Department of Chemistry

Enantioselective analysis of chiral pharmacologically active compounds in urban water

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AIMS

- To introduce the phenomenon of chirality
- To present methodological advances in the analysis of chiral drugs at enantiomeric level in environmental matrices
- To discuss results of enantiomeric profiling of chiral drugs in environmental matrices

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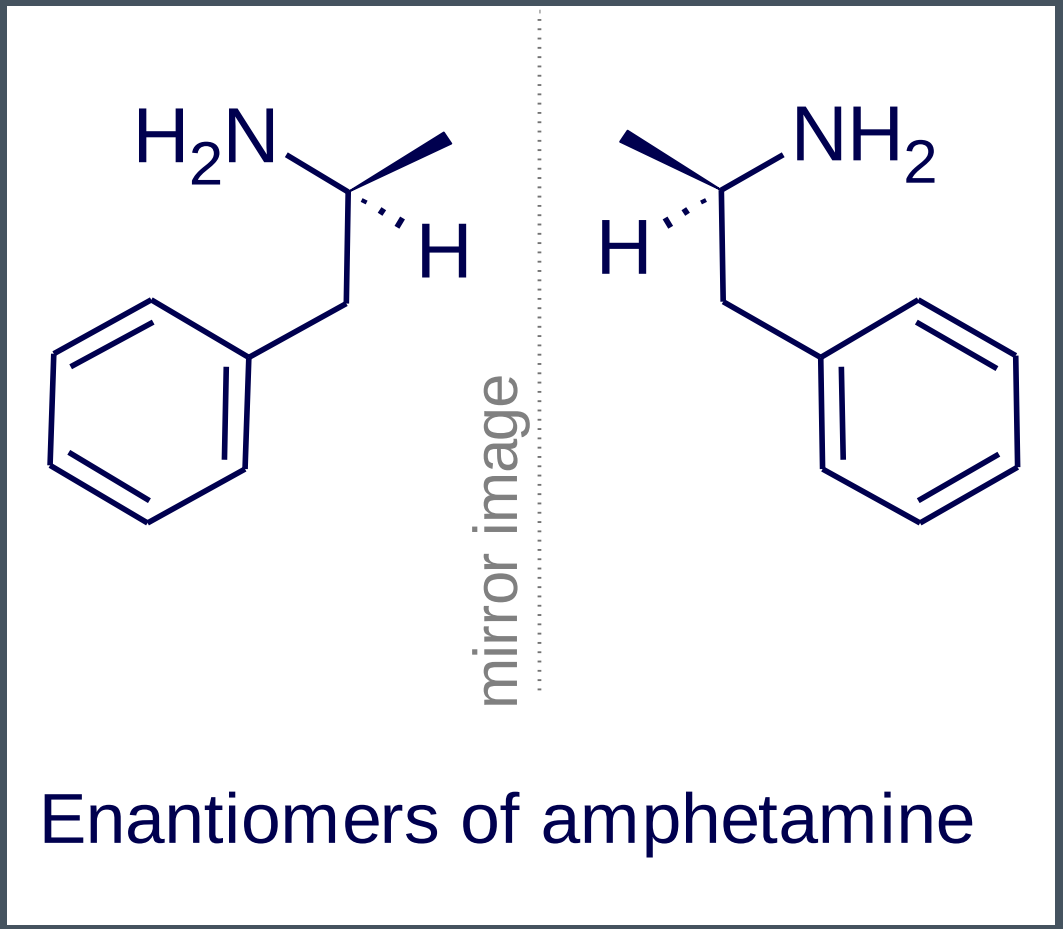
Phenomenon of chirality



Enantiomers of amphetamine

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PRINCIPLES OF CHIRALITY

Enantiomers of the same chiral molecule have similar physico-chemical properties but may differ in their biological properties

Enantiomers of chiral drugs reveal different potency:

- *S*(+)-enantiomers of AMPH-derived drugs have much higher stimulant activity than R(-)-enantiomers
- *S*(+)-MDMA is more amphetamine-like stimulant and *R*(–)-MDMA is more hallucinogenic
- *S*-ibuprofen is 100x more potent than *R*-ibuprofen
- *S*(-)-enantiomers of beta-blockers reveal much higher potency in humans
- Cardiovascular effects of verapamil are mediated by *S*(-)-enantiomer

Chiral drugs have stereoselective disposition in the body:

- *S*(+)-enantiomers of amphetamine-derived drugs metabolise faster in human than *R*(-)-enantiomers

ENVIRONMENTAL LIFE-CYCLE OF PACs

Stereoselective metabolism

CONSUMPTION

Racemic drug

EXCRETION
Non-racemic drug, metabolites

WASTEWATER TREATMENT

DISCHARGE
Non-racemic drug, metabolites, by-products

ENVIRONMENT DEGRADATION

WATER WITHDRAWAL
Non-racemic drug, metabolites, by-products

DRINKING WATER TREATMENT

CONSUMPTION
Non-racemic drug, metabolites, by-products

EMERGING ISSUES

- The enantiomeric composition of a chiral molecule can change throughout its environmental life-cycle
- The very same chiral molecule might have different activity/toxicity at different stages of its environmental life cycle, which will depend on its origin and exposure to environmental factors

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- **NSAIDs, analgesics and anaesthetics**
- **CNS drugs**: antipsychotic drugs, antidepressants, sedative/hypnotics, CNS stimulants and drugs used for ADHD, drugs used in neurological disorders
- **Cardiovascular drugs**: lipid regulating drugs, beta-adrenoceptor blocking drugs, antihypertensives, anticoagulants, calcium channel blockers, anti-arrhythmic drugs
- **Respiratory drugs**: bronchodilators, antihistamines, decongestants
- **Gastro-intestinal drugs**: proton pump inhibitors, H2-receptor antagonists
- **Antimicrobials**
- **Antineoplastics**
- **Illicit drugs**

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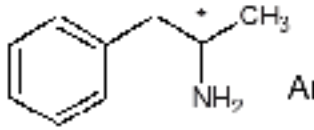
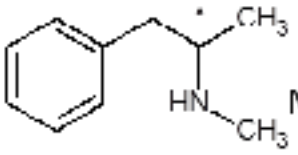
STUDIED CHIRAL DRUGS

