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Field and laboratory studies of the fate and enantiomeric enrichment of venlafaxine and *O*-desmethylvenlafaxine under aerobic and anaerobic conditions

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ABSTRACT

The stereoselectivity of *R,S*-venlafaxine and its metabolites *R,S*-*O*-desmethylvenlafaxine, *N*-desmethylvenlafaxine, *O,N*-didesmethylvenlafaxine, *N,N*-didesmethylvenlafaxine and tridesmethylvenlafaxine was studied in three processes: (i) anaerobic and aerobic laboratory scale tests; (ii) six wastewater treatment plants (WWTPs) operating under different conditions; and (iii) a variety of wastewater treatments including conventional activated sludge, natural attenuation along a receiving river stream and storage in operational and seasonal reservoirs. In the laboratory and field studies, the degradation of the venlafaxine yielded *O*-desmethylvenalfaxine as the dominant metabolite under aerobic and anaerobic conditions. Venlafaxine was almost exclusively converted to *O*-desmethylvenlafaxine under anaerobic conditions, but only a fraction of the drug was transformed to *O*-desmethylvenlafaxine under aerobic conditions. Degradation of venlafaxine involved only small stereoisomeric selectivity. In contrast, the degradation of *O*-desmethylvenlafaxine yielded remarkable *S* to *R* enrichment under aerobic conditions but none under anaerobic conditions. Determination of venlafaxine and its metabolites in the WWTPs agreed well with the stereoselectivity observed in the laboratory studies. Our results suggest that the levels of the drug and its metabolites and the stereoisomeric enrichment of the metabolite and its parent drug can be used for source tracking and for discrimination between domestic and nondomestic wastewater pollution. This was indeed demonstrated in the investigations carried out at the Jerusalem WWTP.

1. Introduction

The large and growing scientific attention to micropollutants and their occurrence in aquatic systems (Richardson, 2010) has brought about an increasing amount of research activity in the field of micropollutant stereoisomers. Toxicity, metabolic pathways and kinetics may largely depend on chirality (Sheldon et al., 2009; Smith, 2009) as does ecotoxicity (Badaloni et al., 2003; Stanley et al., 2006; Stanley et al., 2007; Wharfe et al., 2010). Stereoisomers are rather common in natural as well as in synthetic chemicals. 25% of the agrochemicals are chiral (Williams, 1996), and 50% of the chemicals listed in USEPA Method 1694, (USEPA, 2007) for the analysis of pharmaceuticals and personal care products in water and soil have stereoisomers. Several reviews and comprehensive reports on stereoisomeric selectivity and chiral enrichment of micropollutants in effluents and polluted streams have appeared in recent years (Buser et al., 1999; Nikolai et al., 2006; MacLeod et al., 2007; Celiz et al., 2009; Barreiro et al., 2010; Kasprzyk-Hordern, 2010; MacLeod and Wong, 2010), and much effort is being devoted to the development of analytic methods for enantiomeric separation in polluted aquatic systems (Fono and Sedlak, 2005; Lua et al., 2006; Araujo et al., 2008; Perez and Barcelo, 2008; Kasprzyk-Hordern et al., 2010).

An appealing aspect of the observed chirality of micropollutants is its possible utilization for source tracking, and what chirality can teach about the degradation mechanism. Fono and Sedlak, 2005 showed that chiral enrichment can at times be used to discriminate between pollution from untreated and treated wastewater. The enantiomeric enrichment of naproxen and ibuprofen in a conventional WWTP and in constructed wetlands was reported by Matamoros et al. (2010) and Hijosa et al. (2010) to be conversion dependent. Several recent publications report the enantiomeric enrichment of metabolites of chiral drugs and hormones (Durhan et al., 2006; Selke et al., 2010). The current research shows that chiral enrichment of drug metabolites can be affected by the metabolic pathway and can be used to track the history of the pollution. Furthermore, when combined with metabolite and reactant concentrations it can at times be used as an additional tool for source tracking.

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In this research, we have targeted the biotransformation of venlafaxine (VNF), a phenylethylamine derivative that affects brain neurotransmission by blocking presynaptic reuptake of serotonin and noradrenaline. VNF is a serotonin-norepinephrine reuptake inhibitor drug (SNRI), as are duloxetine, milnacipran and *O*-desmethylvenlafaxine, a metabolite of VNF. *O*-desmethylvenlafaxine is presently marketed as a new SNRI drug, desvenlafaxine. The human metabolism of VNF involves phase I and phase II metabolisms. Phase I is largely carried out by P450 isozymes CYP2D6 and CYP1A2, which are responsible for desmethylation at the nitrogen or oxygen sites. Phase II metabolism is hydrophilic adduct formation by reaction of the drug with glucuronic acid or subsequent conjugation of a phase I metabolite with hydrophilic substitution agents. The distinction between the two phases may have environmental significance since phase II metabolism may be reversed by biodegradation in the treatment system. Only 4.7% of the administered dose of VNF is excreted by the renal route, with *O*-desmethylvenlafaxine (*O*-DMV) as the dominant metabolite.

SNRI drugs are marketed as racemic mixtures, which is significant for the current research and their use for source tracking. A racemic mixture corresponds to a 1:1 ratio between the two enantiomers, i.e. the enantiomer fraction (EF) = 0.5. EF is calculated by the formula EF = E1/(E1 + E2), where E1 and E2 correspond to the concentrations of the two stereoisomers. Following the customary notation of isotopic enrichment, the enantiomer ratio, ER = E1/E2, is used in this article. ER = 1 corresponds to EF = 0.5. ER can be smaller or larger than 1, and there is no convention regarding which enantiomer should be in the denominator.

