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Mechanism for the primary transformation of acetaminophen in a soil/water system

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ABSTRACT

The transformation of acetaminophen (APAP) in a soil/water system was systematically investigated by a combination of kinetic studies and a quantitative analysis of the reaction intermediates. Biotransformation was the predominant pathway for the elimination of APAP, whereas hydrolysis or other chemical transformation, and adsorption processes made almost no contribution to the transformation under a dark incubation. *Bacillus aryabhattai* strain 1-Sj-5-2-5-M, *Klebsiella pneumoniae* strain S001, and *Bacillus subtilis* strain HJ5 were the main bacteria identified in the biotransformation of APAP. The soil-to-water ratio and soil preincubation were able to alter the transformation kinetic pattern. Light irradiation promoted the overall transformation kinetics through enhanced biotransformation and extra photosensitized chemical reactions. The transformation pathways were strongly dependent on the initial concentration of APAP. The main primary transformation products were APAP oligomers and *p*-aminophenol, with the initial addition of 26.5 and 530 μM APAP, respectively. APAP oligomers accounted for more than 95% of transformed APAP, indicating that almost no bound residues were generated through the transformation of APAP in the soil/water system. The potential environmental risks of APAP could increase following the transformation of APAP in the soil/water system because of the higher toxicity of the transformation intermediates.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are released into the aquatic environment through the daily discharge of domestic wastewater. Soil residues of PPCPs result from the irrigation and fertilization of agricultural land by contaminated excrement (Thiele-Bruhn, 2003; Tolls, 2001). Acetaminophen (APAP), also known as paracetamol, is widely used as a pain and fever medication and is one of the most frequently detected human pharmaceuticals in the environment (Muir et al., 1997). The reported aquatic concentrations of APAP range from 0.003 to 30 μg/L in stream water, sewage treatment plant influents, and effluents (Campanha et al., 2015; Robles-Molina et al., 2014; Scott et al., 2014; Stamatis and Konstantinou, 2013). The mean concentration of APAP in agricultural soils is reported to be approximately 0.4 μg/kg (Aznar et al., 2014).

After entering the environment, APAP can undergo adsorption/desorption, hydrolysis, chemical oxidation, phototransformation, and biotransformation. There is little adsorption of APAP to silica, alumina, aquifer sand, and sediment (Löffler et al., 2005; Lorphensri et al., 2007). The hydrolysis of APAP is pH- and temperature-dependent and is negligible at near neutral pH levels (Chen et al., 2002; Koshy and Lach, 1961). The phototransformation of APAP has been widely studied in aquatic environments. Direct UV photolysis of APAP was found to be wavelength-dependent (Martignac et al., 2013; Peuravuori, 2012; Pozdnyakov et al., 2014). The toxicity of the primary transformation intermediate (1-(2-amino-5-hydroxyphenyl) ethanone) was much higher than that of APAP (Kawabata et al., 2012). The environmental fate of APAP is also influenced by the photosensitized transformation in surface water, because APAP has a high reactivity with many inorganic radicals including •OH, N_3,• CO_3^{-}•, and triplet sensitizers (Bisby and Tabassum, 1988; De Laurentiis et al., 2014).

Although the pharmacokinetic transformation of APAP has been widely studied in pharmacology, the biotransformation of APAP has

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not been well investigated, and, in particular, the transformation intermediates are not well known. Using soil isolated bacteria as APAP-degrading microorganisms, hydroquinone was identified as the main intermediate for the biotransformation of APAP in a membrane bioreactor (De Gusseme et al., 2011). During APAP transformation in three bacterial strains isolated from aerobic granular sludge, *p*-aminophenol was found to be the predominant intermediate (Zhang et al., 2013). Biotransformation was found to be the predominant attenuation path of APAP in soil and sediment (Löffler et al., 2005; Lam et al., 2004; Lin et al., 2010; Yamamoto et al., 2009). High levels of bound residues were found during the transformation of APAP in a sediment/water system; however, the transformation intermediate was not identified (Löffler et al., 2005). Li et al. (2014a) reported that 3-hydroxyacetaminophen, hydroquinone, *N*-acetyl-*p*-benzoquinone imine (NAPQI), and *p*-acetanisidide were the primary intermediates of APAP transformation in soils. It was found that the bound residues accounted for approximately 65–80% of the transformation of APAP in a variety of soils, which resulted in a less than 20% mineralization after 120 days of reaction. However, the reason for the generation of high levels of bound residues remains unclear. It is known that the microorganism population and its activity in soil or sediment may be influenced by the presence of water, solar irradiation, and the concentration and composition of pollutants (Jacobs and Sundin, 2001; Panikov, 1999). However, the subsequent effects of micro-environmental changes on the transformation of APAP in soil/water systems have not been investigated.

