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Effects of prometryne on early life stages of common carp (*Cyprinus carpio* L.)

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ABSTRACT

Toxicity of prometryne to early life stages of common carp was assessed. On the basis of accumulated mortality in the experimental groups lowest observed-effect concentration (LOEC) was estimated as 1100 μg/l; and no observed-effect concentration (NOEC) was 850 μg/l. Fulton's condition factor was significantly lower than in controls in fish exposed to 4000 μg/l after 7, 14, and 21 days. By day 14, fish exposed to 4000 μg/l prometryne showed significantly lower mass and total length compared to controls. Fish exposed the 1200 and 4000 μg/l showed delay in development, severe hyperaemia in gill, liver, and caudal and cranial kidney. Subchronic prometryne exposure of early-life stages of common carp at concentrations of 1200 and 4000 μg/l affected their survival, growth rate, early ontogeny, and histology.

1. Introduction

The aquatic environment continues to be under threat by the use of pesticides, resulting in high risk to non-target organisms [1]. Pesticides used in agro-ecosystems and forests enter aquatic environments such as streams, rivers, and lakes if applied in adjacent areas or if an accidental spill occurs [2]. Such pesticides are carried into aquatic environments by surface runoff from sites of application and can negatively affect the health of aquatic organisms [3–8].

Eight s-triazines (atrazine, cyanazine, prometryne, propazine, sebuthylazine, simazine, terbuthylazine, and terbutryne) have been identified as relevant for testing, based on a compilation of freshwater monitoring data in the member states of the European Community [9]. Prometryne [2,4-bis (isopropylamino)-6-methylthio-s-triazine], a selective herbicide of the s-triazine chemical family, has been utilized for pre- and post-emergence control of annual grasses and broadleaf weeds in a variety of crops, including cotton, celery, pigeon peas, and dill [10]. Prometryne was first registered in the United States of America by Ciba Crop Protection in 1964 [11]. Prometryne persists in the soil from one to three months and has a soil half-life of 60 days. With multiple annual applications, prometryne activity can persist for 12–18 months following the most recent application [12]. It is slightly to moderately toxic to fish. Acute toxicity 96 h LC50 for common carp (*Cyprinus carpio* L.) is reported as 8 mg/l [13].

Although prometryne has been banned in Europe since 2004 [14], it can still be found in surface and ground waters. Prometryne has been reported in European surface waters at concentrations from 0.01 to 4.40 μg/l [15–17]. Prometryne is still being widely used in China [14], Australia, Canada, New Zealand, South Africa, and the United States [18].

Although the effects of acute and subchronic exposure of juvenile and adult fish to prometryne, another s-triazine herbicide, have been well documented, there is a dearth of data on the subchronic toxicity of prometryne at environmentally realistic concentrations in embryos and larvae of common carp. Prometryne caused changes in haematological, biochemical profile, caudal kidney [19], and antioxidant enzymes in juvenile carp [20,21]. Fish metamorphose in surface waters, and it is presumed that the embryo-to-larva transformation is sensitive to chemicals in the environment [22]. The aim of the present study was to describe the effects of prometryne on embryos and larvae of common carp, a well-established model species for testing chemical effects on early development [23]. The toxicity of prometryne was assessed on the basis of mortality, early ontogeny, growth rate, Fulton's condition factor (FCF), and occurrence of morphological anomalies during, and at the conclusion of the test.

2. Materials and methods

2.1. Experimental animals

Fertilized eggs of carp were obtained from the breeding station of the Faculty of Fisheries and Protection of Waters Vodnany, Czech Republic. Eggs were fertilized according to standard methods

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described by Kocour et al. [24]. The study was conducted according to the principles of the Ethical Committee for the Protection of Animals in Research of the Faculty of Fisheries and Protection of Waters Vodnany, based on the EU-harmonized animal welfare act of Czech Republic.

2.2. Water parameters

Eggs and larvae were maintained in aerated tap water with the following parameters: dissolved oxygen, >91%; temperature, 18.7–20.6 °C; pH, 7.1–8.1; acid neutralization capacity ($ANC_{4.5}$), 0.99 mmol/l; chemical oxygen demand (COD_{Mn}), 1.1 mg/l; total ammonia, 0.01 mg/l; NO_3^-, 6.75 mg/l; NO_2^-, <0.02 mg/l; $Ca^{2+} + Mg^{2+}$, 8.2 mg/l. The test baths were continuously gently aerated. Temperature, oxygen saturation and pH were measured daily.

To ensure agreement between nominal and actual compound concentrations, water in the aquaria was analysed during the experimental period by liquid chromatography–tandem mass spectrometry (LC–MS/MS) [25]. Water samples were collected from the aquaria immediately before (24 h after application) and after renewing the test solutions (0 h). The mean concentration of prometryne in the water samples was always within differentiation 2% of the intended concentration.

