

**EFFECT OF 2-PHENOXYETHANOL AND ETOMIDATE ON CARDIAC
AND RESPIRATORY FUNCTIONS AND BEHAVIOUR OF COMMON CARP,
CYPRINUS CARPIO L. (ACTINOPTERYGII, CYPRINIFORMES, CYPRINIDAE),
DURING GENERAL ANAESTHESIA**

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Background. Assessments of the efficacy of anaesthetics are usually based on observations of fish behaviour or changes in blood parameters. In this study we attempted to assess the process of anaesthesia, caused by 2-phenoxyethanol and Propiscin (0.2% etomidate), based on recording the heartbeat and the respiratory frequency.

Materials and Methods. The effect of two concentrations of 2-phenoxyethanol ($0.4 \text{ mL} \cdot \text{L}^{-1}$ and $0.8 \text{ mL} \cdot \text{L}^{-1}$) and two concentrations of Propiscin ($1.0 \text{ mL} \cdot \text{L}^{-1}$ and $2.0 \text{ mL} \cdot \text{L}^{-1}$) on heartbeat and respiratory frequency of common carp, *Cyprinus carpio* L., were studied using ECG. Concurrently, during general anaesthesia, the fish behaviour was also observed.

Results. The anaesthetic potential of 2-phenoxyethanol and Propiscin at the applied concentrations was comparable. The behavioural reactions caused by the agents were not identical. At the beginning of the exposure, 2-phenoxyethanol produced locomotor agitation and an irritation-like reaction of the respiratory system. Propiscin had no such effect. Both agents induced a decrease in the ventilatory and cardiac frequencies. The reductions caused by Propiscin were simultaneous. The inhibitory effect of 2-phenoxyethanol at the concentration of $0.8 \text{ mL} \cdot \text{L}^{-1}$ was much stronger on respiration than on heart rate.

Conclusion. The ECG method can be an important and objective tool supplementing data acquired during visual observation of responses to anaesthesia. Its major advantage is the comparativeness of data.

Keywords: 2-phenoxyethanol, Propiscin, anaesthesia of fish, common carp

INTRODUCTION

Sedative and anaesthetic agents are very useful in aquaculture. They reduce fish activity, limit oxygen consumption, and facilitate such routine handling operations as weighing, measuring, sorting, manual spawning, marking, and veterinary procedures. Their main advantage is to minimize stress reactions during high density storage and transport. Anaesthetics most commonly used in aquaculture are MS-222, benzocaine, etomidate, metomidate, 2-phenoxyethanol, quinaldine, quinaldine sulphate, and eugenol (Hajek and Kłyszajko 2004, Dziaman et al. 2005, Cotter and Rodnick 2006, Hajek et al. 2006). All these agents affect fish in a similar way, their effect being concentration dependant. High concentrations induce general anaesthesia, resulting in sedation or unconsciousness, analgesia, cessation of defensive reflexes, and loss of muscle tone. Small concentrations induce only sedation.

Anaesthetics can also cause adverse effects in fish. Risk is especially related to overdosage or prolonged

exposure in the anaesthetising solution. This can lead to hypoxia or even death of the fish, resulting from respiratory and cardiac disorders.

Molecular mechanisms of anaesthetic action in animals are not fully recognized. Usually, research on anaesthesia is based on haematological analyses and observations of behavioural responses. In fish, assessments of anaesthetic effects are usually based on changes of behaviour, keeping or losing of equilibrium, visual observations of opercular rate, reactivity to external stimuli, etc.

This report presents visual observations of behavioural responses of common carp to anaesthesia with 2-phenoxyethanol and Propiscin completed with simultaneous electronic registration of heart rate and respiratory rate.

