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Katedra anatomie a fyziologie hospodářských zvířat

DISERTAČNÍ PRÁCE

Toxikologie ryb a monitoring cizorodých látek

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Prohlášení

Prohlašuji, že jsem disertační práci vypracoval samostatně na základě vlastních zjištění a za pomoci uvedené literatury.

J. Velíšek

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děkování

Rád bych poděkoval své školitelce prof. MVDr. Zdeňce Svobodové, DrSc. za skvělé vedení, ktické rady a bohaté zkušenosti, které mi předala během realizace jednotlivých částí ertační práce. Rovněž bych chtěl poděkovat Výzkumnému ústavu rybářskému a lrobiologickému ve Vodňanech za umožnění realizace této disertační práce. Především pak oucímu Oddělení vodní toxikologie a nemocí ryb Ing. Tomáši Randákovi a všem ostatním covníkům tohoto oddělení, jejichž pomoci si velice vážím. Zvláštní dík patří prof. dr. Terese asow a dr. wet. Piotru Gomulkovi za spolupráci a pomoc při stanovení vlivu anestetik bíčkového oleje a 2-phenoxyethanolu na sumce velkého a stanovení toxického vlivu ethroidního přípravku Alimetrin 10 EC na pstruha duhového. V neposlední řadě bych chtěl ěkovat vedení a sekretariátu Katedry anatomie a fyziologie hospodářských zvířat ZF JU v kých Budějovicích za pomoc poskytnutou v průběhu studia.

SAH **strana**

UHRN

Předkládaná práce je zaměřena na toxikokinetický vliv anestetik 2-phenoxyethanolu a bíčkového oleje na ryby. Dále je pozornost věnována hodnocení toxických účinků ethroidů na ryby a monitoringu zvýšených koncentrací dusitanů ve vodě a jejich vlivu na y.

xikokinetický vliv anestetik na ryby

Hodnocení toxikokinetického vlivu anestetik (2-phenoxyethanolu a hřebíčkového oleje) bylo vedeno u tří důležitých druhů ryb [kapr obecný (*Cyprinus carpio* L.), pstruh duhový *ncorhynchus mykiss*) a sumec velký (*Silurus glanis* L.)] na základě stanovení letálních ncentrací a vlivu anestetik na hematologický a biochemický profil krve a histologického etření tkání.

Zjištěné výsledky ukázaly, že anestetika 2-phenoxyethanol a hřebíčkový olej jsou bezpečná anestézii kapra obecného (*Cyprinus carpio* L.), pstruha duhového (*Oncorhynchus mykiss*) a nce velkého (*Silurus glanis* L.).

xický vliv pyrethroidů na ryby

Hodnocení toxického vlivu pyrethroidů (Decis Flow 2,5; Decis EC 50 a Alimethrin 10 EC) o provedeno u dvou druhů ryb [kapr obecný (*Cyprinus carpio* L.) a pstruh duhový *ncorhynchus mykiss*)] na základě stanovení letálních koncentrací a vlivu pyrethroidů na matologický a biochemický profil krve a histologického vyšetření tkání.

Na základě zjištěných hodnot 96hLC50 byly přípravky Decis Flow 2,5; Decis EC 50 a methrin 10 EC zařazeny do skupiny látek vysoce toxických pro ryby.

onitoring zvýšených koncentrací dusitanů ve vodě a jejich vliv na ryby

Cílem kapitoly bylo zhodnotit vliv dusitanů na hematologický a biochemický profil krve a tologický stav žaber a pomocí hodnot těchto parametrů zhodnotit míru ochrany, kterou oridy mohou poskytnout kapru obecnému (*Cyprinus carpio* L.) proti jedovatým účinkům sitanů.

Výsledky našich pozorování potvrdily, že zvýšené koncentrace dusitanů s nízkými ncentracemi chloridů ve vodě mohou způsobit značné změny v hematologických ukazatelích. ochemické ukazatele v krvi ryb nebyly ovlivněny. Hlavní makroskopické a histologické ěny byly pozorovány na žábrách ryb. Méně změn ve všech stanovených parametrech bylo zorováno u ryb vystavených dusitanům s vyšší koncentrací chloridů ve vodě. To potvrdilo zor, že vyšší koncentrace chloridů ve vodě pozitivně ovlivnily rezistenci ryb k dusitanům.

MMARY

The present study was focused on the toxicokinetic effect of anaesthetics 2-phenoxyethanol l clove oil on fish. Futher attentions paid to the presentation of toxic effects of pyrethroids on ı and monitoring occurrence increased nitrite concentrations in water and their effect on fish.

e toxicokinetic effects of anaesthetics on fish

The assessment of the toxicokinetic effect of anaesthetics (2-phenoxyethanol and clove oil) ; performed on three important fish species [common carp (*Cyprinus carpio* L.), rainbow ıt (*Oncorhynchus mykiss*) and European catfish (*Silurus glanis* L.)] on the basis of the ermination of lethal concentrations and the effects of anaesthetics on haematological and chemical blood profile, and histological examination of tissues.

Results of the examinations suggest that the use of anaesthetics (2-phenoxyethanol and clove is safe for common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss*) and opean catfish (*Silurus glanis*), too.

toxic effect of pyrethroids on fish

The assessment of the toxic effect of pyrethroids preparations (Decis Flow 2.5, Decis EC 50, Alimethrin 10 EC), was performed on two important fish species [common carp (*Cyprinus pio* L.) and rainbow trout (*Oncorhynchus mykiss*)] on the basis of the determination of lethal centrations and effects of pyrethroid preparations on haematological and biochemical blood file, and histological examination of tissues.

The pesticide preparations Decis Flow 2.5, Decis EC 50 and Alimethrin 10 EC were classified ighly toxic for fish.

nitoring of increased concentration of nitrite in water and their effect on fish

The aim of the study was to assess the effects of nitrite on haematological and biochemical file of blood, and histological picture of the gills, and, using these parameters values, to luate the protection that chloride may provide for common carp (*Cyprinus carpio* L.) from c effects of nitrite.

The results of our observation confirmed that elevated nitrite concentrations at low chloride centrations in water might cause marked changes in heamatological indices. The biochemical ces measured in the study reported here, however, were not affected. Major macroscopic and ological changes were observed on fish gills. Less marked changes in all the indices stigated were observed in fish exposed to nitrite when higher chloride concentrations in er were used. This carroborates the assumption that elevated chloride concentrations in water tively influence fish resistance of nitrite.

1. ÚVOD

Ryby představují největší a také nejvýznamnější skupinu obratlovců žijících ve vodním prostředí. Jsou zde konečným článkem potravního řetězce a současně hospodářsky významnými vodními organismy. Snad právě proto je prvořadá pozornost vodní toxikologie věnována rybám.

Rybářství je jednou ze specializovaných složek živočišné výroby, zaměřené na různě intenzivní využívání přirozených nebo hospodářským úsilím vytvořených zásob ryb a ostatních vodních organismů k přímé nebo nepřímé spotřebě člověkem. Během vývoje se vyvinulo v záměrnou, vysoce organizovanou činnost.

Intenzifikace výroby s sebou téměř vždy přináší řadu problémů a negativních průvodních jevů. Jsou to problémy technického rázu, ale hlavně potíže ekologického charakteru, neboť intenzifikační faktory většinou negativně ovlivňují životní prostředí. Dochází k porušení rovnováhy v ekosystému. S narušením těchto systémů, jejichž nedílnou součástí je také vodní prostředí, pak velmi úzce souvisí zdravotní stav rybích obsádek.

Předkládaná disertační práce „Toxikologie ryb a monitoring cizorodých látek" si neklade za cíl postihnout celou šíři změn ve vodním prostředí. Omezuje se především na výběr vhodného anestetika pro ryby, změny vyvolané dusitany na rybách v recirkulačních systémech a posouzení vlivu insekticidních přípravků na bázi pyrethroidů na ryby.

Disertační práce je předkládána jako soubor publikovaných a z části též nepublikovaných prací. Nepublikované práce byly zaslány do redakcí příslušných časopisů, prošly kladným oponentním řízením a podle připomínek oponentů byly připraveny do tisku nebo jsou připravovány k publikování.

Tématicky je práce rozdělena do 3 následujících okruhů:

- Toxikokinetický vliv anestetik na ryby
- Toxický vliv pyrethroidů na ryby
- Monitoring zvýšených koncentrací dusitanů ve vodě a jejich vliv na ryby

. TOXIKOKINETICKÝ VLIV ANESTETIK NA RYBY

Jedním z významných požadavků ochrany zdraví ryb z hlediska zdravotního stavu i podle ožadavků Zákona na ochranu zvířat proti týrání (č. 246/1992 Sb.) je zabránit nešetrné anipulaci a následnému mechanickému poškození ryb. Intenzita mechanického poškození ůže být různá, od poranění slizové vrstvy až po hluboké rány ve svalovině, na ploutvích, řípadně poškození žaber. Přestože u ryb existuje velká schopnost regenerace žaber, ploutví, le i ostatních tkání, nelze působení mechanických vlivů podceňovat. Velkou pozornost je eba věnovat šetrnému a odbornému zacházení s generačními rybami, zejména při umělém ýtěru. Jde o velmi cenné a v té době velmi citlivé ryby, se kterými se ve výtěrové sezóně pakovaně manipuluje. Rovněž při značkování nebo při veterinárních zákrocích (aplikace ormonálních přípravků, léčiv, odběr krve, mechanické odstraňování parazitů atd.) je častou řekážkou velká pohyblivost ryb, která může mít za následek poranění a následnou infekci. by se zabránilo mechanickému poranění, doporučuje se před vlastní manipulací provést ehybnění ryb pomocí anestetik (Čítek et al. 1997; Mundey a Wilson 1997; Ross a Ross 999).

Anestézie není pouze součástí prevence mechanického poškození, ale je především oučástí prevence manipulačního stresu. V první fázi odpovědi organismu na působení anipulačního stresu se objevují endokrinní změny (primární reakce). Ty se dále uplatňují při zení organismu a způsobují metabolické, osmotické a další změny (sekundární reakce), které edou ke snížení nespecifické odolnosti a následně ke zhoršení zdravotního stavu ryb. ředevším reprodukce představuje v životním cyklu všech druhů ryb období, ve kterém ochází k vysokému zatížení organismu, v mnoha aspektech charakteru stresu s následnými egativními účinky (Svoboda a Kolářová 1999).

Při zákrocích na akvarijních rybách, ať již při hormonální stimulaci reprodukce, při likaci léčiv sondou nebo injekčně, je velké nebezpečí mechanického poškození, anipulačního stresu a následného zhoršení zdravotního stavu někdy velmi cenných ryb.

nestézie se uplatňuje také při přepravě ryb, kdy snižuje reakci ryb na vnější podněty a labuje metabolické procesy. To má za následek pokles spotřeby kyslíku a menší hromadění nečných produktů metabolismu (oxid uhličitý, amoniak) ve vodě. To dává možnost zvýšení apacity přepravovaných ryb. Při tomto použití anestetik vzniká nebezpečí, které nastává při lném zklidnění ryb. Tyto ryby klesnou na dno, kde se mohou nahromadit na sebe a udusit se Laise a Vine 1998).

K anestézii ryb byla postupem doby používána celá řada různých látek. Nejprve to bylo užití látek ovlivňujících nervovou činnost člověka, např. etylalkohol, který nemá na ryby statečný účinek, nebo éter, který je velmi těkavý a ohrožuje uživatele snadnou zápalností i ímým účinkem par. Bylo upuštěno od používání uretanu, chlorbutanolu, tribrometanolu a o náročnost na technické zařízení i od elektronarkózy. Všechny vyjmenované způsoby byly ekonány speciálním narkotikem pro poikilotermní obratlovce švýcarské firmy SANDOZ, ámým pod jménem Sandoz MS 222. Tento přípravek jako první splňoval všeobecné žadavky na moderní narkotikum. V posledním období se v rybářské praxi začal velmi enzivně používat 2-phenoxyethanol a hřebíčkový olej jako anestetikum.

lem prací předkládaných v této kapitole je:

zhodnotit pomocí výsledků letálních koncentrací, hematologického, biochemického profilu krevní plazmy a histologického vyšetření tkání vliv anestetik 2-phenoxyethanolu a hřebíčkového oleje na kapra obecného (*Cyprinus carpio* L.), pstruha duhového (*Onchorhynchus mykiss*) a sumce velkého (*Silurus glanis* L.).

výsledky vlivu anestetik na ryby jsou příspěvkem k registraci těchto přípravků v České republice.

Prezentace výsledků:

Vliv anestetik 2-phenoxyethanolu a hřebíčkového oleje na ryby byl prezentován na 4. a 5. konferenci: Využití doplňkové a nekonvenční péče o zdraví zvířat v Českých Budějovicích a na 12th International Conference „Diseases of Fish and Shellfish" Copenhagen, Dánsko.

Výsledky vlivu anestetik na ryby byly publikovány v časopisech Acta Vet. Brno, Vet. Med-Czech, ve sbornících 4. a 5. konference „Využití doplňkové a nekonvenční péče o zdraví zvířat", JU České Budějovice a ve sborníku abstraktů z 12. mezinárodní konference EAFP.

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Anaesthesia of Common Carp (*Cyprinus carpio* L.) with 2-phenoxyethanol: Acute Toxicity and Effects on Biochemical Blood Profile

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Abstract

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The aim of the present study was to assess acute toxicity of the 2-phenoxyethanol anaesthetic in carp, and assess changes in their tissues using the biochemical blood profiles. Acute toxicity values of 2-phenoxyethanol in the carp fry determined by the OECD 203 method "Acute toxicity test in fish" were 10minLC50 0.39; 10minLC0.1 0.30; 10minLC99.9 0.50 $ml \cdot l^{-1}$, 96hLC50 0.17; 96hLC0.1 0.13; and 96hLC99.9 0.26 $ml \cdot l^{-1}$.

A biochemical analysis of blood plasma (VETTEST 8008 analyser, Medisoft Co.) was used to determine changes in the biochemical profile of blood in carp stockfish due to anaesthesia by 2-phenoxyethanol. The analyses were made immediately prior the anaesthesia and after a 10 min and 24 h exposure to the anaesthetic at a concentration of 0.30 $ml \cdot l^{-1}$. A total of 30 two-year-old carp were examined. A significant increase in blood plasma alanine aminotransferase (ALT) levels from 0.15 $\mu kat \cdot l^{-1}$ to 0.35 $\mu kat \cdot l^{-1}$ ($P < 0.05$) was found 24 h after anaesthesia. The other indices monitored [cortisol, glucose (GLU), total proteins (TP), triglycerides (TRIG), ammonia (NH3), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and creatine kinase (CK)] showed no significant differences among the groups of fish examined.

The results showed that 2-phenoxyethanol at a concentration of 0.30 $ml \cdot l^{-1}$ is not completely safe for carp fry and two-year old carp. In view of different sensitivities in different age group mentioned above, the authors assume that concentration will be safe for broodstock carp.

LC50, blood plasma, cortisol, glucose, proteins, enzymes

The anaesthetic 2-phenoxyethanol is used not only for short-term immobilization of fish before artificial propagation, but also whenever fish is handled outside water, e.g. during veterinary interventions. The generally recommended concentration is 0.20 $ml \cdot l^{-1}$ water bath. For large brood fish (e.g. wels), the concentration of 0.30 $ml \cdot l^{-1}$ water bath is recommended. At the recommended concentrations, anaesthesia takes effect within 5 - 10 min. When the fish are transferred to clean water, their physiological reflexes are restored in 10 min.

Modern anaesthetics are expected to be highly soluble in water, quick to take effect, safe for both fish and humans, with wide margins of safety, offer the possibility to increase the degree of anaesthesia as required and to spontaneously recover from anaesthesia. Also, modern anaesthetics should leave no residues in fish (Brožová and Svobodová 1986; Brown 1988; Ross and Ross 1999). The effect of 2-phenoxyethanol on carp was studied by Adámek et al. (1993).

At present, 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, effects of 2-phenoxyethanol on rainbow

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trout were studied (Velíšek and Svobodová 2004). The aim of the present study was to assess acute toxicity of 2-phenoxyethanol in anaesthetized carp and changes in their tissues using biochemical blood profile values.

Materials and Methods

2-phenoxyethanol characteristics

The active substance of 2-phenoxyethanol is ethylene glycol monophenyl ether. Its summary formula is $C_8H_{10}O_2$, the molar weight 138.17 $g \cdot l^{-1}$, 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 $g \cdot l^{-1}$) but readily soluble in ethanol. The anaesthetic affects fish through skin and gills.

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

Acute toxicity test

Carp fry (mirror carp M 72) of 21.9 g mean body mass (15.7 - 28.1 g) and 11.1 cm mean standard length (10.2 - 12 cm), Fulton index 1.6 (1.5 – 1.7), were used to ascertain LC50 of 2-phenoxyethanol after 96 h and 10 min exposures to the anaesthetic.

In the test to establish the 96-hour LC50 of 2-phenoxyethanol, fish were exposed to 2-phenoxyethanol concentrations of 0.10; 0.12; 0.15; 0.18; 0.20; 0.25; 0.30; and 0.35 $ml \cdot l^{-1}$ in diluting water (pH 7.81; acid neutralization capacity – $ANC_{4.5}$ 1.15 $mmol \cdot l^{-1}$; total ammonia 0.04 $mg \cdot l^{-1}$; $N0_3^-$ 11.5 $mg \cdot l^{-1}$; NO_2^- 0.005 $mg \cdot l^{-1}$; $PO4_3^-$ 0.01 $mg \cdot l^{-1}$; chemical oxygen demand - CODMn 1.6 $mg \cdot l^{-1}$), and the controls were exposed to diluting water without the substance tested. Ten carp fry were used per every concentration and for a control group; a total of 90 carp fry were used. The state and behaviour of the fish, as well as temperature, pH and oxygen saturation of water at each concentration and in the control aquarium were checked throughout the test. Medium lethal concentrations of 2-phenoxyethanol for 96 hour exposure (96hLC50) as well as 96hLC0.1 and 96hLC99.9 were computed from those values using the probit analysis (EKO-TOX 5.1 software).

In the test to determine the LC50 in a 10 min exposure, concentrations of 0.30; 0.35; 0.40; 0.50 $ml \cdot l^{-1}$ 2-phenoxyethanol dissolved in water were used. A total of 40 carp fry were used, ten per each concentration. The diluting water had the same characteristics as water used in the 96hLC assay. Changes in the physiological status of the fish, the number of dead fish and the recovery time after the fish were transferred to clean water were recorded. Medium lethal concentrations for the 10 min exposure (10minLC50, 10minLC0.1 and 10minLC99.9) were computed from the values using the probit analysis (EKO-TOX 5.1 software).

The following four stages were observed in the changing physiological status of the anaesthetized fish (Thienpoint and Niemegeers 1965):

1. an increase and a subsequent decrease in the frequency of respiratory movements, a partial inhibition of reactions to external stimuli
2. loss of balance, very slow respiratory movements, fish still react to strong stimuli
3. total loss of reflexes, fish lay on bottom of the tank, irregular respiration, fish do not react to handling
4. respiratory movements cease completely, fish die if left in the bath.

Biochemical examination of blood plasma

Carp stockfish (mirror carp M 72) of 367 g mean body mass (227 - 507 g) and 25.5 cm mean standard length (19 - 32 cm), Fulton index 2.21 (1.58 – 3.30), were used for the determination of biochemical profile of blood plasma. A total of 30 carp were used in the examinations before anaesthesia (10 carp), after 10-min exposure to the anaesthetic (10 specimens) and 24 h exposure to anaesthetic (10 specimens).

The anaesthetic was administered at the concentration of 0.30 $ml \cdot l^{-1}$ 2-phenoxyethanol for 10 min. The blood was collected by a heparinized needle from the heart (after the fish were stunned by a blow to the head). Aqueous solution of heparin sodium salt was used for blood stabilization (Heparin SPOFA inj.) at a concentration of 5000 i.u. sodium salt heparin/ml. To stabilize 1 ml blood, 0.01 ml of aqueous solution of heparin was used (Svobodová et al. 1986).

Blood samples were centrifuged in a cooled centrifuge (4 °C, 837 × g). The biochemical indices determined in blood plasma included cortisol, glucose (GLU), total proteins (TP), ammonia (NH_3), triglycerides (TRIG), aspartate aminotransferase (AST), alanine aminotranspherase (ALT), alkaline phosphatase (ALP), and creatine kinase (CK). For the creatine kinase determination, blood plasma was diluted at the 1:12 ratio with physiological saline. For the biochemical analysis of blood plasma, VETTEST 8008 analyser (IDEXX Laboratories Inc. U.S.A.) was used. The analyzer utilizes dry chemical technology and a colorimetric reaction. The sample analysis was performed on selective testing discs (Multi–layer film slides, Kodak) by means of laser reading the bar codes.

For the statistical test, the analysis of variance was used (ANOVA – Tuckey Test).

Results

Acute toxicity of 2-phenoxyethanol

In the LC50 tests of 96-hour exposure to 2-phenoxyethanol, water temperature, pH and oxygen saturation ranges were 18.3-19.6 °C, 7.48-8.24, and 69-103%, respectively. Based on

Table 1
The effect of carp anaesthesia with 2-phenoxyethanol on blood plasma indices

Indices	Control x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 h after anaesthesia x ± SD (n = 10)
Kortizol (μmol·l^{-1})	667 ± 13^{a}	668 ± 15^{a}	670 ± 21^{a}
GLU (μmol·l^{-1})	5.55 ± 0.31^{a}	5.61 ± 0.28^{a}	5.8 ± 0.58^{a}
TP (g.l^{-1})	34.4 ± 0.78^{a}	34.5 ± 0.90^{a}	35.8 ± 1.48^{a}
NH_3 (μmol·l^{-1})	524 ± 2.03^{a}	508.3 ± 2.21^{a}	501 ± 1.70^{a}
TRIG (μmol·l^{-1})	0.50 ± 0.09^{a}	0.50 ± 0.10^{a}	0.47 ± 0.08^{a}
AST (μkat·l^{-1})	2.04 ± 0.02^{a}	2.05 ± 0.02^{a}	2.09 ± 0.03^{a}
ALT (μkat·l^{-1})	0.15 ± 0.01^{a}	0.19 ± 0.01^{a}	0.35 ± 0.03^{b}
ALP (μkat·l^{-1})	0.20 ± 0.02^{a}	0.21 ± 0.03^{a}	0.19 ± 0.02^{a}
CK (μkat·l^{-1})	162.9 ± 0.35^{a}	161.9 ± 0.72^{a}	163.1 ± 0.53^{a}

Note: 1 For the determination of CK, blood plasma at 1:12 dilution with physiological saline was used. Groups with different alphabetic superscripts differ significantly at $p < 0.05$ (ANOVA).

the results of the tests, lethal concentrations of 2-phenoxyethanol in carp at 96-hour exposure to the anaesthetic are 96hLC50 0.17 ml·l^{-1}; 96hLC0.1 0.13 ml·l^{-1}; 96hLC99.9 0.26 ml·l^{-1}.

A *post mortem* examination of the fish performed after the acute toxicity tests showed increased presence of aqueous mucous. Gills were dark in colour and bled readily when injured. The body cavity contained excess moisture, and an increased injection of visceral vessels was also observed. These results indicated that the fish became more sensitive to mechanical injury after 2-phenoxyethanol exposure.

In the LC50 tests of 10 minute exposure to 2-phenoxyethanol, water temperature, pH and oxygen saturation were 20 °C, 7.65, and 95%, respectively. The effect of 2-phenoxyethanol concentrations on the onset of individual phases of anaesthesia and recovery is shown in Fig. 1. Based on the results of the acute 2-phenoxyethanol toxicity test on carp, lethal concentrations at 10 min exposure to the anaesthetic are 10minLC50 0.39 ml·l^{-1}, 10minLC0.1 0.30 ml·l^{-1} and 10minLC99.9 0.50 ml·l^{-1}.

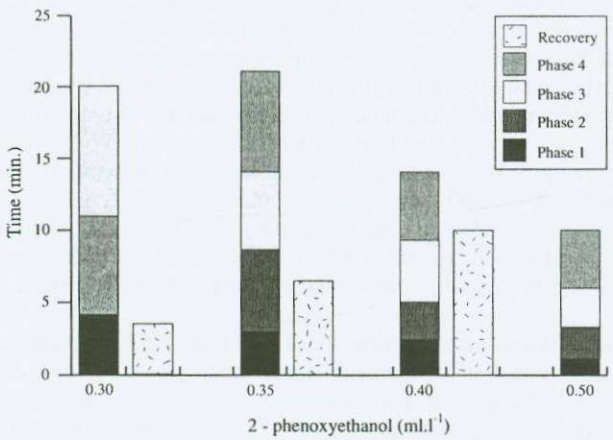

Fig. 1. The effect of 2-phenoxyethanol concentrations on the onset of individual phases of anaesthesia and recovery in carp

Biochemical profile of blood plasma after exposure to 2-phenoxyethanol

Effects of 2-phenoxyethanol exposure on the biochemical profile of blood plasma in the carp are shown in Table 1. At 0.30 $ml \cdot l^{-1}$ concentration of 2-phenoxyethanol and 10 min exposure, the concentration of alanine aminotrasferase (ALT) increased from 0.15 ± 0.01 $\mu kat \cdot l^{-1}$ to 0.35 ± 0.03 $\mu kat \cdot l^{-1}$ within 24 h post anaesthesia. The difference is significant at $p < 0.05$ level. Other indices (cortisol, GLU, TP, TRIG, NH_3, ALP, AST, CK) did not differ significantly among the groups.

Discussion

The 96LC50 value of 0.17 $ml \cdot l^{-1}$ ranks 2-phenoxyethanol among relatively harmless substances. In our experiments, fish were exposed to the anaesthetic for 10 min only. The 10minLC50 value in carp was roughly two times higher than the 96hLC50 value. The therapeutic index (i.e. the ratio between the therapeutic and the lethal concentration) was, however, very low (0.39: 0.30). Although the 10minLC0.1 value found in the present study (0.30 $ml \cdot l^{-1}$) is higher than the recommended concentration for anaesthesia purposes, it is important that procedures recommended for the administration of the anaesthetic be adhered to meticulously in order to prevent injury to anaesthetized carp. The 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 (Svobodová et al. 1987).

The most important factor affecting the efficiency of 2-phenoxyethanol for fish, however, is water temperature: the higher the temperature, the higher the efficiency of the anaesthetic for fish. Repeated use of 2-phenoxyethanol increases the tolerance of the fish 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 Fig. 1 that the onset of individual phases of anaesthesia and recovery times depended on 2-phenoxyethanol concentrations used. At the 0.50 $ml \cdot l^{-1}$ concentration, Phase 4 began 10 min after the administration of the anaesthetic and was followed by the death of the fish. The same effect of anaesthetic concentration on to the onset of anaesthesia was described by Myszkowski et al. (2002) and Weyl et al. (1996).

In the study of the carp, lower 2-phenoxyethanol concentrations of 96hLC50 (0.1; 99.9) and 10minLC50 (0.1; 99.9) were found than in the study with rainbow trout (Velíšek and Svobodová 2004), although the two studies were performed under identical conditions with the exception of water temperature. It might be hypothesized that toxicity of an anaesthetic administered to carp at artificial spawning or when providing veterinary care at temperatures above 20 °C may be even slightly higher.

The biochemical profile of blood may be a source of important information on the organism's internal environment (Masopust 2000). Anaesthetizing carp with 2-phenoxyethanol at 0.30 $ml \cdot l^{-1}$ had no effect on the values of stress factors (cortisol, glucose) in blood plasma. Hseu et al. (2000), however, described increased cortisol concentrations during anaesthesia of sea bass with 2-phenoxyethanol at a concentration of 400 ppm.

With the exception of ALT, no other biochemical indices examined (TP, TRIG, NH_3, ALP, AST, CK) showed any significant differences between carp before and after anaesthesia. Increased levels of blood plasma ALT in carp 24 h post anaesthesia with 2-phenoxyethanol at a concentration of 0.30 $ml \cdot l^{-1}$ testify to parenchymatous tissue damage.

To our knowledge, no other data on biochemical blood profile in carp anaesthetized with 2-phenoxyethanol are available in literature. In a study of rainbow trout anaesthetized with

0.30 ml·l^{-1} 2-phenoxyethanol, however, no significant negative effects of anaesthesia on parenchymatous tissues were found (Velíšek and Svobodová 2004a).

Adámek et al. (1993) studied effects of different concentrations of 2-phenoxyethanol on haematological parameters of carp fry, and found a decrease in blood pH at the 0.25 ml·l^{-1}, and an increase in erythrocyte and haemoglobin levels at the 0.56 ml·l^{-1} concentration of 2-phenoxyethanol anaesthesia.

McCartes (1992) reported no negative effects of anaesthesia with 0.20 ml·l^{-1} 2-phenoxyethanol on spermatozoa and their motility in grass carp (*Ctenopharyngodon idella*) and silver carp (*Hypophthalmichthys molitrix*).

The 2-phenoxyethanol concentration recommended by the authors for carp broodstock anaesthesia is 0.30 ml·l^{-1}, which has also been recommended by Kaiser and Vine (1998) for silver crucian carp.

When 2-phenoxyethanol is used, it is necessary to strictly observe safety regulations because 2-phenoxyethanol is toxic and dangerous for humans, and in poorly ventilated rooms, it may cause fatigue and drowsiness (Svoboda and Kolářová 1999).

Anestezie kapra obecného (*Cyprinus carpio* L.) 2-phenoxyethanolem: akutní toxicita a vliv na biochemický profil krve

Cílem práce bylo posoudit akutní toxicitu a pomocí hodnot biochemického profilu krve zhodnotit změny ve tkáních kapra obecného po působení anestetika 2-phenoxyethanol. Ke stanovení akutní toxicity byla použita metoda OECD 203 „Test akutní toxicity na rybách". Zjištěné hodnoty akutní toxicity 2-phenoxyethanolu pro plůdek kapra obecného byly následující 10minLC50 0.39; 10minLC0.1 0.30; 10minLC99.9 0.50 ml.·l^{-1}; 96hLC50 0.17; 96hLC0.1 0.13; 96hLC99.9 0.26 ml·l^{-1}.

Stanovení změn biochemického profilu krve bylo provedeno vyšetřením krevní plazmy na analyzátoru VETTEST 8008 firmy Medisoft. Biochemický profil krve byl hodnocen u násady kapra obecného před, ihned po 10minutové anestézii a 24 hodin po anestézii v koncentraci 0.30 ml·l^{-1} 2-phenoxyethanolu. Celkem bylo vyšetřeno 30 kusů ryb K_2. Významné zvýšení alanin aminotransferázy (ALT) v krevní plazmě bylo zaznamenáno 24 hodin po anestézii z hodnoty 0.15 μkat·l^{-1} na 0.35 μkat·l^{-1} ($p < 0.05$). U ostatních sledovaných ukazatelů [(kortizol, glukóza (GLU), celkové bílkoviny (TP), triglyceridy (TRIG), amoniak (NH_3), alkalická fosfatáza (ALP), aspartát aminotransferáza (AST), kreatinkináza (CK)] nebyly zjištěny rozdíly mezi posuzovanými skupinami.
Výsledky ukázaly, že 2-phenoxyethanol v koncentraci 0.30 ml·l^{-1} pro plůdek a dvouletého kapra obecného není zcela bezpečná. Vzhledem k diskutované rozdílné věkové citlivosti předpokládáme bezpečnost této koncentrace pro generační kapry.

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Anaesthesia of Rainbow Trout *(Oncorhynchus mykiss)* with 2-phenoxyethanol: Acute Toxicity and Biochemical Blood Profile

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Abstract

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The aim of the present study was to assess acute toxicity of 2-phenoxyethanol in rainbow trout, and to assess changes in their tissues using biochemical blood profile indices. Acute toxicity values of 2-phenoxyethanol for six-month old rainbow trout ascertained by the OECD 203 „Test of Acute Toxicity for Fish" were 0.46 (10minLC50), 0.34 (10minLC0.1), 0.92 ml·l^{-1} (10minLC99.9), 0.25 (96hLC50), 0.21 (96hLC0.1) and 0.30 ml·l^{-1} (96hLC99.9).

Changes in the biochemical blood profile were determined by the biochemical blood plasma analysis using VETTEST 8008 analyser (Medisoft Co.). Biochemical blood profile of rainbow trout was evaluated before anaesthesia with 2-phenoxyethanol at a concentration of 0.30 ml·l^{-1}, immediately after a 10-min anaesthesia and 24 h after anaesthesia. A total of 30 fish were examined. While blood plasma glucose (GLU) concentrations immediately after anaesthesia and total proteins (TP), albumins (ALB) and total globulins (GLOB) 24 h after anaesthesia showed a significant increase, asparate aminotransferase (AST) concentrations immediately after anaesthesia showed a significant decrease. All the changes were significant at the $p < 0.05$ level. In other indices [triglycerides (TRIG), cholesterol (CHOL), ammonia (NH_3), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca^{2+}), anorganic phosphate (PHOS)], no differences between the groups studied were found.

The recommended anaesthetic concentration of 0.30 ml·l^{-1} 2-phenoxyethanol can be considered safe for rainbow trout.

LC50, blood plasma, glucose, proteins, enzymes, minerals

One of important pre-requisites for the protection of fish health and for compliance with the Animal Welfare Act 246/1992 Coll. (in the Czech code of laws) is to provide for safe handling of fish and for the prevention of fish injury (damage to the mucosa, deep muscle injuries, gill injuries).

Much attention should be paid to careful and proper handling of brood fish, especially during artificial spawning procedures when fish are handled repeatedly at the time when they are particularly sensitive to mechanical injury. The use of anaesthetics should help prevent mechanical injury to fish, and also facilitate the handling of big fish during veterinary procedures (Čítek et al. 1997; Ross and Ross 1999).

Modern fish anaesthetics must meet a number of general requirements, e.g. they must be highly soluble in water, take effect quickly, be safe for both fish and humans, have broad safety margins, allow an *ad libitum* intensification of anaesthesia with a possibility of spontaneous recovery, and they must leave no residues in the fish (Brožová and Svobodová 1986; Brown 1988; Ross and Ross 1999).

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The first use of 2-phenoxyethanol to anaesthetize salmonids was reported in 1963 from Canada (Sehdev et al. 1963; Beel 1964). At present, 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 generally recommended concentration is 0.20 $ml \cdot l^{-1}$ of water bath. For big breeding fish, the recommended concentration is 0.30 $ml \cdot l^{-1}$ of water bath. At the recommended concentrations, anaesthesia is induced within 5 to 10 min. When transferred to clean water, fish will recover within 10 min (Svoboda and Kolářová 1999; Hamáčková et al. 2001).

The aim of the present study was to assess acute toxicity of 2-phenoxyethanol in rainbow trout, and to assess changes in their tissues using biochemical blood profile indices.

Materials and Methods

2-phenoxyethanol characteristics

The active agent of 2-phenoxyethanol is ethylene glycol monophenyl ether ($C_8H_{10}O_2$). The molar mass of the anaesthetic is 138.17 $g \cdot l^{-1}$, its density is 1.107 – 1.108, the peroxide content is less than 0.005 %, and the boiling point is 245 °C. The anaesthetic is slightly soluble in water (26.7 $g \cdot l^{-1}$), but readily soluble in ethanol. The anaesthetic affects fish through skin and gills. Its recommended concentrations are 0.20 – 0.30 $ml \cdot l^{-1}$, with the exposure period between 5 and 10 min. It is recommended that the anaesthetic be first mixed well with a small quantity of water before it is poured to the bath. Bath water should have the same characteristics including temperature as the water where the fish were kept. Fish are placed to the bath either individually, or in groups. 2-phenoxyethanol may be administered by a veterinary surgeon or a person authorized by a veterinarian.

The anaesthetic is manufactured by MERCK - Schucherd, 85 662 Hohenbrunn, Germany, and supplied in 2.5 and 1 litre canisters, or in other volumes on request.

Acute toxicity test

Rainbow trout (camloops) of 13.25 g mean body mass (11.2 – 15.3 g) and 11.09 cm mean standard length (10.5 - 12 cm), Fulton index 1.01 (0.95 – 1.07) were used to ascertain LC50 of 2-phenoxyethanol after 96 h and 10 min exposures to the anaesthetic.

In 96-hour LC50 tests, fish were exposed to a range of 2-phenoxyethanol concentrations (0.05, 0.10, 0.20, 0.30, 0.40 and 0.50 $ml \cdot l^{-1}$) in diluting water (pH 7.81; acid neutralization capacity – $ANC_{4.5}$ 1.15 $mmol \cdot l^{-1}$; total ammonia 0.04 $mg \cdot l^{-1}$; $N0^{3-}$ 11.5 $mg \cdot l^{-1}$; NO^{2-} 0.005 $mg \cdot l^{-1}$; PO_4^{3-} 0.01 $mg \cdot l^{-1}$; chemical oxygen demand - COD_{Mn} 1.6 $mg \cdot l^{-1}$), and the controls were placed in diluting water containing no anaesthetic. The fish and its behaviour, water temperature, pH and oxygen saturation were monitored throughout the tests at individual concentrations and in the control aquarium. Mean lethal concentrations LC50 for 96-hour exposure (96hLC50) and 96hLC0.1 and 96hLC99.9 were computed from those values using the probit analysis (EKO-TOX 5.1 software).

In the 10 min exposure LC50 tests, fish were exposed to 0.20, 0.25, 0.30, 0.35, 0.40, 0.45 and 0.50 $ml \cdot l^{-1}$ of 2-phenoxyethanol in diluting water for 10 min. Diluting water of the same parameters as in the 96hLC trials was used. For 10 min, changes in the physiological status, the number of dead fish, and the recovery times of fish placed to clean water were recorded. Mean lethal concentrations LC50 for 10-min exposure (10minLC50) and 10minLC0.1 and 10minLC99.9 were computed from those values using the probit analysis (EKO-TOX 5.1 software).

Changes in the physiological status of anaesthetized fish were assessed in four consecutive stages (Thienpoint and Niemegeers 1965):

1. acceleration and subsequent deceleration of breathing movements, a partial loss of reactivity to external stimuli
2. loss of balance, breathing movements very slow, fish still reactive to strong stimuli
3. total loss of reactivity, fish are lying at 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.

