Fisheries Management and Ecology

Fisheries Management and Ecology, 2008, **15**, 163–166

Management and Ecological Note

Acute toxicity of preservative chemicals in organic baits used in carp, *Cyprinus carpio*, recreational fishing

T. RAPP

Humboldt-University of Berlin, Germany

T. MEINELT & A. KRÜGER

Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

R. ARLINGHAUS

Leibniz-Institute of Freshwater Ecology and Inland Fisheries and Humboldt-University of Berlin, Germany

Boilies are specialised baits used in carp, *Cyprinus carpio* L., recreational fishing. They are made from different meals (e.g. fish meal, soya bean meal), eggs, flavours, feeding stimulants and are boiled in water for hardening (Niesar, Arlinghaus, Rennert & Mehner 2004; Arlinghaus & Niesar 2005). As a result of the popularity of specialised carp fishing throughout Europe, boilies are introduced into water bodies in large amounts, potentially contributing to eutrophication (Arlinghaus & Mehner 2003; Niesar *et al*. 2004).

In addition to nutrients such as phosphorus, some boilie types, particularly those produced commercially, contain preservative chemicals (PC) to prevent deterioration and fungal infestation. The two main preservative chemicals (PCs) used in many ready-made boilies are benzoic acid (BA) and potassium sorbate (PS), or a combination thereof (Anonymous, personal communication 2005). Several studies describe the toxicity of BA to aquatic organisms ranging from bacteria to fish (e.g. Zhao, Ji, Cronin & Dearden 1998; Saha, Bhunia & Kaviraj 2006). Potassium sorbate is the salt of sorbic acid, which is also toxic to multiple aquatic organisms including fish, but lethal concentrations of sorbic acid for fishes are up to 6.5 times higher than those of BA (Koch 1994; Roth 1999). No studies describe the toxicological risk of BA and PS used as preservatives in angling baits. The objective of the present study was to assess the acute toxic effects of boilies containing BA and PS by measuring the lethal concentrations where 50% of embryos of the model organism zebrafish, *Danio rerio* (L.), died within 48 h (48-h, LC_{50}).

Adult zebrafish were kept in an 80-L glass aquarium at 26 °C with a day–night rhythm of 12:12 h. A metal grate was installed at the bottom of the aquarium, through which fertilised eggs could pass, thereby reducing potential egg predation by adults. Below this grate, a funnel led into a tube through which the eggs were collected. After sampling, the eggs were cleaned with reconstituted water at 26 °C. Reconstituted water contained 294.0 mg L^{-1} $CaCl_2$, 123.3 mg L^{-1} $MgSO_4$, 63.0 mg L^{-1} $NaHCO_3$ and 5.5 mg L^{-1} KCl.

Two series of toxicological tests, range-finding-tests with pure substances and tests with boilies containing BA (Merck Darmstadt, Germany) and PS (Fluka Chemie Neu Ulm, Germany), were conducted according to a German standard using the fish egg model zebrafish described in detail in DIN-Norm 38415-T6 (2001). The range-finding-tests were done because the reported toxicity range of BA to zebrafish eggs was very wide (DIN-Norm 38415-T6 2001) and data about

Correspondence: Dr Robert Arlinghaus, Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Müggelseedamm 310, 12587 Berlin, Germany (e-mail: arlinghaus@igb-berlin.de)

doi: 10.1111/j.1365-2400.2008.00598.x

the toxicity of PS were not available. In the range-finding-tests, BA was tested at concentrations of 10–100 mg L^{-1}. Only one concentration of PS (1000 mg L^{-1}) was preliminarily investigated because limited toxicological impact below this concentration (Passino & Smith 1987). However, since BA and PS are often used together in boilies at a proportion of 3:2 (Anonymous, personal communication), a further test of combined pure substances was performed to detect possible additive or antagonistic effects. Based on the range of toxicity of BA from the range-finding-test described above, concentrations of BA from 90–100 mg L^{-1} adjusted to a pH of 7.0 ± 0.2 were examined to determine the 48-h, LC_{50}. Concentrations of PS ranged between 60 and 66 mg L^{-1}. Ten zebrafish eggs in the four- to eight-cell stage were placed in cell wells and incubated for 48 h at a constant temperature of 26 °C. Death was defined as coagulation of eggs, absence of heart beat, non-appearance of somites and no detachment of the tail from the yolk sac.

