Occurrence and transformation of benzodiazepine pharmaceuticals in the environment

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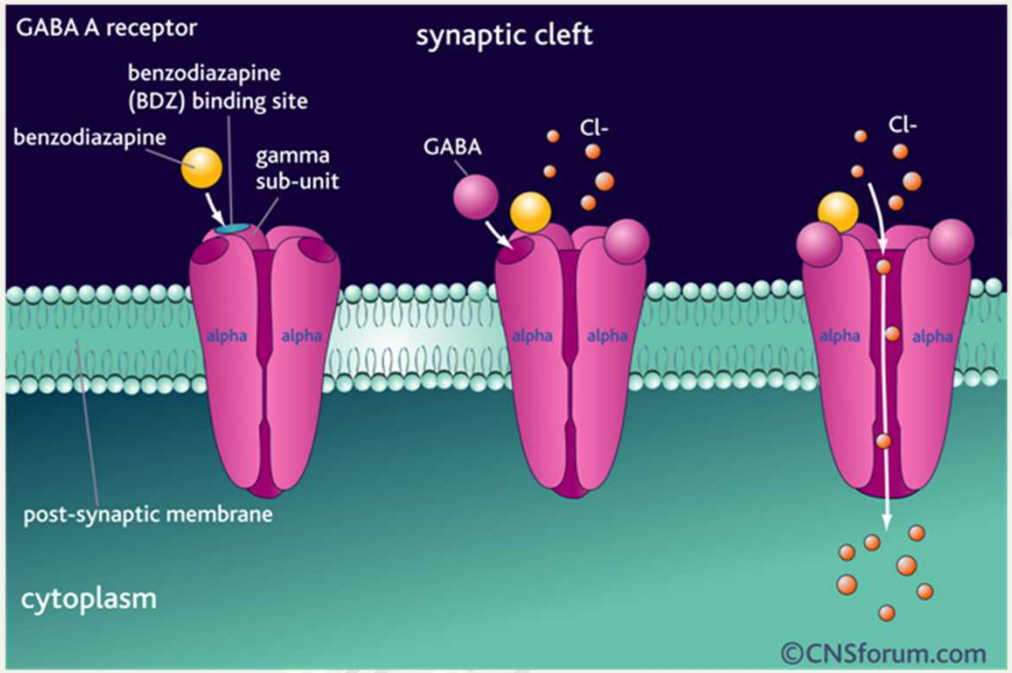
Benzodiazepines

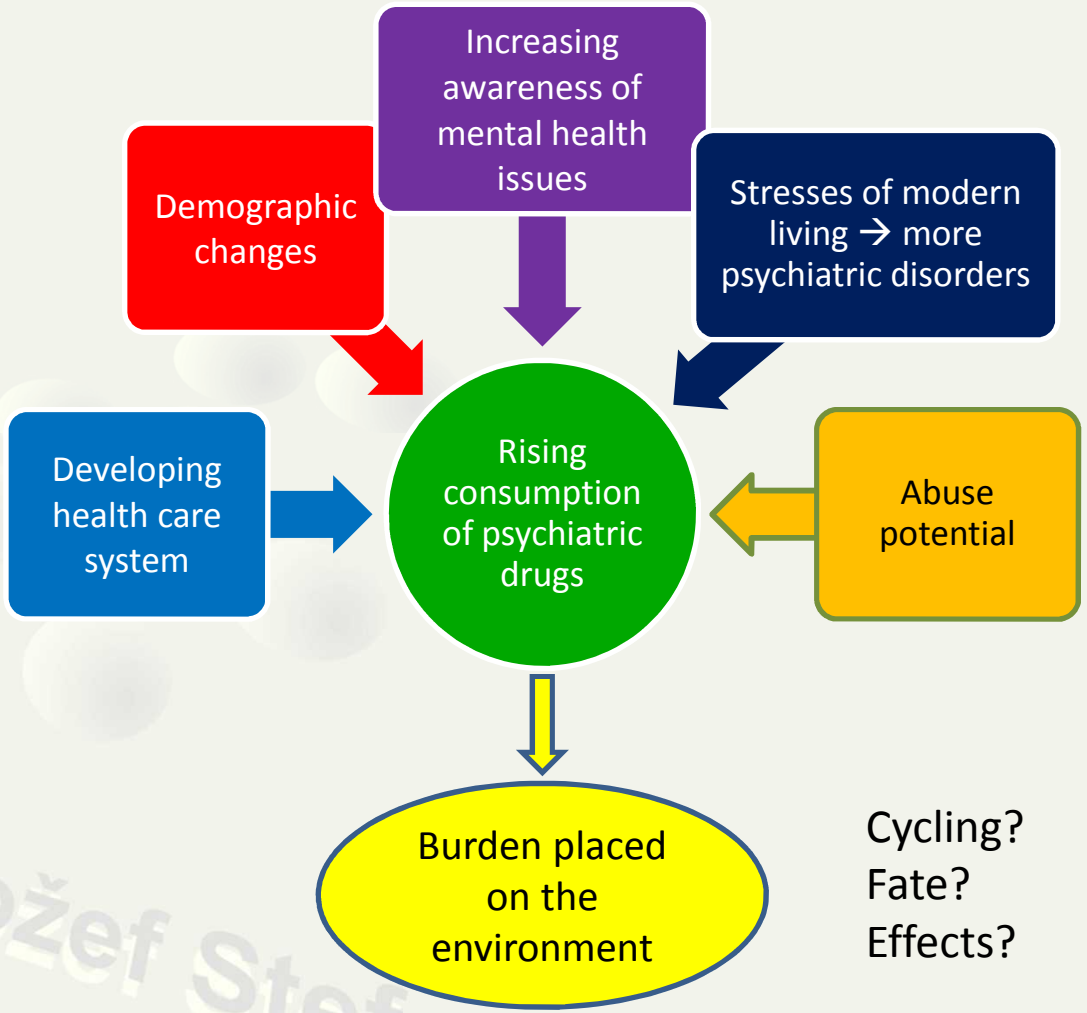
tranquilizers, anxiolytics, anticonvulsants



