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Characterization of a Bisphenol A Specific Yeast Bioreporter Utilizing the Bisphenol A-Targeted Receptor

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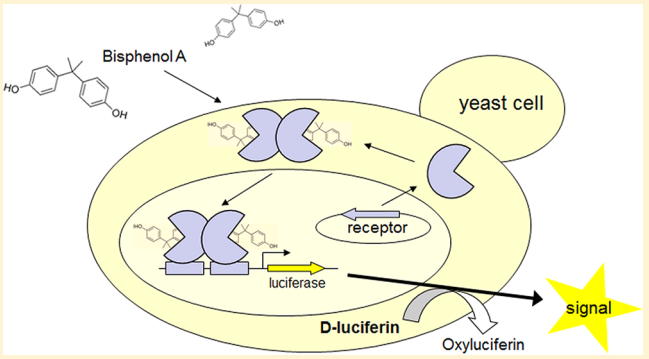
Supporting Information

ABSTRACT: The first *Saccharomyces cerevisiae* yeast bioreporter for analysis of a single endocrine disrupting compound, bisphenol A (BPA), was developed. The bioreporter contains mutated human estrogen receptor α (hERα), called bisphenol A-targeted receptor (BPA-R). The BPA-R bioreporter was characterized with mixtures of estrogenic chemicals and tested with spiked influent wastewater samples. The detection limit for BPA was 4.2-fold lower (0.107 μM, i.e., 24 μg L^{-1}), while that of the native hormone 17β-estradiol (E2) (1 μM, i.e., 272 μg L^{-1}) was 166,000-fold higher compared to the wild type hERα bioreporter. The BPA-R bioreporter responded only to BPA in a chemical cocktail and spiked concentrated wastewater samples with high concentrations of other estrogenic chemicals. As a conclusion, wastewater and other environmental water samples can be concentrated and specifically analyzed for BPA without risk of the mixture effect caused by other estrogenic chemicals. The BPA-R bioreporter is a robust and cost-efficient choice for high-throughput monitoring of BPA and its bioavailability in complex samples.



Bisphenol A (BPA) is a high production volume chemical that is used in numerous products such as polycarbonate plastics and resins, food and beverage packaging, dental sealants, and thermal paper. Over 1 million tons of BPA is produced in the EU annually.[1] BPA is also a well-known xenoestrogen. Although the exposure level and effects of BPA on humans and wildlife still remains under debate, there is growing evidence on adverse effects on both.[2−4]

Biomonitoring studies indicate that humans are constantly exposed to BPA.[5] While the main routes of BPA exposure for humans occur via food and drink, BPA emissions to the environment take place mainly in water phase.[1] BPA is indeed one of the most frequently detected contaminants in environmental waters and wastewaters.[6−9]

BPA has been shown to degrade reasonably well in wastewater treatment processes[10−12] and under aerobic conditions.[13] However, in anaerobic conditions, BPA has been shown to degrade poorly.[13,14] It has been suggested that BPA is in fact pseudopersistent in nature: although it degrades rather rapidly, the input rate is continuous.[3]

There are several liquid and gas chromatography methods coupled with mass spectrometry detection for BPA and other estrogenic chemicals (reviewed by Wille et al.[15]). Although these methods are sensitive, they usually require extensive sample purification and pretreatment, expensive instrumentation, and high level technical expertise. In addition, they are generally poorly suited for high throughput screening of a large number of samples.

Although less sensitive than chemical analysis methods, cell-based bioreporters, such as yeast or mammalian cells, are cost-efficient and easily operated alternative methods for environmental monitoring and chemical testing.[16] Bioreporter assays are particularly useful when bioavailability is assessed. Yeast-cell-based bioreporters have proven to be especially useful in detection of endocrine disrupting potential of pure chemicals as well as contaminated environmental water and wastewater.[17−23]

As a eukaryotic organism, yeast is able to express functional animal nuclear hormone receptors, such as estrogen receptor (ER). The activation of a chemical-bound receptor is coupled to the expression of a suitable reporter protein or enzyme. As a result, a dose−response curve of the chemical versus reporter signal can be obtained. By comparison to this dose−response curve, the activity of an unknown sample can be translated into an equivalent concentration of the reference compound.

