

## The effect of carbendazim on embryonic Prussian carp (*Carassius gibelio*) development and hatching

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**Abstract.** The aim of the study was to investigate the effect of different concentrations of carbendazim, a commonly used fungicide, on the embryonic development and hatching of Prussian carp, *Carassius gibelio* (Bloch). The egg samples were incubated with different concentrations of carbendazim – 0.001, 0.006, 0.036, 0.216, and 1.296 mg l<sup>-1</sup>, for 98 hours. The results of the data showed that carbendazim at concentrations of 0.036 mg l<sup>-1</sup> and higher were lethal for Prussian carp embryos. There were no significant differences in the hatching rate, percentage of hatched larvae, or in the percentage of deformed larvae exposed to the two lowest concentrations of carbendazim tested (0.001 and 0.006 mg l<sup>-1</sup>). The results of the present study showed that carbendazim is harmful to Prussian carp embryos at concentrations of 0.036 mg l<sup>-1</sup> and higher.

**Keywords:** carbendazim, embryonic development, hatching success, Prussian carp

Carbendazim (methyl-1-H-benzimidazol-2-yl-carbamate) is one of the most widely used systemic fungicides and is a representative of the benzimidazole family. It is efficient at

low doses, and it inhibits the development of wide variety of fungi. Therefore, its use is increasing. Carbendazim is used in agriculture, horticulture, forestry, and home gardening. It is also known that carbendazim is the main degradation product of benomyl and thiophanate-methyl fungicides in the environment (WHO 1993). Thus, the amount of carbendazim in the environment could increase. Carbendazim can contaminate fresh waters as a result of run-off. Moreover, it is relatively stable in the aquatic environment. In an aqueous medium, the photodegradation and adsorption of carbendazim into the organic matter in sediments occurs. Carbendazim has a half-life of 6 to 25 weeks in the water phase, and more than one month in sediments (Mazellier et al. 2002, Vega et al. 2005).

Despite the wide application of fungicides, published information about their effects on ecosystems is insufficient since carbendazim is highly toxic to aquatic organisms. The LC<sub>50</sub> value is 91 µg l<sup>-1</sup> for *Daphnia magna* (Van Wijngaarden et al. 1998), 0.007-0.56 mg l<sup>-1</sup> for channel catfish, *Ictalurus punctatus* (Rafinesque) 0.1-1.8 mg l<sup>-1</sup> for rainbow trout, *Oncorhynchus mykiss* (Walbaum), and higher than 3.2-55 mg l<sup>-1</sup> for bluegill, *Lepomis macrochirus* Rafinesque (WHO 1993). Chronic toxicity includes effects on the male mammalian reproductive system, embryotoxicity, and teratogenesis (Hao et al. 2000). To the best of our knowledge, there is no published data that reflect the influence of carbendazim on fish

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reproduction and early stages of development. The aim of this study was to determine whether carbendazim has detectable adverse effects on the embryonic development and hatching of Prussian carp, *Carassius gibelio* (Bloch). Sexually mature female *C. gibelio* and male common carp, *Cyprinus carpio* L., were obtained from the Experimental Fisheries Station of the University of Agriculture for the experiment. Gametes were obtained from five Prussian carp females that had been stimulated with GnRH-analog (Sigma Chemical Co. USA) and four common carp males. The eggs from each female were divided into two Petri dishes (about 150 eggs per dish) for each experimental group and activated with common carp sperm (10  $\mu$ l of sperm per dish) in clean water. After three minutes, the water in all the groups was replaced, and the medium for the experimental groups included carbendazim (Institute of Organic Chemistry, Poland). There were five experimental groups: 0.001 (group E1), 0.006 (group E2), 0.036 (group E3), 0.216 (group E4), and 1.296 mg l<sup>-1</sup> (group E5) of carbendazim exposure, and a control group (group C without carbendazim). The experimental concentrations were determined based on LC<sub>50</sub> values. Each dish contained 50 ml of medium. The medium was changed twice daily. The water used for preparing the experimental medium was aerated, dechlorinated, with a temperature of 23°C ( $\pm$ 1°C), a pH of 7.8, a hardness of 18°n, an oxygen concentration of 9 mg l<sup>-1</sup>, and was stored in a tank. Carbendazim was prepared from a stock solution weighed in a glass vessel, and was then transferred to a volumetric flask containing the experimental medium. Dilutions of this stock solution were used for the tests, and the stock solutions were renewed every 12 h. The control group received acetone at the maximum acetone volume used in the dilution of the dosing concentrations. The acetone concentration used in the experiment was far from harmful, so it was not necessary to use an acetone-free control. After 24 h of incubation, the number of activated eggs was counted. After 72 hours of incubation, the number of normal and deformed (vertebral curvature, yolk sac malformation) hatched larvae were observed and counted every 3 h of the experiment. The number of

