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RESEARCH PAPER

Monitoring bisphenol A and estrogenic chemicals in thermal paper with yeast-based bioreporter assay

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Abstract Bioluminescent *Saccharomyces cerevisiae* yeast-based bioreporters were used to monitor bisphenol A and other estrogenic chemicals in thermal paper samples collected mainly from Finland on two occasions in 2010/2011, and 2013. The bisphenol A-targeted (BPA-R) and the human oestrogen receptor (hERα) bioreporters were applied to analyse both non-treated and extracted paper samples. Bisphenol A was readily bioavailable to the yeast bioreporters on the non-treated paper samples without any pre-treatment. Detected concentrations ranged from a detection limit of 9–142 μg/g to over 20 mg/g of bisphenol A equivalents in the thermal papers. Low bisphenol A like activities were detected in many samples, and were considered to be caused by residual bisphenol A or other types of bisphenols, such as bisphenol S. Most of the thermal paper samples were toxic to the yeast bioreporters. The toxicity did not, however, depend on the bisphenol A concentration of the samples. The yeast bioreporters were demonstrated to be a robust and cost-efficient method to monitor thermal paper samples for their bisphenol A content and estrogenicity. Thermal paper was considered as a potential BPA source for both human exposure and environmental emission.

Keywords Bioavailability · Bioreporter · Bisphenol A · Endocrine-disrupting compounds · *Saccharomyces cerevisiae* · Thermal paper

Abbreviations

BPA	Bisphenol A
BPA-R	Bisphenol A targeted receptor
BPF	Bisphenol F
BPS	Bisphenol S
hERα	Human oestrogen receptor α
LOD	Limit of detection
LOQ	Limit of quantification

Introduction

Bisphenol A (BPA) can be regarded as an everyday chemical for people in Western countries. It is one of the most highly produced chemicals in the world, and it is used in numerous different applications. These include, for example, plastic containers for food and beverages, coatings of food cans and water pipes, dental sealants, medical devices, paints, components in electrical industry, thermal papers, polyvinyl chloride products, flame retardants and polyester and polysulfone resins [1, 2].

Not surprisingly, the majority of people are constantly exposed to BPA [3, 4]. Human exposure to BPA is thought to occur mainly via food and drink [5, 6]. As BPA is the key monomer used to manufacture polycarbonate and resin polymers, any un- or depolymerized BPA is readily leached into the contents. In addition to human exposure, BPA is also widely detected in the environment [7–10], possibly due to

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leaching from BPA-containing products at, for example, landfills [11, 12].

Bisphenol A is a classic example of an endocrine-disrupting compound. It has been shown to be able to function as a xenoestrogen in several organisms, causing effects such as disturbed reproduction, organogenesis, metabolism and neurodevelopment [13–15]. While the present tolerable daily intake is set to 50 microgram/kg bw by the US Environmental Protection Agency and European Food Safety Authority [2, 16], there is an ongoing debate about possible low-dose effects of BPA below this limit [14, 17].

Over 90 % of BPA produced in the European Union is used to for polycarbonate plastics and epoxy resin [1]. However, in addition to oral exposure, other routes have gained attention recently. Especially dermal absorption of BPA due to handling BPA-containing thermal paper has been suggested to have significant role in public and especially occupational BPA exposure [18–23].

Thermal paper is frequently used in cash registers, portable printers and many others for printing receipts, tickets, coupons, labels and stickers. The paper is coated with a solid mixture of chemicals including dyes, developers, solvents and sensitizers. BPA is preferred as a developer in thermal paper due to its low cost. The use of BPA in thermal paper, however, has raised concern since it is used in high quantities, from several milligrams per gram up to 21 mg/g of paper [19–22, 24]. Since BPA is applied as monomeric form on the paper, it is more readily leached from the paper than from plastic products. BPA has been shown to be absorbable via skin [19, 25], and it is easily transferred to other contact material such as paper currency [20, 26].

