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RESEARCH ARTICLE

Acute and Chronic Toxicity of the Coccidiostat Amprolium to *Daphnia magna* and Its Implications for Aquatic Contamination from Livestock Waste

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Abstract

Pharmaceutical residues from livestock production are increasingly detected in aquatic systems where they may persist at low concentrations and affect non-target organisms. Among these, Amprolium is a coccidiostat extensively used in poultry farming with residues capable of reaching surface waters via runoff from manure-amended soils and wastewater effluents. Despite its widespread use, ecotoxicological data for freshwater invertebrates are limited, restricting reliable environmental risk assessment. This study aimed to evaluate both acute and chronic effects of Amprolium on *Daphnia magna*, a sensitive and widely used model organism in aquatic toxicology. Acute toxicity tests, performed according to OECD protocols, exposed neonates (<24 h old) to five concentrations (100–300 mg/L), producing a clear concentration- and time-dependent response, with a 48-h EC_{50} of 48.71 mg/L. Chronic 21-day exposures at environmentally relevant concentrations (0.0625 and 0.125 mg/L) significantly reduced survival, heart rate and reproductive output relative to controls ($P<0.05$). Statistical analyses demonstrated that both exposure level and duration strongly influenced physiological and reproductive endpoints. These findings reveal that even trace levels of Amprolium may disturb population dynamics and ecosystem functioning. The results highlight the scientific and practical importance of incorporating ecotoxicity data into livestock waste management strategies and support the need for regulatory measures to limit pharmaceutical emissions to aquatic environments.

Keywords: Aquatic life, Amprolium, *Daphnia magna,* Micropollutant

INTRODUCTION

Pharmaceutical residues originating from human and veterinary use are increasingly recognized as micro-pollutants in aquatic environments [1]. These substances can persist at low concentrations in surface and ground waters, exerting biological activity on non-target organisms and potentially altering ecosystem functions [2,3]. The combined input from wastewater treatment plants, agricultural runoff and the application of animal manures create continuous exposure scenarios for aquatic biota [4,5].

An additional notable cause of drug contamination arises from utilizing fecal matter from animals like pigs, poultry, and humans as a source of nutrients. As highlighted by Suryanto et al.[6], some antibiotics may have a longer half-life in water and can lead to adverse effects on the fish species. Reports have highlighted the adverse effects of antibiotics on aquatic organisms, including impacts on survival, growth, reproductive capabilities, and changes in biochemical indicators, potentially disrupting the entire aquatic food chain.

In large-scale animal farming, veterinary drugs such as coccidiostats and anthelmintics are commonly given to livestock. These chemicals can enter the aquatic environment through animal waste from outdoor animals or when contaminated liquid manure is applied to farmland. Through processes like surface runoff, leaching, and drift, these substances can lead to both acute and chronic toxicity in aquatic organisms, potentially disrupting biodiversity and affecting the functioning of ecosystems [7,8].

Amprolium is one of the widely used pharmaceuticals in poultry production as a coccidiostat, and residues have the potential to reach surface waters via runoff from manure-amended fields or direct discharges from intensive farms. The paucity of standardized acute and chronic ecotoxicity data for Amprolium constrains robust risk assessment for freshwater invertebrates [9].

Daphnia magna, used in this study, is a widely recognized model organism in aquatic toxicology, frequently employed in both acute and chronic assays due to its high sensitivity to pollutants and practical advantages over vertebrate species. Its use facilitates pre-screening of chemicals, supports alternative testing strategies, and contributes to the development of more efficient and sustainable approaches in environmental risk assessment and toxicological research.

This study aims to fill that gap by quantifying both short-term (12-48 h EC_{10}-EC_{50}) and long-term (21-day reproductive and survival) effects of Amprolium on *Daphnia magna*, a standard model organism in aquatic toxicology. By coupling conventional endpoints (mortality, reproduction) with physiological measures we provide a more comprehensive toxicological profile and discuss the ecological implications for aquatic systems impacted by livestock waste.

Material and Methods

Ethical Statement

The study does not require approval from the Local Animal Experiments Ethics Committee.