Furthermore, yeast bioreporters are well suited for high throughput screening of large numbers of samples. They can be easily adapted to high density 384- and even 1536-well microplates.[24,25]

However, the main drawback of bioreporters in environmental analysis is their lack of specificity toward single chemicals.[16] For example, wastewater can contain several different estrogenic chemicals.[8,26] Usually, any compound

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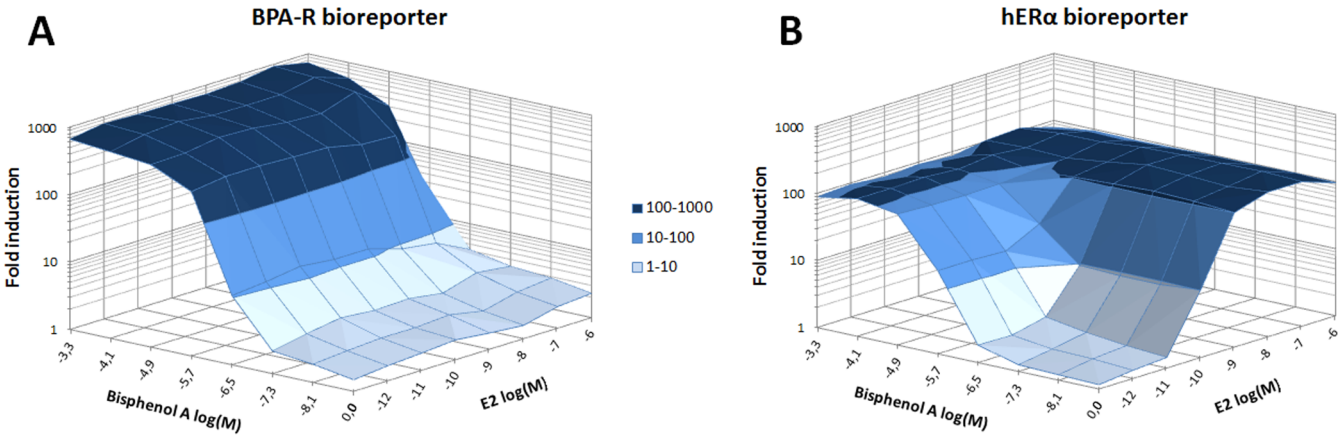

Figure 1. Response surfaces showing the fold inductions of BPA-R (A) and hERα (B) bioreporters with binary mixtures of bisphenol A and 17β-estradiol. Concentrations are given as the total analyte concentrations in a mixture with yeast cells. Values represent the mean of two independent experiments, each comprising four parallel data points.

binding to the studied receptor is able to cause activation of the receptor. For this reason, only the sum of hormonal activity can be measured, and no conclusions about the individual chemicals can be done without other chemical analysis methods.

In order to bring new tools for bioreporter-based high-throughput monitoring of single chemicals, a novel *Saccharomyces cerevisiae* yeast bioreporter for specific detection of BPA was developed. This was accomplished by using the bisphenol A-targeted receptor (BPA-R), created previously by mutagenesis of the human estrogen receptor α (hERα).[27] Ligand-inducible activity of the BPA-R bioreporter was compared to the original wild type hERα bioreporter by testing binary mixtures of BPA and the native hormone 17β-estradiol (E2) and a chemical cocktail consisting of the most frequently occurring environmental estrogens and xenoestrogens. Applicability of the BPA-R bioreporter was also assessed by measuring complex wastewater influent samples spiked with BPA and E2.

EXPERIMENTAL SECTION

Media and Chemicals. Synthetic minimal medium (SD) consisted of 6.7 g L^{-1} yeast nitrogen base w/o amino acids (Beckton Dickinson, Erembodegem, Belgium) supplemented with 2.5% final concentration of D-glucose (Amresco, Solon, OH), histidine (20 mg L^{-1}), leucine (100 mg L^{-1}), adenine (50 mg L^{-1}), and tryptophan (20 mg L^{-1}, only for the BMA64/luc strain), all from Sigma- Aldrich, Schnelldorf, Germany. Bisphenol A, 17β-estradiol, ethinyl estradiol, estriol, estrone, propyl paraben, and nonylphenol were purchased from Sigma-Aldrich, Schnelldorf, Germany. D-Luciferin (BioThema) was purchased from Aboatox (Turku, Finland).

Wastewater Samples and Sample Preparation. Effluent and influent wastewater samples were obtained from the Viikinmäki wastewater treatment plant in Helsinki, Finland. The treatment plant is the largest in Finland, and it processes the wastewaters of the Helsinki area (in total about 800,000 inhabitants). Roughly 270,000 m^3 wastewater flows through the plant each day. Of the incoming wastewater, about 85% is domestic and 15% is industrial wastewater. Samples were taken on two separate days in order to obtain knowledge on short-term variability of water quality and its impact on detection efficiency of bioreporters. Water sample 1 was taken on December 7, 2011 and sample 2 on December 13, 2011. By visual inspection, sample 1 appeared to contain more particulate matter than sample 2.

Samples were stored in 1 L PE bottles in 4 °C before dividing into 30 mL aliquots in 50 mL Falcon tubes with flip to cap (Becton Dickinson, USA). Influent samples of both days were spiked in following manner: 30 μL of 0.2 M BPA stock solution was added to a final concentration of 0.2 mg L^{-1} (0.88 μM); 30 μL of 800 nM E2 stock solution was spiked to a final concentration of 0.8 nM; 15 μL of double strength concentrations of BPA and E2 was added to final concentrations of 0.2 mg L^{-1} (0.88 μM) and 0.8 nM, respectively.

