Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2021.27038

221 (2021) 359–366
May

Toxicity tests using flurbiprofen, naproxen, propranolol, and carbamazepine on *Lepidium sativum*, *Daphnia magna,* and *Aliivibrio fischeri*

Süheyla Tongur, Sevil Yıldız*

Department of Environmental Engineering, Konya Technical University, Konya, Turkey, emails: sevilyildiz0612@gmail.com (S. Yıldız), stongur@ktun.edu.tr (S. Tongur)

Received 27 April 2020; Accepted 16 January 2021

ABSTRACT

The residues of active pharmaceutical ingredients present risk to the environment and can cause health problems. In this study, the toxicities of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine, all of which belong to the most frequently used analgesic, beta-blocker, and anti-epileptic drugs, were investigated. Toxicity tests were conducted using a watercress (*Lepidium sativum*), a freshwater invertebrate (*Daphnia magna*), and a luminescent bacterium (*Aliivibrio fischeri*). The various biological test methods used in this study were compared in terms of the sensitivity of these species to the drugs tested. When the three toxicity-test results were analyzed, different sensitivities were determined in the synthetic pharmaceutical wastewater samples that had different characteristics. In general, *D. magna* was the most sensitive among the test organisms, *A. fischeri* was the second most sensitive, and *L. sativum* was the least sensitive. The results showed that all four micropollutants were capable of causing toxicity in luminescent bacteria, a freshwater invertebrate and in watercress with a 50% effective concentration of ~1.9 to 50 mg/L. In particular, "flurbiprofen" had a more toxic effect than the substances in all the toxicity tests.

Keywords: Acute toxicity; Ecotoxicology; Pharmaceuticals; Toxicity tests; Toxic unit

1. Introduction

In the late 1990s, pharmaceuticals and personal care products (PPCPs), which are widely used in daily life and include several chemical classes, were identified as presenting a potential risk to wildlife [1]. Pharmaceuticals are used primarily to prevent or treat human and animal diseases; whereas, personal care products are used to improve the quality of daily life and include products such as moisturizers, lipsticks, shampoos, hair colors, deodorants, and toothpastes. The chemical and pharmaceutical industries make valuable contributions to our health and high standards of living; however, PPCP use is often associated with environmental pollution [2]. The concern about PPCPs contaminating the environment was part of the agenda of governments after the studies were published that showed the endocrine-disrupting effects on fish from exposure to estrogenic substances [3,4]. These studies were important factors that helped to trigger concerns on the environmental risks of PPCPs, which include antimicrobials, anti-inflammatories, contraceptives, antidepressants, and anti-epileptic drugs [5]. The growing consumption and production of PPCPs have resulted in their being more frequent components of wastewater and the environment [6]. The main route by which PPCPs are introduced into the environment is the disposal of treated and untreated domestic or hospital wastewater. The second environmental route is by leaching, which is connected to growth-enhancer drugs administered to animals for breeding [7,8]. The preoccupation has involved especially, although not exclusively, the aquatic biota, because waterbodies are the final destination of many of these substances [9].

* Corresponding author.

The concentration of PPCPs in aqueous environments usually ranges from levels of ng/L to μg/L [10]. These products are designed to exert specific positive physiological effects on humans and livestock; however, they can adversely affect aquatic organisms. Active pharmaceutical ingredients (APIs) are detected throughout the environment in water, soil, sediment, and sludge as well as in the drinking water in some countries [11]. The main concern with these microcontaminants is related to their persistence in the environment, from a combination of inherent characteristics, which include toxicity in human and animal health. In addition, many residual pharmaceuticals are resistant to conventional water and wastewater treatments, which means that they are only partially removed [12,13]. Many pharmaceuticals are designed to be persistent and lipophilic to enable them to retain their chemical structure in the intended organisms long enough to be therapeutic; consequently, after they are excreted in urine and feces, these chemicals can persist in the environment and enter the food chain through bioaccumulation and biomagnification [14]. Ubiquitous occurrence of pharmaceuticals such as nonsteroidal anti-inflammatory drugs (NSAIDs), lipid regulators, beta-blockers, and psychiatric drugs has been reported in rivers, lakes, and reservoirs throughout the world [15,16]. Flurbiprofen is a member of the phenyl alkanoic acid derivative family of NSAIDs. This drug is indicated primarily as a preoperative antibiotic in both an ophthalmic solution and orally for arthritis or dental pain. Its effects are analogous to those of ibuprofen. The propionic acid derivative naproxen Na (sodium salt) is an NSAID used to treat pain, inflammatory diseases such as rheumatoid arthritis, and fever. Propranolol is a nonselective beta-blocker widely prescribed to treat high blood pressure and a number of heart dysrhythmias. Carbamazepine is one of the most widely used anticonvulsants given to treat tonic–clonic seizures. It selectively depresses responses from the central nervous system, without causing harm or respiratory failure [17]. Carbamazepine is fairly stable in water; therefore, it is found at concentrations ranging from 1 to 3,000 ng/L in rivers receiving wastewater treatment effluents [18]. Propranolol is also quite stable in water and can be found at 10–60 ng/L in surface water [19]. Ibuprofen is commonly found in environmental matrices, such as surface waters and sediments, in concentration units ranging from pg/L to μg/L and pg/g to μg/g, respectively [20,21].

