

Video Article

Screening for Endocrine Activity in Water Using Commercially-available *In Vitro* Transactivation Bioassays

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Abstract

In vitro transactivation bioassays have shown promise as water quality monitoring tools, however their adoption and widespread application has been hindered partly due to a lack of standardized methods and availability of robust, user-friendly technology. In this study, commercially available, division-arrested cell lines were employed to quantitatively screen for endocrine activity of chemicals present in water samples of interest to environmental quality professionals. A single, standardized protocol that included comprehensive quality assurance/quality control (QA/QC) checks was developed for Estrogen and Glucocorticoid Receptor activity (ER and GR, respectively) using a cell-based Fluorescence Resonance Energy Transfer (FRET) assay. Samples of treated municipal wastewater effluent and surface water from freshwater systems in California (USA), were extracted using solid phase extraction and analyzed for endocrine activity using the standardized protocol. Background and dose-response for endpoint-specific reference chemicals met QA/QC guidelines deemed necessary for reliable measurement. The bioassay screening response for surface water samples was largely not detectable. In contrast, effluent samples from secondary treatment plants had the highest measurable activity, with estimated bioassay equivalent concentrations (BEQs) up to 392 ng dexamethasone/L for GR and 17 ng 17 β -estradiol/L for ER. The bioassay response for a tertiary effluent sample was lower than that measured for secondary effluents, indicating a lower residual of endocrine active chemicals after advanced treatment. This protocol showed that *in vitro* transactivation bioassays that utilize commercially available, division-arrested cell "kits", can be adapted to screen for endocrine activity in water.

Video Link

The video component of this article can be found at <http://www.jove.com/video/54725/>

Introduction

Current water quality monitoring is predicated on the ability to accurately and precisely measure the occurrence of chemical contaminants as a proxy for exposure to wildlife and humans. However, this chemical-by-chemical monitoring and assessment paradigm cannot keep pace with the ever-changing chemical universe that we face. As we learn more about the fate and effects of synthetic and natural chemicals, we continue to search for measurement tools that address expected biological impacts, and that at the same time are immune to changes in chemical production, usage and environmental input. Such tools are especially relevant for understanding whether unknown or new chemicals, and transformation products, deserve our attention. Moreover, complex mixtures of chemicals present in water are poorly addressed by individual chemical monitoring. Thus, we face the challenge of modernizing the existing monitoring toolbox to better address these issues in surface waters that receive discharge of treated wastewater effluent and urban/stormwater runoff.

In recent years, bioanalytical techniques have shown promise as screening tools for water quality assessment. In particular, *in vitro* bioassays that respond to chemicals acting via known, specific modes of action^{1,2} are of great interest to the environmental monitoring community³. Numerous investigations have employed *in vitro* bioassays to quantify the endocrine activity of drinking, surface and wastewaters⁴⁻⁶. Moreover, a number of bioassays target molecular initiating events (e.g., receptor activation) which can potentially be linked to deleterious effects via adverse outcome pathway analyses^{7,8}.

The evolution of bioscreening for water quality assessment has been relatively rapid, with hundreds of different *in vitro* bioassay endpoints having been evaluated for their utility^{9,10}. Currently, only a handful of bioassays have been shown to achieve good measurement precision (within laboratories) while demonstrating the ability to differentiate among water qualities^{5,6}. For treated wastewater effluent in particular, the occurrence of estrogens and glucocorticoid steroids has been successfully accounted for using *in vitro* transactivation assays^{11,12}. However, most studies to date have employed bioassays whose cell lines are proprietary (and thus not widely available), require continuous care and manipulation, or both. As a result, the ability to standardize protocols, perform inter-laboratory calibration exercises, and ultimately to transfer this screening technology to the water resources community remains hindered.

At least one supplier of *in vitro* bioassays vetted through the U.S. ToxCast program is commercially available¹³ in easy to use "freeze and thaw" formats. These division-arrested cell "kits" have been shown to be robust in measuring the activity of chemicals extracted from water representing different levels of treatment¹⁴. Although vendor protocols are available to screen the bioactivity of individual chemicals or mixtures, some of them require modification before they can be applied to water samples. Treated wastewater effluent¹⁵, stormwater runoff¹⁶, receiving waters^{17,18} and more recently recycled water^{19,20} are prime examples of aqueous media that are of interest to the water quality community.