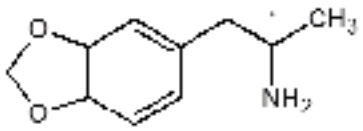
Amphetamines



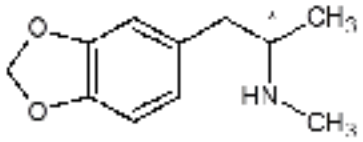
Amphetamine



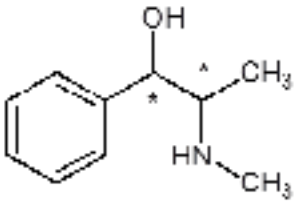
Methamphetamine



MDA



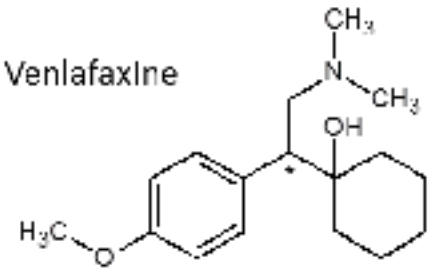
MDMA



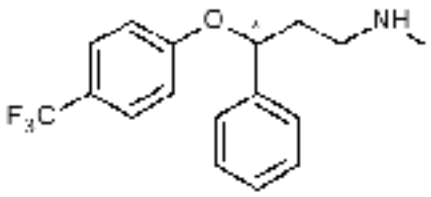
Ephedrine/
Pseudoephedrine

Antidepressants

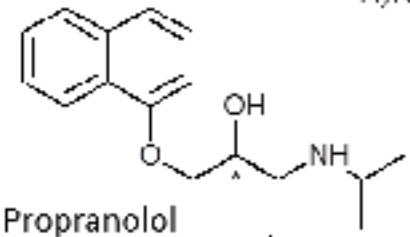
Venlafaxine



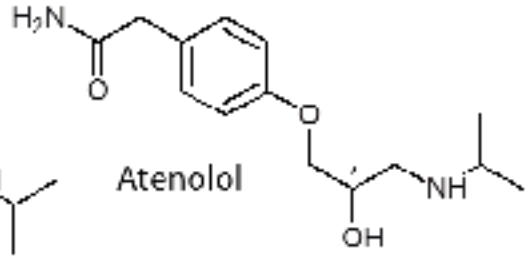
Fluoxetine



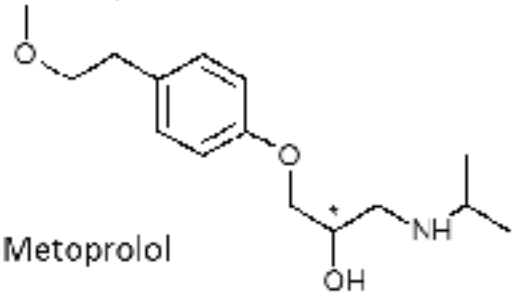
Beta-blockers



Propranolol

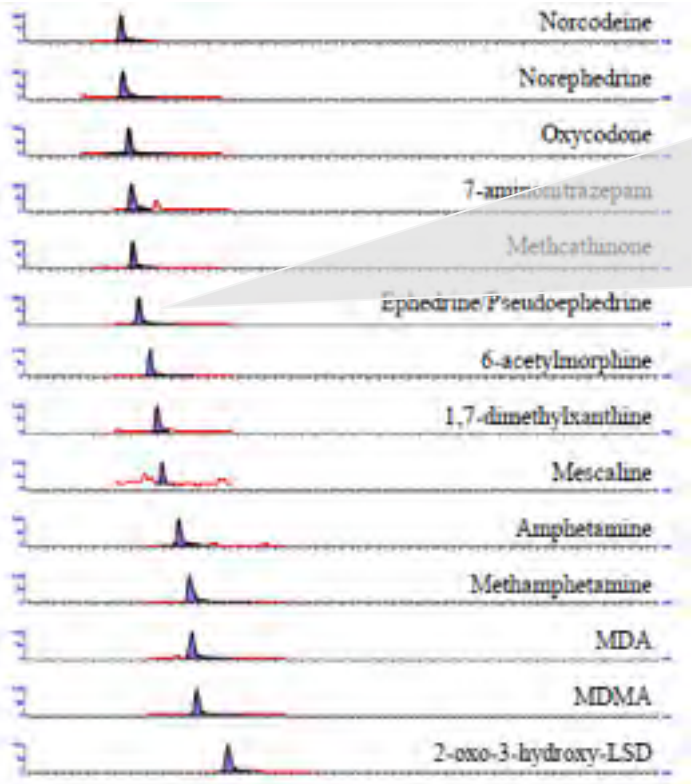


Atenolol

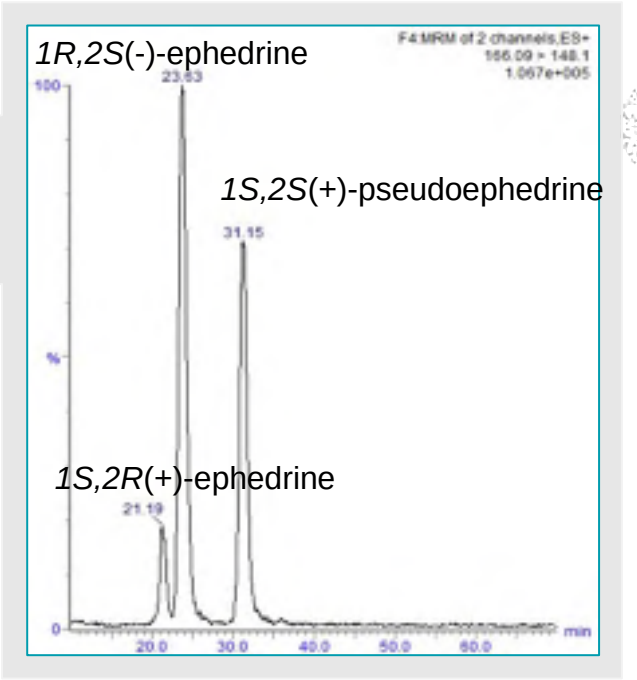


Metoprolol

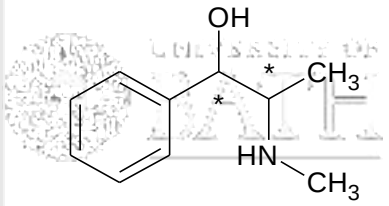
….AND MANY MORE…



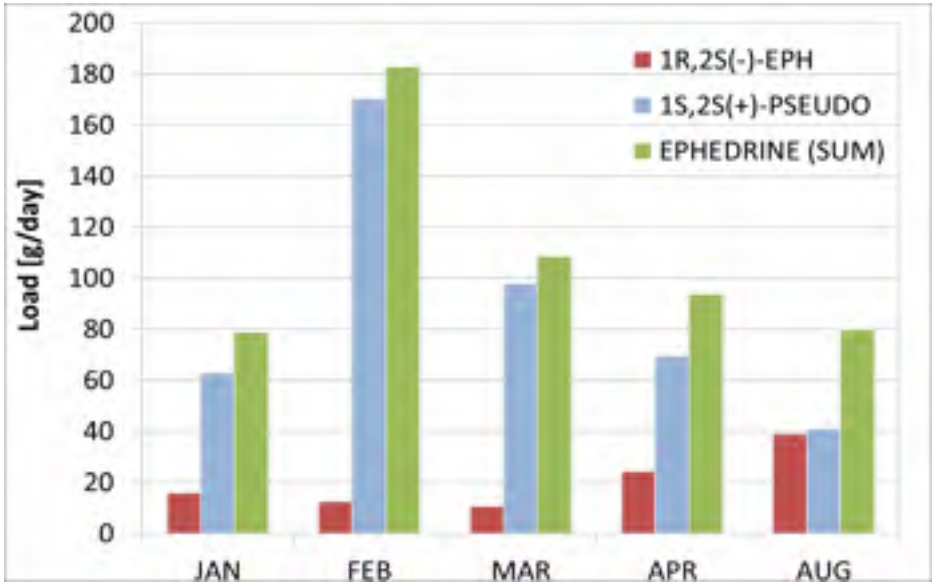
RP (C18)-UPLC-QqQ



RP-chiral (CBH) LC-QqQ



Ephedrine/ Pseudoephedrine



1R,2S(-)-Ephedrine and *1S,2S*(+)-pseudoephedrine loads in sewage (sum of loads from 7 WWTPs)

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EMERGING ISSUES – ENVIRONMENT

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ANALYSIS OF CHIRAL DRUGS IN LIQUID MATRICES

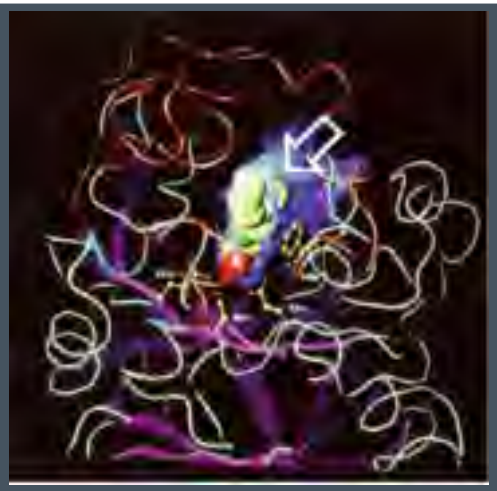
SPE (HLB, 60mg)

Condition: 4mL MeOH, 4mL H_2O; Sample load: 100mL wastewater; 500mL river water; Elution: 4mL MeOH; Evaporation and reconstitution to 0.5mL with mobile phase

UPLC

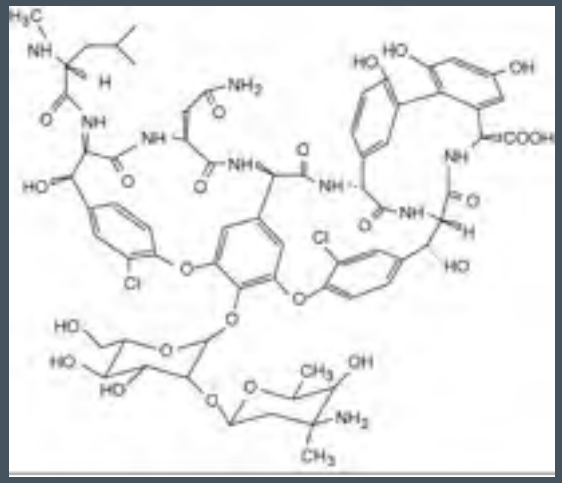
Column 1: CBH

(100x2.1mm; 5um); 90% H_2O, 10% IPA, 1mM NH4OAc; 25°C, 0.075 mL/min; isocratic



Column 2: Chirobiotic V

(250x2.1mm; 5um); 100% MeOH, 4mM NH4OAc, 0.005% HCOOH; 25°C, 0.1 mL/min; isocratic



Mass Spectrometry

MS1:
(ESI+) **QqQ** (Waters TQD)

MS2:
(ESI+) **QTOF** (micrOTOFQ, Bruker)

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Validation			
Parameters:	**UPLC (CBH)-QqQ**	**UPLC (CBH)-QTOF**	**UPLC (Chirobiotic V-QTOF**
Accuracy	<20%	<20%	<20%
Precision	<10%	<10%	<10%
MQLs (wastewater)	1.2-5.8 ng/L (INFL)	-	1.3-86 ng/L (EFFL)
MQLs (river)	0.2-1.2 ng/L	9.1-51.7 ng/L	0.3-39 ng/L
Linearity range	0.05-500 µg/L	0.5-500 µg/L	0.25-100 µg/L 5-500 µg/L