MATERIALS AND METHODS

Experiments were carried out on 44 common carp, *Cyprinus carpio* L., ($250 \pm 35 \text{ g}$) taken from a cage-culture facility located on the warm-water discharge canal of

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the “Dolna Odra” power plant at Stare Czarnowo (Gryfino, near Szczecin, Poland). Fish were acclimatized to the new environment for 14 days in a large 1000 L aquarium, in aerated tap water, at $20 \pm 1^\circ\text{C}$ (pH = 7.5). Fish were subjected to general anaesthesia using 2-phenoxyethanol (manufactured by ICN Biomedicals Inc., Ohio, US) at concentrations $0.4 \text{ mL} \cdot \text{L}^{-1}$ and $0.8 \text{ mL} \cdot \text{L}^{-1}$ or Propiscin (0.2% etomidate) at concentrations $1.0 \text{ mL} \cdot \text{L}^{-1}$ and $2.0 \text{ mL} \cdot \text{L}^{-1}$ (manufactured by Department of Fish Immunology and Pathology in Żabieniec, Inland Fisheries Institute in Olsztyn).

Each fish was treated on an individual basis. Heart rate and respiratory rate were recorded with the aid of ECG equipment manufactured by CED (Cambridge Electronic Design Limited, England). The copper electrodes used were elastic and 1 mm in diameter. Their electrical insulation was removed along the last 5 mm, 6 cm from its free ends. The electrodes penetrated the abdominal wall with their bare parts placed in the pericardium and their isolated proximal ends protruding outside the fish. The elastic connection of the electrodes to the recording unit enabled the fish to move freely within the experimental aquarium. The electrodes were implanted following general anaesthesia with Propiscin at $1.5 \text{ mL} \cdot \text{L}^{-1}$. Then fish was placed in an experimental tank, made of dark glass and illuminated from above by a glow-tube. One of the tank walls was a Venetian mirror, which facilitated discrete observation of the fish. After 24 h, the recording units were switched on and left in a constant monitoring mode. The first 15 min recorded served as control. Subsequently one of the anaesthetics was added to the water. After further 15 min the anaesthetised fish were transferred to an identical aquarium, with clean, clear and well aerated water without anaesthetic, where vital parameters were monitored for another 15 min. The experiment with each concentration was carried out on 11 fish. The results were processed statistically using STATISTICA®6.0 PL. To assess the significance of differences between control values and the values for individual minutes of the experiment, a non-parametric Mann–Whitney test was used. The magnitudes of rela-

tions between the respiratory and heartbeat rhythms were determined using Pearson’s linear correlation coefficient.

The experimental procedure of the presently reported study has been approved by the Local Ethical Committee for Experiments on Animals (Szczecin, Poland).

RESULTS

The 15 min exposure at 2-phenoxyethanol concentrations (0.4 and $0.8 \text{ mL} \cdot \text{L}^{-1}$) resulted in progressive anaesthesia (Table 1). The 2-phenoxyethanol induced strong respiratory reactions in anaesthetised fishes. The main symptoms were: temporary apnoea directly following the addition of the anaesthetic and total respiratory arrest at $0.8 \text{ mL} \cdot \text{L}^{-1}$. After transferring to a recovery tank with clean, well aerated water, the fish immediately regained locomotor equilibrium and increased respiratory rate close to the initial (control) values.

The concentration of $0.4 \text{ mL} \cdot \text{L}^{-1}$ caused almost simultaneous reductions in the heart and ventilation rates (Fig. 1). The exposure to higher dose of the agent resulted in deep depression of respiration (Fig. 2). The 15 min exposure at Propiscin concentrations (1.0 and $2.0 \text{ mL} \cdot \text{L}^{-1}$) resulted in progressive anaesthesia (Table 2). The concentration of $1.0 \text{ mL} \cdot \text{L}^{-1}$ caused almost simultaneous reductions in the heart and ventilation rates (Fig. 3). The exposure to higher dose of the agent resulted in deep depression of respiration (Fig. 4).