This study presents a single, standardized protocol to measure the endocrine activity in water samples using commercially available, division-arrested *in vitro* transactivation bioassays. We demonstrated robustness of the protocol through a comprehensive assessment of background, dose responsivity and repeatability of response for two endpoints of particular interest Estrogen and Glucocorticoid Receptor transactivation (ER and GR, respectively). The protocol was applied to screen samples of treated wastewater effluent and surface water from freshwater systems in California.

Protocol

1. Collect and Process Water Sample (Modified from Escher *et al.*⁹)

1. Fill a clean 1 L amber glass bottle containing 1 g sodium azide and 50 mg ascorbic acid to the top with water sample of interest. Store sample at 4 °C and process within 72 hr.
NOTE: Sodium azide is highly toxic and must be handled with caution. Use protective gear (eye/face, gloves, clothing) and weigh in a properly functioning fume-hood. Do not use a metal spatula for weighing.
2. Pass the sample through a 1.6 µm glass fiber filter and then through a preconditioned Solid Phase Extraction (SPE) cartridge at a flow rate of 5-10 ml/min. Adjust the pressure of the vacuum pump to control the flow rate.
3. Vacuum dry the cartridge for 15 min.
4. Elute the cartridge with 10 ml of methanol, followed by 10 mL of acetone:hexane (1:1, v/v).
5. Concentrate eluate to ~1 ml under a gentle stream of high purity nitrogen. Solvent exchange by adding 500 µl of dimethyl sulfoxide (DMSO) and evaporating the extract down to 500 µl.
6. Transfer the extract to an amber glass autosampler vial. Store at -20 °C.

2. Prepare Dilutions of the Assay Specific Reference Chemical and Water Extract

1. **Prepare 9 dilutions for the calibration curve.**
 1. Make a stock solution of the assay specific reference chemical in 100% DMSO. The final concentration must be 2 µM 17β-estradiol for the ER assay, and 100 µM dexamethasone for the GR assay. Store the stock solutions at -20 °C.
 2. Add 15 µl of the appropriate reference chemical stock to 285 µl of assay medium in a sterile tube (tube #1). Mix the sample by pipetting up and down.
 3. Add 200 µl of a solution of 5% DMSO in assay medium in 8 additional tubes (tubes #2-9).
 4. Transfer a 100 µl aliquot from tube #1 to tube #2 through tube #9 to perform a 3-fold dilution series. Each diluted sample must be mixed thoroughly with a pipette before taking an aliquot and adding it to the next tube.
2. Prepare a solvent control sample by mixing 10 µl of DMSO in 190 µl of assay medium.
3. Prepare four dilutions for each water extract.
 1. In the first tube, add 5 µL of water extract in 95 µL of assay medium and mix thoroughly. Add 50 µl of 5% DMSO in assay medium in three other tubes.
 2. Perform a 2-fold dilution by transferring 50 µl of solution from one tube to the next.

3. Prepare the Cell Suspension to Conduct the FRET Bioassay

1. Prepare the assay specific medium according to the manufacturer's instructions. Assay medium must be stored at 4 °C and warmed to 37 °C in a water-bath before use.
2. Take a vial of ER or GR division-arrested cells out of the cryogenic freezer and thaw the cells quickly by placing the vial in a 37 °C water-bath for 2 min with gentle agitation. Decontaminate the vial with 70% ethanol and place it in a Class II biological safety cabinet.
3. Open the vial using aseptic techniques and transfer the cells into 10 ml of assay medium.
4. Centrifuge at 200 x g for 5 min.
5. Aspirate the supernatant using a sterile glass pipet and resuspend the cell pellet in 6 ml of assay medium.
6. Mix 5 µl of cell suspension with 5 µl of a vital stain solution and add an aliquot into a counting chamber.
7. Count the number of live cells using a light microscope or automated cell counter and estimate the density of live cells in the cell suspension. Dilute if necessary to obtain a final density of 550,000 live cells per ml of assay medium.

