Pharmaceutical residues removal in a pilot wasterwater treatment plant

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Presentation Outline

Introduction

Experimental

Identification

- Target compounds
- Analytical procedure

Elimination

- Pilot wastewater treatment plant
- Identification of degradation products
- Biomass adaptation

Results and Discussion

Conclusions and Future work

Pharmaceuticals: importance and characteristics

- indispensable element of modern life
- highly biologically active
- often highly water soluble
- not readily (bio)degradable → chemical stability needed for pharmacological action

MOBILITY IN WATER

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Pharmaceuticals: purchase

- **Germany:** human pharmaceuticals **6500 t/year** (78g per capita per year)
- consumption expected to increase (demographic changes):
  - ✓ growing life expectancy at birth
  - ✓ higher % of old population
- approx. 90% dispensed in retail pharmacies
  - ✓ 70 % prescribed by doctors
  - ✓ 20 % bought for self-medication (over-the-counter = OTC drugs)

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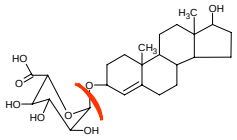
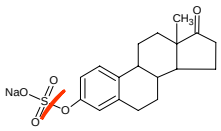
Pharmaceuticals: characteristics

- metabolism in human body → combination of parent compounds and metabolites → release into aquatic environment
- 2nd phase metabolite → in WWTP often degraded back into parent compound / 1st phase metabolite



testosterone glucuronide



estrone sulphate

- combinations: synegistic / additive effects???

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Risks

- occurence:
  - ✓ environmental persistence
  - ✓ high consumption

- risks deriving from chronic low-dosage exposure to pharmaceutical residues: not yet evaluated
- reported aquatic toxicity:
  - ✓ estrogenic effects of contraceptive drugs;
  - ✓ antibiotic resistance;
  - ✓ vulture decline from diclofenac

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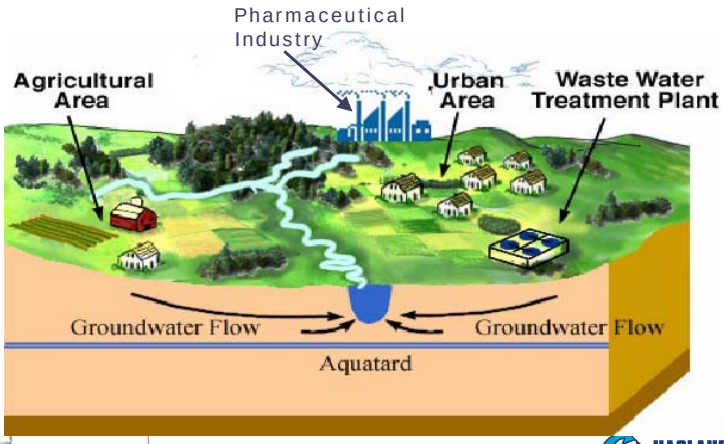
Exposure

Sources of pharmaceuticals in the environment



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Goals of Our Study

Criteria for selection of pharmaceuticals: widespread use in Central Europe

Members of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Relatively well known acute toxicity

Poorly investigated chronic effects of low dosages; possible synergistic effects

PRESENCE IN THE ENVIRONMENT????

PERSISTENCE IN THE ENVIRONMENT?????

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Pharmaceutical Residues in Slovene Surface Waters

sample	Date of sampling	koncentracija (ng L^{-1})			
		naproksen	diklofenak	ketoprofen	ibuprofen
KRKA 1	jul.04	-	-	-	-
	sep.04	-	-	-	-
	apr.05	-	-	-	-
KRKA 2	jul.04	310	280	-	-
	sep.04	60	50	-	-
	apr.05	280	310	120	-
LJUBLJANICA 1	jul.04	-	-	-	-
	sep.04	-	-	-	-
LJUBLJANICA 2	jul.04	70	-	-	-
	sep.04	-	-	-	-
SAVA	jul.04	80	10	-	-
	sep.04	-	-	-	-
MURA	jul.04	50	40	-	-
DRAVA 1	jul.04	50	30	-	-
	sep.04	-	-	-	-
DRAVA 2	jul.04	20	30	-	-
	sep.04	40	-	-	-
PŠATA	jul.04	20	-	-	-