Pharmaceutical compounds can bioaccumulate and can then affect aquatic organisms by altering their physiological and reproductive functions [22]. The main focus of ecotoxicological research lies on the identification of substances that pose a high risk to the environment; however, testing facilities are limited and cannot assess the tremendous number of known micropollutants and also try to determine those of the future. Ecotoxicological tests can be a useful tool to determine a method by which toxic compounds can be removed during wastewater treatment [23]. Bioassays are sensitive, quick, and reliable; however, advantages, rapid tests could result in a weaker answer from the tested organisms. Their use is preferred for the substances that are soluble in water and that reacts quickly and provides a quick response. The toxic effects are measured in specific organisms in terms of, for example, immobility or mortality (*Daphnia magna*), decreased luminescence (*Aliivibrio fischeri*), growth inhibition (*Lepidium sativum*), or reproduction inhibition, which depends on the time of exposure and the concentration or dilution of the substance tested. *L. sativum* is an economically viable plant used in toxicity tests. The great popularity of Daphtoxkit F™ (using *D. magna* as the tested organism) and Microtox® (using *A. fischeri*) is connected to their unquestionable advantages [24]. Tests on these three species are useful because they represent different trophic levels. Toxicity testing has steadily increased in recent years, a useful bioassay in environmental risk assessment.

The aims of the present study were (1) to evaluate the toxic effect of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine on specific organisms and (2) to compare the results in terms of the sensitivities to these substances of three widely used test organisms such as *L. sativum*, *D. magna*, and *A. fischeri*.

2. Materials and methods

2.1. Reagents

Flurbiprofen (CAS No. 5104-49-4), naproxen Na (CAS No. 22204-53-1), propranolol HCl (CAS No. 525-66-6), and carbamazepine (CAS No. 298-46-4) (Fig. 1) were prepared using the drugs in tablet form. Separate solutions were prepared for each drug active ingredient in equal concentrations. That is, separate solutions have been prepared in equal concentrations by calculating for each tablet containing flurbiprofen (one pill includes 100 mg flurbiprofen), naproxen Na (one pill includes 550 mg naproxen Na), propranolol HCl (one pill includes 40 mg propranolol HCl), and carbamazepine (one pill includes 200 mg carbamazepine)

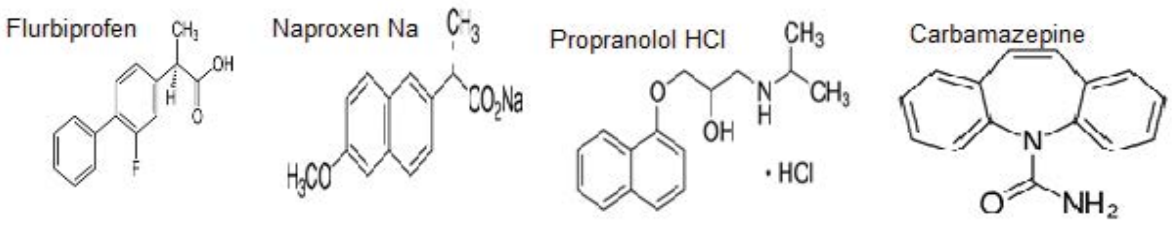

Fig. 1. Chemical structures of compounds used in this study.

APIs. Other excipients in the active pharmaceutical ingredients are given in Table 1. The synthetic pharmaceutical solutions of 1,000 mg/L were prepared in deionized water and stored at 4°C–8°C. Ultrapure Millipore water was used when necessary. Those with low solubility in water were prepared with hydroalcoholic (<1% ethanol) solution. Since ethanol concentration is not more than 1%, it is stated that it is not toxic in international procedures for test organisms (ISO 11348/1-2-3.2007). The pH was measured using the Jenway 3010 pH-meter; electrical conductivity (EC) was read using a Hach portable conductivity and TDS meter (Loveland, CO, USA). Dissolved oxygen was measured using a portable dissolved oxygen meter (Hach). The physicochemical properties of APIs are provided in Table 2. The compounds were typed as the representatives of different classes of drugs such as anti-inflammatory, analgesic, beta-blocker, and anti-epileptic.

2.2. Toxicity tests

The selected test protocols are provided in Table 3. *L. sativum*, a terrestrial plant, was chosen to represent the