- treatment of anxiety, sleep disorders, convulsive states; centrally acting muscle relaxants, premedication and as inducing agents in anaesthesiology

- bind to the GABA-A receptor → allosteric modification of the receptor → increase the frequency of channel opening events → increase in chloride ion conductance & inhibition of the action potential

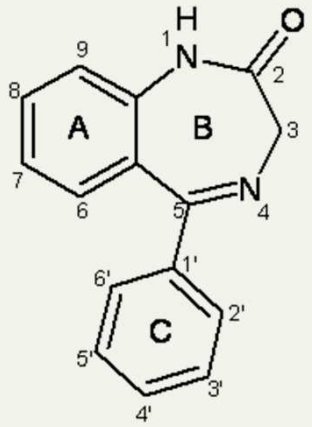
Why benzodiazepines?

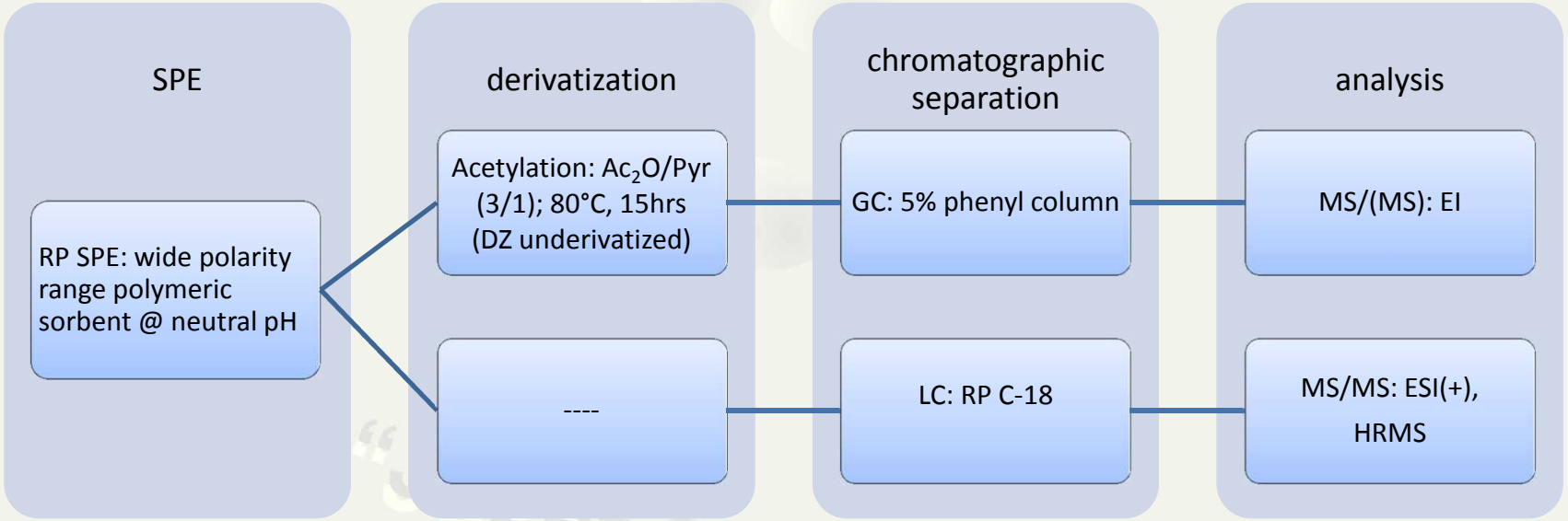


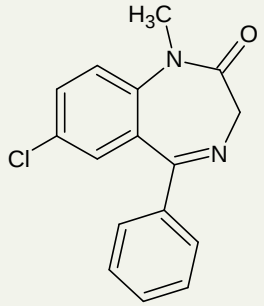
Cycling?
Fate?
Effects?

