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The Impact of Different Drugs on Daphnias Capacity to Survive, Growth, and Reproduce

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Abstract: ***There may be drugs in many of the world's surface waters, but not much is known about how these chemicals affect aquatic life. We used Daphnia magna, a common species of freshwater zooplankton, to test how dangerous single drugs and drug combinations are in water. During both the short-term and long-term pharmacological bioassays, we looked at the life span, appearance, size, number of eggs laid at rest, number of offspring, and percentage of males in the population. We also looked at the rates of death among newborns. The acute stage only lasted six days, but the chronic stage lasted thirty (sex ratio). The Daphnia with a single drug at concentrations ranging from 1 to 100 lg/l didn't change how the organism normally worked. When clofibric acid was given to Daphnia at a concentration of 10 lg/l, their ability to reproduce was much better than when they were given fluoxetine at a concentration of 36 lg/l. Both of these outcomes were better than expected. When fluoxetine at 36 lg/l and clofibric acid at 100 lg/l were given together, they killed a lot of people. When the same dose of fluoxetine was mixed with 10 lg/l of clofibric acid, it caused the carapaces to be misshapen and the setae to move around. The number of males to females in a Daphnia culture changed after it was given three to five antibiotics at doses between 30 and 500 lg/l.***
Keywords: Bioassay; Daphnia; Pharmaceuticals; Hormesis; Sex ratio, Drug, Abnormalities, Antibiotics

I. INTRODUCTION

As more people take medicines and technology gets better at finding them, dozens of human and animal drugs have been found in surface waters all over the world. People, animals, farming, and industrial practices all add medications to aquatic environments. The safest way to get drugs into the environment is probably through the biological route, which is when people poop them out. After going to the doctor in the United States, most people take at least one prescription with them. After being taken in, these drugs can leave the body either as the parent drug or as an active metabolite. By flushing unused or expired prescriptions, people can put drugs into the sewers on purpose. Ternes (1998) says that when sewage is cleaned, medicines are not taken out of the waste stream well enough. The result could be that fresh water sources get dirty. When antibiotics are given to cattle, there are worries that they could get into waterways through runoff or waste from aquaculture. Because making drugs is expensive and the government has strict controls, there aren't that many drugs that end up in the environment through industrial waste. More and more people are worried about where different chemicals end up and how they get there, especially so-called "emerging contaminants" like pharmaceuticals for people and animals, industrial and household wastewater, and steroidal and reproductive hormones. But there hasn't been much research done on how these compounds, especially medicines, affect aquatic animals that they aren't meant to affect.

We did toxicity tests in the lab on a single species of the freshwater zooplankton Daphnia magna to find out how harmful different medications found in the environment are. Daphnia are often used as test animals in aquatic toxicity bioassays because they are very sensitive to chemicals in their environment, have a high rate of reproduction, and play an important role in freshwater ecosystems. Studies of toxicity that only look at whether or not an organism lives may miss effects that are less obvious but still have a big effect on the ecosystem. Some of the effects are changes in how people reproduce and slower rates of growth. At concentrations that are many orders of magnitude below the lethal limit, it is common for Daphnia's normal life processes to be affected in ways that are not lethal. Changes in the way daphnia live their lives can cause changes at the level of a community or ecosystem. Daphnia are important parts of aquatic food webs because they move nutrients from plants to fish.

Bioassays on Daphnia looked at adult and newborn viability and morphology, as well as adult length, production of resting eggs (ephippium), brood size (fecundity), and the percentage of male broods. We did two different bioassays on Daphnia to test it (sex ratio). We did acute exposure bioassays on adult female Daphnia to find out what the non-lethal effects of a drug or drug combination were. These bioassays were done to simulate a sudden change in the environment, such as agricultural runoff, an accidental spill, or the release of wastewater.

We did chronic exposure bioassays with both single drugs and combinations of drugs to find out more about the effects of long-term drug use. A lot of aquatic animals don't know that they are being given a mix of medicines in microgram amounts.

Drugs like erythromycin, fluoxetine, lincomycin, sulfamethoxazole, triclosan, and trimethoprim were chosen for testing because they are widely used in medicine, treat a wide range of conditions, and have been found in waterways at concentrations ranging from a few nanograms per liter to several micrograms per liter. Also, there is evidence that fluoxetine, erythromycin, and lincomycin may help. Several acute and long-term bioassays were done with 1–100 lg/mL concentrations of a single drug or a mix of drugs. Even if test results are often higher than what is known to be in the environment, the overall concentration of all medicines (especially those that work in the same way) may be higher in any given sample than we know. Bioassays in the lab, which will be talked about in this section, are needed to figure out how medicines affect the environment as a whole. We talk about the complicated and unexpected effects of drug combinations, and we stress how important timing and length of exposure are for an important aquatic animal. All of these things are new to the literature. These results will be used as a starting point for more research on how dangerous the environment is.