Table 1
A list of excipients of each formulation

Active pharmaceutical ingredients	Contents of pharmaceuticals
Flurbiprofen	Lactose, microcrystalline cellulose, croscarmellose sodium, hydroxypropyl cellulose, colloidal silicon dioxide, magnesium stearate, hydroxypropyl methyl cellulose, titanium dioxide (E171)
Naproxen Na	Avicel PH-102, povidone, talc, magnesium stearate, distilled water, hydroxypropyl methyl cellulose E-15, Carbowax 4000, Opaspray K-1-4210 A (SDA 3-A ethyl alcohol, FD&C blue indigo carmine aluminum lacquer, titanium dioxide, hydroxypropyl cellulose), methylene chloride, methyl alcohol, carnauba wax
Propranolol HCl	Mannitol, alginic acid, gelatin, magnesium stearate, stearic acid
Carbamazepine	Aerosil 200 (silica aerogel, colloidal anhydride), Aquacoat ECD, solid (solid residue of aqueous polymeric dispersion of ethyl cellulose), Avicel PH-102 (cellulose), Eudragit ED solid (copolymer based polyacrylic/methacrylic esters), magnesium stearate, sodium CMC XL, talc, cellulose-HP-M 603 (hydroxypropyl methylcellulose), Kremopor RH 40 (glyceryl polyoxyethylene glycol stearate), red iron oxide, yellow iron oxide, titanium dioxide

Table 2
Physicochemical properties of active pharmaceutical ingredients

Active pharmaceutical ingredients	pH	Temperature (°C)	Electrical conductivity (μS/cm)	Dissolved oxygen (mg/L)
Flurbiprofen	7.7	24	510	8.6
Naproxen Na	7.6	24	510	8.4
Propranolol HCl	7.5	24	516	9.0
Carbamazepine	7.7	24	514	8.8

Table 3
Properties of selected test protocols

Test	Trophic level	Type of organisms/plants	Type of test	Test duration	Test criterion	Test principles
			Aquatic			
Microtox® (*Aliivibrio fischeri*)	Decomposer	Bacteria	Acute	5 and 15 min	Inhibition of luminescence	Luminescence
Daphtoxkit F™ (*Daphnia magna*)	Primary consumer	Crustaceans	Acute	24 and 48 h	Immobility/mortality	Count of dead/live crustacean
			Terrestrial			
Plant test (*Lepidium sativum*)	Producer	Garden cress	Acute	72 h	Root length	Root length

trophic level of producers; *A. fischeri*, a luminescent bacterium, was chosen to represent the decomposers; and *D. magna*, a crustacean, was chosen to represent the primary consumers. According to these methods, the 50% effective concentration (EC_{50}) was calculated for the different test samples. The four samples were evaluated as toxic units (TUs). Ready-made Persoone toxicity test kits were supplied from Belgium and were used in this study [25].

2.2.1. Lepidium sativum toxicity test

The test using *L. sativum* was conducted according to Devare and Bahadir [26,27]. *L. sativum* toxicity test was conducted using 3 controls and 10 different concentrations of synthetic pharmaceutical solution. Petri dishes were placed onto two pieces of filter paper and 5 mL deionized water was added in the control petri dishes. Each of the 25 *L. sativum* seeds were distributed equally to each petri dish and the lids were closed.

Prepared pharmaceutical sample were placed into the petri dishes of diluted concentrations. Three petri dishes were used for each of the different concentrations. In the same way, experimental procedure was repeated. The covered petri dishes were left in a dark room and incubated at 25°C for 72 h. At the end of the test period for each petri dish with seeds of *L. sativum* the best growth was 20 roots, and the hypocotyl and root lengths were measured. At the end of test period, the mean root length was measured in the control petri dishes, and the percentage of height inhibition was compared with the values and EC_{50} [28].

2.2.2. Daphnia magna toxicity test

The *D. magna* toxicity test was conducted according to the standard test procedure (OECD, 2004; test #202) [29,30]. The solution of the synthetic samples prepared for the experiment was placed in the test containers such that the concentration volumes were gradually increased. For each sample at different concentrations, five *D. magna* (Cladocera, Crustacea) were placed in the cells in the test plate. In the bioassays, a control group was formed and five *D. magna* were placed in the control group. At the end of the 24 and 48 h incubation periods, the immobilized and dead *D. magna* in each experimental vessel were counted. Accordingly, the EC_{50} values were calculated by the graphical interpolation using % inhibition rate.

2.2.3. Aliivibrio fischeri toxicity test

A. fischeri luminescent bacteria were stored in a freezer until use and then kept in a water bath for 2 min before the experiment to reach room temperature. A reactivation solution at 15°C was poured onto the bacteria and remained for 15 min to first activate the bacteria. Drug samples at different concentrations were prepared in a series of dilutions directly in the test vials of the initial concentration with the aid of a diluent for each of the samples. The prepared bacterial solution was transferred into a series of cuvettes and the drug solutions used in the toxicity tests were transferred to another series of cuvettes. The light emission intensity (I_0) was measured before each bacterial solution and transferred into the solution containing the drug according to the test procedures (EN ISO 11348-2 (1998)). *A. fischeri* luminescence was measured at 15°C and 490 nm. Any decreasing light emissions from this species of bacteria in the presence of toxic substances indicated a toxic effect. The results are expressed as EC_{50} disappears at 5 (I_5) and 15 (I_{15}) min [31,32].

2.3. Data analysis

An EC_{50} value is affected over a given period of time. Based on the EC_{50} values obtained from the toxicity tests, TU values were calculated according to the following formula:

$$TU = \left(\frac{1}{EC_{50}}\right) \times 100 \quad (1)$$

According to the acute toxicity classification system reported by Persoone et al. [25], toxicity was determined as follows: class I (TU = 0), "no acute toxicity"; class II (0 < TU < 1), "slightly toxic"; class III (1 < TU < 10), "toxic"; and class IV (11 < TU < 100), "very toxic".

