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Multi-species, Multi-tissue Meta-analysis of Bisphenol A Transcriptome Studies Reveals Species-and Tissue-specific Molecular Perturbations

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Multi-species, Multi-tissue Meta-analysis of Bisphenol A Transcriptome Studies Reveals Species-and Tissue-specific Molecular Perturbations

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Environmental Health Sciences

by

Anthony Crymes

2018

ABSTRACT OF THE THESIS

Multi-species, Multi-tissue Meta-analysis of Bisphenol A Transcriptome Studies Reveals Species- and Tissue-specific Molecular Perturbations

by

Anthony Crymes

Master of Science in Environmental Health Sciences

University of California, Los Angeles, 2018

Professor Michael D. Collins, Co-Chair

Professor Xia Yang, Co-Chair

Abstract**:**

The potential health impact of Bisphenol A (BPA) exposure remains a hotly debated issue. To explore the molecular actions of BPA in a data-driven manner, which may inform on pathway perturbations and health consequences, a systematic analysis of publicly available transcriptomic data was conducted. Species- and tissue-specific differential gene expression, biological pathways and processes, as well as transcriptional were reviewed across 19 datasets of BPA

exposed tissues from rat, mouse, and human studies. A comparison of the data within and between species or tissues was conducted, revealing tissue-/species-specific molecular perturbation such as IL7 in human uterine tissue, or Wnt4 in mouse testis, or Tgfbr2 in rat breast tissues in response to exposure to BPA. In addition to such unique annotations, there were a few responses that were shared between tissues and between species such as common CYP26b1 or ALDH1a7 gene dysregulation, oxidative stress and insulin sensitivity related pathways in liver, and cytokine receptor activity in breast tissue, with differentially regulated genes such as IL1A being common across species that may point to a more conserved molecular response to BPA. In general, our results not only demonstrate complex species- and tissue-specific molecular activities of BPA, but also highlight some consistent transcription factors like SUZ12 and other PcG associated transcription factors as potential BPA targets across tissues and species. It is also apparent that there is a need for more comprehensive and systematic molecular investigations of BPA to better understand its mechanisms of action.

The thesis of Anthony Crymes is approved.

Wendie A. Robbins

Michael D. Collins, Committee Co-Chair

Xia Yang, Committee Co-Chair

University of California, Los Angeles

2018

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Acknowledgements

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Introduction

Bisphenol A (BPA) is an industrial chemical most commonly used in the production of polycarbonate (PC) and polyvinyl chloride (PVC) plastics as well as epoxy resins such as those used to coat the inside of food cans, preventing rust (1, 2). As such, BPA and its metabolites can be identified in greater than 90% of the human population (3). The benefits of such products cannot be understated; however, the use of these products is not without risk (4, 5). With such widespread exposure, BPA has been the focus of risk assessment studies leading to it being associated with numerous human pathologies such as obesity and reproductive dysfunction (6-8). Regulations regarding BPA exposure have been established due to its implication in developmental harm (9) and recent concern that early exposure may lead to negative health effects later in life, consistent with Barker's hypothesis and the developmental origins of health and disease (DOHaD) hypothesis (10-12). Accordingly, regulations have included the removal of BPA from use in products such as baby bottles and children's toys in many western countries (13-15). Despite the implicated perturbations in numerous human biological systems, the exact mechanism of BPA exposure is yet fully understood (16-18). Several studies demonstrate that the doses of BPA that the population is exposed to, currently assumed to cause no harm, may not be

as safe as presumed (19, 20). Unfortunately, directly assessing the in vivo effects of BPA exposure has been a major hurdle (13). Adding to this impedance to the understanding of the effects of BPA are: the observed variances in study designs, intraspecies and interspecies responses, and the nonmonotonic dose response curve of BPA - making comparisons of conclusions between studies difficult (21-23).

Evaluation of safety through measurements of physiological or pathological parameters at the phenotypic level can be difficult to dissect because each phenotype can be determined by complex interactions of many molecular processes. In contrast, molecular signatures of BPA can be accurately measured with modern genomic technologies and have the capacity to faithfully reflect the detailed biological processes involved in BPA activities. With the current tools at the disposal of the toxicological community, and in congruence with such initiatives as Toxicology in the 21st Century (Tox21) and the Precision Medicine Initiative (PMI), innovative approaches need to be taken to identify toxicological risks and detrimental human health endpoints. To this end, analysis of the transcriptome provides a powerful lens for predicting in vivo toxicological endpoints (24, 25). We use the currently available transcriptomic data to make inferences about

potential mechanisms by which BPA may act on individual tissues in vivo. To date, there have been no comprehensive reviews of the genomic research published regarding BPA, and systematic comparisons across studies in various species and tissues are lacking. This study aims to review and meta-analyze the existing transcriptomic investigations of BPA exposure to assess consistency and discrepancy in the genes and pathways revealed across studies. The main purpose of the meta-analysis across studies stratified by species and tissue is to filter out noise in individual studies and retrieve the most robust molecular signals. Here we emphasize in vivo studies in rodent model systems and ex vivo / in vitro human studies (no human in vivo studies are available), aiming to derive a systems-level understanding of the molecular processes involved in BPA activities in a species- and tissue-specific manner. Our findings inform on the value of genomic studies and point to the need for more systematic omics research in the toxicogenomics field.