T. Kosjek, E. Heath, A. Krbavčič; Environment International 31 (2005) 679-685.

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Pharmaceutical Residues in sediments

	River water (14.4.2005) (ng L^{-1})		Sediment (14.4.2005) (ng g^{-1})	
	River Krka (**upstream**)	River Krka (**downstream** the pharmaceutical factory and urban area)	River Krka (**upstream**)	River Krka (**downstream** the pharmaceutical factory and urban area)
ibuprofen	udl	udl	udl	udl
naproxen	udl	308	udl	26
ketoprofen	udl	281	udl	320
diclofenac	udl	89	udl	udl

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Hypothesis

Pharmaceutical residues have been reported in surface, ground and drinking waters.

How to diminish / prevent their entrance into the environment?

A. proper handling of excess / expired pharmaceuticals

B. diminishing the discharge of pharmaceuticals at the point-sources of pollution:

- **municipal & hospital WWTP**
- **effluents from pharmaceutical industry**

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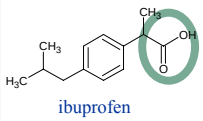
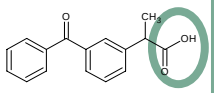
Experimental

Acidic pharmaceuticals

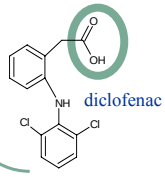
Nonsteroidal antiinflammatory drugs (NSAIDs)



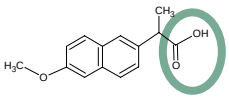
ibuprofen



ketoprofen

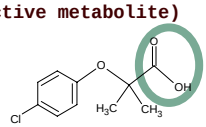


diclofenac



naproxen

Blood lipid regulator (active metabolite)



clofibric acid

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Pharmaceuticals: potential environmental effects

Nonsteroidal antiinflammatory drugs (NSAIDs):

represenatives
Diclofenac (Voltaren®), naproxen, ketoprofen, ibuprofen

side effects
Abdominal pain, gastrointestinal reflux, asthma, prolonged blood coagulation

- ✓ diclofenac: **resistant to biodegradation**
- ✓ reported **environmental toxicity**
- ✓ applied in high amounts; **available without prescription**
- ✓ interactions with numerous pharmaceuticals: syneristic, additive, toxic effects

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Experimental

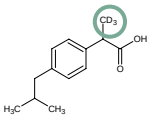
Analytical procedure for determination of pharmaceuticals in water samples (1)

1. Sample Preparation

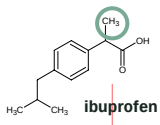
A. sample pre-preparation (acidification, filtration,…)

B. internal standard addition:

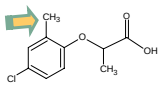
d3-ibuprofen



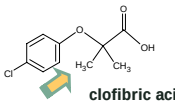
ibuprofen



mecoprop



clofibric acid



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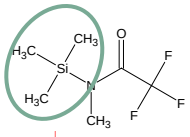
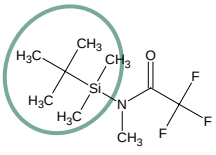
Experimental

Analytical procedure for determination of pharmaceuticals in water samples (2)

C. solid phase extraction (SPE): Strata™ X, Oasis® HLB extraction cartridges

D. derivatisation: MSTFA (N-methyl-N-trimethylsilyl trifluoracetamide), MTBSTFA (N-methyl-N-(*tert*-butyldimethylsilyl) trifluoracetamide





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Experimental

Analytical procedure for determination of pharmaceuticals in water samples (3)

2. Chemical Analysis: GC-MSD

A. separation: gas chromatography: HP 6890 GC, capillariy column Zebron ZB-5 (Phenomenex, 30m x 0,25mm x 0,25um); carrier gas: He; velocity 37 cm s^{-1}; injection: "splitless" mode; T injector: 250°C; volume inj.: 1µL