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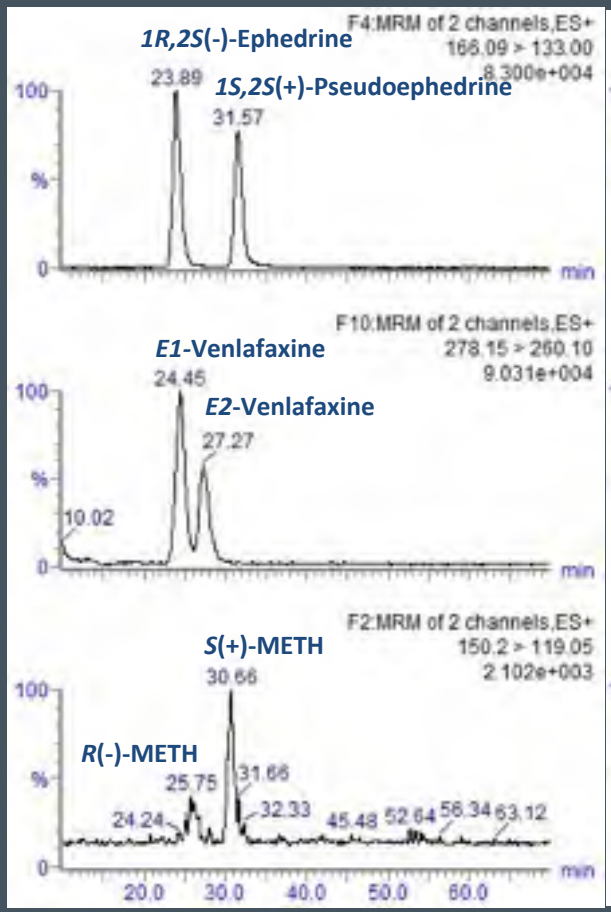
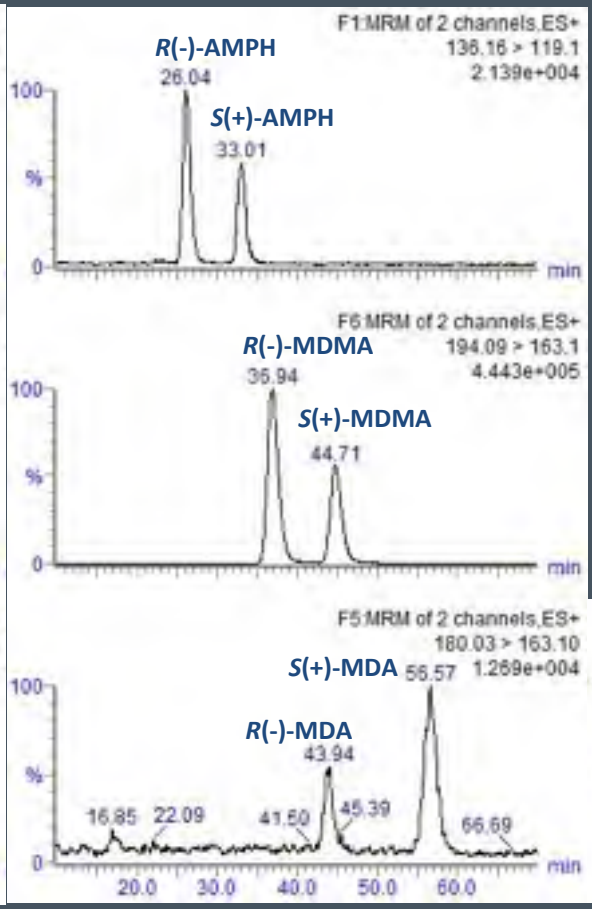
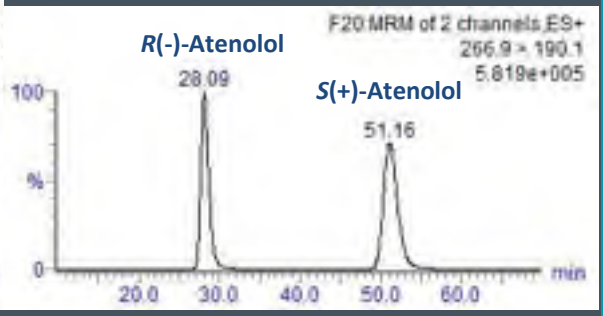




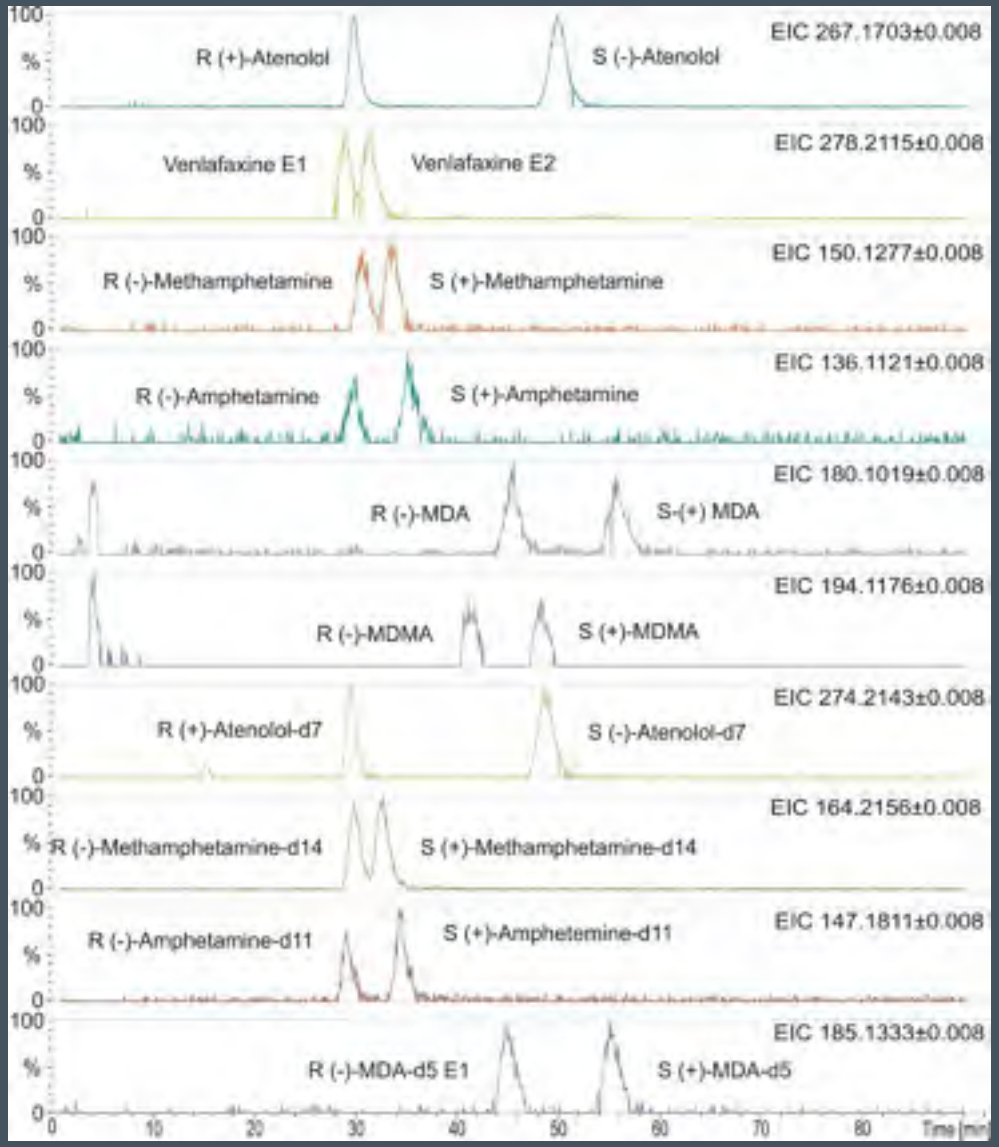


LC-MS/MS chromatograms of chiral drugs in wastewater influent

Column:
CBH (100x2.1mm; 5um);
90% H_2O, 10% IPA, 1mM NH4OAc;
25°C, 0.075 mL/min; isocratic