Material and methods

Identification of studies/data

Initial criteria for inclusion in the present study were: a) public availability, b) in vivo design, c) transcriptomic data, and d) experimentally controlled BPA exposure. These criteria were selected to provide the proper foundation for a quantitative analysis of the current research landscape. To identify such qualifying studies, a search was conducted using National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). This international public repository (26) allows researchers to deposit their collected genomic (including all array-based applications and some high-throughput sequencing data) data in a central, freely accessible database. In addition to being publicly available, we required data to be from in vivo studies. BPA is known to have far reaching systemic effects after exposure, that may be more accurately identified from in vivo studies and more precisely extrapolated to identify the direct implication for human health. We focused on transcriptome data, as this is the most widely studied genomic aspect, hence there were approximately 10 times more transcriptomic datasets available compared to other data types (e.g. miRNA expression, DNA methylation), allowing comparison across studies to derive more reliable conclusions. Lastly, experimental BPA exposure was

required, excluding studies that implied BPA exposures with uncontrolled doses, such as epidemiological studies, which would have many confounding exposures to identify the BPA contribution to genomic expression changes. These selection criteria were amended after assessing the data that was available to be collected. Although the in vivo criteria held for other species, the lack of human data in the analysis was noted, which prompted us to include in vitro and ex vivo human data.

The selection of data (**Figure 1**) was done by using the search terms: 1) "BPA", 2) "Bisphenol A", and 3) "4,4'-(propane-2,2-diyl)diphenol", then filtering by "series". Data were considered from all available years at the time of search, attempting to increase the breadth of the current study, with a range of April 2006 (earliest available data) to September 2017 (conclusion of data acquisition) being included. Data additionally did not need to be included in a published study if the study design (e.g. species information, exposure dosage, sample size) based on data annotation in GEO met our search criteria. These data were then screened against inclusion criteria as described previously. As most studies passing our inclusion criteria were from rat and mouse models, we removed data for other mammals, such as 2 monkey studies and 22 studies

from 9 non-mammalian models, due to the limited numbers of studies available for comparison. There were also studies identified that did not provide accessible/interpretable data formats and were hence excluded. Due to this screening, 19 studies were ultimately identified for inclusion in the final meta-analysis (**Table 1**).

Obtaining BPA signatures across different organ systems and organisms from BPA public high-throughput data

We identified 19 BPA high throughput transcriptome datasets from NCBI GEO. Using R (27) for our analysis, datasets were downloaded using the "GEOquery" R package (28) and organ systems were annotated by Brenda Ontology (29). Obtained dataset matrices were first log normalized, followed by ranking of each gene with the GeoDE package (30), an algorithm specified for identifying treatment related genes. Because of heterogeneity among datasets such as sample size and profiling platform, we decided to use a non-parametric rank based representation in each dataset, followed by aggregation of gene ranks from the same tissue/organ system and the same organism by using the Robust Rank Aggregation method (31) with false discovery rate (FDR) < 0.05 to identify differentially expressed genes (DEGs). The rank

aggregation process was done for the upregulated or downregulated direction separately to obtain DEGs and their enrichment p-values for both directions.

Functional Annotation of DEGs

We annotated both the upregulated and downregulated DEGs with biological pathways and processes from the Gene Ontology (GO) (32), Kyoto Encyclopedia of Genes and Genomes (KEGG) (33) and Disease Ontology (DO) (34) databases using the Clusterprofiler package (35) with false discovery rate (FDR) < 0.05 as the cutoff. To reduce similar terms in Gene Ontology and increase interpretability, we use the "simplify" function implemented in Clusterprofiler with a similarity cutoff of 0.7 to filter out similar terms. Gene symbols of rat and mouse DEGs were converted into human genes based on Ensembl homology (36) for the enrichment analysis. To predict potential transcription factors (TFs) for the identified DEGs, the online tool ENRICHR was used to assess the overlap of DEGs with TF information collected from TRANSFAC JASPAR PWMs (37) and ChEA 2016 (38). TFs reaching Benjamini-Hochberg adjusted p-values of less than 0.05 were considered significant (39). Additional pathways from WikiPathways (40) was also collected via ENRICHR (41, 42), supplementing the aforementioned pathway databases

to provide a more comprehensive analysis. TFs were analyzed to determine potential upstream regulators of downstream pathway and gene dysregulation caused by BPA exposure.

Results

Species and tissue coverage of GEO datasets

As summarized in **Table 1**, the GEO datasets passing our selection criteria include four human tissue types – namely adipose, breast, prostate, and uterine tissue, four mouse tissues – breast, liver, testicular, and uterine, and two rat tissues – breast and liver. A subset of tissues including liver, breast, and uterine were represented by data from more than one species, allowing between species comparison. Other tissues, however, were only examined in one species and prevent us from conducting cross-species comparison.

BPA exhibits tissue-specific and species-specific transcriptome signatures

We identified upregulated or downregulated differentially expressed genes (DEG) at FDR<0.05 for each tissue in each species, resulting in approximately 1600 DEGs on average per tissue (min = 638, max = 1981).

