová 2004b) were studied.

The aim of this study was to assess changes in haematological profile of common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*) after the exposure of anaesthetic 2-phenoxyethanol.

Materials and Methods

Characteristics of 2-phenoxyethanol

The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. Its summary formula is $C_8H_{10}O_2$, the molar weight 138.17 g·l^{-1}, density 1.107 - 1.108 g·dm^{-3}, peroxide content less than 0.005% and the boiling temperature is 245 °C. The anaesthetic is slightly soluble in water (26.7 g·l^{-1}) at 25 °C but readily soluble in ethanol. The anaesthetic affects fish through the skin and gills.

The preparation is marketed by MERCK - Schucherd, 85 662 Hohenbrunn, Germany in 2.5 and 1 litre packages, or in other volumes on request.

Haematological profile

For the haematological profile tests, carp (mirror carp M 72) of 365 ± 49.28 g mean body weight and 255.0 ± 61.92 mm mean body length, and rainbow trout (Camloops farm) of 112.5 ± 38.57 g average body mass and 200.0 ± 41.31 mm average body length were used.

A total of 40 fish from each of two experimental species were divided into four groups and examined: Control I (before the anaesthetic administration), Experiment I (immediately after 10 min of anaesthesia at the concentration of 0.30 ml·l^{-1}), Experiment II (24 h after 10 min of anaesthesia) and Control II (controls examined in parallel with Experiment II). The fish were anaesthetised for 10 min by 2-phenoxyethanol at the concentration of 0.30 ml·l^{-1}. Heparinised injection needles were used to take blood samples from heart of fish stunned by a blow with a blunt object over the head. To stabilize the blood samples, aqueous solution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodová et al. 1991).

The indices used to evaluate the haematological profile included the erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram). The procedures were based on Unified methods for haematological examination of fish (Svobodová et al. 1991).

Results of haematological examinations were tested by the analysis of variance using the Statistica 6.0 (ANOVA - Tuckey Test) software.

Results

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

Indices	Control I (before anaesthesia) x ± SD (n = 10)	Experimental I (immediately after anaesthesia) x ± SD (n = 10)	Experimental II (24 hrs after anaesthesia) x ± SD (n = 10)	Control II (after 24 hrs) x ± SD (n = 10)
RBC (T·l^{-1})	1.66 ± 0.28 ^{a}	1.92 ± 0.30 ^{a}	1.54 ± 0.16 ^{a}	1.65 ± 0.21 ^{a}
Hb (g·l^{-1})	74.25 ± 11.29 ^{a}	86.71 ± 9.0 ^{a}	78.35 ± 8.26 ^{a}	81.0 ± 8.83 ^{a}
MCV (fl)	164.61 ± 19.69 ^{a}	192.42 ± 17.67 ^{a}	190.24 ± 17.76 ^{a}	176.82 ± 27.09 ^{a}
MCH (pg)	45.14 ± 4.74 ^{a}	45.60 ± 4.24 ^{a}	51.25 ± 6.28 ^{a}	49.59 ± 6.67 ^{a}
MCHC (g·l^{-1})	275.53 ± 21.88 ^{a}	236.99 ± 6.96 ^{a}	269.14 ± 16.02 ^{a}	281.54 ± 18.22 ^{a}
Leuko (G·l^{-1})	27.10 ± 9.20 ^{a}	34.40 ± 10.59 ^{a}	33.30 ± 14.18 ^{a}	28.10 ± 9.34 ^{a}

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

Common carp

Effects of 2-phenoxyethanol on the haematological profile of carp are shown in Tables 1 and 2. The 10-min exposure to 2-phenoxyethanol at the concentration of 0.30 ml·l^{-1} caused

a significant ($p < 0.01$) increase of the haematocrit value (Fig. 1), relative and actual count of monocytes (Fig. 2) immediately after anaesthesia. The value of haematocrit, relative and actual count of monocytes had returned back to normal within 24 hours. The rest of the indices (RBC, Hb, MCV, MCHC, MCH, Leuko) were at comparable levels in all groups.

Table 2. Effects of 2-phenoxyethanol anaesthesia on differential leukocyte count in common carp

Indices		Control I (before anaesthesia) x ± SD (n = 10)	Experiment I (immediately after anaesthesia) x ± SD (n = 10)	Experiment II (24 hrs after anaesthesia) x ± SD (n = 10)	Control II (after 24 hrs) x ± SD (n = 10)
Lymphocytes	%	81.56 ± 7.52 [a]	75.20 ± 9.59 [a]	80.30 ± 6.24 [a]	75.60 ± 10.67 [a]
	$G \cdot l^{-1}$	22.32 ± 8.15 [a]	25.87± 6.24 [a]	27.14 ± 27.14 [a]	24.24 ± 8.94 [a]
Neutrophile granulocytes segment	%	4.67 ± 4.66 [a]	5.80 ± 4.94 [a]	4.80 ± 3.62 [a]	7.70 ± 4.42 [a]
	$G \cdot l^{-1}$	1.27 ± 0.54 [a]	1.99 ± 0.76 [a]	1.60 ± 0.64 [a]	1.80 ± 0.79 [a]
Neutrophile granulocytes bands	%	4.22 ± 2.54 [a]	6.50 ± 5.44 [a]	4.60 ± 2.37 [a]	5.40 ± 3.31 [a]
	$G \cdot l^{-1}$	1.14 ± 0.89 [a]	2.26 ± 2.03 [a]	1.53 ± 1.26 [a]	1.51 ± 1.12 [a]
Developmental phases – myeloid sequence	%	7.89 ± 7.49 [a]	9.80± 6.46 [a]	9.70 ± 7.12 [a]	7.90 ± 7.36 [a]
	$G \cdot l^{-1}$	2.12 ± 1.42 [a]	2.89± 1.98 [a]	2.34 ± 1.69 [a]	2.32 ± 1.32 [a]