Test Organism and Culture Conditions

Cultures of *Daphnia magna* were maintained in the laboratory before acute and chronic tests. For all toxicity tests, Daphnids (<24 h old) were obtained from these stock cultures and acclimated to test conditions. All toxicity tests were performed at 21°C±0.5°C in a temperature-controlled laboratory, consistent with standard Daphnia test conditions. Dissolved oxygen and pH were measured in all treatments and controls and were kept within acceptable ranges (dissolved oxygen ≥6 mg/L in stock; during tests, dissolved oxygen was maintained and reported for each group). The water temperature was maintained at 26°C±1°C in the laboratory where the research was conducted. Lighting was provided with a photoperiod providing 12 h of light and 12 h of darkness using fluorescent lamps placed on the water surface and automatically turning on and off. Each box contained an air stone that provided 8-10 ppm of oxygen. Test solutions were refreshed three times a week. Daphnia was fed with 0-100 µ size Inve Aquaculture brand feed daily. The feed was given in 0.1 g powder form by dissolving it in water.

Preparation of Amprolium

Ampronet oral solution, containing 250 mg/mL of Amprolium, was obtained from a local pharmaceutical warehouse in Tekirdağ, Türkiye. The solution, with Amprolium as its active ingredient, was then directly added to the experimental aquarium.

Acute Toxicity Tests

Acute toxicity tests expose *Daphnia magna* to elevated concentrations of a substance for a brief period, usually between 24 and 48 h. The acute toxicity test of Amprolium was conducted following standard OECD protocols for *D. magna* acute test [10] under laboratory conditions that were consistent with the rearing procedures. Acute toxicity assays were conducted using Daphnids (<24 hrs old) to calculate EC_{10} and EC_{50} values. In addition to EC_{50}, EC_{10} values were calculated to provide a more ecologically relevant threshold for sublethal effects. While EC_{50} represents the concentration at which 50% of the organisms show a response, EC_{10} is a more sensitive indicator of early physiological or behavioural changes that may occur at environmentally realistic concentrations. This parameter is frequently recommended in regulatory ecotoxicology as it better reflects no-effect or low-effect levels, which are critical for risk assessment and environmental protection standards.

Five test concentrations (100, 125, 200, 225 and 300 mg/L) plus a control were tested in triplicate, with 20 Daphnids per replicate (60 per concentration). The experiment employed 100 mL glass beakers as test containers; each filled with 25 mL of the test solution. The trial was conducted in the absence of aeration. Throughout the experiment, the test organisms did not receive any food to avoid affecting water quality. Immobility of Daphnia samples was assessed by gently shaking test tubes and the dead were observed to sink to the bottom motionless. pH and oxygen levels were assessed in the control and the test concentrations. Water change was done every 24 hours. Immobilization was recorded at 12, 24, 36 and 48 h by gently agitating the beakers and noting organisms that were unable to swim after agitation. No food was supplied during acute tests to avoid water-quality changes. pH and dissolved oxygen were monitored and reported for each sampling time.

Chronic Toxicity Tests

A total of 180 Daphnia magna were used for chronic toxicity tests consisting of three groups, one control and two trial groups, each containing 60 daphnids. Each group was kept in separate 9 boxes to consist of 3 replicate subgroups. The Daphnia divided into groups were obtained from the same aquarium and distributed according to the random sampling method. Chronic toxicity assays involve extended exposure of *Daphnia magna* to lower concentrations of a substance, typically over several generations. The chronic assessments of *Daphnia magna* were conducted over 21 days, adhering to established OECD protocols [11] and maintaining the same temperature and photoperiod outlined in the rearing procedures. Daphnids, aged less than 24 hours at the start of the test, were exposed for 21 days to two different concentrations of Amprolium 0.0625 and 0.125 mg/L. These concentrations were determined considering concentrations likely to be present in practical wastewater conditions, with the European Food Safety Authority (EFSA) referring to predicted environmental concentrations of Amprolium in groundwater and surface water as 0.036 and 0.012 mg/L [12]. One group was kept in control and not exposed to Amprolium. The count of living offspring generated per animal per day was monitored from the initial offspring's first day. The Daphnids were transferred to freshly prepared pharmaceutical dilutions every alternate day and received daily feeding. Over the 21 days, the creatures were observed daily for mortality and reproductive status. Survival and offspring production were evaluated each time the solutions were refreshed, with pH and oxygen levels measured concurrently. The animals were not fed during the test times. The number of deaths and eggs were also recorded on the same test days.