Of the greater than 6000 genes identified across the four tissues examined in human, only 1070 (17%) were differentially expressed in more than one tissue and five were differentially expressed amongst all four tissues (**Figure 2A)**. Namely, Acetyl-CoA Synthetase Long Chain Family Member 1 (ACSL1) is implicated in lipogenesis (43). Insulin-like Growth Factor Binding Protein 6 (IGFBP6) is involved in cell growth regulation (44) . BPA having been identified as an obesogenic compound can be reflected in these perturbations. The other genes that were shared amongst all tissue included Prostaglandin F Receptor (PTGFR), Spermatogenesis Associated 9 (SPATA9), and Olfactory Receptor Family 1 Subfamily D Member 2 (OR1D2) and reflect the potential for diverse effects of BPA across a variety of organ systems, such as meiotic dysregulation, and cell sensory responses to hormones and xenobiotic compounds.

Mouse samples were from four different tissues. Only breast and uterine tissues were common between mouse and human while testicular and liver tissues were unique to the mouse (**Figure 2A vs 2B**). Approximately 4600 genes were identified as DEGs from mouse tissues, and 608 (13%) were differentially expressed in greater than one tissue with three being differentially

expressed in all four tissues. Those 3 genes were insulin-like growth factor binding protein 1 (Igfbp1), guanylate cyclase activator 2B (Guca2b) which has been linked to diet-induced obesity and both of which are responsible for regulating the cell cycle (45, 46) as well as Aldehyde dehydrogenase 1, family member A1 (Aldh1a1) which has a critical homeostatic role, with over- and under-expression leading to various pathologies (47, 48)

Liver and breast tissues were the only ones identified by our search in rat and about 2600 DEGs were identified. Of these, 101 (4%) were differentially expressed in both tissues (**Figure 2C**). Breast tissue was a tissue type that was identified in all three species, and a comparison of the identified DEGs revealed 129 (4%) of the 3000 DEGs to be overlapping between mouse and rat but none were shared with human breast tissue. When comparing rat liver and mouse liver tissues, 144 (6%) of the approximately 2600 DEGs were share between species. There were no shared DEGs of the uterine tissue between mouse and human.

In summary, seen in these comparisons is a large degree of tissue specificity in response to BPA exposure at the gene level and to a much greater extent species-specificity in gene response. Due

to the potential for noise when comparing DEGs, we next conducted analyses of pathway enrichment among the DEGs, which is less sensitive to noise, with the goal to bring further insight to the identified patterns of gene expression.

BPA affects diverse molecular pathways in a tissue- and species-specific manner

Pathway annotations of DEGs from human tissues showed tissue-specificity (**Figure 3A; Table 2**). The adipose pathways were broadly involved in cell cycle regulation and inflammatory response such as ERK1/2 cascade, p53 signaling, IL-1 response, and chronic inflammatory response. The pathways identified in breast tissue generally reflected cell cycle perturbations with related annotations being insulin-like growth factor receptor binding, DNA replication, and mitotic spindle checkpoint. Breast tissue shared 28 pathways with adipose (the most of any comparison within human tissues). Regarding human reproductive tissue, less than 40 pathways were identified from the prostate and uterine tissue samples. Pathway annotations of the prostate DEGs included G-protein couple receptor and Ubiquitin like protein conjugation enzyme binding, suggesting cell signaling interruption, while in the uterus pathway perturbations related to cytokine binding and ligand-receptor processes were identified.

In mouse (**Figure 3B; Table 2**), most of the identified pathway annotations were also unique to each tissue. Nevertheless, 128 (13%) of the pathways were shared between two or more tissues and two pathways, receptor ligand activity and steroid metabolic process, were shared among all four of the mouse tissues. In the breast samples, pathway annotations included hedgehog and WNT signaling. Dysregulation of metabolic pathways is most notable in the liver tissue, from which we identified fatty acid, carbohydrate, and steroid synthesis pathways as being perturbed. In the reproductive tissues, the testis and uterus, we identified processes related to cellular responses to extracellular stimuli such as hormone and BMP stimuli, G protein signaling, WNT signaling and ERK1/2 signaling.

From the rat liver data, we identified annotations including AGE-RAGE signaling in diabetic complications, MAPK signaling pathway and TGF signaling, as well as insulin-like growth factor binding (**Figure 3C; Table 2**), being affected by BPA exposure. This is an affect that is noted in other species to varying degrees. The rat breast data reflected alterations to cytokine receptor activity and G-protein coupled chemoattractant receptor activity. The only shared

annotation between these two rat tissues was small molecule biosynthesis. This annotation was noted as upregulated in liver and downregulated in uterus with four genes (Egr1, Gpd1, Aldh1a3, Scd) specifically being shared between the two annotations. Interesting to note is that this represents another member of the aldehyde dehydrogenase family that was differentially expressed after BPA exposure, as was seen in humans previously.

