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Acute toxicity of mixture of acetaminophen and ibuprofen to Green Neon Shrimp, *Neocaridina denticulate*

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ARTICLE INFO

Article history:
Received 18 December 2013
Received in revised form
4 April 2014
Accepted 8 April 2014
Available online 10 May 2014

Keywords:
Neocaridina denticulate
Ibuprofen
Acetaminophen
Drug residues
Median lethal concentration

ABSTRACT

In recent years, numerous studies have indicated that various long-term use drugs, such as antibiotics or analgesics, not only cannot be completely decomposed *via* sewage treatment but also exhibit biological toxicity if they enter the environment; thus, the release of these drugs into the environment can damage ecological systems. This study sought to investigate the acute toxicity of two commonly utilized analgesics, ibuprofen (IBU) and acetaminophen (APAP), to aquatic organisms after these drugs have entered the water. To address this objective, the acute toxicity (median lethal concentration, LC_{50}, for a 96-h exposure) of IBU alone, APAP alone, and mixtures containing different ratios of IBU and APAP in green neon shrimp (*Neocaridina denticulata*) were measured. The results of four tests revealed that the 96-h LC_{50} values for IBU and APAP alone were 6.07 mg/L and 6.60 mg/L, respectively. The 96-h LC_{50} for a 1:1 mixture of IBU and APAP was 6.23 mg/L, and the toxicity of this mixture did not significantly differ from the toxicity of either drug alone ($p < 0.05$). The experimental results for mixtures containing unequal ratios of IBU and APAP indicated that mixtures with high APAP concentrations and low IBU concentrations exhibited markedly greater toxicity in *N. denticulata* (LC_{50} = 4.78 mg/L) than APAP or IBU alone. However, mixtures with high IBU concentrations and low APAP concentrations exhibited lower toxicity in *N. denticulata* (LC_{50} = 6.78 mg/L) than IBU or APAP alone. This study demonstrated that different mixtures of IBU and APAP were associated with different toxic effects in green neon shrimp.

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http://dx.doi.org/10.1016/j.etap.2014.04.014

1. Introduction

Pollutants generated by human activity, such as heavy metals and pesticides, negatively impact aquatic organisms after entering bodies of water. This issue has become an important research topic in recent investigations of the effects of human activities on water ecosystems. In recent years, numerous studies have revealed that bodies of water contain not only heavy metals, pesticides, and other common pollutants but also increasing residual concentrations of human pharmaceuticals (Jorgensen, 2010; Halling-Sørensen et al., 1998). A study by the U.S. Environmental Protection Agency indicated that drugs that should not be present in water at detectable levels are currently detectable in certain bodies of water (Whitacre, 2012; Pal et al., 2010; Kolpin et al., 2002). This phenomenon has mainly occurred because no currently available sewage treatment system can remove these drugs and as a result untreated wastewater containing these drugs enters natural bodies of water (Santos et al., 2010). However, there is currently a lack of information regarding how these drugs affect aquatic organisms; therefore, pharmaceuticals and personal care products (PCPPs) are likely to produce unpredictable impacts on the aquatic ecosystems in which they enter (Santos et al., 2010; Sanderson et al., 2003).

