Evaluation and Comparison of Bisphenol A Analog Activity Using ToxCast Data

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Background

- Bisphenol A (BPA) is used in consumer products and industrial applications, primarily in plastics, and has been detected in the environment, human urine, blood, and breast milk.
- Mainly studied as an endocrine disruptor, other toxicities, including obesity, metabolic conditions such as diabetes, and neurodevelopmental effects have also been associated with exposure to BPA, indicating that its effects may not be limited to estrogenicity.
- In addition, a number of BPA analogs are in use and may exhibit other additional toxicities.

Objective

Determine the relative activity of BPA and 20 BPA analogs using ToxCast data in order to gain a greater understanding of their activity not only as possible endocrine disruptors but also across a wide spectrum of potential biological targets

Methods

- We used the Toxicological Priority Index (ToxPi) software to create graphical representations of BPA analog activity and to calculate dimensionless index scores for each analog's activity.
- Two sets of ToxPi's were produced. The first set was created using a selection of ToxCast/Tox21 assays related to 7 "gene sets", where each gene set consists of a number of *in vitro* assays that are targeted to a specific gene: estrogen receptor (ER), androgen receptor (AR), thyroid hormone receptor (TR), peroxisome proliferator-associated receptor (PPAR), pregnane X receptor (PXR), aryl hydrocarbon receptor (AHR), and aromatase (AROM). The second set of ToxPi's was created using all 482 ToxCast assays that have an associated gene target. Each gene target was mapped to a general protein family using the KEGG BRITE hierarchy in order to obtain a broader understanding of the BPA analog activity.
- For the 7 gene sets consisting mostly of nuclear receptors, we also calculated "gene scores", which use the sum of the –logAC50s averaged over the number of assays in the gene set to derive a dimensionless value that is reflective of the chemical's activity in that gene set[1]. For our purposes, we also normalized by the number of assays tested for each chemical as all chemicals were not tested in every assay.
- We also explored the structure-activity relationships of the analogs to the gene sets in order to determine how predicted models of their binding compares to our *in vitro* data using TIMES models in the OASIS Pipeline Profiler. We compared these results to ER and AR model scores as well as the ER model scores from the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP), both available at the Endocrine Disruptor Screening Program (EDSP) dashboard (http://actor.epa.gov/edsp21/).
- We used the Chemical and Product Categories (CPCat) database (http://actor.epa.gov/cpcat) to compare the relative amount of consumer use for the BPA analogs in order to gauge the likelihood of consumer exposure. In addition, we used the High-Throughput Toxicokinetics (httk) R package to calculate half-lives and volumes of distribution for compounds that had available clearance and fraction unbound in plasma data available.
- Finally, we summarized the cytotoxicity of the BPA analogs by reporting the median logAC50 value for active cytotoxicity assays.

Conclusions and Future Directions

- We found that patterns of activity in ToxCast assays vary widely amongst BPA analogs. Many, but not all, of the analogs are ER agonists. Additionally, they are also active towards a number of other nuclear receptors and across a broad spectrum of gene targets.
- Several analogs stand out as being particularly broadly active, including BPAF, BPB, TBBPA, TCBPA, bis(2-hydroxy ethyl) ether, DMB-BPA, and DMBPA.
- Many of these compounds are cytotoxic, often at concentrations at which activity in other assays is observed, potentially confounding these results.
- Comparison of these analogs should take into account their consumer use patterns and toxicokinetics in order to understand the likelihood and duration of exposure. There is a broad range both in amount of consumer use and in half-lives, with half-lives ranging from hours to weeks.
- Our gene scores, the EDSP model scores, and TIMES binding model predictions were in relatively good agreement for ER but not AR. Structural analyses are in the preliminary stage and analyses are ongoing, particularly in how physicochemical properties and specific R-groups affect activity. Analogs included in the current study were chosen based on other comparative studies [2] with the addition of several other analogs. In the future, we will incorporate all chemicals with available data that have bisphenyl structural features into the analysis.
- These broad-based screening approaches allowed us to identify a wide spectrum of potential biological targets and build more comprehensive toxicity profiles of BPA and its analogs in order to better evaluate their potential health effects.