The time between the beginning of exposure to the anaesthetic and the moment the fish lost equilibrium was regarded as the duration of anaesthesia induction. In common carp, $1.0 \text{ mL} \cdot \text{L}^{-1}$ of Propiscin induced loss of equilibrium during no more than 5 min while $0.4 \text{ mL} \cdot \text{L}^{-1}$, 2-phenoxyethanol required an average of 5.6 min (Table 3). Doubling the concentration of either anaesthetic resulted in loss of equilibrium within slightly more than 3 min. Following the exposure to 2-phenoxyethanol, the fish quickly regained locomotor equilibrium, within an average of 6.9 min and 9.3 min, at 0.4 and $0.8 \text{ mL} \cdot \text{L}^{-1}$, respectively. Following exposure to Propiscin, at the

Table 1

The sequences of behavioural responses of common carp exposed to 2-phenoxyethanol at concentrations 0.4 and $0.8 \text{ mL} \cdot \text{L}^{-1}$

Stage of anaesthesia	Sequence of behavioural responses	
	2-phenoxyethanol ($0.4 \text{ mL} \cdot \text{L}^{-1}$)	2-phenoxyethanol ($0.8 \text{ mL} \cdot \text{L}^{-1}$)
I	<ul style="list-style-type: none"> • distinct agitation • apnoea (several seconds) • increased opercular rate 	<ul style="list-style-type: none"> • intensive agitation • distinct restlessness • cessation of opercular movements • increased, irregular opercular rate • swimming up to the surface and gasping for air
II	<ul style="list-style-type: none"> • loss of equilibrium • shallow breathing • sinking to the bottom of aquarium 	<ul style="list-style-type: none"> • loss of equilibrium • tilting on side • initially irregular breathing, finally ceased
III	<ul style="list-style-type: none"> • deep anaesthesia • lying on the side • no defensive reflexes • irregular, shallow breathing 	<ul style="list-style-type: none"> • deep anaesthesia • sinking to the bottom of aquarium • no defensive reflexes • cessation of respiration

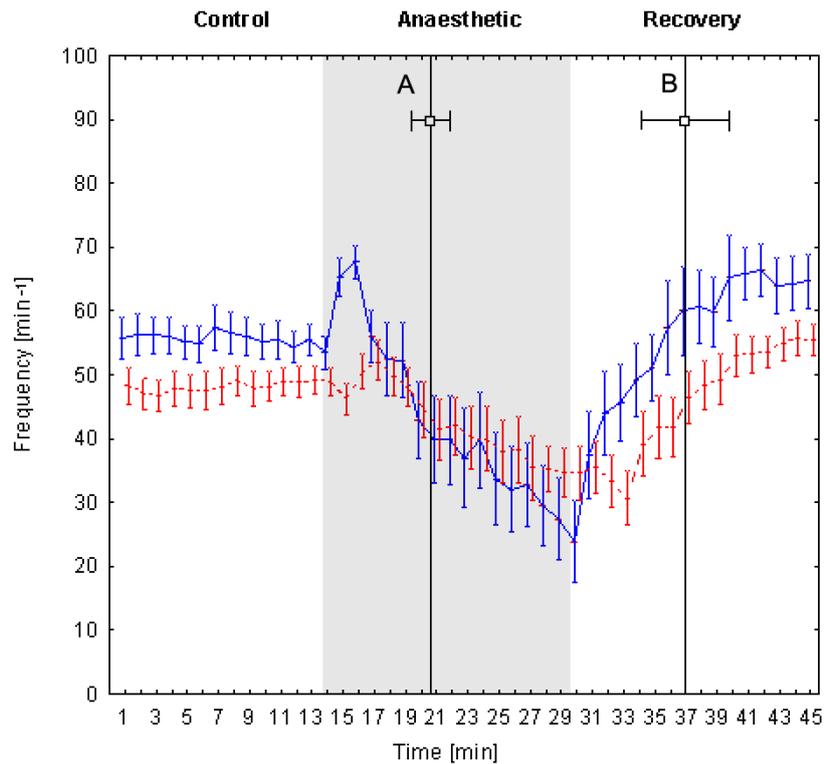


Fig. 1. The effect of 2-phenoxyethanol ($0.4 \text{ mL} \cdot \text{L}^{-1}$) on heartbeat (red dotted line) and respiratory rhythm (blue solid line) (mean values for individual minutes of the experiment \pm standard deviation); A = loss of locomotor equilibrium; B = return to locomotor equilibrium (mean \pm confidence interval)