We also evaluated cross-species comparisons of similar tissues. Between mouse and rat liver tissues, we identified 19 shared pathways, reflecting BPA effects on cellular responses to cAMP, insulin, interferon-gamma, and oxidative stress. This reflects a possibility for a shared immune response in the liver as well as an impact to metabolic processes (**Figure 4A**). These processes were also reflected in shared annotations of both biosynthetic and catabolic pathways, with example annotations including steroid metabolic process – Cyp26b1 was a common gene share between the two species for this annotation), small molecule biosynthesis – with no genes being shared between these two annotations, though two Aldh members were identified (Aldh1a3 in rat and Aldh1a1 in mouse) in this annotation. Most noted is the number of Cyp family genes that are included in the dysregulated pathways in the liver annotations. The breast tissue (**Figure 4B**)

was represented by datasets from all three species. However, none of the pathways were shared amongst all three, though mouse shared 5 annotations with human – reflecting cell receptor activity – and 3 with rat, reflecting ion transmembrane transport. In summary, the breast tissue samples seemed to have experienced alterations in their ability to respond to extracellular stimuli be it endogenous or exogenous signals. (**Figure 4C**).

BPA targets tissue-specific transcription factors (TFs)

To better understand how BPA is perturbing such a diverse array of pathways, we attempted to identify dysregulated TFs with the idea that these upstream disruptions may help to explain the broad and variable downstream DEGs response to exposure to BPA that are observed. Top ranked TFs for each tissue in each species are shown in **Table 3**.

In human (**Figure 5A**), a limited number of tissue-specific TFs were identified, such as cell cycle regulators RELA and FOXM1 in adipose tissue, estrogen receptors ESR1/2 in breast tissue which are known to be effected by BPA, and SUZ12 in prostate tissue, which has been noted for its role in tumorigenesis in previous studies (49). No TFs were identified in human uterine tissue.

Mouse tissue data led to the identification of 48 TFs shared across the four tissues, with no TF being identified as shared amongst all four tissues, and only pair wise overlap of TFs between tissues. (**Figure 5B**). This highlights further the tissue-specific effects of BPA. Between uterine and testicular tissue, NUCKS1, where its role in DNA repair in vitro has been noted as well as its regulation of metabolic effects and response to insulin in vivo, suggesting potential for critical pathological changes in these reproductive tissues (50) The uterus shared TF FOXM1 with liver, which was a TF also identified in human. Liver shared more (4) TFs with testis than other tissues in mouse, which included TCF3, important for determination of cell fate, along with TCF7 (51-53) **,**as well as well-characterized TFs ESR1 and RXR. The testis and the breast shared TFs – SUZ12, JARID2, BMI1, and EZH2 – that are associated with polycomb group protein complex (PcG) function (54, 55).

In rat liver (**Figure 5C)** we identified TFs including LXR, RXR, which are key lipid regulators, as well as ESR1, TP53, and PPAR family TFs. Many, including CREB1, reflect the cell signaling disruptions noted in the pathway analysis with downstream impact on cell cycle progression.

Breast tissue results were reflective of PcG complex TFs including SUZ12, EZH2, and RING1B with such TFs being important for cellular pluripotency, particularly during embryonic development. However, we continue to see a tissue-specific response to BPA in rat emphasized by the fact that zero TFs were shared between the tissues.

Comparison of TFs across species found some similarity across species when comparing like tissues. In liver tissues, from mouse and rat, 17 TFs are shared between the two representing 70% of liver TFs in mouse, though representing less than 20% of the TFs identified in rat **(Figure 6A)**. The shared TFs included several that have been previously noted such as FOXM1, ESR2, LXR, and RXR. PPARA and FOXO1 were identified, which are involved in lipid metabolism and insulin sensitivity (56). In breast tissue only SUZ12 and EZH2 were shared between species – and only between mouse and rat, not human. These, as previously stated, are important in regulating the pluripotency of cells. No other transcription factors were shared in breast tissue making these TFs markedly tissue-specific **(Figure 6B)**.

Some TFs were identified across species but from different tissues, such as SUZ12 being

identified in human prostate, mouse breast and testis, and rat breast and liver. BACH1 was found in human adipose, and mouse testis and rat liver with RELA being found in human adipose and rat liver. These are a few examples of the cross-cutting TFs that were identified in this analysis, however, only 22 (18%) TFs were impacted by BPA exposure in this manner of comparison. Largely, TFs were tissue-specific and species-specific in their dysregulation.

Discussion

Our meta-analysis of existing transcriptome datasets across species demonstrated that numerous genes and pathways are affected by BPA exposure. This was seen in both in vitro and in vivo studies with mouse, rat and human tissues being represented. Of the thousands of genes that were identified to be affected within each species, only a small percentage of genes were found to be perturbed in more than a single tissue sample. This reflects a tissue specific impact of BPA on these organisms. BPA has been implicated as an estrogenic compound (57) as well as an obesogenic compound (58), with the results from our study supporting that BPA can play a role in the dysregulating genes involved in the maintenance of metabolic functions (59), as most prominently noticed by the in vitro human and in vivo mouse studies analyzed. The genes that

have been identified as differentially expressed from BPA exposure reflect extreme heterogeneity in BPA effects on gene expression between tissues and between species. For this reason, we attempted a more holistic approach by looking at what systemic pathways this change in gene expression represented.