MS:
TQD

B.Kasprzyk-Hordern, V.V.R. Kondakal, D.R. Baker, J. Chromatogr 1217 (2010) 4575

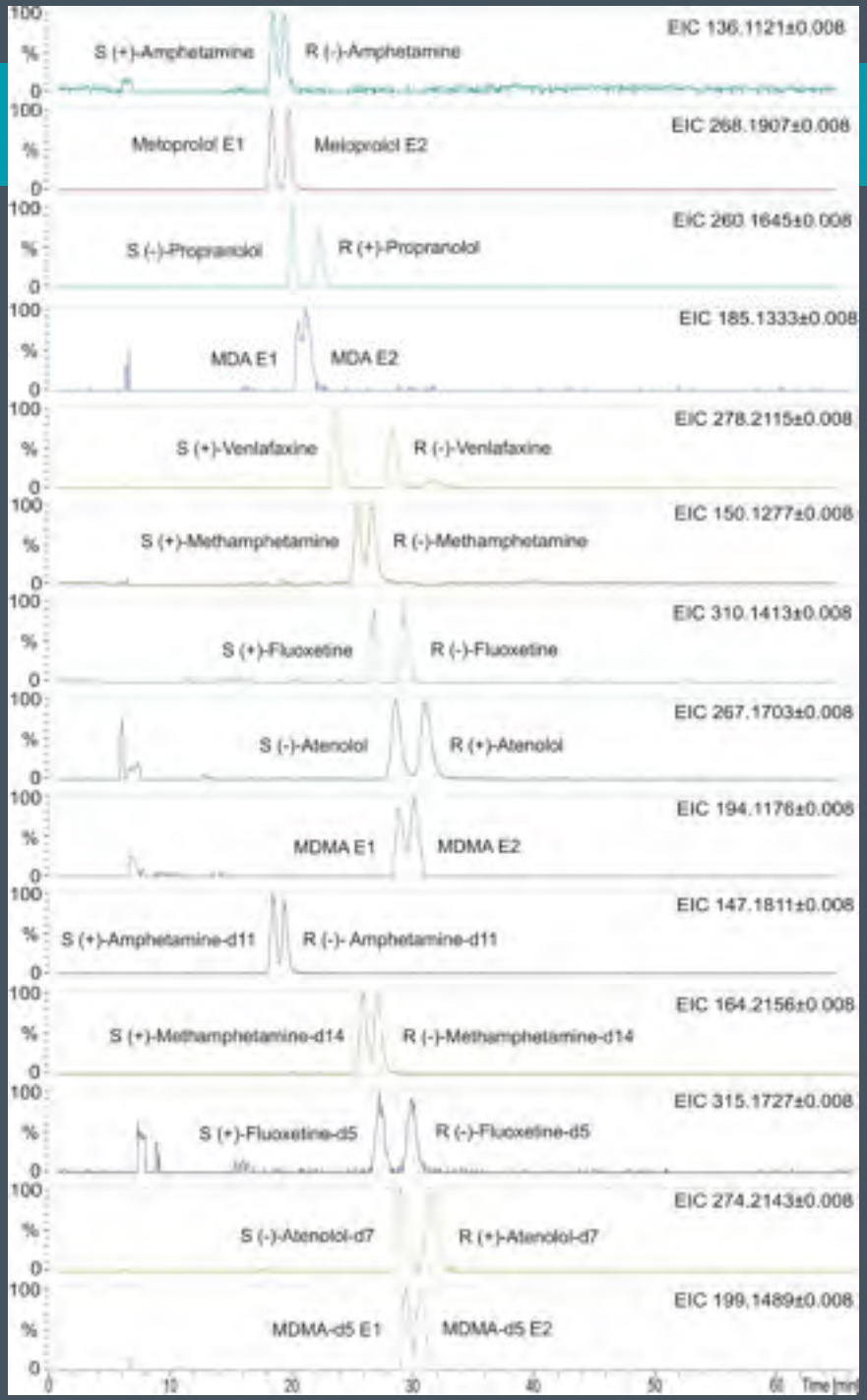


LC-MS/MS chromatograms of chiral drugs in river water spiked with chiral drugs and extracted by SPE (concentration, 100 ng/L)

Column:
CBH (100x2.1mm; 5um);
90% H_2O, 10% IPA, 1mM NH4OAc;
25^oC, 0.075 mL/min; isocratic

MS:
QTOF

J. Bagnall, S.E. Evans, M.T. Wort, A.T. Lubbens, B. Kasprzyk-Hordern, J. Chromatogr doi:10.1016/j.chroma.2012.06.012



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LC-MS/MS chromatograms of chiral drugs in river water spiked with chiral drugs and extracted by SPE (concentration, 100 ng/L)

Column:
Chirobiotic V (100 x 2.1mm; 5um);
100% MeOH, 4mM NH4OAc; 0.005%FA
25^{o}C, 0.1 mL/min; isocratic

MS:
QTOF

J. Bagnall, S.E. Evans, M.T. Wort, A.T. Lubbens, B. Kasprzyk-Hordern, J. Chromatogr
doi:10.1016/j.chroma.2012.06.012

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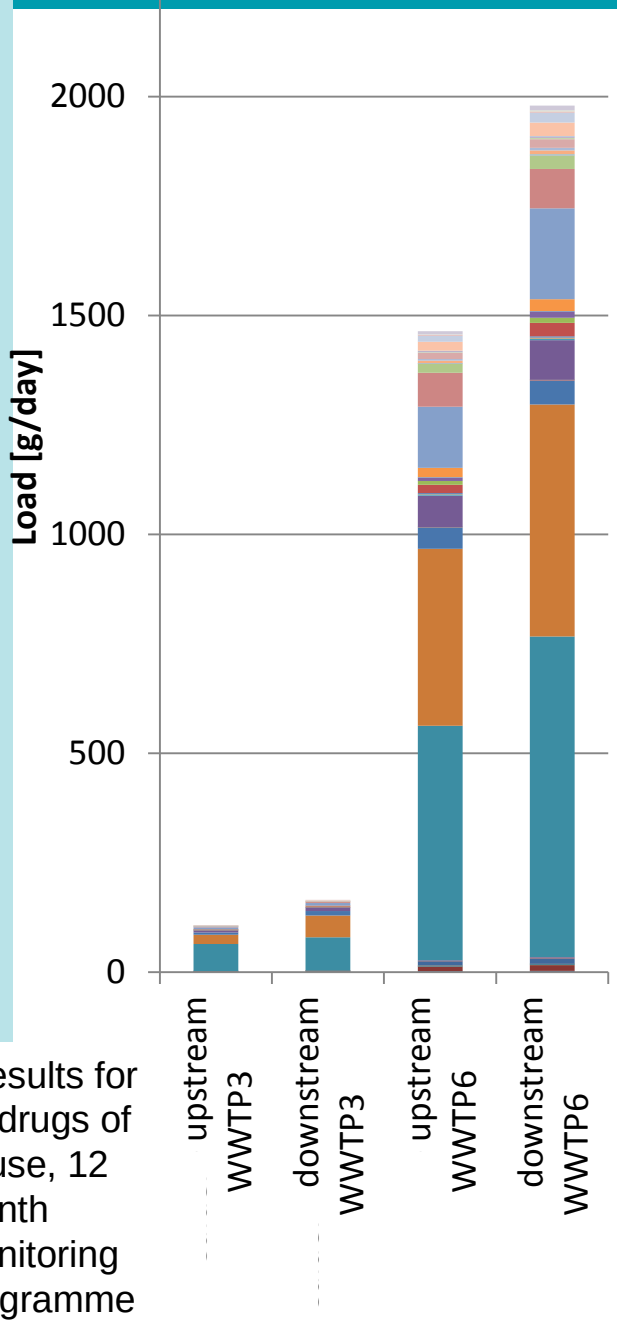
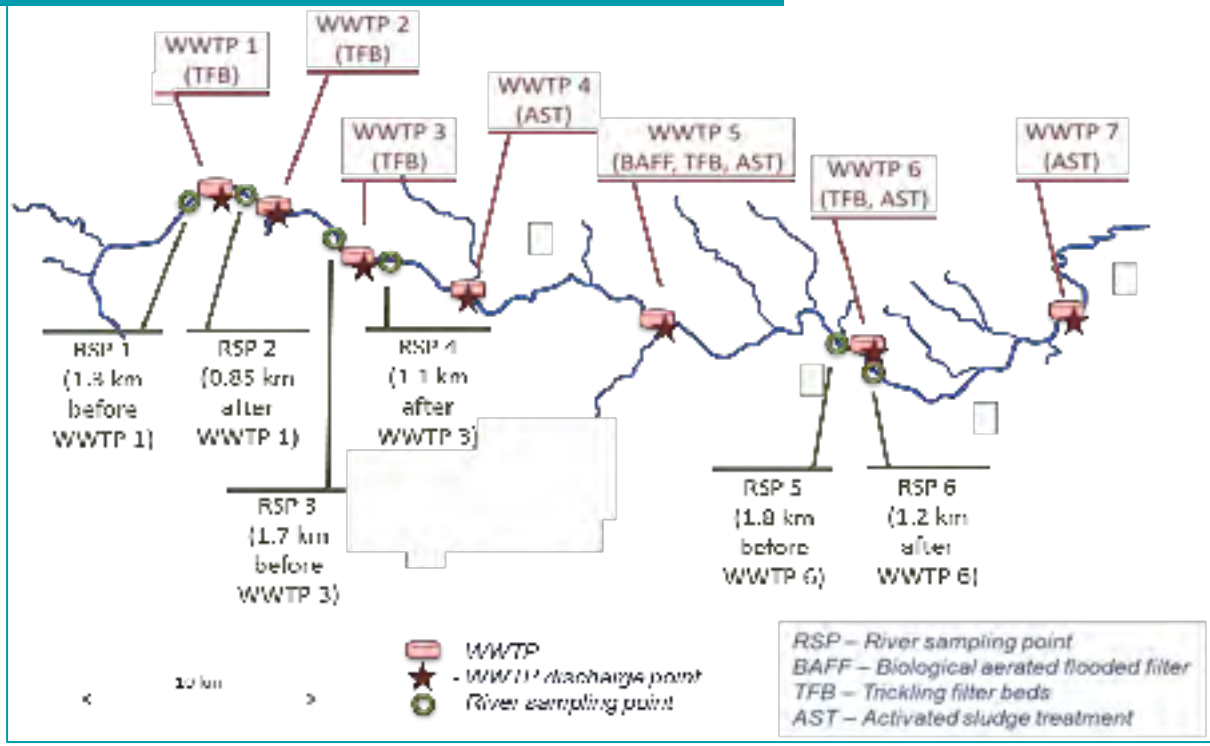
ENANTIOMERIC PROFILING OF CHIRAL DRUGS IN ENVIRONMENTAL MATRICES AND ITS APPLICATIONS:

- Wastewater treatment – enantiomeric profiling via targeted analysis with chiral-UPLC-TQD
- Environmental monitoring – enantiomeric profiling via targeted analysis with chiral-UPLC-TQD
- Mechanisms of degradation of chiral drugs in microcosm experiments – screening of unknowns with chiral UPLC-QTOF
- Enantiomeric profiling in human epidemiology