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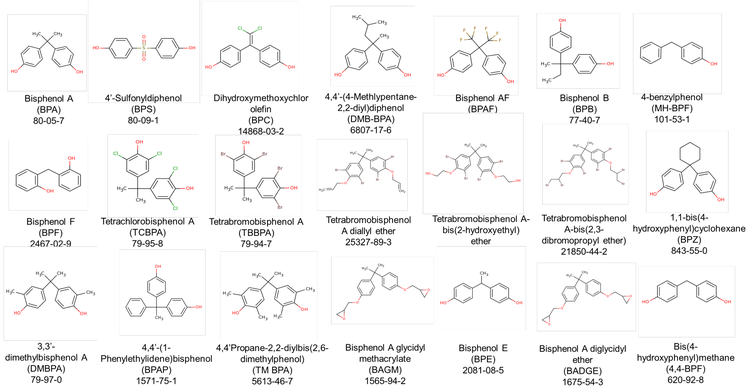
Bisphenol A Analogs



Consumer Use, Toxicokinetics, and Cytotoxicity

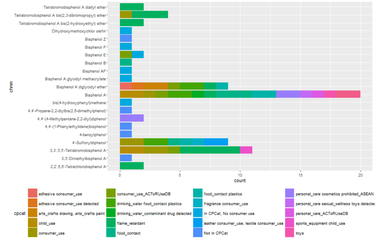


Figure 1. Consumer use of BPA analogs. Data from CPCat database. Count indicates the number of entries in CPCat per given consumer use category.

Chemical Name	Active Cytotoxicity Assays	Median LogAC50	MAD
4,4-BPF	3	0.255	1.55
DMBPA	15	1.21	0.0866
TBBPA bis(2-hydroxyethyl) ether	24	1.34	0.403
BAGM	7	1.39	0.0921
BPB	16	1.39	0.225
BPAF	28	1.48	0.375
TCBPA	19	1.51	0.570
TBBPA	28	1.55	0.214
DMB-BPA	17	1.62	0.131
BPC	3	1.62	0.00850
BPAF	8	1.67	0.496
TM BPA	10	1.71	0.0951
BPZ	11	1.72	0.0711
BADGE	7	1.75	0.157
BPE	1	3	0
TBBPA bis(2,3-dibromopropyl ether)	0	3	NA
BPF	0	3	NA
TBBPA diallyl ether	0	3	NA
BPS	0	3	NA
MH-BPF	0	3	NA

Table 2. Cytotoxicity of BPA analogs. Active Cytotoxicity Assays=the number of cytotoxicity assays in which the compound is active, Median LogAC50t=the median logAC50 of the chemical in these assays, MAD=median absolute deviation. Median LogAC50=3 is inactive.

	Half-life (h)	Volume of Distribution (L/kg)
TBBPA	805	841
BPB	139	45.6
BPAF	138	6.41
BPA	115	0.580
BPF*	3.55	4.13
BPS*	0.564	0.433

Table 1. Half-life (h) and volume of distribution (L/kg body weight) as calculated using the High-Throughput Toxicokinetics (httk) R package. *Rat clearance rates used to calculate these values instead of human.

References

(1) Martin, M.T., et al., *Predictive Model of Rat Reproductive Toxicity from ToxCast High Throughput Screening.* Biology of Reproduction, 2011. **85**(2): p. 327-339.

(2) Stossi, F., et al., *Defining Estrogenic Mechanisms of Bisphenol A Analogs through High Throughput Microscopy-based Contextual Assays.* Chemistry & biology, 2014. **21**(6): p. 743-753.

Results

- The most active compounds in the gene set analysis were BPAF (ER, AROM, AHR), TMBPA (AR), BAGM (TR), TBBPA (PPAR) and BPB (PXR) (Figure 2). In general, activity was not restricted to the ER, with the most active compounds showing high potency in AR, AHR, PPAR and PXR as well. In general, less activity was seen in the TR and AROM gene sets.
- The compounds with highest ToxPi scores in all ToxCast assays with gene targets were BPAF, BPA, TBBPA, and BPB. These analogs were particularly enriched in the nuclear receptor, ion channel, mitochondrial biogenesis, transporter, and transcription factor protein families (Figure 3).
- 15 of the 21 compounds exhibit cytotoxicity in the tested concentration range (Table 1)
- Using the OASIS Pipeline Profiler set of models, we found good predictivity of binding for ER (~19/21) but not for AR (~9/21 correct, 5/21 could not be predicted) (Table 3) . These data are shown compared to the EDSP database and CERAPP model scores (ER only). For the AR model, predictions could not be made for five compounds that fell outside of the applicability domain-three fell outside of the domain as characterized by physicochemical parameters MW and $\log K_{ow}$ and two fell outside of the structural domain as characterized by atom-centered fragments.
- We also explored which physicochemical parameters discriminated for activity in the ER set and found that high molecular weight and extreme hydrophobicity were associated with inactivity.
- We found that several BPA analogs (BPA, BADGE, TBBPA and BPS) have high counts of consumer use (Figure 1). Half-lives ranged from hours to weeks, with TBBPA having the highest half-life (Table 1).