3. Results and discussion

3.1. Results of the toxicity tests

3.1.1. Lepidium sativum toxicity test results

L. sativum toxicity test results were determined at the end of 72 h for flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine. TU values were determined according to the calculated EC_{50}. EC_{50} calculated for the toxicity test results are provided in Table 4 and TUs are shown in Fig. 2.

In the samples of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine; % inhibition of the root lengths was higher than that of the hypocotyl lengths; therefore, hypocotyl growth was greater than root growth for each sample. These results indicated that APIs inhibited root growth more than hypocotyl growth. The TUs of flurbiprofen, naproxen

Table 4
Concentration at which 50% of the test population of *Lepidium sativum* (mg/L) is affected (EC_{50})

Plant characteristic	Active pharmaceutical ingredient			
	Flurbiprofen	Naproxen Na	Propranolol HCl	Carbamazepine
Root (mg/L)	72.41	183.40	132.0	94.39
Hypocotyl (mg/L)	61.24	246.87	247.0	190.17

Na, propranolol HCl, and carbamazepine for roots were as follows: $TU_{flurbiprofen}$: 1.38; $TU_{carbamazepine}$: 1.05; $TU_{propranolol\ HCl}$: 0.75; and $TU_{naproxen\ Na}$: 0.54, respectively. TUs for the hypocotyls were $TU_{flurbiprofen}$: 1.63; $TU_{carbamazepine}$: 0.52; $TU_{propranolol\ HCl}$: 0.40; and $TU_{naproxen\ Na}$: 0.40, respectively. When classified according to TU values, "flurbiprofen" was "toxic" for both the root and the hypocotyl; "carbamazepine" was "toxic" for the root and "slightly toxic" for the hypocotyl; and "propranolol HCl" and "naproxen Na" were "slightly toxic" for both the root and the hypocotyl values.

3.1.2. *Daphnia magna* toxicity test results

D. magna toxicity test results were determined at the end of the 24 and 48 h test periods for flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine. The EC_{50} calculated from the toxicity test results are provided in Table 5 and the TUs are shown in Fig. 3.

For all samples of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine, the TU values obtained after 48 h were higher than those obtained after 24 h. This indicated that, although the concentrations of APIs remained constant, TU values increased with an increase in contact time with the living organisms, which also leads to toxicity. The TUs of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine after 24 h were as follows: $TU_{flurbiprofen}$: 9.3; $TU_{naproxen\ Na}$: 7.9; $TU_{propranolol\ HCl}$: 6.7; and $TU_{carbamazepine}$: 3.6, respectively; those for 48 h were $TU_{flurbiprofen}$: 16.9; $TU_{naproxen\ Na}$: 14.3; $TU_{propranolol\ HCl}$: 14.1; and $TU_{carbamazepine}$: 10.5, respectively. According to these values, for the 24-h test results, TU classifications were "toxic" for all APIs; and those for the 48-h test results were "very toxic". The acute toxicity of "naproxen Na" and "propranolol HCl" appeared similar. "Flurbiprofen" was found to be more toxic to *D. magna* than other APIs.

3.1.3. *Aliivibrio fischeri* toxicity test results

A. fischeri toxicity test results were determined at the end of 5 and 15 min test period for flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine. The EC_{50} values calculated from the test results are provided in Table 6 and the TUs are shown in Fig. 4.

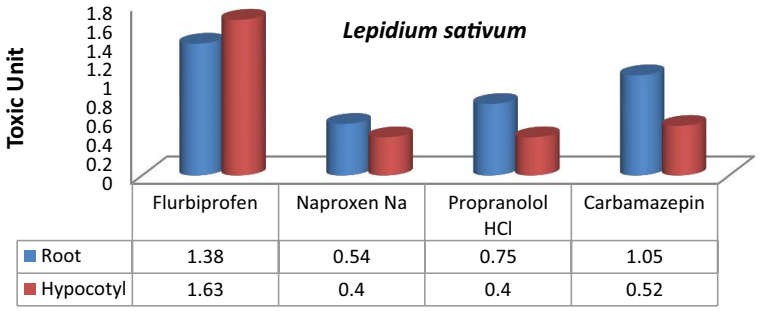

Fig. 2. *Lepidium sativum* toxicity test in toxic units.