Biochemical examination of blood plasma

Rainbow trout (camloops) of 27.2 g mean body mass (20.4 - 34 g) and 14.86 cm mean standard length (13.5 - 17 cm), Fulton index 0.98 (0.95 – 1.01), were used for the determination of biochemical profile of blood plasma. The biochemical blood plasma profile in rainbow trout was studied before anaesthesia (10 trout), immediately after a 10-min exposure to the anaesthetic (10 trout), and 24 hours after anaesthesia (10 trout). A total of 30 rainbow trout were examined. The fish were anesthetized for 10 min by 2-phenoxyethanol at a concentration of 0.30 $ml \cdot l^{-1}$. Blood was collected by a heparinized needle from the heart (after stunning the fish by blow to the head). Aqueous solution of heparin sodium salt was used for blood stabilization (Heparin SPOFA inj. - 1ml aqueous solution contains 5000 i.u. of heparin sodium salt). To stabilize 1 ml of blood, 0.01 ml of aqueous solution of heparin was used (Svobodová et al. 1986).

Blood samples were centrifuged in a cooled centrifuge (4 °C, 837 × g). Biochemical indices determined in blood plasma included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3),

triglycerides (TRIG), cholesterol (CHOL), aspartate aminotransferase (AST), alanin aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca^{2+}) and inorganic phosphate (PHOS). For the biochemical analysis of blood plasma, VETTEST 8008 (IDEXX Laboratories Inc. U.S.A.) analyser (Medisoft Co.) was used. The apparatus is based upon dry chemical technology and colorimetric reaction. Sample analysis is carried out on selective testing discs (Multi – laier film slides, Kodak) by means of laser reading the bar codes. For the statistical evaluation, the analysis of variance was used (ANOVA – Tukey Test).

Results

Acute toxicity of 2-phenoxyethanol

In the 2-phenoxyethanol 96hLC50 tests, water temperature, pH and oxygen saturation ranges were 15.3 – 17.5 °C, 7.30 – 8.10 and 83 - 102%, respectively. On the basis of tests of acute toxicity to rainbow trout, the 96-hour lethal concentrations of 2-phenoxyethanol were determined as 0.25 $ml \cdot l^{-1}$ (96hLC50), 0.21 $ml \cdot l^{-1}$ (96hLC0.1) and 0.30 $ml \cdot l^{-1}$ (96hLC99.9).

Table 1
Effects of 2-phenoxyethanol anaesthesia on blood plasma indices in rainbow trout.
Note: Groups with different alphabetic superscripts differ significantly at $p < 0.05$ (ANOVA).

Indices	Control x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 h after anaesthesia x ± SD (n = 10)
GLU ($mmol \cdot l^{-1}$)	6.85 ± 0.86 ^{a}	8.36 ± 1.25 ^{b}	6.17 ± 0.74 ^{a}
TP ($g \cdot l^{-1}$)	29.25 ± 0.64 ^{a}	31.17 ± 0.81 ^{a}	43.50 ± 1.11 ^{b}
ALB ($g \cdot l^{-1}$)	4.0 ± 1.0 ^{a}	4.0 ± 1.0 ^{a}	11.0 ±1.0 ^{b}
GLOB ($g \cdot l^{-1}$)	25.25 ± 1.00 ^{a}	27.17 ± 1.00 ^{a}	32.5 ± 1.0 ^{b}
NH_3 ($mmol \cdot l^{-1}$)	757.25 ± 9.16 ^{a}	765.33 ± 11.30 ^{a}	736.83 ± 10.40 ^{a}
TRIG ($mmol \cdot l^{-1}$)	0.82 ± 0.64 ^{a}	0.82 ± 0.48 ^{a}	0.70 ± 0.61 ^{a}
CHOL ($mmol \cdot l^{-1}$)	1.97 ± 0.22 ^{a}	1.87 ± 0.22 ^{a}	1.96 ± 0.24 ^{a}
AST ($mkat \cdot l^{-1}$)	5.16 ± 0.04 ^{a}	4.10 ± 0.04 ^{b}	5.15 ± 0.04 ^{a}
ALT ($mkat \cdot l^{-1}$)	0.37 ± 0.03 ^{a}	0.34 ± 0.04 ^{a}	0.39 ± 0.04 ^{a}
ALP ($mkat \cdot l^{-1}$)	0.56 ± 0.02 ^{a}	0.54 ± 0.02 ^{a}	0.49 ± 0.03 ^{a}
LDH ($mkat \cdot l^{-1}$)	18.20 ± 0.19 ^{a}	18.00 ± 0.16 ^{a}	18.10 ± 0.17 ^{a}
PHOS ($mmol \cdot l^{-1}$)	3.70 ± 0.04 ^{a}	3.70 ± 0.07 ^{a}	3.40 ± 0.12 ^{a}
Ca^{2+} ($mmol \cdot l^{-1}$)	2.93 ± 0.07 ^{a}	2.86 ± 0.27 ^{a}	2.85 ± 0.24 ^{a}

A *post mortem* examination of the fish after acute toxicity tests showed increased presence of aqueous mucous and a dark discolouration of the dorsum. The gills were mat dark in colour and bled readily when injured. The body cavity contained excess moisture, and an increased injection of visceral vessels was also observed. These findings indicated that anaesthetized fish were more sensitive to mechanical injury.

During the 10-min LC50 tests, water temperature was 15 °C, pH was 7.75 and the water oxygen saturation was 98%. The effect of 2-phenoxyethanol concentrations on the onset of individual phases of anaesthesia and recovery is shown in Fig. 1. On the basis of tests of acute toxicity to rainbow trout, lethal concentrations of 2-phenoxyethanol at 10-min exposure were computed (10minLC50: 0.46 $ml \cdot l^{-1}$, 10minLC0.1: 0.34 $ml \cdot l^{-1}$ and 10minLC99.9: 0.92 $ml \cdot l^{-1}$).

Biochemical blood plasma profile after 2-phenoxyethanol exposure

Effects of 2-phenoxyethanol on the blood plasma biochemical profile of rainbow trout are given in Tab. 1. At a 10 minute exposure to 2-phenoxyethanol at the concentration of 0.30 $ml \cdot l^{-1}$, the total proteins (TP) values increased from 29.25 ± 0.64 $g \cdot l^{-1}$ to 43.5 ± 1.11 $g \cdot l^{-1}$ in 24 hours after anaesthesia. Albumin (ALB) values increased markedly from 4 ± 1.0 $g \cdot l^{-1}$ to 11 ± 1.0 $g \cdot l^{-1}$ in 24 hours after anaesthesia. Total globulins (GLOB) values 24 hours after anaesthesia were up to 32.5 ± 1.0 $g \cdot l^{-1}$ from 25.25 ± 1.0 $g \cdot l^{-1}$. Glucose (GLU) values

increased from 6.85 ± 0.86 mmol·l^{-1} to 8.36 ± 1.21 mmol·l^{-1} immediately after anaesthesia. Alanine aminotransferase (AST) values decreased markedly from 5.16 ± 0.04 μkat·l^{-1} to 4.10 ± 0.04 μkat·l^{-1} immediately after anaesthesia. All the changes were significant at the $p < 0.05$ level. In other indices (TRIG, NH_3, ALT, ALP, LDH, CHOL, Ca^{2+} and PHOS), no statistically significant differences between experimental groups were observed.

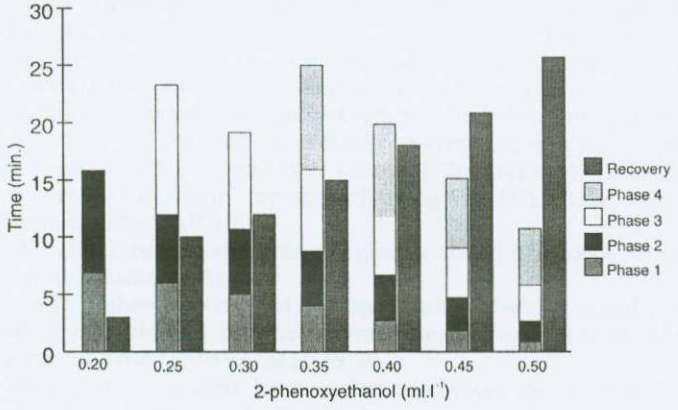

Fig 1. The effect of concentration of 2-phenoxyethanol on the onset of individual phases of anaesthesia and recovery in rainbow trout (*Oncorhynchus mykiss*).

Discussion

The 96LC50 value of 0.25 ml·l^{-1} found in the study suggests that 2-phenoxyethanol is relatively harmless for fish. The fish, however, were exposed to the anaesthetic for 10 min only. The 10minLC50 value in rainbow trout is about twice as high as the 96hLC50 value. In spite of that, the therapeutic index, i.e. the ratio between the therapeutic and the lethal concentrations, is very low (0.46 : 0.30). The 10minLC0.1 value found in the present study (0.34 ml·l^{-1}) is also below the concentration threshold recommended for anaesthesia (0.30 ml·l^{-1}). For that reason, it is essential that recommended procedures for sedation be strictly observed to prevent injury to fish.

Another aspect in favour of using 2-phenoxyethanol for rainbow trout at the concentration of 0.30 ml·l^{-1} is that the 10minLC50 values were recorded for water temperature of 15 °C. A majority of both breeding and veterinary interventions in rainbow trout takes place at much lower temperatures (5–7 °C). Under such conditions, 10minLC50 values will probably be higher. Weyl et al. (1996) and Hamáčková et al. (2001) stated that the most important factor influencing 2-phenoxyethanol efficacy in fish is temperature, i.e. the higher the temperature, the higher the efficiency of the anaesthetic for fish.

Sensitivity to anaesthetics may also be influenced by fish health and physical condition. Sensitivity to anaesthetics is also influenced by oxygen concentrations in the sense that oxygen deficit enhances the anaesthetic efficiency (Svobodová et al. 1987).

Because juvenile fish are more sensitive to 2-phenoxyethanol than adult fish, lower concentrations of the anaesthetic should be used for the former, as they provide much wider safety margins for anaesthesia (Barton and Helfrich 1981).

The same authors (Barton and Helfrich 1981) recommend that 0.30 – 0.40 ml·l^{-1} and 0.25 ml·l^{-1} of 2-phenoxyethanol for 10 minutes be used to anaesthetize adult and juvenile rainbow trout, respectively. Noga (1996), recommends the same concentrations but shorter exposure times (2 - 4 min).

It has been demonstrated that onset times of individual stages of 2-phenoxyethanol anaesthesia as well as recovery times (Fig. 1) were concentration-dependent. The influence of anaesthetic concentrations on anaesthesia induction times has been corroborated by Myszkowski et al. (2002) and Weyl et al. (1996).

The biochemical profile of blood can provide important information about the internal environment of the organism (Masopust 2000).

Increased blood plasma glucose concentrations immediately after 2-phenoxyethanol anaesthesia indicate that the procedure caused some stress in the trout, but the values returned to normal in 24 hours. The AST decrease in blood plasma indicates that the anaesthetic does not damage parenchymatous tissues in rainbow trout.

Torta et al. (2002) reported that 2-phenoxyethanol anaesthesia did not reduce cortisol levels in rainbow trout, did not block stress and had no negative effect on blood indices, i.e. the haematocrit value (PCV), haemoglobin content (Hb), erytrocyte count (RBC), mean corpuscular volume (MVC), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Ortuno et al. (2002) reported an increase in glucose and cortisol values in *Sparus aurata* anaesthetized with 2-phenoxyethanol.

Anaesthesia with 2-phenoxyethanol at the concentration of 400 ppm and 2 min exposure time had no effect on tryptophan, 5-hydroxytryptamine, dopamine or norepinephrine levels in the brain of rainbow trout (Sloley et al. 1986).

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

Anestezie pstruha duhového (*Oncorhynchus mykiss*) 2-phenoxyethanolem: akutní toxicita a vliv na biochemický profil krve

Cílem práce bylo posoudit akutní toxicitu a pomocí hodnot biochemického profilu krve zhodnotit změny ve tkáních pstruha duhového po působení anestetika 2-phenoxyethanolu. Ke stanovení akutní toxicity byla použita metoda OECD 203 „Test akutní toxicity na rybách". Zjištěné hodnoty akutní toxicity 2-phenoxyethanolu pro pstruha duhového ($Pd_{1/2}$) byly následující 10minLC50 0.46; 10minLC0.1 0.34; 10minLC99.9 0.92 $ml \cdot l^{-1}$; 96hLC50 0.25; 96hLC0.1 0.21; 96hLC99.9 0.30 $ml \cdot l^{-1}$.

Stanovení změn biochemického profilu krve bylo provedeno biochemickou analýzou krevní plazmy na analyzátoru VETTEST 8008 firmy Medisoft. Biochemický profil krve byl hodnocen u pstruhů duhových před, ihned po 10minutové anestézii a 24 hodin po anestézii v koncentraci 0.30 $ml \cdot l^{-1}$ 2-phenoxyethanolu. Celkem bylo vyšetřeno 30 kusů ryb $Pd_{1/2}$. Významné zvýšení hodnot ukazatelů v krevní plazmě bylo zjištěno u glukózy (GLU) ihned po anestézii a u celkových bílkovin (TP), albuminů (ALB) a globulinů (GLOB) 24 hodin po anestézii a výrazné snížení koncentrace aspartát aminotransferázy (AST) ihned po anestézii. Všechny rozdíly byly na hladině významnosti $p < 0.05$. U ostatních sledovaných ukazatelů [(triglyceridy (TRIG), cholesterol (CHOL), amoniak (NH_3), alanin aminotransferáza (ALT), alkalická fosfatáza (ALP), laktát dehydrogenáza (LDH), kalcium (Ca^{2+}), anorganický fosfát (PHOS)] nebyly zjištěny rozdíly mezi posuzovanými skupinami.

Koncentrace 0.30 $ml \cdot l^{-1}$ 2-phenoxyethanolu doporučovaná k anestézii se pro pstruha duhového jeví jako bezpečná.

Acknowledgement

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

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tract: The aim of the study was to investigate acute toxicity of 2-phenoxyethanol for sheatfish and, using es of haematological and biochemical profiles of blood and histological tissue examinations, to assess the cts of the fish exposure to that anaesthetic. Acute toxicity values of 2-phenoxyethanol for sheatfish found e 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 , and 96hLC99.9 0.41 ml/l. The 10-min exposure to 2-phenoxyethanolu at a concentration of 0.30 ml/l sed a significantly higher values ($P < 0.05$) of haematocrit (PCV), mean erythrocyte volume (MCV), glucose U) and albumins (ALB) immediately after anaesthesia. A significant decrease ($P < 0.05$) in the level of in aminotransferase (ALT) and a significant increased ($P < 0.05$) in the values of mean corpuscular moglobin concentration (MCHC) were found 24 hrs post anaesthesia. Histological examination showed llary ectasia of gill filaments immediately after 2-phenoxyethanol anaesthesia. Twenty-four hours after esthesia, no ectasia was observed. No histopathological changes were demonstrated in other tissues (liver, en, cranial and caudal kidneys) following anaesthesia. Results of the examinations suggest that the use of 2-noxyethanol at a concentration of 0.30 ml/l does not cause irreversible damage in sheatfish.

words: Acute toxicity, haematological profile, biochemical profile of blood, histological examination of es

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

Guilderhus and Marking (1987) defined three criteria that an anaesthetic applied in culture must fulfill. It must be effective, safe and inexpensive. Their criteria for efficacy s follows: fish must be sedated at 3 min after 15 min of exposure to anaesthetic, fish must in normal swimming in 10 min, and all anaesthetized fish must survive. The effect of an sthetic on fish depends on a number of factors, including concentration of the anaesthetic, r temperature, fish size and species (McFarland, 1960; Fereira et al., 1984).

Although the efficacy of 2-phenoxyethanol has been documented for many fish ies: sockey salmon (Sehdev et al., 1963); rainbow trout (Guilderhus and Marking, 1987; ma et al., 1988); platyfish (Guo et al., 1992); grass carp and silver carp (McCarter, 1992); py (Teo and Chen, 1993); black porgy (Hseu et al., 1996); goldfish (Weyl et al., 1996; se and Vine, 1998); perch (Hamackova et al., 2001); sea bream (Torth et al., 2002); tench yszkowski et al., 2003; Hamackova et al., 2004).

2-phenoxyethanol is used in the Czech Republic for short-term immobilization of fish ore artificial spawning and whenever fish is handled outside water. The recommended

entration for anaesthetic purposes is 0.30 ml/l water bath (Svoboda and Kolarova, 1999; ackova et al., 2001). At present, effects on 2-phenoxyethanol on commercially produced are studied in a project regarding the application of principles of pharmacovigilancy in culture in the Czech Republic. In the first stage of the project, effects of 2-oxyethanol on common carp (Velisek and Svobodova, 2004a) and rainbow trout (Velisek Svobodova, 2004b) were studied. In the second stage of the project, effects of clove oil on ow trout (Velisek et al., 2005a) and common carp (Velisek et al., 2005b) were studied. aim of the present study was to investigate acute toxicity of 2-phenoxyethanol in tfish and, on the basis of haematological indices, biochemical blood profile values and logical examinations, to assess the changes in the organism of sheatfish induced by the sthetic.

TERIALS AND METHODS

henoxyethanol characteristics

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

The anaesthetic is marketed by MERCK - Schucherd, 85 662 Hohenbrunn, Germany .5 and 1 litre packages, or in other volumes on request.

te toxicity of 2-phenoxyethanol

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

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

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

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

eleration and subsequent deceleration of opercular movements, a partial loss of ity to external stimuli

s of equilibrium, opercular movements very slow, fish still reactive to strong stimuli
al loss of reactivity, fish are lying at the tank bottom and do not respond to handling
mplete 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
sis using EKO-TOX 5.1 software

matological blood profile after exposure to 2-phenoxyethanol

For the haematological blood profile tests, sheatfish of 94.90 ± 55.23 g average weight
253.0 ± 74.60 mm average body length were used. A total of 40 fish divided into four
ps were examined: Control I (before the anaesthetic administration), Experiment I
nediately after 10 min anaesthesia at the concentration of 0.30 ml/l), Experiment II (24
after 10 min anaesthesia) and Control II (controls examined in parallel with Experiment
The fish were anesthetized for 10 min by 2-phenoxyethanol at a concentration of 0.30
. Heparinized injection needles were used to take samples of blood from hearts of fish
ned by a blow with a blunt object over the head. To stabilize blood samples, aqueous
tion 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 the erythrocyte count
), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV),
an corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin
tent (MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram).
e procedures were based on Unified methods for haematological examination of fish
obodova et al., 1986).

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

ochemical blood plasma profile after exposure to 2-phenoxyethanol

For biochemical profile of blood plasma tests, sheatfish of 94.90 ± 55.23 g average eight 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, 7 x g). Biochemical indices determined in blood plasma included glucose (GLU), total otein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols RIG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate hydrogenase (LDH), creatinkinase (CK), calcium (Ca^{2+}) and inorganic phosphate (PHOS). r the biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX boratories Inc. U.S.A.) manufactured by Medisoft was used.

Results of biochemical examination were tested by the variance analysis using the atistica 6.0 (ANOVA – Tuckey Test) software.

stological examination of tissues

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

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

ESULTS

cute toxicity of 2-phenoxyethanol

During the 96-hour LC50 tests, the mean water temperature was 19.7 – 20.4 °C, pH as 7.41 – 7.66 and water oxygen levels were 96 – 103% saturation. On the basis of tests of

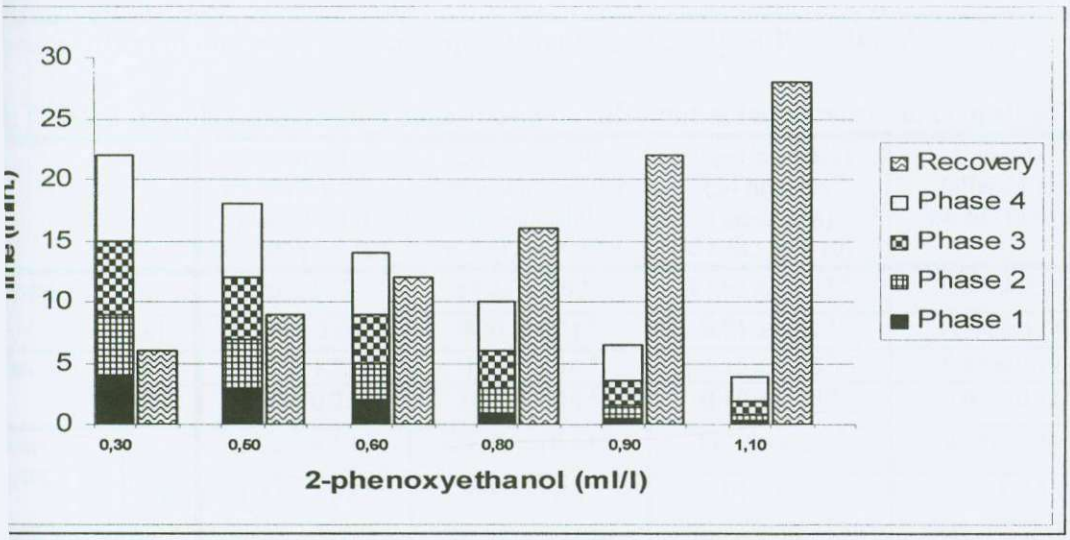
toxicity to sheatfish, the 96-hour lethal concentrations of 2-phenoxyethanol were ıined (96hLC50 0.29 ml/l, 96hLC0.1 0.20 ml/l, and 96hLC99.9 0.41 ml/l).

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

During 10-min LC50 tests, water temperature was 19.7 °C, pH was 7.69 and water :n level was at 96% saturation. On the basis of tests of acute toxicity to sheatfish, the 10- ethal concentrations of 2-phenoxyethanol were determined (10minLC50 0.77 ml/l, ıLC0.1 0.42 ml/l and 10minLC99.9 1.90 ml/l).

Effects of 2-phenoxyethanol concentrations on the time of onset of anaesthesia, on of its individual stages and the course of recovery are showed on Fig. 1.

: Effects of 2-phenoxyethanol concentrations on the onset of individual phases of hesia and recovery in sheatfish.



ıtological blood profile after exposure to 2-phenoxyethanol

Effects of 2-phenoxyethanol on the haematological profile of sheatfish are showed in and 2. The 10-min exposure to 2-phenoxyethanolu at a concentration of 0.30 ml/l a significant ($P < 0.05$) increase PVC and MCV immediately after anaesthesia. A

ant ($P < 0.05$) increase in the value MCHC 24 hrs post anaesthesia were found. The the indices (Er, Hb, MCH, Leuko and leukogram) were at comparable levels in all

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

	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)
	0.82 ± 0.23 [a]	0.56 ± 0.21 [a]	0.50 ± 0.24 [a]	0.76 ± 0.25 [a]
	38.54 ± 6.84 [a]	43.69 ± 9.22 [a]	43.32 ± 9.27 [a]	39.51 ± 5.89 [a]
	0.21 ± 0.04 [a]	0.27 ± 0.04 [b]	0.19 ± 0.03 [a]	0.20 ± 0.03 [a]
	266.94 ± 138.52 [a]	440.39 ± 549.73 [b]	392.55 ± 220.85 [a]	229.19 ± 160.49 [a]
)	53.0 ± 24.38 [a]	68.93 ± 115.55 [a]	84.43 ± 59.62 [a]	50.04 ± 46.35 [a]
/l)	177.56 ± 31.42 [a]	161.29 ± 27.50 [a]	226.19 ± 28.53 [b]	171.67 ± 37.66 [a]
l)	20.40 ± 4.04 [a]	13.60 ± 5.55 [a]	14.20 ± 5.78 [a]	14.0 ± 7.50 [a]

vith different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA).

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

		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)
tes	%	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]
	%	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]
e es	%	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]
e es	%	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]
ntal yeloid	%	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]
es	%	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]
	%	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]

h different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA).

emical blood plasma profile after exposure to 2-phenoxyethanol

Effects of 2-phenoxyethanol on the blood plasma biochemical profile of sheatfish are in Tab. 3. The 10-min exposure to 2-phenoxyethanolu at a concentration of 0.30 ml/l ł a significant ($P < 0.05$) increase in the concentration of glucose and albumins diately after anaesthesia. Their values returned back to normal within 24 hours. The of alanin aminotransferase were found decrease 24 hrs post anaesthesia. The rest of the s (TP, GLOB, NH_3, TRIG, AST, LDH, CK, Ca^{2+} and PHOS) were at comparable levels groups.

3: Effects of 2-phenoxyethanol anaesthesia on biochemical indices of blood plasma in ish.

	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)
nmol/l)	7.24 ± 2.63 [a]	10.52 ± 3.43 [b]	6.52 ± 2.26 [a]	6.91 ± 1.90 [a]
	35.30 ± 4.0 [a]	36.40 ± 2.37 [a]	34.20 ± 1.17 [a]	32.50 ± 2.70 [a]
/l)	3.80 ± 1.83 [a]	6.50 ± 0.92 [b]	3.20 ± 0.98 [a]	2.70 ± 1.27 [a]
(g/l)	31.60 ± 2.11 [a]	32.80 ± 1.40 [a]	31.30 ± 0.90 [a]	29.60 ± 1.69 [a]
nol/l)	931.60 ± 68.09 [a]	946.80 ± 66.33 [a]	939.40 ± 70.03 [a]	936.79 ± 76.11 [a]
mol/l)	1.68 ± 0.39 [a]	1.83 ± 0.50 [a]	1.27 ± 0.11 [a]	1.07 ± 0.25 [a]
kat/l)	7.43 ± 0.60 [a]	7.21 ± 0.86 [a]	7.38 ± 0.79 [a]	7.53 ± 0.71 [a]
kat/l)	0.19 ± 0.09 [a]	0.18 ± 0.11 [a]	0.09 ± 0.09 [b]	0.18 ± 0.10 [a]
kat/l)	8.96 ± 4.18 [a]	8.78 ± 5.66 [a]	8.67 ± 5.12 [a]	8.89 ± 6.65 [a]
at/l)	44.82 ± 2.89 [a]	43.11 ± 3.26 [a]	44.64 ± 4.01 [a]	44.08 ± 4.34 [a]
mol/l)	2.30 ± 0.21 [a]	2.31 ± 0.14 [a]	2.13 ± 0.23 [a]	2.08 ± 0.08 [a]
mmol/l)	1.16 ± 0.14 [a]	1.35 ± 0.23 [a]	1.28 ± 0.22 [a]	1.37 ± 0.18 [a]

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

ogical examination of tissues

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

CUSSION

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

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

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

The most important factor affecting the efficiency of 2-phenoxyethanol for fish, ver, is water temperature: the higher the temperature, the higher the efficiency of the thetic for fish. Repeated use of 2-phenoxyethanol increases the tolerance of the fish to naesthetic (Weyl et al., 1996). Because young fish are more sensitive to 2-oxyethanol than old fish, lower concentrations of the anaesthetic should be used for the r, as they provide much wider safety anaesthesia margins (Barton and Helfrich, 1981). ows from Fig. 1 that the onset of individual phases of anaesthesia and recovery times ded on 2-phenoxyethanol concentrations used.

To evaluate haematological and biochemical profiles of blood and histopathological es in tissues of sheatfish, 2-phenoxyethanol concentration of 0.30 ml/l was used in the t study. Haematological and biochemical profiles of blood can provide important nation about the internal environment of the organism (Masopust, 2000). Values ined in the present study suggest that internal organs and tissues of sheatfish are not

l by 2-phenoxyethanol anaesthesia. That conclusion was also confirmed by the result of ogical examination of parenchymatous organs.

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

2-Phenoxyethanol a common fish anesthetic, is widely applied in closed transport ns of fish (Guo et al., 1992; Teo et al., 1993; Kaiser and Vine, 1998). Compared with e, MS-222, and metomidate is 2-phenoxyethanol most effective in decreasing metabolic y and mortality of transported fish (Guo et al., 1992).

The disadvantage of 2-phenoxyethanol is its relatively low therapeutic index, i.e. the between the therapeutic and the toxic concentrations. The generally reported optimum is 1:4 or higher (Svobodova and Vykusova, 1991). A comparison between the ntration used in a 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 se the anaesthetic is toxic and harmful to humans. In poorly ventilated rooms, it may fatigue and drowsiness of the staff (Svoboda and Kolarova, 1999).

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„Je více cest, ale jeden cíl“

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4. VĚDECKÁ KONFERENCE S MEZINÁRODNÍ ÚČASTÍ

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EFFECT OF TWO ANAESTHETICS, 2-PHENOXYETHANOL AND OVE OIL, ON BIOCHEMICAL PROFILE OF BLOOD PLASMA OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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ACT

goal of this study was to assess changes in tissues of rainbow trout after the effects of anaesthetics, 2-phenoxyethanol and clove oil, by means of values of biochemical le of blood plasma. The determination of changes in the biochemical profile of blood performed by means of biochemical analysis of blood plasma with VETTEST 8008 yser (Medisoft Co.). The biochemical profile of blood was assessed in rainbow trout e-, immediately after 10 – min anaesthesia and 24 hours after the anaesthesia with nmended concentrations of 0.30 $ml.l^{-1}$ 2-phenoxyethanol and recommended entrations of 30 $mg.l^{-1}$ clove oil. The following biochemical indices were determined ood plasma: glucose (GLU), total proteins (TP), albumins (ALB), total globulines)B), ammonia (NH_3), triglycerides (TRIG), aspartate aminotransferase (AST), alanine otransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), um (Ca^{2+}) and anorganic phosphate (PHOS). The application of 2-phenoxyethanol d an increase in GLU concentration in blood plasma immediately after anaesthesia, as as of TP, ALB and GLOB 24 hours after anaesthesia, and an expressive decrease in concentration immediately after anaesthesia. The clove oil caused increased entrations of GLU and NH_3, as well as decreased AST concentration either diately after, or 24 hours after anaesthesia.

ORDS: ANAESTHESIA, BLOOD PLASMA, GLUCOSE, PROTEINS, ES, MINERALS

uction

revention from inconsiderate handling followed by injuries of the fish (from damage nucous layer to deep injuries in musculature or on fins) is one of important ients of fish health protection from the point of view of fish health, as well as g the act for animal protection against cruelty (No. 246/1992 in the Czech code of

reat attention has to be paid to careful and professional handling of brood fish, during the artificial propagation. Fish in the spawning period are very sensitive to cal injuries and they are subjected to repeated handling during the spawning season. ication of anaesthetic should prevent the fish from mechanical injuries and it should easier handling larger fish species during veterinary interventions (Čítek et al. 1997; Ross 1999).

V

he first use of 2-phenoxyethanol anaesthetic for salmonids in Canada was described (Sehdev et al. 1963; Beel 1964). At present, the 2-phenoxyethanol is used in the

ı Republic for a short-term immobilization of fish before artificial propagation, as well ïore any other handling out of water. The generally recommended concentration is 0.20 of water bath. For large brood fish, the recommended concentration is 0.30 $ml.l^{-1}$ of bath. When observing the recommended concentration, the onset of anaesthesia occurs ı 5 - 10 min. When transfered into clean water, the fish recover from anaesthesia within n (Svoboda and Kolářová 1999; Hamáčková et al. 2001).

At present, the clove oil is used in the Czech Republic for a short-term immobilization ı before artificial propagation, as well as before any other handling out of water. The ılly recommended concentration is 30 $mg.l^{-1}$ of water bath. (Svoboda and Kolářová, Hamáčková et al., 2001).

ERIAL AND METHODS

Rainbow trouts of 27.2 g mean weight (20.4 - 34 g) and 14.86 cm mean body length - 17 cm) were used for determination of biochemical profile of blood plasma after the of 2-phenoxyethanol. Rainbow trouts of 123 g mean weight (103 – 145 g) and 23 cm body length (21 – 26 cm) were used for determination of biochemical profile of blood ı after the effect of clove oil. It was assessed in trout prior to the anaesthesia (10 ıens), immediately after the anaesthesia, i.e. after 10 min effect (10 specimens) and 24 h ıe anaesthesia (10 specimens). Thirty specimens were examined in total for each of the ıaesthetics tested. The anaesthesia was carried out using 0.30 $ml.l^{-1}$ concentration of 2-ćyethanol and 30 $mg.l^{-1}$ concentration of clove oil, each of them for 10 min.

Fish blood was sampled by means of heart punction (after stunning the fish by blow to ad) by heparinized needle. Aequous solution of heparine sodium salt was used for ːation (Heparin SPOFA inj. - 1ml of aequous solution contains 5000 i.u. heparine ı salt). The amount of 0.01 ml of aequous solution of heparine was used for ːation of 1 ml blood (Svobodová et al. 1986).

Blood samples were centrifuged in a cooled centrifuge (4 °C, 837 x g). The following ınical indices were determined in blood plasma: glucose (GLU), total proteins (TP), ns (ALB), total globulines (GLOB), ammonia (NH_3), triglycerides (TRIG), aspartate ransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), ı (Ca^{2+}) and anorganic phosphate (PHOS). Biochemical analysis of blood plasma was ıed on VETTEST 8008 (IDEXX Laboratories Inc. U.S.A.) analyser by Medisoft Co. ıparatus is based upon dry chemical technology and colorimetric reaction. Sample s is carried out on selective testing discs (Multi – laier film slides, Kodak) by means of ading the bar codes.

cal test employed the analysis of variance (ANOVA – Tuckey Test).

ŁTS AND DISCUSSION

oxyethanol

Results of the effect of 2-phenoxyethanol anaesthetic on biochemical profile of blood in rainbow trout are shown in Table 1. The 0.30 $ml.l^{-1}$ concentration of 2-ıyethanol used with 10 min exposure affected the total protein content (TP) which ːd 24 h post anaesthesia from 29.25 ± 0.64 $g.l^{-1}$ to 43.5 ± 1.11 $g.l^{-1}$. The level of ıe (ALB) increased markedly just after anaesthesia from 4 ± 1.0 $g.l^{-1}$ to 11 ± 1.0 $g.l^{-1}$. ːl of globulines (GLOB) was found increased 24 h post anaesthesia from 25.25 ± 1.0 32.5 ± 1.0 $g.l^{-1}$. Glucose level (GLU) increased just after anaesthesia from 6.85 ± 0.86 $^{-1}$ to 8.36 ± 1.21 $mmol.l^{-1}$. Value of asparate aminotransferase (AST) considerably just after anaesthesia from 5.16 ± 0.04 $\mu kal.l^{-1}$ to 4.10 ± 0.04 $\mu kal.l^{-1}$. All diferences

e found significant with P< 0.05. Other indices (TRIG, NH_3, ALT, LDH, Ca^{2+}, PHOS) did differ significantly among the groups.

le 1: The effect of rainbow trout anaesthesia with 2-phenoxyethanol on the blood plasma ces.

ec	Control x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 h after anaesthesia x ± SD (n = 10)
(mmol.l^{-1})	6.85 ± 0.86 ^{a}	8.36 ± 1.25 ^{b}	6.17 ± 0.7 4 ^{a}
.l^{-1})	29.25 ± 0.64 ^{a}	31.17 ± 0.81 ^{a}	43.5 ± 1.11 ^{b}
(g.l^{-1})	4 ± 1.0 ^{a}	4 ± 1.0 ^{a}	11 ± 1.0 ^{b}
B (g.l^{-1})	25.25 ± 1.0 ^{a}	27.17 ± 1.0 ^{a}	32.5 ± 1.0 ^{b}
(µmol.l^{-1})	757.25 ± 9.16 ^{a}	765.33 ± 11.30 ^{a}	736.83 ± 10.40 ^{a}
(mmol.l^{-1})	0.82 ± 0.64 ^{a}	0.82 ± 0.48 ^{a}	0.70 ± 0.61 ^{a}
(µkat.l^{-1})	5.16± 0.04 ^{a}	4.10 ± 0.04 ^{b}	5.15 ± 0.04 ^{a}
(µkat.l^{-1})	0.37 ± 0.03 ^{a}	0.34 ± 0.03 ^{a}	0.39 ± 0.03 ^{a}
(µkat.l^{-1})	18.2 ± 0.19 ^{a}	18.0 ± 0.16 ^{a}	18.1 ± 0.17 ^{a}
S (mmol.l^{-1})	3.7 ± 0.04 ^{a}	3.7 ± 0.07 ^{a}	3.4 ± 0.12 ^{a}
(mmol.l^{-1})	2.93 ± 0.07 ^{a}	2.86 ± 0.27 ^{a}	2.58 ± 0.24 ^{a}

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

Biochemical profile of blood plasma is an important informative value on the status of nal milieu of the organism (Masopust 2000).

The increased level of glucose in blood plasma immediately after anaesthesia with 2-ıoxyethanol gave the evidence for slight stress effect to the rainbow trout. However, the es were found balanced 24 hours after anaesthesia. Increased levels of TP, ALB and)B, as well as decreased AST in blood plasma after anaesthesia showed that the sthesia did not damage tissues of rainbow trout.

ve oil

Results of the effect of clove oil anaesthetic on biochemical profile of blood plasma in ow trout are shown in Table 2. The 0.30 ml.l^{-1} concentration of clove oil used with 10 exposure affected the glucose content (GLU) which increased markedly just after sthesia from 5.92 ± 1.36 mmol.l^{-1} to 8.87 ± 1.41 mmol.l^{-1}. The level of ammonia (NH_3) ased markedly just after anaesthesia from 264 ± 47 µmol. l^{-1} to 403 ± 72 µmol.l^{-1}. The es of alanine aminotranspherase (AST) immediately after anaesthesia and 24 hours later lower compared to the control (2.12 ± 2.08 µmol. l^{-1} vs. 3.69 ± 2.09 µmol. l^{-1} and 2.09 ± µmol. l^{-1} vs. 3.79 ± 0.44 µmol. l^{-1}). All diferences were found significant with P< 0.05. r indices (TRIG, TP, ALB, GLOB, ALT, LDH, Ca^{2+}, PHOS) did not differ significantly ng the groups.

