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Abamectin in the aquatic environment

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Abstract Abamectin, widely used as a veterinary anthelmintic, medicine against a variety of animal parasites and insects, can runoff from the sites of application and becomes an aquatic pollutant. The aim of this study was to identify the toxicity of abamectin on bacteria, algae, daphnids, and fish. An extremely high toxicity of avermectin to the survival and reproduction of *Daphnia magna* was observed in 21-day exposure tests. Zebrafish and the algae *Scenedesmus subspicatus* are less sensitive to avermectin. The compound is expected to have adverse effects on the aquatic environment due to its high toxicity, even at very low concentrations, to daphnids and to fish.

Keywords Veterinary pharmaceuticals · Abamectin · Aquatic organisms · Toxicity

Introduction

In the last decade, the large-scale use of pharmaceuticals and their residues in the environment have become an emerging research area in environmental studies. Occurrence of pharmaceuticals in the environment has been investigated in systematic studies and data are now available for the USA, Canada and for some European countries (Kümmerer, 2004). However, a limited number of investigations deal explicitly with the fate and environmental effects of the active compounds (Kümmerer, 2001; Boxall et al. 2003a, b). Consequently, the environmental fate of many pharmaceuticals is not well known (Halling-Sorensen et al. 1998).

Veterinary drugs such as the avermectins (e.g. ivermectin, abamectin, doramectin) are commonly used in veterinary medicine as anthelmintics against internal and external parasites of cattle, pigs, and horses, sheep, and goats (Campbell and Benz 1984; Suarez 2002). In most cases up to 98% of avermectins are excreted through faeces either unchanged or as active metabolites (McKellar and Benchaoui 1996; Lumaret and Errouissi 2002). The avermectins, natural fermentation products of the soil-dwelling actinomycete *Streptomyces avermitilis*, are highly lipophilic, poorly soluble in water but readily soluble in most organic solvents (Roth et al. 1993). They have been shown to have effects on reproduction, biological function and survival of non-target aquatic and terrestrial organisms (Halley et al. 1993; Moore et al. 1993; Steel and Wardhaugh 2002; Wislocki et al. 1989).

The primary target of avermectins is the nervous system of parasites. They interact with the glutamate-gated chloride channels and GABA (γ-amino butyric acid)-gated chloride channels in arthropods and nematodes causing strong chloride influx, which results in disrupted neural signal transmission (Martin 1997; Martin et al. 2002). The mode of action of avermectins is not however specific to parasitic arthropods and nematodes and consequently may affect other organisms in the environment (Mc Kellar 1997). In fish the avermectins can also pass the blood/brain barrier and could cause toxic effects (Høy et al. 1990).

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Administration of abamectin to animals results in its indirect introduction to water bodies, leading to vulnerability of aquatic organisms. Avermectins are rapidly photodegraded in water to less bioactive compounds by oxidative and photo-oxidative mechanisms ($t_{1/2}$ = 4–21 h) (Halley et al. 1993). They are readily adsorbed by organic matter, soil and sediment particles and are unlikely to leach or contaminate groundwater. Nevertheless, they could still pose a risk to the aquatic environment, especially when used frequently in large numbers of animals. The predicted environmental concentrations (PEC) of doramectin at the worst-case scenario range from 0.011 μg/l in surface runoff to 18 μg/l in wet feedlot waste (Pfizer 1996). There is little data on repeated applications of avermectins or on the effect of climate.

In view of this paucity of reports on the environmental fate of abamectin and its possible adverse effects on aquatic organisms (Wislocki et al. 1989), the aim of this study was to determine the toxicity of abamectin on aquatic organisms. A battery of targets including bacteria, green algae, daphnids, and fish was used to evaluate the acute and chronic toxicity of abamectin.

