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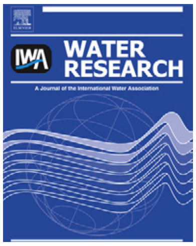

Environmental occurrence, fate and transformation of benzodiazepines in water treatment

T. Kosjek [a], S. Perko [a], M. Zupanc [a,b], M. Zanoški Hren [c], T. Landeka Dragičević [c], D. Žigon [a], B. Kompare [d], E. Heath [a,*]

[a] Jožef Stefan Institute, Department of Environmental Sciences, Ljubljana, Slovenia
[b] Ecological Engineering Institute IEI, Maribor, Slovenia
[c] Faculty of Food Technology and Biotechnology, University of Zagreb, Zagreb, Croatia
[d] Faculty of Civil and Geodetic Engineering, University of Ljubljana, Ljubljana, Slovenia

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ABSTRACT

Benzodiazepine derivatives are prescribed in large quantities globally and are potentially new emerging environmental contaminants. Unfortunately, a dearth of data exists concerning occurrence, persistence and fate in the environment. This paper redresses this by reviewing existing literature, assessing the occurrence of selected benzodiazepine anxiolytics (diazepam, oxazepam and bromazepam) in wastewater influent and effluent and surface water from Slovenia, evaluating their removal during water treatment and identifying the transformation products formed during water treatment. Their occurrence was monitored in hospital effluent, river water and in wastewater treatment plant influent and effluent. The study reveals the presence of benzodiazepine derivatives in all samples with the highest amounts in hospital effluents: 111 ng L^{-1}, 158 ng L^{-1} and 72 ng L^{-1} for diazepam, bromazepam and oxazepam, respectively. Removal efficiencies with respect to biological treatment of diazepam were 16–18% (oxic), 18–32% (anoxic → oxic), 53–76% (oxic → anoxic) and 83% (oxic → anoxic → oxic → anoxic cascade bioreactors), while the removal oxazepam was 20–24% under anoxic conditions. Coupled biological and photochemical treatment followed by the adsorption to activated carbon resulted in a removal efficiency of 99.99%. Results reveal the recalcitrant nature of benzodiazepine derivatives and suggest that only combinational treatment is sufficient to remove them. In addition, eight novel diazepam and four novel oxazepam transformation products are reported.

1. Introduction

Benzodiazepines are the drugs of choice in the pharmacotherapy of anxiety and related emotional disorders, sleep disorders, status epileptics, and other convulsive states. They are also used as centrally acting muscle relaxants, for premedication and as inducing agents in anaesthesiology (Neumeyer and Booth, 1995). Their potential shortcomings include tolerance, withdrawal symptoms, and their abuse potential (Riss et al., 2008; Kosjek and Heath, 2011).

Diazepam (DZ, Valium®) is perhaps the most known drug among this group and is long acting because of its active metabolites that have long half-lives (Baldessarini et al., 1996). In humans it is metabolized into either N-desmethyldiazepam or nordazepam (half-life of up to 100 h). Nordazepam is then further 3-hydroxylated into oxazepam (OXA). The presence of

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[*] *Corresponding author*. Tel.: +386 1 4773584.
E-mail address: ester.heath@ijs.si (E. Heath).

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the hydroxyl group enables rapid glucuronidation and excretion with the urine and explains its significantly shorter half-life (4–15 h) when compared to both DZ and nordazepam (Neumeyer and Booth, 1995; Benzodiazepine Equivalents Table, 2011). Nordazepam and OXA are marketed as Calmday® and Serax®, respectively. According to Fürst et al. (2006), besides DZ, the most commonly prescribed anxiolytics in Slovenia are bromazepam (BZ, half-life: 10–20 h) and alprazolam (half-life: 6–12 h). Fürst et al. (2006) also reported significant differences in the use of high doses of DZ and BZ between the individual regions of Slovenia, what is suggestive of their potential abuse.

In environmental research, benzodiazepines are among those pharmaceuticals less commonly addressed (Ternes et al., 2001). Diazepam has been detected in wastewater (WW) originating from hospitals as well as in effluents from municipal wastewater treatment plants. Ternes et al. (2001) and Martínez Bueno et al. (2007) found levels of DZ as high as 53 ng L^{-1} and 87 ng L^{-1}, respectively, in wastewater treatment plant effluent. DZ was further found at 33 ng L^{-1} in German rivers (Ternes et al., 2001), up to 21 ng L^{-1} in rivers in the region of Madrid (Martínez Bueno et al., 2010) and Zuccato et al. (2000) found up to 23.5 ng L^{-1} DZ in drinking water. Further, Heberer (2002) reports the presence of OXA (0.25 μg L^{-1}) in a wastewater treatment plant (WWTP) effluent, while Baker and Kasprzyk-Hordern (2011) found OXA in majority of WWTP effluent, influent and river water samples. In addition, BZ, OXA and DZ were determined in the Llobregat River (north eastern Spain – a source of potable water at mean concentrations of 7 ng L^{-1}, 20 ng L^{-1} and 3 ng L^{-1}, respectively) (Huerta-Fontela et al., 2011).