Due to the concern of possible health risks of BPA, BPA-free products, for example, baby bottles and thermal papers, have been introduced into the market in recent years. BPA has been replaced with alternative chemicals, such as bisphenol S (BPS, Fig. 1). Also bisphenol F (BPF, Fig. 1) has been used as a resin component similarly as BPA [27].

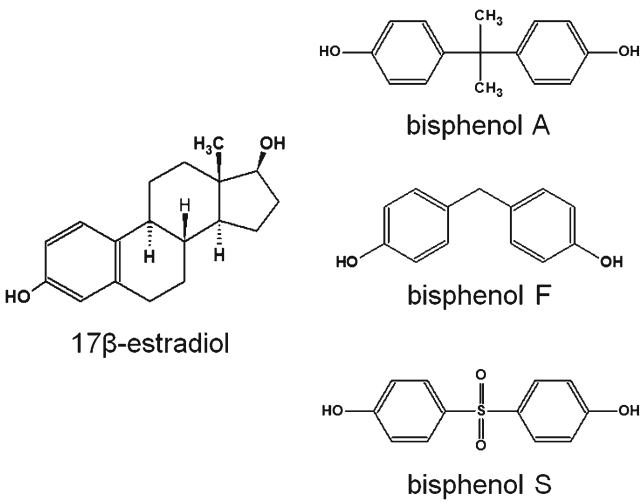

Fig. 1 Chemical structures of oestrogen hormone (17β-estradiol) and bisphenols A, F and S

Several studies have measured BPA in thermal paper using analytical chemical methods such as gas and liquid chromatography [19–22, 24]. These methods, however, usually require extensive sample preparation and purification, and experienced users. They are also only able to measure compounds of known chemical structure, and cannot assess total biological activity in the sample.

Living cell-based bioreporters in turn are able to measure total biological activity of complex samples. In addition, they are usually time and cost-efficient, suitable for high-throughput screening and require little sample purification or pre-treatment [28].

In this study, living cell-based bioreporter assays were used to monitor the quantity and bioavailability of BPA and total estrogenic activity in thermal paper samples. Bioluminescent yeast bioreporters utilising BPA-targeted receptor (BPA-R) [29] and human oestrogen receptor α (hERα) [30] were used. The BPA-R bioreporter expresses a mutated hERα receptor which has an enhanced affinity towards BPA and reduced affinity towards 17β-estradiol and other estrogenic compounds. Both BPA-R and hERα bioreporters are based on the activation of firefly luciferase expression upon target chemical binding to the receptors.

A total of 70 samples were collected and analysed in two sampling time periods in the turn of years 2010 and 2011, and in 2013 and analysed with the yeast bioreporters. In addition, liquid chromatography coupled with mass spectrometry was used to compare the results of the bioreporters to the content of different bisphenols in 10 samples.

Experimental

Media and chemicals

Synthetic minimal medium (SD) consisted of 6.7 g/L yeast nitrogen base without amino acids (Beckton Dickinson, and Company, Erembodegem, Belgium) supplemented with 2.5 % final concentration of D-glucose (AMRESCO, Solon, OH), histidine (20 mg/L), leucine (100 mg/L), adenine (50 mg/L), and tryptophan (20 mg/L, only for the BMA64/luc yeast strain), all from Sigma-Aldrich, Schnelldorf, Germany. Bisphenols A, F, and S were purchased from Sigma-Aldrich, Schnelldorf, Germany. D-luciferin (BioThema) was purchased from Aboatox (Turku, Finland). Mass-labelled ^{13}C-BPA was obtained from Santa Cruz Biotechnology Inc. (Dallas, USA), and methanol (HPLC grade) from J.T. Baker (Deventer, the Netherlands).

Yeast strains

The bisphenol A-targeted receptor (BPA-R) *Saccharomyces cerevisiae* yeast strain has been characterised previously [29]. This strain contains a mutated human oestrogen receptor α with enhanced affinity towards BPA and low affinity towards 17β-estradiol and other estrogens. Wild-type human oestrogen receptor hERα yeast strain, and the constitutively luminescent control strain BMA64/luc have also been characterised previously in literature [30].