Table 5
Concentration at which 50% of the test population is affected (mg/L) for *Daphnia magna* toxicity test

Incubation period	Active pharmaceutical ingredient			
	Flurbiprofen	Naproxen Na	Propranolol HCl	Carbamazepine
24 h (mg/L)	10.74	12.63	14.74	27.21
48 h (mg/L)	5.89	6.94	7.08	9.53

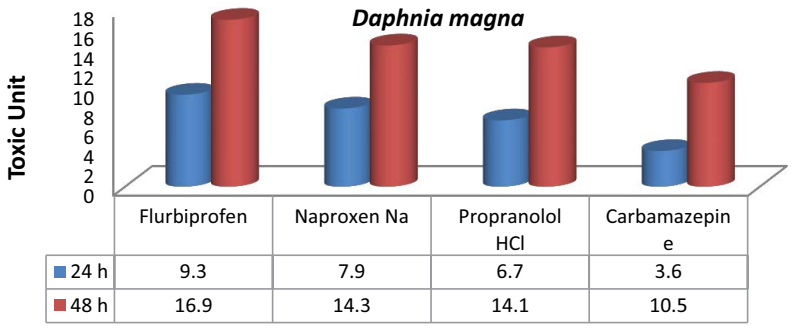

Fig. 3. *Daphnia magna* toxicity test in toxic units.

Table 6
Concentration at which 50% of the test population of *Aliivibrio fischeri* is affected

Incubation period	Active pharmaceutical ingredient			
	Flurbiprofen	Naproxen Na	Propranolol HCl	Carbamazepine
5 min (mg/L)	3.97	9.61	51.7	62.5
15 min (mg/L)	1.90	8.13	33.9	36.1

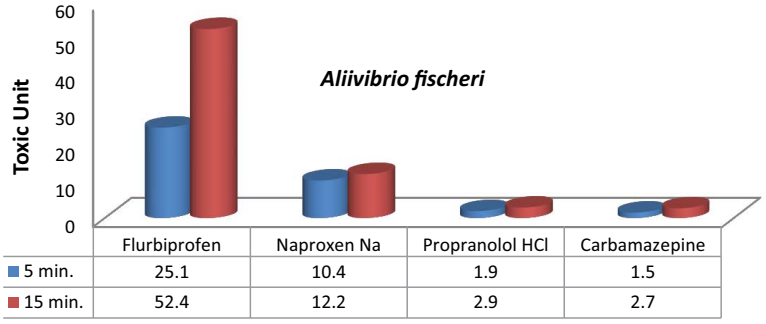

Fig. 4. *Aliivibrio fischeri* toxicity test in toxic units.

The % effect values obtained at the end of 15 min for all samples were higher or closer to those obtained at the end of 5 min; therefore, although the concentrations of APIs remained constant, the increased time in contact with the microorganism also led to increased toxicity. The TUs of flurbiprofen, naproxen Na, propranolol HCl, and carbamazepine at 5 min were as follows: $TU_{flurbiprofen}$: 25.1; $TU_{naproxen\ Na}$: 10.4; $TU_{propranolol\ HCl}$: 1.9; and $TU_{carbamazepine}$: 1.5, respectively; the TUs at 15 min were $TU_{flurbiprofen}$: 52.4; $TU_{naproxen\ Na}$: 12.2; $TU_{propranolol\ HCl}$: 2.9; and $TU_{carbamazepine}$: 2.7, respectively. According to these values, at 5 min, flurbiprofen and naproxen Na were classified as "very toxic"; propranolol HCl and carbamazepine were classified as "toxic". Similarly, according to the results at 15 min, API classifications were the same as those for 5 min. Flurbiprofen was found to be more toxic to *A. fischeri* than the other APIs.

3.2. Evaluation of toxicity test results

When the toxicity test results in the study were evaluated, we found that the three organisms showed different sensitivities to the drugs having different properties. In this study, the physicochemical properties of APIs were measured as pH, temperature, EC, and dissolved oxygen (Table 1). The physicochemical parameters did not influence growth, immobility, mortality, and luminescence during the toxicity tests [33]. Although the TUs from the *D. magna* and *A. fischeri* toxicity tests were different, the API order according to TUs for both the *D. magna* and the *A. fischeri* toxicity tests was the same and ranked as $TU_{flurbiprofen} > TU_{naproxen\ Na} > TU_{propranolol\ HCl} > TU_{carbamazepine}$. For the 72-h test on *L. sativum*, the API order unit ranking was the same for both the root and the hypocotyl parameters, and the TU ranking was $TU_{flurbiprofen} >> TU_{carbamazepine} > TU_{propranolol\ HCl} > TU_{naproxen\ Na}$. Considering TU rankings for all toxicity tests for *L. sativum*, *D. magna*, and *A. fischeri*, we observed that API with the highest toxicity was "flurbiprofen". When the results of the *L. sativum* toxicity test was evaluated according to the TU results; we observed more root inhibition than hypocotyl inhibition (except for that in the results of the flurbiprofen test). This phenomenon can manifest from the toxic substance's suppression of growth hormones and the inhibitory effects in the seed roots that affect the growth of the plant [33].

Considering the EC_{50} values, *A. fischeri* was determined to be more sensitive to "flurbiprofen" in the toxicity test than *D. magna*. In the literature, API both "ibuprofen" and "flurbiprofen" is a chemical structure-like propionic acid derivative and belongs to the therapeutic drug group. This API inhibits the growth of fungi and Gram-negative bacteria, such as *A. fischeri*, because it has a high antimicrobial activity potential [34]. Accordingly, the high potential for "flurbiprofen" to cause bacterial inhibition is the reason for more sensitive results for *A. fischeri* than for *D. magna* in the toxicity tests.