Material and methods

A standard stock solution of abamectin was prepared by dissolving an abamectin reference standard obtained from Dr. Ehrenstorfer, Augsburg (Abamectin, standard purity 95.0%) in ethanol (absolute GR for analysis, Merck, Germany). Appropriate volumes of the standard stock solution were added to dilution water to obtain the desired concentrations. In the case of the toxicity test with algae a standard solution was prepared using acetone p.a. (Riedel de Haen, Germany), which is less toxic than ethanol to algae (Ma and Chen 2005).

In each definitive toxicity experiment five concentrations and a control in two replicates were tested. To exclude the possible toxic effects of the solvents, the highest concentrations used were tested in each toxicity test (a negative control). At least one preliminary and two definitive trials for each test species were conducted.

Luminescent bacteria

The luminescence of the freeze-dried bacteria *Vibrio fischeri* NRRL-B-11177 obtained from the manufacturer (Dr. Lange GmbH, Düsseldorf, Germany) was measured on a LUMIStox 300 luminometer according to the ISO standard (International Organisation for Standardization 1998). The luminescent bacteria were exposed to 0.08, 0.16, 0.31, 0.63, 1.25 and 2.5 mg/l of abamectin for 30 min at 15 ± 0.2°C and the percentage of inhibition was calculated for each concentration relative to the control. The 30 min EC_{20}, EC_{50}, and EC_{80} values were calculated using a standard log-linear model supported by computer software (Lange 2000). The EC_{20} was taken as a toxicity threshold.

Activated sludge

In the acute toxicity test, low concentration of microorganisms of activated sludge (150 mg/l of suspended solids) from the aeration tank of the municipal laboratory waste water treatment plant were used (International Organisation for Standardization 1986). The concentrations tested were 100, 200, 300, 400, 600, 800 and 1000 μg/l of abamectin. The concentration of ethanol in water did not exceed 2 v/v%. Oxygen consumption was measured with an oxygen electrode (WTW Oximeter, OXI 96) following biochemical degradation of meat extract, peptone, and urea every 30 min during 3 h. The oxygen consumption rates were calculated for each concentration, a control and negative control of ethanol alone.

Algae

The green, unicellular alga *Scenedesmus subspicatus* Chodat 1926 (CCAP 276/20) was obtained from the Culture Collection of Algae and Protozoa, Cumbria, United Kingdom. A stock culture of alga was maintained in a nutrient solution according to Jaworski (Thompson et al. 1988) at a constant room temperature of 21 ± 1 °C and under continuous fluorescent illumination (4,000 lux) provided by four 20 W cool-white fluorescent lights (Osram). To encourage gas exchange, flasks were agitated at 150 rpm for 15 min, alternating with 15 min resting, on an orbital shaker (Tehtnica Železniki, type EV 403). Algal growth was determined by counting cells in a Bürker counting chamber after 72 h of exposure. Test flasks were constantly shaken at the same frequency as stock flasks and they were illuminated with four 40 W cool-white fluorescent lights giving an illumination of 7000 lux. The inhibition of specific growth rates for each concentration was calculated in comparison to the control and the percentages of inhibition were plotted against concentration on semi-logarithmic paper. The 72 h EC10, EC50, and EC90 were estimated using linear regression analysis (International Organisation for Standardization 1989).

Acute and chronic toxicity tests with daphnids

Daphnia magna Straus 1820 obtained from the Institut für Wasser, Boden und Lufthygiene des Umweltbundesamtes, Berlin were cultured at 21 ± 1°C in 3-l aquariums covered with glass plates containing 2.5 l of modified M4 medium (Kühn et al. 1984), illuminated with fluorescent bulbs (approx. 1800 lux) for 12 h per day. They were fed daily a diet of the alga *S. subspicatus* Chodat 1926 corresponding to 0.13 mg carbon/daphnia. One day before the start of the experiments reproductive daphnids were isolated and young neonates (aged about 24 h) were used.