4. Plate Cells in a 96-well Black Wall Clear-bottom Plate and Add Diluted Water Extract

1. Create a plate layout that includes a 9-point assay specific calibration curve, QA/QC samples and multiple dilutions for each water extract. An example of a 96-well plate layout is shown in **Figure 1**.
2. Add 90 µl of assay medium to the replicate cell-free control wells.
3. Pour the cell suspension in a sterile pipetting reservoir and add 90 µl of cell suspension to the other wells using a multichannel pipette.
4. Add 10 µl of the diluted samples prepared during step 2 to the appropriate wells. The final concentration of DMSO per well must be 0.5% maximum.

- Cover the plate with a lid and place it in a 5% CO₂ incubator at 37 °C for 16 hr.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Assay specific reference chemical, 9 dilutions									DMSO control	Cell only control	Assay medium
B												
C												
D	Sample 1, dilution 1	Sample 1, dilution 2	Sample 1, dilution 3	Sample 1, dilution 4								
E	Sample 2, dilution 1	Sample 2, dilution 2	Sample 2, dilution 3	Sample 2, dilution 4								
F	Sample 3, dilution 1	Sample 3, dilution 2	Sample 3, dilution 3	Sample 3, dilution 4								
G	Sample 4, dilution 1	Sample 4, dilution 2	Sample 4, dilution 3	Sample 4, dilution 4								
H	Sample 5, dilution 1	Sample 5, dilution 2	Sample 5, dilution 3	Sample 5, dilution 4								

Figure 1: Example of a 96-well Plate Layout. The multi-well plate is designed to include an assay specific calibration curve, 3 types of QA/QC controls (media only, cells in clean medium and cells in DMSO spiked medium) and 4 dilutions per water extract. Each control and dilution of water extract is analyzed in triplicate wells. [Please click here to view a larger version of this figure.](#)

5. Prepare the Loading Solution and Add to Each Well

- After incubation, allow the plate to equilibrate to RT. For this FRET bioassay, steps 5.2 through 5.6 should be conducted in the absence of direct light.
- Prepare a 6x loading solution according to the manufacturer's instructions.
- Add 20 µl of the 6x loading solution to each well.
- Add 10 µl of cell viability reagent to each well to evaluate the cytotoxicity of the diluted water extract.
- Seal the plate with aluminum adhesive film.
- Incubate the plate in the dark at RT for 2 hr.

6. Measure Cytotoxicity and Endocrine Activity Response

- Set up the microplate reader with bottom-reading capabilities following the manufacturer's instructions.
- For the FRET transactivation bioassay, measure fluorescence in the blue (409/460 nm, excitation (Ex)/emission (Em) wavelength) and green (409 Ex/530 Em nm) channels.
- For the cytotoxicity assay, measure fluorescence at 560 Ex/590 Em nm.

7. Assess QA/QC Checks to Determine the Quality of the Data

- Compare the average raw fluorescence of the cell-free (media only) and cells-only (cells in clean assay medium) controls. The media-only background fluorescence should be at least 25% lower than the response of the cells-only control.
- Process the raw FRET data. For both 'blue' and 'green' datasets, subtract the average fluorescence of the cell-free control wells from all cell containing well. Calculate the blue/green ratio for each experimental well.
- Compare blue/green ratio of cells-only and cells with DMSO controls. Fluorescence values of these controls should be within 15% Relative Standard Deviation (RSD).
- Plot the assay specific calibration curve as blue/green ratio against the sample concentration expressed as a log molecular mass (log M). Calculate the slope, R² and log EC₅₀ (50% Effect Concentration). Calibration parameters should be within the expected range listed in **Table 1**.
- Calculate the RSD for all triplicate wells. The variability among replicates should be less than 20%.
- For the cytotoxicity data (560 Ex/590 Em nm), subtract the cell-free control average (i.e. media background) from all cell-containing wells.
- Calculate the average background-subtracted fluorescence for each sample. Plot the resulting fluorescence data as a percent of the DMSO control. Sample dilutions should not exhibit more than 20% cell mortality compared to the cells-only and DMSO controls.

