Transformation and Fate of the Pharmaceutical Carbamazepine as a Model For Pharmaceuticals and Personal Care Products (PPCPS) in Agricultural and Aquatic Environments

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ABSTRACT

It is well documented that pharmaceuticals and personal care products (PPCPs) are incompletely removed during the treatment of wastewater. Treated wastewater and sewage sludge generated during wastewater treatment represent two sources leading to PPCPs to become persistent in both agriculture and aquatic environments. With PPCPs becoming more persistent in these environments, it is important to understand the fate and transformation of these compounds. In this review, we will focus on the pharmaceutical compound carbamazepine (CBZ) as a model for PPCPs as environmental contaminants. CBZ is commonly detected in both agriculture and aquatic environments, but there remains a lack of understanding in its fate and transformation. There is also a paucity of information around the risk factors involved with the fate and transformation of PPCPs, such as toxicity and endocrine disruption. Future research will be aimed toward understanding the toxicological potential of PPCPs and transformation products (TPs).

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Introduction

Pharmaceuticals belong to a class of active compounds widely used in human and veterinary medicine, agriculture and aquaculture purposes. Due to the purpose of these pharmaceuticals, they are designed to remain stable in the harsh conditions of human and animal bodies, making them difficult to remove during wastewater treatment[1]. The anticonvulsant medication carbamazepine and its transformation product, carbamazepine-10,11-epoxide, are relatively stable compounds that are recalcitrant in many environmental niches and thus pose a problem to wastewater treatment and environments impacted by wastewater end products[2]. The potential for adverse risk in the environment by pharmaceutically active compounds and trace organic compounds has long been known, but the extent has yet to be adequately investigated[3]. In many parts of the world, the unregulated use of antibiotics in livestock breeding and aquaculture is much greater than regulated human use, and thus contributes to the presence of antibiotics in surface-, ground- and wastewater systems. Only a few effects have been described for pharmaceutically active compounds, such as bioaccumulation, endocrine disruption, certain kinds of diseases, acquisition of antibiotic-resistance genes in bacteria and changes to microbial populations or biomagnification[4]. Largely, however, the full extent and depth of PPCPs on local flora and fauna as well as the effects that this accumulation has on the health of each ecosystem as a whole remains obscure.