2: The effect of rainbow trout anaesthesia with clove oil on the blood plasma indices.

cec	Control x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 h after anaesthesia x ± SD (n = 10)
(mmol.l^{-1})	5.59 ± 1.36 ^{a}	8.87 ± 1.41 ^{b}	5.96 ± 1.02 ^{a}
g.l^{-1})	37 ± 7.02 ^{a}	37.7 ± 24.30 ^{a}	38.0 ± 11.20 ^{a}
(g.l^{-1})	8.7 ± 1.11 ^{a}	8.6 ± 1.96 ^{a}	9.0 ± 1.87 ^{a}
B (g.l^{-1})	28.5 ± 4.52 ^{a}	29.1 ± 1.66 ^{a}	29.0 ± 2.61 ^{a}
(μmol.l^{-1})	264 ± 47 ^{a}	403 ± 72 ^{b}	263 ± 38 ^{a}
(mmol.l^{-1})	1.07 ± 0.31 ^{a}	1.07 ± 0.11 ^{a}	1.08 ± 0.26 ^{a}
(μkat.l^{-1})	3.69 ± 2.09 ^{a}	2.12 ± 2.08 ^{b}	2.09 ± 1.09 ^{b}
(μkat.l^{-1})	0.18 ± 0.06 ^{a}	0.18 ± 0.07 ^{a}	0.17 ± 0.05 ^{a}
(μkat.l^{-1})	18.2 ± 1.09 ^{a}	18.5 ± 1.02 ^{a}	18.0 ± 1.25 ^{a}
S (mmol.l^{-1})	3.32 ± 0.23 ^{a}	3.40 ±0.17 ^{a}	3.41 ± 0.28 ^{a}
(mmol.l^{-1})	2.89 ± 0.23 ^{a}	2.91 ± 0.09 ^{a}	2.87 ± 0.13 ^{a}

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

The increased level of glucose in blood plasma immediately after anaesthesia with oil gave the evidence for slight stress effect to the rainbow trout. However, the values found balanced 24 hours after anaesthesia. Decreased levels of AST in blood plasma anaesthesia showed that the anaesthesia did not damage tissues of rainbow trout.

CLUSION

The results showed that changes in biochemical profile of blood plasma of fish ed by effects of the two anaesthetics, 2-phenoxyethanol and clove oil, were only short – ones from which the fish recovered within 24 hours. It was therefore possible to mend either 2-phenoxyethanol, and clove oil for anaesthesia of rainbow trout.

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THE EFFECT OF TWO ANAESTHETICS, IENOXYETHANOL AND CLOVE OIL, ON BIOCHEMICAL PROFILE BLOOD PLASMA OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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from inconsiderate handling followed by injuries of the fish (from damage of the mucous layer to deep injuries in musculature s one of important requirements of fish health protection from the point of view of fish health, as well as observing the act for ection against cruelty (No. 246/1992 in the Czech code of laws).

tion of anaesthetic should prevent the fish from mechanical injuries and it should facilitate easier handling larger fish species inary interventions

MATERIALS AND METHODS

s mykiss

The biochemical profile of blood was assessed in rainbow trout before-, immediately after 10 – min anaesthesia and 24 hours after the anaesthesia with recommended concentrations of 0.30 ml.l^{-1} 2-phenoxyethanol and recommended concentrations of 30 mg.l^{-1}

The following biochemical indices were determined in blood plasma: glucose (GLU), total proteins (TP), albumins (ALB), total globulines (GLOB), ammonia (NH_3), triglycerides (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), calcium (Ca^{2+}) and anorganic phosphate (PHOS).

RESULTS

2-PHENOXYETHANOL

1: The effect of rainbow trout anaesthesia 2-phenoxyethanol with on the blood plasma indices

	Control X ± SD (n =10)	Immediately after anaesthesia X ± SD (n =10)	24 h after anaesthesia X ± SD (n =10)
)	6.85 ± 0.86 ᵃ	8.34 ± 1.20 ᵇ	6.17 ± 0.74 ᵃ
	29.25 ± 0.64 ᵃ	31.17 ± 0.81 ᵇ	30.5 ± 1.11 ᵇ
	4.0 ± 1.0 ᵃ	4.0 ± 1.0 ᵃ	11.0 ± 1.0 ᵇ
	25.25 ± 1.0 ᵃ	27.17 ± 1.0 ᵇ	32.5 ± 1.0 ᶜ
	757.25 ± 9.16 ᵃ	765.33 ± 11.30 ᵃ	736.83 ± 10.40 ᵃ
¹)	0.82 ± 0.64 ᵃ	0.82 ± 0.48 ᵃ	0.70 ± 0.61 ᵃ
)	5.16 ± 0.04 ᵃ	6.98 ± 0.04 ᵇ	5.15 ± 0.04 ᵃ
)	0.37 ± 0.03 ᵃ	0.34 ± 0.03 ᵃ	0.39 ± 0.03 ᵃ
)	18.2 ± 0.19 ᵃ	18.0 ± 0.16 ᵃ	18.1 ± 0.17 ᵃ
¹)	3.7 ± 0.04 ᵃ	3.7 ± 0.07 ᵃ	3.4 ± 0.12 ᵇ
	2.93 ± 0.07 ᵃ	2.86 ± 0.27 ᵃ	2.58 ± 0.24 ᵇ

ation of 2-phenoxyethanol (0.30 ml.l^{-1}) caused an GLU concentration in blood plasma immediately after , as well as of TP, ALB and GLOB 24 hours after , and an expressive decrease in AST concentration y after anaesthesia. All diferences were found with P< 0.05. Other indices (TRIG, NH_3, ALT, LDH,) did not differ significantly among the groups.

CLOVE OIL

Tab. 2: The effect of rainbow trout anaesthesia with clove oil on the blood plasma indices

Indices	Kontrol X ± SD (n =10)	Immediately after anaesthesia X ± SD (n =10)	24 h after anaesthesia X ± SD (n =10)
GLU (mmol.l^{-1})	5.59 ± 1.36 ᵃ	8.07 ± 1.42 ᵇ	5.96 ± 1.02 ᵃ
TP (g.l^{-1})	37.0 ± 7.02 ᵃ	37.7 ± 24.30 ᵃ	38.0 ± 11.20 ᵃ
ALB (g.l^{-1})	8.7 ± 1.11 ᵃ	8.6 ± 1.96 ᵃ	9.0 ± 1.87 ᵃ
GLOB (g.l^{-1})	28.5 ± 4.52 ᵃ	29.1 ± 1.66 ᵃ	29.0 ± 2.61 ᵃ
NH_3 (µmol.l^{-1})	264.0 ± 47.0 ᵃ	405.0 ± 75.0 ᵇ	263 ± 38.0 ᵃ
TRIG (mmol.l^{-1})	1.07 ± 0.31 ᵃ	1.07 ± 0.11 ᵃ	1.08 ± 0.26 ᵃ
AST (µmol.l^{-1})	3.69 ± 2.09 ᵃ	5.23 ± 2.08 ᵇ	3.90 ± 1.80 ᵃ
ALT (µmol.l^{-1})	0.18 ± 1.09 ᵃ	0.18 ± 0.07 ᵃ	0.17 ± 0.05 ᵃ
LDH (µmol.l^{-1})	18.2 ± 1.09 ᵃ	18.5 ± 1.02 ᵃ	18.0 ± 1.25 ᵃ
PHOS (mmol.l^{-1})	3.32 ± 0.23 ᵃ	3.40 ± 0.17 ᵃ	3.41 ± 0.28 ᵃ
Ca^{2+} (mmol.l^{-1})	2.89 ± 0.23 ᵃ	2.91 ± 0.09 ᵃ	2.87 ± 0.13 ᵃ

The application of clove oil 30 mg.l^{-1} caused an increased concentrations of GLU and NH_3, as well as decreased AST concentration either immediately after, or 24 hours after anaesthesia. All diferences were found significant with P< 0.05. Other indices (TRIG, TP, ALB, GLOB, ALT, LDH, Ca^{2+}, PHOS) did not differ significantly among the groups.

CONCLUSIONS

ts showed that changes in biochemical blood plasma of fish induced by effects of naesthetics, 2-phenoxyethanol and clove nly short – term ones from which the fish within 24 hours. It was therefore possible nend either 2-phenoxyethanol, and clove esthesia of rainbow trout.

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BOOK OF ABSTRACTS

European Association of Fish Pathologists

12th International Conference "Diseases of Fish and Shellfish"

11-16 September 2005
Copenhagen, Denmark

ECTS OF 2 ANAESTHETICS (2-PHENOXYETHANOL AND CLOVE OIL) TO NBOW TROUT (*ONCHORHYNCHUS MYKISS*) AND COMMON CARP *RINUS CARPIO*).

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itute of Chemical Technology, Prague, Czech Republic

portant requirement of fish health protection is to prevent the fish from inconsiderate ng followed by injuries. The application of anaesthetic should prevent the fish from nical injuries and it should facilitate easier handling larger fish species during ary interventions. Current anaesthetics should meet the following general ements: high solubility of the substance, rapid effect, harmless for fish and man, wide n of safety, arbitraty deepending of anaesthesia with a possibility of spontaneous ry, no residues left in fish.

-phenoxyethanol (active substance ethylene glycol monophenyl ether – C8H10O2) is ot only for short-term immobilization of fish before artifical propagation, but also ver fish is handled outside water. Cove oil is a distillate of flowers, stalks and leaves of ve tree *Eugenia aromatica* (active ingredience is eugenol – 4-allyl-2-methoxyphenol) is used in same indications as 2-phenoxyethanol.

m of the study was to investigate acute toxicity by means of OECD acute toxicity test), heamatological and biochemical profiles of blood, histological tissue examinations, ination of residues in muscle with skin.

s of the examinations suggest that the application of both anaestetics does not cause sibile damage. The 2-phenoxyethanol and clove oil may become useful anaestetics for ajor problem of their use is they are not registered yet.

search was supported by the USB RIFCH no. MSM6007665809 and the Ministry of lture the Czech Republic (NAZV Project No. QF3029).

ECT OF 2 ANAESTHETICS (2-PHENOXYETHANOL AND CLOVE OIL)

BOW TROUT *(Oncorhynchus mykiss)* AND COMMON CARP *(Cyprinus carpio)*.

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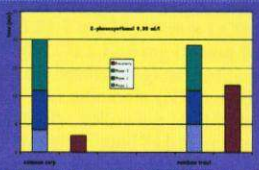
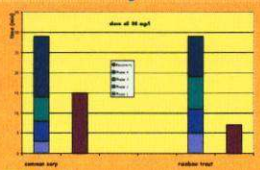
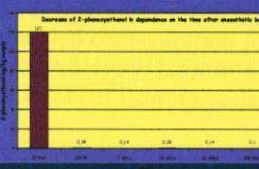
equirement of fish health protection is to prevent the fish from inconsiderate handling followed by injuries. The application of anaesthetic should prevent the fish from uries and it should facilitate easier handling larger fish species during veterinary interventions. Current anaesthetic should meet the following general requirements: high e substance, rapid effect, harmless for fish and man, wide margin of safety, arbitrary deepening of anaesthesia with a possibility of spontaneous recovery, no residues left in

Material + Method	2-Phenoxylsthanol	Clove oil
	chemical substance with exact defined chemical structure, active substance: ethylene glycol monophenyl ether - $C_8H_{10}O_2$, it is slightly solubile in water	natural substance with indefined chemical structure, clove oil is a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (active ingredient is eugenol - 4-allyl-2-methoxyphenol), it is not completely water solubile,
ic dose = tested concentration	0,3 ml.l^{-1}	30 mg.l^{-1}
sthethic on the onset of individual phases of d recovery weight 255-410g)-water temperature-13°C eight 227-507g)-water temperature-20°C		
by means of OECD acute toxicity test (LC50)	common carp 10 minLC50.....0,39 ml.l^{-1} common carp 96hLC50.....0,17 ml.l^{-1} rainbow trout 10 minLC50.....0,46 ml.l^{-1} rainbow trout 96hLC50.....0,25 ml.l^{-1}	.....74 mg.l^{-1} .....15 mg.l^{-1} .....81 mg.l^{-1} .....14 mg.l^{-1}
dex	common carp .....1,3 rainbow trout 1,5	common carp .....*2,5* rainbow trout 2,7
risc phrase	no append (*Daphnia magna* 48 EC50 = 0,21 ml.l^{-1})	R51 (toxic for aquatic org. - *Daphnia magna* 48 EC50 = 4 mg.l^{-1})
profiles of blood (anaesthethic bath 10 min) ter anaesthetic bath and 24 hrs after , Hb, PCV, MCV, MCH, MCHC, Leu, Leukogram	in rainbow trout no effect in common carp significant increase of PCV immediately after anaesthesia (24 hrs after anaesthesia - no observed)	no effect in both fish species
files of blood plasma (anaesthethic bath 10 ly after anaesthetic bath and 24 hrs after U, TP, ALB, GLOB, NH3, TAG, AST, ALT, LDH, 5 (VETTEST 8008 analyzer)	rainbow trout: significant increase of GLU and decrease of AST immediately after anaesthetic bath (24 hrs after anaesthesia - both parameters was in physiol. norm) + significant increase of TP, ALB, GLOB only 24 hrs after anaesthesia common carp: significant increase of ALT immediately after anaesthetic bath and 24 hrs after anaesthesia too	rainbow trout: significant increase of GLU and NH3 immediately after anaesthetic bath (24 hrs after anaesthesia - no changes was observed) + decrease of AST immediately after anaesthetic bath and 24 hrs after anaesthesia too common carp: significant increase of GLU and PHOS immediately after anaesthetic bath (24 hrs after anaesthesia - no changes was observed)
sue examinations: gills, liver, cranial and caudal skin	in rainbow trout no observed effect in common carp immediately after anaesthesia changes in gills on secundary lamellas: edema of epithelium, hyperplasia and hypertropia of eosiniphilous cells on the basis (24 hrs after anaesthesia - no changes was observed)	in both fish species capillary ectasia of gill filaments immediately after anaesthesia (24 hrs after anaesthesia - no ectasia was observed).
f residues in muscle with skin - preliminary		significant levels of clove oil was determined in sampels taken 10 minuts after anaesthesia, in later taken sampels (after 14 hr, 7-14-24-28 days) was found marked decrease

remark: significant = at $p<0,05$ level

examinations suggest that the application of both anaestetics does not cause irreversible damage. The 2-phenoxyethanol and clove oil may become useful anaestetics r problem of their use is that they are not registered yet. Veterinary may administer they anaesthetic "off label" with maximal protection time befor supply to human consumption: 500 diurnal degree (In Czech Rep.: Act No. 166/1999 Coll., on Veterinary Care).

This research was supported by the USB RIFCH no. MSM6007665809 and the Ministry of Agriculture of the Czech Republic (NAZV Project No. QF3029).

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Effects of Clove Oil Anaesthesia on Rainbow Trout *(Oncorhynchus mykiss)*

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Abstract

Velíšek J., Z. Svobodová, V. Piačková: Effects of Clove Oil Anaesthesia on Rainbow Trout *(Oncorhynchus mykiss)*. Acta Vet. Brno 2005, 74: 139-146.

The aim of the study was to investigate acute toxicity of clove oil for rainbow trout and, using values of haematological and biochemical profiles of blood and histological tissue examinations, to assess the effects of the fish exposure to that anaesthetic. Acute toxicity values of clove oil for rainbow trout found were 10 min LC50 81.1 $mg \cdot l^{-1}$; 10 min LC0.1 63.9 $mg \cdot l^{-1}$; 10 min LC99.9 100.1 $mg \cdot l^{-1}$; 96 h LC50 14.1 $mg \cdot l^{-1}$; 96 h LC0.1 12.5 $mg \cdot l^{-1}$, and 96 h LC99.9 16.2 $mg \cdot l^{-1}$.

Clove oil anaesthesia had no effect on the heamatological profile of blood. A significant increase in the concentration of glucose (GLU) and ammonia (NH_3), and a significant decrease in the aspartate aminotransferase (AST) activity following a 10 min anaesthesia were found. A significantly decreased AST activity was also found 24 h after anaesthesia. Clove oil anaesthesia had no effect on other biochemical indices.

Histological examination of the fish following anaesthesia revealed sporadic ectasia in gill lamellae 24 h after anaesthesia in 20% of fish. No histopathological changes were demonstrated in other tissues (liver, spleen, cranial and caudal kidneys).

Results of the examinations suggest that the use of clove oil at a concentration of 30 $mg \cdot l^{-1}$ does not cause irreversible damage in rainbow trout.

Acute toxicity, haematological profile, biochemical profile, blood, histology, examination, tissues

Rapid growth of aquaculture in the world and technological advances applied in it make exacting demands on newly introduced chemicals and preparations. Chemicals used in aquaculture are nowadays subject to strict control, particularly with regard to their safety and efficacy (Taylor and Roberts 1999). Anaesthetics are among important and broadly used veterinary medicines.

An modern fish anaesthetics must meet a number of general requirements, e.g. they must be highly soluble in water, have short induction time, be non-toxic for both fish and humans, have a large safety factor, allow an *ad lib* intensification of anaesthesia with a possibility of spontaneous recovery, and they must leave no residues in fish (Brožová and Svobodová 1986; Brown 1988; Ross and Ross 1999).

Clove oil is used as an anaesthetic before handling or treating fish in breeding, artificial propagation, blood sampling or for some other veterinary interventions. The use of an anaesthetic facilitates the handling of too big or too agile fish species (Trzebiatowski et al. 1996; Iwama et al. 1997; Wagner et al. 2002). At present, clove oil 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 30 $mg \cdot l^{-1}$ water bath (Svoboda and Kolářová 1999; Hamáčková et al. 2001).

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree

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Eugenia aromatica (Sato and Burhanuddin 1995). According to Isaacs (1983), Briozza et al. (1989) and Keene et al. (1998), clove oil is distilled from stems, leaves and flower buds of *Eugenia caryophyllata*, and its active ingredient, i.e. eugenol (4-allyl-2-methoxyphenol), makes up 70 to 90% by weight of clove oil. Clove oil also contains eugenol acetate (> 17%) and kariofilen 5 (> 12%).

In fish, anaesthetics are absorbed and excreted mainly through gills (Locke 1969; Hunn and Allen 1974; Houston and Woods 1976; Ferreira et al. 1984). Eugenol and its compounds and metabolites are quickly removed from the blood bed and tissues of fish (Fisher et al. 1990), and the presence of these substances in muscle tissues of fish or other animals is not considered toxic or mutagenous (Liu and Gibson 1977; Maura et al. 1989; Fisher et al. 1990; Philips 1990; Zheng et al. 1992).

Clove oil has been used as a mild anaesthetic in humane medicine since antiquity (Nagababu and Lakshmaiah 1992; Ross and Ross 1999; Taylor and Roberts 1999). Eugenol inhibits the synthesis of prostaglandin H (PHS), which accounts for the analgesic effect of clove oil (Dewhirst and Goodson 1974; Thomson and Eling 1989; Pongprayoon et al. 1991).

The aim of the present study was to investigate acute toxicity of clove oil in rainbow trout and, on the basis of haematological indices, biochemical blood profile values and histological examinations, to assess the changes in the organism of rainbow trout induced by the anaesthetic.

Materials and Methods

In the study, clove oil marketed by the Kulich Company (Jan Kulich, Hradec Králové/Říčany, CR) in 10 ml and 50 ml containers was used.

Acute toxicity of clove oil

Acute toxicity of clove oil was ascertained by the OECD 203 „Fish, acute toxicity test". For the 96 h and 10 min LC50 trials, rainbow trout (kamloops) of 40 ± 10 g (mean ± SD) average weight and 150 ± 20 mm average body length were used.

The 96-h LC50 test: Experimental fish were exposed to concentrations 7, 10, 12, 14, 16, 18 and 20 $mg \cdot l^{-1}$ clove oil dissolved in diluting water (pH 7.51; acid neutralization capacity – $ANA_{4.5}$ 1.29 $mmol \cdot l^{-1}$; total ammonia 0.03 $mg \cdot l^{-1}$; NO_3^- 7.45 $mg \cdot l^{-1}$; NO_2^- 0.003 $mg \cdot l^{-1}$; PO_4^{3-} 0.02 $mg \cdot l^{-1}$; chemical oxygen demand – COD_{Mn} 1.5 $mg \cdot l^{-1}$), and controls were placed in diluting water with no tested substance added. Ten rainbow trout were used for each concentration and for the control group. The fish and its behaviour, water temperature, pH and oxygen saturation were monitored throughout the tests at individual concentrations and in the control aquarium. Mean lethal concentration (96 h LC50) and also 96 h LC0.1 and 96 h LC99.9 were calculated from mortality rates over the period of 96 hours.

The 10-min LC50 test: For 10 min, the fish were exposed to concentrations of 50, 60, 70, 80, 90 and 100 $mg \cdot l^{-1}$ of clove oil dissolved in diluting water. Ten rainbow trout 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 trout had been moved to clean water, the time of their recovery from anaesthesia was determined. Mean lethal concentrations (10 min LC50) and also 10 min LC0.1 and 10 min C99.9 were calculated from mortality rates over the period of 10 min.

In the tests, the onsets of individual phases of anaesthesia and recovery rates were studied. Evaluations were made in four consecutive phases (Thienpoint 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 at 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 clove oil

For the haematological blood profile tests, rainbow trout (kamloops) of 102.5 ± 20 g (mean ± SD) average weight and 200 ± 40 mm average body length were used. A total of 40 fish divided into four groups were examined: Control I (before the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia at the concentration of 30 $mg \cdot l^{-1}$), Experiment II (24 hrs after 10 min anaesthesia) and Control II (controls examined in parallel with Experiment II). The fish were anesthetized for 10 min by clove oil at a concentration of 30 $mg \cdot l^{-1}$. Heparinized injection needles were used to

take samples of blood from hearts of fish stunned by a blow with a blunt object over the head. To stabilize 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 (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean colour concentration (MCHC), erythrocyte 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 variance analysis using the Statgraphics (ANOVA – Tukey Test) software.

Biochemical blood plasma profile after exposure to clove oil

For biochemical profile of blood plasma tests, rainbow trout (kamloops) of 123 ± 20 g (mean ± SD) average weight and 230 ± 40 cm average body length were used.

Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4 °C, 837 x g). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols (TAG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), creatinkinase (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. The analyzer uses dry chemical and colorimetric analysis techniques. Selective test discs (Multi-layer film slides, Kodak) are used for the evaluation by a laser reading bar codes.

Results of biochemical examination were tested by the variance analysis using the Statgraphics (ANOVA – Tukey Test) software.

Histological examination of tissues

For histological examination of tissues, rainbow trout (kamloops) of 123 ± 20 g (mean ± SD) average weight and 230 ± 40 cm average body length were used.

After blood sampling, samples of gills, liver, cranial and caudal kidneys and spleen were taken for histological examinations. The samples taken 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 clove oil

During the 96-hour LC50 tests, the mean water temperature was 13.7 – 15 °C, pH was 7.41 – 7.86 and water oxygen levels were 76-96% saturation. On the basis of tests of acute toxicity to rainbow trout, the 96-hour lethal concentrations of clove oil were determined (96 h LC50 14.1 $mg \cdot l^{-1}$, 96 h LC0.1 12.5 $mg \cdot l^{-1}$ and 96 h LC99.9 16.2 $mg \cdot l^{-1}$).

The autopsy performed after the acute toxicity test revealed increased amounts of watery mucous 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 obtained.

During 10-min LC50 tests, water temperature was 14 °C, pH was 7.76 and water oxygen level was at 98% saturation. On the basis of tests of acute toxicity to rainbow trout, the 10-min lethal concentrations of clove oil were determined (10 min LC50 81.1 $mg \cdot l^{-1}$, 10 min LC0.1 63.9 $mg \cdot l^{-1}$ and 10 min LC99.9 100.1 $mg \cdot l^{-1}$).

Effects of clove oil concentrations on the time of onset of anaesthesia, duration of its individual stages and the course of recovery are shown in Fig. 1.

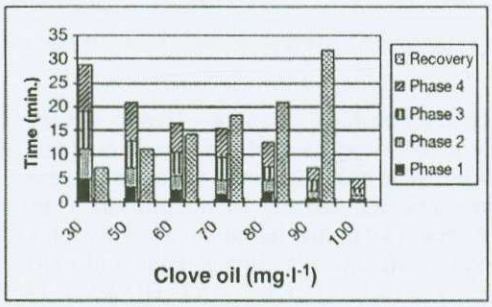

Fig. 1. Effects of clove oil concentrations on the onset of individual phases of anaesthesia and recovery in rainbow trout

Haematological blood profile after exposure to clove oil

Effects of clove oil on the haematological profile of rainbow trout are showed in Tables 1 and 2. The 10-min exposure to the anaesthetic at a concentration of 30 $mg \cdot l^{-1}$ had no effect on the haematological indices studied (Er, Hb, PCV, MCV, MCHC, MCH, Leuko and Leukogram).

Table 1. Effects of clove oil 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)	Experimenatal II (24 hrs after anaesthesia) x ± SD (n = 10)	Control II (after 24hrs) x ± SD (n = 10)
Er ($T \cdot l^{-1}$)	1.43 ± 0.23 [a]	1.28 ± 0.26 [a]	1.16 ± 0.25 [a]	1.06 ± 0.25 [a]
Hb ($g \cdot l^{-1}$)	61.32 ± 10.08 [a]	61.99 ± 12.73 [a]	58.42 ± 10.66 [a]	55.91 ± 6.84 [a]
PCV ($l \cdot l^{-1}$)	0.43 ± 0.05 [a]	0.44 ± 0.07 [a]	0.39 ± 0.01 [a]	0.39 ± 0.03 [a]
MCV (fl)	305.68 ± 51.24 [a]	344.05 ± 57.67 [a]	339.79 ± 56.96 [a]	378.09 ± 90.91 [a]
MCH (pg)	43.67 ± 9.65 [a]	49.15 ± 11.55 [a]	51.06 ± 9.75 [a]	52.44 ± 10.80 [a]
MCHC ($g \cdot l^{-1}$)	141.99 ± 13.32 [a]	141.71 ± 10.79 [a]	150.46 ± 15.87 [a]	140.19 ± 14.41 [a]
Leuko ($G \cdot l^{-1}$)	23.70 ± 11.31 [a]	23.70 ± 4.69 [a]	19.60 ± 9.96 [a]	17.6 ± 6.42 [a]

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

Table 2. Effects of clove oil anaesthesia on differential leukocyte counts 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 24hrs) x ± SD (n = 10)
Lymphocytes	%	74.30 ± 15 [a]	74.70 ± 13 [a]	78.70 ± 9.40 [a]	78.30 ± 11 [a]
	$g \cdot l^{-1}$	18.02 ± 9.49 [a]	19.01 ± 5.26 [a]	13.77 ± 4.55 [a]	15.73 ± 8.77 [a]
Monocytes	%	2.20 ± 2.20 [a]	2.80 ± 3.40 [a]	1.60 ± 1.70 [a]	3.20 ± 2.30 [a]
	$g \cdot l^{-1}$	0.47 ± 0.35 [a]	0.71 ± 0.91 [a]	0.34 ± 0.49 [a]	0.58 ± 0.46 [a]
Neutrophile granulocytes segment	%	18.7 ± 15 [a]	13.6 ± 9.60 [a]	14 ± 8.0 [a]	12 ± 9.50 [a]
	$g \cdot l^{-1}$	4.16 ± 2.91 [a]	3.04 ± 1.86 [a]	2.51 ± 1.74 [a]	3.55 ± 4.29 [a]
Neutrophile granulocytes rods	%	0.20 ± 0.42 [a]	0.10 ± 0.32 [a]	0.10 ± 0.32 [a]	0.30 ± 0.48 [a]
	$g \cdot l^{-1}$	0.05 ± 0.10 [a]	0.03 ± 0.05 [a]	0.02 ± 0.01 [a]	0.05 ± 0.07 [a]
Developmental phases – myeloid sequence	%	4.60 ± 2.07 [a]	3.80 ± 4.20 [a]	6.20 ± 2.87 [a]	5.60 ± 3.24 [a]
	$g \cdot l^{-1}$	0.92 ± 0.76 [a]	1.11 ± 0.68 [a]	1.16 ± 0.92 [a]	1.15 ± 0.52 [a]

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

Biochemical blood plasma profile after exposure to clove oil

Effects of clove oil on the blood plasma biochemical profile of rainbow trout are given in Tab. 3. The 10-min exposure to clove oil at a concentration of 30 $mg \cdot l^{-1}$ caused a significant ($p < 0.05$) increase in the concentration of glucose and ammonia immediately after anaesthesia. Their values returned back to normal within 24 hours. AST levels were down compared with control groups immediately and 24 hours after anaesthesia ($p < 0.05$). The rest of the indices (TP, ALB, GLOB, TAG, ALT, LDH, CK, Ca^{2+} and PHOS) were at comparable levels in all groups.

Table 3. Effects of clove oil anaesthesia on biochemical indices of blood plasma 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)
GLU ($mmol \cdot l^{-1}$)	5.59 ± 1.36 [a]	8.87 ± 1.41 [b]	5.96 ± 1.02 [a]	5.84 ± 0.85 [a]
TP ($g \cdot l^{-1}$)	37 ± 7.02 [a]	37.7 ± 24.30 [a]	38.0 ± 11.20 [a]	36.3 ± 2.66 [a]
ALB ($g \cdot l^{-1}$)	8.7 ± 1.11 [a]	8.6 ± 1.96 [a]	9.0 ± 1.87 [a]	8.6 ± 2.87 [a]
GLOB ($g \cdot l^{-1}$)	28.5 ± 4.52 [a]	29.1 ± 1.66 [a]	29.0 ± 2.61 [a]	28 ± 1.66 [a]
NH_3 ($\mu mol \cdot l^{-1}$)	264 ± 47 [a]	403 ± 72 [b]	263 ± 38 [a]	266 ± 31 [a]
TAG ($mmol \cdot l^{-1}$)	1.07 ± 0.31 [a]	1.07 ± 0.11 [a]	1.08 ± 0.26 [a]	1.03 ± 0.32 [a]
AST ($\mu kat \cdot l^{-1}$)	3.69 ± 2.09 [a]	2.12 ± 2.08 [b]	2.09 ± 1.09 [b]	3.79 ± 0.44 [a]
ALT ($\mu kat \cdot l^{-1}$)	0.18 ± 0.06 [a]	0.18 ± 0.07 [a]	0.17 ± 0.05 [a]	0.19 ± 0,04 [a]
LDH ($\mu kat \cdot l^{-1}$)	18.2 ± 1.09 [a]	18.5 ± 1.02 [a]	18.0 ± 1.25 [a]	18.3 ± 0.92 [a]
CK ($\mu kat \cdot l^{-1}$)	13.1 ± 2.05 [a]	12.8 ± 1.23 [a]	13.4 ± 3.25 [a]	13.0 ± 1.65 [a]
Ca^{2+} ($mmol \cdot l^{-1}$)	2.89 ± 0.23 [a]	2.91 ± 0.09 [a]	2.87 ± 0.13 [a]	2.89 ± 0.18 [a]
PHOS ($mmol \cdot l^{-1}$)	3.32 ± 0.23 [a]	3.40 ± 0.17 [a]	3.41 ± 0.28 [a]	3.35 ± 0.36 [a]

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

Histological examination of tissues

All rainbow trout showed capillary ectasia of gill filaments immediately after clove oil anaesthesia. Twenty-four hours after anaesthesia, sporadic ectasia was only demonstrated at the ends of gill lamellae in 20% of the fish. No histopathological changes were demonstrated in other tissues (liver, spleen, cranial and caudal kidneys) following anaesthesia.

Discussion

Acute toxicity of clove oil to fish is investigated from the point of view of clove oil use as an anaesthetic, and of risk of water contamination with anaesthetizing baths. The 10 min LC50 (LC0.1; LC99.9) values characterize clove oil toxicity in the case of a 10 min exposure to the anaesthetic. Taylor and Roberts (1999) determined 10 min LC50 of clove oil to *Oncorhynchus tchawytcha* and rainbow trout (*Oncorhynchus mykiss*) at 62 $mg \cdot l^{-1}$ and 250 $mg \cdot l^{-1}$, respectively. The 10 min LC50 of clove oil found in the present study of rainbow trout (81.1 $mg \cdot l^{-1}$) is comparable with the figure reported for *Oncorhynchus tchawytcha*, and it is about three time lower than the figure reported for rainbow trout.

To assess the anaesthetic from the point of view of water contamination risks, 96 h LC50 values are used. In their study of juvenile rainbow trout, Keene et al. (1998) reported LC50 for the 8 to 96 h period at approximately 9 ppm (10 $mg \cdot l^{-1}$). That value is in good agreement with 96 h LC50 of 14.1 $mg \cdot l^{-1}$ for rainbow trout fry found in the present study.

The generally reported clove oil concentration as a fish anaesthetic is 30 $mg \cdot l^{-1}$ (Svoboda and Kolářová 1999). Prince and Powell (2000) also recommended the concentration of 30 $mg \cdot l^{-1}$ clove oil for effective and safe anaesthesia of adult rainbow trout. Drawing on the results of their study into clove oil effects on rainbow trout, Keene et al. (1998) concluded that clove oil was a suitable anaesthetic for aquaculture purposes. The concentrations used (40-60 ppm) successfully induced anaesthesia in juvenile rainbow trout with a relatively short recovery period. Griffiths (2000) recommended a concentration of 40 $mg \cdot l^{-1}$ clove oil for rainbow trout anaesthesia. At that concentration, anaesthesia is induced in 4 min and the fish will recover in 14 min. Soto and Burhanuddin (1995) and Anderson et al. (1997) used clove oil at concentrations 33-120 $mg \cdot l^{-1}$ in their studies with *Siganus lineatus* and rainbow trout. At the concentration of 33 $mg \cdot l^{-1}$, rainbow trout lost equilibrium in 150 sec and were totally immobilized within 190 s.

To evaluate haematological and biochemical profiles of blood and histopathological changes in tissues of rainbow trout, clove oil concentration of 30 $mg \cdot l^{-1}$ 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). Values determined in the present study suggest that internal organs and tissues of rainbow trout are not altered by clove oil anaesthesia. That conclusion was also confirmed by the result of histological examination of parenchymatous organs.

In their study of anaesthetized Atlantic salmon (*Salmo salar*), Iverzen et al. (2003) found no change in the concentration of glucose and increased concentrations of lactate and cortisol following clove oil anaesthesia. An increase in cortisol levels related to clove oil anaesthesia has also been reported by Wagner et al. (2002) in rainbow trout. On the other hand, Holloway et al. (2004) found decrease of cortisol concentration in rainbow trout. In our experiments with rainbow trout, a significant increase ($p < 0.05$) in blood plasma glucose and ammonia immediately after the 10-min clove oil anaesthesia was observed. Increased glucose and ammonia levels returned to normal 24 hours after anaesthesia. These findings are in keeping with results of Holloway et al. (2004) who also detected increase of glucose concentration following clove oil anaesthesia.

Clove oil meets seven out of eight criteria for an ideal anaesthetic (Marking and Meyer 1985). Its main advantage is its low price. The use of clove oil, however, requires that general principles of safe handling of chemicals be observed. The authors know from their own experience that a stay in a poorly ventilated room where clove oil is used may cause headache, nausea and fatigue in vulnerable persons. The disadvantage of clove oil is its relatively low therapeutic index, i.e. the ratio between the therapeutic and the toxic concentrations. The generally reported optimum ratio is 1:4 or higher (Svobodová and Vykusová 1991). A comparison between the concentration used in a 10-min anaesthesia of fish (30 $mg \cdot l^{-1}$) and the 10 min LC50 values found (81.1 $mg \cdot l^{-1}$) suggests that the clove oil therapeutic index is 1:2.7.

Vliv anestetika hřebíčkového oleje na pstruha duhového *(Oncorhynchus mykiss)*

Cílem práce bylo posoudit akutní toxicitu hřebíčkového oleje pro pstruha duhového a pomocí hodnot hematologického vyšetření, biochemického profilu krve a histologického vyšetření tkání posoudit stav tkání pstruhů duhových po působení tohoto anestetika. Akutní toxicita hřebíčkového oleje pro pstruha duhového byla následující: 10 min LC50 81.1 $mg \cdot l^{-1}$; 10 min LC0.1 63.9 $mg \cdot l^{-1}$; 10 min LC99.9 100.1 $mg \cdot l^{-1}$; 96 h LC50 14.1 $mg \cdot l^{-1}$; 96 h LC0.1 12.5 $mg \cdot l^{-1}$; 96 h LC99.9 16.2 $mg \cdot l^{-1}$. Hřebíčkový olej neovlivnil hematologický profil krve pstruha duhového. Po 10 min anestézie hřebíčkovým olejem bylo zaznamenáno významné zvýšení ($p < 0.05$) koncentrace glukózy (GLU) a amoniaku (NH_3) a významné snížení ($p < 0.05$) aktivity aspartátaminotransferázy (AST). Ta byla významně snížena i za 24 hodin po anestézii.

Histologické vyšetření ryb po anestézii prokázalo výskyt ektázií na žaberních lístečcích, 24 hodin po anestézii byly ektázie prokázány ojediněle u 20% kusů. V ostatních tkáních (játra, slezina, kraniální a kaudální ledvina) nebyly zjištěny histopatologické změny.

Výsledky ukázaly, že hřebíčkový olej v koncentraci 30 $mg \cdot l^{-1}$ se jeví pro pstruha duhového bezpečný.

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ects of clove oil anaesthesia on common carp *prinus carpio* L.)

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RACT: The aim of the study was to investigate acute toxicity of clove oil for common carp and, using values matological and biochemical profiles of blood and histological tissue examinations, to assess the effects of h exposure to that anaesthetic. Acute toxicity values of clove oil for carp were found 10 minLC50 74.3 mg/l; LC0.1 51.6 mg/l; 10minLC99.9 110.1 mg/l; 96hLC50 18.10 mg/l; 96hLC0.1 15.45 mg/l; and 96hLC99.9 ng/l. The fish were divided into four groups for haematological and biochemical examinations of blood stological examinations of tissues. The groups were Control I (before the anaesthetic administration), ment I (immediately after 10 min anaesthesia at the concentration of 30 mg/l), Experiment II (24 hrs after anaesthesia) and Control II (controls examined in parallel with Experiment II). A total of 40 carp were ied. Clove oil anaesthesia had not effect on the haematological profile. The 10-min exposure to clove oil ncentration of 30 mg/l caused a significant ($P < 0.01$) increase in the concentration of glucose (GLU) and nic phosphate (PHOS) immediately after anaesthesia. Clove oil anaesthesia had not effect on other bio- al indices. Histological examination showed capillary ectasia of gill filaments immediately after clove oil esia. Twenty-four hours after anaesthesia, no ectasia was observed. No histopathological changes were strated in other tissues following anaesthesia. Results of the examinations suggest that the use of clove oil ncentration of 30 mg/l does not cause irreversible damage in carp.

rds: acute toxicity; haematological profile; biochemical profile of blood; histological examination of tis-

sthetics are used with increasing frequency aculture, mainly to reduce the stress and to t mechanical damage to fish during handling. ase is particularly common in stripping, g, biometry, health checks, etc. (Mundey lson, 1997; Ross and Ross, 1999). The use of ietics is also one of foremost requirements Protection of Animals Against Torture Act 92 Sb.

esent, clove oil is used in the Czech Republic rt-term immobilization of fish before artificial spawning. The recommended concentration for anaesthetic purposes is 30 mg/l water bath (Svoboda and Kolarova, 1999; Hamackova et al., 2001).

