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Increased acute toxicity to fish caused by pharmaceuticals in hospital effluents in a pharmaceutical mixture and after solar irradiation

Shih-Wei Li, Angela Yu-Chen Lin *

Graduate Institute of Environmental Engineering, National Taiwan University, 71, Chou-Shan Rd., Taipei 106, Taiwan

HIGHLIGHTS

- Irradiation significantly increased the acute toxicity of the pharmaceutical mixtures to fish.
- Mixing of pharmaceuticals caused a synergistic increase in toxicity.
- The pre-irradiated pharmaceutical mixture induced strange behaviors in the fish.
- Reference threshold of mixing pharmaceuticals toxicity has been tested.

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ABSTRACT

Hospital effluents are an important source of residual drugs and other classes of pharmaceuticals in aquatic environments. The raw wastewater from the studied hospital exhibited acute toxicity to vertebrate organisms, and *Cyprinus carpio* was the most sensitive organism tested. A mixture of 19 commonly used pharmaceuticals caused acute toxicity to *C. carpio* with an LC_{50} value of 60.68 mg L^{-1} after 96 h. This study demonstrated that irradiation for 1–5 days significantly increased the acute toxicity of the pharmaceuticals to fish, leading to increased mortality after a 2-h exposure and approximately 40% of the surviving fish died within 28 days. The pre-irradiated pharmaceutical mixture also induced strange behaviors in the fish that survived the test. The synergistic increase in toxicity caused by the photolysis and mixing of pharmaceuticals cannot be ignored and warrants further examination.

1. Introduction

Pharmaceuticals are detected constantly in aquatic environments, including surface water, ground water and drinking water (Tixier et al., 2003; Kim et al., 2007; Kasprzyk-Hordern et al., 2008; Lin et al., 2008; Lin and Tsai, 2009; Watkinson et al., 2009). These compounds cannot be adequately removed by conventional wastewater treatment plants and drinking water treatment systems (Joss et al., 2005; Verlicchi et al., 2010). In this regard, hospital effluents are an important point source of residual drugs and other classes of pharmaceuticals in aquatic environments (Kümmerer and Al-Ahmad, 1997; Brown et al., 2006). Hospital wastewater is often discharged into public sewer systems, collected at wastewater treatment plants and co-treated with urban wastewater without any specific pretreatment (Altin et al., 2003; Vieno et al., 2007; Pauwels et al., 2008). Hospital effluents have been highlighted as exhibiting genotoxicity toward bacteria (Hartmann et al., 1998) and toxic effects on other aquatic organisms (Emmanuel et al., 2004). Drug-resistant bacteria have also been observed at the effluent discharge sites of wastewater treatment plants (Reinthaler et al., 2003) and in hospital effluents (Tuméo et al., 2008). In addition, pharmaceutical have been observed in liquid manure and pig fattening through the food chain (Berger et al., 1986; Grote et al., 2004). Low concentrations of pharmaceuticals alter community structures and thereby affect the food chains in aquatic environments (Hernando et al., 2006). The bioaccumulation of pharmaceuticals in fish has been also demonstrated (Subedi et al., 2012; Liu et al., 2015). These phenomena are potentially threatening to organisms in surface waters and aquatic ecosystems.

Pharmaceuticals are used for medical purposes and, when evaluated individually, are believed to have no significant toxicity to humans and other organisms. However, Neuwoehner et al. (2009) demonstrated that fluoxetine inhibits growth and photosynthesis in algae (*Pseudokirchneriella subcapitata*). Veterinary diclofenac residues caused a decline in the vulture population in Pakistan, which adversely affected the environment (Oaks et al.,

* Corresponding author.
E-mail address: yuchenlin@ntu.edu.tw (A.Y.-C. Lin).

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2004). Triebskorn et al. (2007) demonstrated that diclofenac, carbamazepine and metoprolol have chronic and cytological effects in fish (*Oncorhynchus mykiss* and *Cyprinus carpio*) at environmentally relevant concentrations (1 μg L^{-1}). Brian et al. (2005, 2007) reported that 5 estrogenic chemicals (estradiol, ethynylestradiol, nonylphenol, octylphenol and bisphenol A) did not induce a significant response in fish (fathead minnows) individually; however, these authors observed that a mixture of the estrogenic chemicals had additive effects on reproductive performance in environmentally relevant concentrations (μg L^{-1} range) with increasing levels of biological complexity.

Previous studies have attempted to model the toxicity of a pharmaceutical mixture of β-blockers and nonsteroidal anti-inflammatory drugs (NSAIDs) (Altenburger et al., 2003; Escher et al., 2006) by QSAR; however, the model approach was not appropriate for simulating realistic scenarios such as the inclusion of complex metabolic mechanisms. In general, biological assays are more effective for evaluating the overall toxicity of a pharmaceutical mixture. Certain toxicity bioassays and aquatic organisms (such as the in vivo bioassay with fish, duckweed, water flea and algae) are indicators of the presence of toxic materials in effluents and surface water and represent an important source of information for developing source-control regulations. Ecotoxicological bioassays provide direct evidence of significant toxicity or biological risks in water samples.