Samples in Falcon tubes were frozen at −80 °C after which the flip top caps were half opened and placed into the freeze dryer (Edwards Super Modulyo, Severn Science, Bristol, UK). The samples were freeze-dried for about 40 h until near dryness and resuspended into 3 mL of Milli-Q water (total 10-fold concentration).

After freeze-drying, three 10-fold concentrated influent samples (3 mL) were spiked by adding 10 μL of BPA or E2 stock solutions to final concentrations of 2 mg L^{-1} and 8 nM, respectively, and 5 μL of double concentrated stock solutions of both chemicals. The 10-fold concentrated samples were stored at −20 °C until being analyzed with the yeast bioreporters.

Yeast Strains. The bisphenol A specific receptor (BPA-R) yeast strain was constructed previously using a mutated hERα receptor P4E C8 expressed in *Saccharomyces cerevisiae*.[27] The mutant receptor has 7 base mutations corresponding to 5 amino acid mutations on helix 11 of the hERα ligand binding domain. Wild type human estrogen receptor hERα yeast strain and the constitutively luminescent control strain BMA64/luc have also been characterized previously in literature.[17]

Bioreporter Assay Procedure. The bioreporter assay was performed in the 384-well microplate format using automated liquid handling as described previously.[24] Briefly, an overnight culture of yeast cells grown in 5 mL of SD medium and incubated overnight at 30 °C with 250 rpm shaking was diluted in the morning in fresh SD medium to OD_{600} of 0.4. The yeast culture was further grown for about 2 h until OD_{600} reached 0.6−0.7. Ten mM D-luciferin stock solution in 0.2 M sodium citrate buffer (pH 5) was added to the yeast culture to a final concentration of 0.5 mM D-luciferin. All subsequent liquid handling was performed robotically by Biomek NXP

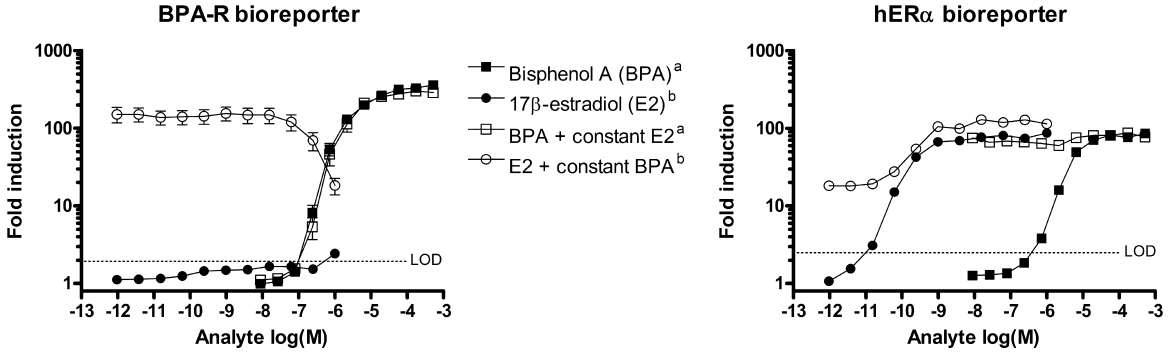

Figure 2. Dose–response curves of bisphenol A (BPA), 17β-estradiol (E2), and constant background mixtures measured with BPA-R and hERα yeast bioreporters. Concentrations are given as the total analyte concentrations in the mixture with yeast cells. The BPA background concentration in the E2 + BPA constant sample was 3 μM. The E2 background concentration in the BPA + E2 constant sample was 0.5 nM. Values represent the mean ± standard error of the mean of five independent experiments, each comprising four parallel data points. a: Curves plotted against BPA concentration. b: Curves plotted against E2 concentration. LOD: limit of detection.[28]

Laboratory Automation Workstation (Beckman Coulter, Munich, Germany): 5 μL of 10-fold concentrated wastewater sample (or standard chemical solution) was dispensed into white 384-well microplates (Optiplate-384, PerkinElmer Inc., USA), and 45 μL of yeast culture was added. Each plate was incubated for 3 h at 30 °C, and luminescence was measured using Victor3 1420 Multilabel Counter (PerkinElmer/Wallac, Turku, Finland) set in luminescence mode for 1 s/well.

Data Analysis. Fold inductions and toxicity correction factors were calculated as previously described.[17] Briefly, fold induction values were calculated for BPA-R and hERα yeast bioreporters by dividing the luminescence signal (expressed as relative light units) of the sample divided by the background (solvent control) signal. The correction factor for the constitutive control strain BMA64/luc was calculated by dividing the solvent background luminescence by the luminescence signal of the sample. Fold induction values of the samples measured with BPA-R and hERα yeast bioreporters were then corrected by multiplying each by the corresponding correction factor. Sigmoidal dose–response curves were fitted, and the detection limits and half-maximal effective concentrations (EC_{50} values) were calculated using the GraphPad Prism 4 software (GraphPad Software, San Diego, California). Detection limits (LOD) with 97.7% confidence thresholds were calculated using twice the coefficient of variation, as described by Hynninen et al.[28]

RESULTS AND DISCUSSION

Responses of BPA-R and hERα yeast bioreporters toward bisphenol A (BPA) and 17β-estradiol (E2) alone and in different binary mixtures were tested in order to create response surfaces. The chemicals were tested in 7 dilutions in a wide concentration range of 525 μM to 8.4 nM of BPA and of 1 μM to 1 pM of E2 (total concentrations in mixture with yeast cells, i.e., 1 part sample + 9 parts yeast culture) in order to obtain full dose–response curves for both bioreporter strains.