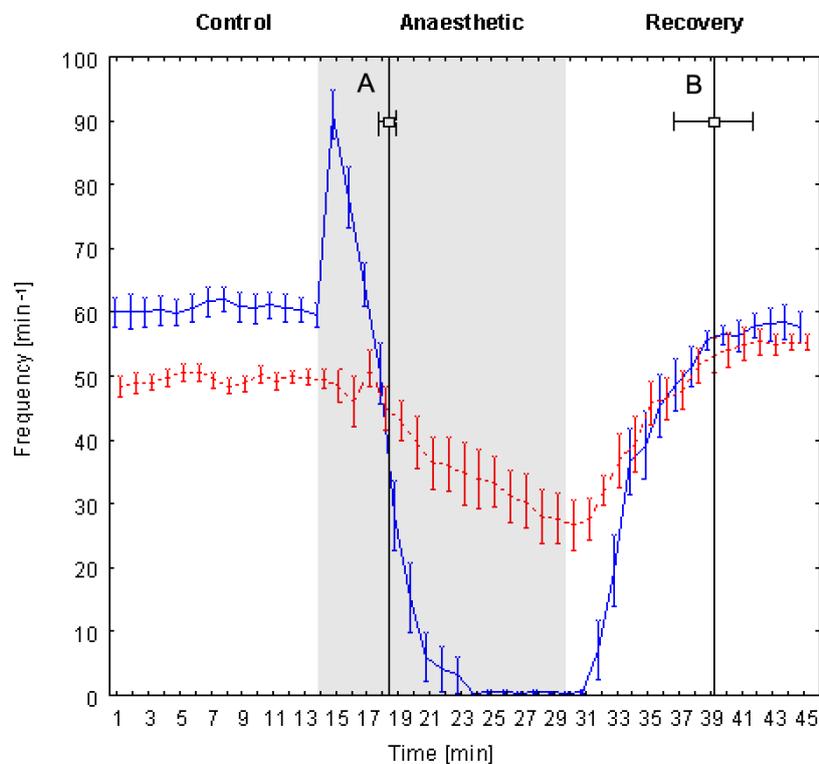


Fig. 2. The effect of 2-phenoxyethanol ($0.8 \text{ mL} \cdot \text{L}^{-1}$) on heartbeat (red dotted line) and respiratory rhythm (blue solid line) (mean values for individual minutes of the experiment \pm standard deviation); A = loss of locomotor equilibrium; B = return to locomotor equilibrium (mean \pm confidence interval)

Table 2

The sequences of behavioural responses of common carp exposed to Propiscin at concentrations 1.0 and 2.0 mL · L⁻¹

Stage of anaesthesia	Sequence of behavioural responses	
	propiscin (1.0 mL · L ⁻¹)	propiscin (2.0 mL · L ⁻¹)
I	• calming down, decreased activity	• calming down, decreased activity
II	• loss of equilibrium, shallow breathing	• loss of equilibrium, shallow breathing • sinking to the bottom of aquarium
III	• deep anaesthesia, the fish lie on the bottom of aquarium, exhibit no defensive reflexes • shallow and irregular breathing	• deep anaesthesia, fish lie on the bottom of aquarium, exhibit no defensive reflexes • shallow and irregular breathing