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove tree *Eugenia aromatica* (Soto and Burhanuddin, 1995). Clove oil is also distilled from stems, leaves and flower buds of *Eugenia caryophyllata*, and its active ingredient, i.e. eugenol (4-allyl-2-methoxyphenol), makes up 70 to 90% by weight (Isaacs, 1983; Briozzo et al.,

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Keene et al., 1998). Besides eugenol acetate
5) and cariofilen 5 (> 12%), clove oil also con-
a very broad range of terpene compounds
ive the oil its characteristic smell and taste
and Ross, 1999; Taylor and Roberts, 1999).
se of its properties, eugenol is used in a va-
)f different applications, e.g. as an antioxi-
Kremer, 1985; Nagababu and Lakshmaiah,
Pulla Reddy and Lokesh, 1992; Rajakumar
ıo, 1993), antimycotic (Bullerman et al., 1977;
inar, 1990) and an antibacterial (Karapinar
ktug, 1987; Briozzo et al., 1989; Moleyar and
imham, 1992), but also as an additive used in
ı cigarettes (Voie et al., 1986; Guidotti, 1989).
et al. (1972) described the use of clove oil as
ıesthetic in warm-blooded animals.
resent, effects on clove oil on commercially
ced fish are studied in a project regarding the
ation of principles of pharmacovigilancy in
ulture in the Czech Republic. In the first stage
project, effects of clove oil on rainbow trout
tudied (Velisek et al., 2005). The aim of the
ıt study was to investigate acute toxicity of
)il in carp and, on the basis of haematologi-
lices, biochemical blood profile values and
)gical examination of tissues, to assess the
es in the organism of carp induced by the
hetic.

ERIAL AND METHODS

ıe study, clove oil marketed by the Kulich
any (Jan Kulich, Hradec Kralove/Ricany, CR)
nl and 50 ml containers was used.

toxicity of clove oil

te toxicity of clove oil was ascertained by the
) 203 "Test of acute toxicity for fish". For the
nd 10 min LC50 trials, carp (mirror carp M
15.0 ± 5.0 g (mean ± SD) body weight and
21 mm body length were used.

96-h LC50 test: Experimental fish were
ed to concentrations 5, 10, 13, 15, 18, 20
) mg/l clove oil dissolved in diluting wa-
H 7.62; acid neutralization capacity – $ANC_{4.5}$
mol/l; total ammonia 0.04 mg/l; NO_3^- 7.35 mg/l;
.002 mg/l; PO_4^{3-} 0.02 mg/l; chemical oxygen
ıd – COD_{Mn} 1.6 mg/l), and controls were
in diluting water with no tested substance added. Ten carp were used for each concentration and for the control group. The fish and its 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.

The 10-min LC50 test: For 10 min, the fish were exposed to concentrations of 30, 50, 70, 90 and 110 mg/l of clove oil dissolved in diluting water. Ten carp 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 carp 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 minutes.

Within the tests, the onsets of individual phases of anaesthesia and recovery rates were studied. Evaluations were made in four consecutive phases (Thienpoint 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 at 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 profile after exposure to clove oil

For the haematological profile tests, carp (mirror carp M 72) of 525 ± 43 g (mean ± SD) body weight and 320 ± 30 mm body length were used. A total of 40 fish divided into four groups were examined: Control I (before the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia at the concentration of 30 mg/l), Experiment II (24 hrs after 10 min anaesthesia) and Control II

rols examined in parallel with Experiment II). rinized injection needles were used to take les of blood from hearts of fish stunned by a with a blunt object over the head. To stabilize samples, aqueous solution of heparin sodium 000 U/ml at 0.01 ml per 1 ml blood was used odova et al., 1991).

indices used to evaluate the haematological e included the erythrocyte count (Er), haebin concentration (Hb), haematocrit (PCV), erythrocyte volume (MCV), mean colour ntration (MCHC), erythrocyte haemoglobin l), leukocyte count (Leuko) and the differenukocyte count (leukogram). The procedures based on Unified methods for haematological ination of fish (Svobodova et al., 1991).

ults of haematological examinations were l by the variance analysis using the Statgrafics VA – Tuckey Test) software.

emical blood plasma profile exposure to clove oil

biochemical profile of blood plasma tests, (mirror carp M 72) of 525 ± 43 g (mean ± ody weight and 320 ± 30 mm body length used.

od plasma was obtained by centrifuging blood les in a cooled centrifuge (4°C, 837 × g). emical indices determined in blood plasma led glucose (GLU), total protein (TP), albumin), total globulins (GLOB), ammonia (NH_3), lglycerols (TAG), aspartate aminotransferase), alanin aminotransferase (ALT), lactate degenase (LDH), creatin 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 variance analysis using the Statgrafics (ANOVA – Tuckey Test) software.

Histological examination of tissues

For histological examination of tissues, carp (mirror carp M 72) of 525 ± 43 g (mean ± SD) body weight and 320 ± 30 mm body length were used.

After blood sampling, samples of gills, liver, cranial and caudal kidney, and spleen and skin were taken for histological examinations. The samples taken 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 clove oil. During the 96-hour LC50 tests, the mean water temperature was 20.0 to 21.1°C, pH was 7.43–7.71 and water oxygen levels were 80–98% saturation. On the basis of tests of acute toxicity to carp, the 96-hour lethal concentrations of clove oil were determined (96hLC50 18.10 mg/l, 96hLC0.1 15.45 mg/l and 96hLC99.9 19.80 mg/l).

The autopsy performed after the acute toxicity test revealed increased amounts of watery mucous on body surfaces, and the gills were matt dark in

1. Effects of clove oil anaesthesia on haematological indices in carp (n = 10)

	Control I (before anaesthesia)	Experiment I (immediately after anaesthesia)	Experiment II (24 hrs after anaesthesia)	Control II (after 24 hrs)
)	1.66 ± 0.28[a]	1.65 ± 0.37[a]	1.58 ± 0.21[a]	1.65 ± 0.21[a]
)	74.25 ± 11.29[a]	80.0 ± 10.17[a]	84.22 ± 4.14[a]	81.0 ± 8.83[a]
/l)	0.27 ± 0.04[a]	0.32 ± 0.05[a]	0.33 ± 0.05[a]	0.29 ± 0.03[a]
fl)	164.61 ± 19.69[a]	197.21 ± 36.91[a]	210.57 ± 28.45[a]	176.80 ± 27.09[a]
pg)	45.14 ± 4.74[a]	50.04 ± 8.81[a]	53.90 ± 6.43[a]	49.59 ± 6.67[a]
(g/l)	275.53 ± 21.88[a]	255.45 ± 27.61[a]	256.62 ± 12.10[a]	281.54 ± 18.22[a]
G/l)	27.10 ± 9.20[a]	33.0 ± 10.85[a]	23.50 ± 7.98[a]	28.10 ± 9.34[a]

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

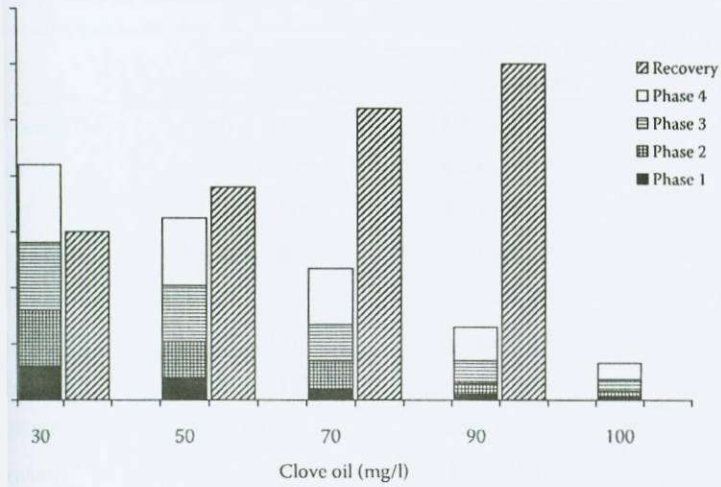

Figure 1. Effects of clove oil concentrations on the onset of individual phases of anaesthesia and recovery in carp

r. The body cavity contained excess moisture, n increased injection of visceral vessels was btained.

ing 10-min LC50 tests, water temperature 9.9°C, pH was 7.50 and water oxygen level t 98% saturation. On the basis of tests of toxicity to carp, the 10-min lethal concenns of clove oil were determined (10minLC50 ıg/l, 10minLC0.1 51.6 mg/l and 10minLC99.9 mg/l).

cts of clove oil concentrations on the time et of anaesthesia, duration of its individual and the course of recovery are showed on 1.

Haematological profile after exposure to clove oil. Effects of clove oil on the haematological profile of carp are showed in Tables 1 and 2.

The 10-min exposure to the anaesthetic at a concentration of 30 mg/l had no effect on the haematological indices studied (Er, Hb, PCV, MCV, MCHC, MCH, Leuko, leukogram).

Biochemical blood plasma profile after exposure to clove oil. Effects of clove oil on the blood plasma biochemical profile of carp are given in Table 3. The 10-min exposure to clove oil at a concentration of 30 mg/l caused a significant ($P < 0.01$) increase in the concentration of glucose and inorganic phosphate immediately after anaesthe-

2. Effects of clove oil anaesthesia on differential leukocyte counts in carp ($n = 10$)

		Control I (before anaesthesia)	Experiment I (immediately after anaesthesia)	Experiment II (24 hrs after anaesthesia)	Control II (after 24 hrs)
ocytes	%	82.50 ± 7.69[a]	79.70 ± 5.93[a]	79.60 ± 7.54[a]	75.60 ± 10.67[a]
	G/l	22.32 ± 8.15[a]	26.44 ± 9.73[a]	18.91 ± 7.07[a]	29.20 ± 7.98[a]
ytes	%	1.50 ± 1.35[a]	1.30 ± 1.06[a]	1.50 ± 1.51[a]	2.40 ± 1.35[a]
	G/l	0.38 ± 0.36[a]	0.40 ± 0.28[a]	0.31 ± 0.31[a]	0.83 ± 0.34[a]
phile granulocytes	%	4.20 ± 4.64[a]	4.0 ± 2.31[a]	5.80 ± 4.92[a]	7.70 ± 4.42[a]
t	G/l	1.19 ± 1.28[a]	1.40 ± 1.01[a]	1.25 ± 0.89[a]	3.16 ± 2.34[a]
phile granulocytes	%	3.80 ± 2.74[a]	3.70 ± 2.71[a]	3.90 ± 2.69[a]	5.40 ± 3.31[a]
	G/l	1.12 ± 1.01[a]	1.40 ± 1.01[a]	0.98 ± 1.01[a]	1.98 ± 1.14[a]
mental phases	%	8.00 ± 2.35[a]	11.30 ± 3.55[a]	9.20 ± 2.12[a]	8.90 ± 4.31[a]
id sequence	G/l	2.17 ± 0.64[a]	3.75 ± 1.17[a]	2.16 ± 0.50[a]	2.50 ± 1.21[a]

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

e 3. Effects of clove oil anaesthesia on biochemical indices of blood plasma in carp (n = 10)

es	Control I (before anaesthesia)	Experiment I (immediately after anaesthesia)	Experiment II (24 hrs after anaesthesia)	Control II (after 24 hrs)
(mmol/l)	6.56 ± 1.23[a]	8.87 ± 1.02[b]	6.97 ± 1.41[a]	6.98 ± 1.18[a]
;/l)	34.20 ± 5.10[a]	34.70 ± 4.68[a]	33.90 ± 5.26[a]	34.30 ± 5.48[a]
(g/l)	7.80 ± 1.20[a]	7.90 ± 0.89[a]	7.40 ± 1.11[a]	8.0 ± 1.02[a]
B (g/l)	26.40 ± 2.48[a]	26.80 ± 3.10[a]	26.60 ± 2.21[a]	26.0 ± 2.68[a]
(μmol/l)	534.0 ± 2.03[a]	529.0 ± 2.59[a]	526.0 ± 3.54[a]	530.0 ± 2.69[a]
(mmol/l)	1.21 ± 0.11[a]	1.20 ± 0.24[a]	1.23 ± 0.16[a]	1.22 ± 0.13[a]
(μkat/l)	1.40 ± 0.19[a]	1.70 ± 0.23[a]	1.57 ± 0.14[a]	1.53 ± 0.18[a]
(μkat/l)	0.23 ± 0.05[a]	0.25 ± 0.04[a]	0.23 ± 0.03[a]	0.22 ± 0.05[a]
(μkat/l)	4.05 ± 0.44[a]	4.73 ± 0.61[a]	3.99 ± 0.51[a]	4.21 ± 0.60[a]
μkat/l)	13.50 ± 0.09[a]	13.50 ± 0.10[a]	13.70 ± 0.08[a]	13.60 ± 0.09[a]
(mmol/l)	2.48 ± 0.21[a]	2.52 ± 0.11[a]	2.46 ± 0.32[a]	2.48 ± 0.18[a]
S (mmol/l)	1.26 ± 0.11[a]	3.41 ± 0.42[b]	1.34 ± 0.16[a]	1.41 ± 0.12[a]

ps with different alphabetic superscripts differ significantly at $P < 0.01$ (ANOVA)

Their values returned back to normal within ours. The rest of the indices (TP, ALB, GLOB, ,TAG, AST, ALT, LDH, CK, and Ca^{2+}) were at parable levels in all groups.

istological examination of tissues. All speci- s of common carp showed capillary ectasia of ilaments immediately after clove oil anaesthe- Twenty-four hours after anaesthesia, no ectasia observed. No histopathological changes were onstrated in other tissues (liver, spleen, cranial caudal kidneys) following anaesthesia.

CUSSION

kasa et al. (1986) recommended 25–100 ppm e oil as effective anaesthesia for the common (*Cyprinus carpio*). It has been demonstrated onset times of individual stages of clove oil sthesia as well as recovery times (Figure 1) concentration-dependant. The same effect naesthetic concentration levels on anaesthe- nset times has been described by Hirata et al. 0) for the crucian carp (*Carassius carassius*) by Hamackova et al. (2004) for the tench (*Tinca*).

aterstrat (1999) reported 100 mg/l clove oil as e concentration for anaesthesia of the channel sh (*Ictalurus punctatus*), adding that exposures longer than 15 min prolonged recovery times and increased mortality. Walsh and Pease (2002) recommended 60–80 mg/l clove oil for anaesthesia of anguillid eels (*Anguilla reinhardti*) because it is effective, relatively inexpensive, and poses little risk to human health.

To evaluate haematological and biochemical profiles of blood and histopathological changes in tissues of carp, clove oil concentration of 30 mg/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). Values determined in the present study suggest that internal organs and tissues of carp are not altered by clove oil anaesthesia. That conclusion was also confirmed by the result of histological examination of parenchymatous organs.

In our experiments with carp, a significant increase ($P < 0.01$) in blood plasma glucose immediately after the 10-min clove oil anaesthesia was observed. Increased glucose level returned to normal 24 hours after anaesthesia. Increased blood plasma glucose level after anaesthesia indicates that the procedure caused some stress in the carp. These findings are in accord with results of Holloway et al. (2004) and Velisek et al. (2005) who also detected increase of glucose concentration in rainbow trout (*Oncorhynchus mykiss*) following

e oil anaesthesia. On the other hand, Iverzen et 003) found no change in the concentration of ose in Atlantic salmon (*Salmo salar*) following e oil anaesthesia.

e disadvantage of clove oil is its relatively low apeutic index, i.e. the ratio between the thera- ic and the toxic concentrations. The generally rted optimum ratio is 1 : 4 or higher (Svobodova Vykusova, 1991). A comparison between the entration used in a 10-min anaesthesia of fish g/l) and the 10minLC50 values found (74.3 mg/ ggests that the clove oil therapeutic index is 1 : According to Taylor and Robers (1999) clove oil efficient and relatively safe anaesthetic.

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5. VĚDECKÁ KONFERENCE S MEZINÁRODNÍ ÚČASTÍ

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IE EFFECT OF CLOVE OIL ANAESTHETIC ON HEAMATOLOGICAL AND BIOCHEMICAL PROFILE OF SHEATFISH (*Silurus glanis* L.)

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TRACT

The goal of this study was to asses the effect of clove oil anaesthesia on the sheatfish e, expressed by means of some haematological and biochemical indices. The atological and biochemical blood profile was examinated before and immediately after in anaesthesia and 24 hours after the anaesthesia with recommended concentration of 30 ^{1} clove oil.The determination of changes in the biochemical profile of blood was rmed by means of biochemical analysis of blood plasma with VETTEST 8008 Analyser isoft Co.).

The 10-min exposure to clove oil at a concentration of 30 mg.l^{-1} caused a significant (*p* 5) decrease in mean corpuscular haemoglobin concentration (MCHC) immediately after thesia. The leukocyte count (Leuko) was decreased 24 hours after anaesthesia. The rest indices [erythrocyte number (Er), haemoglobin concentration (Hb), haematocrit (PCV), cellular volume (MCV) and mean corpuscular haemoglobin (MCH)] were at arable levels in all groups.

The 10-min exposure to clove oil at a concentration of 30 mg.l^{-1} caused a significant (p)5) increase in the concentration of triacylglycerols and alanin aminotransferase diately after anaesthesia. Their values returned back to normal within 24 hours. The of lactate dehydrogenase were found increased 24 hrs post anaesthesia. The rest of the es [glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), onia (NH_3), aspartate aminotransferase (AST), creatinkinase (CK), calcium (Ca^{2+}) and anic phosphate (PHOS)] were at comparable levels in all groups.

words: ***anaesthesia, blood plasma, glucose, proteins, enzymes, minerals***

RODUCTION

Prevention from indesirable injuries of the fish (from damage of the mucous layer to injuries in musculature or on fins) following handling is one of important requirements h health protection from the point of view of fish health, as well as meets the act for l protection against cruelty (No. 246/1992 in the Czech code of laws).

Great attention has to be paid to careful and professional handling of brood fish, ly during the artificial propagation. Fish in the spawning period are very sensitive to anical injuries and they are subjected to repeated handling during the spawning season. pplication of anaesthetic should prevent the fish from mechanical injuries and it should ate easier handling larger fish species during veterinary interventions (Čítek et al. 1997; and Ross 1999).

IEW

Clove oil is used as an anaesthetic before handling or treating fish in breeding, ial propagation, blood sampling or for some other veterinary interventions. The use of aesthetic facilitates the handling of too big or too agile fish species (Trzebiatowski et al. Iwama et al. 1997; Wagner et al. 2002). At present, clove oil is used in the Czech blic for short-term immobilization of fish before artificial spawning and whenever fish is ed outside water. The recommended concentration for anaesthetic purposes is 30 $mg.l^{-1}$ bath (Svoboda and Kolářová 1999; Hamáčková et al. 2001).

Clove oil is a dark-brown liquid, a distillate of flowers, stalks and leaves of the clove *ugenia aromatica* (Sato and Burhanuddin 1995). According to Isaacs (1983), Briozza et 989) and Keene et al. (1998), clove oil is distilled from stems, leaves and flower buds of *ia caryophyllata* too, and its active ingredient, i.e. eugenol (4-allyl-2-methoxyphenol), s up 70 to 90% by weight of clove oil. Clove oil also contains eugenol acetate (> 17%) ariofilen 5 (> 12%).

ERIAL AND METHODS

atological profile after exposure to clove oil

Sheatfish of 94.9 g mean weight (29.7 – 154.0 g) and 25.3 cm mean body length – 32.9 cm) were used for determination of biochemical and haematological profile after oil exposure. A total of 40 fish divided into four groups were examined: Control I e the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia at ncentration of 30 $mg.l^{-1}$), Experiment II (24 hrs after 10 min anaesthesia) and Control II ols examined in parallel with Experiment II). The fish were anesthetized for 10 min by oil at a concentration of 30 $mg.l^{-1}$.

Heparinized injection needles were used to take samples of blood from hearts of fish d by a blow with a blunt object over the head. To stabilize blood samples, aqueous n of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodová et al. 1986).

dices used to evaluate the haematological profile included the erythrocyte count (Er), globin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean cular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content) and leukocyte count (Leuko). The procedures were based on Unified methods for ological examination of fish (Svobodová et al. 1986).

Results of haematological examinations were tested by the variance analysis using the fics (ANOVA – Tuckey Test) software.

mical blood plasma profile after exposure to clove oil

Blood plasma was obtained by centrifuging of blood samples in a cooled centrifuge 37 x g). Biochemical indices determined in blood plasma included glucose (GLU), otein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols , aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate genase (LDH), creatinkinase (CK), calcium (Ca^{2+}) and inorganic phosphate (PHOS). biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX ories Inc. U.S.A.) manufactured by Medisoft was used. The analyzer uses dry l and colorimetric analysis techniques. Selective test discs (Multi–layer film slides, are used for the evaluation by a laser reading bar codes.

Results of biochemical examination were tested by the variance analysis using the fics (ANOVA – Tuckey Test) software.

ULTS AND DISCUSSION

matological profile after exposure to clove oil

ts of clove oil on the haematological profile of sheatfish are showed in Tab 1. The 10-exposure to clove oil at a concentration of 30 $mg.l^{-1}$ caused a significant ($p < 0.05$) ease in mean colour concentration immediately after anaesthesia. The leukocyte count decreased 24 hours after anaesthesia. The rest of the indices (Er, Hb, PCV, MCV and H) were at comparable levels in all groups.

e 1: Effects of clove oil anaesthesia on haematological indices in sheatfish

es	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)
$.l^{-1}$)	0.82 ± 0.23 [a]	0.83 ± 0.29 [a]	0.73 ± 0.16 [a]	0.76 ± 0.25 [a]
$.l^{-1}$)	38.54 ± 6.84 [a]	34.92 ± 4.11 [a]	42.96 ± 6.74 [a]	39.51 ± 5.89 [a]
($l.l^{-1}$)	0.21 ± 0.04 [a]	0.22 ± 0.02 [a]	0.195 ± 0.03 [a]	0.195 ± 0.03 [a]
(fl)	266.94 ± 138.50 [a]	269.48 ± 147.40 [a]	249.95 ± 111.80 [a]	229.19 ± 160.40 [a]
(pg)	53.00 ± 24.38 [a]	47.09 ± 23.60 [a]	59.88 ± 18.11 [a]	50.04 ± 46.35 [a]
C ($g.l^{-1}$)	177.56 ± 31.42 [a]	157.56 ± 24.50 [b]	215.41 ± 30.86 [a]	219.67 ± 37.66 [a]
) ($G.l^{-1}$)	20.40 ± 4.04 [a]	14.00 ± 5.86 [a]	9.20 ± 6.59 [b]	14.00 ± 7.50 [a]

esponding values with different alphabetic superscripts differ significantly at $p< 0.05$ DVA).

hemical blood plasma profile after exposure to clove oil

Results of the effect of clove oil anaesthetic on biochemical profile of blood plasma in fish are shown in Table 2. The 10-min exposure to clove oil at a concentration of 30 ^{1} caused a significant ($p < 0.05$) increase in the concentration of triacylglycerols and n aminotransferase immediately after anaesthesia. Their values returned back to normal n 24 hours. The level of lactate dehydrogenase were found increased 24 hrs post thesia. Other indices (GLU, TP, ALB, GLOB, NH_3, AST, CK, Ca^{2+}, PHOS) did not significantly among the groups.

2: Effects of clove oil anaesthesia on biochemical indices of blood plasma in sheatfish

s	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)
mmol.l^{-1})	7.24 ± 2.63 [a]	6.92 ± 1.98 [a]	4.94 ± 1.01 [a]	5.01 ± 1.90 [a]
$^{-1}$)	35.30 ± 4.0 [a]	34.80 ± 1.60 [a]	36.30 ± 2.15 [a]	32.50 ± 2.70 [a]
$.l^{-1}$)	3.80 ± 1.83 [a]	3.50 ± 0.81 [a]	3.90 ± 1.30 [a]	3.70 ± 1.27 [a]
($g.l^{-1}$)	31.60 ± 2.11 [a]	31.70 ± 0.90 [a]	32.40 ± 1.20 [a]	29.60 ± 1.69 [a]
mol.l^{-1})	931.60 ± 68.09 [a]	949.21 ± 78.44 [a]	923.41 ± 81.55 [a]	936.79 ± 76.11 [a]
mmol.l^{-1})	1.28 ± 0.39 [a]	1.90 ± 0.51 [b]	0.94 ± 0.39 [a]	1.07 ± 0.25 [a]
kat.l^{-1})	7.43 ± 0.60 [a]	7.56 ± 0.66 [a]	7.50 ± 0.59 [a]	7.53 ± 0.71 [a]
kat.l^{-1})	0.19 ± 0.09 [a]	0.10 ± 0.11 [b]	0.17 ± 0.13 [a]	0.18 ± 0.10 [a]
kat.l^{-1})	8.96 ± 4.18 [a]	9.06 ± 5.44 [a]	11.07 ± 6.79 [b]	8.89 ± 6.65 [a]
at.l^{-1})	44.82 ± 2.89 [a]	43.21 ± 3.06 [a]	44.99 ± 3.26 [a]	44.08 ± 4.34 [a]
mol.l^{-1})	2.30 ± 0.21 [a]	2.21 ± 0.15 [a]	2.13 ± 0.12 [a]	2.08 ± 0.08 [a]
mmol.l^{-1})	1.16 ± 0.14 [a]	1.40 ± 0.27 [a]	1.35 ± 0.20 [a]	1.37 ± 0.18 [a]

ponding values with different alphabetic superscripts differ significantly at $p< 0.05$ VA).

NCLUSION

The results showed that changes in biochemical and haematological profile of fish ced by effects of the anaesthetic clove oil were only short – term ones from which the recovered within 24 hours. It was therefore possible to recommend either clove oil for sthesia of sheatfish.

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THE EFFECT OF CLOVE OIL ANAESTHETIC HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF SHEATFISH (*SILURUS GLANIS*)

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n from inconsiderate handling followed by injuries of the fish (from damage of the mucous layer to deep injuries in musculature) is one of important requirements of fish health protection from the point of view of fish health, as well as observing the act for otection against cruelty (No. 246/1992 in the Czech code of laws).

Silurus glanis

MATERIALS AND METHODS

The haematological and biochemical profile was assessed in sheatfish before, immediately after 10 min. anaesthesia and 24 hours after anaesthesia with recommended concentrations of 30 mg.l^{-1}.

The indices used to evaluate the haematological profile included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and leukocyte count (Leuko).

ving biochemical indices were determined in blood plasma: glucose (GLU), total proteins (TP), albumins (ALB), total globulins ammonia (NH_3), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase tate dehydrogenase (LDH), calcium (Ca^{2+}) and anorganic phosphate (PHOS).

RESULTS

le 1: Effects of clove oil anaesthesia on haematological indices in sheatfish

Control I. (before anaesthesia) x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 hrs after anaesthesia x ± SD (n = 10)	Control II. (after 24 hrs) x ± SD (n = 10)
0.82 ± 0.23 [a]	0.83 ± 0.29 [a]	0.73 ± 0.16 [a]	0.76 ± 0.25 [a]
38.54 ± 6.84 [a]	34.92 ± 4.11 [a]	42.96 ± 6.74 [a]	39.51 ± 5.89 [a]
0.21 ± 0.04 [a]	0.22 ± 0.02 [a]	0.195 ± 0.03 [a]	0.195 ± 0.03 [a]
266.94 ± 138.50 [a]	269.48 ± 147.40 [a]	249.95 ± 111.80 [a]	229.19 ± 160.40 [a]
53.00 ± 24.38 [a]	47.09 ± 23.60 [a]	59.88 ± 18.11 [a]	50.04 ± 46.35 [a]
177.56 ± 31.42 [a]	157.56 ± 24.50 [b]	215.41 ± 30.86 [a]	219.67 ± 37.66 [a]
20.40 ± 4.04 [a]	14.00 ± 5.86 [a]	9.20 ± 6.59 [b]	14.00 ± 7.50 [a]

exposure to clove oil at the concentration of 30 mg.l^{-1} ignificant ($p < 0.05$) decrease in mean corpuscular n concentration immediately after anaesthesia. The ount was decreased 24 hours after anaesthesia. The indices (Er, Hb, PCV, MCV and MCH) were at levels in all groups.

Table 2: Effects of clove oil anaesthesia on biochemical indices of blood plasma in sheatfish

Indices	Control I. (before anaesthesia) x ± SD (n = 10)	Immediately after anaesthesia x ± SD (n = 10)	24 hrs after anaesthesia x ± SD (n = 10)	Control II. (after 24 hrs) x ± SD (n = 10)
GLU (mmol.l^{-1})	7.24 ± 2.63 [a]	6.92 ± 1.98 [a]	4.94 ± 1.01 [a]	5.01 ± 1.90 [a]
TP (g.l^{-1})	35.30 ± 4.0 [a]	34.80 ± 1.60 [a]	36.30 ± 2.15 [a]	32.50 ± 2.70 [a]
ALB (g.l^{-1})	3.80 ± 1.83 [a]	3.50 ± 0.81 [a]	3.90 ± 1.30 [a]	3.70 ± 1.27 [a]
GLOB (g.l^{-1})	31.60 ± 2.11 [a]	31.70 ± 0.90 [a]	32.40 ± 1.20 [a]	29.60 ± 1.69 [a]
NH_3 (µmol.l^{-1})	931.60 ± 68.09 [a]	949.21 ± 78.44 [a]	923.41 ± 81.55 [a]	936.79 ± 76.11 [a]
TRIG (mmol.l^{-1})	1.28 ± 0.39 [a]	1.90 ± 0.51 [b]	0.94 ± 0.39 [a]	1.07 ± 0.25 [a]
AST (µkat.l^{-1})	7.43 ± 0.60 [a]	7.56 ± 0.66 [a]	7.50 ± 0.59 [a]	7.53 ± 0.71 [a]
ALT (µkat.l^{-1})	0.19 ± 0.09 [a]	0.10 ± 0.11 [b]	0.17 ± 0.13 [a]	0.18 ± 0.10 [a]
LDH (µkat.l^{-1})	8.96 ± 4.18 [a]	9.06 ± 5.44 [a]	11.07 ± 6.79 [a]	8.89 ± 6.65 [a]
CK (µkat.l^{-1})	44.82 ± 2.89 [a]	43.21 ± 3.06 [a]	44.99 ± 3.26 [a]	44.08 ± 4.34 [a]
Ca^{2+} (mmol.l^{-1})	2.30 ± 0.21 [a]	2.21 ± 0.15 [a]	2.13 ± 0.12 [a]	2.08 ± 0.08 [a]
PHOS (mmol.l^{-1})	1.16 ± 0.14 [a]	1.40 ± 0.27 [a]	1.35 ± 0.20 [a]	1.37 ± 0.18 [a]

The 10 min exposure to clove oil at the concentration of 30 mg.l^{-1} caused a significant ($p < 0.05$) increase in the concentration of triacylglycerols and alanin aminotransferase immediately after anaesthesia. Their values returned back to normal within 24 hours. The level of lactate dehydrogenase was found increased 24 hrs post anaesthesia. The other indices (GLU, TP, ALB, GLOB, NH_3, AST, CK, Ca^{2+}, PHOS) did not differ significantly among the groups.

CONCLUSIONS

ts showed that changes in haematological and al profile of fish induced by the effects of e clove oil were only short – term ones from which ecovered within 24 hours. It was therefore possible end either clove oil for anaesthesia of steatfish.

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Effects of Clove Oil Anaesthesia on European Catfish (*Silurus glanis* L.)

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Abstract

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The aim of the study was to investigate acute toxicity of clove oil for European catfish and, using values of haematological and biochemical profiles of blood and histological tissue examinations, to assess the effects of the fish exposure to that anaesthetic. Acute toxicity values of clove oil for European catfish found were 10minLC50 76.70 $mg \cdot l^{-1}$; 10minLC0.1 49.60 $mg \cdot l^{-1}$; 10minLC99.9 118.50 $mg \cdot l^{-1}$; 96hLC50 18.40 $mg \cdot l^{-1}$; 96hLC0.1 10.70 $mg \cdot l^{-1}$; and 96hLC99.9 31.90 $mg \cdot l^{-1}$. Individual phases of anaesthesia and recovery were determined.

The 10-min exposure to clove oil at a concentration of 30 $mg \cdot l^{-1}$ caused a significant ($p < 0.05$) increase in the concentration of triacylglycerols (TRIG), alanin aminotransferase (ALT) and decreased ($p < 0.05$) in mean corpuscular haemoglobin concentration (MCHC) immediately after anaesthesia. The leukocyte counts were significantly ($p < 0.05$) decreased 24 hours after anaesthesia. A significant ($p < 0.05$) decrease of percentage distribution lymphocytes was found immediately after anaesthesia. On the other hand, percentage and absolute count of myeloid cells were increased. Increased percentage count of eosinophils outlasted 24 hours after anaesthesia, absolute counts of these cells were consistents with control. Histological examination 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 following anaesthesia. Results of the examinations suggest that the use of clove oil at a concentration of 30 $mg \cdot l^{-1}$ does not cause irreversible damage in European catfish.

Acute toxicity, haematological profile, biochemical profile of blood, histological examination of tissues

Anaesthesia, euthanasia and sedation of both wild and captive fish are common requirements in aquaculture and fisheries research around the world. These clinical techniques facilitate a wide variety of activities such as sorting, grading, transportation, tagging, gamete collections, health monitoring, weight/length measurements, blood sampling and invasive surgery to name a few. In all countries with animal care legislation, anaesthetics are routinely required during procedures that are deemed stressful or painful to fish.

The first reports of the use of eugenol containing products as fish anaesthetics date back more than 30 years (Endo et al. 1972) but the use of clove oil as an anaesthetic has been examined more closely in recent years (Soto and Burhanuddin 1995; Anderson et al. 1997; Munday and Wilson 1997; Keene et al. 1998; Peake 1998; Wagner et al. 2002; Iversen et al. 2003) and some variation in efficacy amongst fish species and life stages seems to occur.

Clove oil is derived from the stem, leaves and buds of the *Eugenia caryophylatta* tree, and it contains the active ingredient eugenol (4-allyl-methoxyphenol) in concentrations of 80 -

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90% by volume. Eugenol is commonly used as an analgesic and antiseptic agent in human dentistry (Curtis 1990), and as a food additive for flavouring (Maura et al. 1989), and has been demonstrated to be extremely safe for humans (Miller et al. 1983). Eugenol can be used as an antimycotic agent in warm water fish aquaculture, but for salmonids it is highly toxic (Hussein et al. 2000).

Clove oil 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 30 $mg \cdot l^{-1}$ water bath (Svoboda and Kolářová 1999; Hamáčková et al. 2001). At present, effects on clove oil 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 (Velíšek and Svobodová 2004a) and rainbow trout (Velíšek and Svobodová 2004b) were studied. In the second stage of the project, effects of clove oil on rainbow trout (Velíšek et al. 2005a) and common carp (Velíšek et al. 2005b) were studied. The aim of the present study was to investigate acute toxicity of clove oil in European catfish and, on the basis of haematological indices, biochemical blood profile values and histological examinations, to assess the changes in the organism of European catfish induced by the anaesthetic.

Materials and Methods

In the study, clove oil marketed by the Kulich Company (Jan Kulich, Hradec Králové/Říčany, CR) in 10 ml and 50 ml containers was used.

Acute toxicity of clove oil

Acute toxicity of clove oil was ascertained by the OECD 203 "Fish, acute toxicity test". For the 96 h and 10 min LC50 trials, European catfish of 4.50 ± 1.07 g (mean ± SD) average weight and 83 ± 25 mm average body length were used.

The 96-h LC50 test:

Experimental fish were exposed to concentrations 5, 10, 16, 18, 30 and 40 $mg \cdot l^{-1}$ clove oil dissolved in diluting water (pH 7.51; acid neutralization capacity – $ANC_{4.5}$ 1.29 $mmol \cdot l^{-1}$; total ammonia 0.03 $mg \cdot l^{-1}$; NO_3^- 7.45 $mg \cdot l^{-1}$; NO_2^- 0.003 $mg \cdot l^{-1}$; PO_4^{3-} 0.02 $mg \cdot l^{-1}$; chemical oxygen demand – COD_{Mn} 1.5 $mg \cdot l^{-1}$), and controls were placed in diluting water with no tested substance added. Ten piece European catfish were used for each concentration and for the control group. The fish and its 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.

The 10-min LC50 test:

For 10 min, the fish were exposed to concentrations of 30, 50, 60, 80, 90, and 110 $mg \cdot l^{-1}$ of clove oil dissolved in diluting water. Ten European catfish 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 European catfish had been moved to clean water, the time of their recovery and fish kill (in period 30 min.) 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.

In the tests, the onsets of individual phases of anaesthesia and recovery rates were studied. Evaluations were made in four consecutive phases (Thienpoint 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 at 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 and biochemical blood plasma profile after exposure to clove oil

For the haematological and biochemical blood profile tests and histological examination of tissues, European catfish of 94.90 ± 55.23 g average weight and 253.0 ± 74.60 mm average body length were used. A total of 40 fish divided into four groups were examined: Control I (before the anaesthetic administration), Experiment

l [immediately after 10 min anaesthesia (exposure) at the concentration of 30 mg·l^{-1}], Experiment II (24 hrs after 10 min anaesthesia) and Control II (controls examined in parallel with Experiment II). The fish were anesthetized for 10 min by clove oil at a concentration of 30 mg·l^{-1}. Heparinized injection needles were used to take samples of blood from hearts 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. 1986).

Results of heamatological and biochemical examination were tested by the variance analysis using the Statistica 6.0 (ANOVA – Tuckey Test) software.

Haematological profile

The indices used to evaluate the haematological profile included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (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. 1986).

Biochemical blood plasma profile

Blood plasma was obtained by centrifuging blood samples in a cooled centrifuge (4 °C, 837 × *g*). Biochemical indicators determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), creatin 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. The analyzer uses dry chemical and colorimetric analysis techniques. Selective test discs (Multi–layer film slides, Kodak) are used for the evaluation by a laser reading bar codes.