The response surface of BPA-R bioreporter showed no clear induction with E2, whereas the response toward BPA was similar despite the presence of E2 (Figure 1A). However, some inhibition by E2 on BPA response in the highest three E2 concentrations could be seen. BPA-R bioreporter also exhibited higher maximal fold induction level compared to hERα. The hERα bioreporter, in turn, was induced with both E2 and BPA in an additive manner (Figure 1B).

The possible inhibitory and additive effects of BPA and E2 on BPA-R and hERα bioreporters were further characterized using conventional dose–response curves with 11 dilutions of the chemicals in the same range as above in the response surface experiment. Dose response curves of BPA and E2 with and without a constant background of the other compound were analyzed. The BPA gradient with a constant E2 background contained a high E2 concentration of 0.5 nM (over EC_{50} of hERα), and the E2 gradient with a constant BPA background contained a rather low BPA concentration of 3 μM (less than EC_{50} of hERα).

The constant E2 background of 0.5 nM (136 ng L^{-1}) had no clear additive effect on BPA-R bioreporter compared to BPA alone, whereas this concentration was enough to keep the hERα bioreporter on its maximal induction level (Figure 2, open and solid squares). BPA background in turn showed a clear additive effect on E2 response of hERα bioreporter (Figure 2, open circle).

The hERα bioreporter was about 5 orders of magnitude more sensitive toward E2 than BPA (Figure 2), whereas E2 induced BPA-R bioreporter only in the highest tested concentrations of 1 μM (190 μg L^{-1}) (Figure 2, solid circle) which is about 166,000-fold compared to the detection limit of hERα bioreporter (in this study 0.006 ± 0.003 nM). As in the response surface (Figure 1), E2 had a clear inhibitory effect on the BPA-R bioreporter (Figure 2, open circle). When expressed as sample concentrations, the lowest E2 concentration showing inhibition was 625 nM (i.e., 170 μg L^{-1}), and the calculated IC_{50} was 2.2 μM. Although E2 still shows potency on the BPA-R receptor, these effective concentrations are significantly higher than the highest reported E2 concentrations in influent wastewater (150 ng L^{-1}[18,29]). Thus, E2-containing wastewater samples can be concentrated even up to 100- or 1000-fold without having any effect on BPA-R bioreporter response.

Detection limits for BPA based on the BPA dose–response curves (Figure 2) were calculated (Table 1). BPA-R bioreporter had 4.2-fold greater potency toward BPA than hERα bioreporter in respect to the limits of detection (Table 1). EC_{50} values had only about 2.5-fold difference. This was explained by a steeper curve slope of the BPA-R bioreporter compared to hERα bioreporter (Figure 2). In addition, the efficacy (i.e., the maximal induction levels) of BPA-R bioreporter was clearly higher than that of hERα (Figure 1), which was explained by a higher maximal signal level and lower background level (Table 1). Furthermore, the overall variation

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Table 1. Characteristics of the Bisphenol A Response of the BPA-R and hERα Assays[a]

bioreporter	limit of detection (μM)[b]	EC_{50} (μM)[c]	background (RLU)	maximal signal level (RLU)
BPA-R	0.107	2.0 ± 1.3	50	13,000
hERα	0.454	4.8 ± 1.4	100	11,000

[a]Concentrations are given as the total analyte concentrations in the mixture with yeast cells (1 part sample + 9 parts yeast culture). RLU: relative light unit. [b]Limit of detection with 97.7% confidence threshold.[28] [c]Values represent the mean ± standard deviation of five independent experiments, each comprising four parallel data points.

of BPA-R bioreporter was lower compared to hERα bioreporter (data not shown), resulting in slightly lower fold induction level for the detection limit (Figure 2).

Some existing detection methods for BPA are listed in Table 2. The most sensitive ones include liquid and gas chromatography coupled with mass spectrometric detection. These methods are generally several orders of magnitude more sensitive compared to cell-based assays. However, cell-based assays have many advantages, such as less sample pretreatment requirements, possibility to assess bioavailability, and detection of unknown bioactive compounds and mixture toxicity. In analyzing bisphenol A, the BPA-R bioreporter has about 10-fold lower detection limit compared to other yeast bioreporter assays and similar sensitivity with mammalian cell assays.