Studies have found that streams in Taiwan contain detectable levels of effluent water from pharmaceutical manufacturers, medical institutions, and sewage treatment plants. Contaminants found included the following: waste water from animal husbandries; antibiotics; non-steroidal anti-inflammatory drugs (NSAIDs); estrogens; lipid regulators; antihypertensive drugs; hypoglycemic agents; and other common pharmaceuticals. Acetaminophen (APAP) and ibuprofen (IBU) are NSAIDs that are heavily utilized in Taiwan (Lin et al., 2009, 2010). Both APAP (14–1600 ng/L) and IBU (313–3777 ng/L) have already been detected at ng/L to μg/L levels in Taiwanese streams (Lin et al., 2008, 2009). Moreover, even higher concentrations of APAP (0.17–27 mg/l) and IBU (0.3–3.3 mg/l) have been found in the effluents of certain hospitals and sewage treatment plants (Chen, 2008). Various experiments have determined that exposure to APAP or IBU alone can affect aquatic organisms (Carlsson et al., 2006a,b). For example, in fish, death, liver damage, and abnormal embryonic development can result from exposure to APAP (David and Pancharatna, 2009a; Weigt et al., 2010). IBU exposure inhibits the growth of the mollusk *Planorbis carinatus*, reduces the reproductive capacity of the crustacean *Daphnia magna*, and causes abnormal behavior in *Gammarus pulex*; moreover, IBU concentrations in water of greater than 100 mg/L are known to be fatal to the fish *Oryzias latipes* (Sanderson et al., 2003; Pounds et al., 2008). In the environment, drugs and chemicals typically exist as mixtures rather than as pure substances. The ways in which various interactions among these substances affect organisms remain largely unexplored. For example, IBU exposure causes higher mortality among *D. magna* in the presence of other NSAIDs than IBU alone (Cleuvers, 2003). The NSAIDs APAP and IBU are both frequently detected in the rivers and streams of many regions. The toxic effects of these two substances after entering an organism merit additional investigation.

Damage to or contamination of the environment will impact the physiology and reproduction of various aquatic organisms, including primary producers, primary consumers, secondary consumers, and decomposers (Schweer, 2002; Huang, 2010). In various bodies of water, there exist sufficient quantities of certain arthropods, such as green neon shrimp (*Neocaridina denticulata*) and freshwater shrimp, to affect water quality and the size of fish populations; thus, these arthropod populations are highly significant to their ecological environments (Huang and Chen, 2004; Huang et al., 2006). In addition, these arthropods are frequently used as animal models for toxicity tests because they are not only easy to raise and control in the laboratory but also exhibit rapid and sensitive responses to toxic substances. This study sought to investigate the acute toxicities of APAP alone and IBU alone on *N. denticulata* and to explore how the acute toxicities of these drugs change for various mixtures of APAP and IBU.

2. Materials and methods

2.1. Animal collection and maintenance

Green neon shrimp (*N. denticulata*) were taken from rivers in Tainan County, southern Taiwan for laboratory testing. They were transferred to a 50-L glass aquarium after being identified. This aquarium was equipped with a water-cycling device; the pH was maintained at 7.4–7.8; the dissolved oxygen concentration was greater than 7.3 mg/L; the hardness was 38–45 mg $CaCO_3$/L; the temperature was maintained at $25 \pm 1\,^{\circ}C$; and a 12-h light-dark photoperiod was used. Under these conditions, shrimp were fed twice a day and acclimated for 2 weeks before testing.

2.2. Chemicals preparation

Acetaminophen (APAP), ibuprofen (IBU) and sodium chloride (NaCl) were purchased from Sigma (St. Louis, MO, USA). Stock solutions of ibuprofen (100 mg/L) and acetaminophen (100 mg/L) were prepared in dimethyl sulfoxide (DMSO) and stored in refrigeration (4 °C) for use.

2.3. Establishment of lethal concentration

Acute toxicities were studied as previously described with water renewal methods based on the Standard Guide for Conducting Acute Tests with Green Neon Shrimp (EPA/ROC, 2013). Newly hatched shrimp (10–14 day old, 1.5 mm in body length) were used for the median lethal tests. Shrimp were divided into ten groups (20 shrimp/group) in 1-L glass beakers. APAP and IBU exposures were at 0.2, 0.6, 1, 1.5, 2, 4 and 8 mg/L. APAP/IBU combinations I, II, and III were 1:1, X + 0.5 mg/L, and 0.5 mg/L + Y, respectively. X is the concentration of APAP at 0.1, 1, 2, 4, and 7.5 mg/L, and Y is the concentration of IBU at 0.1, 1, 2, 4, and 7.5 mg/L. Sodium chloride (1, 2, 3, 4, and 5 g NaCl/L) was used as the reference chemical to gauge the shrimp situation in each test. This experiment was repeated four times, and each test was performed in duplicate for all treatments. During the experiment, dead individuals were removed, and shrimp mortality was recorded at the 2nd, 6th, 24th, 48th, and