Histological examination of tissues

After blood sampling, samples of gills, skin, 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 clove oil

During the 96-hour LC50 tests, the mean water temperature was 19.7 - 20.2 °C, pH was 7.41 - 7.86 and water oxygen levels were 76 - 96% saturation. On the basis of tests of acute toxicity to European catfish, the 96-hour lethal concentrations of clove oil were determined (see Table 1).

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 obtained.

During 10-min LC50 tests, water temperature was 19.8 °C, pH was 7.64 and water oxygen was at 95% saturation. On the basis of tests of acute toxicity to European catfish, the 10-min lethal concentrations of clove oil were determined (see Table 1).

Table 1. Acute toxicity of clove oil in European catfish

Lethal concentration	Clove oil (mg·l^{-1})
10minLC0.1	49.60
10minLC50	76.70
10minLC99.9	118.50
96hLC0.1	10.70
96hLC50	18.40
96hLC99.9	31.90

Effects of clove oil concentrations on the time of onset of anaesthesia, duration of its individual stages and the course of recovery are shown in Fig. 1.

Haematological blood profile after exposure to clove oil

Effects of clove oil on the haematological profile of European catfish are shown in Tables 2 and 3. The 10-min exposure to clove oil at a concentration of 30 mg·l^{-1} caused a significant

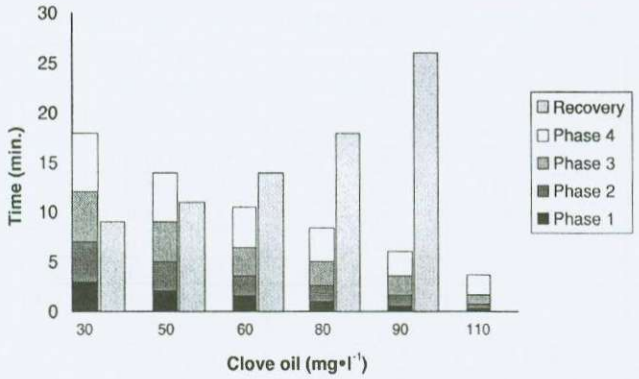

Fig. 1. Effects of clove oil concentrations on the onset of individual phases of anaesthesia and recovery in European catfish

($p < 0.05$) decrease in mean corpuscular haemoglobin concentration immediately after anaesthesia. The leukocyte counts were significantly ($p < 0.05$) decreased 24 h after anaesthesia. A significant ($p < 0.05$) decrease of percentage count of lymphocytes was found immediately after anaesthesia. On the other hand, percentage and absolute counts of myeloid cells were increased. Increased percentage count of eosinophils outlasted 24 h after anaesthesia, absolute counts of these cells were consistent with controls. The rest of the indicators (Er, Hb, PCV, MCV and MCH) were comparable in all groups.

Table 2. Effects of clove oil anaesthesia on haematological indices in European catfish

Indicators	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)
Er ($T \cdot l^{-1}$)	0.82 ± 0.23 [a]	0.83 ± 0.29 [a]	0.73 ± 0.16 [a]	0.76 ± 0.25 [a]
Hb ($g \cdot l^{-1}$)	38.54 ± 6.84 [a]	34.92 ± 4.11 [a]	42.96 ± 6.74 [a]	39.51 ± 5.89 [a]
PCV ($l \cdot l^{-1}$)	0.21 ± 0.04 [a]	0.22 ± 0.02 [a]	0.195 ± 0.03 [a]	0.195 ± 0.03 [a]
MCV (fl)	266.94 ± 138.52 [a]	269.48 ± 147.40 [a]	249.95 ± 220.80 [a]	229.19 ± 160.49 [a]
MCH (pg)	53.0 ± 24.38 [a]	47.09 ± 23.60 [a]	59.88 ± 18.11 [a]	50.04 ± 46.35 [a]
MCHC ($g \cdot l^{-1}$)	177.56 ± 31.42 [a]	157.56 ± 24.50 [a]	215.41 ± 30.86 [a]	171.67 ± 37.66 [a]
Leuko ($G \cdot l^{-1}$)	20.40 ± 4.04 [a]	14.0 ± 5.86 [a]	9.20 ± 6.59 [a]	14.0 ± 7.50 [a]

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

Biochemical blood plasma profile after exposure to clove oil

Effects of clove oil on the blood plasma biochemical profile of European catfish are given in Table 4. The 10-min exposure to clove oil at a concentration of 30 $mg.l^{-1}$ caused a significant ($p < 0.05$) increase in the concentration of triacylglycerols and alanine aminotransferase immediately after anaesthesia. Their values returned to physiological values within 24 h. The rest of the indicators (GLU, TP, ALB, GLOB, NH_3, AST, LDH, CK, Ca^{2+}, PHOS) were comparable in all groups.

Histological examination of tissues

All specimens of European catfish showed capillary ectasia of gill filaments immediately after clove oil anaesthesia. Twenty-four hours after anaesthesia, no ectasia was observed.

Table 3. Effects of clove oil anaesthesia on differential leukocyte counts in European catfish

Indicators		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)
Lymphocytes	%	66.20 ± 10.27 [a]	43.75 ± 7.49 [a]	73.00 ±10.40 [a]	78.70 ± 7.06 [a]
	G·l^{-1}	13.50 ± 2.09 [a]	6.13 ± 1.05 [a]	6.71 ± 0.96 [a]	11.04 ± 1.00 [a]
Monocytes	%	0.90 ± 1.22 [a]	0.35 ± 0.78 [a]	0.30 ± 0.33 [a]	0.45 ± 0.79 [a]
	G·l^{-1}	0.18 ± 0.25 [a]	0.05 ± 0.09 [a]	0.03 ± 0.03 [a]	0.06 ± 0.11 [a]
Neutrophil granulocytes segments	%	17.0 ± 6.54 [a]	16.75 ± 5.37 [a]	6.10 ± 3.90 [b]	5.35 ± 2.34 [b]
	G·l^{-1}	3.47 ± 1.33 [a]	2.35 ± 0.75 [a]	0.56 ± 0.36 [b]	0.75 ± 0.33 [b]
Neutrophil granulocytes rods	%	10.95 ± 8.64 [a]	29.25 ± 6.55 [b]	13.90 ± 5.42 [a]	13.65 ± 6.57 [a]
	G·l^{-1}	2.23 ± 1.76 [a]	4.09 ± 0.92 [b]	1.28 ± 0.50 [a]	1.91 ± 0.92 [a]
Developmental phases – myeloid sequence	%	1.40 ± 1.24 [a]	2.25 ± 2.16 [a]	1.20 ± 0.64 [a]	0.90 ±0.58 [a]
	G·l^{-1}	0.27 ± 0.23 [a]	0.32 ± 0.30 [a]	0.11 ± 0.21 [a]	0.13 ± 0.10 [a]
Eosinophils	%	2.80 ± 2.30 [a]	7.65 ± 4.27 [b]	5.4 ± 3.25 [b]	0.85 ± 0.95 [a]
	G·l^{-1}	0.57 ± 0.47 [a]	1.07 ± 0.98 [a]	0.50 ± 0.41 [a]	0.11 ± 0.21 [a]
Basophils	%	0.75 ± 1.15 [a]	0.0 ± 0.0 [a]	0.10 ± 0.20 [a]	0.10 ± 0.30 [a]
	G·l^{-1}	0.15 ± 0.23 [a]	0.0 ± 0.0 [a]	0.0 ± 0.01 [a]	0.01 ± 0.04 [a]

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

Table 4. Effects of clove oil anaesthesia on biochemical indices of blood plasma in European catfish

Indicators	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)
GLU (mmol·l^{-1})	7.24 ± 2.63 [a]	6.92 ± 1.98 [a]	4.94 ± 1,01 [a]	5.01 ± 1.90 [a]
TP (g·l^{-1})	35.30 ± 4.0 [a]	34.80 ± 1.60 [a]	36.30 ± 2.15 [a]	32.50 ± 2.70 [a]
ALB (g·l^{-1})	3.80 ± 1.83 [a]	3.50 ± 0.81 [a]	3.90 ± 1.30 [a]	2.70 ± 1.27 [a]
GLOB (g·l^{-1})	31.60 ± 2.11 [a]	31.70 ± 0.90 [a]	32.40 ± 1.20 [a]	29.60 ± 1.69 [a]
NH_3 (µmol·l^{-1})	931.60 ± 68.09 [a]	949.21 ± 78.44 [a]	923.41 ± 81.55 [a]	936.79 ± 76.11 [a]
TRIG (mmol·l^{-1})	1.18 ± 0.39 [a]	1.90 ± 0.51 [b]	0.94 ± 0.39 [a]	1.07 ± 0.25 [a]
AST (µkat·l^{-1})	7.43 ± 0.60 [a]	7.56 ± 0.66 [a]	7.50 ± 0.59 [a]	7.53 ± 0.71 [a]
ALT (µkat·l^{-1})	0.19 ± 0.09 [a]	0.30 ± 0.11 [b]	0.17 ± 0.13 [a]	0.18 ± 0.10 [a]
LDH (µkat·l^{-1})	8.96 ± 4.18 [a]	9.06 ± 5.44 [a]	10.07 ± 6.79 [a]	8.89 ± 6.65 [a]
CK (µkat·l^{-1})	44.82 ± 2.89 [a]	43.21 ± 3.06 [a]	44.99 ± 3.26 [a]	44.08 ± 4.34 [a]
Ca^{2+} (mmol·l^{-1})	2.30 ± 0.21 [a]	2.21 ± 0.15 [a]	2.13 ± 0.12 [a]	2.08 ± 0.08 [a]
PHOS (mmol·l^{-1})	1.16 ± 0.14 [a]	1.40 ± 0.27 [a]	1.35 ± 0.20 [a]	1.37 ± 0.18 [a]

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

No histopathological changes were demonstrated in other tissues (liver, skin, spleen, cranial and caudal kidneys) following anaesthesia.

Discussion

The generally reported clove oil concentration as a fish anaesthetic is 30 mg·l^{-1} (Svoboda and Kolářová 1999). Waterstrat (1999) and Small (2003) reported 100 mg·l^{-1} clove oil as a safe concentration for anaesthesia of the channel catfish (*Ictalurus punctatus*), adding

that exposures longer than 15 min prolonged recovery times and increased mortality. The main advantages of clove oil are its low cost and relative safety to both fish and fish-handling humans.

Increasing the anaesthetic dose significantly decreased induction times and resulted in increased recovery times for fish. Similar findings have been reported for carp (Endo et al. 1972; Hikasa et al. 1986), rainbow trout (Keene et al. 1998) and Atlantic salmon (Iversen et al. 2003). The effect of clove oil concentrations on induction and recovery times is shown in Fig. 1.

Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust 2000). Values determined in the present study suggest that internal organs and tissues of European catfish are not altered by clove oil anaesthesia. This conclusion was also confirmed by the result of histological examination of parenchymatous organs.

In our experiments with European catfish, a significant increase ($p < 0.05$) in blood plasma triacylglycerols and alanin aminotransferase immediately after the 10-min clove oil anaesthesia was observed. Increased triacylglycerols and alanin aminotransferase returned to physiological values within 24 h anaesthesia.

On the other hand, Velíšek et al. (2005ab) found changes in the concentration of glucose and inorganic phosphate in common carp (*Cyprinus carpio*), increased concentration of glucose, ammonia and a significant decrease in the aspartate aminotransferase in rainbow trout (*Oncorhynchus mykiss*) following clove oil anaesthesia.

In the study of anaesthetized common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*), Velíšek et al. (2005ab) found no change on the haematological profile following clove oil anaesthesia. In our experiments with European catfish, a significant ($p < 0.05$) decrease in mean corpuscular haemoglobin concentration, percentage distribution of lymphocytes and increased percentage and absolute count of myeloid cells immediately after anaesthesia. A significantly ($p < 0.05$) decreased leukocyte count and increased percentage distribution of eosinophils 24 h after anaesthesia.

The disadvantage of clove oil is its relatively low therapeutic index, i.e. the ratio between the therapeutic and the toxic concentrations. The generally reported optimum ratio is 1:4 or higher (Svobodová and Vykusová 1991). A comparison between the concentration used in a 10-min anaesthesia of fish (30 $mg \cdot l^{-1}$) and the 10minLC50 values found (76.70 $mg \cdot l^{-1}$) suggests that the clove oil therapeutic index is 1:2.6. Results of our examinations suggest that the use of clove oil at a concentration of 30 $mg \cdot l^{-1}$ does not cause irreversible damage in European catfish. According to Taylor and Robers (1999) clove oil is an efficient and relatively safe anaesthetic.

Vliv anestetika hřebíčkového oleje na sumce velkého *(Silurus glanis* L.)

Cílem práce bylo posoudit akutní toxicitu hřebíčkového oleje pro sumce velkého a pomocí hodnot hematologického vyšetření, biochemického profilu krve a histologického vyšetření tkání posoudit stav tkání sumce velkého po působení tohoto anestetika. Akutní toxicita hřebíčkového oleje pro sumce velkého byla následující: 10minLC50 76,70 $mg \cdot l^{-1}$; 10minLC0,1 49,60 $mg \cdot l^{-1}$; 10minLC99,9 118,50 $mg \cdot l^{-1}$; 96hLC50 18,40 $mg \cdot l^{-1}$; 96hLC0,1 10,70 $mg \cdot l^{-1}$ a 96hLC99,9 31,90 $mg \cdot l^{-1}$. Byl popsán časový průběh anastézie a jejího odeznění.

Po 10 min. anestézii hřebíčkovém oleji v koncentraci 30 $mg \cdot l^{-1}$ bylo zaznamenáno významné zvýšení ($p < 0,05$) koncentrace triacylglycerolu (TRIG), alanin aminotransferázy (ALT) a signifikantní ($p < 0,05$) snížení střední barevné koncentrace (MCHC) ihned po anestézii. Počet leukocytů (Leuko) byl signifikantně ($p < 0,05$) snížen 24 hodin po anestézii. Procentuální zastoupení lymfocytů bylo signifikantně ($p < 0,05$) sníženo ihned po anestézii.

Naproti tomu zastoupení myeloidních buněk se zvýšilo. Zvýšené procentuální zastoupení eosinofilních granulocytů přetrvalo i 24 hodin po anestézii, absolutní počet těchto buněk byl shodný s kontrolou. Histologické vyšetření ryb po anestézii prokázalo výskyt ektázií na žaberních lístcích, 24 hodin po anestézii ektázie nebyly prokázány. V ostatních tkáních (játra, kůže, slezina, kraniální a kaudální ledvina) nebyly zjištěny histopatologické změny. Výsledky ukázaly, že hřebíčkový olej v koncentraci 30 $mg \cdot l^{-1}$ je pro sumce velkého bezpečný.

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TOXICKÝ VLIV PYRETHROIDŮ NA RYBY

Už více než sto let se jako insekticidy používají přírodní pyrethriny, alkaloidy získané
pretiny starčkolisté (*Chrysanthenum cinerariaefolium*) a příbuzných druhů. V posledních
ti letech je postupně nahrazují jejich syntetické analogy, zejména vzhledem k jejich
stabilitě. Rozpustnost pyrethroidů ve vodě je velmi nízká, naopak liposolubita je velmi
oká. Přítomnost halogenů v některých pyrethroidech přispívá k větší perzistenci,
cytující lepší reziduální aktivitu vůči hmyzu, ale také ke zvýšení potencionálního
ativního účinku na životní prostředí (Bradbury a Coats 1989a).

Pyrethroidy je možno rozdělit na základě jejich chemické struktury a mechanismu účinku
lvou typů. Pyrethroidy typu I (neobsahují α-kyano skupinu, např. permethrin) reverzibilně
ují sodíkové kanály nervových vláken, tím prodloužují fázi jejich depolarizace, což se
edně projeví jako tremor. Pyrethroidy typu II (obsahují α-kyano 3-fenoxybenzyl skupinu,
. cypermethrin, deltamethrin, fenvalerát) reverzibilně blokují sodíkové kanály nervových
en a navíc ovlivňují GABA receptory v nervových vláknech (Reddy et al. 1991; Hayes
4).

Insekticidy na bázi pyretroidů se používají proti obaleči dubovému na semenných
stech dubů a zvažuje se i jejich použití při rozsáhlém zasažení dubů na hrázích rybníků.
mto případě je třeba vzít v úvahu reálné nebezpečí částečného zasažení vodní hladiny
íků a možného kontaktu ryb s pyrethroidy.

V chovech ryb jsou pyrethroidy (zejména na bázi deltamethrinu) používány ve
dinávských zemích k tlumení parazitárních onemocnění, např. vyvolaných
eophtheirus salmonis ve farmových chovech lososovitých druhů ryb (Noga 1996).
eské republice se zatím tyto látky k léčení ryb nepoužívají.

Zvažovalo se i použití pyrethroidů jako náhrady organofosforečného pesticidu Soldep,
žívaného k redukci hrubého dafniového zooplanktonu při ohrožení rybí obsádky
íkovým deficitem v silně eutrofních rybnících. Vzhledem k vysoké toxicitě pyrethroidů
pro dafnie, tak pro ryby se od této eventuality upustilo a náhrada se hledá dále ve skupině
nofosforečných pesticidů (Svobodová et al. 1987).

V posledních deseti až patnácti letech se velmi intenzivně rozšířilo používání přípravků na
pyrethroidů jako insekticidů a antiparazitik. Růst spotřeby pesticidů v moderním světě
i stále větší obavy z vážného znečišťování prostředí, k němuž jejich používáním dochází.
V současné době zaujímají významné místo v pořadí příčin úhynů ryb, příkladem je úhyn
na Balatonu v roce 1995 (Nemcsok et al. 1999). V nižších koncentracích nemusí mít
prostřední dopad na rybí obsádku, ale mohou dlouhodobě negativně ovlivňovat nejranější
ojová stadia ryb (Lahr et al. 2000; Drastichová et al. 2002).

Insekticidy na bázi syntetických pyrethroidů mají značná pozitiva, protože jsou vysoce
né v nízkých koncentracích a málo toxické pro savce a ptáky (Maund et al. 1998). Jejich
ni nízká toxicita pro teplokrevné obratlovce je předurčuje pro aplikaci ve zdravotnictví,
avinářství a zemědělství. Pyrethroidy jsou však vysoce toxické pro ryby (hodnota LC50 je
ně pod 10 µg/l), proto je nebezpečné jejich použití v blízkosti vodních toků (Bradbury a
ts 1989b). Nicméně obavy vyvolává potenciální riziko pro vodní organismy (zvláště pro
a vodní členovce) vzhledem k jejich vysoké toxicitě prokázané ve standardních
ratorních testech. Situace v přírodních podmínkách je odlišná od uměle udržovaných
stantních podmínek v průběhu laboratorních testů. Důležitým faktorem je rychlá absorbce
throidů na rostliny, sediment a organický materiál, která výrazně snižuje biologickou
upnost pesticidů a tím i riziko pro vodní organismy (Hill et al. 1994). Přesto je nebezpečí
a reálné, jak ukázal případ úhynu ryb v důsledku otravy pesticidním přípravkem na bázi
amethrinu na jezeře Balaton v roce 1995 (Nemcsok et al. 1999).

V souvislosti se vstupem do Evropské Unie došlo ke změnám v procesu registrace ticidních přípravků. Pro stanovení ekotoxikologického rizika pesticidů jsou základem je o toxicitě přípravků pro necílové organismy a předkládaná koncentrace účinné látky kosystému. Do skupiny necílových vodních organismů patří rovněž ryby, vodní bezobratlí sy (Rauscherová et al. 1999).

em prací předkládaných v této kapitole je:

stanovit akutní toxicitu přípravků na bázi pyrethroidů na kapra obecného (*Cyprinus carpio* L.) a pstruha duhového (*Oncorhynchus mykiss*).

stanovit vliv přípravků na bázi pyrethroidů na biochemický, hematologický profil krevní plazmy a histologické změny tkání.

získané výsledky budou podkladem pro hodnocení rizik použití insekticidů v životním prostředí (environmental risk assessment).

zentace výsledků:

kané výsledky týkající se vlivu deltamethrinu na hematologický a biochemický profil ra obecného (*Cyprinus carpio* L.) byly prezentovány na konferenci EUROTOX 2005 v kově (Polsko).

ledky hodnocení přípravků na bázi pyrethroidů byly publikovány v časopise Toxicology ers, a byly přijaty do časopisu Bulletin of Environmental Contamination and Toxicology a slány do redakce Vet. Med-Czech a Environmental Toxicology and Pharmacology.

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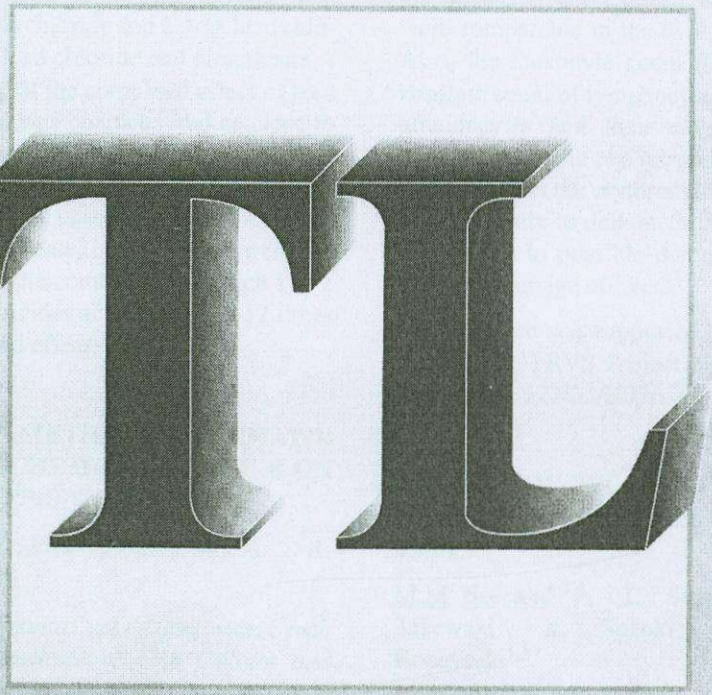

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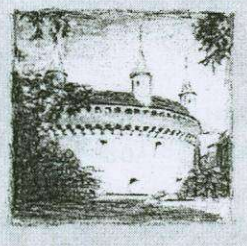

EUROTOX

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same time data on a character of combined on an organism of pesticides and heavy metals rature is extremely limited. In this connection logical experiments were conducted for deter- the character of combined effect on organisms m-blooded animals (rats) of primary contami- for the environment: pesticides (2.4-D, copper te, deltamethrin and dimethoate) and heavy met- thylmercury and lead chloride). At that our own l of mathematical planning of the experiment ed (Rakitski, 1985). Each combination of a pes- nd a heavy metal was studied in nine series of with different components correlation at levels 6, LD33 and LD50 or LD2.5, LD9.25 and LD16 e of effect intensification). As the result corre- ng mathematical models of the studied processes veloped for the combined effects of methylmer- d 2.4-D, methylmercury and copper sulphate, mercury and deltamethrin, methylmercury and oate and also lead chloride and 2.4-D, lead chlo- d deltamethrin, lead chloride and dimethoate.

as founded out that the combined effect of lead e and pesticides was characterized as close to e effect (Kn = 0.8–1.06) and methylmercury and les as more than additive effect (intensifica- Kn = 1.41–3.2). In all cases, for the exclusion of ation with dimethoate, heavy metals were the components of the combinations which 1.5–2 xceeded the pesticides action and 1.3–12 times ed their combined effect.

CTS OF DELTAMETHRIN ON HEMATO- CAL AND BIOCHEMICAL PROFILE ON MON CARP (*CYPRINUS CARPIO*)

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n of this study was to assess the effect of thrin [(*S*)-a-cyano-3-phenoxybenzyl-(1*R*,3*R*)- libromvinyl)-2,2-dimethylcyclo-propancarbox- ylate] on common carp (*Cyprinus carpio*). The effect was assessed on the basis of the results of haematological and biochemical examination of a control and an experimental group exposed to Decis flow 2.5 pesticide preparation (active substance 25 g l^{-1} of deltamethrin) in a concentration of 0.13 mg l^{-1}. The experimental group showed significantly lower values ($p < 0.01$) of erythrocyte count (RBC), haemoglobin content (Hb), haematocrit (PCV) and significantly higher values ($p < 0.01$) of ammonia (NH_3), aspartate aminotransferase (AST) and alanin aminotransferase (ALT) compared to the control group. Values of mean erythrocyte volume (MCV), mean colour concentration (MCHC), erythrocyte haemoglobin (MCH), glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), triacylglycerols (TRIG), lactate dehydrogenase (LDH), creatinkinase (CK), calcium (Ca^{2+}), inorganic phosphate (PHOS) alkalic phosphatase (ALP), lactate and cholinesterase (ChE) were comparable in the two groups during the study. Also, the leukocyte count (Leuko) and relative and absolute count of lymphocytes, monocytes, neutrophil granulocytes and their developmental forms were comparable in the two groups.

Changes in the erythrocyte and biochemical profile after exposure to deltamethrin-based preparation may be referred to possible disruption of haematopoiesis and mild damage of liver.

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P11-15
EFFECTS OF PYRETHROIDS ON DOPAMINE RELEASE AND UPTAKE IN THE RAT STRIATUM

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EFFECTS OF DELTAMETHRIN ON HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF COMMON CARP (*CYPRINUS CARPIO*)

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-15 years the application of pyrethroids as insecticide and antiparasitary preparations has very markedly increased. Thus, re successively replacing organophosphate pesticides. The main advantage of pyrethroids is their photostability, high already in low concentrations, easy disintegration and low toxicity to birds and mammals.

MATERIALS AND METHODS

haematological and biochemical profile was performed on 15 control and 28 experimental -to-two-year-old common carp after 96 h of exposure to Decis flow 2.5 (active substance 25 g.l^{-1} (S)-α-cyano-3-phenoxybenzyl(1R,3R)-3-(2,2-dibromvinyl)-2,2-dimethylcyclo-propancarboxylate]) on of 0.13 mg.l^{-1}.

to evaluate the haematological profile included the erythrocyte count (RBC), haematocrit (PCV),), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular centration (MCHC), leukocyte count (Leuko) and differential leukocyte count (Leukogram).

ochemical indices were determined in blood plasma: glucose (GLU), total proteins (TP), albumins ulines (GLOB), ammonia (NH_3), triglycerides (TRIG), aspartate aminotransferase (AST), alanine (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), calcium (Ca^{2+}), anorganic phosphate hosphatase (ALP), lactate and cholinesterase (ChE).

Fig. 1. *Cyprinus carpio*

RESULTS

Haematological profil after exposure to deltamethrin

The experimental group showed significantly lower values ($p < 0.01$) of erythrocyte count, haemoglobin content and haematocrit compared to the control group.

Values of mean erythrocyte volume, mean colour concentration and erythrocyte haemoglobin were comparable in the two groups during the study. Also, the leukocyte count and relative and absolute count of lymphocytes, monocytes, neutrophil granulocytes and their developmental forms were comparable in the two groups.

Biochemical profil after exposure to deltamethrin

The experimental group showed significantly higher values ($p < 0.05$) of ammonia, aspartate aminotransferase and alanine aminotransferase, compared to the control group.

Values of glucose, total proteins, albumins, total globulins, triglycerides, alkaline phosphatase, lactate dehydrogenase, calcium, anorganic phosphate, alkalic phosphatase, lactate and cholinesterase were comparable in the two groups during the study.

es of carp. Indexes a,b characterize ce between groups (p<0.01).

ontrol D (n =15)	Experiment X ± SD (n =28)
± 0.30 ª	1.60 ± 0.20 ᵇ
± 10.10 ª	85.20 ± 8.14 ᵇ
± 0.04 ª	0.36 ± 0.04 ᵇ
7 ± 35.76 ª	229.23 ± 24.79 ª
5 ± 4.39 ª	54.22 ± 6.54 ª
± 0.02 ª	0.24 ± 0.02 ª
0 ± 4.53 ª	34.21 ± 7.87 ª

Tab. 2. Biochemical indices of carp. Indexes a,b characterize an accordance or a difference between groups (p<0.05).

Indices	Control X ± SD (n =15)	Experiment X ± SD (n =28)
GLU (mmol.l^{-1})	5.15 ± 1.13 ª	5.04 ± 0.41 ª
TP (g.l^{-1})	36.97 ± 5.96 ª	38.60 ± 6.77 ª
ALB (g.l^{-1})	6.42 ± 2.41 ª	6.47 ± 1.09 ª
GLOB (g.l^{-1})	30.50 ± 5.29 ª	32.13 ± 3.91 ª
NH_3 (μmol.l^{-1})	102.83 ± 31.16 ª	233.80 ± 112.13 ᵇ
TRIG (mmol.l^{-1})	2.13 ± 0.25 ª	2.18 ± 0.31ª
AST (μkat.l^{-1})	3.50 ± 0.89 ª	5.68 ± 2.78 ᵇ
ALT (μkat.l^{-1})	0.59 ± 0.20 ª	0.81 ± 0.30 ᵇ
CK (μkat.l^{-1})	810.86 ± 3.86 ª	810.27 ± 4.30 ª
LDH (μkat.l^{-1})	6.78 ± 2.40 ª	6.74 ± 1.88 ª
Ca^{2+} (mmol.l^{-1})	2.56 ± 0.14 ª	2.58 ± 0.13 ª
PHOS (mmol.l^{-1})	1.84 ± 0.25 ª	1.94 ± 0.32 ª
ALP (μkat.l^{-1})	0.23 ± 0.11 ª	0.30 ± 0.11 ª
lactate (mmol.l^{-1})	1.28 ± 0.57 ª	1.47 ± 0.47 ª
ChE (μkat.l^{-1})	2.10 ± 1.03 ª	1.90 ± 1.15 ª

ial count (%) in control (C) and common carp affected by acute

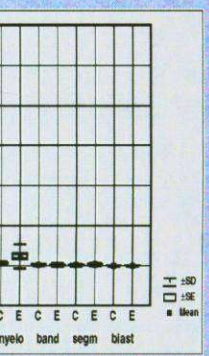
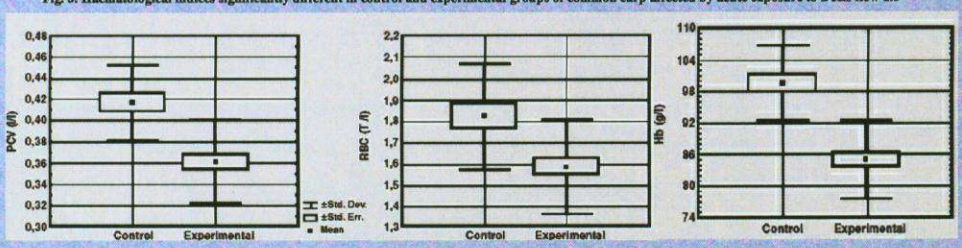


Fig. 3. Haematological indices significantly different in control and experimental groups of common carp affected by acute exposure to Decis flow 2.5



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Effect of Deltamethrin on Biochemical Profile of Common Carp (*Cyprinus carpio* L.)

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The contamination of surface waters by pesticides used in agriculture represents a problem of worldwide importance. The major negative impact of pesticide use upon fisheries is the accumulation of residues in fish tissues. Even though being relatively rapidly degradable, applied pesticides may be toxic for fish. Pyrethroids, synthetic analogues of pyrethrins belong to the chemical group of non-systemic insecticides. They are very toxic to insects, amphibians and fish and are of a very low order of toxicity to birds and mammals (Bradbury and Coats 1989a). These chemicals can be divided into two major classes based on their structure, chemical and neurophysiological properties and toxicological actions. These classes are known as type I and type II pyrethroids. These compounds impact upon the central and peripheral nervous system. Their action is focused on sodium channels within the lipophilic component of the membranes at or close to the Na^+ gate proteins. They act by modulating the opening and closing the channels that can result in synaptic discharge, depolarisation and ultimately death (Roberts and Hudson 1998). Type II pyrethroids have also been found to interact with the GABA-ergic system.

Substances of this class are used to control wide-scale insect infestation in a wide range of crops, ornamentals and trees. They are also of importance to veterinary medicine for their use in ectoparasitics (Wardhaugh 2005; Bradbury and Coats 1989b). In aquaculture, pyrethroids are applied to control some parasitic diseases caused by, e.g. *Lepeophtherius salmonis* in salmon farming (Toovey and Lyndon 2000; Sevatdal and Horsberg 2001).

Deltamethrin is the first potent and photostable insecticide belonging to the type II pyrethroid group. After being used to control mosquito populations, the pesticide caused massive eel kills in Lake Balaton, Hungary, in the summer periods of 1991 and 1995. In 1995, deltamethrin presence was demonstrated in several other fish species and sediment samples taken from the lake (Bálint et al. 1997). Acute toxicity and the effect of deltamethrin on haematological indices of common carp were evaluated by Svobodová et al. (2003). Changes in the erytrocyte profile after exposure to deltamethrin may be referred to possible disruption of haematopoiesis.

The present paper aims to contribute to the assessment of deltamethrin [(S)-a-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromvinyl)-2,2-dimethylcyclo-propan-carboxylate] effects by evaluation of its impact upon blood plasma biochemical indices of two-year-old common carp (*Cyprinus carpio* L.).

MATERIALS AND METHODS

The chemical formulation of the active substance deltamethrin, 25 g/L (Decis flow 2.5, Bayer CropScience GmbH, Germany) was used for testing. Blood plasma biochemical examination of two-year-old common carp (*Cyprinus carpio* L.) (M 72 polyhybrid strains of mirror carp) was performed at the end of a 96-hr acute toxicity test with 0.13 mg/L of Decis flow 2.5 (3.25 µg/L of deltamethrin).

Simultaneously, the control group of carp was examined. The test was designed semistatically, with bath renewal every 24 hr (exposure volume 200 L). Basic physical and chemical indices of the test diluting water were as follows: pH ranged from 7.8 to 7.9, $ANC_{4.5}$ (alkalinity) 1.15 mmol/L, COD_{Mn} 1.6 mg/L, BOD_5 0.79 mg/L, $NH_4^+ + NH_3$ 0.04 mg/L, $N0_3^-$ 11.5 mg/L, NO_2^- 0.005 mg/L, PO_4^{3-} 0.01 mg/L, a sum of Ca + Mg 14 mg/L. During the test, water temperature and oxygen saturation ranged from 20.0 to 21.2 °C and 84 to 99 %, respectively.

Biochemical analyses of plasma involved 14 control carp (C) (557 ± 48 g body weight) and 15 experimental carp (E) (573 ± 57 g body weight). Blood samples were obtained by cardiac puncture. Heparin in the amount of 50 IU sodium salt per 1 mL of blood was used for stabilization. Individual blood samples were centrifuged in a cooled centrifuge at 400 G for 15 min. Determined plasma biochemical indices included glucose (GLU), lactate (LACT), total proteins (TP), albumins (ALB), total globulins (GLOB), triacylglycerols (TAG), ammonia (NH_3), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalic phosphatase (ALP), creatine kinase (CK), cholinesterase (ChE), calcium (Ca^{2+}) and inorganic phosphate (PHOS).

For plasma biochemical analysis, VETTEST 8008 analyser (IDEXX Laboratories Inc., USA; Medisoft Co.) was used. The apparatus is based upon dry chemical technology and colorimetric reaction. Sample analysis was carried out on selective testing discs (Multi-layer film slides, Kodak) by means of laser reading the bar codes. Detection limits of the methods were as follows: GLU (0.01 mmol/L), TP (1.0 g/L), ALB (1.0 g/L), GLOB (1.0 g/L), TAG (0.01 mmol/L), NH_3 (1.0 μmol/L), LDH (0.0167 μkat/L), AST (0.0835 μkat/L), ALT (0.0835 μkat/L), ALP (0.0167 μkat/L), CK (0.0167 μkat/L), Ca^{2+} (0.01 mmol/L) and PHOS (0.01 mmol/L). For the determination of CK activity, plasma was diluted 10 times with a physiological solution (0.6 g/L NaCl). ChE and LACT were determined by a COBAS MIRA automatic analyser (Hoffman, La Roche, Co., Switzerland) using the BioVendor tests No. 12061 and 12351. Detection limits of the methods were 1.35 μkat/L and 0.05 mmol/L for ChE and LACT.

QA/QC measures were consistently applied within experiment. All the measurements described above were carried out according to validated standard operation procedures.

The statistical analysis was conducted by basic descriptive and one-factor analysis of variance (STATISTICA, Version 6.0). The level of significance was calculated at the $p<0.01$ and $p<0.05$ levels. The program also performed categorized box and whiskers plots including box whiskers type mean, standard error of the mean (SE) and 1.96*SE.

Experiments on fish were approved by the Ethical Committee of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology Vodňany (approval No. 3/2004).

RESULTS AND DISCUSSION

During the course of deltamethrin poisoning in experimental carp the following clinical symptoms of choreoathetosis were observed: accelerated respiration, loss of movement and coordination, for example fish laying down at the bottom of the tank and moving on one spot was a common observation. Subsequently a short excitation stage (convulsions, movement in circles) was recorded.

All two-year-old common carp survived at the exposure to 0.13 mg/L of Decis flow 2.5. The results of blood plasma biochemical indices profile in control and experimental carp are given in Table 1, and Figure 1 and 2. The exposition of carp to deltamethrin in the concentration of 3.25 μg/L caused a significant increase of ammonia level ($p<0.01$) and aspartate aminotransferase ($p<0.05$) and alanine aminotransferase ($p<0.05$) activities (given in Fig. 1 and Fig. 2). The rest of the indices monitored was found to be at comparable levels in both groups under study, showing no significant differences between the groups.

An enhanced energy demand caused by short-term pyrethroid stress stimulates the activity of GDH (glutamate dehydrogenase) which induces the glutamate fission to ammonia and α-ketoglutaric acid utilized in the TCA cycle (Philip and Rajasree 1996). In the study of Philip and Rajasree (1996), 5-d and 10-d exposure of common carp to 3.0 μg/L of cypermethrin caused decrease of ammonia concentration in gill, liver, brain and muscle tissues. By comparison of the acute toxicity of deltamethrin and cypermethrin to various fish species, deltamethrin can be supposed more toxic (Gangolli 1999). In our study, 3.25 μg/L of deltamethrin caused an increase of plasma ammonia level, since detoxifying mechanisms were supposedly unable to convert the arisen toxic ammonia to a less harmful substance.

The activities of plasma enzymes are also used as a relevant stress indicator. The enzymes used for the purpose are above all LDH, CK and transaminases (ALT and AST). A significant increase in the concentration of the above mentioned plasma enzymes indicates stress-based tissue impairment (Svoboda 2001). After acute exposure to deltamethrin, a significant increase ($p<0.05$) in AST and ALT levels was found in experimental carp in comparison to control specimens (Fig. 2). Increased activities of both transaminases indicated amplified transamination processes. An increase in transamination occurs due to amino acid input into the TCA cycle in order to cope with the energy crisis during pyrethroid-based stress (Philip et al. 1995).