The toxicological tests were conducted with four types of boilies, two commercially available, ready-made boilies from companies A and B purchased in a local angling shop in Berlin, Germany and self-made boilies. The self-made boilies were made using a commercially available boilie mix lacking PC (M&M Baits, Yellow Birdfoodmix), to which 3% BA and 2% PS, based on fresh weight, were added. Boilies were made as they would be by anglers adding eggs, cooking for 90 s and drying at the air for 24 h. The concentration of BA and PS was chosen because the manager of a large boilie manufacturer indicated that PC is often added to boilie mixes at 5% of the fresh boilie mixture (Anonymous, personal communication). Self-made boilies without PC served as a control. Each type of boilie was tested as whole boilie as well as divided (split) boilie. Many anglers employ split boilies to increase attractiveness of the groundbait location through enhanced leakage of feeding stimulants as a result of increased surface area per boilie (T. Rapp, personal observation). Three to 17 g L^{-1} of whole boilies and 2 to 15 g L^{-1} split boilies in 1 g L^{-1} steps were added to reconstituted water for 24 h to allow leakage of preservatives. Investigations were conducted at a single concentration of 20 g L^{-1} for boilies without preservatives. As a result of high oxygen consumption resulting from microbiological activity in the test with self-made boilies without PC, the eggs were aerated via a thin tube (Pasteur aeration).

For all tests, the 48-h, LC_{50} was calculated by probit analysis using SPSS 9.0 (SPSS Inc., Chicago, IL, USA), except in tests where only one concentration was employed. Sample size per concentration was $n = 10$ eggs. Values are expressed for the 48-h, LC_{50} as mg L^{-1} of the chemical for the range-finding-tests and for the boilie tests in g L^{-1} boilies. Differences in the 48-h, LC_{50} among treatments in the range-finding-tests as well as in the boilie tests were tested statistically as described in Lozán & Kausch (2004, p. 273). In the boilie tests, whole and split boilies of the same type and whole boilies of different types were compared. Significance was judged at $P < 0.05$. All values reported are averages with the 95% confidence interval denoted.

For BA, a mean 48-h, LC_{50} of 96.1 mg L^{-1} (95.6 – 96.6 mg L^{-1}) was determined. Potassium sorbate at a test concentration of 1000 mg L^{-1} caused no toxic effects on zebrafish eggs. For the combination of BA and PS, a 48-h, LC_{50} of 96.5 mg L^{-1} (94.9 – 98.4 mg L^{-1}) of BA was determined, which was not significantly different from the 48-h, LC_{50} of BA when tested as a single substance.

Self-made boilies without PC tested at a concentration of 20 g L^{-1} caused no toxic effects. All boilies with PC (self-made-boilies) and with assumed PC (ready-made boilies) caused acute toxic effects in zebrafish eggs (as measured by 48-h LC_{50}) at average boilie concentrations from 5.8 to 13.3 g L^{-1} (Fig. 1). The highest acute toxicity for both whole and split boilies was detected for ready-made boilies of company B and self-made boilies with BA and PS (Fig. 1). However, the difference between these two boilies was not significant ($P > 0.05$). Toxicity of boilies of company A was significantly lower. In all trials, the toxicity was significantly higher for split boilies (consequently the concentration of boilies at 48-h, LC_{50} was lower) than whole boilies of the same type.

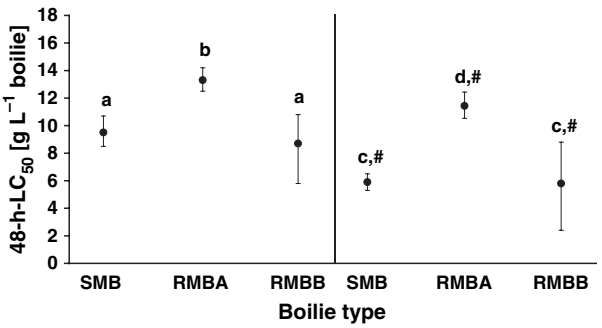

Figure 1. Average of 48-h, LC_{50} and 95% confidence intervals of self-made boilies (SMB) with benzoic acid and potassium sorbate, ready-made boilies of company A (RMBA) and ready-made boilies of company B (RMBB). Values for whole boilies are presented on the left hand side and values for divided boilies on the right hand side of the horizontal line. Different letters display significant differences between boilie types among either whole or divided boilies denote differences within the same boilie type comparing whole and divided boilies.

Toxic concentrations of BA to the embryos of zebrafish in the range-finding-test were within the range of values described previously for zebrafish (15.6 to >100 mg L^{-1}, DIN-Norm 38415-T6 2001). Documented lethal concentrations of BA for other juvenile and adult fish species (Zhao *et al*. 1998; Saha *et al*. 2006) were higher than the values reported in the present study. Eggs are particularly sensitive to environmental perturbations and pollutants (von Westernhagen 1988), which might, in addition to species-specific variability (Fent 1998), explain these patterns. In addition to mortality, BA can induce sublethal effects on fish embryos such as oedemas and deformities that were observed during all tests. Note, endpoints other than mortality might result from exposure fish to BA leaking from angling baits, e.g. growth depression (Saha *et al*. 2006).

Similar to previous research on the toxicity of sorbic acid (Koch 1994; Roth 1999), no acute toxicity of PS was observed in the present study. Coupled with the lack of interactive effects of PS and BA in the range-findings-tests, this suggests that PS is not toxic to fish eggs in the concentrations commonly used in boilies.

