Science of the Total Environment 628–629 (2018) 18–25

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Sorption, plant uptake and metabolism of benzodiazepines

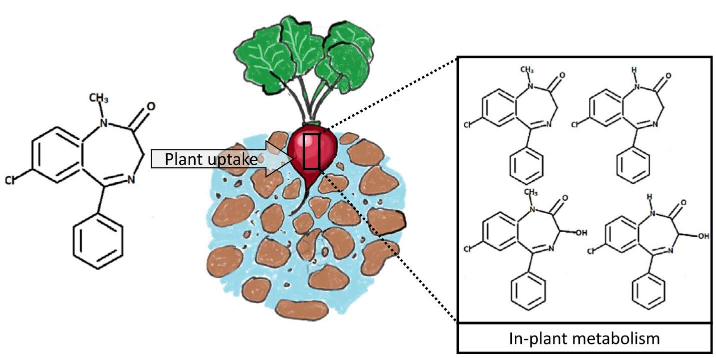
Laura J. Carter [a,b,*], Mike Williams [a], Sheridan Martin [a], Sara P.B. Kamaludeen [c], Rai S. Kookana [a,d]

[a] CSIRO Land and Water, Waite Campus, Adelaide, South Australia 5064, Australia
[b] Environment Department, University of York, York YO10 5NG, UK
[c] Tamil Nadu Agricultural University, Department of Environmental Science, Coimbatore, Tamil Nadu, India
[d] University of Adelaide, Waite Campus, Adelaide, South Australia 5064, Australia

HIGHLIGHTS

- Physicochemical properties of benzodiazepines determine the extent of plant uptake.
- Soil physicochemical properties also influence benzodiazepine plant uptake.
- Plant metabolism of benzodiazepines leads to the formation of biologically active benzodiazepine metabolites.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 31 January 2018
Accepted 31 January 2018
Available online xxxx

Editor: Jay Gan

Keywords:
Soil
Pharmaceutical
Metabolism
Radish
Silverbeet

ABSTRACT

Reuse of treated wastewater for irrigation of crops is growing in arid and semi-arid regions, whilst increasing amounts of biosolids are being applied to fields to improve agricultural outputs. Due to incomplete removal in the wastewater treatment processes, pharmaceuticals present in treated wastewater and biosolids can contaminate soil systems. Benzodiazepines are a widely used class of pharmaceuticals that are released following wastewater treatment. Benzodiazepines are represented by a class of compounds with a range of physicochemical properties and this study was therefore designed to evaluate the influence of soil properties on the sorption behaviour and subsequent uptake of seven benzodiazepines (chlordiazepoxide, clonazepam, diazepam, flurazepam, oxazepam, temazepam and triazolam) in two plant species. The sorption and desorption behaviour of benzodiazepines was strongly influenced by soil type and hydrophobicity of the chemical. The partitioning behaviour of these chemicals in soil was a key controller of the uptake and accumulation of benzodiazepines by radish (*Raphanus sativus*) and silverbeet (*Beta vulgaris*). Benzodiazepines such as oxazepam that were neutral, had low sorption coefficients (K_d) or had pH-adjusted log octanol-water partition coefficients (log D_{ow}, pH 6.3) values close to 2 had the greatest extent of uptake. Conversely, benzodiazepines such as flurazepam that had an ionised functional groups and greater K_d values had comparatively limited accumulation in the selected plant species. Results also revealed active in-plant metabolism of benzodiazepines, potentially analogous to the known metabolic transformation pathway of benzodiazepines in humans. Along with this observed biological transformation of benzodiazepines in exposed plants, previously work has established the widespread presence of the plant signalling molecule γ-amino butyric acid (GABA), which is specifically modulated by benzodiazepines in humans. This highlights the need for further assessment of the potential for biological activity of benzodiazepines following their plant uptake.

* Corresponding author at: Environment Department, University of York, York YO10 5NG, UK.
E-mail address: laura.carter@york.ac.uk (L.J. Carter).

https://doi.org/10.1016/j.scitotenv.2018.01.337

1. Introduction

Benzodiazepines are a class of pharmaceuticals which are among the most highly prescribed psychoactive pharmaceuticals worldwide. One of the most well-known benzodiazepines is diazepam (Valium®) which was in the top 10 psychiatric medicines prescribed in the U.S. in 2011 at 14.6 million prescriptions (Lindsley, 2012). In addition to diazepam, lorazepam, clonazepam, alprazolam and temazepam were also among the top 200 dispensed prescriptions in the U.S. according to IMS Health 2012 (RxList, 2012).

As a class of compounds, benzodiazepines share a common ring structure (Fig. S1) with individual benzodiazepines having variations in the ring substitution, which affects their respective physicochemical properties and pharmacokinetic profile (Charney et al., 2001). Benzodiazepines generally display weak basic properties although their physico-chemical properties such as log K_{ow} values, vary greatly (Fig. S1; Table S1), which will ultimately influence their fate in the environment.

The widespread use of benzodiazepines has led to their recent detection in the environment, where they have been measured at ng/L to low μg/L concentrations in wastewater effluents (Calisto et al., 2011; Fick et al., 2017; Jelic et al., 2011; Kosjek et al., 2012; Kummerer, 2009; Loffler et al., 2005; Mendoza et al., 2014; Nunes et al., 2015; Stein et al., 2008). Even at these relatively low concentrations, there have been reports that have indicated benzodiazepines are also bioactive in aquatic organisms (Brodin et al., 2013; Gagne et al., 2010). A number of benzodiazepines have been reported to be resistant to removal in the environment, as well as interacting weakly with solids (Calisto et al., 2011; Jelic et al., 2011; Loffler et al., 2005; Stein et al., 2008), there is a potential for them to be released into the terrestrial environment through wastewater irrigation which has become an increasingly important means of water recycling (Asano et al., 2007). The fate and effects of these bioactive pharmaceuticals in the terrestrial environment, however, has received little attention. Plant uptake of pharmaceuticals have been reported in a range of vegetable crops including radish, tomato, lettuce, and soybean (Carter et al., 2014; Goldstein et al., 2014; Malchi et al., 2014; Wu et al., 2010; Wu et al., 2014), depending on the physicochemical properties of the compound (Briggs et al., 1982; Carter et al., 2014). Based on the physicochemical properties of benzodiazepines, including having a moderate log K_{ow} and existing as unionised compounds, there is a high potential for them to taken up by plants (e.g. Briggs et al., 1982; Carter et al., 2014).