In the acute toxicity tests, daphnids were exposed to 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, and 1.3 µg/l of abamectin and the immobile daphnids were counted after 24 and 48 h of exposure (International Organisation for Standardization 1996a). The EC_{10}, EC_{50} with corresponding 95% confidence limits and EC_{90} values were obtained using probit analysis (US Environmental Protection Agency 1994).

A chronic toxicity test with *D. magna* was performed in a semi-static exposure system according to the OECD Guideline (Organisation for Economic Cooperation and Development 1998). Individual daphnids were placed in a 100 ml beaker containing 50 ml of solution; ten beakers were used for each concentration and a control. The room temperature was maintained at 21 ± 1°C and a photoperiod of 16 h light: 8 h dark was provided. On 3 alternate days (M, W, F) the surviving daphnids were transferred to freshly prepared test solutions and fed a diet of *S. subspicatus* at a ratio of 0.15 mg C/day per daphnid. Neonates were counted daily and then removed. On alternate days the pH, temperature, and dissolved oxygen concentration were measured. The endpoints of the chronic toxicity test were mortality of daphnids, appearance of the first offspring, and total young per female after 21 days. The daphnid reproduction results were analysed by the linear interpolation method and the one-tailed Dunnett's test to calculate the IC_{25}, and the NOEC and LOEC values, respectively (US Environmental Protection Agency 1994).

Fish

Zebrafish *Danio rerio* Hamilton Buchanan were obtained from a commercial supplier and acclimatised to the test temperature at least 7 days prior to the beginning of an experiment. During acclimatisation the fish were fed daily with commercial fish food and illuminated with fluorescent bulbs for 12 h per day. Brook water determined to be unpolluted and with a total hardness of 140 mg CaO/l, alkalinity 131 mg CaO/l, and pH 8.4 was used as holding and dilution water. The toxicity tests with zebrafish were conducted in both static (International Organisation for Standardization 1996b) and semi-static (International Organisation for Standardization 1996c) exposure systems at 21 ± 1°C in a 3-l aquarium containing 2.5 l of test sample. Test solutions were slightly aerated and every 24 h dead fish were counted and removed. The endpoints of the acute toxicity test were survival of fish and swimming performance during the 96 h of exposure. The percentages of mortality and swimming ability were calculated for each concentration of abamectin. The 96 h LC_{10}/EC_{10}, LC_{50}/EC_{50} with corresponding 95% confidence limits and LC_{90}/EC_{90} values were calculated using probit analysis (US Environmental Protection Agency 1994).

A summary of test conditions in toxicity tests are given in Table 1.

Water analyses

Concentrations of the avermectin B_{1a} component of abamectin were analysed at the beginning and at the end of the experiment. Analyses were performed in duplicate immediately after sampling.

Fifteen millilitre of acetonitrile and 50 µl of triethylamine were added to 35 ml of water sample. The sample was then transferred to a reservoir connected to an activated (5.0 ml of acetonitrile) and conditioned (5.0 ml of 30 v/v% acetonitrile in water) Bakerbond Octyl (C_8)

Table 1 Summarised test conditions in toxicity experiments

Toxicity	Acute			Chronic	
Organism	*V. fischeri*	*D. magna*	*D. rerio*	*D. magna*	*S. subspicatus*
Duration	30 min	24 and 48 h	96 h	21 days	72 h
Exposure	Static	Static	Semi-static	Semi-static	Static
Concentrations in preliminary test	10–5,000 µg/l	0.1–10.0 µg/l	10–100 µg/l	0.0001–0.3 µg/l	0.01–20 mg/l
Concentrations in definitive test	80–2,500 µg/l	0.1–1.3 µg/l	10–80 µg/l	0.0047–0.075 µg/l	1–20 mg/l
Max. solvent concentration definitive test (v/v%)	0.25	0.003	0.008	0.0002	0.2
Stock solution	1 mg/ml	50 µg/ml	1 mg/ml	50 µg/ml	10 mg/ml
Solvent	Ethanol	Ethanol	Ethanol	Ethanol	Acetone
Endpoint	Luminescence	Immobility	Mortality, swimming	Mortality, reproduction	Growth