Pathway annotations that were used included KEGG, DO, GO, and WikiPathways. The goal was to view these annotations and see if there were concerted effects on biological processes reflecting BPA exposure. As we noted in our comparison of the various pathway annotations that were identified, the tissue specificity of BPA we saw at the gene level carried over to this pathway level analysis, as may have been expected. However, there were several themes that could be better extrapolated from this level of analysis. In human adipose tissue, we saw that ERK 1/2 cascade, p53 signaling and inflammatory annotations were noted. With ERK 1/2 signaling being intricately tied to cell fate (60) BPA may have the potential to affect processes involved with cell growth and/or apoptosis. The dysregulation of genes involved in mentioned inflammatory pathways such as HIF1A and IL1A note an immune dysfunction that has previously been associated with metabolic alterations in adipose tissue (61). Other cytokine

signaling and G-Protein signaling pathways were noted in other human tissues, suggesting that BPA may have an ability to induce widespread genetic effect on cell determination in human tissues, as previous studies have demonstrated (62)**.** It has also been seen that BPA may in fact mediate its metabolic effects through an immune response (63, 64), again noting the potential for broad systemic effects.

In mouse tissues, we also saw potential for metabolic dysregulation, most prominently in the liver tissue. Genes associated with the metabolic pathways identified were commonly CYP superfamily genes, such as CYP1A1 or CYP17A1 (associated with the dysregulation of steroid metabolism in mouse liver). CYP genes have previously been identified to be impacted by BPA (65) and with CYP proteins being ubiquitously involved in metabolic processes, there is potential for diverse downstream effects of BPA. In other tissues we noted more prominently pathways impacting cell fate, such as BMP stimulus response, with BPA having been associated with priming BMP signaling pathways to alter cell differentiation (66). Other pathways noted in mouse were also implicated in cell signaling. With genes related to immune response (IL family) and metabolism (CYP family, ALDH family), it is interesting to note that while explicit pathway

annotations did not grossly overlap between mouse and human, similar classes of genes were found to be affected in both species by BPA in our analysis.

Rat liver data continued to corroborate the implication of ERK signaling disruption in response to BPA exposure, as ERK 1/2 signaling was stated to be affected in the mouse and human tissues previously. The rat breast annotations were largely related to receptor activity, with primarily CCR family genes being affected, such as CCR1 – which has been recently implicated in the metastatic potential of breast cancer (67).

As mentioned, TF analysis has the potential to help explain the how a single chemical such as BPA can have such diverse, systemic, and tissue-specific effects. Our human data was limited but did turn up dysregulation of ESR1 (68, 69) and ESR2 (70, 71), which have previously been implicated in response to BPA as well as TFs that have not been studied so intensely, RELA, FOXM1 and SUZ12 – all three of which were identified across a variety of tissues in our analysis suggesting the need to investigate these TFs further. Particularly, with the implication of these TFs in the determination of cells, the importance of their potential impact on various BPA

pathologies should not be understated. While human TFs were limited, there were more mouse TFs identified in our analysis, however, many of them reflected the trends previously stated, namely, TFs identified were responsible for cell cycle regulation, and cellular development. This is a story that was then reflected in the rat data with similar TFs having been identified – SUZ12 and EZH2, for example. Rat liver also reflected BPA's potential to impact TFs characterized for metabolic homeostasis – PPARA (72, 73), LXR (74), and RXR (75).

The TF analysis successfully highlighted some molecular consistencies that crossed species and tissues, however, the specificity in tissue response means that there would remain a great deal of specificity at the TF level, as we saw. Further research can help us fully understand the modality by which these, and potentially other yet-to-be identified, TFs may regulate the systemic impact of BPA

Our study sought to associate BPA exposure with gene, pathway and transcription factor level perturbations. This is not the first study to attempt to make these connections, but this is the first to our knowledge that simultaneously leverages these three levels of analysis, applying them to

previously published exposure studies to understand BPA's effects. Our goal was to identify consistency, seeking a consensus from what is reported as seemingly disconnected data and results. To conclude our comparison, we found that there was generally dysregulation of the cell cycle stemming from BPAs impact on various metabolic processes, cell communication processes, as well as disruption of cell growth, supporting broad and systemic effects of BPA found in other studies. However, it is important to note that many tissues were only represented by one study. Even when tissues were represented by multiple studies, they were under varied experimental conditions, such as different exposure window and dosage, qualities that may have gross effects on gene expression, making conclusions difficult to draw with such low sample size. With it appearing that BPA may influence cellular cycle progression, the window of exposure could be critical to ultimate health endpoints with developing organisms being uniquely susceptible to the chemical since cell proliferation is so great, however, this is unclear and such research is still necessary (7). Additionally, with BPA's nonmonotonic dosage response curve, dose is even more important to integrate into such an analysis, since extrapolating from any single dose can prove difficult. This is not to say there is no merit to the current analysis of the available data, but that there is still much more work to be done, especially regarding the

collection of high-quality data well-annotated for experimental conditions. Furthermore, experimental replication to better identify consistent effects of exposure, critically teasing away experimental variance from qualities such as exposure window and dose response. Increasing the number of genomic datasets through systematic study design will allow for more definitive conclusions about gene expression, pathway perturbation, potential disease progression, and allow for the identification of key regulatory transcription factors in these processes.

Stemming from this need for continued, strategic research into BPA, and the nature of this review of public data, there is a deficit in the number of data samples for the various tissues represented posing a limitation to our analysis. Additionally, all the human data in this study represents in vitro experimental designs. While these are valuable exploratory tools, BPA has systemic implications and thus warrants experimental designs that are sensitive to this fact. Exposure studies are not done on humans for apparent reasons, however, numerous ecological studies have been done to identify the extent of human exposure, and in some cases ex vivo data was collected to compare exposure to exposure response endpoints. Similarly, from these epidemiological designs, transcriptomic data can be collected and matched to identify what

levels of exposure are associated with various homeostatic perturbations. With more data, the BPA contribution to gene expression can be elucidated.

