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PROPISCIN – A SAFE NEW ANAESTHETIC FOR FISH

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ABSTRACT. Anaesthetics are needed when handling fish, especially during tagging. However, most anaesthetics applied at present have a strong toxic effect on fish. For this reason it is only permissible to keep fish anaesthetized for a short time. A new anaesthetic, Propiscin, which allows fish to be anaesthetized for up to 0.5 h, has been successfully tested. It contains a 0.2% stabilized solution of etomidate and can be used as a bath. When administered correctly, the required disappearance of sense perception and motor reflexes in the fish can be obtained in about 2-4 min. The low toxicity of this pharmacological confection has been proved according to a full set of clinical, toxicological, hematological and biochemical criteria. Clinical tests have been conducted on with many fish species, mainly salmonids.

Key words: PROPISCIN, ANAESTHESIA, COMMON CARP, RAINBOW TROUT, OXYGEN CONSUMPTION

INTRODUCTION

Anaesthetics are one of the groups of pharmaceutical preparations. They have been used extensively in intensive fish culture to reduce the effects of stress on fish and to lower mortality after handling and transporting large stocks of fish. General anaesthetics have been applied for many years in fisheries. These agents not only allow handling for breeding or medical purposes to be achieved quickly and simply, but they also decrease stress in the fish during these procedures. General anaesthesia is induced by placing fish in water baths containing the anaesthetic which is absorbed through the gills and partly through the skin. Many anaesthetics are used, including chloroform, 2-phenoxyethanol, methylpentynol, urethane, MS-222, and clove oil. Numerous investigations, however, have shown that the majority of these are strongly toxic to fish, so that the period of general anaesthesia must be kept very short and this usually complicates handling. For instance, chloroform is a protoplasmic toxin, urethane has teratogenic and carcinogenic effects and induces enzymatic processes of pharmaceutical biotransformation, and MS-222 causes chemical stress and has a disadvantageous effect on eggs and sperm (Ball and Cowen 1959, Allison 1961, Crawford and Hulsey 1963, Smit et al. 1979a, b, Siwicki 1984). In Poland, yet another anaesthetic was used, Propanidid, which was considered to be a safe and very effec-

tive anaesthetic for use with fish, but it is no longer being produced (Siwicki 1984, Jeney et al. 1986, Veenstra et al. 1987).

Consequently new, less toxic and more effective preparations are required for inducing general anaesthesia in fish. A new drug, Propiscin, was developed at the Inland Fisheries Institute in Poland. The active ingredient is etomidate, which is commonly used to anaesthetize fish (Morgan et al. 1975, Amend et al. 1982, Escoubet 1982, Limsuwan 1982, Limsuwan et al. 1983, Plumb et al. 1983, Falls et al. 1988, Vermeer and Falls 1988). Propiscin has proved to be very successful, and can induce a short period of general anaesthesia which lasts about 30 min (Szkudlarek and Zakęś 1996, Trzebiatowski et al. 1996). It has all the advantages of general anaesthetic agents: it does not act depressively on the respiratory system; it causes a slight drop in blood pressure; it is safe and, possibly most important, it has not been found to be teratogenic or carcinogenic. Therefore, its application in a water bath to achieve general anaesthesia in fish was investigated. It is the manufacturer's intention that Propiscin be used exclusively on fish, and it has never been tested on humans.

MATERIAL AND METHODS

The objective of our study was to evaluate the anaesthetic potential of Propiscin. We used it with numerous fish species including common carp *Cyprinus carpio* L., grass carp *Ctenopharyngodon idella* (Val.), catfish *Clarias gariepinus* (Burchell), burbot *Lota lota* (L.), rainbow trout *Oncorhynchus mykiss* (Valbaum), huchen *Hucho hucho* (L.), brown trout *Salmo trutta m. fario* L., and grayling *Thymallus thymallus* (L.). The following investigations were conducted directly before Propiscin induced anaesthesia, in the fifteenth minute of anaesthesia, and 1, 2, 12 and 24 h after the fish had been roused from general anaesthesia:

- toxicology including an acute test of toxicity to determine LC_{50} for 15 minutes and 24 h to specify safe doses of the confection for rainbow trout at a temperature of 15°C;
- allergenic responses by, and irritation of, the fish tissues – in vitro study on common carp epithelial cells (EPC) and rainbow trout fibroblasts (RTG-2);
- the effect of the confection on basic water parameters: pH, quantity of dissolved oxygen (O_2), ammonium nitrogen ($N\text{-}NH_4^+$), nitrite nitrogen ($N\text{-}NO_2$), carbon dioxide (CO_2), oxidizing as well as on the oxygen consumption of common carp and rainbow trout during general anaesthesia;

- haematological parameters of rainbow trout blood including red blood cell count (RBC), hematocrit value (HCT) and hemoglobin level (Hb) with the calculation of average red blood cell volume, average hemoglobin contents and average hemoglobin concentration in cells in order to determine its effect on the ability of the blood to transport oxygen and carbon dioxide;
- the pH, pO_2, pCO_2, $pHCO_3^-$, total carbon dioxide (TCO_2) and free ion (H^+, K^+, Na^+) levels in the blood to illustrate the state of the acid-base balance in rainbow trout.

At each sampling time, 10 to 35 fish were used, depending on the needs. A total of 1600 fish of various species weighing from 0.5 to 5,000 g were tested.

Clinical observations of the general anaesthesia were also conducted. The behavioral responses of the fish to Propiscin were observed in order to determine the five major stages of anaesthesia, similar to those used by Schoettger and Julin (1967). The concentration of Propiscin was considered to be effective when the confection induced stage 3 anaesthesia within three minutes and maintained it for at least 30 minutes.

RESULTS AND DISCUSSION

The toxicological investigation to determine the preparation's lethal concentration for rainbow trout in a water bath indicated an LC_{50} of 1.203 and 0.266 ml l^{-1} a for a period of 15 min and 24 h, respectively. The determined safety index (LC_{50}/ED_{50}) of the 15 min long bath was 2.95. There was no allergenic response to Propiscin and no tissue irritation was observed during the in vitro study. The same was observed when etomidate was used (Amend et al. 1982).

When administered in a water bath, Propiscin induced general anaesthesia which was dependant on temperature, fish species and the length of time that fish were kept in the solution. The fish roused when transferred from the solution to clean water, and the shorter the time that they were kept in the Propiscin solution, the quicker they roused.

The effective concentrations of Propiscin were used as recommended doses. The course of the general anaesthesia induced by Propiscin doses are shown in Table 1, and clinical observations of the general anaesthesia are presented in Table 2. When used on other species of fish, a Propiscin concentration of about 0.5 ml l^{-1} should be administered, which is the equivalent of 1 mg of pure etomidate. This is less compared to the dosage required when using glycol-soluble etomidate (Amend et al. 1982, Limsuwan et al. 1983, Plumb et al. 1983).

TABLE 1

Course of general anaesthesia induced by the recommended doses of Propiscin in specific fish species

Species	Size	Water temperature (°C)	Propiscin dose (ml l^{-1})	Time from administration to anaesthesia (min)	Time of anaesthesia (min)	Maximum rousing time (min)
Rainbow trout	Spawners	7.0	0.5	2.5 - 3.0	15	60
	Spawners	7.0	1.0	1.0	15	90
	Commercial fish	4.5	1.0	0.5 - 1.0	10	30
	Commercial fish	4.5	0.5	0.75 - 1.5	10	30
Common carp	2 years old Fingerling	17.0	1.0	3.0	15	20 - 30
	Fingerling	19.0	1.0	1.0 - 1.5	15	20 - 25
Grass carp	Spawners	23.0	1.0	5.0	30	20
	Spawners	23.0	3.0	2.0	10	30
Brown trout	Commercial fish	7.0	0.5	2.5	15	60
	Commercial fish	7.0	1.0	1.0	15	90
Grayling	Spawners	7.0	0.5	2.5 - 3.0	15	60
	Spawners	7.0	1.0	1.0	15	90
Huchen	Spawners	5.0	1.0	2.5 - 3.5	5	40
	Spawners	5.0	1.5	2.0 - 2.5	5	40
	Spawners	4.5	1.0	2.0 - 2.5	30	80
Burbot	Spawners	5.0	0.5	2.5	15	120

In the doses applied in a water bath, Propiscin did not cause a change in pH or CO_2 content of the water, however, statistically significant differences in oxidizing were detected. Decreasing oxygen consumption by the anaesthetized fish was observed. The differences in the dissolved oxygen concentrations are presented in Figs. 1 and 2. During general anaesthesia and for 24 h following it, the blood parameters in the fish, such as RBC, HCT and Hb, did not change. In the blood, the pH, pO_2, pCO_2, $pHCO_3^-$, total carbon dioxide (TCO_2) and free ion (H^+, K^+, Na^+) levels also remained unchanged. During general anaesthesia, gaseous and metabolic acidosis occurred, but the return to the normal balance was quick, as early as 1 h after the fish roused.