Chemical analysis

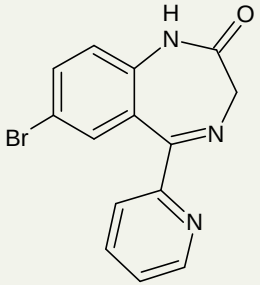
- Confirmatory
- Quantification
- Identification of transformation products



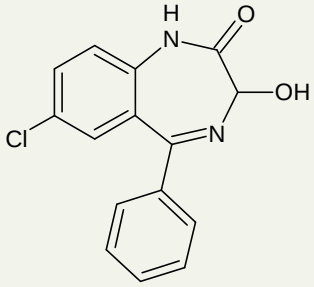




Diazepam (DZ)



Bromazepam (BZ)

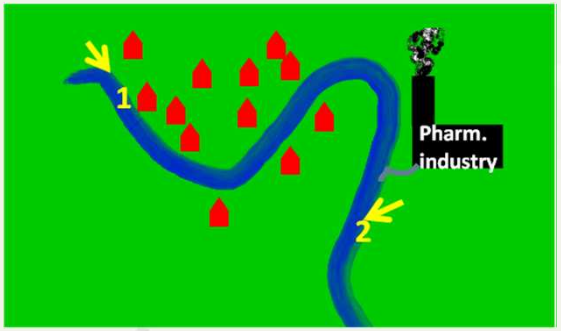


Oxazepam (OXA)

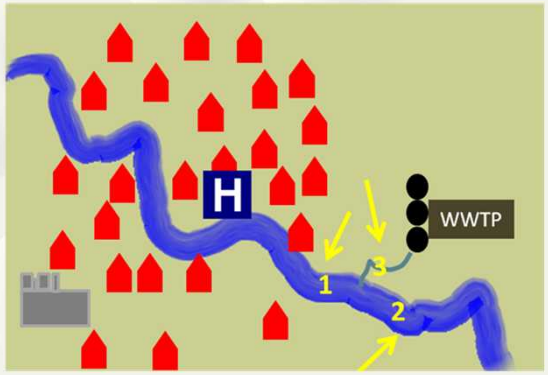
Occurrence

(surface waters, wastewaters)

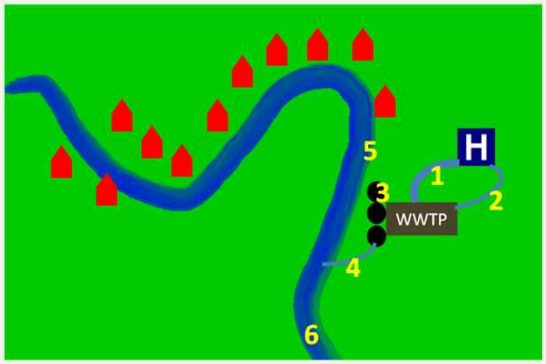
Town A
(22 000 inhabitants)



Town B
(360 000 inhabitants)



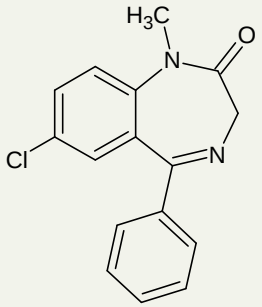
Town C
(18 000 inhabitants)



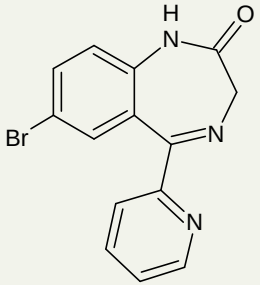
- sampling approach: time proportional (24hrs) or grab
- sampling campaigns : winter and spring 2011

Occurrence *(cont'd)*

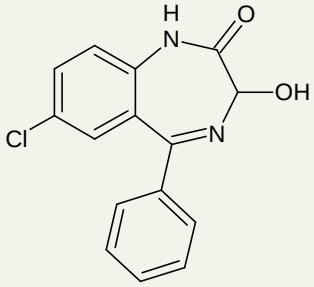
(surface waters, wastewaters)



Diazepam (DZ)



Bromazepam (BZ)



Oxazepam (OXA)

Analyte	WWTP influent			WWTP effluent			River water		
	c_{range} (ngL^{-1})	c_{med} (ngL^{-1})	$\%_{positive}$	c_{range} (ngL^{-1})	c_{med} (ngL^{-1})	$\%_{positive}$	c_{range} (ngL^{-1})	c_{med} (ngL^{-1})	$\%_{positive}$
DZ	17-111	26	100	18-22	22	100	9-69	20	78
BZ	40-158	99	25	--	32	30	6-19	13	50
OXA	41-72	56	67	28-133	65	100	11-31	26	56

• Legend: c_{range}: concentration range, c_{med}: median concentration, % positive: percentage of > LOD samples

Occurrence *(detailed)*

Table 2 – Concentrations (ng L^{-1}) of OXA, DZ and BZ determined in the wastewater and river water samples.