Sample preparations for bioreporter assays

A total of 70 thermal paper and other samples were collected in grocery stores and other sources in two separate time points: at the turn of years 2010 and 2011, and at 2013 in Finland (65 samples), USA (2 samples), Sweden (2 samples) and Estonia (1 sample). Samples were stored in resealable polyethylene plastic bags before treatment. Samples were treated and analysed at the respective times of sampling.

For direct contact measurement, paper pieces with diameter of 3.2 mm were prepared using a small hole punch. Punch pieces were collected and stored in room temperature in micro centrifuge tubes. All samples were analysed in triplicate in one to two independent experiments.

For extraction procedure, paper pieces with 9 mm were taken using a regular hole punch. Punch pieces were weighed in micro centrifuge tubes and extracted with 50 % DMSO. Tubes were vortexed briefly and incubated at room temperature for 30 min. Extracts were then collected in clean micro centrifuge tubes and stored in −20 °C. In year 2010/2011 extract dilutions of 4–128-fold, and in year 2013 dilutions of 5–135-fold were analysed with the yeast bioreporters. All samples were analysed in quadruplicate in two independent experiments.

Bioreporter assay procedure

The bioreporter assay for measuring untreated paper samples (the so-called direct contact method) was performed in white 96 well plates (OptiPlate-96, PerkinElmer Inc., USA) using a regular procedure described previously [30]. Briefly, a culture of yeast cells was grown in 5 mL of SD medium and incubated overnight at 30 °C with 250 rpm shaking. On the morning, the culture was diluted in fresh SD medium to optical density (OD_{600}) of 0.4. The yeast culture was further grown for about 2 h until OD_{600} reached 0.6–0.7. Ten microlitres of 10 % DMSO was pipetted on the well plates, after which paper pieces were applied on the droplet using forceps. This prevented the pieces from escaping the wells. Thereafter, 90 μL of yeast culture was added. The plate was covered with a lid, shaken for 20 s, and incubated at 30 °C for 2.5 h. Subsequently, the plate was shaken for 20 s, and 100 μL of 1-mM D-luciferin in 0.1-M Na-citrate buffer was added. After briefly shaking the plate, luminescence was measured immediately using Victor3 1420 Multilabel Counter (PerkinElmer/Wallac, Turku, Finland) set in luminescence mode for 1 s/well.

The bioreporter assay for measuring paper extracts was performed in the 384-well microplate format using automated liquid handling as described previously [31]. The cultivation steps were done as in the 96-well plate procedure. After cultivation, 10-mM D-luciferin stock solution in 0.2-M sodium citrate buffer (pH 5) was added to the yeast culture into final concentration of 0.5-mM D-luciferin. All subsequent liquid handling was performed robotically by Biomek NXP Laboratory Automation Workstation (Beckman Coulter, Munich, Germany): 5 μL of paper extract dilutions (from 4- to 128-fold dilutions in 2010/2011, and from 5- to 135-fold dilutions in 2013) or standard bisphenol chemical solution was dispensed into white 384-well plates (OptiPlate-384, PerkinElmer Inc., USA) and 45-μL yeast culture mixed with D-luciferin was added. Each plate was incubated for 3 h at 30 °C, and luminescence was measured as above.

Data analysis for bioreporter assays

Fold-inductions and toxicity correction factors were calculated as previously described [30]. 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 correcting toxic and other sample-borne effects on signal was calculated using the constitutive control strain BMA64/luc by dividing the solvent background luminescence with the luminescence signal of the sample. All fold induction values of the samples and reference bisphenol compound solutions 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 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. [32].

Bisphenol A-equivalent concentrations (BPA_{eq}) were calculated by comparing the corresponding fold induction values of each data point to the BPA standard curve prepared on the same day. Concentration was calculated using the sigmoidal dose–response curve equation ($y = \text{bottom} + (\text{top-bottom})/(1+10^{((\log EC50 - x)\times \text{hill slope})})$). The calculated total concentrations were then divided with the weight of each paper sample.