When the sensitivity to toxicity was compared, it was determined from the study that the most sensitive tests that can be used in determining their toxicity are the *D. magna* toxicity test, followed by the *A. fischeri* toxicity test, and then finally the cost-effective *L. sativum* toxicity test. The *D. magna* toxicity test indicates that it is a useful biomarker and a sensitive indicator for assessing the ecotoxicity of drugs in the aquatic environment [35].

For all three toxicity tests, flurbiprofen was determined to have the highest toxic effect among APIs, as indicated by the TU rankings. The EC_{50} value obtained as a result of the tests of flurbiprofen on *D. magna* was 5.89 mg/L at the end of 48 h, and for *A. fischeri* was 1.90 mg/L at the end of 15 min. Studies by Santos et al. [36] and Grabarczyk et al. [37] have reported that the EC_{50} values of ibuprofen for *D. magna*, which is similar in terms of the chemical structure of flurbiprofen within the range of 1–80 mg/L. Alonso et al. [38] have found that this component has a higher risk factor value than other components. In another study, Quinn et

al. [39] have found that the EC_{50} value was 1.65 mg/L in the ibuprofen toxicity test using *Hydra attenuata*. These results are comparable to those in the literature and are significant.

In the present study, the "naproxen Na" toxicity tests on *D. magna* and *A. fischeri* ranked second in TU values. The EC_{50} values obtained as a result of the studies of naproxen Na; on *Daphnia magna* was 6.94 mg/L at the end of 48 h and for *A. fischeri* was 8.13 mg/L after 15 min. Quinn et al. [39] have found that the EC_{50} value for "naproxen" is 2.62 mg/L for *H. attenuata*. Santos et al. [36] and Grabarczyk et al. [37] in their studies on the ecotoxicity of naproxen and its derivatives on the rotifer *Brachionus calyciflorus*, water flea *Ceriodaphnia dubia*, crustacean *D. magna*, luminescent bacteria *Vibrio fischeri*, and shrimp *Thamnocephalus platyurus* EC_{50} values are within the range of 1–100 mg/L. When the results of the studies in the literature were compared with those of the present study, we found that they are meaningful and comparable, and that this active substance is toxic to the test organisms.

In the present study, the TU for "propranolol HCl" ranked third for *D. magna* and *A. fischeri*. The EC_{50} obtained as a result of the studies of propranolol HCl; on *D. magna* toxicity was 7.08 mg/L at the end of 48 h, and on *A. fischeri* was 33.9 mg/L after 15 min. Santos et al. [36] have found that the acute toxicity of propranolol on *D. magna* is an EC_{50} value of 7.7 mg/L after 48 h; however, the highest EC_{50} value obtained in the acute toxicity tests for propranolol was determined to be 438 mg/L. In the present study, the EC_{50} values for the root and the hypocotyl lengths of *L. sativum* were 132 and 247 mg/L, respectively. When the highest value (438 mg/L) detected in the literature and *L. sativum* test results were compared, they were found to be consistent with this value. In another study, Cleuvers [40] has found an EC_{50} value of 7.5 mg/L for propranolol in the toxicity test using *D. magna* (in the present study EC_{50} = 7.08 mg/L).

In the present study, the TU for "carbamazepine" tested on *D. magna* and *A. fischeri* ranked last for toxicity. The EC_{50} values obtained as a result of the studies of carbamazepine; on *D. magna* was 9.53 mg/L at the end of 48 h and for *A. fischeri* was 36.1 mg/L after 15 min. Kim et al. [41] and Tongur and Yıldız [33] using *A. fischeri* have found that the EC_{50} value of carbamazepine at the end of 15 min was 45.8 and 36.1 mg/L, respectively. In other studies, Santos et al. [36] and Tongur and Yıldız [33] have found that the EC_{50} value of carbamazepine on *D. magna* at the end of 48 h was 12.7 and 9.53 mg/L, respectively. When the results obtained from the literature and present study are compared, those for carbamazepine were similar.

As a result, we determined that APIs have acute toxic effects on the organisms tested. According to the results of the toxicity tests on *D. magna* and *A. fischeri* for these components, the EC_{50} for living organisms is within the range of 10–50 mg/L. It is possible that APIs can act as toxic trace components in the environment. Pharmaceutical residues can be transported through the food chain and seriously harm other species.

4. Conclusion

According to the conducted study test results were examined, we observed that they showed different sensitivities for the four different APIs having different characteristics. The reason that the sensitivities differed among the test organisms was that synthetic wastewater containing APIs had different chemical compositions. To be able to determine the sensitivity of the toxicity tests, when the EC_{50} values were based on the periods specified in the test procedures (i.e., 72 h for *L. sativum*, 48 h for *D. magna*, and 15 min for *A. fischeri*), the test with the lowest EC_{50} values (i.e., the highest sensitivity) for APIs, was that conducted on *D. magna*. The results showed that all four micropollutants were capable of causing toxicity in luminescent bacteria, a freshwater invertebrate and in watercress with a 50% effective concentration of ~1.9 to 50 mg/L. The observation that APIs used in the present study have a toxic effect on the water environment is an indication that the natural environment is threatened by the everyday use of these pharmaceuticals. Our study could provide for assessing the potential risks that different pharmaceuticals might bring to the ecosystem. Therefore, considering that conventional wastewater treatment methods are insufficient in the removal of microcontaminants such as pharmaceutical active ingredients, it is important to apply treatment methods including advanced treatment technologies for better removal efficiencies.