	Sampling point	Sampling date	Sampling approach	c(OXA) ng/L	c(BZ) ng/L	c(DZ) ng/L
Town A						
River before municipality	1-A	Winter 2011	Grab	<LOD	<LOD	<LOD
River after municipality and pharmaceutical industry	2-A	Winter 2011	Grab	<LOD	<LOD	<LOD
Town B						
Hospital effluent 1	1-B	Winter 2011	Composite 24 h	<LOD	40	27
	1-B	Spring 2011	Composite 24 h	72	<LOD	49
Hospital effluent 2	2-B	Winter 2011	Composite 24 h	<LOD	<LOD	17
	2-B	Spring 2011	Composite 24 h	41	158	111
WWTP influent	3-B	Winter 2011	Composite 24 h	58	<LOD	21
	3-B	Spring 2011	Composite 24 h	54	<LOD	25
WWTP effluent	4-B	Winter 2011	Composite 24 h	28	32	18
	4-B	Spring 2011	Composite 24 h	46	<LOD	22
Stream before effluent	5-B	Winter 2011	Composite 24 h	11	6	17
	5-B	Spring 2011	Composite 24 h	21	9	28
Stream after effluent	6-B	Winter 2011	Composite 24 h	30	17	21
	6-B	Spring 2011	Composite 24 h	31	19	20
Town C						
River before WWTP discharge	1-C	Spring 2011	Grab	<LOD	<LOD	9
River after WWTP discharge	2-C	Spring 2011	Grab	<LOD	<LOD	69
	2-C	Winter 2011	Grab	<LOQ	<LOD	13
WWTP effluent	3-C	Spring 2011	Grab	84	<LOD	21
	3-C	Winter 2011	Grab	133	<LOD	22