» *CBZ Transformation Pathways*

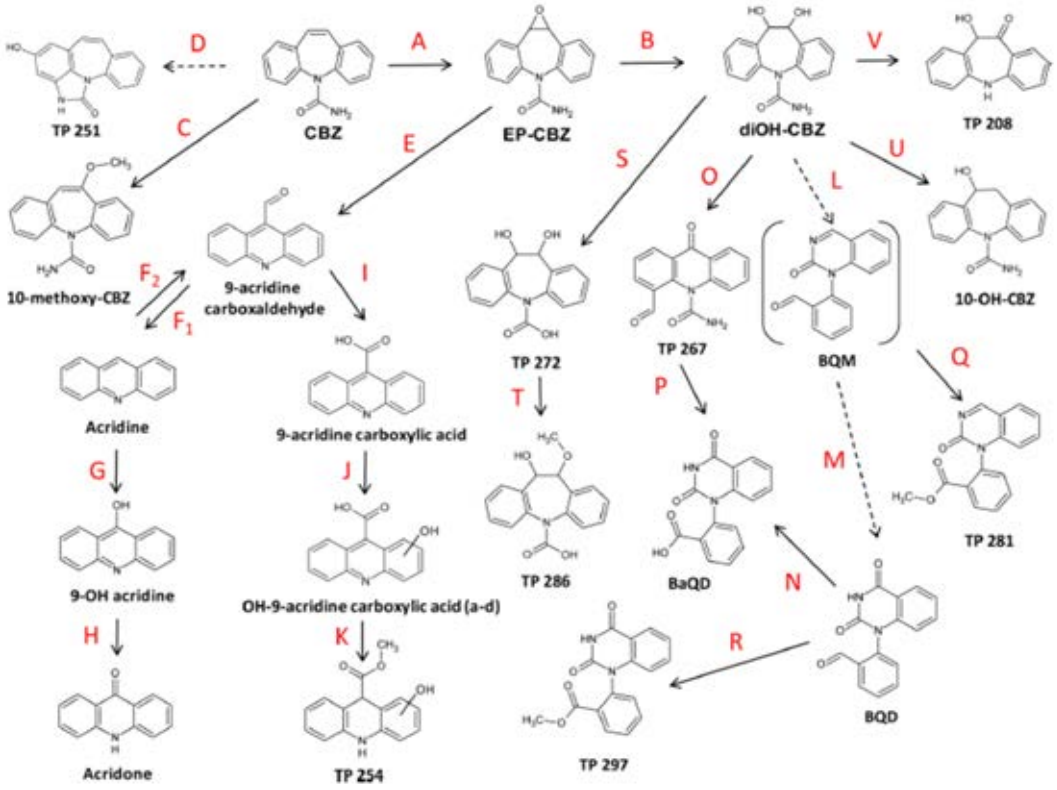

Figure 1: CBZ Transformation Pathways

Carbamazepine and its transformation product, carbamazepine-10,11–epoxide, are pharmaceutically active compounds that contribute to this growing environmental concern. As presented in Figure 1, CBZ can undergo many transformation processes, with the most common occurring in the conversion of CBZ to CBZ-10,11-epoxide (EP-CBZ) in humans and other biota. This can result in the conversion of EP-CBZ to acridine—a DNA intercalating substance—threatening multiple trophic levels of biota in both aquatic and terrestrial ecosystems[5]. The medication is regularly prescribed for the treatment of chronic conditions such as bipolar disorder, epileptic disorders, and certain depressive disorders which, in part, accounts for the large accruement of CBZ and EP-CBZ in wastewater systems across the globe[4]. The medication's molecular composition consists of a tri-heterocyclic aromatic ring structure containing a heteroatom nitrogen. The presence of aromatic structures in this substance confers on it properties related to aromaticity, electrophilic substitution reaction, and resonance stabilization. This means that the medication has reduced bioavailability in the environment resulting in steady accumulation and eventual steady-state concentrations in many niches[6].

» Input and Impact on Freshwater Organisms

PPCPs are detected in the environment at trace concentrations that range from below ng/L to μg/L and typical concentrations in treated wastewater and biosolids range from ng/L to μg/L and μg/kg to mg/kg respectively[8]. The prescription pharmaceutical carbamazepine has been measured in reclaimed wastewater and biosolids destined for land application with average concentrations of 93.6 ng/L and 66.4 μg/kg respectively[9].

CBZ is one of the most frequently detected PPCPs in aquatic environments[10]. While it has been recently shown to cause acute and chronic toxicity in a variety of non-target aquatic organisms, the full extent of the pharmaceutical's effects remain largely unilluminated. *Chen et al.*[11] discusses the acute exposure of CBZ and chronic effects of the drug on populations of *Daphnia similis.* This species of freshwater crustacean native to Lake Taihu, China is regularly subjected to CBZ concentrations ranging from .[24] to 8.74 ng/L. At concentrations higher than 6.25 μg/L, the CBZ seemed to inhibit the release of chitobiase and thus reduced the molting of D. *similis*. While this concentration is significantly greater than those reported in Lake Taihu, the study underlines how PPCPs like CBZ interact with non-target biological systems in unpredictable and demonstrably adverse ways. The study also demonstrated that chronic exposure to CBZ at concentrations of 0.03, 0.3, 3, and 30 μg/L over the course of 21 days reduced the size of broods, average amount of offspring per brood, as well as the mean number of broods per female. The detection rate of CBZ in Lake Taihu, Nanjing, and Yangtze River were all found to be 100%, with the latter containing concentrations as high as 1090 ng/L. While this study was largely focused on populations of D. *similis* in Lake Taihu, the detection rate for CBZ in other populations of organisms in Lake Taihu such as common carp (*Cyprinus carpio*), yellow catfish (*Pelteobagrus fulvidraco*), and crucian carp (*Carassius auratus*) were all found to be at or significantly near 32%8. The waterways of China serve as a model and similar CBZ accumulation and interactions with local biota can be observed in water systems around the globe12,14. In Nigeria, only an alarming 23.4% of respondents reported disposing of their unused medication in complete compliance with the national guidelines[13]. In countries like Germany, negligent disposal can result in the accumulation of PPCPs like CBZ at detection rates well over 1500 ng/L in surface water and STP effluent[14]. While a variety of exposure experiments have been conducted on many different populations of organisms, even a sequestered model like the organisms of Lake Taihu demonstrate the inadequacy of our understanding. In a variety of complex ecosystems across the globe, we understand comparatively little about how CBZ and other PPCPs affect individual organisms. The same is true of the emergent effects of CBZ on the interconnected trophic levels of each ecosystem as a whole, many of which end in the eventual accumulation of these compounds into the human ecosystem.

