# EDC Toolbox 2 (stage 2) Project Newsletter

Issue 5 (Feb 2016)

Global Water Research Coalition

## Project at a glance

Bioanalytical tools (*in vitro* bioassays) are finding increasing utility as screening tools because the chemical nature of endocrine activity in a sample may be unknown and/or difficult to quantify. This is particularly true for those less-studied endocrine endpoints, where the causative chemicals are often unknown. The EDC Toolbox 2 project continues and expands on previous GWRC efforts to develop and validate methods to measure estrogenic activity in water (EDC Toolbox 1) to include a range of substantially less well-studied endocrine endpoints. For more details, please see the literature review produced in stage 1, available in the Members Only area of the GWRC website.

The research team at the Nieuwegein meeting

The project agreement was finally signed off in May 2014, and the project is conducted in four work packages:

1. WP1 will conduct at meta-analysis of androgenic and progestagenic assays;
2. WP2 will develop and validate methods to extract and analyse a variety of endocrine-active compounds from water;
3. WP3 will benchmark different assays for thyroid activity; and
4. WP4 will apply the newly validated GWRC battery to measure endocrine activity in water samples from participating countries, and include three different matrices (treated wastewater, surface and drinking water).

Together, the work packages will fill some of the gaps identified from the stage 1 literature review and enhance the analytical capabilities within the GWRC membership.

The project lead organisation is Water Research Australia, and involves TZW, KWR, Veolia-VERI, Suez-CIRSEE and the Water Research Commission. The project is funded by support from PUB, Stowa, Water Research Australia, TZW, Water RF and the GWRC. In-kind support is kindly provided by: Veolia - VERI, TZW, Suez – CIRSEE, KWR and Griffith University. The South African component of the project is funded by the Water Research Commission. For more information on the project team, please see the first project newsletter (Issue 1).

## Project update

The project is progressing well. Delivery of WP3 is slightly behind schedule due to minor complications in some of the reporting laboratories, but all data has now been received and no further delays are anticipated.

### *WP1: Meta-analysis of androgenic and progestagenic assays*

Status:  Completion: ■■■■■

The working report from WP1 is now available. In brief, while mammalian reporter gene assays and cell proliferation assays are the most sensitive methods, significant sample enrichment will be necessary to detect non-estrogenic endocrine activity in water samples based on current (often incomplete) knowledge.

### *WP2: Extraction methods*

Status:  Completion: ■■■■■

The working report for WP2 is now available. In brief, SPE extraction with a StrataX cartridge at pH 2 was the most efficient method, although extraction with C18 and Oasis HLB cartridges likewise were very efficient.

*WP3: Thyroid assays* Status:  Completion: ■■■■□

All laboratories have now provided their data. From initial analysis, it appears that reporter gene bioassays (nuclear receptor-mediated action) are not responsive with environmental water samples. The data is currently being processed to determine the performance of each assay, and final analysis is expected to be completed by the next reporting period.

*WP4: Application of the battery* Status:  Completion: ■■■■□

All laboratories are currently analysing the 24 environmental samples collected (treated wastewater, surface, drinking and ultrapure water samples from 6 countries - The Netherlands, Germany, Spain, France, Australia and South Africa). Participating laboratories include Veolia, CIRSEE and TZW for chemical analysis and Griffith University, Uni Pretoria, BDS, UFZ-Helmhotz Leipzig and IRCM Montpellier for bioassay analyses. All results are expected by Mar 2016.

Chemical analysis will include the initial screen developed at TZW, but also include various analytes by silylation-GC/MS and LC/MS at Suez-CIRSEE and a LC/MS screening on 112 compounds by Veolia-VERI.

Bioassay endpoints include: estrogen (ER-GeneBLAzer), androgen (AR-GeneBLAzer), progestagen (PR-GeneBLAzer and PR-CALUX), glucocorticoid (GR-GeneBLAzer and GR-CALUX), RXR (RXR-CALUX, HG5LN-RXR), PPAR (HG5LN-PPARg), mineralcorticoid (HG5LN-MR) and thyroid activity (most likely the T-SCREEN, although TBC by WP3 conclusions).

*Final project report* Status:  Completion: ■■□□□

The WP1 and WP2 working reports have been formatted to be incorporated into the final report. Reports from WP3 and WP4 will further be integrated in time, and the final report is currently anticipated on time at the end of April 2016.

## Relevant resources that may be of interest

Recent publications by members of the research team may be of interest to the reader:

- Schriks et al (2015). Selection criteria to select in vitro bioassays for implementation and use. DEMEAU project report (link)
- Brack et al (2015). The SOLUTIONS project: Challenges and responses for present and future emerging pollutants in land and water resources management. *Science Total Environ* 503-504: 22-31 (link)
- Triebskorn et al (2015). Monitoring primary effects of pharmaceuticals in the aquatic environment with mode of action-specific in vitro biotests. *Environ Sci Technol* 49(5): 2594-2595 (link)
- Henneberg et al (2014). Are in vitro methods for the detection of endocrine potentials in the aquatic environment predictive for in vivo effects? Outcomes of the project SchussenAktiv and SchussenAktiv plus in the Lake Constance Area, Germany. Plos ONE, 6(6): e98307 (link)

## Project timeline

| | 2014 | | | | | | | | 2015 | | | | | | | | | | | | 2016 | | | |
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| | M | J | J | A | S | O | N | D | J | F | M | A | M | J | J | A | S | O | N | D | J | F | M | A |
| WP1 (A&P) | | | | | | | | | | | | | | | | | | | | | | | | |
| WP2 (chem) | | | | | | | | | | | | | | | | | | | | | | | | |
| WP3 (T) | | | | | | | | | | | | | | | | | | | | | | | | |
| WP4 (all) | | | | | | | | | | | | | | | | | | | | | | | | |
| Final Report | | | | | | | | | | | | | | | | | | | | | | | | |