In humans, benzodiazepines interact with γ-amino butyric acid (GABA) receptors ($GABA_A$ and $GABA_B$) by potentiating the effects of endogenous GABA already bound to the receptor through increasing the efficiency of the intracellular flow of chloride (Cl^-) ions (Haefely, 1984; Olsen and Sieghart, 2008). This has the effect of decreasing neuronal activity, making benzodiazepines effective anxiolytics and anticonvulsants. Recent physiological and genetic evidence indicates that plants may possess GABA like receptors that have features in common with animal receptors (Kinnersley and Lin, 2000; Kinnersley and Turano, 2000). Indeed it has recently been demonstrated that GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters (Ramesh et al., 2015). Given the importance of GABA signalling in plants it is important to establish the plant uptake of GABA modulating benzodiazepines from soils.

The human metabolic pathway of benzodiazepines has been extensively characterised where a multiphase transformation pathway can result in the formation of active metabolites, including temazepam and oxazepam, which are prescription pharmaceuticals in their own right (Fig. S2). Knowledge relating to the fate and transport of pharmaceutical metabolites, in general, within the terrestrial environment is particularly limited as such studies have primarily focussed on the parent compound. Plant metabolism of pharmaceuticals has not been extensively characterised, although this is an important consideration where biologically active transformation products in plant organs have a similar potency to that of the parent compound (Malchi et al., 2014).

Along with their potential for bioactivity in plants, the various substitutions of the benzodiazepine ring structure also modify the physicochemical properties of this class of pharmaceuticals which are likely to affect their fate in soil systems. This study was therefore designed to evaluate the influence of soil properties on the sorption behaviour and subsequent uptake of a range of benzodiazepines with variable physicochemical properties into two common vegetable crops, radish (*Raphanus sativus*) and silverbeet (*Beta vulgaris*). Analysis was also carried out to consider any potential in-plant metabolism of the benzodiazepine parent compounds. The soil was spiked directly with benzodiazepines, as opposed to a continuous exposure reflecting wastewater irrigation, to ensure maximum uptake by the plant and for findings from the sorption studies to be related to plant uptake behaviour.

2. Materials and methods

Primary standards of chlordiazepoxide, chlordiazepoxide-D_5, clonazepam, clonazepam-D_4, diazepam, diazepam-D_5, flurazepam, oxazepam, oxazepam-D_5, nordiazepam, nordiazepam-D_5, temazepam, temazepam-D_5, triazolam and triazolam-D_4 (≥98% purity) were obtained from Novachem (Melbourne, Australia). Hoaglands No. 2 Basal Salt Mixture was purchased from Sigma-Aldrich (Sydney, Australia). HPLC grade solvents were used for all extractions and Optima LC/MS grade methanol was used (Thermo Fisher Scientific; Sydney, Australia) for LC-MS/MS analysis.

Two soil types with contrasting properties were used for this experiment. Soil was obtained from the Tepko agricultural region (pH 6.3, EC 0.09 dS/m, OC 1%, CEC 5.2 cmol (+)/kg, 8% clay, 3% silt and 89% sand), as well as the Inman Valley region in southern Australia (pH 6.3, EC 0.21 dS/m, OC 5.2%, CEC 23.4 cmol (+)/kg, clay 52%, silt 29%, sand 19%). The soils were not cropped and had not previously received biosolids or wastewater applications. Prior to experimental use the soil was air-dried and then sieved to 2 mm to ensure homogeneity. Radish (*Raphanus sativus*, Cherry Belle variety) and silverbeet (*Beta vulgaris*, Fordhook Giant variety) were obtained from Mr. Fothergills (Sydney, Australia).

2.1. Sorption

The sorption of chlordiazepoxide, clonazepam, diazepam, flurazepam, nordiazepam, oxazepam, temazepam and triazolam was studied in the two soils using an adaption of the batch equilibrium method based on the Organisation for Economic Co-operation and Development (OECD) guideline 106 (OECD, 2000) (see Supporting Information for a detailed method description). Briefly, benzodiazepines were spiked individually into glass tubes containing soils at a 1:5 w:w ratio with 0.01 M $HgCl_2$ solution (to prevent biodegradation of the benzodiazepines) to achieve a final soil concentration of 0.8 mg/kg. Tubes were shaken on a rotating shaker for 16 h, centrifuged at 650g for 45 min and the supernatant was analysed by LC-MS/MS. Sorption coefficients (K_d) were determined as a ratio of between the measured soil and water concentration in the test tubes. Measured soil and pore water concentrations obtained from the plant uptake experiment (see below) were also used to determine 'in pot' sorption coefficients (field K_d).

2.2. Plant uptake experiment

The uptake and potential metabolism of chlordiazepoxide, clonazepam, diazepam, flurazepam, oxazepam, temazepam and triazolam was studied in two test soils (Inman Valley and Tepko). For each benzodiazepine treatment, plastic pots containing 500 ± 5 and 750 ± 5 g soil were prepared in triplicate for the radish and silverbeet exposure respectively. A portion of sand (1% of soil weight) was placed in a culture