The Effects of Tetracycline and Ibuprofen on Common Duckweed, *Lemna minor*

by John Von Drasek

**ABSTRACT**

Pharmaceuticals and chemicals are finding their way into drinking water by means of household and industrial sewage. This study explores the effects of two common pharmaceuticals—tetracycline and ibuprofen—on the growth of Common Duckweed, *Lemna minor*. The number of duckweed per test tube for each treatment was compared after 37 days. Tetracycline was not found to effect clonal growth of duckweed. There was significant difference in duckweed count between the ibuprofen treatment and the control treatment with ibuprofen inhibiting the growth of the duckweed. It is possible that the tetracycline prevented the protein synthesis of harmful bacteria growing with the duckweed, hence promoting duckweed growth. Ibuprofen has thoroughly been shown to restrain reproduction in aquatic mammals, but further studies are needed to investigate the reasons for its growth-inhibitory effects on aquatic plants.

**INTRODUCTION**

Chemical contaminants in drinking water have been associated with a broad array of adverse health effects, including cancer, cardiovascular disease, neurological disease, and miscarriage (Barrett 2014). The United States Environmental Protection Agency sets primary standards for limiting the levels of contaminants in drinking water. However, these “safe” levels of chemicals do not mean that no one, under any conditions, could possibly be harmed (Howd 2002). Some hormonally active chemicals, capable of affecting reproduction and inducing
carcinogenesis in wildlife as well as humans, have been found to be universal contaminants in
the last decade (Fenner-Crisp 1997). Moreover, pharmaceuticals are appearing to be a rising
problem linked to water contaminants (Pomati et al. 2004). These chemicals find their way into
waters via waste water discharge and terrestrial runoff (Halling-Sørensen et al. 1998).
Furthermore, these medicines are excreted from the treated body, either unaltered or as
metabolites, some of which are still bioactive (Sarmah et al. 2006). This would make them
potentially hazardous to bacteria and other organisms in the environment (Jørgensen and
Halling-Sørensen 2000).

Pharmaceutically active compounds are defined as substances used for prevention,
diagnosis, or treatment of a disease and for restoring, correcting or modifying organic functions
(Daughton and Ternes 1999). Among these pharmaceuticals are antibiotics and anti-
inflammatories. Tetracycline is an antibiotic that is effective against a wide range of aerobic and
anaerobic gram-positive and gram-negative bacteria. It is used to treat brucellosis, acne, gum
disease, Lyme disease, and exacerbations of chronic bronchitis (Monteiro and Boxall 2010).
Ibuprofen is a common anti-inflammatory used for relieving pain and alleviating fever. It has
been widely reported to be found in sewage treatment effluents and surface waters (Monteiro and
Boxall 2010).

The objective of the following study was to investigate the effects of tetracycline and
ibuprofen on the growth of Common Duckweed, *Lemma minor* L. (Lemnaceae). Duckweed,
which is universally found, served as an indicator species to other aquatic life, and to a lesser
degree, humans.
METHODS

Tetracycline was obtained from Mars Fishcare North America, Inc. (Chalfont, Pennsylvania). Ibuprofen was acquired in the form of Advil® from Pfizer, Inc. (Kings Mountain, North Carolina). A concentration of 5 μg/mL was made for both tetracycline and ibuprofen using 55 mL of aged water from a fish tank. The concentration reflected concentrations that could be found in drinking water (Pomati et al. 2004). The water was intended to provide nutrients to the duckweed. Sixteen test tubes were concentrated with tetracycline to serve as the tetracycline treatment. Another sixteen test tubes were concentrated with ibuprofen to serve as the ibuprofen treatment. The remaining sixteen test tubes served as the control treatment. Each test tube per treatment contained three duckweed plants. Tetracycline and ibuprofen were added to their respective treatments nine days after the duckweed was added to the test tubes. All treatments were given 14 hours of light and 10 hours of darkness for five weeks inside a windowsill on the campus of College of DuPage, Glen Ellyn, IL. The three treatments were exposed to a constant temperature of 24°C and a photoperiod of 14 hours of light and 10 hours of darkness.

The number of duckweed per test tube for each treatment was compared at the end of 37 days. Mann-Whitney rank tests were used to compare counts of the chemical treatments to the control as assumptions of parametric testing could not be met (Zar 1984).

RESULTS AND DISCUSSION

Counts of duckweed did not vary significantly between the control and tetracycline treatment, but did between the control and ibuprofen treatment (Table 1). Tetracycline is an aminoglycoside that is active against a broad spectrum of prokaryotes via its inhibition of protein
synthesis by preventing the association of aminoacyl-tRNAs specific to bacterial ribosomes (Pomati et al. 2004). The tetracycline may have terminated the protein synthesis of harmful bacteria growing with the duckweed, thereby causing that bacteria to die, ultimately allowing the duckweed to survive. Furthermore, tetracyclines have been reported to induce chlorosis in higher plants (Brain et al. 2007). This property may have caused the duckweed to produce insufficient chlorophyll, thereby decreasing photosynthetic activity in order to grow, but not to the point causing the duckweed to perish. Abscisic acid is a plant hormone involved in bud and seed dormancy, growth regulation, leaf senescence, stomatal opening, and a variety of plant stress responses (Fedoroff 2002). Tetracycline promotes the release of this stress hormone, which may have resulted in lower rates of growth and reproduction of the duckweed (Pomati et al. 2004). This would explain the little variation between the tetracycline and control treatments.

Ibuprofen appears to have a negative growth effect on L. minor. However, the reasons for the negative growth effect of ibuprofen on aquatic plants have been given little attention. Consequently, further studies are needed to verify the effects of ibuprofen on the development of aquatic plants. Nevertheless, the effects of ibuprofen on the maturation of other aquatic life have been examined. Ibuprofen is suspected of influencing sex steroid hormones through steroidalic pathways in both vertebrates and invertebrates (Han et al. 2010). Ibuprofen is known to manipulate the cyclooxygenase pathway (Flippin et al. 2008). The cyclooxygenase enzyme could influence the production of eicosanoids, which are important regulators of reproduction (Hayashi et al. 2008). In Oryzias latipes, ibuprofen affected the reproduction of the fish, including fewer offspring and delayed hatching of eggs (Han et al. 2010). Delayed hatching of eggs ultimately makes the eggs susceptible to predation (Hans et al. 2010). Furthermore, the population growth rate of Daphnia magna was significantly reduced in the presence of ibuprofen, and reproduction
was inhibited at low concentrations of ibuprofen (Heckmann et al. 2007). The aforementioned reproductive effects may be similar situations to that of the duckweed. The ibuprofen may have repressed reproductive hormones produced by the duckweed, causing the duckweed to not grow or die. Further studies are needed to validate this idea.

**LITERATURE CITED**


Han, S., K. Choi, J. Kim, K. Ji, S. Kim, B. Ahn, J. Yun, K. Choi, J.S. Khim, X. Zhang and J.P. Giesy. 2010. Endocrine disruption and consequences of chronic exposure to ibuprofen in
Japanese medaka (*Oryzias latipes*) and freshwater cladocerans *Daphnia magna* and *Moina macrocopa*. Aquatic Toxicology 98: 256-264.


Table 1. Summary (All n=16) of duckweed growth according to treatment. Also provided are Mann-Whitney statistics and probability values from comparisons of the control to the chemical treatment. Significance was determined at P<0.05.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± standard error</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.875 ± 0.256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>4.063 ± 0.266</td>
<td>119</td>
<td>0.734</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>2.500 ± 0.398</td>
<td>46</td>
<td>&lt;0.002</td>
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