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

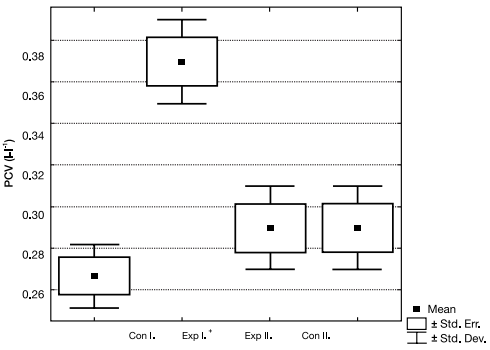

Fig. 1. Effects of 2-phenoxyethanol anaesthesia on haematocrit value in common carp
*Statistical significance $p < 0.01$

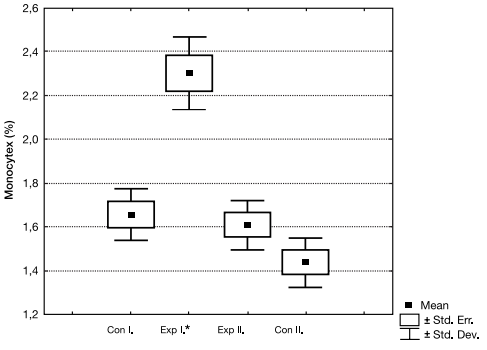

Fig. 2. Effects of 2-phenoxyethanol anaesthesia on relative count of monocytes in common carp
*Statistical significance $p < 0.01$

Rainbow trout

Effects of 2-phenoxyethanol on the haematological profile of rainbow trout are shown in Tables 3 and 4. The 10-min of exposure to the anaesthetic at a concentration of 0.30 $ml \cdot l^{-1}$ 2-phenoxyethanol had no effect on the haematological indices studied (RBC, Hb, PCV, MCV, MCHC, MCH, Leuko and Leukogram).

Discussion

Anaesthetics are necessary for many hatchery procedures in aquaculture. Because species may differ widely in their response to the same anaesthetic, screening of dosage of different anaesthetics is often necessary.

Table 3. Effects of 2-phenoxyethanol anaesthesia on haematological indices in rainbow trout

Indices	Control I (before anaesthesia) x ± SD (n = 10)	Experimental I (immediately after anaesthesia) x ± SD (n = 10)	Experimental II (24 hrs after anaesthesia) x ± SD (n = 10)	Control II (after 24 hrs) x ± SD (n = 10)
RBC ($T \cdot l^{-1}$)	1.43 ± 0.23 [a]	1.32 ± 0.36 [a]	1.30 ± 0.31 [a]	1.06 ± 0.25 [a]
Hb ($g \cdot l^{-1}$)	61.32 ± 10.08 [a]	58.01 ± 13.21 [a]	57.89 ± 12.80 [a]	55.91 ± 6.84 [a]
PCV ($l \cdot l^{-1}$)	0.43 ± 0.05 [a]	0.42 ± 0.04 [a]	0.40 ± 0.06 [a]	0.39 ± 0.03 [a]
MCV (fl)	305.71 ± 51.24 [a]	321.14 ± 63.01 [a]	341.02 ± 76.33 [a]	378.1 ± 90.91 [a]
MCH (pg)	43.67 ± 9.65 [a]	45.98 ± 12.3 [a]	46.36 ± 14.98 [a]	52.44 ± 10.80 [a]
MCHC ($g \cdot l^{-1}$)	141.99 ± 13.32 [a]	139.56 ± 15.09 [a]	141.66 ± 13.74 [a]	140.19 ± 14.41 [a]
Leuko ($G \cdot l^{-1}$)	23.70 ± 11.31 [a]	22.40 ± 13.25 [a]	20.00 ± 17.21 [a]	17.61 ± 6.42 [a]

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

Table 4. Effects of 2-phenoxyethanol anaesthesia on differential leukocyte count in rainbow trout.