8. Data Analyses

- Calculate the assay Limit of Detection (LOD) as the minimum calibration response plus two standard deviations of the mean of that response.
- For samples showing a dose response, derive the EC₅₀ of the water extract using the slope of a linear regression of the calibration curve between 10 and 50% effect concentrations of the reference chemical.
- Calculate the Bioanalytical Equivalent Concentration (BEQ) using the formula: BEQ = EC₅₀ of reference chemical/EC₅₀ of water sample.

Representative Results

In the present study, 4x 24 hr composite samples of treated municipal wastewater effluent, 6 grab samples of surface water from freshwater systems in southern California and a field blank consisting of ultrapure water were selected to illustrate this protocol. 3 of the 4 effluent samples were from conventional activated sludge wastewater treatment plants ("secondary effluent"), and the fourth one from an advanced wastewater treatment plant with sand/carbon filtration added post biological treatment ("tertiary effluent"). Surface water samples were collected from watersheds representing different land use (open, agricultural and urban).

Dilutions of the water extracts showed no apparent cytotoxicity with less than 20% cell mortality observed compared to the DMSO controls (**Table 1**). Cell-free (media only) background was low, and the comparison between cells-only and cells with DMSO responses indicated that the solvent had no measurable impact on the fluorescence measurements. Parameters of the assay specific calibration curves, including slope and log EC_{50} , were within the range of acceptable historical values (**Table 1**) demonstrating a good reproducibility of the calibration curves over time. An example of acceptable calibration curve for the ER assay is shown in **Figure 2**. The selection of 17β -estradiol and dexamethasone as reference chemicals was based on the strength of assay response, likelihood of occurrence in treated wastewater and historical use by other investigators²⁰⁻²². The variability in the response among triplicate samples was less than 20% RSD. Thus, all data were deemed to be of acceptable quality, and were subsequently used to estimate the ER and GR bioactivity (expressed as BEQ in ng/L) in wastewater and surface water samples.

A Relative Enrichment Factor (REF) of 5 to 10 and 4 dilutions per sample appeared suitable for screening of the water samples analyzed in the present study. The majority of WWTP effluent samples showed a dose response above LOD for both ER and GR bioassays; the lowest dilution had a response within the calibration curve and the highest dilution was typically below LOD (**Figure 3**). However, surface water extracts did not show strong ER or GR activity. Despite an REF of 10, the fluorescent responses were often close to or below the LOD. The absence of bioassay responses for the field blank confirmed that the detections noted above LOD were not the result of sampling or procedural lab artifacts.

ER and GR activities were detected in 7 and 4 of the 10 representative water samples, respectively (**Table 2**), with the highest of BEQs of 17 ng E2/L and 392 ng Dex/L found in the secondary effluents. The GR activity in the sample of tertiary effluent was below the bioassay LOD. Likewise, most surface water samples exhibited no GR activity above LOD and much lower levels of ER activity than the secondary effluents.

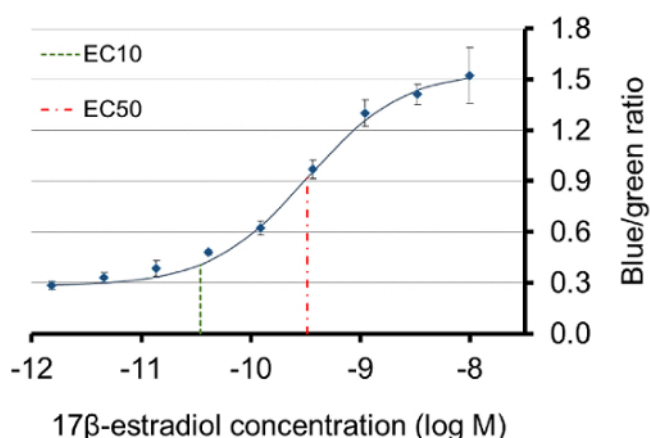


Figure 2: Example of Calibration Curve for the ER Assay. The dose-response curve, plotted as the mean fluorescence ratio (\pm SEM), is sigmoidal and used to determine the EC_{10} and EC_{50} values. This portion of the curve is fitted using linear regression and used to derive a bioassay equivalent concentration. [Please click here to view a larger version of this figure.](#)