cartridge (500 mg, 6 ml; J.T. Baker, Phillipsburg, NJ, USA). After applying the sample, the cartridge was washed with 5 ml 50 v/v% of acetonitrile in water and the analyte was eluted with 5.0 ml of acetonitrile. The eluate was collected in a polypropylene test-tube and evaporated to dryness under nitrogen at 60°C (evaporator Organomation: N-evap No. 111, Berlin, MA, USA). Dry residues were derivatised at room temperature with 100 µl *N*-methylimidazole solution in acetonitrile (1:1, v/v) and with 150 µl trifluoroacetic anhydride solution in acetonitrile (1:2, v/v). After 30 s acetonitrile was added to obtain a final volume of 1 ml (De Montigny et al. 1990). Fifty microlitre of sample was then injected into the HPLC system.

Within the set of sample determinations, two appropriate standard concentrations of the B_{1a} component of abamectin, prepared in acetonitrile (Merck, Darmstadt, Germany), were also analysed employing the same procedure to perform the calibration. The results were evaluated according to the external standard method. The detection limit (LOD) of abamectin was 0.25 ng/ml and the linearity of the method was in the range from 0.25 to 100 ng/ml.

A Varian ProStar HPLC system consisting of a Varian ProStar Solvent Delivery Module, a Varian ProStar Avto-Sampler Model 410 and a Varian ProStar Fluorescence Detector (excitation wavelength 365 nm; emission wavelength 470 nm was used for the simultaneous determination of abamectin in water samples. Separation of abamectin was performed using a Phenomenex Luna 3 µm C_{18} (2) column (150 × 4.6 mm ID; 3 µm particle size) with a Phenomenex pre-column C_{18} (ODS, Octadecyl) (4.0 × 3.0 mm ID; 5 µm particle size) at a column temperature of 28°C. The flow rate of the mobile phase consisting of methanol, acetonitrile, water (475:475:60, v/v/v) was 1.1 ml/min.

Results and discussion

Acute toxicity to bacteria and daphnids

Upon acute exposure, abamectin was found to be highly toxic to daphnids, but less toxic to bacteria. The EC_{20} for bacteria, established as a toxicity threshold for *V. fischeri*, was found to be at 0.39 mg/l (Table 2). In this study, preliminary acute toxicity tests with microorganisms from activated sludge were performed. The highest tested concentration of abamectin was 1 mg/l and no inhibition of biochemical degradation of organic matter was determined. Higher concentrations of abamectin were not tested due to the high toxicity to microorganism (57%) of the ethanol used as a solvent. For this reason *V. fischeri* was used as a test organism. There are no reported data concerning the toxicity of abamectin to aquatic bacteria (Tomlin 1997).

Table 2 EC values of abamectin to bacteria *V. fischeri* and to *D. magna*

	V. fischeri 30 min	*D. magna*	
		24 h	48 h
EC10 (µg/l)	/	0.11	0.12
EC20 (µg/l)	390	/	/
EC50 (µg/l)	690	0.33	0.25
(confidence limits)	(610–770)	(0.21–0.43)	(0.21–0.30)
EC80 (µg/l)	1200	/	/
EC90 (µg/l)	/	0.97	0.50

The concentrations of abamectin were measured in water samples containing 0.1, 0.3, 0.5, 0.7, 0.9 and 1.1 µg/l both initially and at the end of the experiment (Fig. 1).

With the exception of the first sample containing 0.1 µg/l of abamectin, the concentrations of abamectin decreased by about 40% in a few hours and then remained constant for 48 h (Fig. 1). The calculated EC values for daphnids in Table 2 are based on the measured exposure concentrations. In our study, the EC_{50} values based on the mobility of daphnids (Table 2) were similar to those reported by Wislocki et al. (1989) despite the fact that his toxicity endpoint was mortality for which the 48 h LC_{50} of abamectin was reported to be 0.34 µg/l.

