*engineering proceedings*

MDPI

*Proceeding Paper*

# Evaluation of Potential Ecotoxicity of Cefepime Phototransformation Products †

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† Presented at the 2nd International Electronic Conference on Processes: Process Engineering—Current State and Future Trends (ECP 2023), 17–31 May 2023; Available online: https://ecp2023.sciforum.net/.

**Abstract:** Drugs are an important problem of pollution of the aquatic environment. Global drugs consumption, their use in human and veterinary medicine and agriculture are among the main sources of environmental pollution. Among the detected drugs, antibiotics are an important group, as they have high biological activity, and most of them dissolve well in water. The presence of antibiotics in the environment can adversely affect the organisms living in it—leading to reproductive, metabolic or histopathological disorders. Drugs entering aquatic systems can remain unchanged or under the influence of various factors undergo degradation or transformation processes. One of these phenomena is the process of phototransformation as a result of which the resulting derivatives differ in physicochemical, pharmacological properties and toxicity from the parent compounds. The purpose of this preliminary study was to determine the potential ecotoxicity of the degradation products of cefepime, 4th generation cephalosporins. Toxicity was assessed using in silico methods, and then microbiotests were performed: Daphotoxikit and Thamnotoxikit. The used tests allow for a simple and quick assessment of how the tested mixtures of substances affect the survival of bioindicators. In addition, there is no need for continuous culture of test organisms. The tests do not require Ethics Committee approval and comply with the 3Rs principle (Reduction, Replacement, Refinement), which aims to reduce the use of laboratory animals. Data obtained during our preliminary studies indicate that mixtures of the parent compound and their photodegradation products are more toxic to the tested organisms than the parent compound.

**Keywords:** phototransformation; microbiotests; antibiotics

**Citation:** Żandarek, J.; Starek, M.; Dąbrowska, M. Evaluation of Potential Ecotoxicity of Cefepime Phototransformation Products. *Eng. Proc.* **2023**, *37*, x. https://doi.org/10.3390/xxxxx

Published: 17 May 2023



## 1. Introduction

High drug consumption is linked to the entry of pharmaceutical substances into the environment. More and more drugs are being detected in environmental waters. The most commonly detected groups are antibiotics, nonsteroidal anti-inflammatory drugs and hormones. Sources of drugs in the environment are primarily wastewater from both households and the public sector (hospitals, role farms, industry). After ingestion, drugs are metabolized and sequentially excreted in altered or unchanged form along with urine and feces and enter the environment with excrement. Unneeded, out-of-date drugs also often find their way into sewage systems. In addition, a major problem is the misuse of drugs in animal husbandry both to reduce the development of diseases, to cure and to increase food production.

The manure of such animals, rich in medicinal substances, often ends up in farm fields. Wastewater treatment systems are not completely effective. This is confirmed by

the fact that medicinal substances are detected in water flowing out of wastewater treatment plants, resulting in further environmental pollution [1–4]. In addition, the presence of a huge amount of medicinal substances in environmental waters can increase overall environmental toxicity due to interactions [5].

Antibiotics are used to treat diseases of bactericidal origin. They are divided into bactericidal and bacteriostatic agents. Their presence in the aquatic environment is particularly dangerous due to increasing antibiotic resistance. Every year in the European Economic Area there are about 670,000 infections caused by antibiotic-resistant bacteria and as many as 33,000 end in death [6]. In addition, an increasing number of microorganisms are multidrug resistant i.e., resistant to several antibiotics.

As a result, treatment of serious infections can be difficult and even result in failure and lead to patient death. Drugs present in the environment can adversely affect aquatic organisms. Medicinal substances accumulate in animal tissues, which can lead to reproductive disorders, histopathological changes and metabolic disorders [7]. By analyzing the toxicity of montelukast, a leukotriene receptor antagonist, toxic effects on the digestive tract of Daphnia magna were confirmed [8]. There are also reports of toxic effects on plant germination, growth and development [9]. Analyzing the above information, it becomes reasonable to study the toxicity of drug degradation products. Using bioindication, it is possible to assess the degree of environmental pollution based on the effect of a toxic substance on a living organism. Due to the different properties of drugs, the best way is to study toxicity at different trophic levels using more organisms. For example, the algae Raphidocelis subcapitata and Pseudokirchneriella subcapitata, the microorganisms Daphnia magna, Thamnocephalus platyurus, and the luminescent bacteria Vibrio fischeri, which take pollutants from the environment and store them in the body, are used. Toxkits tests provide a simple and quick way to assess the effects of analyzed substances on the survival of bioindicators. The toxicity of drugs, tattoo ink and even the effectiveness of remediation treatments can be assessed using these tests [10]. In surface waters, drugs undergo various transformations and one of the most important is phototransformation. Compounds under the influence of solar or artificial radiation are transformed into other products. This process results in the formation of derivatives with both reduced toxicity and increased toxicity relative to the parent compound. The decomposition of compounds under the influence of light is also important in terms of reducing the accumulation of drugs in the environment [11]. The process of light-dependent transformation is influenced by factors such as the presence of dissolved organic matter in the environment, microbial content, varying pH, season, and depth. Compared to ultrapure water, most often in the environment, due to the content of dissolved organic matter, faster phototransformation processes are observed [12–15]. The toxicity of phototransformation products may turn out to be reduced or increased in relation to the parent substance. The purpose of this study was to evaluate the toxicity of phototransformation products of cefepime, a fourth-generation cefelosporin antibiotic, using the Daphotoxikit F and Thamnotoxikit F microbiotests.