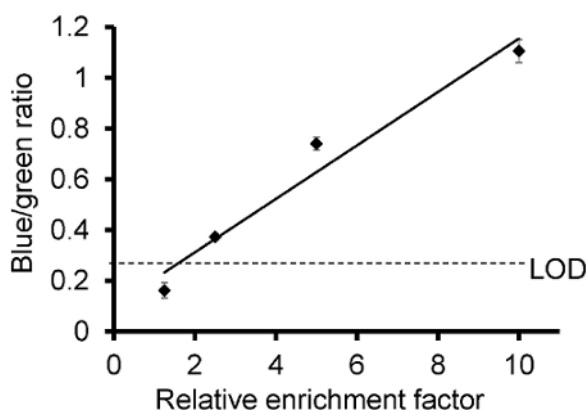


Figure 3: Mean Blue/green Fluorescence Ratio (\pm SEM) of Water Samples Plotted Against the Relative Enrichment Factor. Bioassay Equivalent Concentrations (BEQ) are calculated for samples whose dilution series show a concentration dependent-response with a minimum of two points above the limit of detection (LOD). [Please click here to view a larger version of this figure.](#)

	Estrogen Receptor (ER) Assay		Glucocorticoid Receptor (GR) Assay	
	Study results	Acceptance criteria	Study results	Acceptance criteria
Calibration parameters				
Mean log EC50 (M)	-9.6	-10.1 to -9.1	-8.5	-9.0 to -8.0
Mean slope	1.1	0.9 to 1.5	2.0	1.8 to 2.4
R2	0.99	>0.95	0.99	>0.95
QA/QC controls				
Media background	yes	media only <cell+media	yes	media only <cell+media
Cytotoxicity (% mortality)	0%	<20% mortality	0 to 5%	<20% mortality
Sample precision (%RSD)	2 to 18%	<20%	4 to 13%	<20%

Table 1: Summary of QA/QC Results From Bioanalytical Screening of Treated Wastewater Effluent and Surface Water Samples.

Sample ID and Description	ER-BEQ (ng E2/L)	GR-BEQ (ng Dex/L)
A – final effluent from full secondary treatment plant	6.4	248
B – final effluent from full secondary treatment plant	13	392
C – final effluent from full secondary treatment plant	17	236
D – final effluent from tertiary treatment plant	2.3	<22
E – surface water from agricultural area	<0.5	30
F – surface water from agricultural area	<0.5	<22
G – surface water from urban area	4	<22
H – surface water from urban area	0.9	<22
I – surface water from open field	0.8	<22
J – surface water from open field	<0.5	<22
K – field blank (ultrapure water)	<0.5	<22

Table 2: Bioassay Equivalent Concentrations for Estrogen Receptor (ER-BEQ) and Glucocorticoid Receptor (GR-BEQ) activity. Three types of samples collected in California (USA) are analyzed: municipal wastewater effluent (A-D), surface water (E-J), and field blank (K).

Discussion

The well documented potency of environmental estrogens, such as 17 β -estradiol (E2), warrants screening for these chemicals at ng/L concentrations^{23,24}. In this study, the ER response for wastewater effluents (BEQ range: 2.3 to 17 ng E2/L) was somewhat higher than reported for secondary effluent from Australian WWTPs²⁰, whereas the BEQs for surface water (<0.5 to 4 ng E2/L) were within the range reported for surface and stormwater elsewhere (<1 to 11 ng E2/L)¹⁶. Despite the low levels of ER activity measured in the surface water samples, this assay represents a relevant screening endpoint for water quality assessment. Estrogens such as bisphenol A and alkylphenol surfactants are often detected in the aquatic environment, but they can be difficult to quantify using conventional analytical chemistry. ER-cell assays may also prove useful for screening of currently and newly registered pesticides, as shown by Kojima et al²⁵.