Acute toxicity to fish

The first experiment based on the preliminary experiments; zebrafish were exposed to 60, 70, 80, 90, and 100 µg/l of abamectin in a static exposure system for 96 h. After 24 h, at all tested concentrations, some of fish were lying at the bottom of the aquariums, still alive, but unable to swim. After 48 h, some fish recovered and were able to swim again, the others died. The measurements of abamectin in the water showed that the concentrations of abamectin decreased by 20–29% in a few hours after starting the experiment. After 96 h the abamectin concentrations dropped by ≥90% in all tested samples, hence the recovery of some of the fish. Depletion of oxygen was observed in all tested samples containing abamectin due to intensive degradation of ethanol as a consequence of substantial growth of bacteria. Further experiments were carried out in a semi-static exposure system. Zebrafish were exposed to 10, 20, 30, 40, 50, 60, 70, and 80 µg/l of abamectin; the LC/EC values obtained are given in Table 3.

The same effect of inability to swim as in static exposure was observed and therefore swimming was also taken as a toxicity endpoint. The 96 h LC_{50} and the 96 h EC_{50} were 55.1 and 49.3 µg/l, respectively. Literature data showed that the toxicity of abamectin to fish strongly depends on the species tested. The most sensitive was rainbow trout with a 96 h LC_{50} of 3.2 µg/l. Jenčič et al.

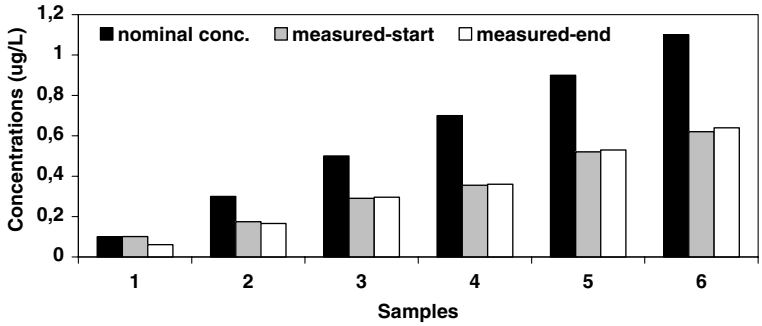

Fig. 1 Nominal (0.1, 0.3, 0.5, 0.7, 0.9, 1.1 μg/l) and measured concentrations of abamectin in water samples at the beginning of the experiment and after 48 h in acute toxicity test with daphnids

Table 3 LC/EC values of abamectin to zebrafish

	Mortality (LC)	Swimming ability (EC)
96 h LC10/EC10 (μg/l)	30.8	21.1
96 h LC50/EC50 (μg/l) (confidence limits)	55.1 (46.1–66.7)	49.3 (37.3–63.3)
96 h LC90/EC90 (μg/l)	98.3	114.8

(2006) studied the toxicological and histopathological effects of abamectin on rainbow trout and based on the measured concentrations of abamectin in the water samples, they established a 58 h LC_{50} of 1.5 μg/l. The least sensitive fish species was carp with a 96 h LC_{50} of 42 μg/l; no data on zebrafish are available (Wislocki et al. 1989). In our experiments, the sensitivity of zebrafish was similar to that of carp.

Abamectin disappeared from water. The measured initial concentrations of abamectin were more than 80% of the nominal concentrations since the maximum decrease in the concentration of abamectin was 13%. However, at the end of the experiment the measured concentrations of abamectin decreased on average by 85% of the nominal concentrations. The results of chemical analysis show that breakdown of the B_{1a} component of abamectin in water occurred during the experiments resulting in markedly lower concentrations than the nominal concentrations. The LC/EC values were calculated using the mean measured initial concentrations of abamectin.