» *Input and Impact on Terrestrial Organisms*

Treated wastewater and sewage sludge (biosolids), two main products of wastewater treatment plants, are frequently applied to the agro-ecosystem. Incomplete removal and/or degradation of PPCPs in wastewater treatment plants is well documented and thus PPCPs are ubiquitous in treated wastewater effluents and biosolids[26]. Once in the agro-ecosystem, PPCPs may undergo several fate-determining steps such as, adsorption, desorption, transport, degradation, transformation, and uptake by plants which in turn can introduce PPCPs into the food chain and lead to potential human exposure[1]. Uptake of CBZ by root and fruit vegetables has been confirmed numerous transformation products (TPs) and carrot cell cultures along different vegetables grown under field conditions have also determined several TPs[15]. Metabolism of organic contaminants in plants is comparable to the liver and can be divided into 3 separate phases. Xenobiotics undergo processes such as oxidation, reduction, and hydrolysis during the first metabolism phase. This usually results in compounds commonly more reactive than the respective parent compound due to the introduction of functional groups. During the second metabolism phase, further conjugation of the reactive compounds occurs. This conjugation process usually increases the hydrophobicity. Increasing the hydrophobicity leads to the third metabolism phase which is detoxification and compartmentation[16].

Pathways, Types of Exposure, and Effects on Aquatic and Terrestrial Wildlife

» *Pathways and Exposure*

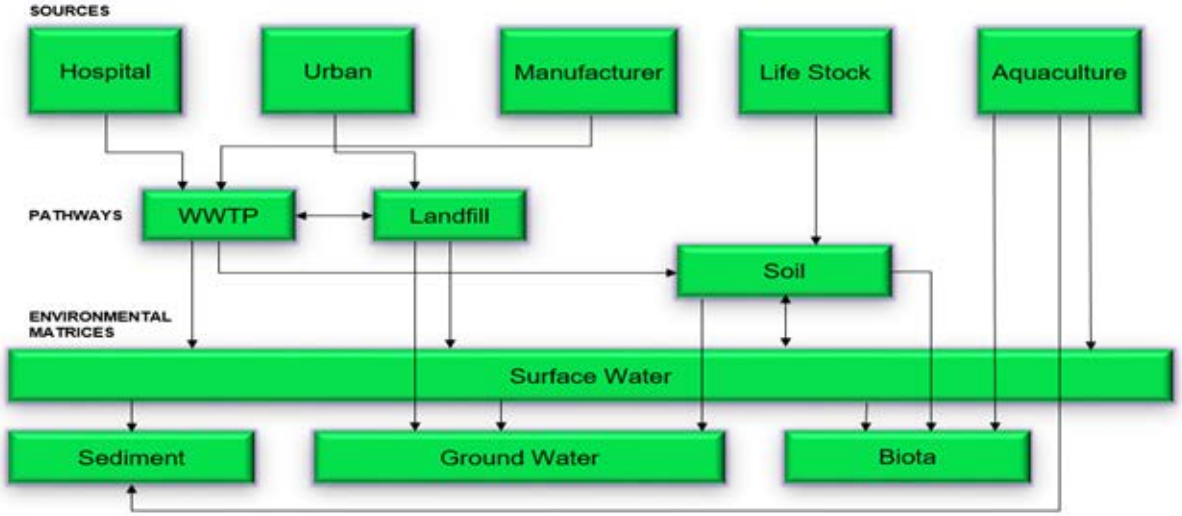

Figure 2: PPCP pathways through the human ecosystem into WWTP and the environment.