Fate

Microbiological

- oxic/anoxic flow-through reactors, suspended biomass

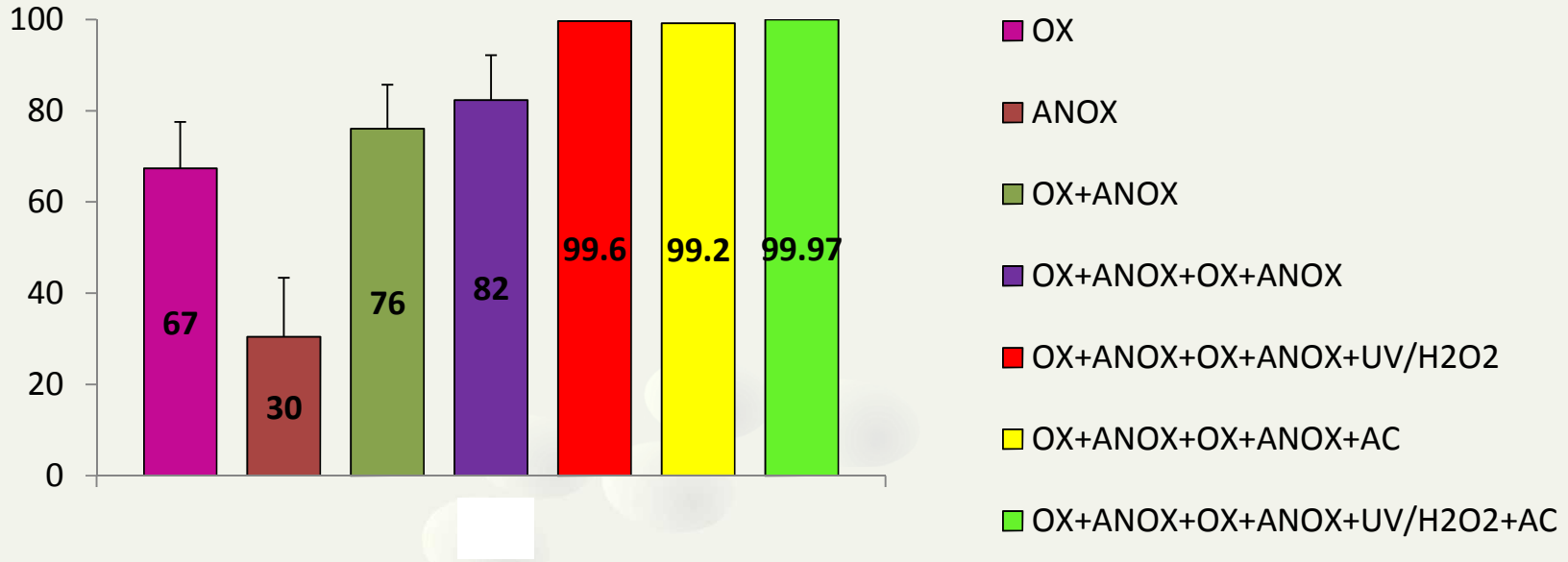
Abiotic

- photolytic / photochemical ($UV+H_2O_2$) degradation

- sorption to activated carbon

Sequential treatment of DZ

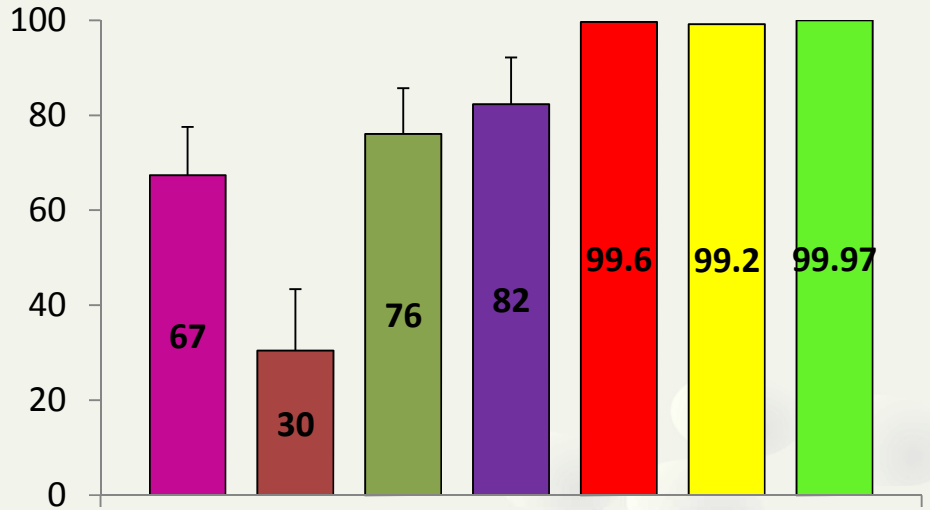
99.9% removal may not be sufficient!



Literature data: low removal of DZ

Sequential treatment of DZ

99.9% removal may not be sufficient!