The transparency of *Daphnia magna* enables clear observation of its internal structures and physiological processes under a microscope. On measurement days, individual test organisms were placed on a single-cavity microscope slide in a 50 µL droplet of aquarium water. One-minute video recordings of their movements under the microscope (Soif BK300-L) were taken and slowed down to 25% of the original speed using VLC Media Player. This approach was chosen because direct microscopic counting of heartbeats is often error-prone due to their rapid contractions. Video analysis provides more precise and reproducible results, reduces observer bias, allows post-experiment verification, and enables reliable detection of subtle physiological changes during long-term exposure.

Statistical Analysis

The software SPSS v27.0 was used for the statistical analyses. A one-way analysis of variance (ANOVA) followed by a Scheffe test was applied to assess the statistical differences between the different pharmaceutical concentrations and the control ($P<0.05$) to determine if the applied concentration of the pollutants had a significant effect on the heartbeats.

The two-way Repeated Measures ANOVA, employing a Huynh-Feldt correction was used for mean heartbeat values across assessment stages.

The chi-square test was used to compare mortality rates between groups, and the weekly egg production numbers were compared using the Kruskal-Wallis test due to non-normal distribution.

The acute EC_{10} (10% mortality) and EC_{50} (50% mortality) assays (measuring immobile organisms) were performed in triplicate for 100, 125, 200, 225 and 300 mg/L concentrations of Amprolium, each treatment containing 60 (20x3) Daphnids. The 95% confidence limits for these assays, as well as for the 12, 24, 36 and 48-h acute test was determined by calculating the effective concentration values and their 95% confidence limits using non-linear regression analysis through Probit analysis. Concentration factors were used as input data concentrations.

Results

The mean of the heartbeat measurements was expressed in heartbeats per minute and averaged for the experimental and control test animals.

Acute Tests

Exposure of *D. magna* to Amprolium at different concentrations for 12, 24, 36 and 48 h resulted in a negative effect on its survival. During the acute tests with Daphnids, no mortality was observed in the control group. The oxygen content remained unaltered and there were no significant pH fluctuations throughout the testing process. *Table 1* presents the concentration levels examined, alongside the calculated EC_{10} and EC_{50} values for 12, 24, 36 and 48 h. A significant increase in deaths over time was observed due to the effect of the applied concentrations. Both EC_{50} and EC_{10} values were determined to provide insight into low effect levels that would occur at environmentally realistic concentrations. *Fig. 1* also demonstrates the concentration effect on survival over time.

Chronic Tests

Results showed that even low concentrations of Amprolium in water led to a decrease in heart rates, while simultaneously increasing death rates, egg numbers, and offspring production ($P<0.05$). pH levels for control, 0.0625 and 0.125 mg/L groups were 7.04 (6.97-7.20), 6.58 (6.40-6.73) and 6.52 (6.40-6.75), respectively while O_2 levels for control, 0.0625 and 0.125 mg/L groups were 6.18 (6.00-6.40), 4.11 (4.00-4.20), 4.05 (4.00-4.20).

Changes in the Heartbeats

Over 21 days, differences in heart rates of *Daphnia magna* exposed to varying concentrations of Amprolium revealed a significant reduction in heart rates with increasing concentrations (*Table 2*, $P<0.05$). The 0.125 g/mL group showed a significant difference from the control group within the first 3 days and differed from the 0.0625 g/mL group on day 6. By days 9 and 12, the trial groups were

Table 1. *Daphnia magna acute EC values for Amprolium with confidence limits (95% probability)*