2.3. Experimental protocol

The trial was carried out using the modified test No. 210 – Fish, Early-Life Stage Toxicity Test OECD [23]. At 24 h post-fertilization, unfertilized eggs were discarded, and 100 fertilized eggs were transferred into each of fifteen glass crystallization basins with the prometryne solution, plus control dishes. Prometryne (chemical purity 99.3%) was obtained from Sigma–Aldrich Corporation (USA). Four concentrations of test solutions and a control were used, each with 100 fertilized eggs, in triplicate. The concentrations were: 0.51 μg/l (environmental concentration in Czech rivera), 80 μg/l, 1200 μg/l, and 4000 μg/l. Prometryne concentrations of 80 μg/l, 1200 μg/l, and 4000 μg/l corresponded to 1% of the 96 h LC50, 15% of 96 h LC50, and 50% of 96 h LC50 for carp [13].

The water for each treatment was renewed daily by gently draining each chamber and adding new solution slowly to avoid disturbing embryos and larvae. Control of hatching, mortality, and behaviour was made twice daily, and dead fish were removed. From 6 day larvae were fed freshly hatched brine shrimp *Artemia salina* nauplii *ad libitum* daily prior to water exchange.

On days 7, 14, 21, 28, and 35 samples of fish (30 per concentration groups and control) were collected to monitor development, occurrence of morphological anomalies, growth rate, FCF, and the length/mass relationship. Determination of development periods and stages followed Penaz et al. [26]. Final evaluations included accumulated mortality, mass and total length (TL) of fish with no deformities. The total length was measured by stereomicroscopy using a micrometer. Mass to 0.1 mg was measured with a Mettler-Toledo balance.

2.4. Trial schedule

The experiment schedule was: day 1, trial initiation (1 day post-fertilization); day 7, hatching complete; day 9, initiation of exogenous feeding; day 35, end of the experiment. To 35 day, the majority of control fish had become first stage juveniles.

2.5. Growth rate evaluation

The mean specific growth rate (SGR) for fish in each experimental group was calculated for the period from day 7 to day 35 and compared with controls using the method described by Kroupova et al. [27].

2.6. Statistical analysis

One-way ANOVA was conducted to compare differences among the test groups using the software program Statistica 12 for Windows (StatSoft). The differences in cumulative mortality among groups were assessed using contingency tables (χ^2) [28].

2.7. Evaluation of 35 day LC50, LOEC, and NOEC

For the evaluation of LC50, lowest observed-effect concentration (LOEC), and no observed effect concentration (NOEC) at the completion of the test, a probit analysis EKOTOX 5.1 software (Ingeo Liberec) was conducted based on mortality at different prometryne concentrations. The day 35 LC5 and day 35 LC10 values were used to express the NOEC and LOEC values, respectively.

2.8. Histopathology

Histopathology was evaluated in all groups at the end of the trial. Six fish from each group and control were placed in 10% buffered formalin, prepared with standard histological techniques, stained with haematoxylin and eosin, examined by light microscopy.

3. Results

3.1. Hatching

Hatching began 5 days following onset of exposure, and the majority of eggs in all treatment groups hatched by day 7. Significantly ($p < 0.01$) lower hatching and embryo viability were found in fish exposed to the two highest prometryne concentrations, 1200 and 4000 μg/l, compared with controls and other concentrations (0.51 and 80 μg/l).

3.2. Cumulative mortality

Significant ($p < 0.01$) differences from controls in cumulative mortality were found in fish exposed to 1200 (15% 96 h LC50) and 4000 μg/l prometryne (50% 96 h LC50) (Fig. 1). Massive mortality in those groups occurred on days 6 and 7. Based on mortality in the experimental groups, prometryne concentrations were estimated at day 35 to be LC50 = 2314 μg/l, LOEC = 1100 μg/l, and NOEC = 850 μg/l with 95% confidence interval.

3.3. Growth parameters

Beginning on day 14 of exposure, fish exposed to prometryne at 4000 μg/l showed significantly ($p < 0.01$) lower mass (Fig. 2) and total length (Fig. 3) than did controls. The FCF values were significantly ($p < 0.01$) lower in the 4000 μg/l group after 7, 14, and 21 days compared to controls (Table 1). Inhibition of specific growth in the group exposed to the 4000 μg/l was 41.68% compared to controls (Table 2).

3.4. Early ontogeny

Fish exposed to 1200 and 4000 μg/l were delayed in development (Table 3) compared with the control group. At the conclusion of the trial the percent of individuals remaining in larval stages (L4b or L6) was elevated with higher concentrations of prometryne, whereas the majority of control fish reached the juvenile stage.