The use of VNF is rapidly growing. Venlafexine ranked 100 in the list of the most prescribed drugs in the USA in the year 2010 with 55 million prescriptions, 5.5 times more than the previous year (http://drugtopics.modernmedicine.com/drugtopics/data/articlestandard//drugtopics/252011/727243/article.pdf). VNF has a K_{OC} = 10.1 at pH 7.4, its boiling point is 398 °C and its pK_a = 9.3 (Cherkaoui et al., 2001). However, until recently VNF has been much less investigated relative to other pharmaceutical and personal care products. Some recent publications underscore the prevalence of VNF in sewage and surface waters (Lamas et al., 2004; Metcalfe et al., 2004; Schultz and Furlong, 2008; Lajeunesse et al., 2008; Calisto and Esteves, 2009; Alonso et al., 2010; Schultz et al., 2010). Only one group, has studied VNF's presence at four WWTPs and found that its enantiomer fraction (EF) ranged from 0.5 to 0.63, corresponding to enanatiomeric ratio, ER of 1–1.7 (Kasprzyk-Hordern et al., 2010).

2. Experimental section

2.1. Microcosm tests in lab scale

Batch tests were carried out in parallel in separate 250 mL beakers filled with 200 mL of wastewater effluent. Samples were collected from the overflow of the Jerusalem WWTP. The samples were charged with 2.5% of activated sludge collected from the aerated reactor to assure the presence of an active mixed bacterial culture. The chemical oxygen demand, COD of the starting suspension was 250 mg L^{-1}. 125 or 150 μL from stock solutions of VNF or *O*-DMV (with a concentration of 1 g L^{-1}) were added, which amounts to addition of 25 or 30 μg L^{-1} of *O*-DMV and VNF, respectively.

The beakers designated for anaerobic biodegradation were purged with nitrogen gas for 20 min to remove oxygen, prior to charging with the reactants. After this procedure the oxygen content was lowered below 0.2 mg L^{-1}. Prior to the degradation tests, the samples were analyzed for VNF and its metabolites. The beakers were sealed with plastic cups. All microcosms were kept in the dark at 28 ± 2 °C. The beakers for the aerobic degradation tests were covered with gauze plugs (Medi elast™, Medipharm, UK) and agitated with magnetic stirrers throughout the experiment to assure sufficient oxygen supply. The level of the waste water was adjusted once a week by adding 2–3 mL of distilled water to compensate for evaporation. The anaerobic microcosms were kept unshaken in the dark. The entire content of each beaker was used for a single analysis, in order to prevent non-representative consecutive sampling of a heterogeneous culture from the same beaker.

2.2. Target WWTPs

The target WWTPs listed in Table S2 of the supplementary material represent vastly different water treatment conditions, covering the most common treatments practiced in Israel (Gasser et al., 2011). The Shafdan WWTP is the largest plant in Israel. It treats approximately 127.1 Mm^3 year of wastewater by conventional treatment (primary clarifier and activated sludge treatment) including nitrification and denitrification. The sludge retention time (SRT) is very short (3 d). One reason for the short SRT is that the sludge is not recycled and the leachate is discarded to the Mediterranean Sea. The west Jerusalem WWTP, the third largest plant in Israel, treats approximately 27 Mm^3 year by conventional treatment. Currently, the plant does not include nitrification–denitrification. The sludge is treated in an anaerobic digester and the leachate is recycled to the aerated reactor thus increasing the BOD loading. Based on the 2010 Jerusalem WWTP annual report, the average COD in the effluents was 66 ± 8.3 mg L^{-1} (n = 12), average TOC was 17.1 ± 5.7 mg L^{-1} (n = 6) and average Kjeldhal-N was 50.8 ± 6.8 mg L^{-1} (n = 12). The secondary effluents are discharged to the Soreq river running for 22 km in an open river bed to the Jerusalem Mountains wastewater reservoir which retains the water for a few weeks prior to storage in the Tal Shahar reservoir. The water is used for irrigation in the summer. The reservoir is not covered and as a result algae growth can be observed. Ayalon, the third WWTP is probably the least rigorous treatment plant involving a treatment system similar to that of Jerusalem, i.e. activated sludge without nitrification and denitrification; and the sludge is treated anaerobically. The sludge retention time averages only 2.5 d. Ra'anana WWTP includes activated sludge treatment with nitrification and denitrification, aerobic treatment of the sludge and tertiary deep bed filtration. Hasharon and Tnovot WWTPs involve conventional activated sludge treatment with nitrification and denitrification. The sludge is not bio-treated rather dewatered and the leachate is recycled to the aerated biological reactor.

2.3. Analytical methods

VNF and its metabolites, *O*-DMV, *N*-DMV, *O*,*N*-DMV, *N*,*N*-DMV and t-DMV samples were analyzed by triple quadruple LC/MS/MS according to EPA method 1694 (USEPA, 2007) for elution of basic compounds from solutions containing less than 1% solids. The maximal TSS of the current studies was 0.2 g L^{-1}. Only a brief description of the method is currently provided; full details are presented in the Supplementary materials. First, a known amount of VNF-D6 was added to the samples. The compounds were then extracted by Solid Phase Extraction (SPE) with Oasis HLB 60 mg cartridges (Waters, Milford, MA) using 200 mL of each sample. Analytes were subsequently eluted with methanol and formic acid solutions, and the mixed extracts were concentrated to a final volume of 5 mL.

Analysis of the concentrated mixed extracts was performed using an Agilent G6410A triple quadruple mass spectrometer with a positive electrospray ionization ion source. Analyte separation was conducted with an Agilent ZORBAX Eclipse Plus C18 (2.1 mm ID, 100 mm length, 3.5 μm particle size). Limits of Quantification (LOQ) calculated at 10 times the background level are denoted in Table 1 along with the recovery at 1.0 μg L^{-1}.