Exposure Time (h)	n	Mortalities	EC	Concentration levels (100-300 mg/L)	LCI[a]	UCI[b]
12	60	43.8	EC_{10}	0.28	NA	NA
			EC_{50}	21.55	NA	NA
24	60	47.6	EC_{10}	6.63	0.02	23.49
			EC_{50}	47.77	5.24	80.45
36	60	51.6	EC_{10}	10.55	0.39	27.83
			EC_{50}	46.65	10.40	73.98
48	60	54.6	EC_{10}	16.04	1.68	34.04
			EC_{50}	48.71	15.60	72.24

a: Lower Confidence Interval, b: Upper Confidence Interval

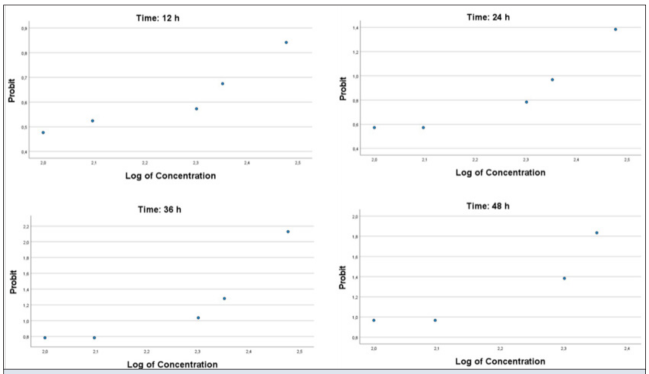

Fig 1. Acute EC values for Amprolium with confidence limits (95% probability) for Daphnia magna at 12, 24, 36 and 48 h

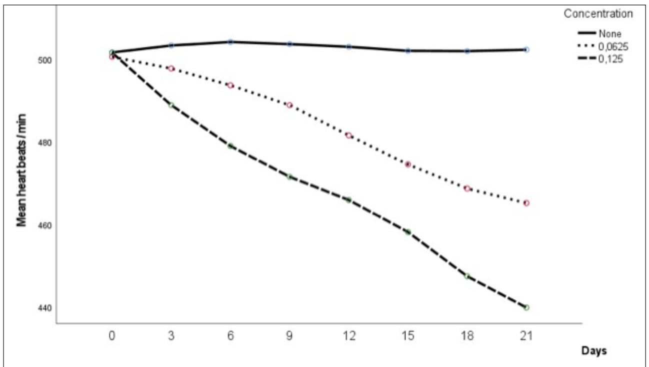

Fig 2. The course of the heart beats over time

significantly different from the control group, with all groups showing significant differences from each other on days 15, 18 and 21 (P<0.05).

After the sixth day, the trial groups differed from the control group (P<0.05), while the control group drew almost a stable curve throughout the entire study *(Fig. 2)*.

The two-way repeated measures ANOVA test revealed that the difference between the repeated measurements depending on time and the interaction of the concentration with time was statistically significant (P<0.05).

Table 2. *One-way ANOVA test results for group comparisons of heartbeats by time (days)*

Days	Concentration	n	$\bar{x}$	$S\bar{x}$	F	Sig.
0	None	60	500.40	3.62	0.637	.530
	0.0625	60	501.25	4.36		
	0.125	60	495.58	3.45		
3	None	60	503.87^{a}	4.47	5.36	.005
	0.0625	60	492.93^{ab}	5.98		
	0.125	60	479.08^{b}	5.52		
6	None	60	507.18^{a}	5.40	6.86	.001
	0.0625	60	498.25^{a}	5.97		
	0.125	59	478.59^{b}	5.31		
9	None	60	500.67^{a}	4.35	9.96	.000
	0.0625	60	474.00^{b}	5.30		
	0.125	57	472.68^{b}	5.37		
12	None	56	505.96^{a}	4.56	12.15	.000
	0.0625	60	483.98^{b}	5.59		
	0.125	50	470.24^{b}	4.86		
15	None	54	502.26^{a}	4.42	20.91	.000
	0.0625	52	475.19^{b}	5.30		
	0.125	44	458.20^{c}	4.91		
18	None	52	501.04^{a}	3.51	35.94	.000
	0.0625	48	470.60^{b}	4.97		
	0.125	42	452.62^{c}	3.56		
21	None	51	502.24^{a}	2.59	103.14	.000
	0.0625	35	465.14^{b}	3.36		
	0.125	29	439.83^{c}	3.72		

P<0.05

The two-way Repeated Measures ANOVA, employing a Huynh-Feldt correction, revealed a statistically significant variance in the mean heartbeat values across assessment stages (0, 3rd, 6th, 9th, 12th, 15th, 18th, and 21st days) (F (6.123, 685.723) = 13.023, P<0.05) *(Table 3, Table 4,)*.