Table 2. Detection Methods for Bisphenol A

method	detection limit	EC_{50}[a]	reference
gas chromatography	0.026 nM		42
liquid chromatography	0.026 nM		43
ELISA assay	0.4−0.9 nM	3.4−5.2 nM	44
yeast ER assay	>1 μM	3.4 μM	22
yeast ER assay (BLYES)		95 μM	31
yeast two-hybrid	0.9 μM (EC_{10})		36
yeast two-hybrid		11 μM	45
yeast estrogen screen (YES)	1.6 μM (PC_{10})	12.6 μM (PC_{50})	46
HeLa cells	0.1 μM (PC_{10})	0.8 μM (PC_{50})	46
CHO-K1 cells		0.2 μM	45

[a]EC_{50}: half-maximal effective concentration.

E2 or BPA are not, however, main contributors to the overall estrogenicity in wastewaters. Several papers have reported that, for example, estrone is also present even in higher concentrations than E2.[8,18,26] Nonylphenol has also been found to be another main cause of wastewater estrogenicity.[8,10] The BPA-R receptor has been tested previously with estrone, estriol, nonylphenol, diethyl stilbestrol, hydroquinone, and β-sitosterol.[27] However, the mixture effect of several estrogenic compounds has not been tested before.

To mimic an extreme situation in which a highly contaminated water sample is concentrated 5-fold, a cocktail with some of the highest reported concentrations of estrogenic compounds in 5-fold concentrations was prepared. The reported concentrations of estrogenic contaminants in water and their final concentrations in the cocktail sample are shown in Table 3. Cocktails without and only with BPA were also prepared.

BPA-R bioreporter only responded to samples with BPA and with an equal level of induction. Importantly, the cocktail without BPA was clearly under the detection limit (Figure 3). The hERα bioreporter exhibited nearly a maximal induction level at cocktails with and without BPA, whereas the sample with only BPA did not reach the detection limit of hERα bioreporter even in this high concentration. Thus, it is possible to concentrate even highly contaminated samples containing the tested estrogenic chemicals above without impairing BPA detection of BPA-R bioreporter. It should be noted, however, that because of their high number, all existing estrogenic and phenolic compounds could not be tested in this study. For this reason, the specificity of BPA-R receptor is not yet fully validated.

Table 3. High Concentrations of Estrogenic Chemicals in Water Samples Reported in the Literature and Their 5-Fold Concentrations Used in Preparation of Cocktail Sample

chemical	reported concentration in water	final concentration in cocktail sample
17β-estradiol	200 ng L^{-1} [39]	1,000 ng L^{-1}
ethinyl estradiol	830 ng L^{-1} [39]	4,150 ng L^{-1}
estrone	360 ng L^{-1} [26]	1,800 ng L^{-1}
estriol	180 ng L^{-1} [31]	900 ng L^{-1}
nonylphenol	40 μg L^{-1} [39]	200 μg L^{-1}
propyl paraben	2.8 μg L^{-1} [41]	14 μg L^{-1}
bisphenol A	85 μg L^{-1} [9]	425 μg L^{-1}

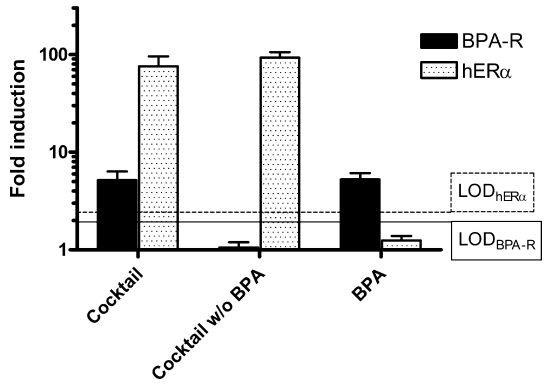

Figure 3. Fold inductions of chemical cocktail sample, cocktail without bisphenol A (BPA), and only BPA measured with BPA-R and hERα bioreporters. Values represent the mean ± standard deviation of two independent experiments, each comprising four parallel data points.

With most environmental samples, it is likely that some sample concentration is needed to reach the detection limit (0.107 μM, i.e., 24 μg L^{-1}) of BPA-R bioreporter. In natural surface waters, most of the detected BPA concentrations have been between 0.5 and 410 ng L^{-1} [6,9,26,29,30] and in wastewater between 2.5 ng L^{-1} and 11.8 μg L^{-1}.[8−12,26,30−32] However, many environmental samples with BPA concentrations above the detection limit of the BPA-R bioreporter have been reported. BPA has been shown to be a major contributor to estrogenicity in landfill leachates and, thus, a significant source of BPA in the environment.[33−36] The highest reported concentrations are ranging from micrograms per liter (53 to 105 μg L^{-1})[9,34,36] to as high as milligrams per liter (5 to 17 mg L^{-1})[33,37] In addition, high BPA concentrations of micro- to milligrams per liter or kilogram have been reported in other environmental samples, such as sediments,[9,26] sewage sludge,[9] compost water,[9] and even some surface waters.[38,39] Crain et al.[2]