II. THE COMPONENTS AND PROCEDURES

A. *Acute Exposure Bioassays*

In each test, one adult female Daphnia was put in a 50-ml glass jar with 30 ml of an artificial lake water medium called Combo. Algae and Daphnia were able to grow in this medium, which had a pH of about 8.2 and a conductivity of about 475 lS. We fed this Daphnia clone a mixture of the algae Selenastrum and Ankistrodesmus to get a concentration of 2.0 x 106 algal cells per milliliter in its solution.

At this concentration, the Daphnia clone did well and had a lot of babies. Algae and Daphnia were both grown in tanks with 16-hour light and 8-hour dark cycles at a constant temperature of 25.1 °C. Daphnia with eggs were put into groups of different ages at random. The treatments were: (a) a control solution with only Combo media and algae (b) Combo media and algae with one drug (c) Combo media and algae with two or more drugs (d). For each treatment, 40 jars were used as experimental units (see following section for details on pharmaceutical treatment make-up). During egg maturation and embryogenesis, when the organism is exposed to medicines, it is most susceptible to the effects of pollutants.

Different treatment groups were given jars of Daphnia, which were then stacked randomly in trays and put in an incubator for one molt cycle.

After molting, Daphnia were moved to a new area, and their young were thrown away. We made sure that the medicine kept getting to the offspring by moving the Daphnia into a new solution after the first molt. This gave them fresh food and gave the medicine another chance to work. On day 4, after the transfer, the offspring were allowed to leave the incubator. They were safe to do so because they had been exposed to the drug throughout their development.

On day six, the contents of each jar were moved to a petri dish with 30 mL of liquid, and the algal suspension was taken out using a pipette with a filter. We used a dissecting microscope with a low magnification to look at the adult and juvenile viability, morphology, length (from just above the compound eye to the base of the tail spine), resting egg production, fecundity, and the number of male broods (also known as the sex ratio). About 10% of the time, this clone will have a mixed brood, which means it will have both male and female babies. Depending on the genes of the clone, it will either only have female babies or only male babies. Each bioassay was done at least twice to make sure it was correct.

B. *Chronic Exposure Bioassays*

After a single female Daphnia gave birth to a brood of at least 26 female Daphnia in their first instar, researchers got them right away. Randomly, the participants were split into two groups: a control group and a group that got drug treatment. One Daphnia instar was given 30 milliliters of a certain treatment and put in a 50 milliliter glass jar. This was the part that was a test. The treatments for both short-term and long-term drug exposure were the same as those described in the bioassays section.

After being put into different treatment groups, jars with Daphnia were moved around and the environment's light and temperature were carefully tracked. Daphnia were measured every day until they had their first set of babies. After that, they were measured every three days when they had a new set of babies.

Every three days, the medium was changed so that there was always enough food and medicine. Up until the end of the experiment, which usually happened on day 30, the number of babies born and their genders were kept track of. Results like growth, fertility, and the number of males to females were looked at in the same way as in the section on rapid bioassays. Each bioassay was done at least twice to make sure it was correct.

C. Chemical Stock Preparation

The erythromycin, clofibric acid, and clofibric acid (2-(pchlorophenoxy)- 2-methylpropionic acid came from Sigma Chemical in St. Louis, Mississippi. The effectiveness of each prescription drug was tested at doses ranging from 1 to 100 lg/l. The values used to detect chemicals in the environment came from the research, and the test concentrations were chosen to match. Each drug had to be diluted a number of times before it could be used for research. The concentrations could only be guessed at, not checked. Every week, new 10 mg/l pharmaceutical stock solutions were made and put in the fridge.

The drug combinations were chosen based on how they would be used to treat the patient and how they would work. We gave D. magna a mixture of clofibric acid (10 or 100 lg/l) and fluoxetine (36 lg/l) to make it look like it was exposed to medicines from different classes at the same time in the environment. The biomedical system is a likely way for these drugs to get into the natural world because of how they are used in therapy (i.e. human excretion). In our bioassays, we used concentrations of erythromycin, lincomycin, triclosan, trimethoprim, and sulfamethoxazole that ranged from 1 to 100 lg/l. The goal of the tests was to find out whether or not drugs with the same way they work were more likely to have a combined effect than drugs with different ways they work. All of these chemicals, except for triclosan, are antibiotics that could get into the environment through medical or agricultural use. Triclosan is an antibacterial agent that people use.

III. CONCLUSIONS

Daphnia magna, an aquatic animal that wasn't supposed to be hurt by the drugs, had trouble reproducing and growing when it was exposed to many chemicals found in the environment. Daphnia are an important part of aquatic ecosystems, and even small changes that don't kill them can cause a chain reaction in other types of aquatic life. If there are less Daphnia in the ocean, it might be harder to see, which could hurt fish and other predators that eat plankton. Sublethal effects on Daphnia haven't been studied yet, and it's not clear how or how much they could spread across a freshwater environment. We think that in the future, risk assessment efforts should not only look at the lowest ambient concentration at which a drug or drug combination is fatal, but also at the lowest concentration at which it has any noticeable effect on reproduction or development. This is because these effects can happen at very low levels of background exposure. Our research shows that mixing medicines may have effects that are different from what you would expect from each treatment on its own.