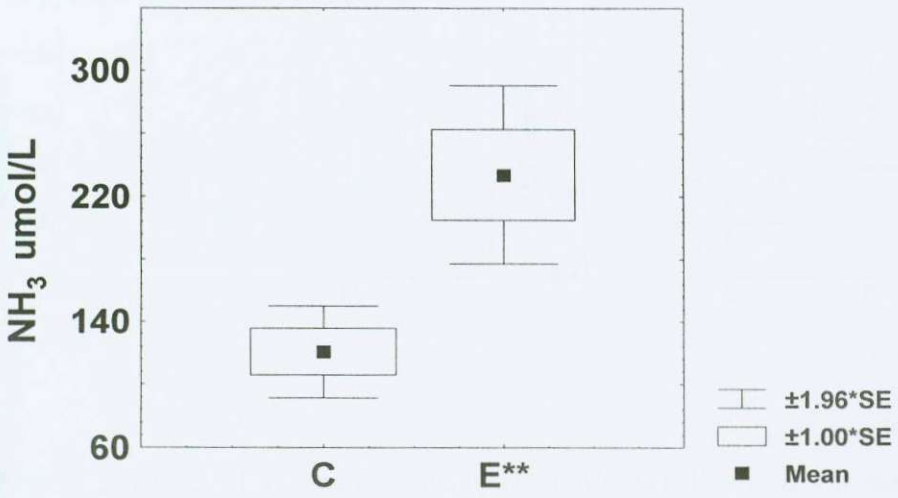

Figure 1. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma ammonia concentration in carp (C - control group, E - experimental group; significance **p<0.01).

It has been suggested that the stress condition in general induces the elevation of the transamination pathway (Natrajan 1985) and is likely to have contributed to the toxic stress induced by deltamethrin and increased transaminase activities in the present study. Similar increases in ALT and AST activities were reported by Philip and Rajasree (1996) in gill, brain and liver of *Cyprinus carpio* during 5-day and 10-day exposure to 3.0 μg/L of the pyrethroid cypermethrin, and by Begum (2005) in gill and liver of freshwater teleost air breathing fish *Clarias batrachus* after 10-d exposure to 0.07 mg/L of cypermethrin. In the study of Szegletes et al. (1995a), 96-hr exposure to 1.0 and 1.5 μg/L of deltamethrin induced the AST activity in carp serum. In carp, the 2.5-fold increase in AST activity after 72-hr exposure to 2 μg/L of deltamethrin was also reported by Bálint et al. (1995).

The mechanism of AChE inhibition by deltamethrin is less known. Deltamethrin-based changes in sodium and chloride flux in various tissues may be connected with the function of nervous system. The secretory and enzymatic properties of nervous tissue may also be influenced (Szegletes et al. 1995b). Bálint et al. (1995) suggest that deltamethrin modifies the catalytic centre of AChE, either directly through the binding to the active site or via modification in enzyme structure.

In response to deltamethrin treatment, the ChE activity remained almost the same as in the control fish. The difference between the groups tested was found to be 10.6 %. In the study of Bálint et al. (1995), the effect of deltamethrin on AChE activity in different tissues of adult common carp was demonstrated using *in vitro* and *in vivo* treatments. *In vitro* kinetic studies showed the inhibition of brain AChE activity. *In vivo*, the carp exposure to 2 μg/L of deltamethrin for 3 days resulted in 21.4% decrease in the AChE activity in serum of carp tested.

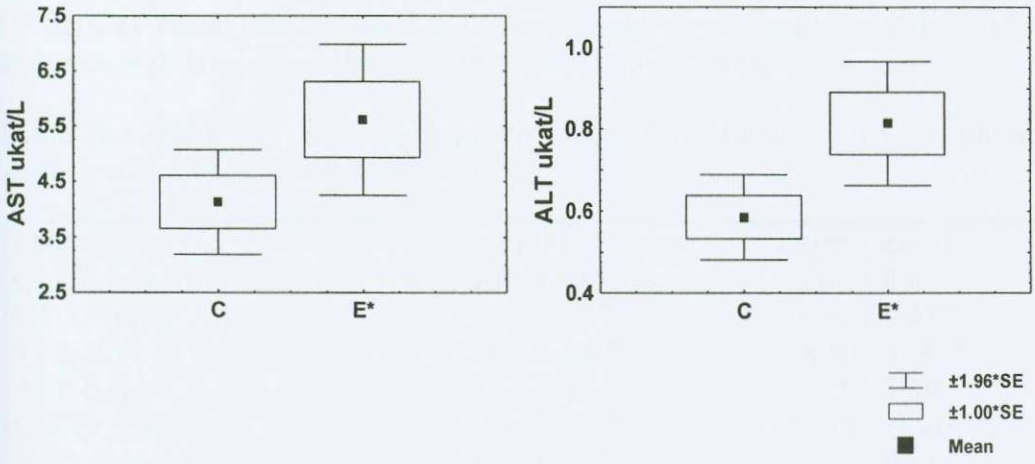

Figure 2. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma AST and ALT concentrations in carp (C - control group, E - experimental group; significance *p<0.05).

Szegletes et al. (1995b) demonstrated that exposure to 2 μg/L of deltamethrin caused the AChE activity decrease of as much as 20 % in blood plasma of *Cyprinus carpio*. The same results were detected by Bálint et al. (1997) in the dying eels in Lake Balaton due to contamination by deltamethrin.

Acute toxicity of pyrethroids for fish is temperature-dependent, i.e. the higher test water temperature the lower acute toxicity of pyrethroids (Bradbury and Coats 1989a). The results of above mentioned studies are in contrast to the results of our study, in which the difference in ChE activity between experimental and control groups was found to be non-significant. The contradiction of the results can be explained by using different test water temperature since in our study fish were tested at mean water temperature of 20.5 °C, while Bálint et al. (1995) and Szegletes et al. (1995b) used water temperature of 12 °C.

The increase of blood glucose concentration demonstrated the response of exposed fish to metabolic stress. The increase of LDH level indicated metabolic changes, i.e. the glycogen catabolism and glucose shift towards the formation of lactate in stressed fish, primarily in the muscle tissue (Simon et al. 1983).

In the study of Bálint et al. (1995), blood plasma LDH and glucose levels of common carp were significantly increased after the 6-hr exposure to 2 μg/L of deltamethrin, with subsequent slight decreases in their levels. The activity of LDH increased 1.5- and 2.5-fold in 6- and 72-hr samples, respectively, and glucose level was by 30 % higher (6-hr sample) as compared to LDH and glucose levels of the control carp. Szegletes et al. (1995a) reported interesting changes in blood glucose content. After 24-hr exposure to 1 μg/L of deltamethrin, fish seemed to be stressed, although the increase of glucose level was not significant. When the fish became adapted to deltamethrin presence, the glucose level decreased, especially after 72 hours. At the same time, the control animals kept in similar conditions

showed a small non-significant decrease. Meanwhile, fish in aquaria containing 1.5 μg/L of deltamethrin reacted to the treatment by increased glucose level after 48 hours, and this did not change until the end of the treatment.

Table 1. Effect of acute exposure to Decis flow (0.13 mg/L) on plasma biochemical indices in carp (mean ± SD).

Indices	Control (C)	Experiment (E)
GLU (mmol/L)	5.15 ± 1.13 [a]	5.01 ± 0.41 [a]
LACT (mmol/L)	1.28 ± 0.57 [a]	1.47 ± 0.47 [a]
TP (g/L)	36.97 ± 5.96 [a]	38.60 ± 6.77 [a]
ALB (g/L)	6.42 ± 2.41 [a]	6.47 ± 1.09 [a]
GLOB (g/L)	30.50 ± 5.29 [a]	32.13 ± 3.91 [a]
TAG (mmol/L)	2.13 ± 0.25 [a]	2.18 ± 0.31 [a]
LDH (μkat/L)	6.78 ± 2.40 [a]	6.74 ± 1.88 [a]
ALP (μkat/L)	0.23 ± 0.11 [a]	0.30 ± 0.11 [a]
CK (μkat/L)	810.86 ± 3.86 [a]	810.27 ± 4.30 [a]
ChE (μkat/L)	5.10 ± 1.03 [a]	4.56 ± 1.15 [a]
Ca^{2+} (mmol/L)	2.56 ± 0.14 [a]	2.58 ± 0.13 [a]
PHOS (mmol/L)	1.84 ± 0.25 [a]	1.94 ± 0.32 [a]

The biochemical profile of blood can provide important information about the internal environment of the organism (Masopust 2000). In our study, ammonia, AST and ALT proved to be the most sensitive parameters in two-year-old common carp. The exposure of carp to the insecticide Decis flow 2.5 in the concentration of 0.13 mg/L (3.25 μg/L of deltamethrin) caused significant increases of the above mentioned indices. Other biochemical indices observed were considered to be less appropriate for the evaluation of pyrethroid-based stress response in common carp.

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ects of Deltamethrin on Rainbow Trout (*Oncorhynchus mykiss*)

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tract

aim of this study was to assess the effect of deltamethrin on rainbow trout (*Oncorhynchus iss*). Control and experimental group of fish were exposed to Decis EW 50 pesticide aration (active substance 50 $g \cdot l^{-1}$ of deltamethrin). The acute semistatical toxicity test ng 96 h was performed on rainbow trout juveniles. The 96hLC50 value of Decis EW 50 0.02 $mg \cdot l^{-1}$. Examination of haematological and biochemical profile and histological ıe examination was performed on one-to-two-year-old rainbow trout after 96 h of ɔsure to Decis EW 50 in a concentration of 0.02 $mg \cdot l^{-1}$. The experimental group showed ificantly lower values ($P<0.05$) of plasma glucose, alanine aminotransferase, inesterase and significantly higher ($P<0.05$) values of erythrocyte count, haemoglobin ent, haematocrit and plasma total protein, albumins, ammonia, aspartate ıotransferase, creatinekinase and calcium compared to the control group. The ımethrin-based Decis EW 50 pesticide preparation was classified among substances ıgly toxic for fish.

vords: Pyrethroids; Acute toxicity; Haematological profile; Biochemical profile of blood; ɔlogical examination of tissues

Introduction

Synthetic analogues of the pyrethrins, extracts from the ornamental *Chrysanthenum rariaefolium*, have been developed to circumvent the rapid photodegradation problem ountered with the insecticidal natural pyrethrins. The widespread use of these pesticides sequently leads to the exposure of manufacturing workers, field applicators, the ecosystem finally the public to the possible toxic effects of these pesticides (Smith and Stratton, 6; Solomon et al., 2001).

During investigations to modify the chemical structures of natural pyrethrins, a certain ıber of synthetic pyrethroids were produced with improved physical and chemical erties and greater biological activity. Several of the earlier synthetic pyrethroids were essfully commercialized, mainly for the control of household insects. Other more recent throids have been introduced as agricultural insecticides because of their excellent ity against a wide range of insect pests and their non-persistence (Casida et al., 1983; mson, 1985). Toxic effects of pyrethroids on nontarget organisms have been reviewed and rted to be in the parts per billion values of toxicity (Smith and Stratton, 1986).

In the environment, synthetic pyrethroids are fairly rapidly degraded in soil and in plants. r hydrolysis and oxidation at various sites on the molecule are the major degradation esses. The pyrethroids are strongly adsorbed on soil and sediments, and are hardly eluted water. There is a little tendency for bioaccumulation in organisms (Haya, 1989).

Deltamethrin is a widely used pesticide based on pyrethroids. It is among the most tive pyrethroid preparations (Bradbury and Coast, 1989). Deltamethrin was synthesized 974, and first marketed in 1977 (Pham et al., 1984). It works by paralyzing the insects' ous system and therefore giving a quick knock-down effect after surface contact or stion. It is used commonly to control caterpillars on apples, pears and hops, and for the rol of aphids, mealy bugs, scale insects, and whiteflies on glasshouse cucumbers, ıtoes, potted plants, and ornamentals (Mueller-Beilschmidt, 1990).

The mechanism of its effectiveness in the case of fishes is the same as that of other throids containing -cyano-3-phenoxybenzyl groups. They block the sodium channels of e filaments, thereby lengthening their depolarisation phase; moreover, they affect the A receptors in the nerve filaments (Bradbury and Coast, 1989; Eshleman and Murray, ; Hayes, 1994).

Fish make intimate contact with the surrounding water through the gills. Due to their hilicity, pyrethroids have a high rate of gill absorption, which in turn would be a

ibuting factor in the sensitivity of the fish to aqueous pyrethroid exposures. Fish seem to ficient in the enzyme system that hydrolyzes pyrethroids. The main reaction involved in netabolism of deltamethrin, cypermethrin, or cyhalothrin in mice and rats is ester age mainly due to the action of carboxyesterase. Metabolism in fish is largely oxidative oute, 1989; Kamalaveni et al., 2003). After short-term deltamethrin exposure, adult ater catfish (*Heteropneustes fossilis*) showed hypocalcaemia and the researchers e this condition to the possible impairment of either net electrolyte influx at the gill or nction. Deltamethrin exposure also caused hypophosphataemia and was linked to the redistribution of electrolytes between intracellular or extracellular compartments and ient of renal function. Deltamethrin may disturb the calcium and phosphate tasis and may lead to an effect on the reproductive state of the fish (Srivastav et al., laund et al., 2002).

assessment of the ecotoxicological risks caused by pesticides to ecosystems is based on the toxicity and effects of pesticide preparations to non-target organisms. Fish are he group of non-target aquatic organisms. The present paper is a contribution to the ent of toxicity and effects of a deltamethrin-based pesticide to fish.

erials and Methods

goal was to assess the effect of deltamethrin [(S)- a-cyano-3-phenoxybenzyl (1R,3R)-ibromvinyl)-2,2-dimethylcyclo-propancarboxylate] on fish. It was tested in the form EW 50 pesticide, the active substance of which was deltamethrin in the amount of 50 e toxic effect was assessed by the results of acute toxicity tests and results of logical, biochemical and histological examination of rainbow trout after exposure to icide.

ute toxicity

acute toxicity test on rainbow trout with Decis EW 50 followed the OECD Direction and Methodical Manual ISO 7346/2. Juveniles of rainbow trout (camloops) of 4.1 ± ean body weight and 65.1 ± 3.61 mm mean body length were used for the test. Six concentrations and a control were used in the basic test. Seven fish specimens were every concentration and also in the control. The test was performed semistatically for e bath was changed every 24 h. Basic physical and chemical indices of diluting water he acute toxicity test were as followed: acid neutralization capacity – $ANC_{4.5}$ 1.15

ıol·l^{-1}; total ammonia 0.04 mg·l^{-1}; $N0_3^-$ 11.5 mg·l^{-1}; NO_2^- 0.005 mg·l^{-1}; PO_4^{3-} 0.01 mg·l^{-1}; :mical oxygen demand - COD_{Mn} 1.6 mg·l^{-1}. Water temperatures in the test ranged from 5 to 16.5 °C, oxygen saturation of water ranged between 101 and 108 %. The LC50, LC0 l LC100 values in the respective time intervals were determined by probit analysis.

. *Haematological profile after exposure to deltamethrin*

Haematological, biochemical and histological examination of rainbow trout (camloops) s performed at the end of 96 h acute toxicity test with Decis EW 50 in concentration of 2 mg·l^{-1}. At the same time, the control group of trout was examined haematologically, chemically and histologically. Rainbow trout (camloops) of 309.18 ± 64.80 g average ght and 307 ± 25 mm average body length were used. The test was performed ıistatically with the bath exchanged every 24 h. Diluting water had the same physical and mical parameters as described above. Water temperatures during the test ranged from 14.5 6.2 °C, oxygen saturation of water was above 60 % (ranging from 70 to 83 %), pH ranged n 7.40 to 7.82. The test was performed in 2 aquaria of 200 l volume. Each aquarium was :ked with 15 specimens of one- to two-year-old rainbow trout (1 control aquarium, 2 aria with Decis EW 50 in the concentration 0.02 mg·l^{-1}).

Heparinised injection needles were used to take samples of blood from hearts of fish ıned by a blow with a blunt object over the head. To stabilize blood samples, aqueous ıtion of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodová et al., 1986). indices used to evaluate the haematological profile included the erythrocyte count (Er), moglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean ıur concentration (MCHC), erythrocyte haemoglobin (MCH), leukocyte count (Leuko) the differential leukocyte count (Leukogram). The procedures were based on Unified hods for haematological examination of fish (Svobodová et al., 1986).

Results of haematological examinations were tested by the variance analysis using the istica 6.0 (ANOVA – Tukey Test) software.

Biochemical blood plasma profile after exposure to deltamethrin

For biochemical profile of blood plasma tests, rainbow trout (camloops) of 309.18 ± 64.80 ıerage weight and 307 ± 25 mm average body length were used. Blood was sampled by ns of cardiac puncture as mentioned above. Individual blood samples of all investigated were centrifuged (4°C, 837 x g) to obtain blood plasma samples. Biochemical indices rmined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB),

al globulins (GLOB), ammonia (NH_3), triacylglycerols (TRIG), aspartate aminotransferase ST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatinekinase (CK), cium (Ca^{2+}), lactate (LACT), cholinesterase (ChE) and inorganic phosphate (PHOS). For biochemical analysis of blood plasma, the VETTEST 8008 analyzer (IDEXX Laboratories . U.S.A.) manufactured by Medisoft was used. The analyzer uses dry chemical and orimetric analysis techniques. Selective test discs (Multi–layer film slides, Kodak) were d for the evaluation by a laser reading bar codes. ChE and LACT were determined by a BAS MIRA automatic analyser (Hoffman, La Roche, Co., Switzerland) using the Vendor tests No. 12061 and 12351.

Results of biochemical examination were tested by the variance analysis using the tistica 6.0 (ANOVA – Tukey Test) software.

Histological examination of tissues

histological examination of tissues, rainbow trout (camloops) of 309.18 ± 64.80 g average ght and 307 ± 25 mm average body length were used. After blood sampling, samples of in, gills, skin, liver, cranial and caudal kidney and spleen were taken for histological minations. The samples taken were immediately fixed in 10% formaldehyde, drained and bedded in paraffin. Sections were made of the paraffin blocks and stained with matoxylin-eosin.

Results

Acute toxicity

On the basis of tests of acute toxicity to rainbow trout, the 96-hour lethal concentrations of is EW 50 were determined (96hLC50 0.02 $mg \cdot l^{-1}$, 96hLC0 0.01 $mg \cdot l^{-1}$ and 96hLC100 5 $mg \cdot l^{-1}$). The 96hLC50 is the basic value in the acute toxicity test. For rainbow trout eniles the 96hLC50 value was 0.02 $mg \cdot l^{-1}$ of Decis EW 50 preparation, which esponded to 1 $\mu g \cdot l^{-1}$ of deltamethrin. In the course of deltamethrin poisoning in rainbow t, the following clinical symptoms were observed: accelerated respiration, loss of vement coordination, fish lay-down at their flank and are moving in this position. sequent short excitation stage (convulsions, jumps above the water surface, movement in les) changes into a resting stage, and an other short-time excitation follows again. In the , fish fall into damp, move mainly at their flank. Respiration is slowed down, the damp se and subsequent agony are very long.

Haematological profile

esults of erythrocyte profile and leukocyte profile of the control and experimental ɔw trout under study are given in Table 1, 2. Compared to the control specimens, those the acute exposure to deltamethrin had significantly higher ($P<0.05$) erythrocyte count, ɔglobin content and haematocrit. Values recorded for MCV, MCH, MCHC and gram were comparable in both groups under study.

1: Derived haematological parameters in rainbow trout affected by acute exposure to EW 50.

:s	Units	Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
	$T \cdot l^{-1}$	1.10 ± 0.22 [a]	1.20 ± 0.27 [b]
	$g \cdot l^{-1}$	60.41 ± 10.79 [a]	63.33 ± 6.83 [b]
	$l \cdot l^{-1}$	0.38 ± 0.06 [a]	0.41 ± 0.04 [b]
	fl	362.15 ± 95.70 [a]	352.73 ± 71.98 [a]
	pg	56.83 ± 14.22 [a]	55.29 ± 12.23 [a]
C	$g \cdot l^{-1}$	158.11 ± 15.96 [a]	156.88 ± 15.79 [a]
	$G \cdot l^{-1}$	22.00 ± 9.62 [a]	25.87 ± 11.73 [a]

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

e 2: *Leukocyte differencial count in rainbow trout affected by acute exposure to Decis* *0.*

s	Units	Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
ocytes	%	83.11 ± 11.24 [a]	79.50 ± 13.95 [a]
	$g \cdot l^{-1}$	18.89 ± 7.32 [a]	20.67 ± 3.53 [a]
ytes	%	1.93 ± 1.62 [a]	1.80 ± 2.36 [a]
	$g \cdot l^{-1}$	0.42 ± 0.32 [a]	0.46 ± 0.61 [a]
hile granulocytes	%	9.87 ± 8.41 [a]	11.67 ± 8.61 [a]
ts	$g \cdot l^{-1}$	2.17 ± 1.85 [a]	3.01 ± 2.12 [a]
hile granulocytes	%	0.08 ± 0.02 [a]	0.05 ± 0.03 [a]
	$g \cdot l^{-1}$	0.01 ± 0.004 [a]	0.01 ± 0.003 [a]
mental phases –	%	4.99 ± 3.49 [a]	4.53 ± 3.39 [a]
l sequence	$g \cdot l^{-1}$	1.09 ± 0.77 [a]	1.17 ± 0.89 [a]

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

ochemical blood plasma profile

ılts of biochemical blood plasma profile of the control and experimental rainbow trout udy are given in Table 3. The experimental rainbow trout exposed to acute effects of ımethrin-based pesticide showed a significantly ($P<0.05$) decreased concentration of alanine aminotransferase, cholinesterase and significantly ($P<0.05$) increased total albumins, ammonia, aspartate aminotransferase, creatinekinase and calcium in blood The rest of the indices (GLOB, TRIG, LDH, LACT and PHOS) were comparable in groups during the study.

ıle 3: Derived biochemical indices of blood plasma in rainbow trout affected by acute ıosure to Decis EW 50.

ices	Units	Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
U	$mmol \cdot l^{-1}$	5.27 ± 1.42 [a]	4.87 ± 0.59 [b]
	$g \cdot l^{-1}$	43.80 ± 4.18 [a]	49.87 ± 2.45 [b]
B	$g \cdot l^{-1}$	6.07 ± 2.91 [a]	11.27 ± 1.34 [b]
OB	$g.l^{-1}$	37.80 ± 2.37 [a]	37.93 ± 2.67 [a]
l_3	$\mu mol \cdot l^{-1}$	946.73 ± 195.78 [a]	1122.0 ± 235.24 [b]
IG	$mmol \cdot l^{-1}$	0.54 ± 0.21 [a]	0.89 ± 1.11 [a]
T	$\mu kat \cdot l^{-1}$	4.20 ± 0.73 [a]	5.57 ± 0.84 [b]
T	$\mu kat \cdot l^{-1}$	0.18 ± 0.09 [a]	0.12 ± 0.04 [b]
H	$\mu kat \cdot l^{-1}$	32.59 ± 5.24 [a]	32.79 ± 2.01 [a]
	$\mu kat \cdot l^{-1}$	18.56 ± 5.07 [a]	25.66 ± 4.03 [b]
+	$mmol \cdot l^{-1}$	2.61 ± 0.24 [a]	2.98 ± 0.19 [b]
CT	$mmol \cdot l^{-1}$	2.47 ± 0.95 [a]	3.51 ± 2.20 [a]
rtisol	$nmol \cdot l^{-1}$	203.96 ± 125.79 [a]	193.68 ± 108.06 [a]
E	$\mu kat \cdot l^{-1}$	3.46 ± 1.35 [a]	2.14 ± 1.06 [b]
OS	$mmol \cdot l^{-1}$	3.84 ± 0.16 [a]	3.93 ± 0.35 [a]

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

e: [1] For the determination of NH_3, LHD and CK, blood plasma at 1:2 dilution with siological saline was used.

Histological examination of tissues

No histopathological changes were demonstrated in tissues (brain, gills, skin, liver, spleen, ial and caudal kidney) of rainbow trout following after exposure to deltamethrin.

Discussion

In the course of 96 h toxicity test of deltamethrin-based pyrethroid preparation Decis EW n rainbow trout juveniles, there was no mortality of fish in the control aquarium. Oxygen ration of water did not drop below 60 % in any concentration tested, nor in the control

p. Presence of the substance tested (above 80 % of the nominal concentration) was ided by means of daily exchange of the testing bath. Fulfilling these conditions, the test be considered valid. On the basis of the observed value of 96hLC50 (0.02 $mg \cdot l^{-1}$), the aration Decis EW 50 can be included in a group of substances that are highly toxic for the risk sentence R50 states the value of 96hLC50 less than 1 $mg \cdot l^{-1}$. The value of .C50 for Decis EW 50, 0.02 $mg \cdot l^{-1}$ essentially corresponds to 1 $\mu g \cdot l^{-1}$ deltamethrin. The :s observed by us were in agreement with those reported by other authors who have mined the toxicity of deltamethrin for various species of fish. Bradbury and Coast 9); Haug and Hofman (1990); Viran et al. (2003) report the mean lethal toxicity for us fish species in laboratory conditions as varying between LC50 0.001 and 0.01 $mg \cdot l^{-1}$, w and Godzi (1994) state the value of LC50 0.0037 $mg \cdot l^{-1}$ for gibel carp. Žlábek (1999) the value of LC50 0.099 $mg \cdot l^{-1}$ Decis flow 2.5 for rainbow trout. Gangolli (1999) reports alues of 96hLC50 for common carp and rainbow trout as varying between 0.0005 and 8 $mg \cdot l^{-1}$.

1 the course of deltamethrin poisoning in rainbow trout, the following clinical symptoms observed: accelerated respiration, loss of movement coordination, fish lay-down at their and are moving in this position. Subsequent short excitation stage (convulsions, jumps e the water surface, movement in circles) changes into a resting stage, and an other short- excitation follows again. In the end, fish fall into damp, move mainly at their flank. iration is slowed down, the damp phase and subsequent agony are very long. Similar ges in the clinical symptoms are also reported by Svobodová et al. (2003) in carp ving acute poisoning with cypermethrin. Bradbury and Coats (1989) reported signs of lerate poisoning in fish, which included loss of schooling behaviour, swimming near the surface, hyperactivity, erratic swimming, seizures, loss of buoyancy, elevated cough increased gill mucus secretions, flaring of the gill arches, head shaking, and listlessness e death.

he main haematological response of rainbow trout to the acute effect of deltamethrin- preparation was a significant ($P<0.05$) increase in erythrocyte count, haemoglobin nt and haematocrit compared to the control group. Svobodová et al. (2003) reported ite changes in the red blood picture in carp after acute exposure to deltamethrin. In this , there were no changes in the white blood picture of rainbow trout. Sopinska and Guz) observed decrease in total leucocyte count and neutrophil granulocyte count in carp ving acute poisoning with permethrin.

The main biochemical response of rainbow trout to the acute effect of deltamethrin-based paration was significant ($P<0.05$) decrease in plasma glucose, alanine aminotransferase, linesterase and significant ($P<0.05$) increase in plasma total protein, albumins, ammonia, artate aminotransferase, creatinekinase and calcium compared to the control group. Balint al. (1995) found an increase in the concentration acetylcholinesterase, lactate ydrogenase and glucose in common carp (*Cyprinus carpio*) after exposure to deltamethrin. nalaveni et al. (2001) found a decrease in the activity of succinate dehydrogenase (SDH) a increase of glucose-6-phosphate dehydrogenase (G6PD) in common carp.

No histopathological changes were demonstrated in tissues of rainbow trout after acute osure to deltamethrin. Eisler (1992) observed histological damage to gill surfaces by valerate, which was attributed to high accumulations in gills, irritation due to elevated cus secretion, increased ventilation volume, and decreased gill-oxygen uptake efficiency.

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ects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*)

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RACT: The aim of this study was to assess the effect of cypermethrin [(R,S)-α-cyano-3-phenoxybenzyl -cis,tra-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate] on rainbow trout (*Oncorhynchus*). The effect was assessed on the basis of the results of acute toxicity tests and on the comparison of of haematological, biochemical and histopathological tissue examinations of a control and experimental exposed to Alimetrine 10 EC pesticide preparation (active substance 100 g/l of cypermethrin). The acute atical toxicity test lasting 96 h was performed on rainbow trout juveniles. The 96hLC50 value of thrine 10 EC was 31.4 µg/l. Examination of erythrocyte, leukocyte and biochemical profile and athological tissue examination was performed on 15 control and 15 experimental specimens of one-to-two-ld rainbow trout after 96 h of exposure to Alimetrine 10 EC in the concentration of 31.4 µg/l. The mental group showed significantly higher values ($P < 0.01$) of plasma ammonia (NH_3), aspartate ransferase (AST), lactate dehydrogenase (LDH), creatinekinase (CK), lactate (LACT) and significantly ($P < 0.01$) values of alkaline phosphatase (ALP) compared to the control group. Also, a significant e in count of developmental forms of myeloid sequence, and segmented neutrophile granulocytes in the mental group was found. Teleangioectasiae of secondary gill lamellae and degeneration of hepatocytes bserved with histopathological examination. No histopathological changes were demonstrated in tissues

spleen, cranial and caudal kidney) of rainbow trout following exposure to cypermethrine. The methrine-based Alimethrineee 10 EC pesticide preparation was classified among substances strongly toxic ;h.

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ords: pyrethroids, acute toxicity, haematological profile, biochemical profile of blood, histopathology

ypermethrin is a widely used pesticide based on pyrethroids. It is among the most ive pyrethroid preparations (Bradbury and Coats, 1989a). The mechanism of its iveness in the case of fish is the same as that of other pyrethroids containing -cyano- 3- xybenzyl groups. They block the sodium channels of nerve filaments, thereby ening their depolarisation phase; moreover, they affect the GABA receptors in the nerve nts (Bradbury and Coats, 1989b; Hayes, 1994).

permethrin is a synthetic pyrethroid used for the control of ectoparasites which infest sheep, poultry and some companion animals. Recently, the compound has been used as ıotherapeutic agent for the control of ectoparasite infestations (*Lepeophtheirus salmonis ıligus elongatus)* in marine cage culture of Atlantic salmon, *Salmo salar* (Richards, Roth et al., 1993; Hart et al., 1997; Boxaspen and Holm, 2001; Treasurer and orth, 2004).

ermethrin is very toxic for fish (in laboratory tests 96-h LC50 were generally within ge of 0.4 - 2.8 µg/l), and aquatic invertebrates LC50 in the range of 0.01 - 5 µg/l anson, 1982; Sarkar et al., 2005). Fish sensitivity to pyrethroids may be explained by latively slow metabolism and elimination of these compounds. The half-lives for ion of several pyrethroids by rainbow trout are all longer than 48 hours, while

ination half-lives for birds and mammals range from 6 to 12 hours (Bradbury and Coats,)b).

he assessment of the ecotoxicological risks caused by pesticides to ecosystems is based ata on the toxicity and effects of pesticide preparations to non-target organisms. Fish are ıg the group of non-target aquatic organisms. The present paper is a contribution to the sment of toxicity and effects of a cypermethrin-based pesticide to fish.

ERIAL AND METHODS

permethrin [(RS)-α-cyano-3-phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-hyl cyclopropane carboxylate] was tested in the form of Alimethrine 10 EC pesticide, ning 100 g/l of active compound. The toxic effect was assessed through the results of toxicity tests and results of haematological, biochemical and histopathological nation of rainbow trout after exposure to this pesticide.

toxicity

: acute toxicity test on rainbow trout with Alimethrine 10 EC followed the OECD ion No. 203 and Methodical Manual ISO 7346/2. Juveniles of rainbow trout (camloops) 71 ± 1.06 g (mean ± SD) mean body weight and 88.9 ± 14.3 mm mean body length sed for the test. Six various concentrations and a control were used in the basic test. ;h specimens were used for every concentration and also in the control. The test was ned semistatically for 96 hrs. The bath was changed every 24 hrs. Basic physical and al indices of diluting water used in the acute toxicity test were as follows: acid isation capacity – $ANC_{4.5}$ 1.15 mmol/l; total ammonia 0.04 mg/l; NO_3^- 11.5 mg/l; NO_2^- ng/l; PO_4^{3-} 0.01 mg/l; chemical oxygen demand - COD_{Mn} 1.6 mg/l. Water temperature est ranged from 15.1 to 16.6 °C, oxygen saturation of water ranged between 94 and 98

The LC50, LC0 and LC100 values in the respective time intervals were determined by ›bit analysis.

ıematological, biochemical and histopathological examination

Haematological, biochemical and histopathological examination of rainbow trout ımloops) was performed at the end of 96 h acute toxicity test with Alimethrine 10 EC in the ncentration of 31.4 µg/l. At the same time, the control group of trout was examined. The st was performed semistatically with the bath exchanged every 24 hrs. Diluting water had e same physical and chemical parameters as described above. Water temperatures during the st ranged from 14.2 to 15.5 °C, oxygen saturation of water was above 60 % (ranging from to 93 %), pH ranged from 8.30 to 8.54. The test was performed in three 400 l aquaria. Each uarium was stocked with 30 specimens of one- to two-year-old rainbow trout (1 control uarium, 2 aquaria with Alimethrine 10 EC in the concentration 31.4 µg/l).

For the haematological, biochemical and histopathological examination, rainbow trout ımloops) of 144.50 ± 44.82 g average weight and 241.77 ± 26.0 mm average body length ere used.

ıematological profile

Heparinised injection needles were used to take samples of blood from the hearts of fish ınned by a blow with a blunt object over the head. To stabilize blood samples, an aqueous lution of heparin sodium salt at 0.01 ml per 1 ml blood was used (Svobodova et al., 1991).

The indices used to evaluate the haematological profile included the erythrocyte count BC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume ICV), mean colour concentration (MCHC), erythrocyte haemoglobin (MCH), leukocyte

Leuko) and the differential leukocyte count (Leukogram). The procedures were based ïed methods for haematological examination of fish (Svobodova et al., 1991).

results of haematological examinations were tested by variance analysis (ANOVA – ; test) using the Statistica 6.0 software.

nical blood plasma profile

ɔd plasma was obtained by the centrifugation of blood samples in a cooled centrifuge 37 x g). Biochemical indices determined in blood plasma included glucose (GLU), ɔtein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate genase (LDH), creatinekinase (CK), alkaline phosphatase (ALP), calcium (Ca^{2+}), LACT), cholinesterase (ChE) and inorganic phosphate (PHOS). For the biochemical of blood plasma, the VETTEST 8008 analyser (IDEXX Laboratories Inc., U.S.A.) :tured by Medisoft was used. The analyser uses dry chemical and colorimetric techniques. Selective test discs (Multi–layer film slides, Kodak) are used for the ɔn by a laser reading bar codes. ChE and LACT were determined by a COBAS MIRA ic analyser (Hoffman, La Roche, Co., Switzerland) using the BioVendor tests No. ıd 12351.

:esults of biochemical examination were tested by variance analysis (ANOVA – test) using the Statistica 6.0 software.

thological examination of tissues

blood sampling, samples of gills, liver, skin, cranial and caudal kidney and spleen en for histopathological examinations. The taken samples were immediately fixed in naline, drained and embedded in paraffin. Sections were made of the paraffin blocks ed with haematoxylin-eosin.

SULTS

te toxicity

)n the basis of the acute toxicity tests with rainbow trout, the 96-hour lethal concentrations limethrine 10 EC were determined (96hLC50 31.4 µg/l, 96hLC0 19.8 µg/l and 96hLC100 µg/l).

The 96hLC50 is the basic value in the acute toxicity test. For rainbow trout juveniles the LC50 value was 31.4 µg/l of Alimethrine 10 EC preparation, which corresponded to 3.14 of cypermethrin. In the course of deltamethrin poisoning in rainbow trout, the following cal symptoms were observed: accelerated respiration, loss of movement coordination, lay-down at their flank and are moving in this position. Subsequent short excitation stage vulsions, jumps above the water surface, movement in circles) changes into a resting , and another short-time excitation follows again. In the end, fish fall into damp, move ly at their flank. Respiration is slowed down, the damp phase and subsequent agony are long.

natological profile

ıe results of erythrocyte profile of the control and experimental rainbow trout under the are given in Tab 1. Compared to the control specimens, those after the acute exposure to methrin at the concentration of 3.14 µg/l had no effect on the haematological indices d (RBC, Hb, PCV, MCV, MCHC, MCH and Leuko).

1: Haematological parameters in rainbow trout affected by acute exposure to thrine 10 EC.

	Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
T/l)	0.80 ± 0.15 [a]	0.78 ± 0.24 [a]
)	41.71 ± 6.39 [a]	42.69 ± 10.30 [a]
/l)	0.36 ± 0.04 [a]	0.39 ± 0.05 [a]
fl)	460.57 ± 88.82 [a]	568.15 ± 291.13 [a]
pg)	53.31 ± 10.87 [a]	59.81 ± 23.85 [a]
(g/l)	115.96 ± 12.36 [a]	108.72 ± 17.68 [a]

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

It was evident that the acute exposure to cypermethrin resulted in a significant decrease in ınt of developmental forms of myeloid sequence and the segmented neutrophilic nulocytes in the experimental group. The results of examinations of the leukocyte profile :ontrol and experimental rainbow trout, are given in Tab 2.

ıle 2: Leukocyte differencial count in rainbow trout affected by acute exposure to nethrine 10 EC.