Despite the limitations discussed above, it is apparent that BPA has a variety of effects on cellular development and functions in various tissues and species, as we identified through our comprehensive meta-analysis. Cascades of events led to many metabolic pathways being affected, with a handful of key transcription factors having been associated with these dysregulations. From this, we can better understand what the potential targets of BPA are and thus, even with nearly ubiquitous exposure, understand through which means BPA may possibly impact human health, providing for a clearer lens to view preventions and treatments of the health outcomes that may be present.

Table 1. Datasets included in the analysis. * = Primary progenitor cells harvested then differentiated in vitro (1 = male child, 2 = female adult, 3 = male adult)

GSE #	Tissue	Organism	Exposure Window	Exposure Route	Study Type	Dosage	Citation
56085	Adipose	Human (3T3-L1)	Postnatal	Culture	In Vitro	50μM, 25μM	(76)
58516	Adipose	Human (*1)	Postnatal	Culture	In Vitro	10nM	(77)
98680	Adipose	Human (*2)	Postnatal	Culture	In Vitro	10nM, 10μM	(78)
26884	Breast	Human (MCF-10F)	Postnatal	Culture	In Vitro	10^{-5}or10^{-6} M	(79)
50705	Breast	Human (MCF-7)	Postnatal	Culture	In Vitro	$1.95x10^{-12}$ – $1.00x10^{-4}$ M	(80)
59345	Breast	Human (MCF-7)	Postnatal	Culture	In Vitro	50nM	N/A
85350	Breast	Human (MCF-7)	Postnatal	Culture	In Vitro	400nM	N/A
87701	Breast	Human (MCF-7)	Postnatal	Culture	In Vitro	N/A	N/A
62953	Prostate	Human (*3)	Postnatal	Culture	In Vitro	10, 200, 1000nM	N/A
17624	Uterine	Human (Ishikawa)	Postnatal	Culture	In Vitro	1nM, 100nM, 10μM, 100μM	(81)
44387	Breast	Mouse	Prenatal	Osmotic Pump	In Vivo	250 ng/kg/d	(82)
26728	Liver	Mouse	Postnatal	Food	In Vivo	50 or 5000 μg/kg	(83)
43977	Liver	Mouse	Postnatal	Gavage, IP injection, or Food	In Vivo	N/A	N/A
14774	Testis	Mouse	Perinatal	Drinking Water	In Vivo	160 μg, 16 mg or 64 mg / kg	(84)
24525	Uterine	Mouse	Postnatal	Subcutaneous Injection	In Vivo	30 mg/kg	(85)
86923	Uterine	Mouse	Prenatal	Osmotic Pump	In Vivo	5 mg/kg/d	(86)
68973	Breast	Rat	Prenatal	Gavage	In Vivo	25 or 250 μg/kg	(87)
57815	Liver	Rat	Postnatal	Gavage	In Vivo	100 mg/kg	(88)
8251	Liver	Rat	Postnatal	Gavage	In Vivo	610 mg/kg	(89)

Table 2. Top 5 over-represented pathways among differentially expressed genes for each tissue in each species.

Species	Adipose	Breast	Liver	Prostate	Testis	Uterus
Human	Olfactory transduction, cytokine activity, Receptor ligand activity, *Receptor regulator activity*, Olfactory receptor activity	*Chromosome segregation*, Sister chromatid segregation, Organelle fission, Mitotic nuclear division, Chromosome (centromeric region)	N/A	Chromosome (centromeric region), Condensed chromosome, Borderline personality disorder, Spindle, Ubiquitin-like protein conjugating enzyme binding	N/A	Neuroactive ligand-receptor interaction, Substrate-specific channel activity, Tertiary granule, Neuropeptide receptor activity, Metal ion transmembrane transporter activity
Mouse	N/A	Cornified envelope, Epidermis development, Keratinocyte differentiation, Peptide cross-linking, Receptor ligand activity	Arachidonic acid epoxygenase, Oxioreductase activity (acting on), Epoxygenase P450 pathway, Heme binding, Retinol metabolism	N/A	*Receptor ligand activity*, *Receptor regulator activity*, ERK1 and ERK2 cascade, Kidney development, Positive regulation of ion transport	*Chromosome segregation*, Spermatogenesis, Condensed chromosome (centromeric region), Spindle, Mitotic sister chromatid segregation
Rat	N/A	Myofibril, Myofibril assembly, Muscle system process, Sarcoplasmic reticulum membrane, Striated muscle cell development	Cellular response to peptide, Response to acid chemical, Cell-substrate adheren junction, Cell-substrate junction, Focal adhesion	N/A	N/A	N/A

Table 3. Top 5 predicted transcription factors for the differentially expressed genes for each tissue in each species.