The presented results show that Propiscin has little effect on the basic physiological processes of fish so that it can be regarded as a low-toxicity agent with negligible side effects. Since this preparation is well-tolerated by many fish species, Propiscin can be used in a water bath to anaesthetize fish. It has been observed that Propanidid, etomidate and metomidate produce similar anaesthetic effects in different fish spe-

TABLE 2

Clinical progression of general anaesthesia

	Period	Time course
1.	Tranquillity period	10-20 s
2.	Excitation period (stadium *excitationis*)	10-20 s
	- unrest	
	- accelerated movements of gills	
	- intensive defence reactions	
3.	General anaesthesia period (stadium *anaesthesiae*)	
	3.1. Shallow general anaesthesia	0.5-3 min
	- limited or lack of consciousness	
	- slow turning to one side	
	- no defence reflex	
	- no reaction to pricking	
	- regular movements of gills	
	3.2. Full general anaesthesia	50-60 min
	- lying on one side	
	- twitching of eyeballs	
	- regular movements of gills	
	- acoustic reflex	
4.	Suffocation period (stadium *asphyxiae*) which is the result of overdosing the injection or keeping the fish in a water bath too long, i.e. more than 1 h	
	- irregular and decreasing movements of gills	
	- no acoustic reflex	
	- excrement discharge	

cies (Amend et al. 1982, Limsuwan et al. 1983, Plumb et al. 1983, Siwicki 1984, Jeney et al. 1986, Veenstra et al. 1987, Mattson and Riple 1989).

Until recently, the general anaesthetics used, such as MS-222 and 2-phenoxyethanol, allowed the fish to be held for only a short time in the agent solution and did not cause full myorelaxation. Propiscin applied in a water bath can provide up to 1 h of general anaesthesia during which the fish may be handled freely. The lack of allergy and irritation allows repeated treatment of the fish with this confection. Similar effects were observed with Propanidid (Siwicki 1984).

However, some differences in the reaction and behavior of particular fish species during general anaesthesia were noted. Salmonids, for instance, become anaesthetized more rapidly than cyprinids do, but both cyprinids and salmonids rouse nearly at the same time. There was no difference in the rousing time when administering

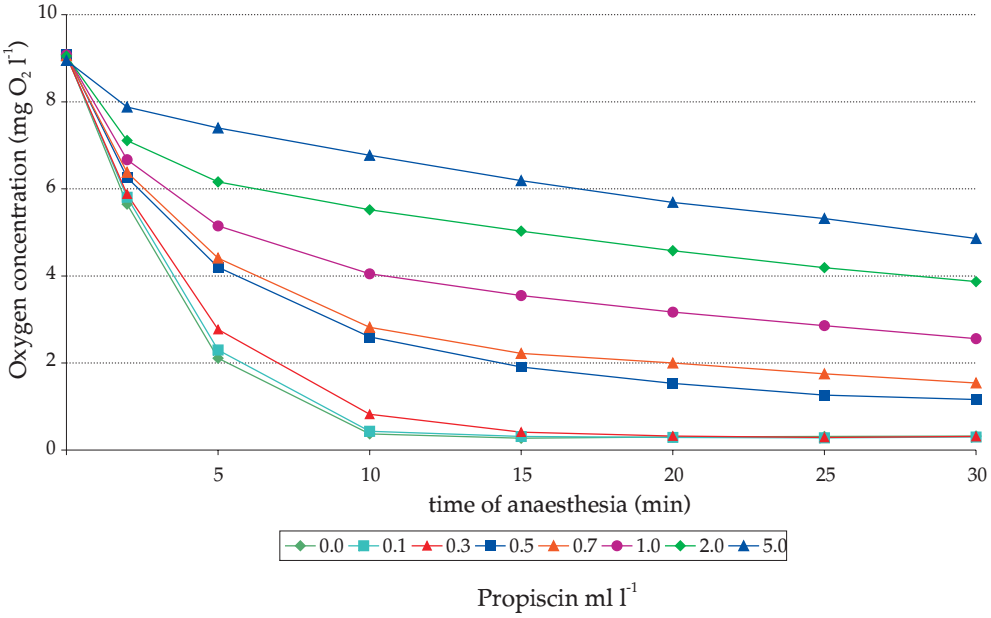

Fig. 1. Differences of the dissolved oxygen concentration during the application of various Propiscin doses with common carp.