Self-made boilies containing a mixture of BA and PS were highly toxic (Fig. 1). Toxicity of the boilies from company A was slightly lower. This commercial boilie type contained only small amounts of BA (Rapp 2006), which, according to the range-finding-tests, were too low to account for the observed mortality. Toxicity observed in the ready-made boilies of company B was as high as self-made boilies with BA and PS, but these boilies did not include BA and only contained the non-toxic PS (Rapp 2006). This suggests that there are other unknown substances used in commercially available boilies that may be toxic to fish eggs. It remains unclear which substances are involved.

Toxicity was significantly higher in split boilies than in whole boilies of the same type, as indicated by lower 48-h, LC_{50}. This was probably because of increased surface area that facilitates more rapid leakage of PC. Consequently, toxic effects might be greater in small boilies with comparatively large surface, although this needs further confirmation.

From a managerial perspective, it is unlikely that the acute toxic concentrations of 5.8–13.3 g L^{-1} determined in the present study will be achieved at the level of the entire water volume in intensive carp or other coarse fisheries (Rapp 2006). However, PCs in boilies may still have undesirable impacts on organisms, both lethally (this study) or sublethally (Saha *et al*. 2006), for example if leakage of toxic substances from the baits accumulates in the water–sediment interface or in macrophyte beds. Since toxic effects of PC contained in boilies cannot be ruled out under particular conditions, alternative methods for preservation of boilies such as freezing, drying or salting should be considered by anglers and bait-supply enterprises. Reducing the input of toxic chemicals into aquatic ecosystems is in agreement with the precautionary approach to fisheries management.

Acknowledgments

The Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) provided funding for this study within the project 'Principles for Sustainable Recreational Fisheries Management'. We thank all the people who provided information on the use of PCs in angling baits, as well as Angelika Stüber and Jörg Gelbrecht from IGB for supporting chemical analyses.

References

Arlinghaus R. & Mehner T. (2003) Socio-economic characterisation of specialised common carp (*Cyprinus carpio* L.) anglers in Germany, and implications for inland fisheries management and eutrophication control. *Fisheries Research* **61,** 19–33.

Arlinghaus R. & Niesar M. (2005) Nutrient digestibility of angling baits for carp, *Cyprinus carpio*, with implications for groundbait formulation and eutrophication control. *Fisheries Management and Ecology* **12,** 91–97.

DIN-Norm 38415-T6 (2001) *Suborganismische Testverfahren (Gruppe T), Teil 6: Bestimmung der nicht akut giftigen Wirkung von Abwasser auf die Entwicklung von Fischeiern über Verdünnungsstufen (T6) Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung*. Weinheim: Verlag Wiley-VCH, 15 pp (In German).

Fent K. (1998) *Ökotoxikologie*. Stuttgart, New York: Georg Thieme Verlag, 288 pp (In German).

Koch C.S. (1994) Ökotoxikologische Daten von Kosmetik-Konservierungsstoffen. *Seifen-Öle-Fette-Wachse-Journal* **120,** 655–660.

Lozán J.L. & Kausch H. (2004) *Angewandte Statistik für Naturwissenschaftler*, 2nd edn. Hamburg: Parey Verlag, 287 pp (In German).

Niesar M., Arlinghaus R., Rennert B. & Mehner T. (2004) Coupling insights from carp, *Cyprinus carpio*, angler survey with feeding experiments to evaluate composition, quality and phosphorus input of groundbait in coarse fishing. *Fisheries Management and Ecology* **11,** 225–235.

Passino D.R.M. & Smith S.B. (1987) Acute bioassays and hazard evalution of representative contaminants detected in great lakes fish. *Environmental Toxicology and Chemistry* **6,** 901–907.

Rapp T. (2006) *Toxische Wirkung von Konservierungsstoffen in Angelfuttermitteln: der Einfluss von Benzoesäure und Kaliumsorbat auf die Eientwicklung beim Zebrabärbling*. Bachelor Thesis, Berlin: Humboldt-University of Berlin, 86 pp (In German) http://www.adaptfish.igb-berlin.de.

Roth L. (1999) *Wassergefährdende Stoffe, Ausgabe 5/99*. Landsberg: ecomed Verlagsgesellschaft AG & Co. KG (CD-ROM, In German).

Saha N.C., Bhunia F. & Kaviraj A. (2006) Comparative toxicity of three organic acids to freshwater organisms and their impact on aquatic ecosystems. *Human and Ecological Risk Assessment* **12,** 192–202.

von Westernhagen H. (1988) Sublethal effects of pollutants on fish eggs and larvae. In: W.S. Hoar & D.J. Randall (eds) *Fish Physiology*. London: Academic Press, pp. 253–346.

Zhao Y.H., Ji G.D., Cronin M.T.D. & Dearden J.C. (1998) QSAR study of the toxicity of benzoic acids to *Vibrio fischeri*, *Daphnia magna* and carp. *The Science of the Total Environment* **216,** 205–215.