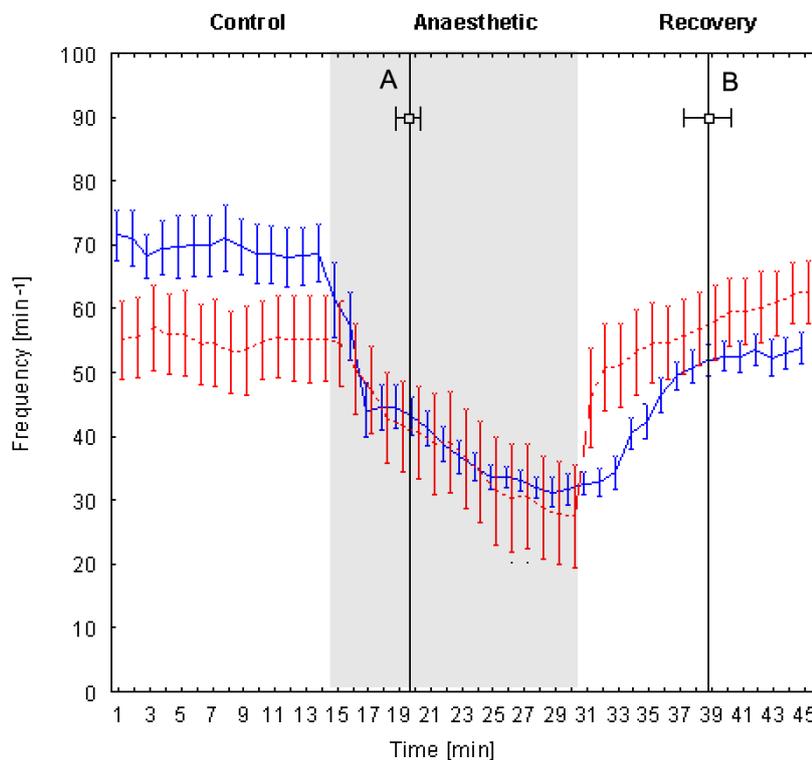


Fig. 3. The effect of Propiscin (1.0 mL · L⁻¹) on heartbeat (red dotted line) and respiratory rhythm (blue solid line) (mean values for individual minutes of the experiment ± standard deviation); A = loss of locomotor equilibrium; B = return to locomotor equilibrium (mean ± confidence interval)

lower concentration (1.0 mL · L⁻¹) the fish regained equilibrium within average 8 min, and in case of doubled concentration (2.0 mL · L⁻¹)—during average 12 min.

DISCUSSION

Traditional visual monitoring of the course of anaesthesia in common carp revealed that locomotor agitation was the first sign of the 2-phenoxyethanol-induced narcosis. According to Molinero and Gonzalez (1995), and Takashima et al. (1983), agitation is a characteristic stress reaction of fish, resulting in the increase of cortisol and glucose levels in serum.

Agitation was usually associated with a short period of apnoea (a few seconds), followed by the fish gasping for

air—a result of hypoxia. The gasping for air might have been an attempt to reduce the flow of water with a foreign chemical through the gills, considering that during the experiment the water was well aerated but 2-phenoxyethanol has a characteristic rosy smell.

The fast retreat of general anaesthesia symptoms observed at the 2-phenoxyethanol concentration of 0.8 mL · L⁻¹ might have resulted from a relatively short exposure to the anaesthetic (15 min) and from the fact observed by other authors, that 2-phenoxyethanol is quickly eliminated by fish (Imamura-Kojima et al. 1987, Mattson and Rippe 1989). Unlike 2-phenoxyethanol, Propiscin caused no agitation. The induction of anaesthesia progressed calmly, the fish activity decreased,