Problems concerning loss of doramectin during aquatic toxicity testing were discussed in a Pfizer risk assessment report (1996). We observed similar behaviour of the closely related abamectin in water samples (Fisher and Mrozik 1992). Davies et al. (1997) also reported that the physico-chemical properties and the adsorption characteristics of ivermectin complicated the 96-h toxicity test using the mysid *Neomysis integer*. Since the molecular weight of avermectin B_{1a}, the major component of abamectin is high, the water solubility is low (7.8 μg/l) and its K_{ow} is 9900 (Wislocki et al. 1989; Halley et al. 1993), it could be presumed to bioconcentrate in aquatic organisms. However, van den Heuvel et al. (1996) found that abamectin does not bioconcentrate strongly in bluegill sunfish due its rapid elimination during the depuration period.

Chronic toxicity to algae

Initially, a stock solution of abamectin of 1 g/l was prepared in 96% ethanol as for all other toxicity tests. Samples with 10, 100, and 200 μg/l of abamectin and the corresponding negative controls without abamectin were tested. In the highest tested samples containing 100 and 200 μg/l of abamectin growth inhibition was due to ethanol toxicity since ethanol alone inhibited growth at the same level as abamectin. There was no growth inhibition at 10 μg/l of abamectin and the amount of ethanol in the negative control, (ethanol without abamectin) corresponded to 0.001 v/v%. The maximum recommended concentration of solvent in samples is 100 μl/l (0.01%) (International Organisation for Standardization 1999). We found that 0.01% of ethanol in the samples was still toxic to *S. subspicatus* and the established 72 h EC_{50} of ethanol was 0.043 v/v%. Ma and Chen (2005) studied the effects of various organic solvents on the growth of *Chlorella pyrenoidosa* and found the 96 h EC_{50} of ethanol to be 0.18 v/v%-less toxic than in our experiment. Since ethanol is clearly toxic to *S. subspicatus*, we used acetone in place of ethanol; the results of toxicity tests with solutions in acetone are given in Table 4.

At the start of the experiment, the measured concentrations were 80% or more of the nominal concentrations of abamectin in the water samples; however, the measured concentrations of abamectin dropped by 70% and more after 72 h. Adsorption on algal cells plays an important role as well as adsorption of abamectin on glass test vessels (Organisation for Economic Cooperation and Development 2000). It was stated that adsorption is important at low concentrations, i.e. less than 1 mg/l, which was also confirmed by our measurements as the highest decrease of concentration was detected in the sample with 1 mg/l of abamectin. The 72 h EC_{50} was found to be 4.4 mg/l of

Table 4 Toxicity of abamectin to algae *S. subspicatus*

Nominal concentration of abamectin (mg/l)	Growth inhibition (%)	
	Abamectin	Acetone
1	10	/
5	45	/
10	66	/
15	73	/
20	100	10

Table 5 Final data of chronic toxicity of abamectin to *S. subspicatus* and *D. magna*

S. subspicatus		*D. magna*	
72 h EC10 (mg/l)	0.71	21 days LOEC (µg/l)	0.0094
72 h EC50 (mg/l)	4.4	21 days NOEC (µg/l)	0.0047
72 h EC90 (mg/l)	21.3	21 days IC25 (µg/l)	0.0074

abamectin (Table 5) considering the measured concentrations at the start of the experiment. Ma et al. (2002) reported 96 h EC_{50} values of 9.89 mg/l and 7.31 mg/l for *Scenedesmus obliqnus* and *C. pyrenoidosa*, respectively. In comparison to these results, *S. subspicatus* was found to be more sensitive to abamectin than *S. obliqnus* and *C. pyrenoidosa.*

The algae were intensively illuminated during the experiments, but the measured concentrations of abamectin in the water samples at the end of the experiments with algae were essentially the same as those in the daphnid tests, which were not illuminated. As a result, it was assumed that photodegradation does not play an important role in the elimination of abamectin; the most important factor is probably adsorption on the glass test vessels.