For example, when clofibric acid and fluoxetine were used together on Daphnia, they caused it to die and become deformed. Neither treatment alone had any noticeable effect. Temperature, diet, and dangerous chemicals, especially those that mess with hormones, have all been the focus of a lot of research and are well-known environmental stressors. Ecological risk assessments should take into account the fact that organisms are constantly exposed to a wide range of natural or human-made stressors (such as medications, pesticides, nutrients, etc.). Based on what we found, we think that the dose and length of time Daphnia magna was exposed to the drug play a role in how toxic it is. Bioassays with Daphnia can be used to find out how a chemical or combination of chemicals affects the environment, but it is important to do both short-term (6-day) and long-term (30-day) bioassays. Because acute bioassays give the most accurate results, this is the case. The fact that Daphnia can respond to and/or get rid of certain medicines may explain why the effects of these different types of bioassays often have different results. Because Daphnia's ability to adapt to the many biologically active chemicals means a lot for the environment, more research needs to be done. We expect that future studies on the ecological effects of medications will show that different kinds of changes will happen to the development and reproduction of invertebrate populations. This is something we think will happen.

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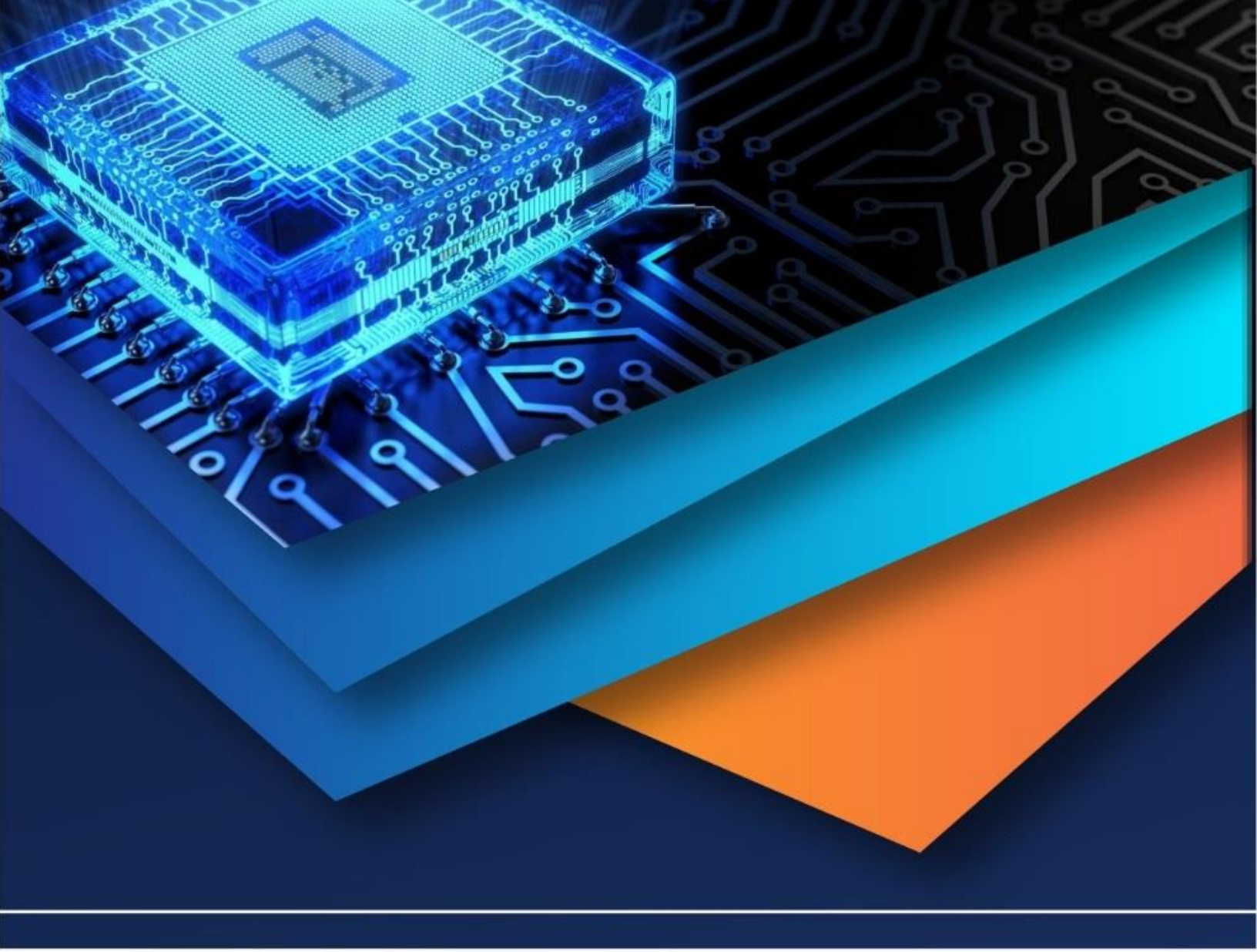

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