Treated Effluent as Environmental Flows: Concerns and Issues from an Australian Perspective

Anu Kumar, Marianne Woods, Mike Williams, Ali Shareef, Hai Doan and Rai Kookana
What are EDCs?

“an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism or its progeny, or (sub)populations”
### Emerging Contaminants in US Waters

<table>
<thead>
<tr>
<th>Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>139</td>
<td>Streams in 30 States</td>
</tr>
<tr>
<td>62</td>
<td>Intense AFO Activities</td>
</tr>
<tr>
<td>52</td>
<td>Intense Urbanization</td>
</tr>
<tr>
<td>17</td>
<td>Mixed Land Use</td>
</tr>
<tr>
<td>8</td>
<td>Minimally Developed</td>
</tr>
<tr>
<td>22</td>
<td>Antibiotics</td>
</tr>
<tr>
<td>14</td>
<td>Prescription Drugs</td>
</tr>
<tr>
<td>5</td>
<td>Nonprescription Drugs</td>
</tr>
<tr>
<td>15</td>
<td>Hormones and Steroids</td>
</tr>
<tr>
<td>39</td>
<td>Household and Industrial</td>
</tr>
</tbody>
</table>

*Widespread occurrence of biologically active chemicals*

Sources of Endocrine-Active Chemicals

- Energy
- Agriculture
- Consumer Products
- Domestic Effluent
- Livestock
- Mining
- Electronic Waste
- Phytochemicals
Toxicology

ppm (mg/L) → Death/Disease

Endocrinology

ppt (ng/L) → Physiology

Defined Safety Threshold

No Defined Safety Threshold
Canadian whole-lake experiments (Kidd et al. 2007)

- A 7-yr long whole lake study
- Ethynylestradiol (EE2) 5-6 ng/L
- 3 times/week for 20 wks (3 yr)
- Lake trout, fathead minnow,
- Intersex in males
- Altered oogenesis in females
- Near extinction of fathead minnow
Challenges & Differences of the Australian Environment

- Very seasonal water flows
- Some systems are effluent dominated
- Rivers highly regulated
- Unique fauna
- Climate change predictions

Photo: Tony Auld, DECC
In some waterways, no flow all year without the STP effluent.
Recycling and reuse of waste and wastewater in Australia - major environmental initiatives
Australian Guidelines for Water Recycling

Risk Management Framework

Assessing Risk
-What might happen?
-Likelihood of happening?
-Consequence of happening?

Managing Risk
-What can be done to prevent?

Monitoring Risk
-Does management system work?
For better understanding info required:

1. Occurrence
2. Sources and Source Pathways
3. Transport and Fate
4. Ecological Effects
List of estrogenic compounds

- Compounds selected – ranked as being the most significant based on their relative potency to E2, prevalence and observed biological effects

<table>
<thead>
<tr>
<th>Class</th>
<th>Compound</th>
<th>Relative Potency to E2 (in vitro)</th>
<th>[WWTP effluent] (ngL⁻¹)</th>
<th>Example of reported biological effects (in vivo)</th>
<th>Concentration of effects (ngL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural hormones</td>
<td>17β-estradiol (E2)</td>
<td>1.0 ±</td>
<td>1-48 c</td>
<td>Reduced sexual behaviour and impregnation success in adult male mosquitofish, <em>Gambusia holbrooki</em> <em>g</em> VTG induction in adult zebrafish, <em>Danio rerio</em> <em>h</em></td>
<td>20 [84 d]</td>
</tr>
<tr>
<td></td>
<td>Estrone (E1)</td>
<td>0.22 ±</td>
<td>1-76 c</td>
<td></td>
<td>204 [21 d, EC50]</td>
</tr>
<tr>
<td>Pharmaceuticals</td>
<td>17α-ethylxynestradiol (EE2)</td>
<td>1.03 ±</td>
<td>0.2-7 c</td>
<td>Skewed sex ratios and ova-testis in fathead minnow, <em>Pimephales promelas</em> <em>i</em></td>
<td>4 [305 d]</td>
</tr>
<tr>
<td>Phenols</td>
<td>Bisphenol A (BPA)</td>
<td>0.0001 ±</td>
<td>&lt; 1000 c</td>
<td>Altered sex ratios in African clawed toad, <em>Xenopus laevis</em>, larvae exposed from 2 days post-hatch <em>j</em></td>
<td>23 [84 d]</td>
</tr>
<tr>
<td>Alkylphenols</td>
<td>4-n-tert-octylphenol (4-t-OP)</td>
<td>0.0004 ±</td>
<td>nd-500 c</td>
<td>Reproductive malformations (additional female organs) in adult freshwater snails, <em>Marisa cornuarietis</em> <em>k</em></td>
<td>1 000 [140 d]</td>
</tr>
<tr>
<td></td>
<td>4-n-nonylphenol (4-n-NP)</td>
<td>0.000009±</td>
<td>170-37000 c</td>
<td>Induction of VTG in immature juvenile Atlantic salmon, <em>Salmo salar</em> <em>l</em></td>
<td>50 000 [7 d]</td>
</tr>
<tr>
<td></td>
<td>4-n-nonylphenol (4-NP)</td>
<td>0.00014 ±</td>
<td>nd-332000 c</td>
<td>Intersex condition in Japanese medaka, <em>Oryzias latipes</em> exposed from 1 day post-hatch <em>m</em></td>
<td>300 000 [100 d]</td>
</tr>
<tr>
<td></td>
<td>Nonylphenol ethoxylates (NP1EO, NP2EO)</td>
<td>0.000006±</td>
<td>nd-332000 c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Exposure assessment