B. identification: mass spectrometry

- **quantitative determination: sim mode with characteristic ion fragments (internal standard addition).** m/z for MTBSTFA derivatisation: ibuprofen *m/z* = 263, ibuprofen-D3 *m/z* = 266, naproxen *m/z* = 287, ketoprofen *m/z* = 311, diclofenac *m/z* = 352 & 354
- **identification of degradation products: scan mode**

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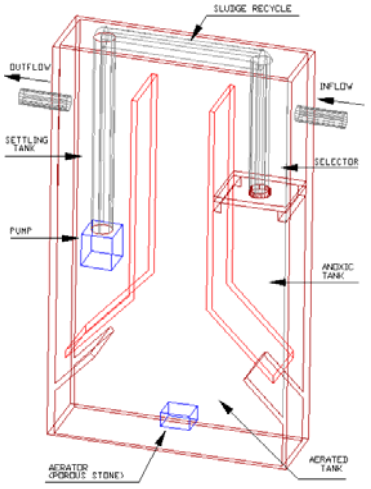
Persistence / removal in PWWTP?

PWWTP design

- aerobic and anaerobic / anoxic segment
- continuous supply with pharmaceuticals at flow rate 2 L day^{-1}
- residence time = 48 hours
- reactor volume = 4 L
- active sludge origin: Slovene municipal wastewater treatment plant
- sampling: influent & effluent



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Persistence / removal in PWWTP?

Pilot wastewater treatment plant (PWWTP)

- R0 = blank
- R1 = 0,05 mg L^{-1} of each pharmaceutical
- R2 = 0,005 mg L^{-1} of each pharmaceutical

$$\%\ el.eff. = \frac{(c_{X-INF} - c_{X-EFF})}{c_{X-INF}} \times 100$$

- PWWTP operation: since june 2004
- Sampling: first 6 months on a weekly basis, later on a monthly basis

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Results

Removal of pharmaceuticals in PWWTP

PHARMACEUTICAL	REACTOR	AV. REMOVAL RATE (%)	STDEV
IBUPROFEN	R1	95,6	4
	R2	96,4	6
NAPROXEN	R1	96,1	3
	R2	88,8	8
KETOPROFEN	R1	94,9	5
	R2	90,3	6
DICLOFENAC	R1	54,4	23
	R2	56,4	36
CLOFIBRIC ACID	R1	24,2	22
	R2	29,5	20

- High and constant removal of ibuprofen, naproxen and ketoprofen
- Poor and variable removal of diclofenac and clofibric acid

- Removal mechanisms: (bio)degradation and/or adsorption

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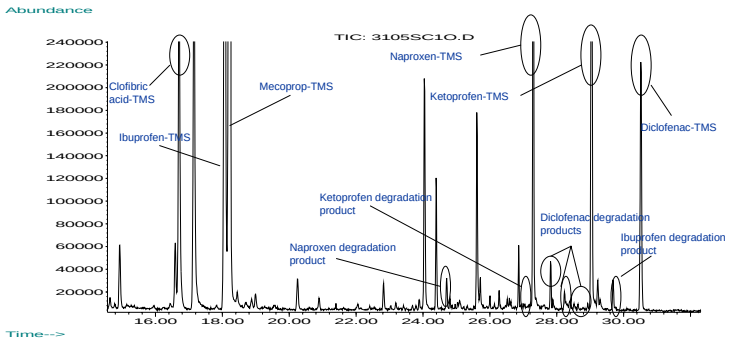
Results

Identification of degradation products (1)



- trimethylsilyl esters of residual pharmaceuticals and internal standard
- degradation products are signposted