Chronic toxicity to daphnids

An experiment was performed with concentrations of 0.6, 0.3, 0.15, 0.075 and 0.0375 µg/l of abamectin; all daphnids died within 14 days. The abamectin concentrations were measured in the water samples after 48 h (in the semi-static test samples were replaced every 2 days) and the nominal concentrations were found to have decreased by between 23 and 33%. Daphnids were exposed to lower nominal abamectin concentrations, indeterminate due to the extremely low concentrations, below the detection limit of the analytical method (0.25 µg/l). Abamectin was very toxic to daphnids since a sample with 0.0094 µg/l still caused mortality and inhibited the reproduction of daphnids (Fig. 2). The final data for chronic toxicity are given in Table 4.

The NOEC was detected at 0.0047 µg/l of abamectin and the LOEC was 0.0094 µg/l (nominal concentrations). The IC_{25}, proposed by Chapman et al. (1996) as a replacement of the NOEC, was 0.0074 µg/l (Table 5). Daphnids are filter feeders and a possible reason for the extremely high toxicity of abamectin to daphnids is the uptake of abamectin via algae. The result obtained demonstrated that the daphnids exposed in our study were approximately 10 times more sensitive than those reported by Wislocki et al. (1989), who reported a NOEL of 0.03 µg/l of abamectin. The sensitivity of *Mysidopsis bahia* to abamectin [NOEL 0.0035 µg/l (Wislocki et al. 1989)] is similar to that of daphnids.

Conclusions

Abamectin is poorly soluble in water, readily degraded and is adsorbed on particles and glass. For these reasons it may

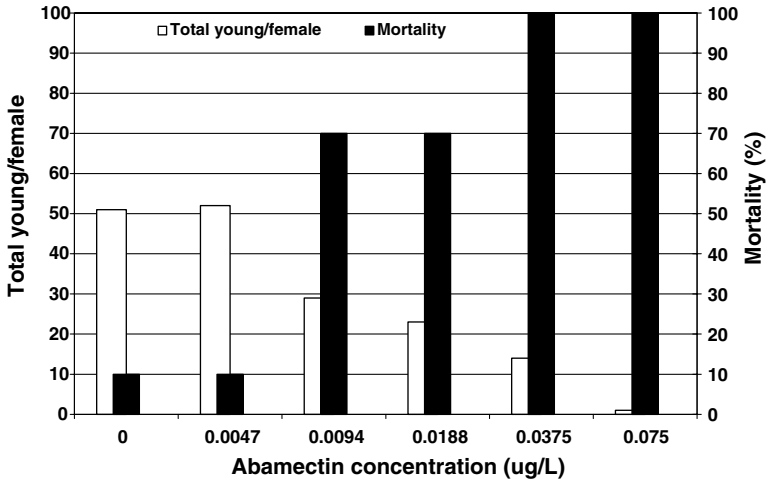

Fig. 2 Mortality and reproduction of daphnids after 21 days of exposure to abamectin

be supposed that aquatic organisms will not encounter abamectin in significant concentrations and are therefore unlikely to be harmed by abamectin in the environment. Abamectin is however a potent acute and chronic toxin to *D. magna*; the 48 h EC_{50} and 21 days NOEC were found to be as low as 0.25 and 0.0047 μg/l, respectively. Zebrafish are also highly sensitive to abamectin with a 96 h EC_{50} of 50.4 μg/l. The least sensitive species in our study were the algae *S. subspicatus* with a 72 h EC_{50} at 4.4 mg/l. Based on its experimental toxicity to bacteria, algae, daphnids and fish, abamectin should be classified as very toxic to aquatic organisms according to the criteria for the EU classification of dangerous substances (EC 2001). Despite the fact that low concentrations of abamectin are expected in water bodies, abamectin could cause toxic effects on some sensitive aquatic organisms at very low concentrations.

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