Compounds in hospital wastewater undergo various degradation pathways, including biotic (biodegradation, bioaccumulation) and abiotic (hydrolysis, photolysis, sorption, oxidation) degradation, when released into environmental surface waters. NSAIDs and sulfonamide antibiotics have been reported to rapidly photodegrade in aqueous media (half-life = 2.4 min–1 h under sunlight) (Owen et al., 2007; Matamoros et al., 2009). Photochemical reactions may be important in pharmaceutical degradation, particularly considering the subtropical location of Taiwan with its relatively shallow rivers. However, previous results have indicated that photolysis may result in byproduct mixtures that exhibit an even greater toxicity on *Vibrio fischeri* (Li et al., 2011; Wang and Lin, 2012) and *Daphnia magna* (Trovó et al., 2009) as well as acute and chronic toxicity on algae, rotifers and microcrustaceans (Isidori et al., 2005).

This study aimed to investigate the occurrence and acute toxicity of hospital wastewater and its receiving surface waters. The acute toxicity and reference threshold of pharmaceutical mixtures were evaluated. The toxicity of the pharmaceutical mixture due to photolysis reactions was also evaluated. In addition, we observed the behaviors of the surviving tested organisms. These results clarify our understanding of the toxicity of pharmaceutical mixtures and their potential effects on the test organisms in aquatic environments.

2. Materials and methods

2.1. Chemicals and standards

All standards were of at least 98% purity, and all chemicals were LC or ACE grade. The suppliers and purities of the compounds are provided in the Supplementary Information (Text S1).

2.2. Hospital effluents and surface-water sampling

The samples were collected from the Zhudong Veterans' Hospital (24°43′22.9″N, 121°05′59.4″E) and the Toucian River in Taiwan (the sampling time data are provided in the Supplementary Information, Table S1). The Toucian River is 63.03 km long and drains an area of 565.94 km^2. The Zhudong Veterans' Hospital, located upstream of the Toucian River, drains its effluents into an agricultural channel that flows into the Toucian River. The samples were collected from the hospital's raw wastewater (influent of the wastewater treatment plant [WWTP]) and from the WWTP effluent either with or without chlorination. In addition, samples from upstream and downstream points of the Toucian River were collected to evaluate the effects of the hospital effluents on aquatic organisms. The distance from upstream to downstream is close to 3 km. Composite samples were obtained during three intervals on September 19, 2011 (8:00–11:30, 12:30–16:00 and 18:30–22:00, which are three peak hours of water use). Each sampling procedure collected 20 l during an interval, and the samples were stored ±4 °C. A total of 60 L of sample was collected from one location with a total of 5 locations. In addition, the dissolved oxygen, pH, water temperature, turbidity, and conductivity were measured at the time of collection. Some samples were mixed to detect pollutants, pharmaceuticals and acute toxicity. Detailed sampling information is available in the Supplementary Information (see Table S7).

2.3. Chemical analysis of the pharmaceuticals

The chemical analytical quantification methods utilized in this study followed those described in our previous work (Lin et al., 2008). Briefly, target pharmaceuticals were chromatographically separated using an Agilent 1200 module (Agilent, Palo Alto, CA, USA) equipped with a Phenomenex Luna C18 column (Agilent, Palo Alto, CA, USA, 150 × 4.6 mm, 5 μm). A binary gradient with a flow rate of 1.0 mL/min was used. Mobile phase A contained 0.1% formic acid (v/v) in water. Mobile phase B contained 0.1% formic acid (v/v) in methanol. Identification of pharmaceuticals was performed with a Sciex API 4000 LC–MS/MS (Applied Biosystems, Foster City, CA, USA) equipped with a turbo ionspray source with multiple reaction monitoring (MRM) using the two highest characteristic precursor ion/product ion transition pairs. Compounds were identified using the LC retention time ±30% of the retention time of a standard. Analyses were performed in negative mode for 14 compounds (ibuprofen, naproxen, fenoprofen, ketoprofen, fenoprop, diclofenac, piroxicam, indomethacin, mepirizole, clofibric acid, gemfibrozil, benzafibrate, pravastatin and oxacillin) and positive mode for all others (16 commonly used and detected pharmaceutical categories, a total of 74 drugs). The method detection limits (MDLs) were determined as the minimum concentration of analyte in the linear range with a signal-to noise ratio of $\geqslant$3:1 in a water matrix. MDLs ranged from 0.1 to 10 ng L^{-1} with linearity >0.9911 (for supplementary Information, see Table S3). Detailed analytical and quantification methods are available in our earlier work (Lin et al., 2008).

2.4. Acute toxicity test

The acute toxicity test was performed primarily according to the United States Environmental Protection Agency's (USEPA) 2002 "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" and the standard methods NIEA B901, B902, B904 and B905 of the Taiwan Environmental Protection Agency. Two vertebrate organisms (*Pseudorasbora parva* and *C. carpio*) were used to characterize the acute toxicity. These tested organisms were purchased from the aquatic environment and ecology laboratory of National Chiayi University before the study. Laboratory culture, food and dilution water preparation can be identified in the standard methods of NIEA B901 (*D. magna*), B902 (*P. parva*), B904 (*C. carpio*) and B905 (*Neocaridina denticulate*) of the Taiwan EPA. The test organisms had mortalities below 10% at least 7 days prior to the toxicity test, and the feeding, behavioral observations and mortality were