References

[1] K. Kümmerer, Treatise on Water Science, *Elsevier*, Oxford, 2011, pp. 69–88.
[2] K. Ji, K. Choi, S. Lee, S. Park, J.S. Kim, E.H. Jo, K.H. Choi, X. Zhang, J.P. Giesy, Effects of sulfathiazole, oxytetracycline and chlortetracycline on steroidogenesis in the human adenocarcinomas (H295R) cell line and freshwater fish oryziaslatipes, J. Hazard. Mater., 182 (2010) 494–502.
[3] S. Jobling, M. Nolan, C.R. Tyler, G. Brighty, J.P. Sumpter, Widespread sexual disruption in wild fish, Environ. Sci. Technol., 32 (1998) 2498–2506.
[4] J.E. Hinck, V.S. Blazer, C.J. Schmitt, D.M. Papoulias, D.E. Tillitt, Widespread occurrence of intersex in black basses (*Micropterus* spp.) from US rivers, 1995–2004, Aquat. Toxicol., 95 (2009) 60–70.
[5] USEPA-United States Environmental Protection Agency, Contaminants of Emerging Concern Including Pharmaceuticals and Personal Care Products, 2015.
[6] J. Marugan, D. Bru, C. Pablos, M. Catala, Comparative evaluation of acute toxicity by *Vibrio fischeri* and fern spore based bioassay in the follow-up of toxic chemicals degradation by photocatalysis, J. Hazard. Mater., 213 (2012) 117–122.
[7] J.B. Ellis, Pharmaceutical and personal care products (PPCPs) in urban receiving waters, Environ. Pollut., 114 (2006) 184–189.
[8] H. Sanderson, D.J. Johnson, T. Reitsma, C.R.A. Brain, J. Wilson, K.R. Solomon, Ranking and prioritization of environmental risks of pharmaceuticals in surface waters, Regul. Toxicol. Pharm., 39 (2004) 158–183.
[9] K.E. Arnold, A.R. Brown, G.T. Ankley, J.P. Sumpter, Medicating the environment: assessing risks of pharmaceuticals to wildlife and ecosystems, Philos. Trans. R. Soc. London, Ser. B, 369 (2014) 1–11.
[10] D.W. Kolpin, E.T. Furlong, M.T. Meyer, Pharmaceuticals, hormones and other organic wastewater contaminants in US Streams, 1999–2000: a national reconnaissance, Environ. Sci. Technol., 36 (2002) 1202–1211.
[11] K. Lees, M. Fitzsimons, A. Tappin, J. Snape, S. Camber, Pharmaceuticals in soils of lower income countries: physicochemical fate and risks from wastewater irrigation, Environ. Int., 94 (2016) 712–723.
[12] P.M. Palmer, L.R. Wilson, P.O. Keefe, R. Sheridan, T. King, S. Chen, Sources of pharmaceutical pollution in the New York City Watershed, Sci. Total Environ., 394 (2008) 90–102.