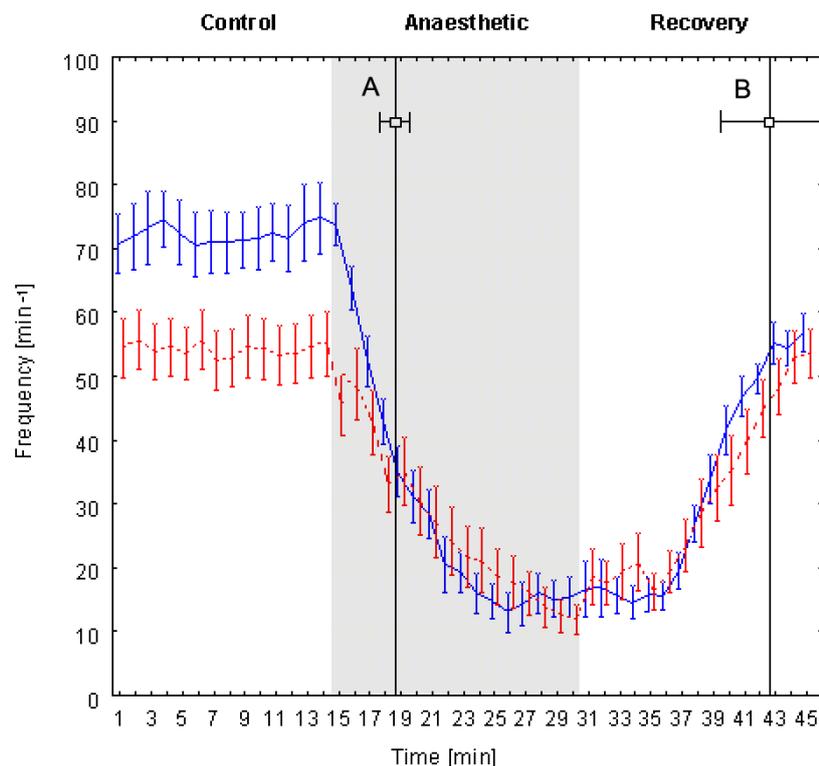


Fig. 4. The effect of Propiscin ($2.0 \text{ mL} \cdot \text{L}^{-1}$) on heartbeat (red dotted line) and respiratory rhythm (blue solid line) (mean values for individual minutes of the experiment \pm standard deviation); A = loss of locomotor equilibrium; B = return to locomotor equilibrium (mean \pm confidence interval)

Table 3

Mean times of loss and recovery of locomotor equilibrium in fish subjected to selected anaesthetics

Anaesthetic	Loss of equilibrium			Recovery of equilibrium		
	mean	confidence interval ($\pm 95\%$)	SD	mean	confidence interval ($\pm 95\%$)	SD
2-phenoxyethanol ($0.4 \text{ mL} \cdot \text{L}^{-1}$)	5.64	4.34 – 6.93	1.92	6.86	4.05 – 9.67	4.18
2-phenoxyethanol ($0.8 \text{ mL} \cdot \text{L}^{-1}$)	3.27	2.74 – 3.81	0.79	9.27	6.72 – 11.82	3.79
Propiscin ($1.0 \text{ mL} \cdot \text{L}^{-1}$)	4.50	3.71 – 5.29	1.18	8.49	6.90 – 10.07	2.36
Propiscin ($2.0 \text{ mL} \cdot \text{L}^{-1}$)	3.55	2.63 – 4.48	1.38	12.45	9.52 – 15.39	4.37

SD = standard deviation.

followed by loss of equilibrium. Rarely, increased ventilation as well as slightly increased heart rate (only in some individuals) observed during the first minutes of induction seem to have been just a reaction to external stimuli (i.e., pouring solution to the tank or movement within the fish's range of vision). Etomidate does not cause a raise in serum cortisol level, which is a characteristic of the stress response (Amend et al. 1982, Davis et al. 1982). Probably the anaesthetic blocks ACTH activity in mesonephron cells, preventing hyperglycaemia and hypochloraemia (Thomas and Robertson 1991). As a consequence it reduces agitation of fish in the first

stage of anaesthesia induction and facilitates gentle anaesthesia induction (Plumb et al. 1983, Limsuwan et al. 1983).