Drugs of abuse in a river catchment

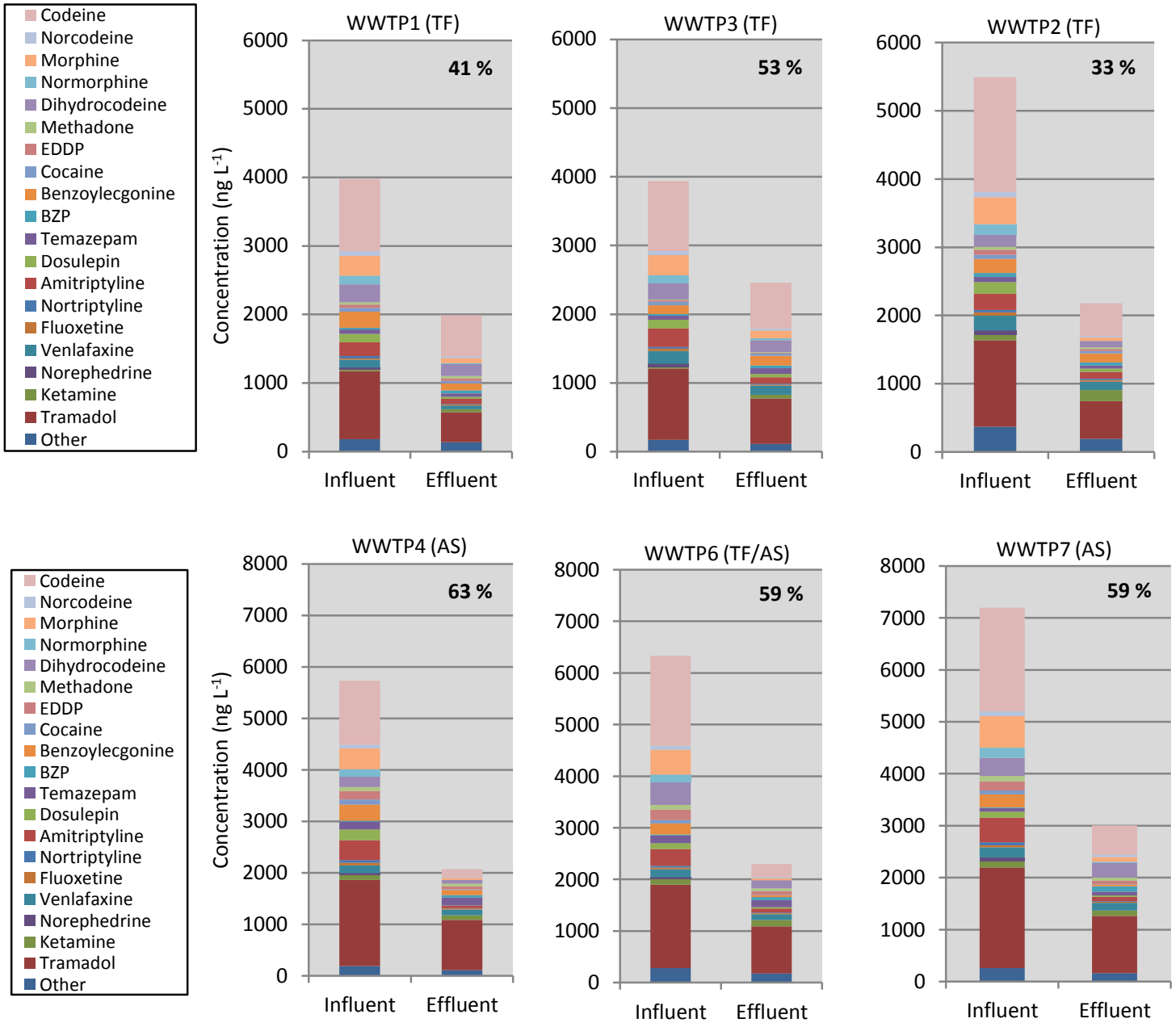


*Results for 64 drugs of abuse, 12 month monitoring programme



- Ephedrine
- Ketamine
- Fluoxetine
- Dosulepin
- Diazepam
- Tramadol
- EDDP
- Morphine
- Codeine
- Nicotine
- MDMA
- BZP
- Cocaethylene
- Cocaine
- Sildenafil
- Venlafaxine
- Nortriptyline
- Oxazepam
- Temazepam
- Norpropoxyphene
- Methadone
- Oxycodone
- 6-acetylmorphine
- 1,7-dimethylxanthine
- MDA
- Methamphetamine
- Norbenzoylecgonine
- Norketamine
- Norfluoxetine
- Amitriptyline
- Nordiazepam
- Nortramadol
- EMDP
- Dihydrocodeine
- Norcodeine
- Continine
- Caffeine
- TFMPP
- Amphetamine
- Benzoylecgonine

Drugs of abuse in WWTPs



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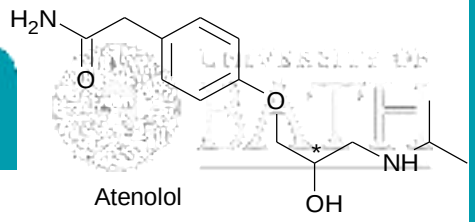
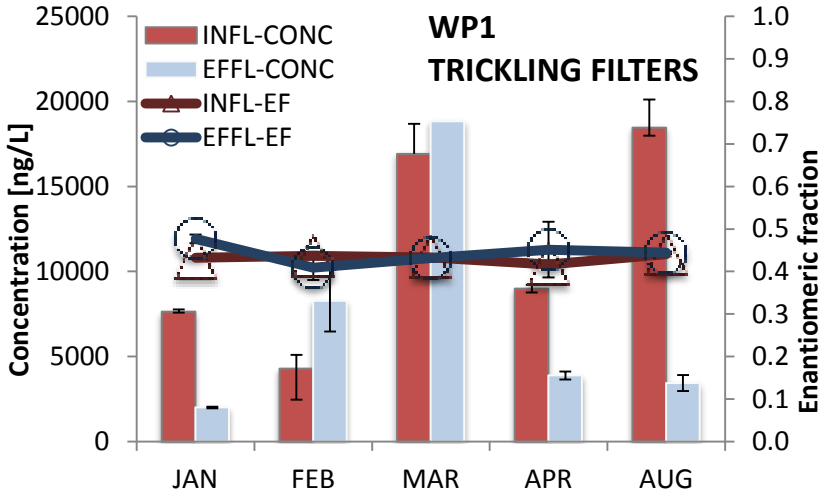
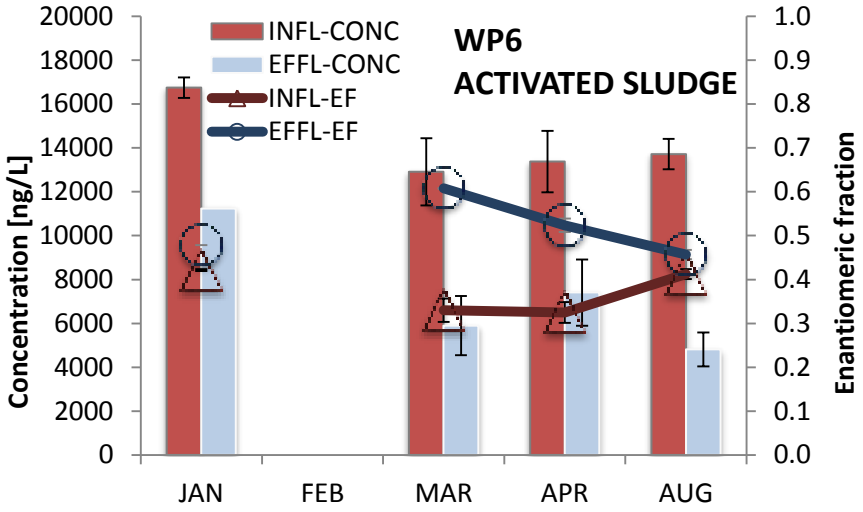
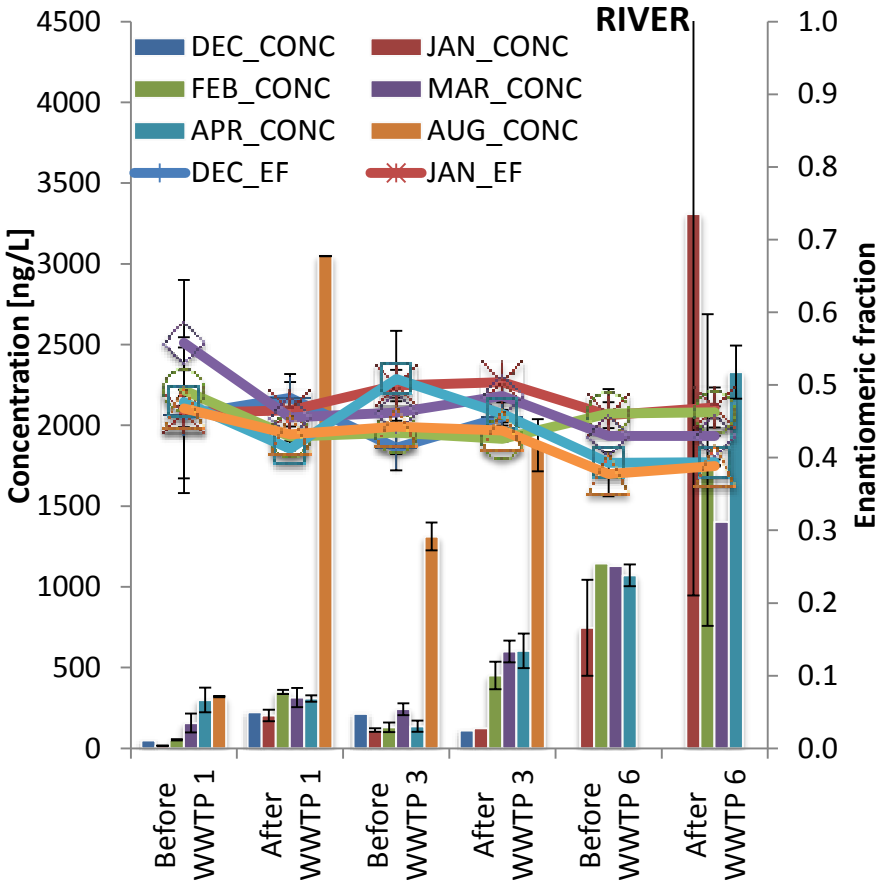