Chemical analysis of paper samples

Ten paper samples of sampling time point 2013 (sample codes 4_2013, 33_2013, 36_2013, 41_2013, 42_2013, 48_2013, 49_2013, 53_2013, 57_2013 and 24_2013) were analysed for their BPA, BPS and BPF concentrations by liquid chromatography–triple quadrupole mass spectrometry (Thermo Scientific UltiMate 3000 Rapid Separation LC system connected to TSQ Quantum Discovery MAX spectrometer) using electrospray ionisation (ESI) in a negative ion mode. Samples for chemical analysis were chosen so that they showed different activity profiles on the bioreporter assays between each other or between the two bioreporters.

Prior to instrumental analysis, the samples were extracted with 2 mL 60 % aqueous methanol (methanol/water, 60:40, *v*/*v*). Bisphenols were separated on a Waters XBridge C18 column (50×2.1 mm i.d., 3.5 μm) with a gradient elution. Chromatograms were recorded by single reaction monitoring (SRM) with a specific transition per analyte. The analytes were quantified using a calibration curve. For BPA, the quantification was carried out using a mass-labelled internal standard, and for BPS and BPF, an external standard method was applied. Linearity of detector response was tested using the concentration range of 2.5–125 ng/mL for individual bisphenols, and was found acceptable ($R2>0.999$). The lowest concentration in the linearity test and calibration curve was considered as the limit of the quantification (LOQ).

Results and discussion

S. cerevisiae yeast-based bioreporters utilising bisphenol A-targeted receptor (BPA-R) [29] and human oestrogen receptor (hERα) [30] were used in the present study. The BPA-R bioreporter is intended for specific detection of bisphenol A, whereas the hERα bioreporter measures combined effects of all estrogenic compounds in a sample. Although 17β-estradiol is the regular reference compound for hERα, BPA was used for both bioreporters for more efficient comparison of results and quantification BPA equivalents in the samples.

Bisphenols A, F and S (BPA, BPS and BPF) were first tested for their potency with the BPA-R and hERα bioreporters. BPA and BPS are commonly used in thermal papers as a developer, whereas BPF has been mainly used in resin manufacturing.

BPA showed highest potency of all bisphenols on the BPA-R bioreporter whereas BPF and BPS were about 1.5 and 2.5 orders of magnitude less potent inducers, respectively (Fig. 2). On hERα bioreporter, BPF showed equal estrogenicity with BPA, whereas BPS was about two orders of magnitude less potent (Fig. 2).

As shown previously, BPA-R bioreporter had about four-fold lower detection limit for BPA compared to hERα bioreporter [29] (Table 1). While the detection limit for BPF was about 10-fold on the BPA-R compared to hERα, the detection limits for BPS were quite similar between the two bioreporters. Thus, BPS has significantly lower potency on both bioreporters compared to BPA. However, only BPA-R shows clear discrimination between BPA and BPF while BPF had equal potency on the hERα bioreporter.

BPF has been shown to exhibit similar estrogenic potency as BPA also on a previously published yeast bioreporter assay, whereas BPS was a clearly weaker xenoestrogen [33]. However, in mammalian cell-based assays BPS has shown comparable estrogenic activity with BPA [34]. The difference between the assays might be due to longer incubation step in the mammalian cell assay, and possibly differing metabolism, for example degradation, of the test substances in yeast and mammalian cells.

Next, thermal paper and other samples were tested for their BPA-like and estrogenic activity using the BPA-R and hERα bioreporters. Thermal paper is used mainly as point-of-sale receipts (50 % in EU [1]). For this reason, majority of tested papers were obtained from cashiers.

Bioavailability of chemicals in the paper was evaluated by analysing non-treated samples. Small pieces of paper (diameter 3.2 mm) were excised using a hole punch, and the pieces were analysed in 96-well plates.

Majority of the samples collected in 2010/2011 and a large proportion of samples in 2013 caused an activity of at least 85 % of the maximal induction level in both yeast bioreporters (Fig. 3). Since the activities of nearly all of these samples were high on both the BPA-targeted BPA-R and the total estrogenicity-detecting hERα bioreporters, they were concluded to contain BPA.