CBZ, and subsequently its TPs, find their way into wastewater systems and landfills chiefly through the municipal waste stream, and from there into the surface waters or ground waters through discharge of treated wastewater and as leachate from landfills[15-16]. The chemical properties of CBZ and many of its TPs make it difficult to remove through conventional methods of wastewater treatment. Coupled with the sheer volume of pharmaceuticals being released into water systems, gives these compounds virtually ubiquitous access to reservoirs of surface- and groundwater18. It is estimated that 50 to 150 g of CBZ is consumed per year in industrialized countries[19]. These estimations were based on prescribing data. Several studies have been conducted to characterize the method of disposal to determine how much of CBZ may be entering landfills and wastewater systems directly from individual households[15-18]. A study in the UK found that 63.2% of 400 households reported disposing of their pharmaceuticals with the rest of their household waste while only 21.8% returned their unused medication to a pharmacy and 11.5% reported disposing of them in a toilet or sink. A similar study conducted in Germany reported that of the nearly 16,000 tons of pharmaceuticals disposed of by Medicaid, 60-80% were disposed of in toilets or alongside household waste as well[14]. These statistics underline a disquieting pattern of systematic negligence among both the inadequately informed populaces and the agencies that have a responsibility to educate them.

» *Effects within Terrestrial and Aquatic Agricultural Systems*

Pharmaceuticals, which are well characterized for human toxicity, are entering the environment and many have significant enough lifetimes to potentially impact non-target organisms[18]. When non-target organisms are exposed to pharmaceuticals like CBZ there is potential for negative effects on exposed individuals. Many TPs of pharmaceuticals are also biologically active and may elicit toxic effects in non-target organisms. Organisms that have been chronically exposed to contaminants may develop increased tolerance or resistance in comparison to non-exposed individuals, but with associated costs, such as oxidative damage[19]. Ecosystems are filled with plant and animal relationships in which herbivores depend on primary producers to survive. There can be negative effects within entire ecosystems if these relationships are interrupted, either by one species not being available to the other or by xenobiotic substances entering the environment and disrupting naturally occurring phenomena[20]. This relationship extends to the health of an agricultural system as well. PPCPs like CBZ are able to accumulate, due to their recalcitrance, in wastewater which is used to irrigate farmland. From there it enters surface waters, which is a drinking water source for livestock or humans, or is indirectly used in large aquaculture environments for the raising of salmon or other fish stock[21]. A study conducted by Hampel et al. in 2013 indicated that, when exposed to concentrations of CBZ approaching 7.85 μg/L over the course of 5 days, populations of Atlantic salmon's expression of mRNA sequences shifted, with the highest changes being in the expression of pituitary hormones' encoding features like somatolactin, prolactin, and somatropin[21]. The results indicated that even relatively low concentrations of CBZ seem to have an effect on brain physiology—targeting similar processes in humans. What's more, the excited gene expression pathways were all associated with mRNA sequences that indicate a high degree of stress in Atlantic salmon. These types of large-scale and chronic exposures to populations of organisms that we depend on for sustenance and livelihood could potentially result in total collapse if

left unchecked. Further research needs be conducted in order to assess the vulnerability of agricultural systems to PPCP exposure, not only in populations of domesticated or raised fauna, but in the maintenance of crops as well.