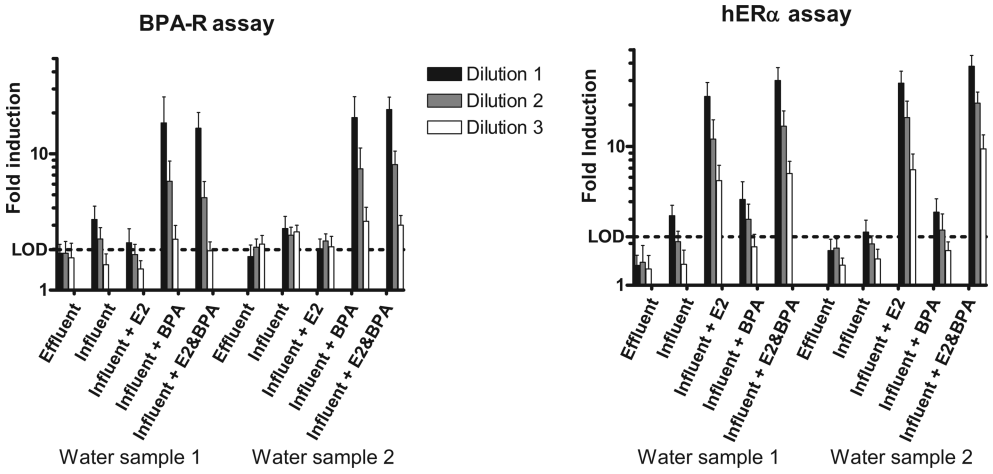

Figure 4. Fold inductions of the freeze-dried nonspiked and spiked wastewater samples 1 and 2 measured with the BPA-R and hERα bioreporters. The 10-fold concentrated wastewaters were diluted 1.65-fold in dilution 1, 2.7-fold in dilution 2, and 4.5-fold in dilution 3. Values represent the mean ± standard deviation of three independent experiments, each comprising four parallel data points. LOD: limit of detection.[28]

claim that, when assessing the risk of environmental concentrations of BPA for wildlife, the maximum detected BPA concentration should be considered because BPA exposure at a critical time point in development can have a permanent effect on the organism.

Next, performance of BPA-R and hERα bioreporters in measuring spiked and unspiked wastewater samples was tested. Samples from the largest wastewater treatment plant (WWTP) in Finland, the Viikinmäki WWTP, were obtained and spiked with BPA, E2, or both. One aliquot of the influent sample was spiked with 0.8 nM E2, a second with 0.88 μM BPA, and a third with both chemicals. The spiked concentrations were chosen so that the E2 concentration was clearly over hERα bioreporter's EC_{50} value of 0.5 nM,[17] and the BPA concentration was less than the EC_{50} of BPA-R bioreporter but still over the LOD of hERα bioreporter. After spiking, the samples were concentrated 10-fold by freeze-drying. Three dilutions (1.65-, 2.7-, and 4.5-fold) of the concentrated sample were tested.

BPA-R bioreporter was induced by the BPA-spiked samples and also with the influent and effluent samples, although at a level close to the detection limit (Figure 4). The samples spiked with E2, however, stayed at the same level with the nonspiked influent samples regardless of the high E2 concentration in the 10-fold concentrated sample (8 nM, i.e., 2.2 μg L^{-1}).

BPA-R bioreporter was able to detect BPA in all dilutions of the sample spiked with BPA (Figure 4). In addition, the samples containing BPA and both BPA and E2 exhibited equal level fold inductions with both water samples, indicating that E2 had no effect on BPA-R bioreporter. Thus, it is evident that E2 does not cause an interference with the BPA-R bioreporter even in concentrated samples.

The hERα bioreporter responded to all spiked samples as well as to the nonspiked influent wastewater samples (Figure 4). The samples spiked with BPA only, however, stayed at very low induction level, with the dilution 3 already below the detection limit. In addition, the hERα bioreporter exhibited high levels of induction with the samples containing E2 and both E2 and BPA (Figure 4). These samples had no difference in their levels of induction. Thus, hERα bioreporter had difficulties in detecting even a rather high concentration of BPA, especially if mixed with additional E2.

BPA-R bioreporter detected the same level of concentrations with overlapping variations in the samples containing BPA and both BPA and E2 (Table 4). The hERα bioreporter, in turn, detected different levels of concentrations between the E2-spiked and E2- and BPA-spiked samples measured in dilution 1, but in other dilutions, the difference was overlapping within variations (Table 4). Thus, BPA-R bioreporter detected only BPA and was not affected by E2 or any other estrogenic chemical in the wastewater sample, whereas hERα bioreporter could detect differences only when the BPA concentration was clearly over the detection limit.