In year 2013, there were somewhat less high-activity and more no-activity samples compared to year 2010/2011. This was anticipated since after the first sampling period, BPA-free thermal paper became more popular in Finnish markets. However, this finding was not statistically confirmed since the sample number was small and the samples were not all obtained from same locations in both sampling time points.

Activities of the samples were corrected for moderate toxicity using the constitutively luminescent control strain BMA64/luc [30] (see Data analysis for bioreporter assays in Experimental). The threshold value for reliable quantification of BPA equivalents (BPA_{eq}) was set to a maximum of three-fold reduction of the luminescence signal of the control strain. However, a great proportion of the non-treated thermal paper samples showed toxicities significantly higher than this: in sampling time period 2010/2011 nearly 40 % and in year 2013 nearly 60 % of the samples (see Supplementary material). Toxicity was not significantly reduced when smaller paper samples (ø 1.6 mm) were tested (data not shown).

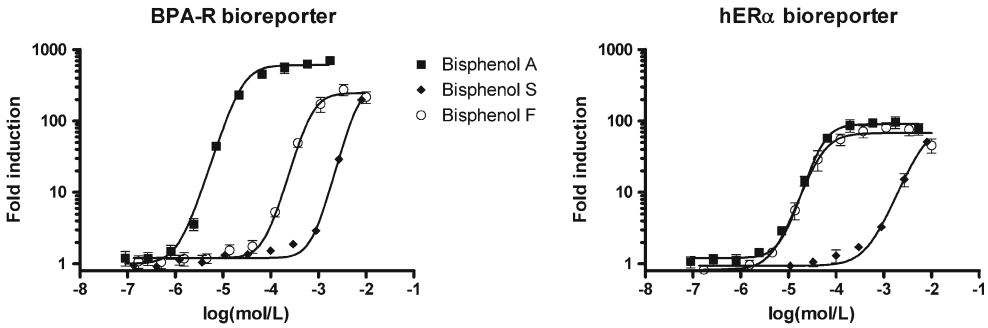

Fig. 2 Dose–response curves of BPA-R and hERα bioreporters for bisphenol A, F and S. Results are given as averages ± standard deviation of two independent experiments

High toxicity prevented the detection of low activities by lowering the responses below LOD, and reliable quantification of high-activity samples due to high correction factor. Toxicity was not due to BPA, BPS or BPF since the yeast was very tolerant towards tested high concentrations of the pure solutions. The reduction of luminescence signal of the control strain was lower than twofold with BPA and BPS up to 1.2 and 2 g/L, respectively, and lower than threefold with BPF up to 0.67 g/L sample concentration (see Electronic Supplementary Material Fig S1). Nor did toxicity correlate with the activity of the samples or the appearance of the paper. The reason could be other chemicals used in the paper, such as dyes or sensitizers. The identity and possible toxicity of these chemicals to other organisms remains to be clarified, but should be of high interest due to the common usage of thermal papers.

The limits of detection (LOD) for BPA_{eq} for the non-treated samples were, on average, 11±2 μg/g on BPA-R bioreporter, and 98±36 μg/g on hERα bioreporter. The EC_{85} values (i.e. the upper quantitative limit) were 328±163 and 1,321±374 μg/g for the BPA-R and hERα bioreporters, respectively. The upper quantitative limits were relatively low compared to the reported BPA concentrations of several milligrams per gram in thermal paper [19–22, 24]. Thus, high activity of samples, as well as toxicity prevented reliable quantitative measurement of BPA_{eq} concentrations in the non-treated thermal paper samples.

In order to gain quantitative results, paper samples were extracted with DMSO, and dilutions of the extracts were measured with the both bioreporters. Half of the analysed paper samples contained at least 1 mg/g BPA_{eq} (Fig. 4). The highest BPA_{eq} concentrations measured in the samples were over 20 mg/g (see Supplementary Material, Table S1). The upper quantitative limits (i.e. EC_{85} levels of induction) were, on average, 10±5 and 23±3 mg/g on the BPA-R bioreporter in years 2010/2011 and 2013, respectively (depending on the greatest extract dilution used in each year), and 53±8 and 71±19 mg/g on the hERα bioreporter.