Human Effects and Risk Assessment

» *Chemical Risk of CBZ and TPs*

Multiple studies have shown that human metabolites (HMs) and TPs can exhibit higher toxicity than the respective parent compound[20]. Carbamazepine is heavily metabolized in the human body and more than 30 metabolites, which are excreted via urine and feces, have been identified. Among these 30 are reactive metabolites, such as arene oxide[21], 9-acridine carboxaldehyde[22] CBZ 10,11-epoxide[23], an iminoquinone metabolite (CBZ-IQ), and an o-quinone metabolite (CBZ-quinone)[24], all of which contribute to the idiosyncratic toxicity associated with CBZ[25]. Indirectly, CBZ and its related TPs may pose a risk to the stability of agricultural systems worldwide. This could potentially result in the acute loss of crops or livestock as well as create degenerative trends in population sizes, representing millions of dollars in property loss and potential food shortages.

Analytical Techniques and Challenges

» *Analytical Techniques, Tools, and Challenges*

Pressurized liquid extraction (PLE) along with Ultra-High Performance Liquid Chromatography coupled with a triple quadruple mass spec (UHPLC/MS/MS) has proven to be a dependable source for the analysis of PPCPs and known TPs. While certainly not the only technique, the utilization of HPLC and mass spectrometry represents just one of the tools being utilized for monitoring PPCPs like CBZ and its TPs[29]. Over the past 25 years many new, highly selective, and robust analytical devices have surfaced and allowed for a greater resolution between parent compounds and their TPs. Peak separation challenges during sample analysis can play a part in analyte detection as TPs can show peak overlap as well as have the same molecular mass as other TPs or the parent compound. Methods involving Orbitrap MS, time of flight (TOF) MS detectors, and increased quadrupole application, such as in a triple-quadrupole system, have allowed for the quantification of even smaller concentrations of pharmaceuticals present in the environment. The coupling of novel sample-extraction methodology has also allowed for the probing of previously problematic environmental matrices like biosolids, landfill leachate, various soils, sewage sludge, and biological tissues. However, even with the advent of modern methodology and technology, the extent of PPCPs and their influence alongside other environmental stressors remains elusive. Considerably less is known about how individual PPCPs interact with multiple facets of an environment as well as how they influence the effects of other PPCPs or TPs that may be existing alongside them. Many of the methods being employed to monitor and identify PPCPs, alongside their TPs, follow similar myopic trends. Furthermore, there is even woefully less known about how the interaction between individual PPCPs may affect other emergent patterns of interaction, such as the accumulation of PPCPs within the trophic cascade, or when framed with effects of acute and chronic conditions after exposure. Additionally, identifying TPs without previously characterized standards has proven to be one of the most difficult aspects of monitoring PPCPs in the environment as well as in understanding the extent to which the compounds morph and engage with biological systems over time.

Gaps in Research and Future Research

It is imperative that our knowledge of the extent of risks posed by PPCPs, like CBZ, be encompassing and detailed. The application of informatics is essential to the understanding of these complex systems involving the nexus of PPCPs, environmental systems, and the human ecosystem. Additionally, TPs and their associated pathways pose risks to the environment and may be underestimated in their involvement with perpetuating contamination by existing in forms that can participate in reversible conjugation pathways back into their parent compound[30]. One of these is the TP of CBZ, Acridine, a known DNA intercalating agent. There are many TPs that have yet to be identified and even more still that may possess mechanisms of interaction with non-target organisms. TPs represent another hurdle that wastewater treatment plants must be aware of and treat for as well. Many of the TPs of CBZ have exhibited similar stability and recalcitrance as their parent molecule. This means that future research should be geared toward the identification of unknown TPs as well as assessing the dangers that both known and unknown TPs might pose to both terrestrial and aquatic environments. Furthermore, additional research assessing the specific risks that CBZ and its TPs have to endemic wildlife should be extensive in its range of survey as well as hierarchical. How PPCPs move through the trophic cascade of an environment, both horizontally between organisms of a similar trophic level and vertically, through predation, remains largely unelucidated. Without this plethora of information, devising efficient and effective removal techniques remains challenging.

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1. Figure 1: CBZ Transformation Pathway; Environ. Sci. Technol. 2015, 49, 20, 12351-12362 Publication Date: September 29, 2015 https://doi.org/10.1021/acs.est.5b02222

2. Figure 2: Pathways Outline; PPCP pathways through the human ecosystem into WWTP and the environment.