Our results confirmed observations of other researchers that induction and recovery caused by 2-phenoxyethanol are relatively short (Kouřil et al. 2001, Ortuño et al. 2002), probably due to rapid elimination from the tissues (Imamura-Kojima et al. 1987). In case of exposure to Propiscin, regaining of locomotor equilibrium is longer. Many authors (Amend et al. 1982, Gilderhus and Marking 1987, Hamáčková et al. 2001, Kouřil et al. 2001, Hajek and Kłyszczko 2004) found that full recovery after

Propiscin-induced anaesthesia was several times longer than anaesthesia caused by other agents. Hajek and Kłyszczko (2004) observed, that 7-min exposure of common carp to Propiscin ($2.0 \text{ mL} \cdot \text{L}^{-1}$) prolonged recovery to 31 min. Kouřil et al. (2001) compared 3 anaesthetics (Propiscin, 2-phenoxyethanol, and clove oil) and observed that the longest time of recovery from anaesthesia was characteristic for Propiscin. Also Hamáčková et al. (2001) discovered that Propiscin-exposed fish remained unconscious significantly longer than following exposure to other anaesthetics (2-phenoxyethanol and clove oil).

Both agents affected the heart rate in common carp. 2-phenoxyethanol caused the short-term increase of heart rate and then bradycardia. Similar observations were made by Yamamitsu and Itazawa (1988). During a 2-h exposure at concentrations 0.6 and $0.8 \text{ mL} \cdot \text{L}^{-1}$, 2-phenoxyethanol induced short-term tachycardia, followed by bradycardia. Heart rate monitoring during Propiscin anaesthesia revealed, that bradycardia was the only effect of etomidate. The gradual change of heart rate was correlated with the respiratory rate decrease indicating a mild course of anaesthesia. At exposure to $2.0 \text{ mL} \cdot \text{L}^{-1}$, heart rate dropped to extremely low values (ca. 15 beats per minute). A gradual increase back to the control values was observed not sooner than in the 7th min after transferring the fish to a clean water. The research on the higher vertebrates revealed etomidate not to cause functional heart disorders (Hughes and MacKenzie 1978, Mazerolles et al. 1996). Also in humans, etomidate dose of $0.2 \text{ mg} \cdot \text{kg}^{-1}$ body weight, used for induction of general anaesthesia, did not induce any functional disorders (Vercruyssen et al. 1976, Aroński et al. 1981, Nestorowicz et al. 1986).

Both, Propiscin and 2-phenoxyethanol, decreased respiratory rhythm in carp, especially when high concentrations were used. Similar observation was reported by Hajek and Kłyszczko (2004). The results also correspond with clinical research on humans. Morgan et al. (1977) in their research on etomidate and respiration observed a short-term depression combined with apnoea. Other authors also observed decreased respiratory rate in humans after etomidate injection (Nestorowicz et al. 1986, Rzymiski et al. 1991).

In fish, respiratory rate decrease, combined with bradycardia and diminished blood flow through the gills, results in hypoxia. The hypoxia may be made more severe by greater blood flow resistance, as erythrocytes swell and accumulate in the gills (Fromm et al. 1971). Hypoxia during narcosis is a result of depressive action of anaesthetics on blood circulation and respiration, and from reduced ability to assimilate oxygen, as the anaesthetics block cellular respiration processes (Holeton and Randall 1967, Fromm et al. 1971).

Forced water flow through the gills seems to be a noteworthy method of reducing the fish hypoxia during anaesthesia (Houston et al. 1971, Tytler and Hawkins 1981). The method can be especially valuable, when anaesthesia must be maintained for some time. Hajek and Kłyszczko

(2004) demonstrated, that forced water flow through the gills of Propiscin-anaesthetized carps immediately increased their heart rate. This might indicate, that the bradycardia observed during anaesthesia with Propiscin was not caused by the anaesthetic directly, but mainly by the cessation of water flow through the gills.

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