The GR activity exceeded the ER activity by an order of magnitude in all 4 effluent samples analyzed in this study (**Table 2**). This trend is consistent with previous studies on wastewater effluent¹⁴, and the range for GR-BEQs reported here is slightly higher but comparable to that reported for other secondary effluents using the same *in vitro* bioassay¹². Using a different cell bioassay, the range for GR-BEQs for effluent from wastewater treatment plants in Japan was lower (<3 to 78 ng Dex/L) than those reported for secondary effluent in the present study²². With the exception of the single surface water sample that registered a maximum GR response of 30 ng Dex/L (**Table 2**, sample E), the GR activity in the remaining surface water samples was consistent with the low activity reported for Dutch surface waters²¹. However, the environmental impact for GR active chemicals is less well characterized than for ER active chemicals²⁶, making assessment of the potential for higher order effects a challenge.

As with conventional chemical analysis, adherence to written protocols and validation of measurements using a performance-based QA/QC approach maximizes data quality and robustness. Although validation of cell bioassays adapted for water quality assessment will continue to evolve and improve, measurement of the QA/QC parameters first outlined in Mehinto *et al.*¹⁴ and applied to this study showed that our results were well within prespecified guideline values (**Table 1**). The calibration curve parameters for these bioassays mirror those used for calibrating other analytical protocols (e.g., GC-MS), with the addition of criteria that assess the viability of the cells (e.g., cytotoxicity) representing the main difference. Another operational difference made possible using cells in a high throughput format is the inclusion of multiple sample dilutions that illustrate the concentration dependent response expected when bioactivity is measured (**Figure. 2**). In reviewing the sample measurements, the lower ER and GR responses for the tertiary effluent (sample D) compared to the three secondary effluent samples (A-C) provides additional

evidence of the relative accuracy of our bioassay screening results in **Table 2**. Incorporation of control or reference samples that represent the matrix of interest, in this case water, would further enhance the ability to compare bioassay results within and especially across measuring entities.

The responses of the ER and GR transactivation cell lines utilized in this study were expressed relative to the activity of the strong agonists E2 and dexamethasone, respectively. This allows for a quantitative estimate of bioactivity expressed as a Bioassay Equivalent Concentration (BEQ). This further allows for investigation of chemicals that contribute to the bioassay response, *i.e.* a direct comparison of BEQs with concentrations of individual agonists determined via conventional analytical chemistry. This concept underlines the added utility of bioscreening in directing diagnostic investigation of causative agents, also known as effects-directed analysis.

Since SPE is routinely used to isolate a wide range of pharmaceuticals and other "emerging contaminants" from water, we employed a previously published protocol^{9,20} that featured a sorbent designed to capture both hydrophilic and hydrophobic chemicals in water. Although we assume quantitative capture of all bioactive organic compounds using this technique, additional work is needed to fully standardize and validate the performance of such extraction methods. The type of cell assay employed in this study may present another limitation. These assays measure the interactions between contaminants and nuclear receptors and do not take into account other possible interactions, such as chemical-membrane receptor. Thus, it is possible that the screening responses measured here were underestimated. Like sample extraction, there are no standardized methods to analyze cell assay data and different approaches may produce different results. The linear regression used in this study to model the response for the lower half of the calibration curve may not be appropriate for all cell assays, particularly if modeled response is extended beyond the relatively narrow range specified in the present study. Additional investigation into the above issues is needed to understand the extent to which these tools can be applied as a robust monitoring tool.

Whereas much of the groundwork is being laid for ER and GR as screens for endocrine active chemicals, researchers are broadening the scope of bioanalytical tools to include other relevant mode of actions, *e.g.*, androgenicity, genotoxicity, neurotoxicity and immunotoxicity^{9,27}. A more complete toolbox will enhance the ability to screen for bioactive chemicals, including transformation products of known and/or unknown chemicals that can occur²⁸, now and into the future, in various aquatic environments¹⁹.