Table 3. *The two-way repeated measures ANOVA - Mauchly's test of sphericity*

Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
					Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Time	.442	89.298	27	.000	.811	.875	.143

b: A correction factor when the assumption of sphericity is violated

Table 4. *Tests of within-subjects' effects on heartbeats*

Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Time	Sphericity Assumed	99468.287	7	14209.755	13.023	.000	.104	91.161	1.000
	Greenhouse-Geisser	99468.287	5.678	17518.467	13.023	.000	.104	73.943	1.000
	Huynh-Feldt	99468.287	6.123	16246.283	13.023	.000	.104	79.734	1.000
	Lower-bound	99468.287	1.000	99468.287	13.023	.000	.104	13.023	.947
Time * Concentration	Sphericity Assumed	60561.686	14	4325.835	3.965	.000	.066	55.504	1.000
	Greenhouse-Geisser	60561.686	11.356	5333.096	3.965	.000	.066	45.021	.999
	Huynh-Feldt	60561.686	12.245	4945.809	3.965	.000	.066	48.546	.999
	Lower-bound	60561.686	2.000	30280.843	3.965	.022	.066	7.929	.701
Error (Time)	Sphericity Assumed	855444.657	784	1091.128					
	Greenhouse-Geisser	855444.657	635.926	1345.195					
	Huynh-Feldt	855444.657	685.723	1247.508					
	Lower-bound	855444.657	112.000	7637.899					

a: Computed using alpha = .05

Table 5. *Survival * concentration crosstabulation*

Survival		Concentration			Total
		None	0.0625	0.125	
Dead	Count	9	25	31	65
	Expected Count	21.7	21.7	21.7	65.0
Alive	Count	51	35	29	115
	Expected Count	38.3	38.3	38.3	115.0
Total	Count	60	60	60	180
	Expected Count	60.0	60.0	60.0	180.0

Table 6. *Chi-square tests for survival * concentration*

Test	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	18.686[a]	2	.000
Likelihood Ratio	20.120	2	.000
Linear-by-Linear Association	17.385	1	.000
N of Valid Cases	180		

a. 0 cells (0.0%) have an expected count less than 5. The minimum expected count is 21.67

Additionally, time and concentration interactions were also found to be statistically significant (P<0.05).

Survival

The survival and mortality data of test organisms throughout the 21-day exposure period are presented in *Table 5*. According to these results, the highest mortality was recorded in the 0.125 g/L treatment group, whereas the lowest mortality occurred in the control group. The statistical significance of the differences among groups was further evaluated using the chi-square test, the outcomes of which are summarized in *Table 6* (P<0.05).

Egg Numbers

Fertility, as indicated by the number of eggs produced per week, was negatively affected over time in the trial groups exposed to different concentrations of Amprolium (*Table 7*).

Table 7. *Kruskal-Wallis Test results for the comparison of the egg numbers*

Time	Concentration	n	Mean	Mean Rank	Kruskal-Wallis H	df	P
Day 0	Control	60	2.92	86.33	1.442	2	.486
	0.625	60	3.37	96.92			
	1.25	60	2.82	88.25			
Day 3	Control	60	3.35	89.78	1.456	2	.483
	0.625	60	3.48	96.47			
	1.25	60	3.00	85.25			
Day 6	Control	60	2.25	116.12	28.486	2	.000
	0.625	60	1.40	86.31			
	1.25	59	.78	67.19			
Day 9	Control	60	2.07	115.40	32,103	2	.000
	0.625	60	1.02	80.01			
	1.25	57	.63	67.48			
Day 12	Control	56	1.70	96.03	9.398	2	.009
	0.625	60	.80	82.15			
	1.25	50	.52	71.09			
Day 15	Control	54	1.56	92.56	19.934	2	.000
	0.625	52	.42	66.82			
	1.25	44	.27	64.82			
Day 18	Control	52	1.58	90.36	24.189	2	.000
	0.625	48	.50	64.31			
	1.25	42	.21	56.37			
Day 21	Control	51	1.49	72.01	22.765	2	.000
	0.625	35	.43	49.20			
	1.25	29	.14	43.98			