## 2. Materials and Methods

### *2.1. UV-VIS Experiments*

Aqueous solutions of cefepime were prepared at the following concentrations: 10, 100 and 1000 μg/mL. Successively, the solutions were transferred to quartz dishes and photodegradation test was carried out. Samples were irradiated using a lamp SUNTEST CPS+ (Atlas) simulating solar radiation for 2, 4, 6 and 8 h. The experiment used radiation of 750 W/cm$^2$ and a temperature of 37 °C. At the same time, control tests were carried out, i.e., samples wrapped in UV-VIS-impermeable foil were exposed.

*2.2. Daphotoxikit F*

Daphotoxikit F acute toxicity microbiotest was carried out according to the OECD protocol. Hatching of test organisms was carried out—ephippia from the test tube were transferred to a microsite, rinsed with tap water and successively transferred to a petri dish.

Hatching was carried out in a previously prepared standard medium containing $NaHCO_3$ (64.75 mg/L), $CaCl_2 \cdot 2H_2O$ (294.00 mg/L), $MgSO_4 \cdot 7H_2O$ (123.25 mg/L) and KCl (5.75 mg/L). Ephippia were incubated under continuous light at 20–22 °C, for 72 h. Before the test, the test organisms were fed spirulina. Multi-well plates were prepared with dilutions of the antibiotic to be tested. The analysis was carried out both on control solutions (samples wrapped in foil that did not transmit UV-VIS radiation) and on solutions exposed for different times. Each concentration was evaluated in 4 replicates. 5 Daphnia were placed on the multiwell plates in each well. Plates prepared in this way were incubated in the dark at 20 °C. After 24 and 48 h, the number of dead and immobilized organisms was counted in comparison with actively swimming ones. Subsequently, using the appropriate software, the effect of percentage mortality and EC50 value were calculated.

*2.3. Thamnotoxikit F*

Thamnotoxikit F microbiotest began with the preparation of a standard medium containing $NaHCO_3$ (96 mg/L), $CaSO_4 \cdot 2H_2O$ (60 mg/mL), $MgSO_4 \cdot 7H_2O$ (123 mg/mL) and KCL (4 mg/mL). Subsequently, the hatching of organisms from cysts was carried out. To the tube with cysts, 1mL of hatching medium was added and shaken for 30 min. After this time, the cysts were transferred to a hatching dish, 10mL of hatching medium was added and gently stirred. They were incubated under continuous light for 22 h at 25 °C. An acute toxicity test was performed. Prepared well plates with dilutions of test solutions were supplemented with test organisms. The finished plate was placed in an incubator and incubated for 24 h in the dark at 25 °C. After this time, the killed larvae were counted under a microscope. Using the software available for the test, crustacean mortality was calculated and the LC50 values were determined.

## 3. Results and Discussion

The toxicity analysis was based on the evaluation of solutions of the parent compounds as well as after-irradiation solutions containing the photoproducts as well as the residue of the parent compound. The resulting products were analyzed as a mixture without separation into individual products. They also occur naturally in the environment in this form. Similarly, the toxicity assessment of a mixture of compounds after irradiation was carried out by Cantalupi et al. [16]. According to our analysis, the toxicity of photodegradation products such as methotrexate [17], ketoprofen [18,19], sertaline [20], citalopram [21], verapamil [22] have been studied so far. Our study is the first to evaluate the ecotoxicity of cefepime. UV-VIS irradiation produced a number of photoproducts, which, when mixed, were found to be more toxic to both *Daphnia magna* and *Thamnocephalus platyurus* organisms.

## 4. Conclusions

The data obtained during our preliminary studies indicate that mixtures of the parent compound and its photodegradation products are more toxic to the test organisms than the parent compound.

This demonstrates the need for continuous analysis of drug fate in the environment.

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