live eggs after 24 h of exposure, the total percentage of deformed larvae, hatching success (percentage of hatched larvae in relation to total number of live eggs after 24 h of exposure), and hatching rate (percentage of hatched larvae per time unit) were determined. The exposure lasted for 98 hours. The results were analyzed with nonparametric the two-tailed Mann-Whitney test (n = 5). The differences between the means were significant at P < 0.05. The share of live eggs after 24 h of incubation was 45.42-78.57% in the control group, 42.72-70.05% in group E1, 50.72-70.06% in group E2, and 0.00-3.86% in group E3. Carbendazim caused 100% mortality in the eggs exposed to high concentrations after 24 h (0.216 and 1.296 mg l<sup>-1</sup>) or after 48 h (0.036 mg l<sup>-1</sup>) of incubation, which was the first evidence noted of the toxic effects of carbendazim on Prussian carp eggs. There was a significant difference in the percentage of live eggs after 24 h of exposure between group E3 and groups C, E1, and E2 (P < 0.05; Fig. 1).

The effects of other pesticides on the early life stages of fish have been examined by many authors, and the development of embryos is influenced by other pesticides. Köprücü and Aydın (2004) report that the number of dead common carp embryos significantly increased in response to different concentrations of deltamethrin (0.005, 0.05, 0.5, 5, 25, and 50  $\mu$ g l<sup>-1</sup>). Furthermore, diazinon exposure resulted in a decreased number of live common carp eggs at the different of concentrations 0.25, 0.5, 1, 2, 4, and 8 mg l<sup>-1</sup> (Aydın and Köprücü 2005). The mortality of zebrafish, *Danio rerio* (Hamilton), embryos was also observed by Haendel et al. (2004) during dithiocarbamate pesticide (sodium metam) exposure (LC<sub>50</sub> was 1.95  $\mu$ M). In contrast, Socha et al. (2012) report that exposing Prussian carp and common carp eggs to a polychlorinated biphenyl mixture – Aroclor 1254 (1, 10, 50 and 100 ng ml<sup>-1</sup>), did not cause significant differences in the percentage of live eggs between treated and control groups. Exposure to TCDD (0-100  $\mu$ g l<sup>-1</sup>) also did not increase the mortality of eggs at each embryonic stage prior to hatching in red seabream, *Pagrus major* (Temminck & Schlegel) (Yamauchi et al. 2006).

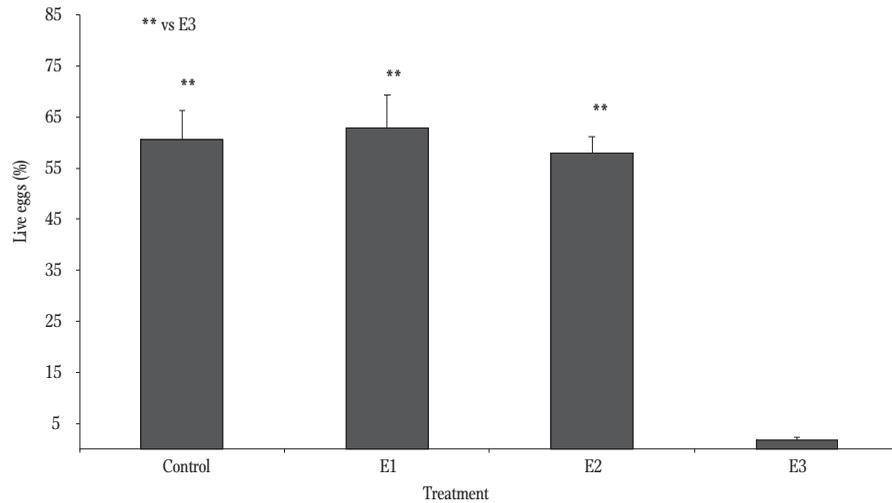


Figure 1. Influence of carbendazim on the percentage of live Prussian carp (*Carassius gibelio*) eggs after 24 h of incubation. Data expressed as means  $\pm$  SEM (n=5).