Species	Adipose	Breast	Liver	Prostate	Testis	Uterus
Human	BACH1, *RELA*, *FOXM1*	*FOXM1*, E2F4, SOX2, AR, E2F7	N/A	*SUZ12*	N/A	**None Identified**
Mouse	N/A	*SUZ12*, JARID2, BMI1, RNF2, EZH2	RXR, PPARA, LXR, RARG, ESR1	N/A	*SUZ12*, OCT4, TP53, *JARID2*, *EZH2*	*FOXM1*, CREB1, *NUCKS1*, *CLOCK*, RUNX2
Rat	N/A	*SUZ12*, *EZH2*, RING1B	*CLOCK*, RXR, LXR, *RELA*, *NUCKS1*	N/A	N/A	N/A

Figure 1. Dataset identification and filtering.

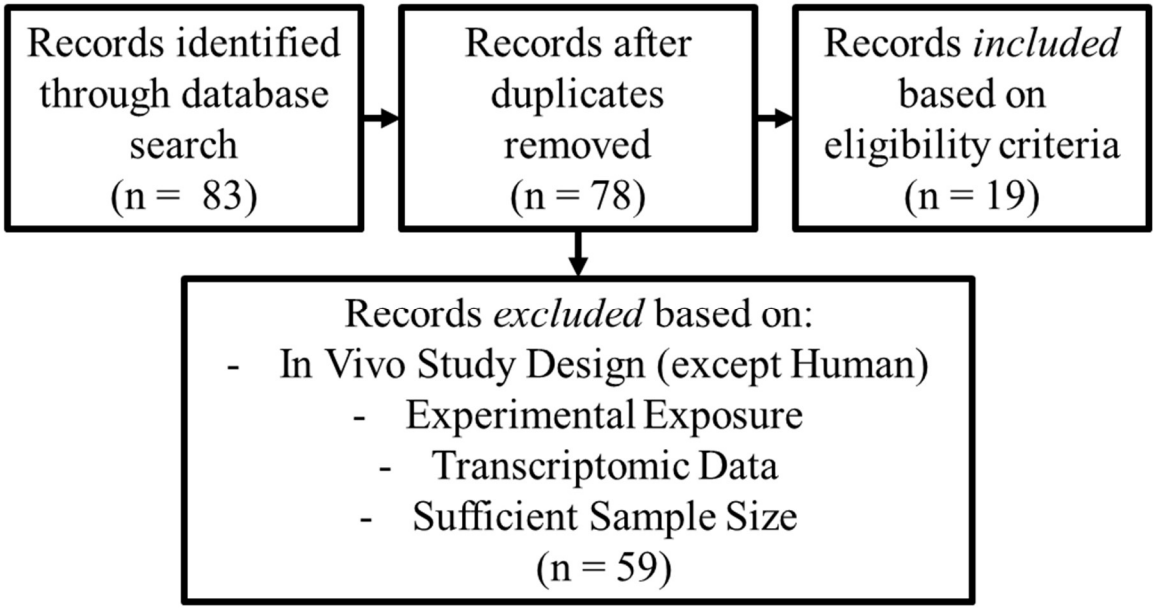
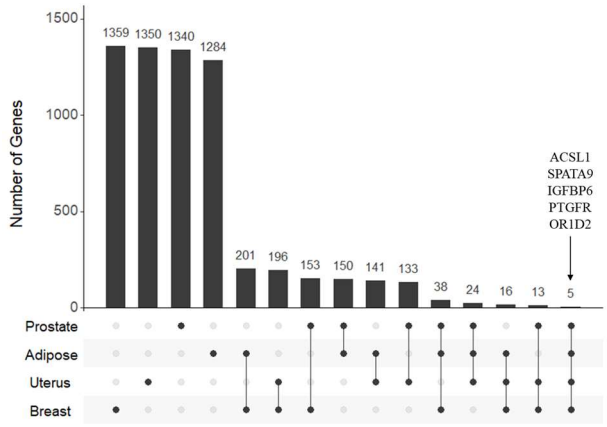
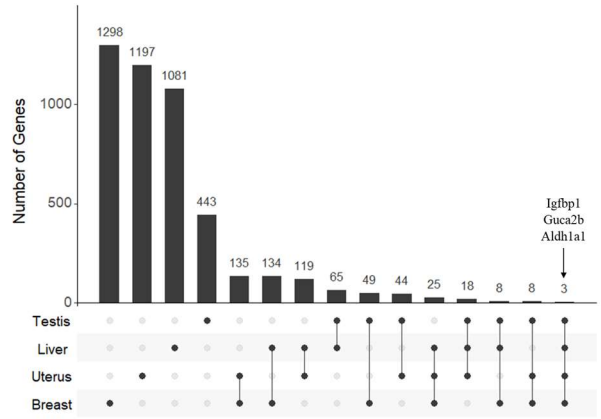


Figure 2. Differentially expressed genes in individual tissues of each species. A) Human. B) Mouse. C) Rat.

A.



B



C.