:es		Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
:o	G/l	13.15 ± 3.56 [a]	10.22 ± 5.03 [a]
phocytes	G/l	12.54 ± 3.11 [a]	9.94 ± 4.08 [a]
ocyte	G/l	0.01 ± 0.01 [a]	0.01 ± 0.03 [a]
rophile granulocytes ients	G/l	0.51 ± 0.19 [a]	0.25 ± 0.21 [b]
rophile granulocytes bands	G/l	0.05 ± 0.07 [a]	0.02 ± 0.04 [a]
lopmental phases- oid sequence	G/l	0.03 ± 0.05 [a]	0.01 ± 0.02 [b]

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

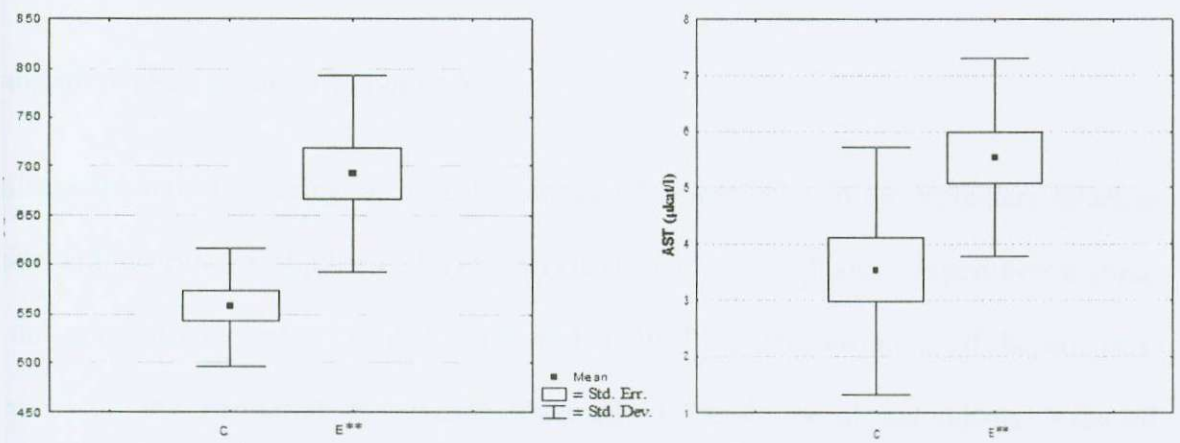
hemical blood plasma profile

he results of biochemical blood plasma profile of the control and experimental rainbow under the study are given in Tab 3 and Figs 1, 2, and 3. The experimental rainbow trout sed to acute effects of the cypermethrin-based pesticide showed significantly ($P < 0.01$) :ased concentration of alkaline phosphatase and a significantly ($P < 0.01$) increased :ntration of ammonia, aspartate aminotransferase, lactate dehydrogenase, creatinekinase ıctate in blood plasma. The rest of the indices (GLU, TP, ALB, GLOB, TRIG, ALT, ChE, PHOS) were comparable in the two groups during the study.

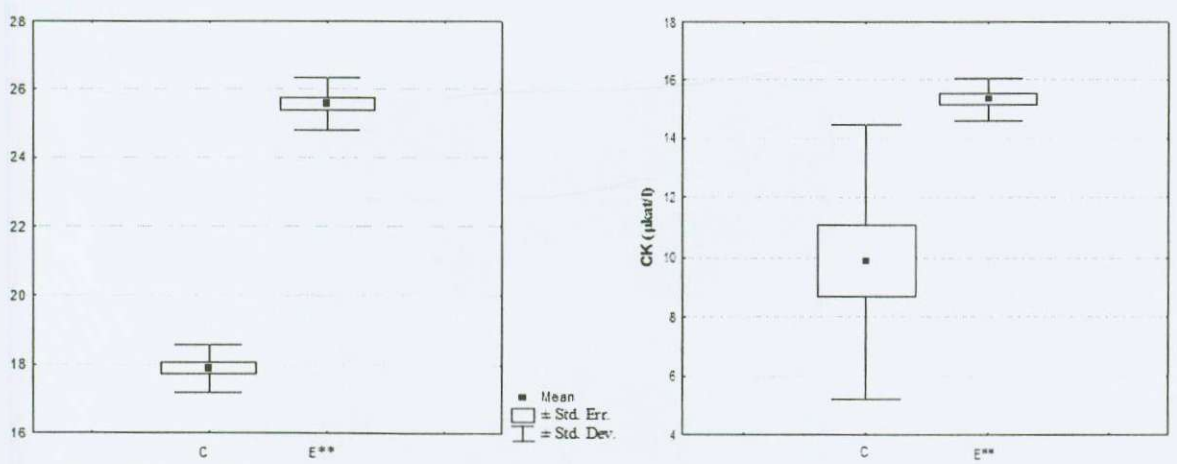
ble 3: Biochemical indices of blood plasma in rainbow trout affected by acute exposure to imethrine 10 EC.

ices	Control group x ± SD (n = 15)	Experimental group x ± SD (n = 15)
U (mmol/l)	3.64 ± 0.75 [a]	4.07 ± 1.84 [a]
(g/l)	36.60 ± 5.14 [a]	39.33 ± 4.30 [a]
B (g/l)	6.80 ± 2.71 [a]	8.60 ± 1.99 [a]
OB (g/l)	29.80 ± 2.81 [a]	30.87 ± 2.47 [a]
IG (mmol/l)	0.97 ± 0.12 [a]	0.86 ± 0.19 [a]
T (μkat/l)	0.08 ± 0.02 [a]	0.08 ± 0.01 [a]
$^{+}$ (mmol/l)	2.53 ± 0.18 [a]	2.81 ± 0.38 [a]
ɜ (μkat/l)	2.03 ± 1.30 [a]	2.52 ± 0.99 [a]
OS (mmol/l)	1.46 ± 0.22 [a]	1.39 ± 0.16 [a]

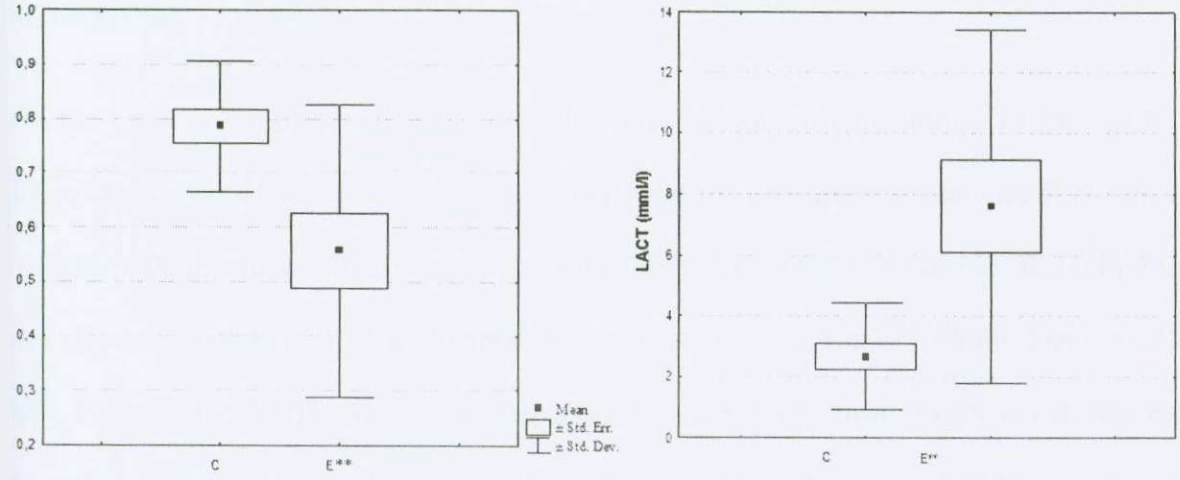
oups with different alphabetic superscripts differ significantly at $P < 0.01$ (ANOVA).



gure 1.: Effect of acute exposure to Alimethrine 10 EC (31.4 μg/l) on plasma NH_3 ncentration and AST activity in rainbow trout (C - control group, E - experimental group; gnificance $^{**}P < 0.01$).



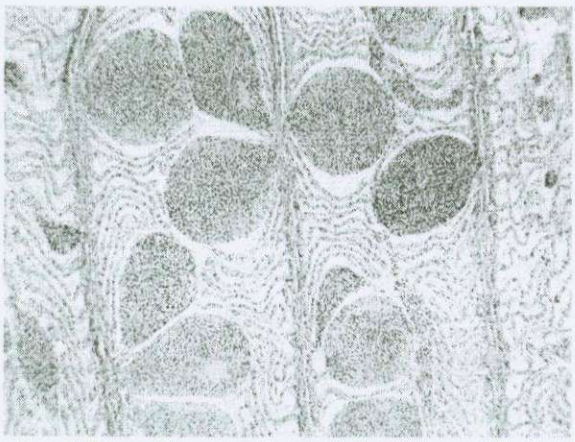
gure 2.: Effect of acute exposure to Alimethrine 10 EC (31.4 μg/l) on plasma LDH and activity in rainbow trout (C - control group, E - experimental group; significance $^{**}P$ .01).



igure 3.: Effect of acute exposure to Alimethrine 10 EC (31.4 µg/l) on plasma ALP and ACT activity in rainbow trout (C - control group, E - experimental group; significance $^{*}P < 0.01$).

stopathological examination of tissues

Histopathological examination revealed severe teleagioectasiae in the secondary lamellae gills with the rupture of pillar cells (Photo 1) in the 60 % individuals of experimental group the concentration of 31.4 µg/l Alimethrine 10 EC). Degeneration of hepatocytes, ecially in the periportal zones, was observed in the 40 % of individuals. Affected atocytes showed pycnotic nuclei and many small or one big vacuole in the cytoplasm. The pe of vacuoles was typical for fatty degeneration of liver. No changes were seen in other mined organs.



o 1: Gills of rainbow trout from the experimental group with the teleangioectasiae in the ndary lamellae. HE, 100 x.

SCUSSION

On the basis of the observed 96hLC50 value, the preparation Alimethrine 10 EC can be luded in a group of substances that are highly toxic for fish: the risk sentence R50 states values of 96hLC50 less than 1 mg/l. The value of 96hLC50 for Alimethrine 10, 31.4 µg/l, entially corresponds to 3.14 µg/l cypermethrin. Bradbury and Coats (1989b); Davis et al. 93); Polat et al. (2002) report a mean lethal toxicity of cypermethrin to various fish species laboratory conditions as values below 10 µg/l. Bradbury and Coasts (1989b) state LC50 0 µg/l and 1.2 µg/l the value for rainbow trout and brown trout (*Salmo trout*), respectively. res (1985) reported the value of 96hLC50 for rainbow trout to be 2.57 µg/l. Whalon et al. 90) state the value of 24hLC50 4.50 µg/l and 20 µg/l for common carp (*Cyprinus carpio* and silver carp, respectively. Pyrethroids are more toxic to fish at lower temperatures and ear to be more toxic to smaller fish than larger ones (Mauck et al., 1976; Hill, 1985; Başer l., 2003).

Haematological and biochemical profiles of blood can provide important information about internal environment of the organism (Masopust, 2000).

n our experiments with rainbow trout, a significant decrease in count of developmental ıs of myeloid sequence and the segmented neutrophilic granulocytes in the experimental p was observed. No significant differences were observed in the levels of RBC, Hb, PCV, V, MCHC, MCH and Leuko. On the other hand, Atamanalp et al. (2002a) and Atamanalp Yanik (2003) found a significant increase ($P < 0.05$) in the levels of RBC and a ficant decrease ($P < 0.05$) in the Hb, MCH, MCHC, thrombocyte count and erythrocyte nentation rate in rainbow trout (*Oncorhynchus mykiss*) following cypermethrin and ozeb acute exposure.

ıe main biochemical blood profile response of rainbow trout to the acute effect of 31.4 of Alimethrine 10 EC was a significantly ($P < 0.01$) decreased concentration of alkaline

osphatase and significantly ($P < 0.01$) increased concentration of NH_3, LDH, AST, CK and ACT in blood plasma.

Cypermethrin caused an increase in plasma ammonia level supposedly due to an increase amino acids catabolism and a failure of ammonia excretion mechanisms (Svoboda, 2001).

The activities of enzymes in blood plasma can be also used as a relevant stress indicator. e enzymes used for the purpose are above all LDH, CK and transaminases (ALT and AST). ignificant increase in the activity of the above mentioned plasma enzymes indicates stress-ed tissue impairment (Svoboda, 2001). After acute exposure to cypermethrin, a significant ease ($P < 0.01$) in AST level was found in experimental trout in comparison to control cimens. Increased activities of both transaminases indicated amplified transamination cesses. An increase in transamination occurs due to amino acid input into the TCA cycle in er to cope with the energy crisis during pyrethroid-based stress (Philip et al., 1995).

The increase in LDH level indicated metabolic changes, i.e. the glycogen catabolism and ose shift towards the formation of lactate in stressed fish, primarily in the muscle tissue ion et al., 1983).

On the other hand, Atamanalp et al. (2002b) found changes in the concentration of calcium phosphor in rainbow trout following cypermethrin exposure. Jee et al. (2005) found an ease in levels of serum glutamic-acid-oxylacetic-acid-transaminase, glutamic-acid-vic-acid-transaminase, glucose and alkaline phosphatase and a decrease in the entration of plasma total protein, albumin, cholesterol and lysozyme in Korean rockfish *astes schlegeli*) exposed to cypermethrin.

We observed teleangioectasiae of secondary lamellae of the gills and degeneration of tocytes in periportal zones in our experiment. Teleangioectasiae comps acute respiratory ess. Degeneration of hepatocytes in periportal zones can imply the influence of toxic ounds in the digestive tract. The biochemical changes in liver profile can relate to

patocytes damage. Sarkar et al. (2005) found significant changes as hyperplasia, integration of hepatic mass, focal coagulative necrosis in *Labeo rohita* exposed to permethrin. Sublethal effects of pyrethroids on fish include damage of gills and behavioral anges. Because they are highly lipophilic (attracted to the non-water soluble components of s), pyrethroids are likely to be strongly absorbed by the gills, even from water containing levels of pyrethroids (Smith and Stratton, 1986). Edwards et al. (1986) reported acute icity symptoms of cypermethrin in rainbow trout such as, gill flailing, hyperactivity, loss buoyancy and inability to remain upright.

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MONITORING ZVÝŠENÝCH KONCENTRACÍ DUSITANŮ VE VODĚ A JEJICH VLIV NA RYBY

Dusitany jsou přirozenou součástí koloběhu dusíku v přírodě. Přítomnost dusitanů v rostředí je problémem z důvodu jejich vysoké toxicity pro živočichy. Toxický vliv dusitanů a ryby je velmi dobře dokumentován v literatuře (Lewis a Morris 1986; Jensen 2003). řestože jsou dusitany toxické, tvoří v malých koncentracích přirozenou složku živočišných ání. Jsou totiž vnitřně produkovány jako metabolit fyziologického přenašeče molekuly xidu dusnatého, který je produkován endotelem cév, nervovými buňkami a aktivovanými akrofágy.

Dusitany zpravidla doprovázejí ve vodách dusičnany a formy amoniakálního dusíku. zhledem ke své chemické a biochemické nestálosti se obvykle vyskytují ve velmi malých a ísto jen stopových koncentracích. V přírodních vodách dusitany mezi anorganickými rmami dusíku nikdy nedominují, protože v oxických podmínkách jsou rychle ansformovány nitrifikací na dusičnany. Na druhé straně přichází v anoxických podmínkách úvahu biologická denitrifikace na elementární dusík, resp. N_2O. Proto lze dusitany často rokázat v nízkých koncentracích jako meziprodukt chemických a biochemických ansformací sloučenin dusíku (Pitter 1999).

V posledních letech škodlivý vliv dusitanů na ryby přitahuje hodně pozornosti. Zvláště v quakulturních zařízeních s recirkulací vody. Zvýšené koncentrace se velmi často objevují v ecirkulačních systémech, zejména bezprostředně po zahájení provozu nebo v důsledku erovnováh v procesu nitrifikace (Kamstra et al. 1996; Avnimelech et al. 1986). Proces trifikace je využíván pro snížení koncentrace amoniaku, který je hlavním produktem usíkatého metabolismu ryb (Wood 1993). Během nitrifikace dochází k biochemické oxidaci moniakálního dusíku na dusitany a následně až na dusičnany, které jsou pro ryby téměř etoxické. Pokud druhá fáze nitrifikace neprobíhá dostatečně rychle, dochází v systému hromadění dusitanů, které bývá příčinou zhoršení zdravotního stavu ryb a mnohdy i jejich asového úhynu (Svobodová et al. 2005). Mezi faktory ovlivňující proces nitrifikace patří H, teplota, koncentrace kyslíku, počet nitrifikačních bakterií nebo přítomnost látek hibujících nitrifikaci, tzn. HNO_2, NH_3, methylenová modř, antibiotika a některé organické oučeniny (anilín, dodecylamin, p-nitrobenzaldehyd) (Russo a Thurston 1991).

Dusitany jsou pro ryby toxické. Vstřebávají se přes tzv. „chloridové" buňky žaber do krve, de se vážou na barvivo hemoglobin za vzniku methemoglobinu. Methemoglobin nemá chopnost přenášet kyslík, a tak se snižuje kapacita krve pro transport kyslíku. Zvýšené nožství methemoglobinu v krvi bývá doprovázeno hnědým zbarvením krve a žaber. Při ýrazném zvýšení obsahu methemoglobinu v krvi jsou ryby malátné, ztrácejí orientaci, ikový reflex, objevují se záškuby až křeče svaloviny (Svobodová et al. 1992). Vysoké odnoty dusitanů nalézaných ve vodě způsobují těžké fyziologické poruchy, včetně iontové gulace a porušení dýchacích, kardiovaskulárních, endokrinních a vylučovacích procesů ebo mohou mít za následek i masové úhyny ryb (Jensen 2003; Kroupová et al. 2005). usitany jsou také akumulovány v některých tkáních, ve kterých vyvolávají poruchy a oškození, a to v žábrách (hyperplazii a vakuolizaci chloridových buněk), játrech, mozku a valech. V těchto orgánech bývá koncentrace dusitanů nižší než v krvi, ale hodnoty naměřené játrech a mozku mrtvých nebo poškozených ryb mohou dosáhnout až 30 násobku hodnoty aměřené v okolní vodě (Margiocco et al. 1983).

Toxicita dusitanů pro ryby značně kolísá a závisí na mnoha vnějších i vnitřních faktorech. ejvýznamnějšími jsou kvalita vody (Crawford a Allen 1977; Russo et al. 1981; Eddy et al. 983; Lewis a Morris 1986), druh ryb (Palachek a Tomasso 1984), věk ryb (Bartlett a eumann 1998), individuální citlivost ryb (Williams a Eddy 1988; Stormer et al. 1996; ggergaard a Jensen 2001) a další. Z fyzikálně-chemických parametrů vody nejvíce ovlivňuje

xicitu dusitanů koncentrace chloridů. Bylo zjištěno, že se zvyšující se koncentrací chloridů esá toxicita dusitanů pro ryby (Máchová et al. 2004). Nebezpečí dusitanů spočívá v tom, že st příjmu chloridů žábrami může být nahrazena právě dusitany (NO_2^- má totiž jistou afinitu iontové výměně Cl^-/HCO_3^-). Proto zvýšením koncentrace chloridů ve vodě ochráníme ryby ed zvýšeným příjmem dusitanů žábrami a tím i před jejich toxickými účinky (Perrone a leade 1977).

Od roku 1977 je známo, že toxicita dusitanů silně závisí na salinitě vody (Crawford a Allen 977). V mořské vodě byla zaznamenána 50 až 100krát nižší mortalita ryb než ve sladké vodě i stejné koncentraci dusitanů. Různí autoři došli k protikladným závěrům o kombinovaném ivu jednotlivých faktorů na toxicitu dusitanů pro ryby. Důležitost jednotlivých faktorů je ále hodnocena.

ílem prací předkládaných v této kapitole je:

stanovit vliv chloridů na toxické účinky dusitanů na ryby, a to pomocí výsledků testu akutní toxicity, vyšetření hematologického a biochemického profilu krevní plazmy a histologického vyšetření tkání.

rezentace výsledků:

ískané výsledky týkající se vlivu chloridů na toxické účinky dusitanů na ryby byly ezentovány na konferenci ICTX 2004 v Tampere (Finsko) a na 6. konferenci „mladých ědeckých pracovníků" Brno (2004).

ýsledky hodnocení vlivu chloridů na toxické účinky dusitanů u ryb byly publikovány v asopisech Aquaculture Research, Toxicology and Applied Pharmacology a ve sborníku z 6. onference „mladých vědeckých pracovníků".

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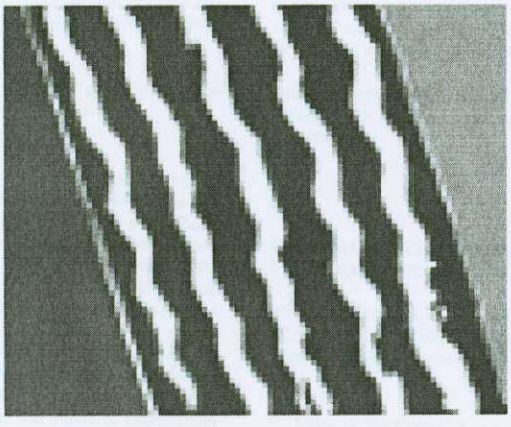
ABSTRACTS

10th International Congres of Toxicology
11-15 July 2004, Tampere, Findland



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analysed for opiates and for cannabis and the concentrations of total morphine ranged between 0.07 and 14.61 ng/mg, (mean 9.8 ng/mg) while for cannabis the concentrations ranged from 0.01 to 1.16 ng/mg (mean 0.07 ng/mg). Samples corresponding to the detention time awaiting trial showed diminished levels of drugs while samples corresponding to the time they were free, presented high drug levels. Segmental hair analysis was performed in cases where more information was necessary (e.g. detainees in custody for several months). The court must be able to discriminate between addiction and casual drug use. Although hair testing for drugs of abuse is not specially referred in any Law in Greece, District Attorneys in Crete demand the hair test in severe cases where medical examination is not able to give answers in legal issues, such as the confirmation of long-term drug use or the severity of abuse. In those cases a segmental hair analysis was conducted and the Courts accepted it.

P12 Ecotoxicology

373

MODULATORY EFFECT OF CADMIUM EXPOSURE ON DELTAMETHRIN INDUCED OXIDATIVE STRESS IN CHANNA PUNCTATA BLOCH

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Modulatory effect of cadmium pre-treatment (0.2 mg/kg b.w. i.p. on alternate days for 7 days) on deltamethrin-induced oxidative stress and antioxidants was studied in freshwater fish *Channa punctata* Bloch. Lipid peroxidation (LPO) was measured as an indicator of oxidative stress. Activities of glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) were measured in liver, kidney and gills. Level of reduced glutathione (GSH) was also measured. As a result of 48 h exposure to deltamethrin (0.75 ppb), LPO values increased significantly in all the tissues when compared with controls. Fish pretreated with cadmium and subsequently exposed to deltamethrin showed significantly reduced LPO values when compared with deltamethrin-exposed fish. Conversely, in kidney, an additive response was observed. Deltamethrin significantly affected glutathione dependent enzymes. In the cadmium pre-treated deltamethrin exposed fish, a restorative response on antioxidant enzymes was observed only in liver and gills. Deltamethrin exposed fish, which were pre-treated with cadmium showed normalization of GSH level in liver and gills but kidney GSH remained elevated. Cadmium alone had no significant effect on various parameters. Metallothionein (MT) induction studied by SDS-PAGE of various organs showed presence of MT-like protein in liver only in cadmium pre-treated group. It was confirmed by molecular weight characterization and cadmium analysis of separated fractions by AAS. These results demonstrate a modulatory role of cadmium on the oxidative stress and other related parameters in fish. This modulation can be attributed to the interactive role of cadmium against deltamethrin-induced toxicity. Findings of the present investigation demonstrate that in an aquatic environment biological response of a toxic chemical may be influenced by the other chemicals and in an ecotoxicological investigation mixture of toxicants may provide more realistic impact assessment.

374

EFFECT OF CHLORIDES UPON FISH TOLERATION TO NITRITE EFFECTS

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In the last years deaths of fish reared in recirculation systems have been frequently diagnosed. This applies to newly built systems or systems put again into operation, in which biological filters are not fully functional yet. The deaths and damages of fish occur due to methaemoglobinaemia that is caused by the effect of increased nitrite concentrations in water. A protective effect of chlorides during activity of increased nitrite concentrations upon health status of two years old carps (*Cyprinus carpio*) was assessed using examination of haematological values, biochemical profile of blood plasma and histological examination of gills. Two groups of carp were exposed to nitrites in concentration 67 mg.l^{-1} NO_2^-, at temperature 20–21 °C during 96 hours. The chloride content in the water of the first group was 71 mg.l^{-1}, of the second group was 11 mg.l^{-1}. Exposition of fish to nitrite was manifested the most markedly by the methaemoglobin formation. The highest methaemoglobin content (90,5 ± 4,38%) was found in the second group of fish, the content of 38,3 ± 13,00% in the first group of fish and 2,01 ± 0,47% in control group (11 mg.l^{-1} chlorides without nitrites). Out of other investigated parameters significant differences of the following values were found: higher red blood cells count, haemoglobin content, white blood cells count in the first group of carp and lower methaemoglobin content compared to the second group of carp. The protective effect of chlorides was confirmed even by the results of erythrograms examination and the results of histological examination of gills.

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375

PHYSICAL AND CHEMICAL CRITERIA FOR PESTICIDES' ECOTOXICOLOGY MONITORING IN ECOSYSTEMS

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Using of the physical and chemical properties of pesticides (including a dipole moment) allow creating of pesticides analytical chemistry monitoring system. The connection between the physical and chemical properties of different classes of organic pesticides and their behavior in environment was studied. The most important and general characteristic of pesticides in order to evaluation complex of their properties is polarity. Polarity is characterized by value of dipole moment (μ), which is a function of the molecule compound's structure. More than 80 pesticides' compounds were determined and divided into 3 groups: non-polar (μ-0-2*D*), slightly polar (μ-2-6*D*) and polar (μ > 6*D*). Dipole moment determination by thin layer chromatography was worked out and theoretically proved. The pesticide splitting up into three groups according their polarity gave the possibility creating the algorithm of the system of pesticide mixture analyze, which comprise 5 stages. The algorithm allows choosing the optimal methods of extraction, purification, quantitative and qualitative determination of pesticides in any mixtures and spheres. The dependence of pesticide degradation rate in soil on Polarity (Dipole moment) was found out. Degradation rate constant of the Dursbane destruction is k-0.07; and half-life is T_{50}-9.9 days. Dursbane dipole moment μ is 0.32. Degradation rate constant of the Stomp is k-0.23; and T_{50}-3.0 days and μ is 2.79. Degradation rate constant of the Oxadixile is k- 0.34; and T_{50}-2.0 days and μ is 5.42. Low polarity pesticides are more persistent and more harmful for the environment. On the ground of field experiments conducted in different soil climatic zones risk assessment simulation model for different pesticides application was derived. It gives the possibility conducting of crops chemical protection with minimizing of pesticides negative effect on environment.

Effect of chlorides upon fish toleration to nitrite effects

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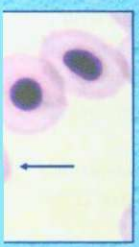


is located in one cell pole.

A protective effect of chlorides during activity of increased nitrite concentrations upon health status of two-year-old carp (*Cyprinus caprio* L.) was assessed using examination of haematological values and biochemical profile of blood plasma.

MATERIALS AND METHODS

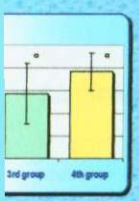
The acute toxicity test on common carp (*Cyprinus carpio* L.) followed the OECD Direction No. 203 and Methodical Manual ISO 7346/2. Four groups of two-year-old carp were exposed to different concentration of nitrites and chlorides during 96 hours, at a temperature of 20-21 °C. Examination of blood parameters and biochemical profile was performed on 28 carps (7 from each group).

1st group: 71 mg/l NO_2^- 71 mg/l Cl^-

2nd group: 71 mg/l NO_2^- 11 mg/l Cl^-

3rd group: 0 mg/l NO_2^- 71 mg/l Cl^-

4th group: 0 mg/l NO_2^- 11 mg/l Cl^-



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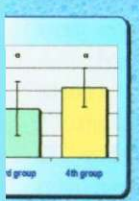

Indexes a,b characterize
roups (p<0.05).

ifR) and control carp (right).

RESULTS

The **second** experimental group of carp showed significantly **lower** haemoglobin content ($p<0.05$), erythrocytes number ($p<0.05$), leukocytes number ($p<0.05$), lymphocytes number ($p<0.05$) and significantly **higher** methaemoglobin content ($p<0.01$) compared to the third and the fourth group. The first group of carp showed significantly higher methaemoglobin content ($p<0.05$) compared to the third and the fourth group, but the percentage of methaemoglobin was lower then in the second group of carp.

The erythrocytes of the **second** group of carp showed significantly **higher** number of elongated erythrocytes with nucleus located in one cell pole compared to the control group. All erythrocytes of the second group of carp had remarkably **pale** cytoplasm compared to the control group.

The concentration of glucose, total protein, triglyceride, ammonia, albumine, phosphorus, calcium and the activity of alanin aminotransferase, aspartate aminotransferase and lactate dehydrogenase were **comparable** in experimental and control groups.

Indices	Units	1st group Mean±SD	2nd group Mean±SD	3rd group Mean±SD	4th group Mean±SD
PCV	l/l	0.21±0.02[a]	0.21±0.02[a]	0.24±0.02[a]	0.27±0.04[a]
MCV	fl	156.71±13.35[a]	192.29±18.73[b]	167.14±35.38[a]	195.43±10.94[a]
MCH	pg	45.65±2.02[a]	47.37±6.39[a]	44.43±4.14[a]	44.63±3.05[a]
MCHC	l/l	0.29±0.01[a]	0.25±0.02[a]	0.28±0.04[a]	0.23±0.01[b]
monocytes	G/l	0.08±0.11[a]	0.07±0.06[a]	0.03±0.08[a]	0.06±0.09[a]
myelocytes	G/l	0.42±0.35[a]	0.28±0.07[a]	0.43±0.27[a]	0.93±0.36[b]
metamyelocytes	G/l	1.99±1.86[a]	1.36±0.72[a]	2.82±1.00[a]	2.44±0.75[a]
band neutrophiles	G/l	0.08±0.16[a]	0.02±0.02[a]	0.04±0.05[a]	0.12±0.16[a]
segmented neutrophiles	G/l	0.00±0.00[a]	0.01±0.02[a]	0.03±0.08[a]	0.00±0.00[a]

Tab 1. Haematological indices of carp. Indexes a,b characterize an accordance or a difference between groups ($p<0.05$).

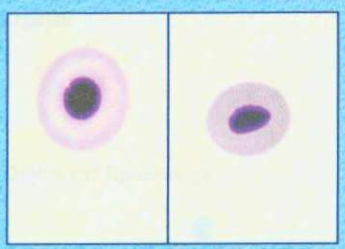

Fig. 2. Erythrocyte of carp afected by nitrites (left) and control carp (right).

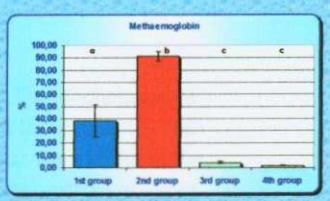

Fig. 6. Methaemoglobin content (%) of carp. Indexes a,b,c characterize an accordance or a difference between groups ($p<0.05$, $p<0.01$).

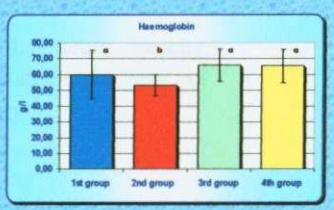

Fig. 7. Haemoglobin content (g/l) of carp. Indexes a,b characterize an accordance or a difference between groups ($p<0.05$).

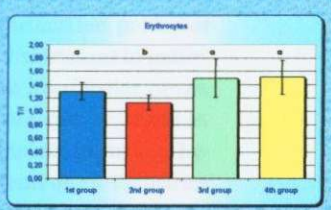

Fig. 8. Erythrocyte count (T/l) of carp. Indexes a,b characterize an accordance or a difference between groups ($p<0.05$).

CONCLUSION

orides was confirmed by the results of changes in the red globin content, erythrocytes number) and white (leukocytes and icture of common carp.

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Haematological and biochemical profiles of carp blood following nitrite exposure at different concentrations of chloride

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Abstract

Haematological parameters of 2-year-old carp (*Cyprinus carpio* L.) were assessed to study the protective effect of chloride on the health of fish exposed to elevated nitrite concentrations. Four groups of carp were exposed to different concentrations of nitrite and chloride for 96 h (group E1: 67 mg L^{-1} NO_2^-, 11 mg L^{-1} Cl^-; group E2: 67 mg L^{-1} NO_2^-, 100 mg L^{-1} Cl^-; group E3: 0 mg L^{-1} O_2^-, 100 mg L^{-1} Cl^- and group C: 0 mg L^{-1} NO_2^-, 11 mg L^{-1} Cl^-). The main haematological response of carp to an acute exposure to nitrite (group E1) was a significant decrease ($P<0.05$) in haemoglobin concentrations (53.40 ± 6.61 g L^{-1}), haematocrit (0.21 ± 0.02 LL^{-1}), erythrocyte count (1.13 ± 0.12 TL^{-1}), leucocyte count (7.1 ± 4.19 GL^{-1}) and lymphocyte count (5.28 ± 2.51 GL^{-1}), and a significant increase in methaemoglobin concentration (90.50 ± 4.38%, $P<0.01$) and mean corpuscular haemoglobin concentration (0.27 ± 0.2 LL^{-1}, $P<0.05$). At higher chloride concentrations (group E2), a lower nitrite toxicity was observed. In group E2 carp, methaemoglobin made up 38.32 ± 13.30%. Erythrocytes in carp exposed to nitrite showed qualitative changes. Compared with the control group C, group E1 carp showed a significantly higher number ($P<0.05$) of elongated erythrocytes, with the nucleus located at one cell pole (0.519 ± 0.388 TL^{-1}). All erythrocytes of group E1 carp had remarkably clear cytoplasms compared with the cytoplasm in the control group C. The biochemical values found were comparable with those found in controls. The main histological lesions were found in the gills of carp exposed to nitrite and consisted of hyperplasia and an elevated number of chloride cells.

Keywords: *Cyprinus carpio* L., acute toxicity, methaemoglobinaemia, qualitative changes in erythrocytes, gill histopathology

Introduction

Nitrite is an intermediate and important product in bacterial nitrification and denitrification processes in the nitrogen cycle. Nitrite concentrations in natural water are typically low in the micromolar range. Elevated concentrations of nitrite can be found in water receiving nitrogenous effluents, in various hypoxic environments or in effluents from industries producing metal, dyes and celluloid (Pitter 1999).

Today, when intensive methods of aquaculture are used, elevated nitrite concentrations cause great problems in aquaculture. Intensive rearing methods are being commonly used today. These methods mostly rely on recirculating water systems to remove waste ammonia from water. If, however, the oxidation of ammonia is incomplete, larger quantities of nitrite may accumulate in the system. As soon as nitrification in biological filters begins or if the nitrification

ess is in a state of imbalance, nitrite concentra- may reach 1 mM or more (Avnimelech, Weber, ıer, Milstein & Zorn 1986; Kamstra, Span & Van rd 1996). This may result in mass fish mortality ɔodová, Máchová, Poleszczuk, Hůda, Hamáčková oupová 2005). Elevated nitrite concentrations in :r also cause great problems in intensive culture ·namental fish, including excessive stock of fish insufficient inflow of fresh water to aquariums řák 2004).

itrite toxicity in fish varies considerably and de- ls on a large number of external and internal ɔrs. Among the most important ones are water ity (such as pH, temperature, and cation, anion oxygen concentrations), length of exposure, fish ies, fish size and age and individual fish suscept- y (Lewis & Morris 1986; Jensen 2003). Problems nitrite in freshwater animals stem from the fact NO_2^- crosses the gill barrier by competing with ride for chloride uptake sites (Gaino, Arillo & si 1984; Williams & Eddy 1986) and is accumu- l in the plasma (Shechter, Gruener & Shuval ; Bath & Eddy 1980). Since 1977, nitrite toxicity been known to depend greatly on the salinity of water in which the nitrite exposure occurred wford & Allen 1977). The relationship between te toxicity and chloride concentration is linear ıso & Thurston 1977; Palachek & Tomasso 1984; 'onnell 1985). It has been established that the ef- of chloride on nitrite toxicity is so significant that :riments in which chloride concentrations are documented are of very little value because they not be meaningfully compared with the results of :r studies (Lewis & Morris 1986).

he aim of the present study was to assess the ef- s of nitrite on the haematological and biochem- profile of blood and histological picture of the , and, using the values of these parameters, to uate the protection that chloride may provide to common carp against toxic effects of nitrite.

terial and methods

n acute toxicity test in common carp (*Cyprinus io* L.), the OECD Direction No. 203 was observed. test was performed in a semi-static assay for ı. Fish were kept in tanks, each containing 200 L est solution. The bath was changed every 24 h. r groups each, containing seven specimens of 2- r-old carp, were exposed to different concentra- ıs of nitrite and chloride:

Group E1: 67 mgL^{-1} NO_2^-; 11 mgL^{-1} Cl^-.
Group E2: 67 mgL^{-1} NO_2^-; 100 mgL^{-1} Cl^-.
Group E3: 0 mgL^{-1} NO_2^-; 100 mgL^{-1} Cl^-.
Group C: 0 mgL^{-1} NO_2^-; 11 mgL^{-1} Cl^- (control).

Each tank was aerated with an air pump attached to aeration stones. The basic physical and chemical indices of diluting water used in the acute toxicity test were as follows: $ANC_{4.5}$ (acid neutralization capacity) = 1.15 mmol L^{-1}; COD_{Mn} (chemical oxygen demand) = 1.5 mg L^{-1}; total ammonia = 0.04 mg L^{-1}; NO_3^- = 7.75 mg L^{-1}; PO_4^{3-} = 0.01 mg L^{-1}; sum of Ca+Mg = 14 mgL^{-1}. Water temperatures in the test ranged from 20 to 21 °C, oxygen saturation of water was above 60% (ranging from 61% to 82%) and pH ranged from 6.22 to 7.30.

A total of 28 carp (seven from each group) were examined to determine the haematological and biochemical profiles of blood plasma and histopathological changes of the gills. Carp body weights in individual groups were as follows: group E1: 678 ± 63.1 g (mean ± SD), group E2: 671 ± 103.9 g, group E3: 692 ± 60.0 g and group C: 744 ± 127 g.

Blood was sampled from the caudal vessels and stabilized by 50 IU sodium heparin per 1 mL blood. Erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), methaemoglobin (MetHb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), leucocyte count (Leuco) and differential leucocyte count (leucogram) were determined in the blood samples (Svobodová, Pravda & Paláčková 1991). Qualitative assessment of erythrocytes was also carried out. In each carp, 300 erythrocytes were assessed for changes in cytoplasm colouring, erythrocyte disintegration, non-nucleated erythrocytes, change in the erythrocyte shape and the nucleus location. The leucogram and morphological changes in erythrocytes were evaluated by examination of blood smears stained with Pappenheim stain.