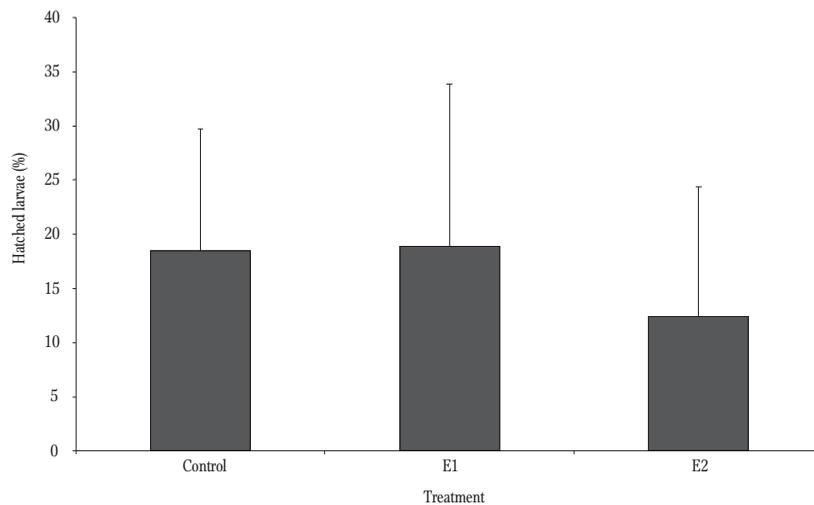


Figure 2. Influence of carbendazim on the percentage of hatched Prussian carp (*Carassius gibelio*) larvae (hatching success). Data expressed as means  $\pm$  SEM (n=5).

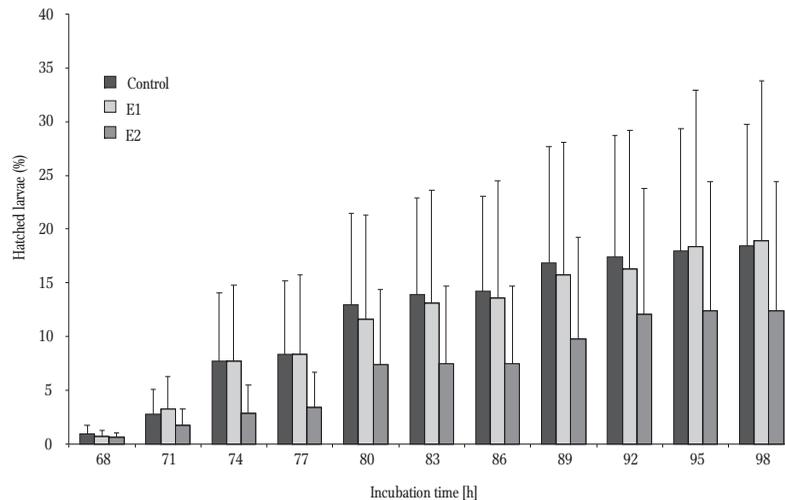


Figure 3. Influence of carbendazim on the hatching rate percentages of activated Prussian carp (*Carassius gibelio*) eggs. Data expressed as means  $\pm$  SEM (n=5).

Hatching is a particularly susceptible period, and larvae are more responsive than embryos, which are protected by the embryonic membrane. The present study showed that low concentrations (0.001 and 0.006 mg l<sup>-1</sup>) of carbendazim did not affect the hatching success of Prussian carp (Fig. 2). However, there are some data that indicate pesticides effect the number of hatched larvae. According to Aydin and Köprücü (2005), diazinon exposure results in decreased common carp hatching success; at concentrations of 0.25, 0.5, 1, 2, 4, and 8 mg l<sup>-1</sup>, the hatching success values were 84.6, 75.2, 54.1, 31.0, 6.0, and 0.0%, respectively. Exposure to deltamethrin resulted in similar effects (Köprücü and Aydin 2004). The results obtained by Fent and Meier (1994) showed that triphenyltin chloride (TPT) caused a significant reduction in the hatching success of *Phoxinus phoxinus* (L.) at a concentration of 0.0159 mg l<sup>-1</sup>. Water pollutants, such as endocrine disruptors, can also affect fish reproduction and impair hatching. Exposing adult male medaka, *Oryzias latipes* (Temminck & Schlegel), to bisphenol-A and nonylphenol resulted in decreased numbers of hatching larvae (Shioda and Wakabayashi 2000).