- OX
- ANOX
- OX+ANOX
- OX+ANOX+OX+ANOX
- OX+ANOX+OX+ANOX+UV/H2O2
- OX+ANOX+OX+ANOX+AC
- OX+ANOX+OX+ANOX+UV/H2O2+AC

INFLUENT
c(DZ) = 94µg/L

EFFLUENT
c(DZ) = 18µg/L
c(OXA) = 2.2µg/L

UV/H_2O_2
c(DZ)=170ng/L
c(OXA)=166ng/L

AC
c(DZ)=16ng/L
c(OXA)=10ng/L

AC
c(DZ)=450ng/L
c(OXA)=136ng/L

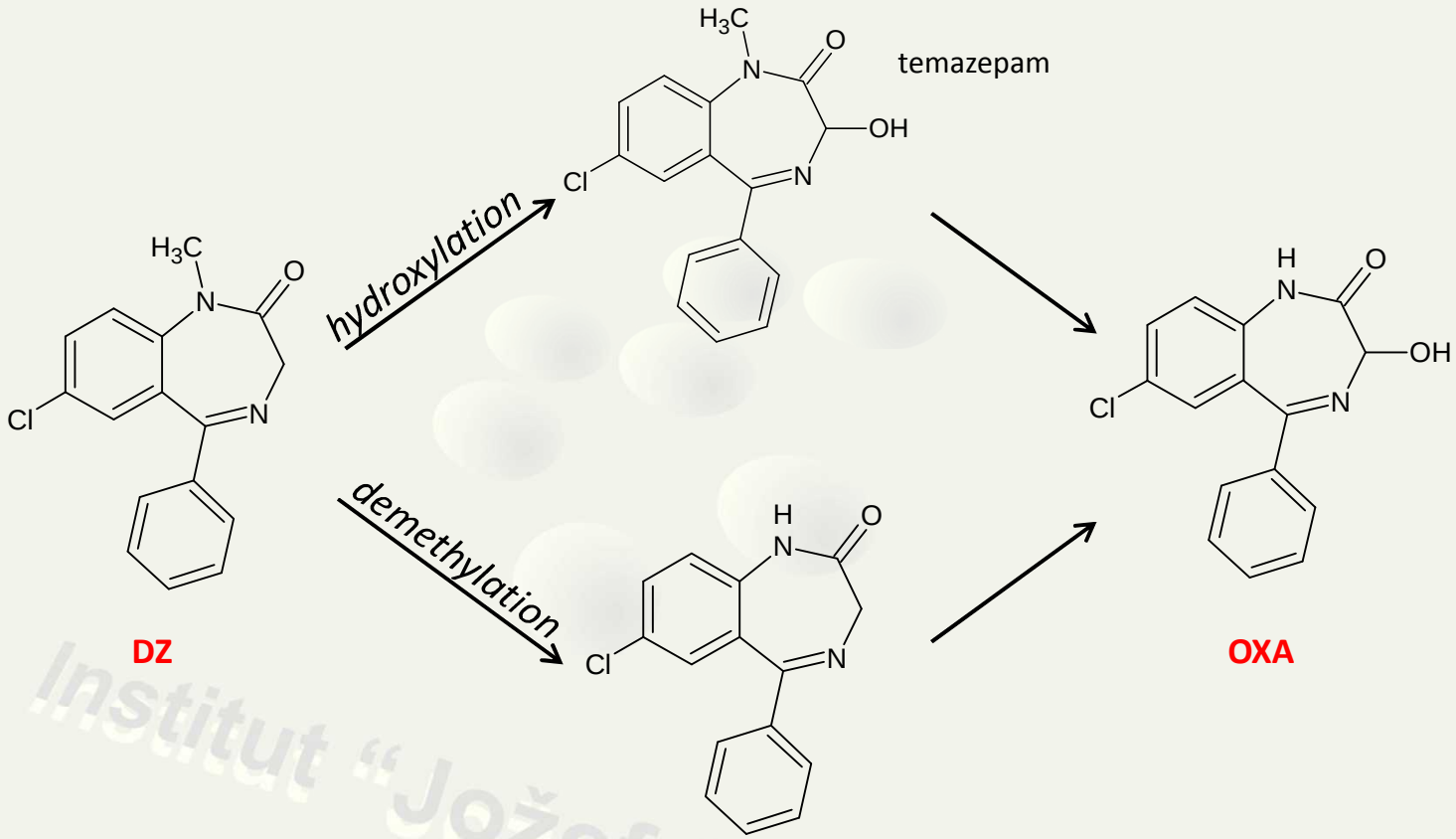
Literature data: low removal of DZ

Not applicable to ”real world”

THE ABATEMENT OF A PARENT PHARMACEUTICAL DOES NOT PROVIDE THE INDICATION OF TREATMENT EFFICIENCY → TRANSFORMATION PRODUCTS (persistence, toxicity?)

Transformation of DZ

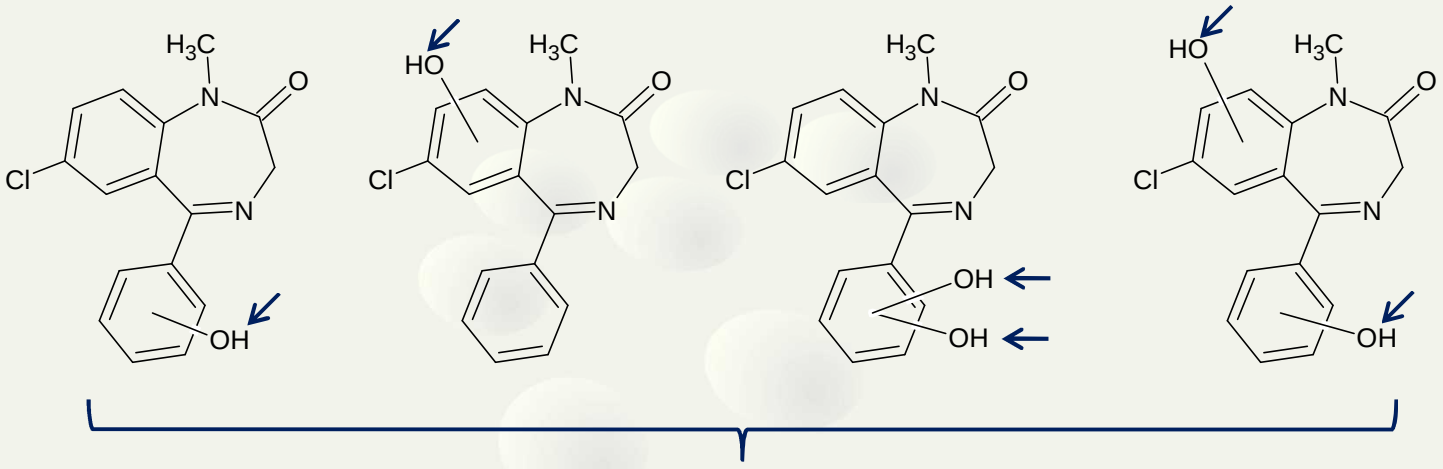
A.\ Mimicking human metabolism:

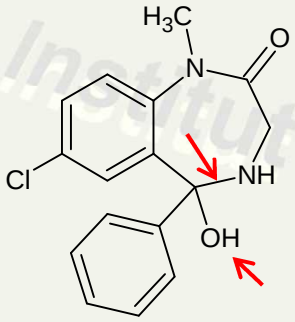


Transformation of DZ *(cont'd)*

B.\ Different than human metabolism

→ novel, previously unrecognized compounds:



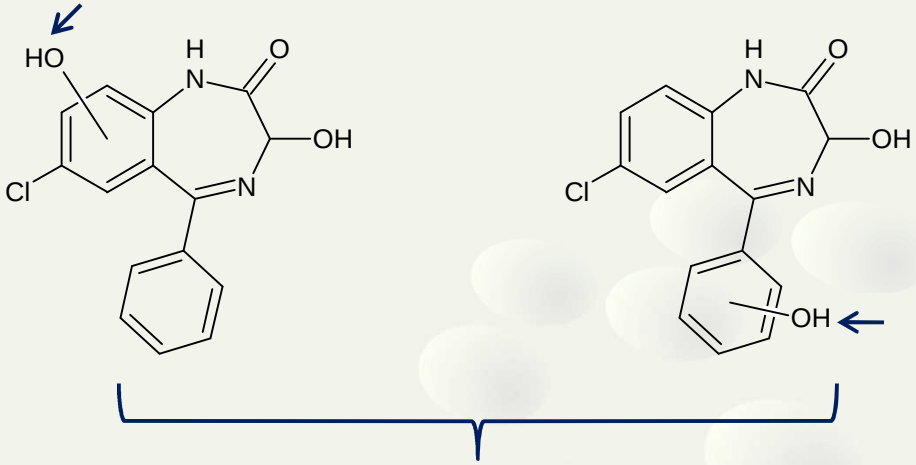


biotransformation

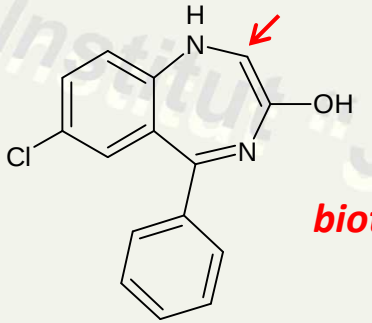
Transformation of OXA

B.\ Different than human metabolism

→ novel, previously unrecognized compounds:

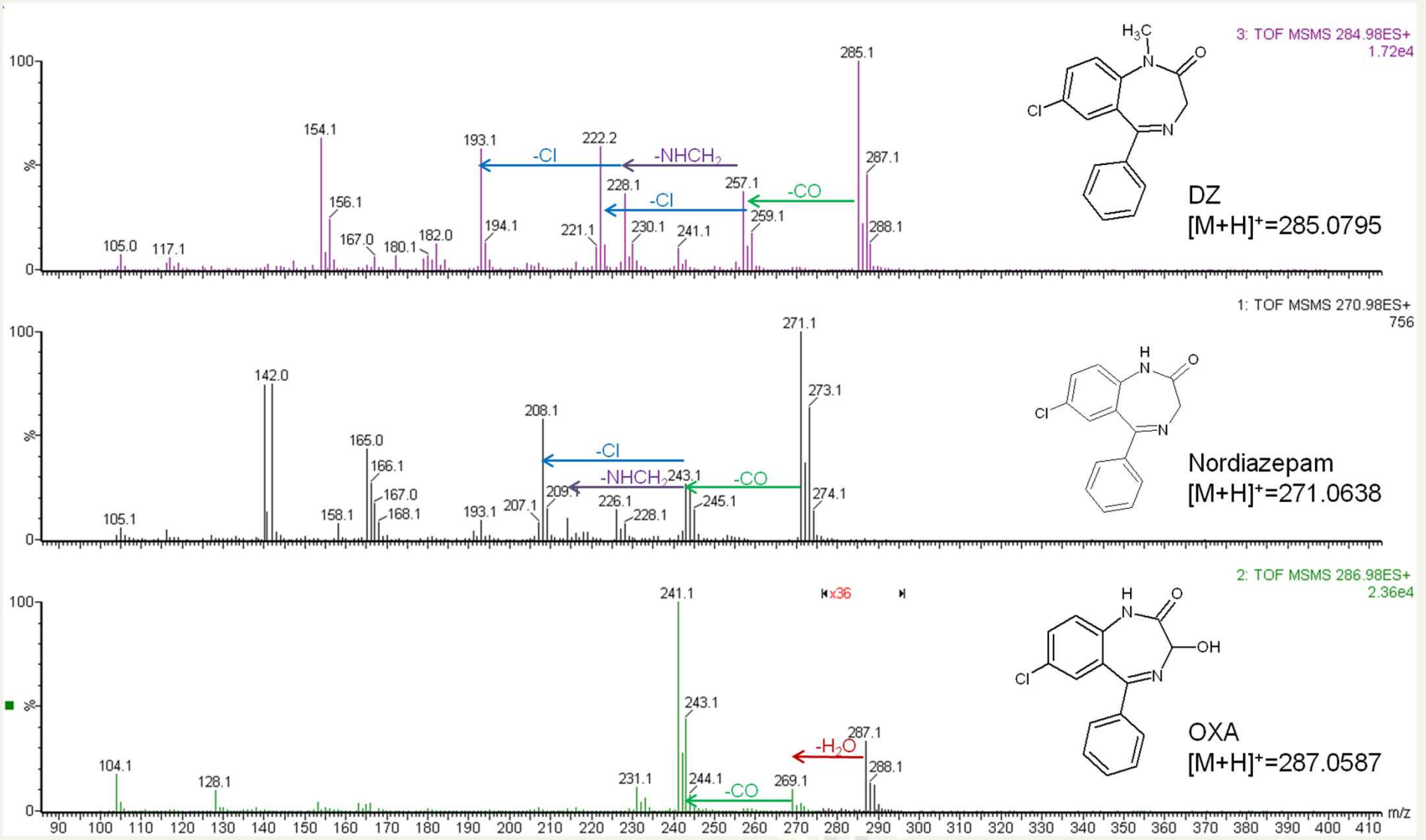


photochemical treatment



biotransformation

Identification: MS/MS and HRMS



In conclusions...