The hERα bioreporter detected a clear excess of BPA in the BPA-spiked sample (Table 4). The reason for this was probably the background estrogenicity of the influent wastewater (Figure 3), corresponding to 0.054 ± 0.012 nM E2-equivalents (i.e., 15 ng L^{-1}). Although the background estrogenicity and the spiked BPA concentrations were both supposed to be below the LOD in dilution 2 (Figure 2, Tables 2 and 3), the mixture effect was enough to cause a detectable induction. This kind of additive effect of sub-LOD concentrations of chemicals has also previously been suspected to cause a positive signal in the yeast ER bioreporter assay.[31]

The detected equivalent concentrations of E2 and/or BPA by both bioreporters were lower than the spiked concentrations (Table 4). The BPA-R bioreporter had detection efficiency of 28−38% in wastewater 1 and 40−49% in wastewater 2, while those of hERα bioreporter were 28−36% and 33−49%, respectively (Table 4). Detection efficiency was slightly higher in the influent wastewater sample 2, which by superficial inspection seemed to have less solid matter.

The effect of sample toxicity, freeze-drying, and unspecific matrix effect were regarded as possible reasons for the low detection efficiencies of the yeast bioreporters. Toxicity of the sample dilutions was expressed as a correction factor, i.e., fold decrease of light signal caused by exposure to sample compared to solvent control, by using the constitutively luminescent control strain BMA64/luc (see Supporting Information Figure S-1). The effect of toxicity of the samples on the bioreporter strains hERα and BPA-R was evaluated by comparing the detection efficiencies in different dilutions (Table 4). The detection efficiencies of both bioreporters were nearly similar in all measured dilutions, with no or only a few percentage points

Table 4. Detection Efficiency of Freeze Dried Spiked Influent Wastewater Samples by BPA-R and hERα Yeast Bioreporters

			BPA-R assay				hERα assay			
			water sample 1		water sample 2		water sample 1		water sample 2	
water sample	sample dilution[a]	spiked analyte concentration[b]	detected analyte concentration[c]	detection, %[d]	detected analyte concentration[c]	detection, %[d]	detected analyte concentration[c]	detection, %[d]	detected analyte concentration[c]	detection, %[d]
influent	nondiluted		0.14 ± 0.04 μM BPA_{eq}		0.12 ± 0.04 μM BPA_{eq}		0.052 ± 0.013 nM $E2_{eq}$		0.039 ± 0.012 nM $E2_{eq}$	
influent + BPA	1	0.53 μM	0.20 ± 0.06 μM BPA_{eq}	38	0.22 ± 0.04 μM BPA_{eq}	41	0.58 ± 0.12 μM BPA_{eq}	109	0.50 ± 0.08 μM BPA_{eq}	95
	2	0.32 μM	0.12 ± 0.02 μM BPA_{eq}	38	0.14 ± 0.02 μM BPA_{eq}	43	0.45 ± 0.08 μM BPA_{eq}	141	0.39 ± 0.09 μM BPA_{eq}	120
	3	0.20 μM	0.06 ± 0.01 μM BPA_{eq}	33	0.08 ± 0.01 μM BPA_{eq}	42	<LOD[f]		<LOD[f]	
influent + BPA and E2	1	0.53 μM + 0.48 nM	0.20 ± 0.01 μM BPA_{eq}	37	0.26 ± 0.03 μM BPA_{eq}	49	0.20 ± 0.03 nM $E2_{eq}$	36[e]	0.24 ± 0.02 nM $E2_{eq}$	44[e]
	2	0.32 μM + 0.29 nM	0.10 ± 0.01 μM BPA_{eq}	32	0.14 ± 0.01 μM BPA_{eq}	45	0.10 ± 0.01 nM $E2_{eq}$	35[e]	0.14 ± 0.01 nM $E2_{eq}$	49[e]
	3	0.20 μM + 0.18 nM	0.05 ± 0.01 μM BPA_{eq}	28	0.08 ± 0.01 μM BPA_{eq}	40	0.06 ± 0.01 nM $E2_{eq}$	32[e]	0.08 ± 0.01 nM $E2_{eq}$	44[e]
influent + E2	1	0.48 nM	0.06 ± 0.02 μM BPA_{eq}		0.06 ± 0.01 μM BPA_{eq}		0.15 ± 0.02 nM $E2_{eq}$	32	0.19 ± 0.01 nM $E2_{eq}$	39
	2	0.29 nM	0.05 ± 0.02 μM BPA_{eq}		0.07 ± 0.01 μM BPA_{eq}		0.09 ± 0.02 nM $E2_{eq}$	30	0.12 ± 0.02 nM $E2_{eq}$	39
	3	0.18 nM	<LOD[f]		0.06 ± 0.01 μM BPA_{eq}		0.06 ± 0.01 nM $E2_{eq}$	28	0.06 ± 0.01 nM $E2_{eq}$	33

[a]The 10-fold concentrated water samples were diluted 1.65-fold in dilution 1, 2.7-fold in dilution 2, and 4.5-fold in dilution 3. [b]Analyte concentration in the original spiked influent sample before freeze-drying. [c]Values represent the mean ± standard deviation of three independent experiments, each comprising four parallel data points. Concentrations are given as either bisphenol A equivalents (BPA_{eq}) or 17β-estradiol equivalents ($E2_{eq}$). [d]Detection percentage was calculated using the average detected concentration. [e]Detection percentage was calculated using the expected sum of estrogenic activity of E2 and BPA for the hERα bioreporter in the mixture. [f]LOD: limit of detection.

difference between the dilutions. Thus, toxicity of the sample had a minor or no effect on the detection efficiency of either bioreporter.