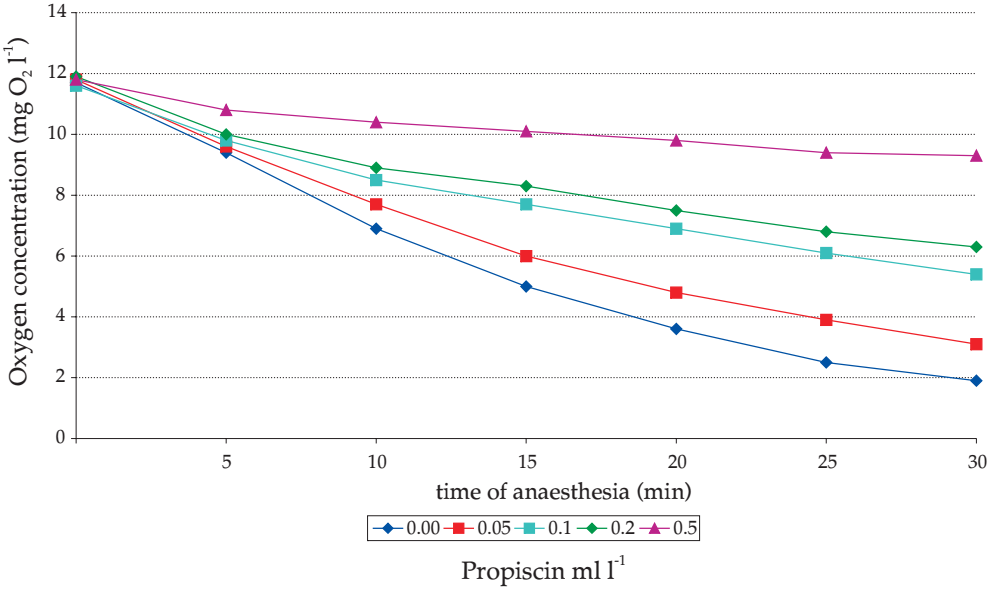

Fig. 2. Differences of the dissolved oxygen concentration during the application of various Propiscin doses with rainbow trout.

Propiscin or etomidate, but it is longer when using MS-222 or quinaldine (Amend et al. 1982, Limsuwan et al. 1983, Plumb et al. 1983).

The practical application of the Propiscin confection has also been tested during controlled spawning, during handling of fish at commercial farms and during tagging experiments. The results of our preliminary study suggested, that Propiscin is a lowly toxic, safe and very effective product for reducing the influence of polyethiological stress in intensive fish culture.

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STRESZCZENIE

PROPISCIN – NOWY BEZPIECZNY PREPARAT DO ZNIECZULENIA OGÓLNEGO RYB

Przeprowadzono szereg badań w celu określenia przydatności preparatu Propiscin do znieczulania ogólnego różnych gatunków ryb. Skontrolowano wpływ tego preparatu na parametry chemiczne wody oraz na wybrane parametry fizjologiczne ryb, z uwzględnieniem toksyczności. Na podstawie wyników obserwacji określono optymalne koncentracje preparatu do stosowania wobec krajowych ryb karpiowatych, łososiowatych i innych w różnych temperaturach wody. Przebieg znieczulenia ogólnego przedstawiono w tabeli 1, obserwowane okresy kliniczne – w tabeli 2. Określane tempo zużycia tlenu przez karpia (*Cyprinus carpio*) i pstrąga tęczowego (*Oncorhynchus mykiss*) przedstawiono odpowiednio na wykresach 1 i 2.

Uzyskane wyniki badań, jak również obserwowana u ryb pełna miorelaksacja, w połączeniu z niską szkodliwością preparatu i łatwością jego stosowania, wskazują na pełną przydatność Propiscinu do znieczulania ogólnego różnych gatunków ryb.

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