The present study revealed that carbendazim (0.001 and 0.006 mg l<sup>-1</sup>) did not effect hatching rates of Prussian carp (Fig. 3). Socha et al. (2012) note a statistically significant higher rate of Prussian carp

hatching at 75 h of exposure to Aroclor 1254 at a concentration of 1 ng ml<sup>-1</sup>. The concentrations of Aroclor 1254 tested (1, 10, 50, and 100 ng ml<sup>-1</sup>) did not affect the hatching rates of common carp larvae. Exposing embryos to toxicity also results in reduced larval numbers and quality. It could also cause decreased larval body sizes and contribute to malformations. The low concentrations of carbendazim (0.001 and 0.006 mg l<sup>-1</sup>) did not increase the percentage of deformed Prussian carp larvae (Fig. 4); however, many organic chemicals did. Atrazine at a concentration of 1.3 mg l<sup>-1</sup> increased the number of deformations in zebrafish (Görge and Nagel 1990). PFOS-contaminated water (1, 3, and 5 mg l<sup>-1</sup>) resulted in various zebrafish body abnormalities such as epiboly deformities, yolk sac and pericardial edema, tail and heart malformations, swim bladder inflation, and spinal curvature (Shi et al. 2008). Socha et al. (2012) report that Aroclor 1254 at a concentration of 10 ng ml<sup>-1</sup> resulted in an increased percentage of deformed common carp larvae. However, no similar effects were noted among Prussian carp at the same concentration. This confirms that the sensitivity of developing embryos to toxic substances varies among species. This could also depend on the degree of environmental contamination. According to Weis et al. (1981), *Fundulus heteroclitus* (Walbaum) eggs from polluted environments were

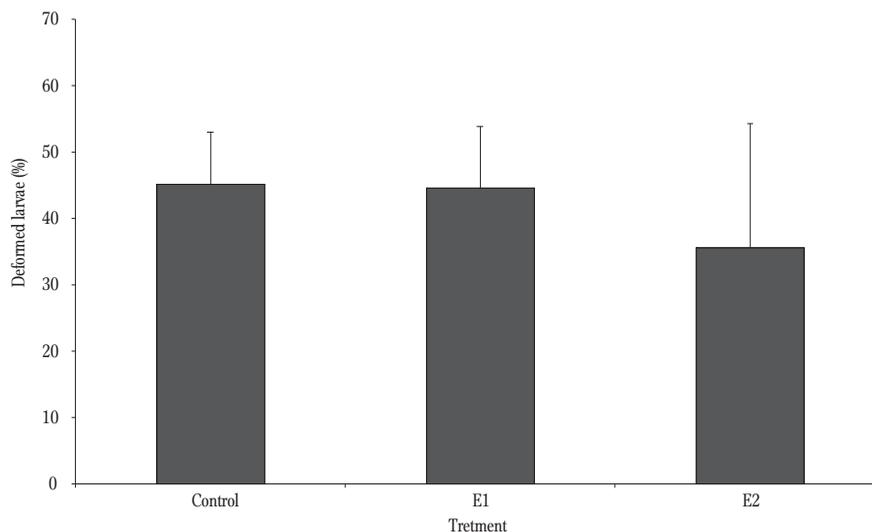


Figure 4. Influence of carbendazim on the number of deformed Prussian carp (*Carassius gibelio*) larvae. Data expressed as means  $\pm$  SEM (n=5).

resistant to the teratogenic effects of 0.005 ppm methylmercury while eggs from non-polluted environments were sensitive to them. Khan and Weis (1987) report that methylmercury increased the percentage of deformed embryos and larvae from eggs exposed to 1 mg l<sup>-1</sup> for 20 min prior to fertilization.

In an age of rapid industrial development, environmental pollution is a persistent problem. Although the available literature is inconclusive, which could result from species differences, there is no doubt that many organic substances have negative impacts on developing fish eggs. This experiment confirmed 100% mortality from exposure to high concentrations of carbendazim. Individuals exposed to low concentrations of this pesticide developed normally; however, a number of data shows that low concentrations of pollutants can affect larvae quality and hatching success. It is necessary to conduct further studies to determine the long-term effects on different fish species of exposure to various carbendazim concentrations.

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**Author contributions.** A.L. participated in the development of the concept, performed the research, and wrote and edited the text; B.B. elaborated the concept of this work, participated in the research and in writing and editing the text; M.S. participated in the research, analyzed the data, and made the graphs. H.L. and K.B.T. corrected the manuscript.

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