- Treatment plants in Qld, SA, ACT
  - Influent
  - Effluent
  - Receiving environments
- Eight EDCs
  - Natural Hormones (E1, E2)
  - Synthetic Hormone (EE2)
  - Alkylphenol ethoxylates (OP, NP, NPEOs)
  - Bisphenol A (BPA)
- Some pharmaceuticals
- Analysed by GC/MS; LC/MS; ELISA
- Bioanalytical tools
  - YES - >0.5 ng/L (EEQ)
  - ER-CALUX - >0.2 ng/L (EEQ)
EDCs in treated effluents

- **Treated effluents**
  - Consistency across sites
  - Alkylphenols – low μg/L
  - Steroid estrogens – low ng/L

- **Dairy effluents**
  - E1 – 39 ng/L
  - E2 - 9 ng/L

**NP1EO, NP2EO, NP >> OP > BPA, E1 > E2 > EE2**
Key messages (2003-2006)

• EDC’s in the Australian environment at levels that have been shown by overseas studies to have detectable effects.

• Australian research shows that EDCs can enter riverine systems through a variety of inputs, including urban and rural waste streams such as households, industries and intensive animal farming.

• Poor treatment & lower dilution in rural streams
  • Implications?
  • Effects on organisms & populations?
    • Laboratory exposures
    • On-site assessment
Mosquitofish

Richard Lim et al (UTS)

Female

Male

Gonopodium

CSIRO. Estrogenic Endocrine Disrupting Chemicals in the Australian Riverine Environment

13th International Riversymposium
Selection of Appropriate Fish Test Model

Zebrafish
- OECD

Fathead minnow
- USEPA

Murray River Rainbowfish (Melanotaenia fluviatilis)
- Australian ecotoxicological test species
- Found in SA, Vic and NSW
- Maintained under laboratory conditions
- Male and females differentiated
Reproduction

- Spawning
- Egg Production
- Fecundity
- Hatching success

CSIRO. Estrogenic Endocrine Disrupting Chemicals in the Australian Riverine Environment
Histology

<table>
<thead>
<tr>
<th>Liver condition</th>
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</thead>
<tbody>
<tr>
<td>Testis-ova development</td>
</tr>
<tr>
<td>Pathological effects</td>
</tr>
<tr>
<td>Secondary sex characteristics</td>
</tr>
<tr>
<td>Presence of spawning tubercles</td>
</tr>
<tr>
<td>Gonadosomatic index</td>
</tr>
<tr>
<td>Hepatosomatic index</td>
</tr>
<tr>
<td>Gross morphology</td>
</tr>
</tbody>
</table>
**Biomarkers**

### Hormones
- E2
- 11-KT
- Testosterone

### Molecular markers
- Vitellogenin
- mRNA
- Zrp
Field Experiment in collaboration with USGS

- 28-Day continuous flow
- Two test species (rainbowfish/zebrafish)
- Two test solutions (WWTP effluent/River water)
- Five dilutions (100%, 50%, 25%, 10%, 0% effluent)
- Four time points (1, 7, 14, and 28 days)
- Three *in-vivo* biological endpoints (mRNA, vitellogenin, histology)
- Two *in-vitro* biological assays (YES/YAS)
- Chemical analysis for EDCs
### Summary of Results – Tiered Approach with Multiple Lines of Evidence

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Analytical</th>
<th>In-vitro</th>
<th>Fish mortality</th>
<th>Fish biomarker</th>
<th>Fish histology</th>
<th>Overall summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Water</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Effluent 100%</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Day 1</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Effluent 100%</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>NA</td>
</tr>
<tr>
<td>Effluent 100%</td>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NA</td>
</tr>
<tr>
<td>Day 14</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Effluent 100%</td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Day 28</td>
<td></td>
<td></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

- **Reference Water:** No effect
- **Effluent 100% Day 1:** Effect
- **Effluent 100% Day 7:** Severe effect
- **Effluent 100% Day 14:** Severely effected
- **Effluent 100% Day 28:** Severely effect

**Legend:**
- **-** No effect
- **+** Minor effect
- **++** Effect
- **+++** Severe effect
- **NA** Not Analysed
Next Steps

Evaluate downstream impact with native/caged fish surveys

Define temporal variability

Test other aquatic systems (treatment technology, stream dilution)
Challenges!!

- Extremely complex systems
- Variability in hydrology, chemistry, biology, engineering
- Analytical detection limits vs biological effects levels
- Multiple modes of biological action
- Logistical coordination
- Limited scientific resources
Thank you