Benzodiazepines are normally halogenated compounds, and it is suggested that the presence of a halogen in a chemical structure significantly reduces its susceptibility to biodegradation (Johnson et al., 2008). Most data concerning the efficiency of WW treatment is for DZ, and reveals that <10% is removed during classical biological treatment, while anaerobic sludge treatment is only slightly more efficient (10–50%); removal is a result of adsorption to activated sludge, rather than degradation (Ternes et al., 2004; Löffler et al., 2005). Similarly, OXA is persistent to both, aerobic or anaerobic biodegradation and field-based experiments also reveal its recalcitrance (Patterson et al., 2010, 2011). To our knowledge, other than the papers cited no other published degradation data on OXA is available.

DZ is considered to undergo photochemical degradation under environmental conditions which may constitute a feasible mechanism for its removal from surface waters (Boreen et al., 2003). In comparison to classical treatment technologies, advanced oxidation methods are more efficient at eliminating DZ (Belden et al., 2007; Calisto and Esteves, 2009). Degradation of DZ was improved in the presence of ferrioxalate, either under black-light or solar irradiation and the removal efficiency was 80% after 60 min irradiation (Bautitz and Nogueira, 2010). DZ is relatively resistant to ozonation (Verlicchi et al., 2010), but it can be oxidised by $\cdot$OH radicals during ozone treatment (Ternes et al., 2004). Unfortunately, there are no published data for the remaining benzodiazepines regarding their persistence to abiotic treatment methods.

Eventhough the K_{ow} of these compounds do not indicate sorption to be an important removal process, recent researches suggest this is not the case. Ternes et al. (2004) classify DZ as easily adsorbable to activated carbon (99% removal, 0.2 mg L^{-1} of activated carbon) while Calisto and Esteves (2009) report that temazepam and OXA may undergo more abiotic losses by sorption to humic substances. Löffler et al. (2005) found DZ to be a highly persistent pharmaceutical with rapid and extensive sorption onto sediments as well as being highly stable in soils, ground waters and during the WWs treatment. In the same study OXA was reported as moderately persistent in water/sediment systems.

It is clear that available data regarding the presence, cycling and fate of benzodiazepine derivatives in the environment are insufficient. This study aims to address this deficiency by (i) identifying and determining the environmental concentrations of benzodiazepines residues, (ii) evaluating their removal during biological and photochemical water treatment and (iii) studying the formation of stable transformation products during wastewater treatment. In addition we also make suggestions about how to improve current wastewater treatment.

2. Experimental

2.1. Chemicals

Authentic compounds DZ and BZ were donated by a cooperating body, while OXA was curtsy of Belupo, d.d. (Croatia). The internal standard [$^2H_5$]-oxazepam (OXA-d5: hydrogen atoms of the C_6H_5 phenyl group were replaced by deuterium atoms, 99.9%) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and [$^2H_5$]-diazepam (DZ-d5: hydrogen atoms of the C_6H_5 phenyl group were replaced by deuterium atoms, 99.9%) was purchased from LGC Standards GmbH (Wesel, Germany). Both deuterated internal standards were used for the trace-level analytical method development and validation, and for the determination of occurrence of the selected pharmaceuticals in the environment. All standards were of highest available purity (>99%). For derivatisation we used acetic anhydride (Sigma Aldrich, USA) and pyridine (Merck, Germany). All applied solvents (ethylacetate, methanol) and chemicals (hydrogen peroxide 30%) were of analytical grade purity.

2.2. Sample preparation

Fourhundred milliliters river water (RW) and 200 mL WW samples were first filtered through glass microfiber prefilters (Machery Nagel, Dueren, Germany) and afterwards through 1.2 μm cellulose nitrate (Whatman, Kent, UK) filters. After filtration the internal standards DZ-d5 and OXA-d5 were added. The concentration of the internal standards was 4.4 nM in the case of treatment experiments; 0.44 nM (DZ-d5) and 1.32 nM (OXA-d5) for determination of actual WW samples; and 0.22 nM (DZ-d5) to 0.44 nM (OXA-d5) for natural water samples. The sample pH was 6.5–7.0 and was not adjusted before the extraction. Samples were extracted by Oasis™ (Waters Corp., Milford, MA, USA) 60 mg/3 mL solid-phase extraction (SPE) cartridges, previously conditioned