Disclosures

The authors have nothing to disclose.

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References

- Dix, D.J., Houck, K.A., Martin, M.T., Richard, M.A., Setzer, R.W., Kavlock, R.J. The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol. Sci.* **95** (1), 5-12 (2007).
- Reif, D.M., *et al.* Endocrine profiling and prioritization of environmental chemicals using ToxCast data. *Environ. Health Perspect.* **118** (12), 1714-1720 (2010).
- Maruya, K.A., *et al.* A tiered, integrated biological and chemical monitoring framework for contaminants of emerging concern (CECs) in aquatic ecosystems. *Integr. Environ. Assess. Manag.* (2015).
- Van der Linden, S.C., *et al.* Detection of multiple hormonal activities in wastewater effluents, surface water, using a panel of steroid receptor CALUX bioassays. *Environ. Sci. Technol.* **42** (15), 5814-5820 (2008).
- Leusch, F.D.L., *et al.* Comparison of five in vitro bioassays to measure estrogenic activity in environmental waters. *Environ. Sci. Technol.* **44** (10), 3853-3860 (2010).
- Jarosova, B., *et al.* Europe-wide survey of estrogenicity in wastewater treatment plant effluents: the need for effect-based monitoring. *Environ. Sci. Pollut. Res.* **21** (18), 10970-10982 (2014).
- Sonneveld, E., *et al.* Comparison of in vitro and in vivo screening models for androgenic and estrogenic activities. *Toxicol. Sci.* **89** (1), 173-187 (2006).
- Piersma, A.H., *et al.* Evaluation of an alternative in vitro test battery for detecting reproductive toxicants. *Reprod. Toxicol.* **38**, 53-64 (2013).
- Escher, B.I., *et al.* Benchmarking organic micropollutants in wastewater, recycled water and drinking water with *in vitro* bioassays. *Environ. Sci. Technol.* **48** (3), 1940-1956 (2014).
- U.S. Environmental Protection Agency (USEPA) Endocrine Disruptor Screening Program. *Prioritization of the endocrine disruptor screening program universe of chemicals for an estrogen receptor adverse outcome pathway using computational toxicology tools*. OCSPP/OW/ORD/NCCT (2012).
- Leusch, F.D.L., *et al.* Assessment of wastewater and recycled water quality: a comparison of lines of evidence from in vitro, in vivo and chemical analyses. *Water Res.* **50**, 420-31 (2014).
- Jia, A., Wu, S., Daniels, K.D., Snyder, S.A. Balancing the budget: accounting for glucocorticoid bioactivity and fate during water treatment. *Environ. Sci. Technol.* **50** (6), 2870-2880 (2016).
- Huang, R., *et al.* Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. *Environ. Health Perspect.* **119** (8), 1142-1148 (2011).
- Mehinto, A.C., *et al.* Interlaboratory comparison of in vitro bioassays for screening of endocrine active chemicals in recycled water. *Water Res.* **83**, 303-309 (2015).