Blood samples were centrifuged in a cooled centrifuge (4 °C, 837 *g*). The biochemical indices determined in blood plasma included glucose (GLU), total proteins (TP), albumins (ALB), total globulins (GLOB), ammonia (NH_3), triacylglycerols (TAG), calcium (Ca^{2+}), inorganic phosphorus (PHOS) and lactate dehydrogenase (LDH). For the biochemical analysis of blood plasma, a VETTEST 8008 Analyser (IDEXX Laboratories, Westbrook, ME, USA) was used.

After blood sampling, the fish were killed and samples of gills were collected for histological examination. The samples collected were immediately fixed in 10% formaldehyde, drained and embedded in par-

affin. Sections of paraffin blocks were made and stained with haematoxylin–eosin.

The statistical tests were performed using the Tukey test, a one-way analysis of variance (ANOVA).

Results

Haematological profile

The most significant changes in the red blood count in the groups of carp compared were found in methaemoglobin concentration levels (Fig. 1). Significantly, the highest concentration of methaemoglobin (90.50 ± 4.38%, $P<0.01$) compared with other groups of carp was found in group E1 after exposure to nitrite (67 mg L^{-1}) at a low chloride concentration (11 mg L^{-1}). The second highest concentration of methaemoglobin (38.32 ± 13.30%) was found in group E2 after exposure of nitrite (67 mg L^{-1}) with a high chloride concentration (100 mg L^{-1}). In this group of carp, methaemoglobin concentration was significantly lower ($P<0.01$) than in carp of group E1 but significantly higher than in carp of groups E3 and C. Methaemoglobin concentrations in groups E3 and C were below 5% (it is 4.22 ± 1.16% and 2.01 ± 0.47% respectively). Haemoglobin concentrations (53.40 ± 6.61 g L^{-1}) and erythrocyte counts (1.13 ± 0.12 TL^{-1}) in group E1 were significantly lower ($P<0.05$) than in other groups of carp (Fig. 1). Haematocrit values (Table 1) were significantly lower ($P<0.05$) in carp of groups E1 (0.21 ± 0.02 LL^{-1}) and E2 (0.21 ± 0.02 LL^{-1}) compared with the control group C (0.25 ± 0.04 LL^{-1}). No significant differences in MCH values (Table 1) between the groups were found. Significantly higher ($P<0.05$) MCV values were found in carp of groups E1 (192.3 ± 18.0 fL) and C (195.1 ± 10.0 fL) compared with groups E2 (158.7 ± 13.0 fL) and E3 (161.1 ± 35.0 fL) (Table 1). The lowest ($P<0.05$) MCHC of all the groups was found in group C (control group) (Table 1).

A qualitative erythrocyte assessment showed a significant increase in the number of elongated oval-shaped erythrocytes with the nucleus at a cell pole (0.519 ± 0.388 TL^{-1}) in group E1 carp exposed to nitrite (67 mg L^{-1}) with a low concentration of chloride (11 mg L^{-1}) (Fig. 2). No elongated erythrocytes were found in the control group C. Similar numbers of erythroplastids were found in all the carp groups

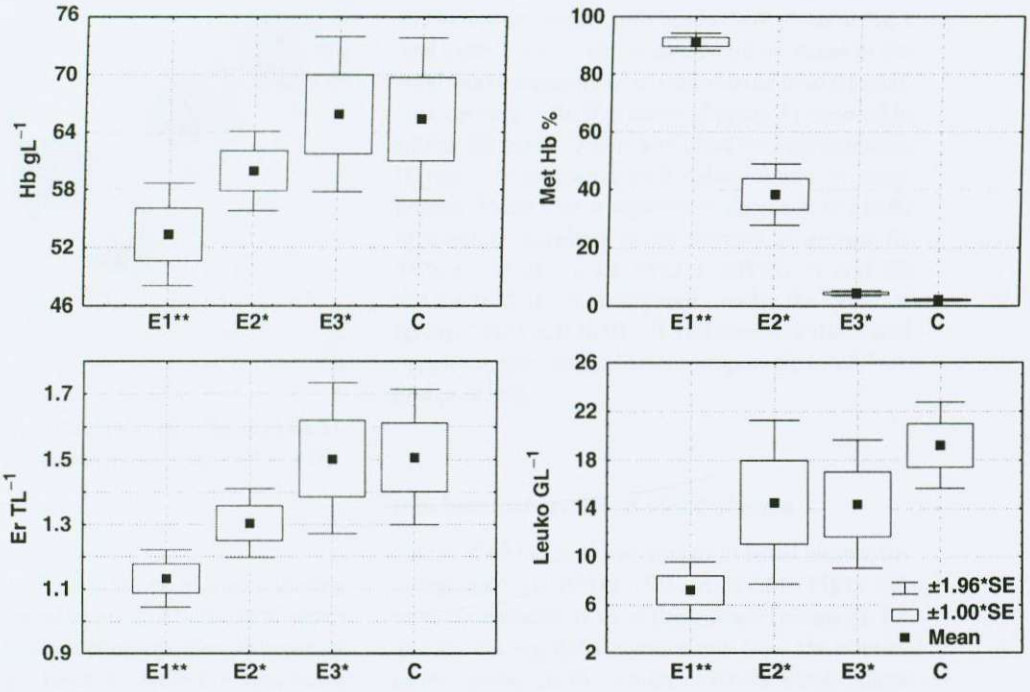
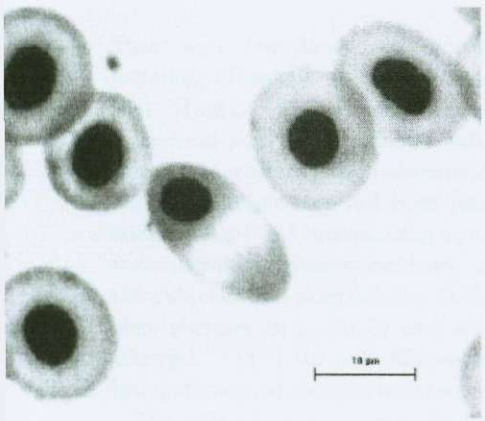


Figure 1 Changes in haematological indices [haemoglobin (Hb), erythrocyte (Er), methaemoglobin (MetHb), leucocyte count (Leuco)] in carp exposed to different baths. Experimental groups E1 (67 mg L^{-1} NO_2^- 11 mg L^{-1} Cl^-) and E2 (67 mg L^{-1} NO_2^- 100 mg L^{-1} Cl^-), E3 (0 mg L^{-1} NO_2^- 100 mg L^{-1} Cl^-) and the control group C (0 mg L^{-1} NO_2^- 11 mg L^{-1} Cl^-). Statistical significance $^*P \leq 0.05$, $^{**}P \leq 0.01$.

le 1 Haematological indices (comparison of red blood counts, qualitative changes in erythrocytes and differential leukocounts) of carp groups studied (groups with different alphabetic superscripts differ significantly at $P < 0.05$)

x	Group E1 67 mg L^{-1} NO_2^- $x \pm SD$ ($n = 7$)	Group E2 100 mg L^{-1} Cl^-, 67 mg L^{-1} NO_2^- $x \pm SD$ ($n = 7$)	Group E3 100 mg L^{-1} Cl^- $x \pm SD$ ($n = 7$)	Group C Control group $x \pm SD$ ($n = 7$)
(LL^{-1})	0.21 ± 0.02^a	0.21 ± 0.02^a	0.24 ± 0.01^a	0.25 ± 0.04^a
(fL)	192.3 ± 18.0^b	158.7 ± 13.0^a	161.1 ± 35.0^a	195.1 ± 10.0^b
(pg)	45.10 ± 6.49^a	45.20 ± 2.59^a	44.40 ± 4.15^a	44.90 ± 3.01^a
IC (LL^{-1})	0.27 ± 0.02^a	0.28 ± 0.01^a	0.28 ± 0.03^a	0.20 ± 0.01^b
itative changes in erythrocytes (%)				
sintegration	0.330 ± 0.353^a	0.189 ± 0.163^a	0.141 ± 0.240^a	0.047 ± 0.115^a
ngation+nucleus at pole	0.519 ± 0.388^b	0^a	0^a	0^a
ythroplastid	0.047 ± 0.032^a	0^a	0.094 ± 0.050^a	0^a
rential leukocyte counts (GL^{-1})				
mphocytes	5.28 ± 2.51^a	12.07 ± 6.79^b	11.00 ± 5.94^b	15.67 ± 4.24^b
nocytes	0.065 ± 0.060^a	0.077 ± 0.110^a	0.034 ± 0.080^a	0.056 ± 0.090^a
rophil granulocytes				
elocytes	0.28 ± 0.07^a	0.42 ± 0.35^a	0.43 ± 0.27^a	0.93 ± 0.36^b
tamyelocytes	1.36 ± 0.72^a	1.99 ± 1.86^a	2.82 ± 1.00^a	2.44 ± 0.75^a
ds	0.017 ± 0.024^a	0.079 ± 0.156^a	0.040 ± 0.048^a	0.119 ± 0.160^a
gments	0.009 ± 0.020^a	0^a	0.034 ± 0.084^a	0^a
m	1.66 ± 0.79^a	2.49 ± 2.13^a	3.32 ± 1.27^a	3.49 ± 0.68^a

haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin entration.



ıre 2 Elongated erythrocytes with the nucleus lod at one cell pole in group E1 carp (67 mg L^{-1} NO_2^-; g L^{-1} Cl^-).

stigated. The highest number of disintegrating hrocytes was found in group E1 carp exposed to ite with a low chloride concentration. Differences veen the groups, however, were not significant le 1). In carp exposed to nitrite (67 mg L^{-1}) with w concentration of chloride (group E1), erythros were characterized by a conspicuously clear cyasm (Fig. 3).

The results of white blood count examinations of individual groups of carps studied are given in Fig. 1 and Table 1. There was a significant decrease in the total leucocyte count ($P < 0.05$) and the total lymphocyte count ($P < 0.05$) in carps of group E1 exposed to nitrite (67 mg L^{-1}) at a low chloride concentration (11 mg L^{-1}) compared with values found in other groups. There was a significant decrease ($P < 0.05$) in absolute numbers of myelocytes in groups E1 (0.28 ± 0.07 GL^{-1}), E2 (0.42 ± 0.35 GL^{-1}) and E3 (0.43 ± 0.27 GL^{-1}) compared with the control (group C, 0.93 ± 0.36 GL^{-1}). The results of the rest of the parameters studied were comparable in all four groups of carp.

Biochemical profile of blood plasma

Values of all biochemical indices in blood plasma investigated (GLU, TP, TAG, NH_3, ALB, LDH, PHOS, Ca) were comparable in all of the groups. Groups E1, E2 and E3 did not differ significantly from the control group (group C). The values of biochemical indices in the control group were as follows: GLU = 8.16 ± 1.08 mmol L^{-1}, TP = 38.81 ± 3.30 g L^{-1}, TAG = 0.97 ± 0.12 mmol L^{-1}, $NH_3 = 603.5 \pm 95.0$ µmol L^{-1}, ALB = 9.45 ± 1.39 U L^{-1}, LDH = 209.1 ± 43.0 U L^{-1},

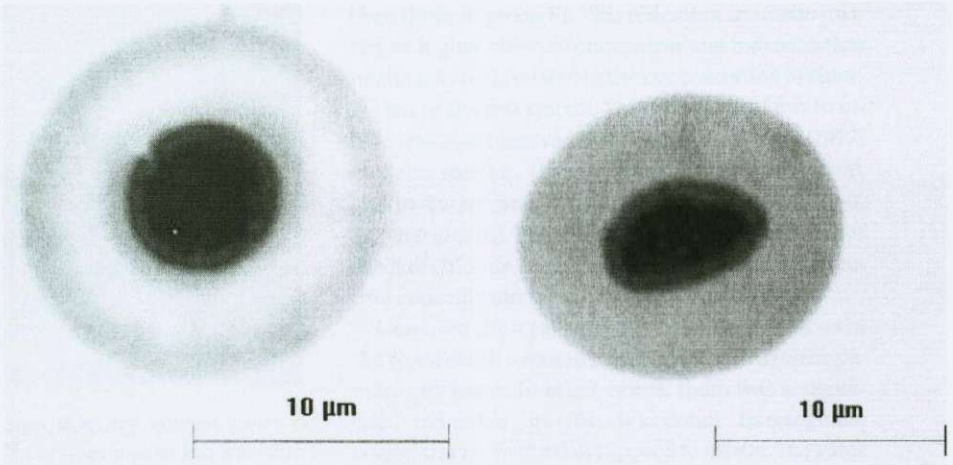


Figure 3 Noticeably clear erythrocyte cytoplasm in group E1 carp (67 mg L^{-1} NO_2^-; 11 mg L^{-1} Cl^-) (left) compared with the erythrocyte cytoplasm in the control group C carp (right).

PHOS = 3.23 ± 0.65 mmol L^{-1} and Ca^{2+} = 2.20 ± 0.14 mmol L^{-1}.

Histopathological changes on the gills of the carp

There were considerable differences in the histopathology of the gills of carp in group E1 (67 mg L^{-1} NO_2^-; 11 mg L^{-1} Cl^-). In some parts, the respiratory epithelium was almost intact;in other parts, there were areas with marked productive changes accompanied by respiratory cell hyperplasia and subsequent filling-in of intralamellar spaces (Fig. 4). In these areas, increased incidence of eosinophilic chloride cells was observed (Fig. 4). The same results were obtained in group E2 carp (67 mg L^{-1} NO_2^-; 100 mg L^{-1} Cl^-), the only difference being that the histopathological changes were less conspicuous.

The gills of carp in group E3 (0 mg L^{-1} NO_2^-; 100 mg L^{-1} Cl^-) showed slight regressive changes consisting in the swelling of cells of the respiratory epithelium and their vacuolization, particularly at lamella tips. A slight increase in the number of eosinophilic chloride cells was also observed.

The gills of carp in group C (controls) (0 mgL^{-1} NO_2^-; 11 mgL^{-1} Cl^-) showed no histopathological changes (Fig. 5). Only two carp displayed hyperaemia and blood capillary congestion, and, in places, slight manifestations of respiratory epithelium desquamation.

Markedly changed macroscopic picture of the gills was found in carp of groups E1 and E2. After nitrite intoxication, the gills of carp in group E2, and especially in group E1, were brown-red to chocolate brown in colour.

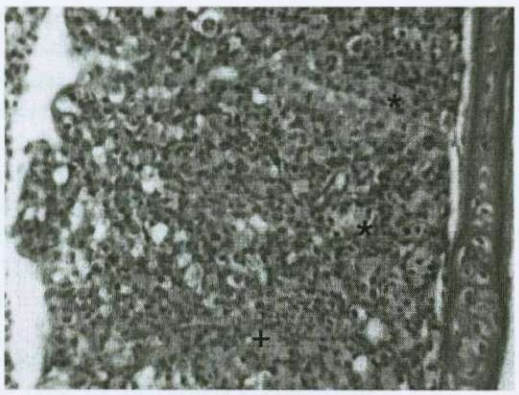
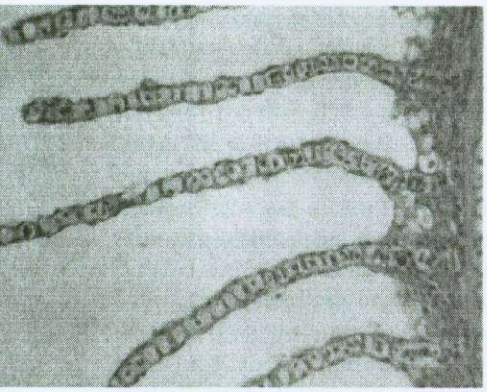


Figure 4 Gills of group E1 carp (67 mg L^{-1} NO_2^-; 11 mg L^{-1} Cl^-). Hyperplasia of the respiratory epithelium with fusion of secondary lamellae (asterisk) and hyperplasia of the eosinophile granular cells (cross). Haematoxylin and eosin (H&E), × 400.

Discussion

The main haematological response of carp to the acute exposure of nitrite (group E1) was a significant decrease ($P < 0.05$) in haemoglobin concentration, haematocrit, erythrocyte count, leucocyte count and lymphocyte count. A significant increase, on the other hand, was observed in methaemoglobin levels ($P < 0.01$) and MCH ($P < 0.05$).



ure 5 Gills of the carp from the control group C $mgL^{-1}NO_2^-$; 11 $mgL^{-1}Cl^-$); secondary lamellae with morphological changes. Haematoxylin and eosin &E), × 400.

Methaemoglobin contains haem iron in the Fe^{3+} te and it lacks the capacity to bind oxygen reversi- (Bodansky 1951; Kiese 1974). In fish, methaemo- binaemia usually results from exposure to high ncentrations of nitrite, although a small amount methaemoglobin can be formed spontaneously in rmal erythrocytes in the absence of nitrite. The re- ted values include 0.9–3.6% for rainbow trout, *corhynchus mykiss* (Walbaum) (Smith & Russo 75), 10.9% for prespawning pink salmon, *Oncor- chus gorbuscha* (Walbaum) (Cameron 1971), and % for channel catfish, *Ictalurus punctatus* (Rafin- ue) (Huey, Simco & Criswell 1980). In the present periment, the methaemoglobin made up, on aver- , 4.2% of total haemoglobin in group E3 and % of total haemoglobin in group C (control group). contrast to the control group, methaemoglobin ncentrations in fish exposed to nitrite (group E1) reased to 90.5% of the total haemoglobin. Ele- ed methaemoglobin levels after acute exposure to rite were also observed by Klinger (1957) in Eur- an minnow, *Phoxinus phoxinus* (L.), by Westin 74) in chinook salmon, *Oncorhynchus tshawytscha* lbaum), and by Huertas, Gisbert, Rodríquez, Car- a, Williot and Castelló-Orvay (2002) in Siberian rgeon, *Acipenser baerii baerii* (Brandt). Methaemo- bin imparts a brownish colour to whole blood. So sible clinical sign of high methaemoglobin levels ish is the brown colouring of blood and gills. The s of carp in group E1 exposed to nitrite were dark- wn.

Although elevated, methaemoglobin levels in up E2 were lower (38.3% of total haemoglobin) than those in group E1. This reduction in nitrite toxicity at higher chloride concentrations indicates that nitrite toxicity is related to the concentration of chloride ion in the test system. This tolerance of fish to nitrite was also observed by Perrone and Meade (1977) in Coho salmon, *Oncorhynchus kisutch* (Walbaum), and by Barlett and Neumann (1998) in brown trout, *Salmo trutta* (L.). These results confirm the protective effect of chloride during an exposure to increased nitrite concentrations in the aquatic environment.

Moreover, the total haemoglobin concentrations in the blood of fish exposed to nitrite (group E1) were significantly lower. In other words, there was a significant reduction in the functional haemoglobin concentration in the fish exposed to nitrite. The same phenomenon was observed by Woo and Chiu (1995) in sea bass, *Lates calcarifer* (Bloch), and by Knudsen and Jensen (1997) in carp, *Cyprinus carpio* (L.). Woo and Chiu (1995) also observed significantly lower MCH and MCHC values.

In this study, qualitative erythrocyte changes were observed in carp after nitrite exposure. Group E1 carp showed a significantly higher number of elongated erythrocytes with the nucleus located at one cell pole compared with the control group C (no elongated erythrocytes found). All erythrocytes of group E1 carp had a remarkably clear cytoplasm compared with the control group. This discoloration in the erythrocyte cytoplasm may indicate reduced haemoglobin concentrations.

The general health of fish is a function of their environment. The presence of various chemicals in water may have adverse effects on the physiological pathways in the fish, including important mechanisms that help protect fish against diseases, i.e. the non-specific defence mechanism (Carballo & Muñoz 1991; Carballo, Muñoz, Cuellar & Tarazona 1995) and the specific immune response (Anderson 1996). Acute stress can cause significant changes in the white blood cell count (Groff & Zinkl 1999; Noga 2000). The response to environmental impacts often leads to leucopenia with lymphopenia and sometimes neutrophilia, which is similar to the classic leucocytic response to stress in mammals (Ainsworth 1992; Noga 2000). In the present experiment, the decrease in leucocyte count and the lymphopenia in the carp exposed to nitrite indicate a reduction in non-specific immunity of the carp. Probably, prolonged stress caused dissipation of leucopoiesis, resulting in reduction in the total leucocyte count. Similar results were found by Das, Ayyappan, Jena and Das (2004) in *Cirrhinus mrigala* (Hamilton).

Previous histopathological studies of fish exposed to pollutants revealed that fish organs are efficient indicators of water quality (Cardoso, Chiarini-Garcia, Ferreira & Poli 1996; Barlas 1999, Cengiz, Ünlü & Balci 2001). The gills are important organs in fish for respiration, osmotic regulation, acid base balance and nitrogenous waste excretion (Heath 1987). Fish gain nitrite ions through an active uptake mechanism associated with the chloride cells of the gills (Lewis & Morris 1986). The main histological lesions that occurred in the gills of nitrite-exposed carp were hyperplasia and an elevated number of chloride cells. Michael, Hilmy, el-Domiaty and Wershana (1987) observed hyperplasia and hypertrophy in the gills of *Clarias gariepinus* (Burchell) chronically exposed to nitrite.

The results of our observations confirmed that elevated nitrite concentrations at low chloride concentrations in water may cause marked changes in haematological indexes. The biochemical parameters measured in the study reported here, however, were not affected. Major macroscopic and histological changes were observed on the gills of the fish. Less marked changes in all the parameters investigated were observed in fish exposed to nitrite when higher chloride concentrations in water were used. This corroborates the assumption that elevated chloride concentrations in water positively influence the nitrite resistance of fish.

Acknowledgments

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VETERINÁRNÍ A FARMACEUTICKÁ UNIVERZITA BRNO

FAKULTA VETERINÁRNÍ HYGIENY A EKOLOGIE
Oborová rada pro hygienu a technologii potravin

VI. KONFERENCE MLADÝCH VĚDECKÝCH PRACOVNÍKŮ S MEZINÁRODNÍ ÚČASTÍ

SBORNÍK

VFU BRNO 2. 6. 2004

Vliv chloridů na toxické účinky dusitanů na ryby

Effect of chlorides upon fish toleration to nitrite effects

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Abstract

A protective effect of chlorides during activity of increased nitrite concentrations upon health status of two yaers old caprs (*Cyprinus carpio*) was assessed using examination of heamatological parameters. Two groups of carp were exposed to nitrites in concentration 67 mg/l during 96 hours. The chloride content in the water of the first group was 71 mg/l, of the second group was 11 mg/l. The third group of carp was exposed only to chlorides in a concentration of 71 mg/l. Exposition of fish to nitrite was manifested the most markedly by the methaemoglobin content. The highest methaemoglobin countet (91,22 ± 3,73 g/l) was found in the second group, the content of (38,32 ± 13,25 g/l) was found in the first group, the content of 4,30 ± 1,12 g/l was found in the third group and the content of 2,00 ± 2,48 g/l was found in control group (11 mg/l of chlorides without nitrites). Out of other investgated parameters significant differences of the following values were found: higher red blood cells count, haemoglobin content, white blood cells count and lower methaemoglobin content in the first group of carp compared to the second group of carp. The protective effect of chlorides was confirmed even by the results of erythrograms examination.

Úvod

Dusitany zpravidla doprovázejí ve dusičnany a formy amoniakálního dusíku, avšak jen v malých koncentracích, protože jsou málo stále. Průměrná koncentrace dusitanového dusíku v pitných vodách podzemního původu z 8 regionů ČR je asi 0,004 mg/l (NO_2^- 0,026 mg/l). V povrchových vodách se koncentrace dusitanového dusíku pohybuje v rozmezí 0,004 – 0,179 mg/l N- NO_2^-. Zvýšené koncentrace dusitanového dusíku lze prokázat (i stovky mg/l) v odpadních vodách za strojírenských závodů (Pirter 1999). Zvýšené koncentrace dusitanů (řádově desetiny, ale i jednotky mg/l N- NO_2^- se mohou vyskytovat ve vodách intenzivních chovech ryb, zejména v recirkulačních systémech, většinou bezprostředně po zahájení provozu, kdy ještě nejsou plně zapracovány biologické filtry a existuje zde nerovnováha v procesu nitrifikace Proces nitrifikace je využíván pro snížení koncentrace amoniaku, který je hlavním produktem dusíkatého metabolismu ryb (Svobodová et al. 2003).

Dusíkaté ionty se dostávají do organismu ryb přes žaberní aparát pomocí chloridových buněk. Dusitany se v krvi váží na hemoglobin za vzniku methemoglobinu, čímž se snižuje transportní kapacita krve pro kyslík (Svobodová et al., 1987). Toxicita dusitanů pro ryby značně kolísá a závisí na mnoha vnitřních i vnějších faktorech (druh a věk ryb, kvalita vody atd.). Podle nejnovějších poznatků mají významný vliv na toxicitu dusitanů (Navrátil et al., 2000).

Cílem práce bylo posoudit ochranný vliv chloridů na toxicitu dusitanů pro kapra obecného.

Materiál a metodika

V práci byl posouzen toxický účinek dusitanů na kapra obecného při různé koncentraci chloridů ve vodě. Toxický účinek byl posuzován na základě výsledků hematologického vyšetření kaprů po akutním působení dusitanu sodného a chloridu sodného.

Hematologické vyšetření dvouletých kaprů obecných (*Cyprinus carpio L.*) bylo provedeno v závěru 96 hodinového testu akutní toxicity s dusitanem sodným. Dvě skupiny kaprů byly vystaveny koncentraci dusitanu sodného 100 mg/l (67 mg/l NO_2^-). Obsah chloridů ve vodě u první skupiny byl 71 mg/l (100 mg/l chloridu sodného) a u druhé 11 mg/l. Třetí skupina kaprů byla vystavena pouze chloridům v koncentraci 71 mg/l (100 mg/l chloridu sodného, bez dusitanů) a čtvrtá skupina kaprů byla kontrolní (bez dusitanů, chloridy 11 mg/l). Test byl proveden semistatickým způsobem s výměnou lázně po 48 hodinách. Základní fyzikálně-chemické parametry ředící vody byly následující: pH 8,21; $KNK_{4,5}$ 1,15 mmol/l; celkový amoniak 0,04 mg/l; NO_3^- 7,75 mg/l; NO_2^- 0,003 mg/l; PO_4^{3-} 0,01 mg/l; $CHSK_{Mn}$ 1,5 mg/l. Teplota vody se testu se pohybovala v rozmezí 20-21°C, nasycení vody kyslíkem bylo nad 60%. Test byl proveden ve 4 akváriích o objemu 200 litrů, v každém akváriu bylo 7 kusů dvouletých kaprů (1 akvárium kontrolní, 1 akvárium s koncentrací chloridů 71 mg/l, akvárium s koncentrací dusitanů 67 mg/l, 1 akvárium s koncentrací dusitanů 67 mg/l a chloridů 71 mg/l).

Laboratorní vyšetření bylo provedeno u 7 kusů kontrolních kaprů (hmotnost 744,29 ± 126,16 g), 7 kusů kaprů vystavených koncentraci chloridů 71 mg/l (592,14 ± 59,99 g), 7 kusů kaprů vystavených koncentraci dusitanů 67 mg/l (670,71 ± 103,94 g) a 7 kusů kaprů vystavených koncentraci dusitanů 67 mg/l a chloridů 71 mg/l (678,57 ± 63,12 g).

Krev byla odebrána kardiální punkcí. Ke stabilizaci krve byl použit heparin v množství 50 m.j. na 1 ml krve. Byl stanoven počet erytrocytů (RBC), hematokrit (PCV), množství methemoglobinu (MetHb), střední objem erytrocytu (MCV), hemoglobin erytrocytu (MCH), střední barevná koncentrace erytrocytu (MCHC), počet leukocytů (Leuko) a diferenciální počet leukocytů. Bylo postupováno podle metodiky Jednotné metody hematologického vyšetřování ryb (Svobodová et al., 1986). Dále bylo provedeno kvalitativní hodnocení erytrocytů. U každé ryby bylo posouzeno 300 erytrocytů, u kterých byly zaznamenány následující změny diferencovaný rozpad erytrocytů, bezjaderné erytrocyty a změna tvaru erytrocytů a polohy jádra.

Statistické zpracování výsledků bylo provedeno pomocí analýzy variance (ANOVA).

Výsledky

Vyšetření červeného krevního obrazu

Výsledky vyšetření červeného krevního obrazu kontrolních a pokusných skupin kaprů jsou uvedeny v tabulce 1. U skupiny kaprů vystavených účinku dusitanů (67mg/l) došlo k signifikantnímu snížení množství hemoglobinu ($p<0,05$), počtu erytrocytů ($p<0,05$), k signifikantnímu zvýšení množství methemoglobinu ($p<0,01$) a střední barevné koncentrace ($p<0,05$) v porovnání s kontrolní skupinou. U kaprů vystavených kombinovanému účinku dusitanů a chloridů došlo k signifikantnímu zvýšení množství hemoglobinu ($p<0,05$) a střední barevné koncentrace ($p<0,05$) a k signifikantnímu snížení středního objemu erytrocytu v porovnání s kontrolní skupinou. U kaprů vystavených účinků chloridů došlo k snížení

ního objemu erytrocytu ($p<0,05$) a k zvýšení střední barevné koncentrace v porovnání trolní skupinou. Další sledované parametry byly u kontrolních a pokusných kaprů atelné.

1: Porovnání ukazatelů červeného krevního obrazu u pokusných a kontrolních kaprů.

atel	NO_2^- x ± SD	$NO_2^- + Cl^-$ x ± SD	Cl^- x ± SD	Kontrola x ± SD
/l)	53,35 ± 6,26 [b]	59,96 ± 15,19 [a]	65,87 ± 10,15 [a]	65,35 ± 10,61 [a]
(l/l)	0,21 ± 0,02 [a]	0,21 ± 0,02 [a]	0,24 ± 0,02 [a]	0,27 ± 0,04 [a]
(T/l)	1,13 ± 0,11 [b]	1,30 ± 0,13 [a]	1,50 ± 0,29 [a]	1,51 ± 0,26 [a]
b (g/l)	91,22 ± 3,73 [b]	38,32 ± 13,25 [b]	4,30 ± 1,12 [a]	2,00 ± 0,48 [a]
(fl)	192,29 ± 18,73 [b]	156,71 ± 13,35 [a]	167,14 ± 35,38 [a]	195,43 ± 10,94 [b]
(pg)	47,37 ± 6,39 [a]	45,65 ± 2,02 [a]	44,43 ± 4,14 [a]	44,63 ± 3,50 [a]
C (l/l)	0,25 ± 0,02 [a]	0,29 ± 0,01	0,28 ± 0,04 [a]	0,23 ± 0,01 [b]

y a,b charakterizují shodu nebo rozdílnost hodnot mezi skupinami (ANOVA $p<0,05$; l)

Kvalitativní hodnocení erytrocytů byl zjištěn u kaprů vystavených účinků dusitanů /l) signifikantní ($p<0,01$) nárůst počtu erytrocytů protáhle oválného tvaru s jádrem ným na jednom pólu buňky. Počet bezjaderných erytrocytů a rozpadajících se cytů byl u kontrolních a pokusných kaprů srovnatelný. Erytrocyty kaprů vystavených dusitanů (67 mg/l) měly nápadně projasněnou cytoplazmu.

ení bílého krevního obrazu

Výsledky vyšetření bílého krevního obrazu kontrolních a pokusných skupin kaprů jsou ny v tabulce č. 2. U skupiny kaprů vystavených účinkům dusitanů (67mg/l) došlo fikantnímu snížení celkového počtu leukocytů ($p<0,05$), absolutního počtu lymfocytů 5) a absolutního počtu myelocytů ($p<0,05$) v porovnání s kontrolní skupinou. U kaprů ených kombinovanému účinku dusitanů (67 mg/l) a chloridů (71 mg/l) a u kaprů ených účinku chloridů (71 mg/l) došlo k signifikantnímu snížení absolutního počtu cytů ($p<0,05$) v porovnání s kontrolní skupinou. Další sledované parametry byly u lních a pokusných kaprů srovnatelné.

Tab. č. 2: Porovnání ukazatelů bílého krevního obrazu u pokusných a kontrolních kaprů.

Ukazatel	NO_2^- x ± SD	NO_2^- + Cl^- x ± SD	Cl^- x ± SD	Kontrola x ± SD
leukocyty (G/l)	7,00 ± 3,12 [b]	14,50 ± 8,46 [a]	14,36 ± 6,46 [a]	19, 21 ± 4,15 [a]
lymfocyty (G/l)	5,28 ± 2,51 [b]	12,07 ± 6,79 [a]	11,00 ± 5,95 [a]	15,67 ± 4,24 [a]
monocyty (G/l)	0,07 ± 0,06 [a]	0,08 ± 0,11 [a]	0,034 ± 0,08 [a]	0,06 ± 0,09 [a]
myelocyty (G/l)	0,28 ± 0,07 [b]	0,42 ± 0,35 [b]	0,43 ± 0,27 [b]	0,93 ± 0,36 [a]
metamyelocyty (G/l)	1,36 ± 0,72 [a]	1,99 ±1,86 [a]	2,82 ± 1,00 [a]	2,44 ± 0,75 [a]
neutrolily-tyčky (G/l)	0,02 ± 0,02 [a]	0,08 ± 0,16 [a]	0,04 ± 0,05 [a]	0,12 ± 0,16 [a]
neutrolily-segmenty (G/l)	0,01 ± 0,02 [a]	0,00 ± 0,00 [a]	0,03 ± 0,08 [a]	0,00 ± 0,00 [a]

Indexy a,b charakterizují shodu nebo rozdílnost hodnot mezi skupinami (ANOVA $p<0,05$; $p<0,01$)

Diskuse

Hlavní hematologickou odezvou kaprů na akutní působení dusitanů bylo signifikantní snížení množství hemoglobinu ($p<0,05$), počtu erytrocytů ($p<0,05$), počtu leukocytů ($p<0,05$), absolutního počtu lymfocytů ($p<0,05$) a absolutního počtu myelocytů ($p<0,05$) a signifikantní zvýšení množství methemoglobinu ($p<0,01$). K podobným výsledkům dospěli také Woo a Chiu (1995) a Huany a Chen (2002). Zvýšené množství methenoglobinu a kaprů vystavených současnému působení dusitanů a chloridů bylo nižší v porovnání se skupinou kaprů vystavěných pouze působení dusitanů. Potvrdil se tak ochranný vliv chloridů na toxicitu dusitanů pro ryby. Stejného výsledku dosáhly také Perrone a Mead (1977).

Kromě kvalitativních změn byly pozorovány také kvalitativní změny erytrocytů u kaprů vystavených působení dusitanů. Nápadně projasnění cytoplazmy erytrocytů u kaprů vystavených působení dusitanů indikuje snížení koncentrace hemoglobinu.

Změny v bílém krevním obraze indikují poškození imunitního systému ryb. Po působení různých polutantů dochází ke snížení celkového počtu leukocytů způsobené především snížením počtu lymfocytů (Iwama a Nakanishi 1996).

Závěr

Z výše uvedených výsledků vyplývá, že dusitany v koncentraci (67 mg/l) způsobily výrazné změny v hodnotách červeného i bílého krevního obrazu. Nejvýznamnější změny se týkají koncentrace hemoglobinu a methemoglobinu, celkového počtu leukocytů a absolutního počtu lymfocytů. Kromě kvantitativních změn erytrocytů byly pozorovány i změny kvalitativní. Dále byl potvrzen ochranný vliv chloridů na toxicitu dusitanů pro ryby.

Poděkování

Zpracování příspěvku bylo provedeno v rámci projektu MŠM č. 126100003.

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5. ZÁVĚR

V posledních letech se stává stále významnějším požadavek Zákona na ochranu zvířat proti týrání (č. 246/1992 Sb.) ochrana zdraví ryb z hlediska zdravotního stavu ale i zabránění nešetrné manipulaci a následnému mechanickému poškození ryb. Jednou ze součástí prevence poškození ryb je použití anestetik. Bylo testováno několik látek (Propiscin, 2-phenoxyethanol, Quinaldin a hřebíčkový olej) jako anestetika pro ryby. Jako nejvhodnější látky pro anestezii ryb se ukázaly 2-phenoxyethanol a hřebíčkový olej. Tyto dvě anestetika splňují všechna kriteria (nízká cena, bezpečnost pro ryby a pracovníky). Cílem práce bylo přispět k registraci anestetik v České republice v rámci projektu NAZV QF3029 Harmonizace s EU v uplatňování principů farmakovigilance v akvakulturních chovech v ČR.

V posledních letech se velmi intenzivně rozšířilo používání pesticidních přípravků na bázi pyrethroidů. Jsou stále vyvíjeny nové pesticidní přípravky, u kterých jsou hodnocena rizika jejich použití pro životní prostředí (environmental risk assessment). Cílem kapitoly bylo zhodnotit toxický vliv přípravků na bázi pyrethroidů (Decis 2.5, Decis 50 EC, Alimethrin 10 EC) na ryby. Pesticidní přípravky byly zařazeny do skupiny látek vysoce toxických pro ryby (R 50 LC 50 < 1 $mg.l^{-1}$).

Negativní vliv dusitanů na ryby stále přitahuje hodně pozornosti, zvláště v akvakulturních zařízeních s recirkulací vody. Vysoké hodnoty dusitanů nalézaných ve vodě mohou způsobit těžké fyziologické poruchy, které mohou přejít až v masový úhyn ryb. V současnosti je snaha lépe porozumět souhrnnému vlivu jednotlivých faktorů ovlivňujících toxicitu dusitanů na ryby. Naše práce potvrdila názor, že vyšší koncentrace chloridů ve vodě pozitivně ovlivňují rezistenci ryb k dusitanům.

Výsledky uvedené v disertační práci byly získané v rámci řešení výzkumných projektů *MSM 6007665809 Biologické, environmentální a chovatelské aspekty v rybářství, NAZV QF3029 Harmonizace s EU v uplatňování principů farmakovigilance v akvakulturních* hovech v ČR, FRVŠ 744/2005/G3 Vliv pyrethroidů na ryby a projektu GAČR 523/03/H076 a Oddělení vodní toxikologie a nemocí ryb ve Výzkumném ústavu rybářském a ydrobiologickém ve Vodňanech.

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