As expected, nearly all of the samples with over 1 mg/g BPA_{eq} caused an activity of >85 % in the bioreporter assays with non-treated paper pieces (Figs. 3 and 4). Thus, BPA in thermal paper was considered to be directly bioavailable to the yeast bioreporters. High bioavailability is in accordance with other studies, where BPA has been shown to readily transfer from thermal paper to skin and other contact materials [19, 23, 25, 26]. Frequent handling of thermal paper by, for example, cashiers, can result in even higher BPA concentration in urine than high consumption of canned food [18].

BPA-R bioreporter detected low BPA_{eq} concentrations of less than 1 mg/g on a greater number of samples than hERα bioreporter (Fig. 4). This was due to higher sensitivity of BPA-R bioreporter towards BPA: the LOD of the BPA-R bioreporter for BPA was on average 9±6 and 29±3 μg/g in years 2010/2011 and 2013, respectively, whereas hERα bioreporter had LODs of 64±21 and 142±31 μg/g.

Majority of the analysed samples were cash register receipts, of which less than half contained over 1 mg/g of BPA_{eq} (Fig. 5). The other receipt samples, such as queuing tickets, bottle return receipts and cash withdrawal receipts, had proportionally more high-concentration samples. In addition, all of the three tested scale stickers for vegetables in supermarkets contained more than 1 mg/g BPA_{eq}. Some of the transportation tickets, such as bus tickets, also contained high concentrations of BPA_{eq}.

The samples were mainly collected in Finland. Two cashier receipts were from Sweden, and they contained very low BPA_{eq} concentrations of about 40 and 9 μg/g (see Supplementary Material, Table S1). Of the two receipts from USA,

Table 1 Detection limits of BPA-R and hERα bioreporters for bisphenol A, F and S

Bioreporter	Detection limits (mg/L)[a]		
	Bisphenol A	Bisphenol F	Bisphenol S
BPA-R	0.3±0.1	12.8±1.3	162.4±3.6
hERα	1.3±0.1	1.2±0.6	110.1±10.6

[a] Concentrations are given as average sample concentrations±standard deviation

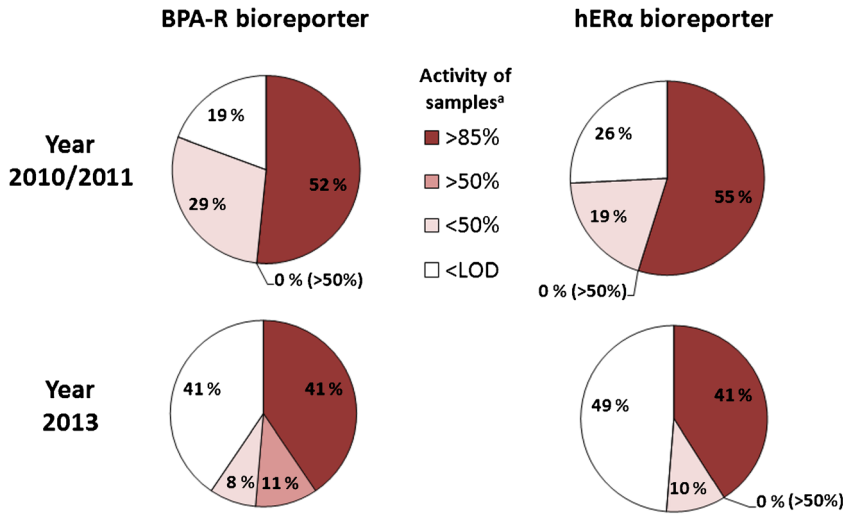

Fig. 3 Activities of non-treated paper samples in direct contact measurement with BPA-R and hERα bioreporters. Both sampling time points are shown separately. [a]Activities of samples are given as percentage of the maximal fold induction level of the bioreporter. *LOD* limit of detection

one had very low BPA_{eq} quantity whereas the other had over 10 mg/g of BPA_{eq}. The cashier receipt from Estonia had a very high content of over 20 mg/g of BPA_{eq}.