Chemical Name	Estrogen Receptor					Androgen Receptor			
	Gene Score	EDSP Agonist Score	EDSP Antagonist Score	TIMES Binding	CERAPP Binding	Gene Score	EDSP Agonist	EDSP Antagonist Score	TIMES Binding
BPAF	1.07×10^{7}	0.552	0.000026	Yes	Active (Weak)	1.43×10^{3}	0.000746	163	Yes
BPB	2.85×10^{6}	0.491	0.00196	Yes	Active (Weak)	741	0	0.134	Yes
BPA	7.98×10^{5}	0.45	0	Yes	Active (Weak)	790	0	0.136	Yes
BPS	1.20×10^{4}	0.263	0	Yes	Active (Very Weak)	3.55	0	0	No
TCBPA	1.44×10^{4}	0.19	0	Yes	Active (Very Weak)	12.3	0	0	Yes
TBBPA	21.4	0	3.81E-07	No	Inactive	99.0	0	0.0159	NA
BADGE	12.9	0	0	No	Inactive	24.9	0	0.0197	Yes
BPF	32.0	0	0	Yes	Active (Very Weak)	3.20	0	0	No
BAGM	3.00	0	0	Yes	Inactive	83.1	0	0	Yes
TBBPA diallyl ether	0	0	0	No	Active (Weak)	0	0	0	NA
DMBPA	6.13×10^{4}	NA	NA	Yes	NA	8.85	NA	NA	Yes
BPAP	5.44×10^{6}	NA	NA	Yes	NA	0	NA	NA	NA
DMB-BPA	7.80×10^{5}	NA	NA	Yes	NA	541	NA	NA	Yes
4,4-BPF	5.16×10^{3}	NA	NA	Yes	NA	7.67	NA	NA	Yes
BPE	5.62×10^{4}	NA	NA	Yes	NA	7.45	NA	NA	Yes
TBBPA bis(2-hydroxyethyl) ether	10.6	NA	NA	No	NA	74.6	NA	NA	NA
MH-BPF	190	NA	NA	Yes	NA	12.2	NA	NA	No
TMBPA	1.64×10^{5}	NA	NA	Yes	NA	1.21×10^{5}	NA	NA	Yes
BPZ	803	NA	NA	Yes	NA	188	NA	NA	Yes
BPC	729	NA	NA	Yes	NA	14.4	NA	NA	Yes
TBBPA-bis(2,3-dibromopropyl ether)	0	NA	NA	No	NA	0	NA	NA	NA

Table 3. Comparison of gene scores, estrogen and androgen receptor model predictions as calculated by the Endocrine Disruptor Screening Program Dashboard using ToxCast data (AUC=area under the curve for the model. NA=data not available) and TIMES model predictions from the OASIS Pipeline Profiler (yes=binds, no=doesn't bind, NA=could not be predicted by TIMES model, yellow indicates a likely incorrect prediction).CERAPP scores are also included for comparison.

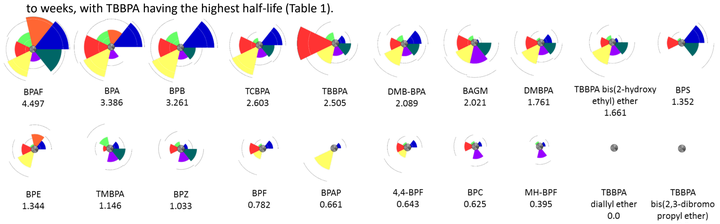


Figure 2. ToxPi images and scores representing activity of BPA analogs in 7 gene sets. The legend includes the number of assays included in each gene set. The length each slice is proportional to the potency of the assays composing the slice. Slices are equally weighted.

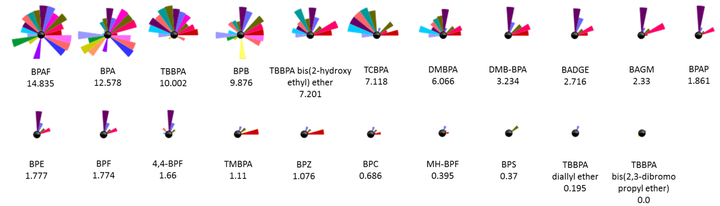


Figure 3. ToxPi images and scores representing activity of BPA analogs in all ToxCast assays that have an associated gene target. Each gene target was mapped to its respective KEGG protein family. The legend includes the number of assays included in each gene set. The length each slice is proportional to the potency of the assays composing the slice. Slices are equally weighted.