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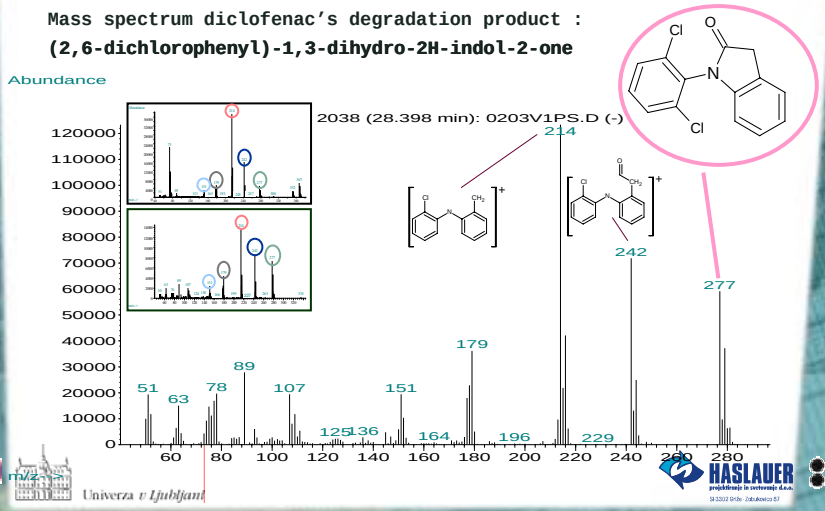
Results

Identification of degradation products (2)

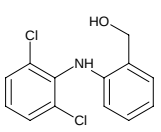
Mass spectrum diclofenac's degradation product :
(2,6-dichlorophenyl)-1,3-dihydro-2H-indol-2-one



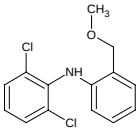
Results

Identification of degradation products (3)

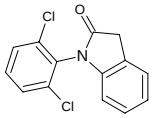
Diclofenac's degradation products:



2-((2,6-dichlorophenyl) -amino) benzyl alcohol

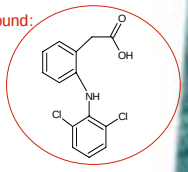


methyl ether of 2-((2,6-dichlorophenyl)-amino) benzyl alcohol

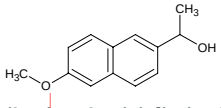


1-(2,6-dichlorophenyl) -1,3-dihydro-2*H*-indol-2-one

Parent compound:

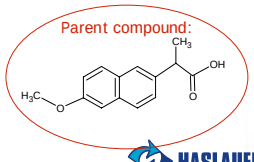


Naproxen's degradation product:



(6-methoxy -2-naphthyl) ethanol

Parent compound:



Results

Study of biomass adaptation (1)

- Two years of continuous operation … → adaptation of the biomass???
- **T-RFLP** (Terminal-Restriction Fragment Length Polymorphism) analysis: differences in bacterial community between R1 compared to R0 and R2 model reactors

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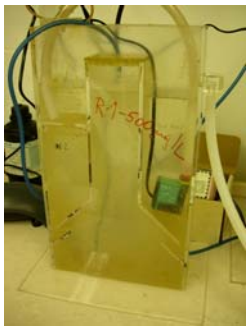
Results

Study of biomass adaptation (2)

- Replication of R1 biomass to:
  - model reactor operating under same concentration 50 µg/L: R1r/50
  - 4-times higher conc., 200 µg/L: R1r/200,
  - 10-times higher conc., 500 µg/L: R1r/500)

→ immediate acclimatization of R1r/50



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Future work

- optimisation of WWTP operation to improve removal of clofibric acid and diclofenac (e.g. integration of anaerobic segment)
- additional methods to improve removal of persistent compounds, e.g. ozonation/H_2O_2, UV treatment, etc…
- determination of removal mechanism of the selected pharmaceutical residues
- influence of light (photolysis) on the removal efficiency

Future work

- wider range of pharmaceuticals involved into the (bio)degradation study (carbamazepine, estrogenic compounds, antitumor drugs,…)
- toxicity (ecotoxicity, genotoxicity, cytotoxicity,…) of pharmaceuticals and their degradation products in collaboration with National Institute of Biology, Slovenia
- the new-developed treatment procedure on larger scale pilot wastewater treatment plant

Acknowledgements

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National Institute of Biology, Slovenia

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- Microbial community: Prof. dr. Ines Mandič Mulec and coworkers