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
Rising consumption of psychiatric drugs

Increasing awareness of mental health issues

Stresses of modern living $\rightarrow$ more psychiatric disorders

Demographic changes

Developing health care system

Rising consumption of psychiatric drugs

Abuse potential

Burden placed on the environment

Cycling?
Fate?
Effects?
**Chemical analysis**

- Confirmatory
- Quantification
- Identification of transformation products

SPE
- RP SPE: wide polarity range polymeric sorbent @ neutral pH

Derivatization
- Acetylation: Ac₂O/Pyr (3/1); 80°C, 15hrs (DZ underivatized)

Chromatographic separation
- GC: 5% phenyl column
- LC: RP C-18

Analysis
- MS/(MS): EI
- MS/MS: ESI(+), HRMS
Occurrence
(surface waters, wastewaters)

- Diazepam (DZ)
- Bromazepam (BZ)
- Oxazepam (OXA)

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)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>WWTP influent</th>
<th>WWTP effluent</th>
<th>River water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(c_{\text{range}})</td>
<td>(c_{\text{med}})</td>
<td>%positive</td>
</tr>
<tr>
<td>DZ</td>
<td>17-111</td>
<td>26</td>
<td>100</td>
</tr>
<tr>
<td>BZ</td>
<td>40-158</td>
<td>99</td>
<td>25</td>
</tr>
<tr>
<td>OXA</td>
<td>41-72</td>
<td>56</td>
<td>67</td>
</tr>
</tbody>
</table>

- Legend: \(c_{\text{range}}\): concentration range, \(c_{\text{med}}\): median concentration, % positive: percentage of > LOD samples
## Occurrence (detailed)

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

**Microbiological**
- oxic/anoxic flow-through reactors, suspended biomass

**Abiotic**
- photolytic / photochemical (UV+H$_2$O$_2$) degradation
- sorption to activated carbon
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**

Literature data: low removal of DZ
Sequential treatment of DZ

**INFLUENT**
- $c(DZ) = 94\mu g/L$
- $c(OXA) = 2.2\mu g/L$

**EFFLUENT**
- $c(DZ) = 18\mu g/L$
- $c(OXA) = 2.2\mu g/L$

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

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

99.9% removal may not be sufficient!

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:

- **DZ** → **N-desmethyldiazepam** / **nordiazepam** → **temazepam** → **temazepam** → **OXA**
Transformation of DZ (cont’d)

B. Different than human metabolism

→ novel, previously unrecognized compounds:
Transformation of OXA

B. Different than human metabolism

→ novel, previously unrecognized compounds:

\[
\text{photochemical treatment}
\]

\[
\text{biotransformation}
\]
Identification: MS/MS and HRMS

DZ
[M+H]^+=285.0795

Nordiazepam
[M+H]^+=271.0638

OXA
[M+H]^+=287.0587
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:

<table>
<thead>
<tr>
<th>Compound / abbreviation</th>
<th>LC – $t_R$ (min)</th>
<th>Accurate mass (calculated) [M+H]$^+$</th>
<th>Elemental composition [M+H]$^+$</th>
<th>Mass error</th>
<th>MS/MS</th>
<th>Treatment conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>DZ</td>
<td>2.97</td>
<td>285.0795</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$OCl</td>
<td>−0.5 ppm</td>
<td>285/287, 257/259, 228/230, 222, 193, 154/156</td>
<td>parent compound</td>
</tr>
<tr>
<td>OXA</td>
<td>2.40</td>
<td>287.0587</td>
<td>C$<em>{15}$H$</em>{12}$N$_2$O$_2$Cl</td>
<td>−0.3 ppm</td>
<td>287/289, 269/271, 257/259, 241/243, 205, 163/165, 151</td>
<td>1. from DZ by biotransformation; 2. used as a parent compound</td>
</tr>
<tr>
<td>temazepam</td>
<td>2.72</td>
<td>301.0744</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$OCl</td>
<td>−1.3 ppm</td>
<td>301/303, 283/285, 271/273, 255/257</td>
<td>photocatalysis, biotransformation</td>
</tr>
<tr>
<td>nordazepam</td>
<td>2.51</td>
<td>271.0638</td>
<td>C$<em>{15}$H$</em>{12}$N$_2$OCl</td>
<td>0.0 ppm</td>
<td>271/273, 243/245, 208, 165/167, 140/142</td>
<td>photocatalysis, biotransformation</td>
</tr>
<tr>
<td>TP-C-301: 3 isomers, hydroxylated DZ (ring »C«)</td>
<td>1.66 2.29 3.39</td>
<td>301.0744</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$OCl</td>
<td>0.0 ppm</td>
<td>301/303, 273/275, 238, 209, 182, 154/156</td>
<td>photocatalysis</td>
</tr>
<tr>
<td>TP-A-301: 2 isomers, hydroxylated DZ (ring »A«)</td>
<td>1.75 2.59</td>
<td>301.0744</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$OCl</td>
<td>2.0 ppm</td>
<td>301/303, 273/275, 238, 209, 198, 170/172, 105</td>
<td>photocatalysis</td>
</tr>
<tr>
<td>TP-C-317: 2 hydroxylated DZ</td>
<td>2.28</td>
<td>317.0693</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$O$_3$Cl</td>
<td>0.9 ppm</td>
<td>317/319, 289/291, 260/262, 254, 225, 179/181, 182/184, 154/156, 123</td>
<td>photocatalysis</td>
</tr>
<tr>
<td>TP-A/C-317: 2 hydroxylated DZ</td>
<td>1.67</td>
<td>317.0693</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$O$_3$Cl</td>
<td>−0.9 ppm</td>
<td>317/319, 289/291, 260/262, 199, 182/184, 105</td>
<td>photocatalysis</td>
</tr>
<tr>
<td>TP-303</td>
<td>1.80</td>
<td>303.0900</td>
<td>C$<em>{16}$H$</em>{14}$N$_2$O$_3$Cl</td>
<td>−1.0 ppm</td>
<td>303/305, 246/248, 228/230, 193</td>
<td>biotransformation</td>
</tr>
<tr>
<td>TP-271</td>
<td>2.89</td>
<td>271.0638</td>
<td>C$<em>{15}$H$</em>{12}$N$_2$OCl</td>
<td>−1.8 ppm</td>
<td>271/273, 253/255, 218, 190</td>
<td>biotransformation</td>
</tr>
<tr>
<td>TP-A/C-303: 3 isomers, hydroxylated OXA (ring »A« or »C«)</td>
<td>1.75 1.99 2.63</td>
<td>303.0536</td>
<td>C$<em>{15}$H$</em>{12}$N$_2$O$_3$Cl</td>
<td>0.0 ppm, 0.7 ppm, 0.3 ppm</td>
<td>303/305, 285/287, 257/259</td>
<td>photocatalysis at pH2</td>
</tr>
</tbody>
</table>