The purpose of identification is not the identification itself, but...

- ✓ To evaluate the effect of water treatment ... Do we produce transformation products with increased toxicity? → **the identified transformation products should be tested** on geno-, eco-, cyto- ... **toxicity**
- ✓ To **upgrade the treatment** in order to **achieve mineralisation** instead of (slight to moderate) structural transformation of mother compounds
- ✓ To contribute to a **comprehensive risk assessment**
- ✓ To **raise the scientific and public awareness** that persistent TPs are, as well as their parent compounds, environmentally relevant emerging pollutants.

Acknowldegements

• Slovenian Research Agency, Postdoctoral project Z1-3677: “Psychoactive pharmaceuticals and their transformation products in water treatment processes”

• Slovenian Research Agency, Research program P1-0143: »Cycling of nutrients and contaminants in the environment, mass balances and modeling of environmental processes and risk analysis«

For more information see:

• Kosjek et al., Environmental occurrence, fate anf transformation of benzodiazepines in water treatment, Water Research 46 (2012) 355-368.

• Kosjek and Heath, Halogenated Heterocycles as Pharmaceuticals, Top. Heterocycl. Chem.: Halogenated heterocycles : synthesis, application and environment (2012) 27: 219–246, ©Springer-Verlag Berlin Heidelberg 2011.

• Heath and Kosjek, Sources, occurrence and fate of halogenated heterocyclic pharmaceuticals in the environment, Top. Heterocycl. Chem.: Halogenated heterocycles : synthesis, application and environment (2012) 27: 247-268, ©Springer-Verlag Berlin Heidelberg 2011.

TPs

Compound / abbreviation	LC – t_R (min)	Accurate mass (calculated) $[M+H]^+$	elemental composition $[M+H]^+$	mass error	MS/MS	treatment conditions
DZ	2.97	285.0795	$C_{16}H_{14}N_2OCl$	–0.5ppm	285/287, 257/259, 228/230, 222, 193, 154/156	parent compound
OXA	2.40	287.0587	$C_{15}H_{12}N_2O_2Cl$	–0.3ppm	287/289, 269/271, 257/259, 241/243, 205, 163/165, 151	1. from DZ by biotransformation; 2. used as a parent compound
temazepam	2.72	301.0744	$C_{16}H_{14}N_2O_2Cl$	–1.3ppm	301/303, 283/285, 271/273, 255/257	photocatalysis, biotransformation
nordazepam	2.51	271.0638	$C_{15}H_{12}N_2OCl$	0.0ppm	271/273, 243/245, 208, 165/167, 140/142	photocatalysis, biotransformation
TP-C-301: 3 isomers, hydroxylated DZ (ring »C«)	1.66 2.29 3.39	301.0744	$C_{16}H_{14}N_2O_2Cl$	0.0ppm 0.1ppm –1.0ppm	301/303, 273/275, 238, 209, 182, 154/156	photocatalysis
TP-A-301: 2 isomers, hydroxylated DZ (ring »A«)	1.75 2.59	301.0744	$C_{16}H_{14}N_2O_2Cl$	2.0ppm –2.7ppm	301/303, 273/275, 238, 209, 198, 170/172, 105	photocatalysis
TP-C-317: 2×hydroxylated DZ	2.28	317.0693	$C_{16}H_{14}N_2O_3Cl$	0.9ppm	317/319, 289/291, 260/262, 254, 225, 179/181, 182/184, 154/156, 123	photocatalysis
TP-A/C-317: 2×hydroxylated DZ	1.67	317.0693	$C_{16}H_{14}N_2O_3Cl$	–0.9ppm	317/319, 289/291, 260/262, 199, 182/184, 105	photocatalysis
TP-303	1.80	303.0900	$C_{16}H_{16}N_2O_2Cl$	–1.0ppm	303/305, 246/248, 228/230, 193	biotransformation
TP-271	2.89	271.0638	$C_{15}H_{12}N_2OCl$	–1.8ppm	271/273, 253/255, 218, 190	biotransformation
TP-A/C-303: 3 isomers, hydroxylated OXA (ring »A« or »C«)	1.75 1.99 2.63	303.0536	$C_{15}H_{12}N_2O_3Cl$	0.0ppm, 0.7ppm, 0.3ppm	303/305, 285/287, 257/259	photocatalysis at pH2