[13] B.F. Silva, A. Jelic, R. Lopez-Serna, A.A. Mozeto, M. Petrovic, D. Barcelo, Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain, Chemosphere, 85 (2011) 1331–1339.
[14] B. Loganathan, M. Phillips, H. Mowery, T.L. Jones-Lepp, Contamination profiles and mass balance macrolide antibiotics and illicit drugs from a small urban wastewater treatment plant, Chemosphere, 75 (2009) 70–77.
[15] J. Osorio, A. Larranaga, J. Acena, S. Perez, D. Barcelo, Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers, Sci. Total Environ., 540 (2016) 267–277.
[16] J. Roberts, A. Kumar, J. Du, C. Hepplewhite, D.J. Ellis, A.G. Christy, S.G. Beavis, Pharmaceuticals and personal care products (PPCPs) in Australia is largest inland sewage treatment plant, and it contribution to a major Australian river during high and low flow, Sci. Total Environ., 541 (2016) 1625–1637.
[17] X. Miao, C.D. Metcalfe, Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography–electrospray tandem mass spectrometry, J. Chromatogr. A, (2009) 5807–5818.
[18] I. Munoz, J.C. Lopez-Doval, M. Ricart, Bridging levels of pharmaceuticals in river water with biological community structure in the Llobreget river basin (northeast Spain), Environ. Toxicol. Chem., 28 (2009) 2706–2714.
[19] D. Bendz, N.A. Paxeus, T.R. Ginn, F.J. Loge, Occurrence and fate of pharmaceutically active compounds in the environment, a case study: Höje River in Sweden, J. Hazard. Mater., 122 (2005) 195–204.
[20] J.L. Zhao, G.G. Ying, Y.S. Liu, F. Chen, J.F. Yang, L. Wang, Occurrence and risks of triclosan and triclocarbon in the Pearl River system, South China: from source to the receiving environment, J. Hazard. Mater., 179 (2010) 215–222.
[21] M.G. Pintado-Herrera, E. Gonzalez-Mazo, P.A. Laro-Martin, Environmentally friendly analysis of emerging contaminants by pressurized hot water extraction-stir bar sorptive extraction-derivatization and gas chromatography-mass spectrometry, Anal. Bioanal. Chem., 405 (2013) 401–411.
[22] D.P. Mohapatra, S.K. Brar, R.D. Tyagi, P. Picard, R.Y. Syrampalli, Carbamazepine in municipal wastewater and wastewater sludge: ultrafast quantification by laser diode thermal desorption-atmospheric pressure chemical ionization coupled with tandem mass spectrometry, Talanta, 99 (2012) 247–255.
[23] J.C. Fisher, J.B. Belden, J.R. Bidwell, Can site-specific heuristic models predict the toxicity of produced water?, Chemosphere, 80 (2010) 542–547.
[24] D.T. Sponza, Toxicity studies in a chemical dye production industry in Turkey, J. Hazard. Mater. A, 138 (2006) 438–447.
[25] G. Persoone, M.P. Gayuaerts, C.R. Janssen, W. de Coen, M. Vangheluwe, Cost-effective Acute Hazard Monitoring of Polluted Waters and Waste Drumps with the Aid of Toxkits, Final Report, CEC Contract ACE 89/BE 2/D3, Vabrap, University of Ghent, Belgium, 1993, 600 p.
[26] M. Devare, M. Bahadir, Biological monitoring of landfill leachate using plants and luminescent bacteria, Chemosphere, 28 (1994) 261–271.
[27] M. Devare, M. Bahadir, Ecotoxicological assessment of inorganic waste disposal in salt mines. Part II: phytotoxicity tests, Fresenius Environ. Bull., 3 (1994) 119–126.
[28] M.E. Aydin, M. Kolb, S. Tongur, Evaluation of Sustainable Toxicity Tests for Industrial Wastewaters, TUBITAK (The Scientific and Technical Research Council Center of Turkey) and JULICH (International Bureau of the German Ministry or Education and Research) Project, Project no. 104136, 2008.
[29] OECD, Guidelines for Testing of Chemicals, *Daphnia* sp. Acute Immobilization Test, 2004, pp. 1–12.
[30] Standard Operational Procedure Daphtoxkit F™ Magna, Crustacean Toxicity Screening Test for Freshwater, Nazareth, Belgium, 2006.
[31] D. Gottlieb, The production and role of antibiotics in soil, J. Chromatogr. A, 1217 (1976) 4212–4222.
[32] Determination of the Inhibitory Effect of Water Samples on the Light Emissions of *Vibrio fischeri* (Luminescent Bacteria Test), Part 2: Method Using Liquid-Dried Bacteria, EN ISO 11348-2, 1998.
[33] S. Tongur, S. Yıldız, Toxicological evaluation of carbamazepine active pharmaceutical ingredient with *Lepidium sativum*, *Daphnia magna* and *Vibrio fischeri* toxicity test methods, Desal. Water Treat., 201 (2020) 438–442.
[34] M. Farre, I. Ferrer, A. Ginebreda, M. Figueras, L. Olivella, L. Tirapu, M. Vilanova, D. Barceló, Determination of drugs in surface water and wastewater samples by liquid chromatography–mass spectrometry: methods and preliminary results and including toxicity studies with *Vibrio fischeri*, J. Chromatogr. A, 938 (2001) 187–197.
[35] C. Li, Y. Wang, A. Lin, Ecotoxicological effect and of ketamine: evidence of acute, chronic and photolysis toxicity to *Daphnia magna*, Ecotoxicol. Environ. Saf., 143 (2017) 173–179.
[36] L. Santos, A.N. Araujo, A. Fachini, Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment, J. Hazard. Mater., 175 (2010) 45–95.
[37] L. Grabarczyk, E. Mulkiewicz, S. Stolte, A. Puckowski, M. Pazda, P. Stepnowski, A. Bialk-Bielinska, Ecotoxicity screening evaluation of selected pharmaceuticals and their transformation products towards various organisms, Environ. Sci. Pollut. Res., 27 (2020) 26103–26114.
[38] E. Alonso, D. Munoz, J. Martin, J.L. Santos, I. Aparicio, Occurrence, temporal evolution and risk assessment of pharmaceutically active compounds in Donana Park (Spain), J. Hazard. Mater., 183 (2010) 602–608.
[39] B. Quinn, F. Gagne, C. Blaise, An investigation into the acute and chronic toxicity of eleven pharmaceuticals (and their solvents) found in wastewater effluent on the cnidarian, *Hydra attenuata*, Sci. Total Environ., 389 (2008) 306–314.
[40] M. Cleuvers, Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects, Toxicol. Lett., 142 (2003) 185–195.
[41] Y. Kim, K. Choi, J. Jung, S. Park, P.G. Kim, J. Park, Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea, Environ. Int., 33 (2007) 370–375.