Of the other tested papers and samples, only the unprinted thermal paper rolls contained over 1 mg/g of BPA_{eq}. Napkin towel, new paper notebooks, regular non-printed paper receipt and the resealable plastic bag used for storing the samples were tested negative. Newspaper and a recycled paper note book showed very low BPA_{eq} content with the BPA-R bioreporter, although at a level close to LOD (see Supplementary Material, Table S1).

BPA and BPS have been previously found in recycled paper products such as newspaper, hand towels, toilet paper and carton products [20, 23, 35]. Thus, the chemicals used in thermal papers are entering also other paper products via recycling.

In EU, however, 70 % of thermal paper does not enter recycling [1]. Especially in landfills, thermal paper waste can be a significant source of BPA contamination in the environment [20, 35]. Although BPA is usually readily degradable, it has been suggested to be, in fact, pseudo-persistent due to continuous emissions to the environment [13].

In order to evaluate the quantitative and qualitative performance of the yeast bioreporters, 10 samples with different activities from 2013 were analysed with instrumental method

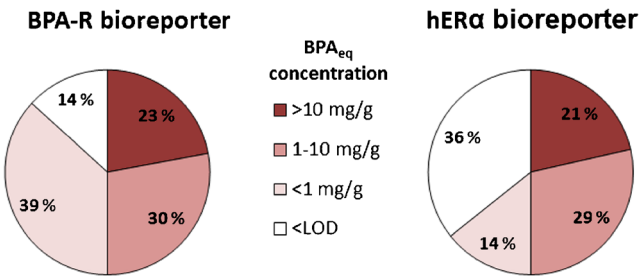

Fig. 4 Bisphenol A equivalent concentrations of samples. Both sampling time points are combined in the figures

(liquid chromatography-mass spectrometry, i.e. LC-MS). The results of the two methods are shown in Table 2.

High BPA equivalent concentration of the yeast bioreporters correlated well with the BPA results of the LC-MS analysis (Table 2). The BPA concentrations were at same order of magnitude, although the results gained from the yeast assays were slightly higher than those from LC-MS. Low BPA_{eq} measured with the yeast assays were not, in contrast, shown to contain significant amounts of BPS or BPF in LC-MS. Some of the low activity samples (codes 49 and 57) had residual BPA in addition to low amounts of BPS. It has been shown previously, that some BPA-free thermal papers can actually contain residual BPA [20]. Residual BPA probably contributed to the activity detected in the yeast assays since the LODs of the yeast assays for BPS were rather high (about 5–15 mg/g, estimated from results in Table 1).

In other samples (codes 4, 41 and 42), however, the low activity could not be explained by residual BPA—nor significant concentrations of BPS. It is possible that these samples contain some other phenolic or estrogenic chemicals which were not tested with the yeast bioreporters in this study. These could include, for example, other types of bisphenols or even parabens [36].

It is still possible that other samples not tested with LC-MS, but on which both bioreporters detected low BPA equivalent concentration, can contain BPS. BPS concentrations in thermal paper have been reported to be at a similar range of several milligrams per gram that has been used for BPA [23], and such concentrations are high enough to cause a low signal in BPA-R and hERα bioreporters.

Although BPA has been widely replaced with BPS, this approach is still controversial. Besides the discussion of its estrogenic potency, BPS has shown lower degradability compared to BPA in certain environmental conditions [37, 38]. This can lead to accumulation of the chemical, and thus the increased xenoestrogenic burden of the environment.