In this study, the influence of the soil/water ratio (mass vs. volume, abbreviated as *m*:*v*), initial APAP concentration, and light irradiation on the transformation of APAP were systematically investigated. APAP-degrading bacterial strains were isolated and identified. The transformation intermediates were identified by liquid chromatography mass spectrometry (LCMS) and gas chromatography mass spectrometry (GCMS), with standard compounds. The primary aim of this work was to quantify the transformation products and identify the main transformation pathway of APAP in a soil/water system. The second objective was to determine to what extent and how soil residues were generated during the transformation of APAP in a soil/water system. The results obtained will provide an in-depth understanding of the environmental fate of APAP and its potential environmental risks.

2. Materials and methods

2.1. Chemicals

APAP (98%) was purchased from Alfa Aesar (Morecambe, UK). APAP dimer (99%) was purchased from Toronto Research Chemicals (Toronto, Canada). Hydroquinone (≥99%), *p*-benzoquinone (≥98%), and *p*-aminophenol (>97%) were purchased from Sinopharm Chemical Reagents (Shanghai China). *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), with 1% trimethylchlorosilane (TMCS), and horseradish peroxidase (EC 1.11.1.7 (type VI-A)) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All organic solvents (HPLC grade) and other chemicals were of the highest purity that was commercially available. APAP trimer was synthesized according to a previously reported method, with some modification of the reactant concentrations (details are provided in the Supporting Information) (Potter et al., 1985). Ultrapure water (resistivity >18.0 MΩ cm) was used during sample preparation.

2.2. Soil and soil extraction solution samples

An agricultural silt loam (10.0% sand, 64.6% silt, 25.4% clay, 1.65% organic matter, pH 7.02, and cation exchange capacity of 11.78 cmol kg^{-1}) was collected from Wuhan (China) at a depth of 10 cm. The soil was air-dried, gently crushed, sieved (2 mm), and stored at room temperature.

Sterilized soil, preincubated soil, and soil extraction solution samples were prepared for the following experiments. Soil sterilization was accomplished by autoclaving three times at 121 °C for 30 min. Preincubation soil samples were obtained by incubating air-dried soil at 25 °C and 40% water holding capacity for 2 weeks. Soil extraction solution was prepared as follows: a 0.4-g sample of air-dried soil was dispersed in 200 mL ultrapure water (*m*:*v* = 1:500) and violently stirred for 3 days. The dispersion was then filtrated through a 0.22-μm mixed cellulose ester membrane, and the filtrated solution was used as the soil extraction solution.

2.3. Batch experiments of APAP transformation under dark incubation

Batch experiments were conducted to investigate the contribution of abiotic transformation (hydrolysis, adsorption, or chemical oxidation), and biotransformation to the elimination of APAP in an air-dried or preincubated soil/water system.

Biotransformation of APAP under dark incubation: The biotransformation of APAP was conducted in a 100-mL Erlenmeyer flask with a silicon stopper. Different dosages (0.5, 0.1, and 0.05 g) of either air-dried or preincubated soils were added to a 50-mL APAP (26.5 μM) aqueous solution resulting in a suspension with a soil:-water ratio of 1:100, 1:500, and 1:1000 (*m*:*v*), respectively. Flasks were then shaken on a reciprocal shaker at 200 rpm in the dark at 25 °C. Aliquots (0.5 mL) were pipetted from the flask at different time intervals (0, 1, 1.6, 2, 2.6, 3 days for an *m*:*v* of 1:100, and 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days for an m:v of 1:500 and 1:1000) to a 2-mL centrifuge tube and immediately centrifuged at 9000 rpm for 15 min to terminate the transformation. The supernatant was then collected to determine the concentration of APAP and its metabolites.

Abiotic transformation of APAP: The hydrolysis control experiment was conducted in the absence of any soil, whereas the adsorption or soil induced chemical transformation was investigated using sterilized soil (*m*:*v* = 1:500) combined with an extra 0.1 mM $HgCl_2$ to inhibit microorganism activity. The details of these experiments were the same as those for the biotransformation experiment described above.

To determine the trace transformation intermediates and to investigate the biotransformation of APAP at a high concentration, a batch experiment was conducted with 530 μM spiked APAP in an air-dried soil/water suspension (*m*:*v* = 1:500).

Batch experiments at each soil water ratio were conducted in triplicate in three independent flasks.

2.4. Transformation of APAP under simulated solar irradiation

A 200 mL aqueous solution containing 26.5 μM APAP and 0.4 g of air-dried soil (*m*:*v* = 1:500) were artificially irradiated in a 250-mL Erlenmeyer flask. The solutions were magnetically stirred under two different irradiation conditions ($\lambda \geq 340$ and ≥ 420 nm). The irradiation reactor was equipped with a Philips Master Color lamp (CDM-T 150 W/942) as a light source. Cutoff filters at 420 nm (Shimadzu, Kyoto, Japan) were used to cut off wavelengths below 420 nm of irradiation lights. The irradiation intensity was approximately 1.9×10^4 and 1.7×10^4 μW/cm^2 under $\lambda \geq 340$ and ≥420 nm irradiation, respectively.

To investigate abiotic APAP transformation under irradiation, experiments were conducted with sterilized air-dried soil (*m*:*v* = 1:500) under $\lambda \geq 340$ nm irradiation with the addition of extra 0.1 mM $HgCl_2$ to inhibit microbial activity. A preliminary