$P<0.05$

Discussion

This study utilized *Daphnia magna* as a sentinel species to examine the environmental and ecological consequences of pharmaceutical residues derived from animal waste. Findings revealed that even at trace concentrations, these contaminants present significant hazards to aquatic biota. In particular, changes in cardiac activity, survival rates, and reproductive output observed in Daphnia exposed to Amprolium emerged as highly sensitive biomarkers. Such endpoints can therefore serve as robust indicators for evaluating the ecological impacts of micropollutants and hold promise as early-warning metrics in aquatic environmental monitoring programs.

The acute toxicity tests conducted with *Daphnia magna* in the current study revealed a significant impact on their survival, demonstrating an increased sensitivity to Amprolium. The EC_{50} value, which indicates the concentration of a chemical toxic to 50% of the test organisms within a specific exposure period was determined to be 48.71 mg/L over 48 h. This finding underscores the heightened susceptibility of *D. magna* to Amprolium under laboratory conditions in a short-term exposure in this study.

Various studies report EC_{50} values for antiparasitic and anticoccidial drugs with different values. Puckowski et al.[13] reported 48-h EC_{50} values for Flubendazole and Fenbendazole as 0.0448 mg/L and 0.0193 mg/L. Yoshimura and Endoh [9] found 48-h EC_{50} values for Amprolium hydrochloride, Levamisole hydrochloride, Pyrimethamine and Trichlorfon as 227 mg/L, 64.0 mg/L, 5.2 mg/L and 0.00026 mg/L, respectively. It is important to recognize that EC_{50} values can vary significantly based on factors like strain, water hardness, pH, temperature, and other environmental conditions. As such, interpreting these values should be done carefully, considering the specific parameters of each study. Furthermore, the rate in surface waters and long-term exposure will lead to significant changes in concentration values. When the effects of mixtures of different wastes are added to this, the table will change completely.

Chronic toxicity assessments provide a comprehensive understanding of potential ecological impacts and aid in determining acceptable long-term exposure levels. Different chemicals can have varying effects on *D. magna*, causing toxicity and affecting mortality and reproduction. In the current research, *D. magna* exposed to different concentrations of Amprolium with increased concentrations significantly reduced the heartbeat rates and this decrease became more pronounced over time (P<0.05, *Table 2)*. In an earlier study, the heartbeat rate proved to be the most sensitive toxicity endpoint [14]. Similar to this study, Vo et al.[15] examined the chronic effects of Ampicillin on *Daphnia magna* and found significant reductions in survival, reproduction, and growth. Several other researchers suggest that substances like herbicides, gasoline pollution, environmental hormones, and ethanol can impact the metabolism of *Daphnia magna* by reducing the heartbeat rate [16-18]. Taken together with literature information, our results indicate that the heart rate of *Daphnia magna* can provide a reliable basis for toxicological studies and testing of aquatic micropollutants.

The methodology applied in this study for calculating heart rate numbers led to different results from the numbers reported in the literature because the same precision was not applied in other similar studies. A remarkable change was seen in the numbers obtained by reducing the video recording speed to 25% in calculating the heartbeat numbers. This method offers the possibility of obtaining healthier values for fast repetitive movements at the microscopic level. In this study, it was determined that the control group had an average of 500 beats per minute, while values around 300 and below are reported in the literature [19]. It was observed that when attempting to count the heartbeats of Daphnia under a microscope by eye, accurate values could not be obtained.