ENANTIOSELECTIVE FATE OF ATENOLOL





$$EF = \frac{R(+)\text{-}ATENOLOL}{R(+)\text{-}ATENOLOL + S(-)\text{-}ATENOLOL}$$



B. Kasprzyk-Hordern, D. Baker, Environ. Sci. Technol., 46 (2012) 1681

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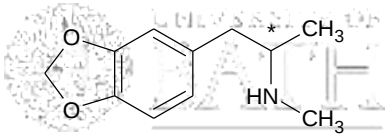
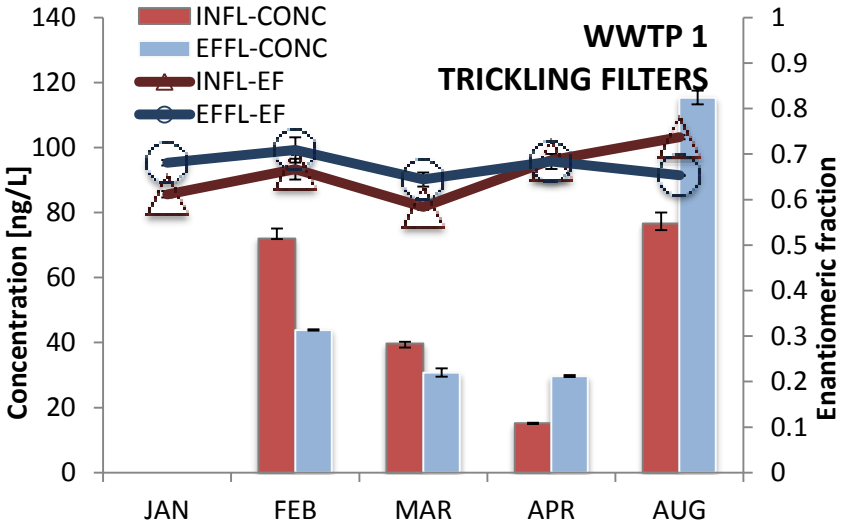
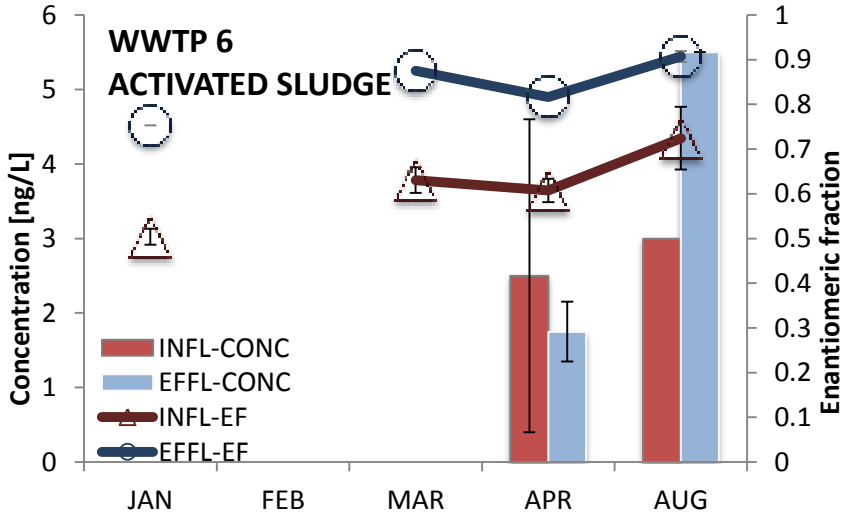


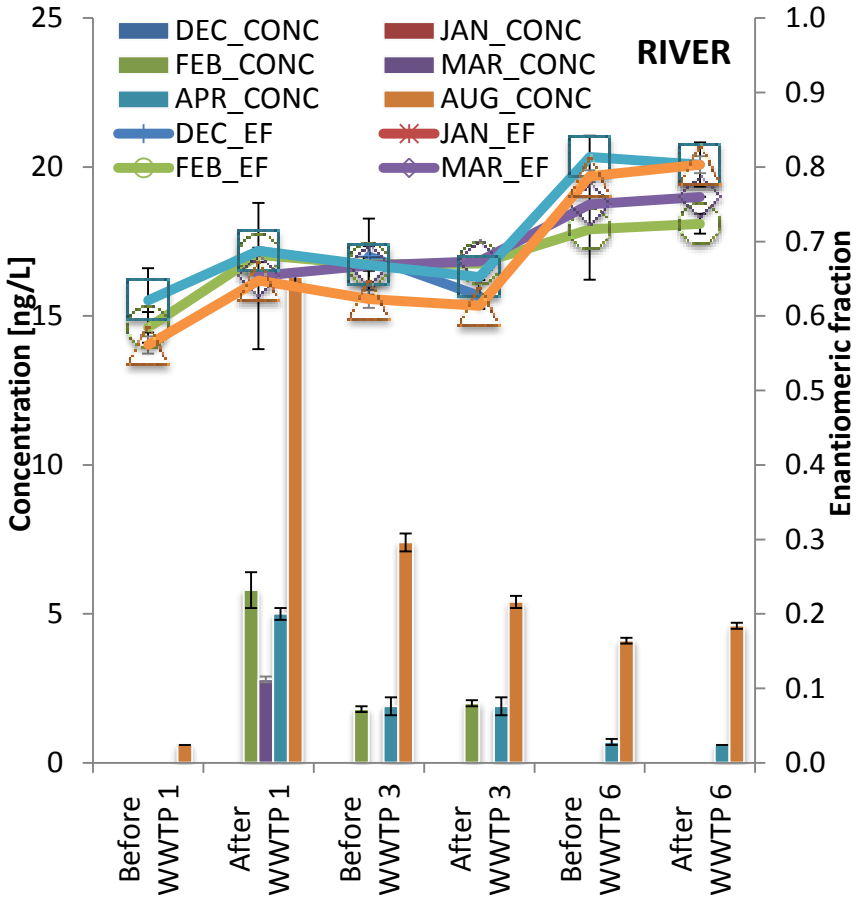
MDMA

ENANTIOSELECTIVE FATE OF MDMA





$$EF = \frac{R(-)\text{-}MDMA}{R(-)\text{-}MDMA + S(+)\text{-}MDMA}$$



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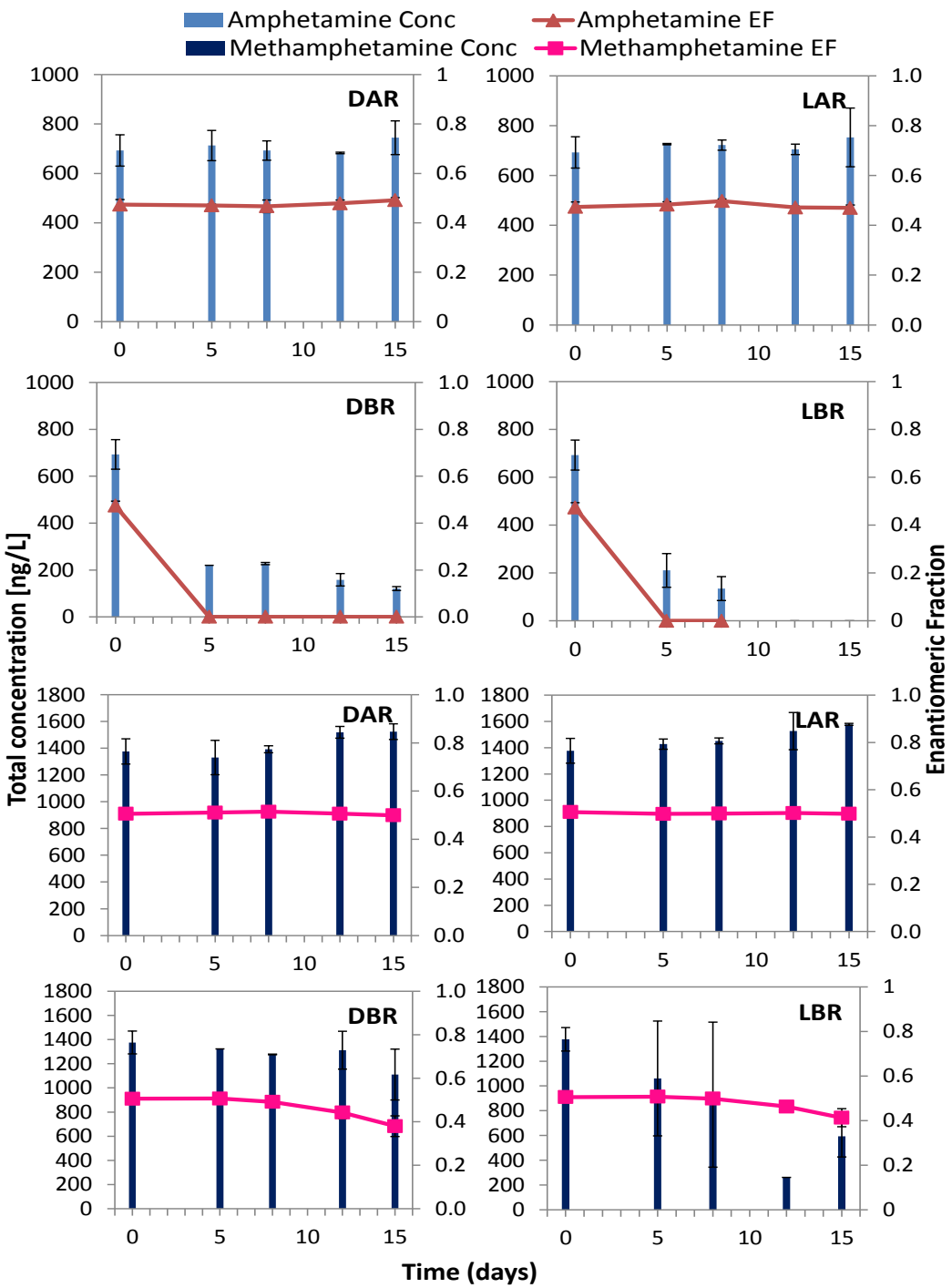
EMERGING ISSUES - ENVIRONMENT