15. Ternes, T.A., Joss, A., Siegrist, H. Scrutinizing pharmaceuticals and personal care products in wastewater treatment. *Environ. Sci. Technol.* **38** (20), 392A-399A (2004).
16. Tang, J.Y.M., *et al.* Toxicity characterization of urban stormwater with bioanalytical tools. *Water Res.* **47**, 5594-5606 (2013).
17. Scott, P.D., *et al.* An assessment of endocrine activity in Australian rivers using chemical and in vitro analyses. *Environ. Sci. Pollut. Res.* **21** (22), 12951-12967 (2014).
18. Vidal-Dorsch, D.E., Bay, S.M., Maruya, K., Snyder, S.A., Trenholm, R.A., Vanderford, B.J. Contaminants of emerging concern in municipal wastewater effluents and marine receiving water. *Environ. Toxicol. Chem.* **31** (12), 2674-2682 (2012).
19. WaterReuse Research Foundation (WRRF). *Direct potable reuse: a path forward*. WRRF, Alexandria, VA (2011).
20. Leusch, F.D.L., *et al.* Assessment of the application of bioanalytical tools as surrogate measure of chemical contaminants in recycled water. *Water Res.* **49**, 300-315 (2014).
21. Schriks, M., *et al.* Occurrence of glucocorticoid activity in various surface waters in the Netherlands. *Chemosphere* **93** (2), 450-454 (2013).
22. Suzuki, G., Sato, K., Isobe, T., Takigami, H., Brouwer, A., Nakayama, K. Detection of glucocorticoid receptor agonist in effluents from sewage treatment plants in Japan. *Sci. Tot. Environ.* **527-528**, 328-334 (2015).
23. Purdom, C.E., Hardiman, P.A., Byea, V. V. J., Enoa, N. C., Tyler, C. R., Sumpter, J.P. Estrogenic effects of effluents from sewage treatment works. *Chemistry and Ecology*. **8** (4), 275-285 (1994).
24. Kidd, K.A., *et al.* Collapse of a fish population after exposure to a synthetic estrogen. *Proc. Natl. Acad. Sci.*, **104** (21), 8897-8901 (2007).
25. Kojima, H., Katsura, E., Takeuchi, S., Niiyama, K., Kobayashi, K. Screening of estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. *Environ. Health Perspect.* **112** (5), 524-531, doi 10.1289/ehp.6649 (2004).
26. Kugathas, S., Sumpter, J.P. Synthetic glucocorticoids in the environment: First results on their potential impacts on fish. *Environ. Sci. Technol.* **45**, 2377-2383 (2011).
27. Van der Linden, S.C., *et al.* Development of a panel of high-throughput reporter-gene assays to detect genotoxicity and oxidative stress. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **760**, 23-32 (2014).
28. Cwiertny, D.M., Snyder, S.A., Schlenk, D., Kolodziej, E.P. Environmental designer drugs: when transformation may not eliminate risk. *Environ. Sci. Technol.* **48**, 11737-11745 (2014).

Materials List for:

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Materials

Name	Company	Catalog Number	Comments
GeneBLazer ER alpha DA assay kit	ThermoFisher	K1393	Kit includes ER division arrested (DA) cells and LiveBLazer FRET loading kit.
GeneBLazer GR DA assay kit	ThermoFisher	K1391	Kit includes GR division arrested (DA) cells and LiveBLazer FRET loading kit.
PrestoBlue cell viability reagent	ThermoFisher	A-13261	
Trypan blue, 0.4% in PBS	Sigma-Aldrich	T8154	Also available at ThermoFisher
Corning 96-well black wall, clear-bottom plate	Corning	3603	Individually wrapped, sterile with lid
Whatman glass fiber filters, GF/A, 1.6 µm	Sigma-Aldrich	WHA1820025	
Microplate aluminum sealing film	E&K Scientific	T592100	
Oasis HLB 6 cc cartridge, 200 mg sorbent	Waters	WAT106202	
17β Estradiol	Sigma-Aldrich	E2758	CAS #50-28-2
Ascorbic acid	Fisher Scientific	A61-100	Also available at Sigma-Aldrich
Dexamethasone	Sigma-Aldrich	D4902	CAS #50-02-2
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D8418	Molecular grade
Solvents (acetone, hexane, methanol)	Fisher Scientific		HPLC grade
Sodium azide	Sigma-Aldrich	S2002	Chemical is highly toxic and must be handled with caution. Use protective clothing and weigh under a fume-hood. Also available at EMD Millipore.
Automated cell counter or hemocytometer	Various*		Suppliers include Bio-Rad, Fisher Scientific, Sigma-Aldrich and ThermoFisher.
Class II biological safety cabinet	Various*		
CO ₂ incubator	Various*		
Cryogenic freezer	Various*		Liquid nitrogen storage dewar is recommended.
Fluorescence microplate-reader	Various*		The reader must have bottom read capabilities.
* No recommended source, the choice of this equipment depends on budget, frequency of use, and lab space.			