Indices		Control I (before anaesthesia) x ± SD (n = 10)	Experiment I (immediately after anaesthesia) x ± SD (n = 10)	Experiment II (24 hrs after anaesthesia) x ± SD (n = 10)	Control II (after 24 hrs) x ± SD (n = 10)
Lymphocytes	%	71.20 ± 13.61 [a]	69.00 ± 14.13 [a]	73.50 ± 10.50 [a]	69.20 ± 12.09 [a]
	$G \cdot l^{-1}$	16.87 ± 3.25 [a]	15.23 ± 3.17 [a]	14.17 ± 2.31 [a]	14.87 ± 2.07 [a]
Monocytes	%	2.80 ± 1.78 [a]	2.60 ± 2.46 [a]	3.00 ± 1.95 [a]	3.09 ± 1.13 [a]
	$G \cdot l^{-1}$	0.49 ± 0.42 [a]	0.58 ± 0.55 [a]	0.60 ± 0.39 [a]	0. 53 ± 0.19 [a]
Neutrophile granulocytes segment	%	21.60 ± 11.40 [a]	24.00 ± 12.88 [a]	22.50 ± 7.24 [a]	25.50 ± 10.89 [a]
	$G \cdot l^{-1}$	5.12 ± 2.17 [a]	5.38 ± 2.89 [a]	4.50 ± 1.45 [a]	4.38 ± 1.87 [a]
Neutrophile granulocytes bands	%	1.50 ± 1.07 [a]	1.05 ± 0.85 [a]	1.02 ± 1.04 [a]	1.01 ± 1.26 [a]
	$G \cdot l^{-1}$	0.36 ± 0.25 [a]	0.24 ± 0.19 [a]	0.24 ± 0.21 [a]	0.27 ± 0.22 [a]
Developmental phases – myeloid sequence	%	4.50 ± 2.06 [a]	4.40 ± 1.96 [a]	4.70 ± 3.64 [a]	5.90 ± 3.80 [a]
	$G \cdot l^{-1}$	1.07 ± 0.49 [a]	0.99 ± 0.44 [a]	0.94 ± 0.73 [a]	1.01 ± 0.65 [a]

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

In our experiments with common carp, the significant increase ($p < 0.01$) of haematocrit value and relative and actual count of monocytes immediately after the 10-min 2-phenoxyethanol anaesthesia was observed. All these values returned to physiological ranges within 24 h after anaesthesia. On the other hand, Adámek et al. (1993) found increased count of RBC and concentration of Hb in common carp (*Cyprinus carpio*) following 2-phenoxyethanol (0.30 $ml \cdot l^{-1}$) anaesthesia.

In our experiments with rainbow trout, 2-phenoxyethanol anaesthesia had no effect on haematological profile. These findings are in accordance with the results of Tort et al. (2002), who also found no change in haematological profile in rainbow trout *(Oncorhynchus mykiss)* following 2-phenoxyethanol (0.50 $ml \cdot l^{-1}$) anaesthesia.

Guo et al. (1995) thought that 2-phenoxyethanol was more suitable than either quinate or MS-222 to sedate non-food fishes (e.g., ornamental fish) during live transport. A major drawback of 2-phenoxyethanol is that it requires rather high anaesthetic doses in fishes. The range of effective anaesthetic doses of 2-phenoxyethanol in most fish species are from about 0.20 to 0.60 $ml \cdot l^{-1}$ (Inoue et al. 2004).

2-phenoxyethanol, which despite of some secondary negative effects (Ortuño et al. 2002), its questionable usefulness (Kaiser and Vine 1998) and potential hazard to the

handler (Morton 1990), is considered very suitable for aquaculture practice because of its easy preparation, low price, rapid action, rapid and uneventful recovery (Pucéa et al. 1989) and bactericidal and fungicidal properties (Jolly et al. 1972). It has been suggested as a good anaesthetic (Ortuño et al. 2002). Furthermore, it should be noted that, as 2-phenoxyethanol is not approved for use in food fish, we do not advocate its use in any fish unless MRL values (EEC Regulation 2377/90) are set and proper licensing is acquired.

Our results showed that 2-phenoxyethanol at 0.30 $ml \cdot l^{-1}$ concentration may be used as an efficient and safe anaesthetic for carp and rainbow trout.

Vliv anestetika 2-phenoxyethanolu na hematologický profil kapra obecného (*Cyprinus carpio*) a pstruha duhového (*Oncorhynchus mykiss*)

Cílem studie bylo posoudit hematologický profil kapra obecného (*Cyprinus carpio*) a pstruha duhového (*Oncorhynchus mykiss*) po působení anestetika 2-phenoxyethanolu. Hematologický profil byl hodnocen před, po 10 min anestézii a 24 hodin po anestézii v doporučené koncentraci 0,30 $ml \cdot l^{-1}$ 2-phenoxyethanolu. 10 minutová anestézie 2-phenoxyethanolem způsobila signifikantní zvýšení ($p < 0,01$) hematokritové hodnoty a relativního a absolutního počtu monocytů u kapra obecného. K poklesu hodnot na úroveň kontrolní skupiny ryb došlo do 24 hodin po anestézii. Anestézie 2-phenoxyethanolem neměla vliv na hematologický profil pstruha duhového. Výsledky ukázaly, že 2-phenoxyethanol v koncentraci 0,30 $ml \cdot l^{-1}$ je pro kapra obecného a pstruha duhového bezpečný.

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Council regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ No L 67 of 7. 3 1997, p. 1)