Stereoselective biodegradation of AMP in river microcosms

$$EF = \frac{S(+)AMPH}{R(-)AMPH + S(+)AMPH}$$

$$EF = \frac{S(+)METH}{R(-)METH + S(+)METH}$$

- DAR- Dark Abiotic Reactor
- DBR- Dark Biotic Reactor
- LAR- Light Abiotic Reactor
- LBR- Light Biotic Reactor



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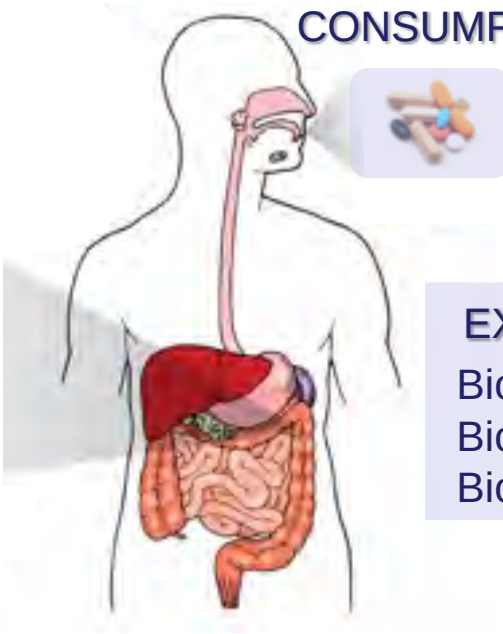
HUMAN EPIDEMIOLOGY using wastewater profiling

CONSUMPTION



Metabolism

EXCRETION

Biomarkers of exposure
Biomarkers of disease
Biomarkers of lifestyle

WASTEWATER TREATMENT

SEWAGE PROFILING

- HEALTH: exposure to environmental & food toxicants
- DISEASE: cancer, infectious disease
- LIFESTYLE: smoking, alcohol use, drugs abuse

COMMUNITY-WIDE HEATH & LIFESTYLE ASSESSMENT

SEWAGE EPIDEMIOLOGY

● CONCEPTUALLY SIMPLE ● METHODOLOGICALLY SOPHISTICATED

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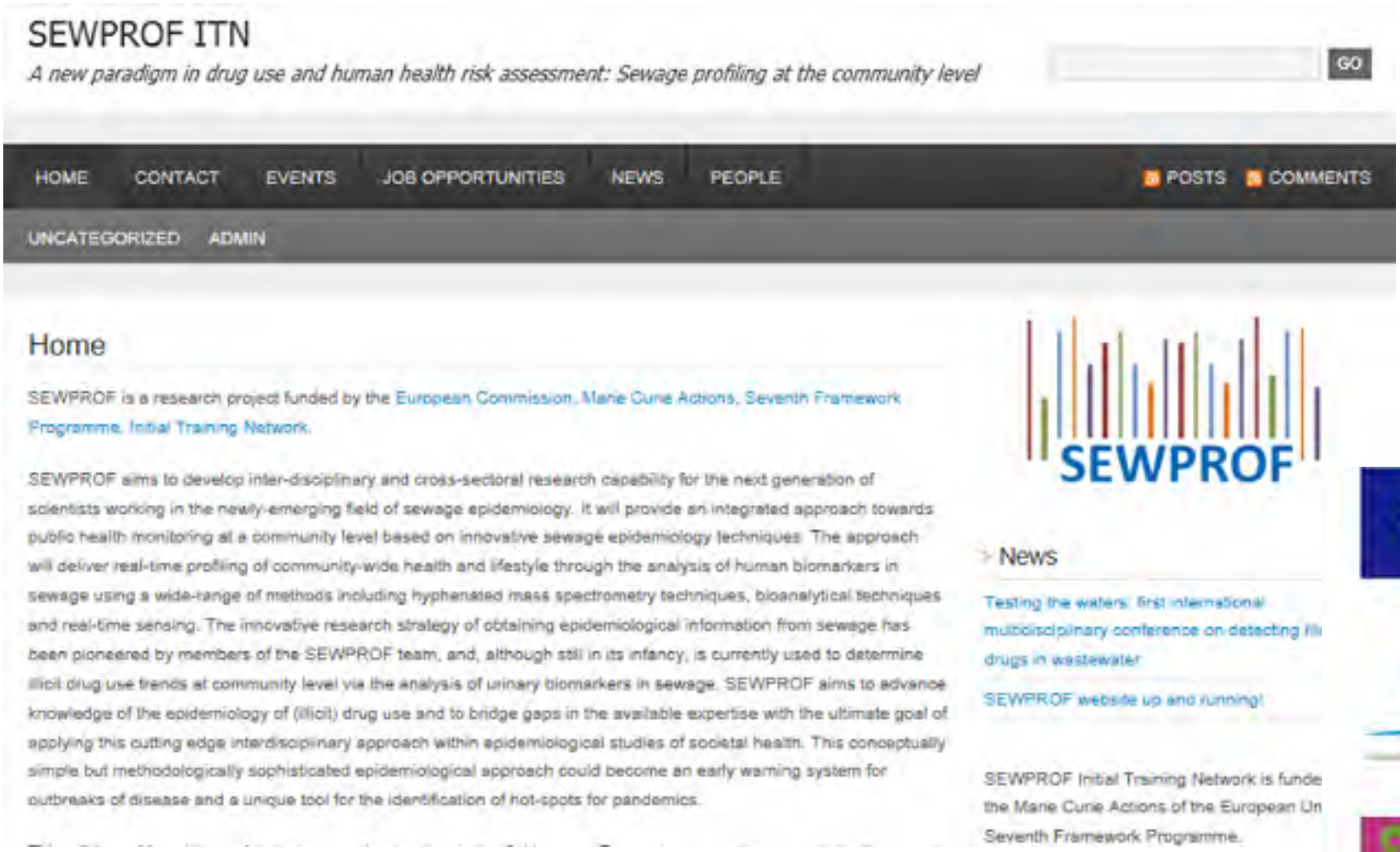

PEOPLE

MARIE CURIE ACTIONS

GO TO: www.sewprof-itn.eu

Daily consumption

$$mg\ day^{-1} 1000\ people^{-1} \frac{\left(\frac{100}{Excretion}\right)\left(\frac{MW_{Par}}{MW_{DTR}}\right)}{\frac{Population}{1000}} - OS$$

Compound	DTR	WWTP Influent Daily usage (mg day^{-1} 1000 people^{-1})	
Cocaine	Benzoylecgonine	250	
Amphetamine	Amphetamine	286	
Methamphetamine	Methamphet.	1	
MDMA	MDMA	73	
Oxycodone	Oxycodone	54 (NHS)	50
Dihydrocodeine	Dihydrocodeine	225 (NHS)	213
Methadone	Methadone	97	
Amitriptyline	Nortiptyline	419 (NHS)	588
Fluoxetine	Fluoxetine Norfluoxetine	266 (NHS)	260 308

Load -amount of DTR arriving at the WWTP (g day^{-1});
Excretion - the percentage excretion of the DTR after relevant forms of administration;
MW_{Par} - the molecular weight of the parent compound and MW_{DTR} - the molecular weight of the DTR.
OS - the amount of DTR that is present in wastewater due to other sources other than the parent compound, if applicable (mg day^{-1} 1000 people^{-1}).