In order to assess the effect of freeze-drying to the detection efficiency, three freeze-dried influent samples were spiked after freeze-drying and analyzed with both bioreporters. The spiked concentration was 10-fold compared to the samples that were spiked before freeze-drying in order to reach the same theoretical concentration in both sets of spiked influent samples.

The detection efficiencies of the samples spiked after freeze-drying by BPA-R bioreporter were on average 31% higher, and those of hERα bioreporter were 37% higher (Table 4 and Supporting Information Table S-1). Thus, freeze-drying had an effect on the detection efficiency of both bioreporters, with somewhat higher effect on the detection of E2 by hERα bioreporter compared to the BPA detection by BPA-R bioreporter.

Freeze-drying has been considered a good method to concentrate water samples because of minimal loss of active material.[18] However, it has been noted before that hydrophobic compounds, such as steroid hormones, might get adsorbed to the walls of plastic containers during freeze-drying, causing loss to the detected chemical.[22] In this study, it was possible that E2 was somewhat more affected by freeze-drying than the more hydrophilic BPA; however, the difference was only a few percentages. The possible adsorption effect should be considered when choosing a method to concentrate water samples. On the basis of our results, freeze-drying is a reasonably good option.

Since the effects of toxicity and freeze-drying did not fully explain the detection efficiencies of the yeast bioreporters, it is probable that the wastewaters exhibited some matrix effect. Detection efficiencies of both bioreporters in influent wastewater sample 1 were lower than in sample 2 (Table 4 and Table S1 in the Supporting Information). Sample 1 contained more organic solid matter that could adsorb part of the analytes and thus lower their bioavailability. Matrix effect has been shown to cause error in bioreporter-based measurement of endocrine disrupting compounds in environmental samples and wastewater.[18,22,40] Even chemical analytical methods can suffer from matrix effect.[15] The effects reducing detection efficiency can be assessed by, for example, using internal standards. When using the BPA-R bioreporter assay, a parallel sample spiked with BPA is strongly recommended.

CONCLUSIONS

The bisphenol A specific receptor (BPA-R) bioreporter characterized in this study is the first yeast-based bioreporter developed for detection of a single chemical. This bioreporter overcomes the commonly acknowledged drawback of nuclear receptor bioreporter assays: the lack of chemical specificity.[16] BPA-R bioreporter assay combines the easiness of bioassays with the unique specificity toward BPA without the need for extensive chemical analysis. BPA was successfully measured in concentrated influent wastewater samples. In addition, there was no interference with the background estrogenicity or even high concentration of spiked E2. The bioreporter assay can be routinely performed in a high-throughput 384-well microplate format combined with automated liquid handling. In addition, BPA-R bioreporter had also a higher signal intensity compared to hERα bioreporter, which makes it more suitable for direct measurement of untreated turbid samples like sediments. All

these features make BPA-R bioreporter a good option for cost-efficient monitoring of BPA in complex samples.

BPA-R yeast bioreporter is a valuable addition to the battery of yeast bioreporter assays used in environmental monitoring. It can be applied for both detection of BPA and, when combined with estrogen receptor bioreporter, assessment of the proportion of estrogenicity caused by BPA in a complex sample. Samples such as wastewater, landfill effluents, sediments, and leachates from solid materials could be measured using BPA-R bioreporter.

It is probable that some sample concentration for BPA measurement with BPA-R bioreporter is needed with most environmental water samples unless high contamination rate is suspected. Freeze-drying is a reasonably good option for BPA water sample concentration. To increase water concentration in measurements, it is also possible to use a 50:50 ratio of sample and yeast instead of 10:90 used in this study.

In spite of the difficulties in detecting single low potency compounds in complex mixtures, the wild type receptor bioreporters, such as the hERα bioreporter, are useful in determining the total hormonal activity of a sample. Compared to chemical analytical methods, bioreporters are capable of, for example, detecting previously unknown chemicals, and the mixture effect of several chemicals without the need to know the chemical composition of the sample beforehand.

■ ASSOCIATED CONTENT

Supporting Information

Wastewater sample toxicity and detected concentrations of the samples spiked after freeze-drying. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

J. Rajasärkkä and M. Virta designed the study; J. Rajasärkkä conducted the experiments, analyzed the data, wrote the manuscript, and acted as corresponding author.

Notes

The authors declare no competing financial interest.

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