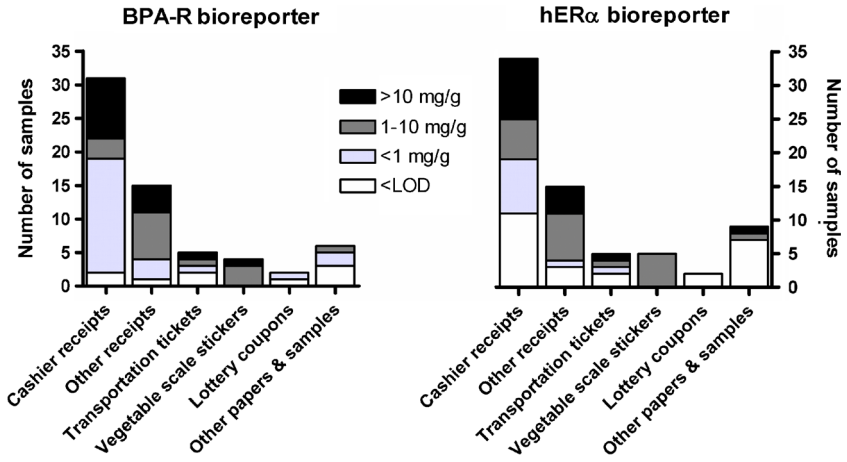

Fig. 5 Equivalent concentrations of bisphenol A in the sample types analysed with BPA-R and hERα bioreporters. Both sampling time points are combined in the figure

Conclusions

The yeast-based bioreporter assays were well applicable to BPA and other estrogenic chemical detection in thermal paper samples. Non-treated samples could be analysed for the bioavailability of BPA, and to distinguish samples with high or low activities. Extraction of samples was needed for quantitative results due to toxicity and high BPA concentrations in the samples. The yeast bioreporters are a user-friendly, inexpensive and robust method for analysing different kind of complex samples. Furthermore, yeast bioreporter assay can be used to high-throughput analysis of samples in 384-well plates.

Equivalent concentrations of BPA were measured by using the BPA-R bioreporter, and effects-based monitoring of estrogenic chemicals was performed by using the hERα bioreporter. By using a combination of these two bioreporters, BPA was concluded to be the main cause of estrogenicity in high-activity samples. The chemical analysis with LC-MS also supported this conclusion. Low activities of the samples were probably due to residual BPA in some of the thermal papers, and BPS or other phenolic or estrogenic chemicals in others. Such chemicals could include, for example, other bisphenol types or dyes and sensitizers used in the chemical coating mixture.

According to our results, BPA is readily bioavailable to the yeast bioreporters. It was also easily dissolved from the paper samples to the extracts. The high bioavailability of BPA in thermal papers detected in this study is in accordance with previous studies.

Due to the high concentration of bioavailable BPA in thermal papers, the use of BPA-containing thermal papers can pose a previously underestimated risk to humans via handling of the papers and residual BPA in recycled paper products. Thermal paper waste can also be a great contributor to the emissions of BPA into the environment. Finally, risks of other possibly toxic chemicals in thermal paper should be further studied.

Table 2 Comparison of the bisphenol A-equivalent concentrations measured with the BPA-R and hERα bioreporters and results of LC-MS analysis

Code	Sample type	BPA_{eq}: yeast assays (mg/g)		Bisphenols: LC-MS (mg/g)		
		BPA-R	hERα	BPA	BPS	BPF
4_2013	Cashier receipt	0.06±0.01	<1.5	<0.010	<0.0001	<0.0001
33_2013		18.4±1.1	11.4±2.1	12.2±0.2	<0.0001	<0.0001
36_2013		<0.04	<1.7	<0.010	0.0009±0.00005	<0.0001
41_2013		0.09±0.02	<0.13	<0.010	<0.0001	<0.0001
42_2013		0.44±0.07	<1.4	<0.010	0.011±0.005	<0.0001
48_2013		>27.3	20.4±2.9	15.1±0.4	<0.0001	<0.0001
49_2013		0.11±0.02	0.13±0.01	0.05±0.01	0.246±0.030	<0.0001
53_2013		13.5±3.9	8.9±1.0	7.7±0.3	<0.0001	<0.0001
57_2013		0.06±0.02	0.56±0.08	0.06±0.02	0.009±0.001	<0.0001
24_2013	Vegetable scale sticker	>7.1	5.6±0.6	8.8±1.1	<0.0001	0.0007±0.00005

BPA bisphenol A, *BPS* bisphenol S, *BPF* bisphenol F

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Conflict of interest The authors declare no conflict of interest.

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