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SEWAGE EPIDEMIOLOGY – IMPORTANCE OF ENANTIOMERIC PROFILING:

- Distinction between legal and illicit use of drugs
- Verification of the method of synthesis of illicit drugs
- Identification of whether drug residue results from consumption of illicit drug or metabolism of other (illicit) drug
- Verification of potency of abused drugs

MDMA AND MDA

- No medical use
- Produced as racemate
- Metabolism favours *S*(+)-enantiomer
- Preferential metabolism of *S*(+)-MDMA leads to enrichment of MDMA with *R*(-)-enantiomer and formation of *S*(+)-MDA (twice as much S(+)-MDA is excreted in urine as compared to R(-)-MDA)

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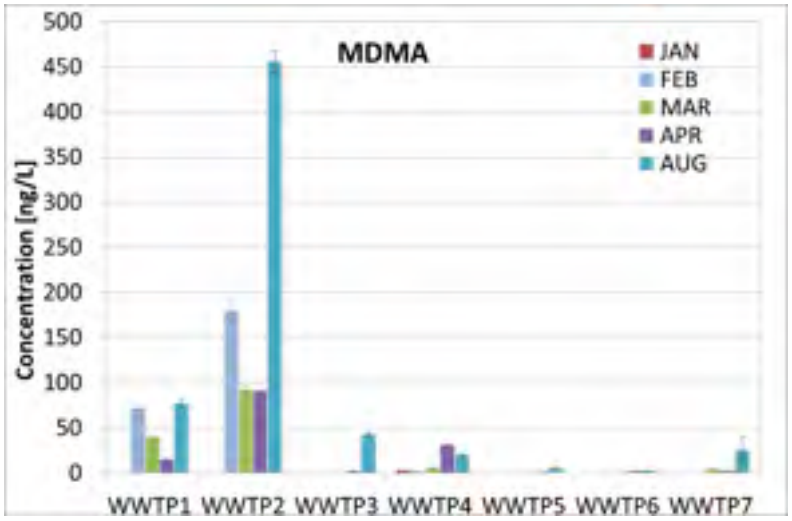
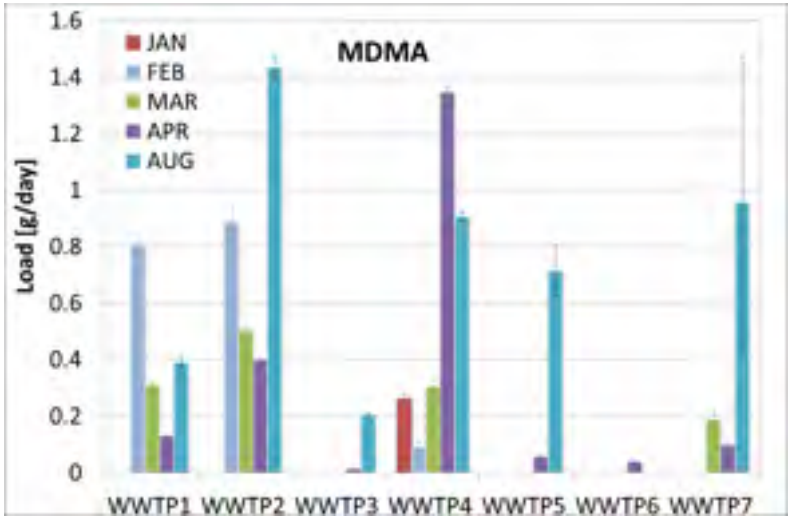
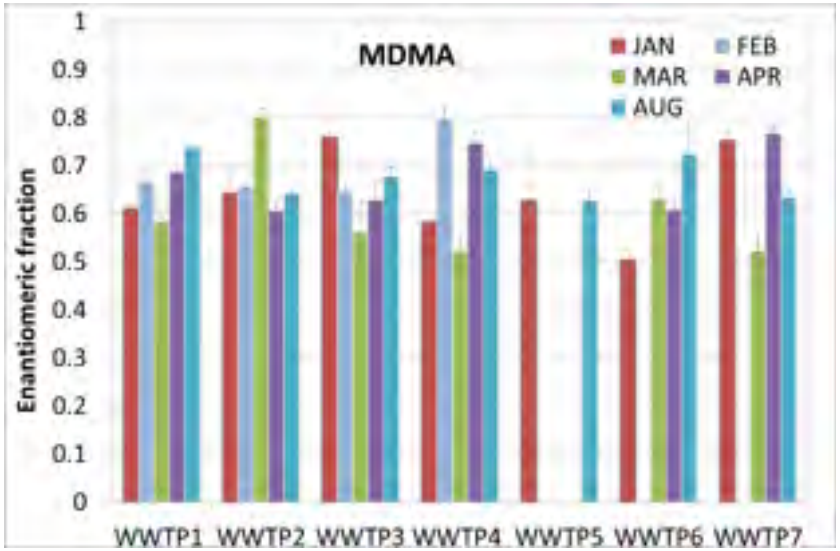
MDMA in 7 WWTPs in England





$$EF = \frac{R(-)MDMA}{R(-)MDMA + S(+)MDMA}$$



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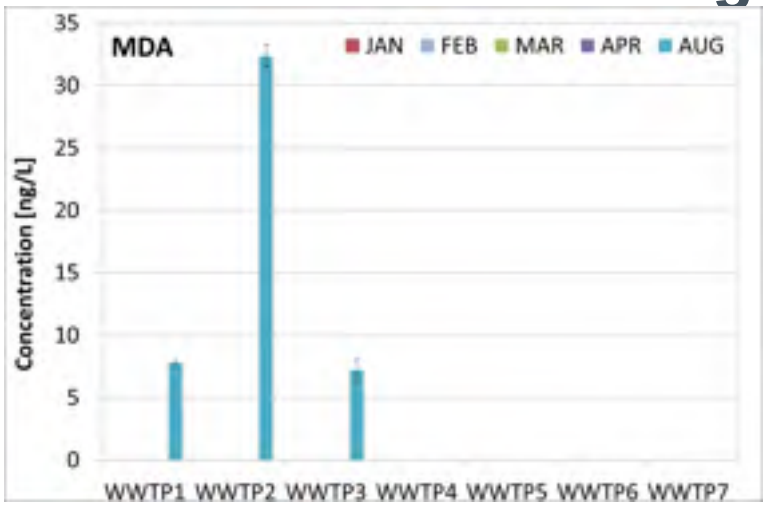
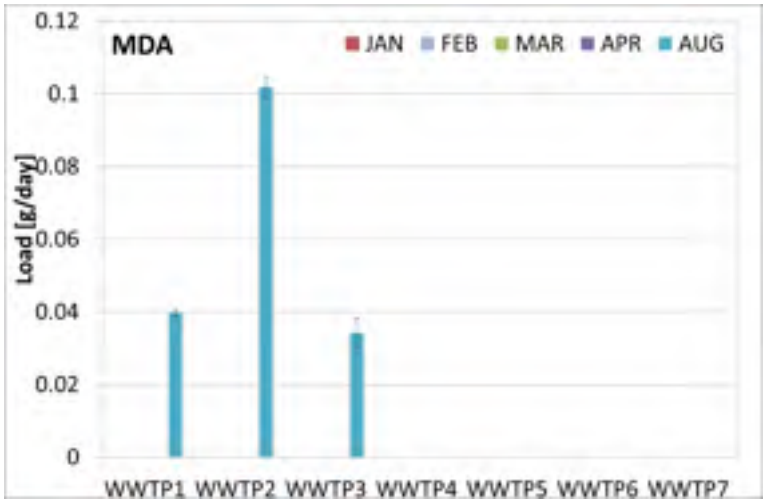
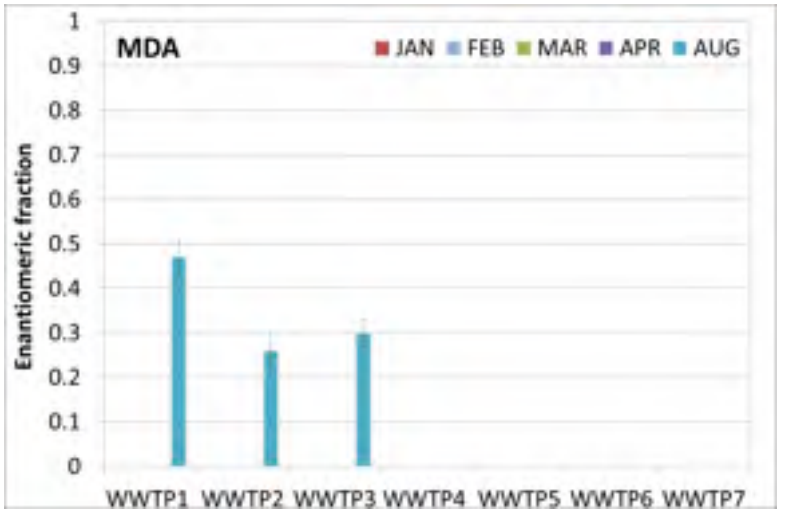
MDA in 7 WWTPs in England





$$EF = \frac{R(-)MDA}{R(-)MDA + S(+)MDA}$$



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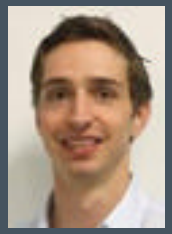
Conclusions

Enantiomeric profiling of chiral drugs in environmental matrices:

- Provides invaluable information about environmental fate and toxicity of chiral drugs
- Should be incorporated into environmental risk assessment of chiral drugs
- Provides yet another dimension to sewage epidemiology as it helps with:
  - Distinction between legal and illicit use of drugs
  - Verification of the method of synthesis of illicit drugs
  - Identification of whether drug residue results from consumption of illicit drug or metabolism of other (illicit) drug
  - Verification of potency of abused drugs

Acknowledgments

Main contributors:



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