Considering the aspect of repeated measurements, significant differences in each measurement period were noticed in the experimental groups, while a stable condition was observed in the control group (*Fig. 1*, *Table 3*, *Table 4*). Time concentration interaction was also found to be statistically significant; differences in values occurred not only depending on time but also depending on concentration.

There was a significant difference between the concentration groups in terms of the number of deaths (P<0.05) during the chronic exposure. On a week-by-week basis, a significant increase in deaths has been observed in the last 3 weeks in the 0.125 and 0.0625 g/L concentration groups in the last week. Considering both the mortality rates and the decreasing number of heartbeats, it can be concluded that a significant number of *Daphnia magna* exposed to Amprolium initially exhibit physiological resistance, but this resistance diminishes over time. This finding is also significant as it highlights the emergence of the resistance threshold.

Amprolium also had a negative impact on fertility in *Daphnia magna*, with a decrease in the number of eggs produced corresponding to the higher concentrations used. It is important to note that, while the presence of residual Amprolium in the water reduces heart rates and egg production in Daphnia over time, it also leads to an increase in mortality.

To mitigate potential risks to the aquatic environment, proper regulations are needed concerning the sale, use, and disposal of medications. Additionally, sanctions should be enforced against those who fail to comply with these regulations.

Since anticoccidials are commonly used in animal husbandry, their residues often end up in animal waste. These drugs are typically absorbed slowly and excreted through the feces. When animal waste is used as fertilizer, anticoccidials can leach into the environment, contaminating soil and water. This is particularly concerning in intensive farming systems, where large numbers of animals are confined and fed medicated feed pellets.

Pharmaceuticals and their metabolites, many of which are ionizable and whose ecotoxicological behavior varies with pH, represent an uncertain environmental risk; in marine aquaculture, their release via fish waste can contaminate coastal waters [20].

Building on the points discussed above, the following section highlights the key implications of our findings and their relevance to environmental risk assessment.

Daphnia studies are crucial for assessing the toxicity of aquatic substances, offering valuable insights into their ecological impacts. As sensitive water quality indicators, Daphnia help monitor environmental changes and evaluate ecosystem risks. The high responsiveness of *D. magna* to various stressors, including chemical pollutants, heavy metals, and pesticides, allows for detecting harmful effects even at low concentrations, making it a key model for evaluating environmental contaminants.

Utilizing both acute and chronic toxicity assays provides a comprehensive overview of a substance's toxicological profile. Acute tests detect immediate hazards, whereas chronic tests uncover subtle, long-term effects. Regulatory agencies often mandate data from both types of assessments for substance approval and classification, highlighting their critical role in environmental risk evaluation.

This study demonstrated that Amprolium had a significant effect on the survival and reproduction of *Daphnia magna*, highlighting its sensitivity as an indicator of micropollutant toxicity. Acute toxicity tests showed a clear increase in mortality over time, with statistically significant differences in repeated measurements and a notable interaction between concentration and time.

While pharmaceuticals are vital for treating infections, their responsible use is essential to minimize environmental damage from yearly pollutant releases into surface waters. Thus, standard laboratory ecotoxicity tests, as demonstrated by this study's findings, could effectively assess ecosystem quality and rank hazards in water bodies affected by urban runoff and wastewater.

In conclusion, the long-term effects of pollutants on aquatic organisms, including reproductive issues, physiological changes, and sensitive species extinction, can disrupt ecosystems and lead to broader community-level consequences. Therefore, we recommend that large-scale treatment plants integrate toxicity assessment with physicochemical parameter monitoring. This dual approach would refine animal waste management, reducing its potential impact on aquatic life before wastewater enters water systems.

Declarations

Availability of Data and Materials: Data will be offered by the corresponding author (MY) on demand.

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Conflicts of Interest: The authors have no conflicts of interest to declare.

Declaration of Generative Artificial Intelligence (AI): No AI tool is used in this research work write-up.

Author Contributions: MY and ÇY designed the study. MY, ÇY and CP helped in the methodology and work plan. MY and ÇY carried out the experiments. MY performed the statistical analysis. MY, ÇY and CP revised the paper.

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