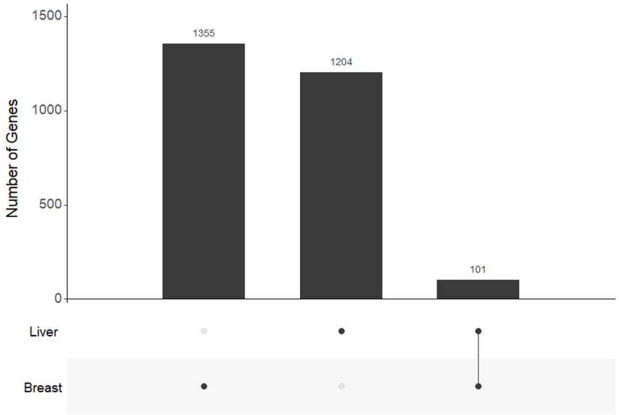
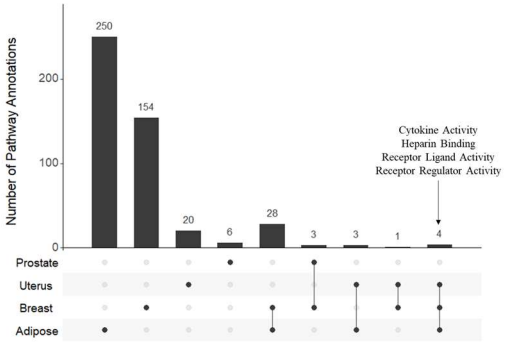
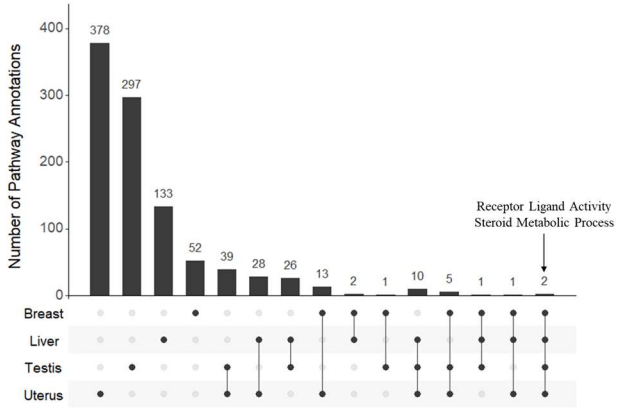


Figure 3. Over-represented molecular pathways among differentially expressed genes in each tissue of each species. A) Human pathways. B). Mouse pathways. C) Rat Pathways.

A.



B.



C.

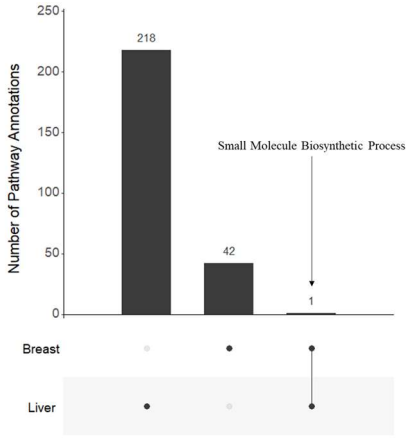
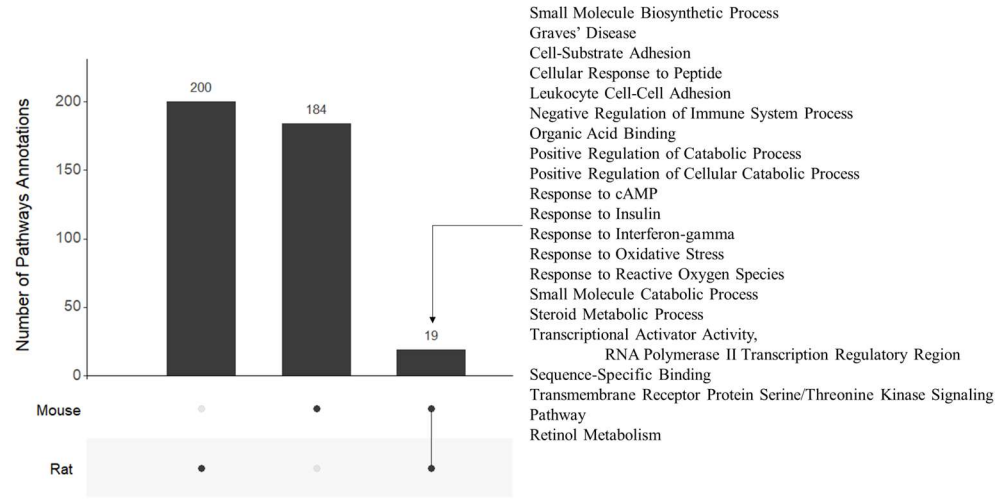
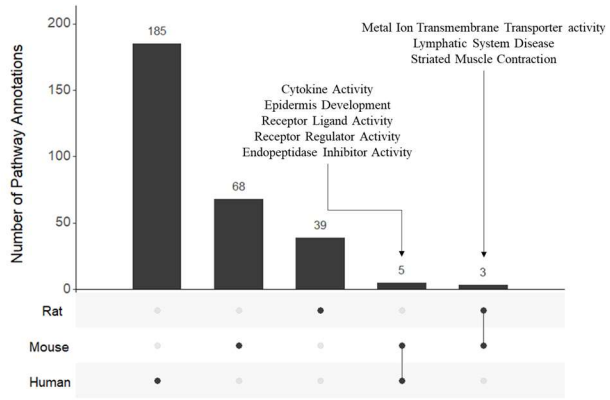


Figure 4. Between-species comparison of pathways for individual tissues. A). Liver B) Breast. C) Uterus.

A



B



C

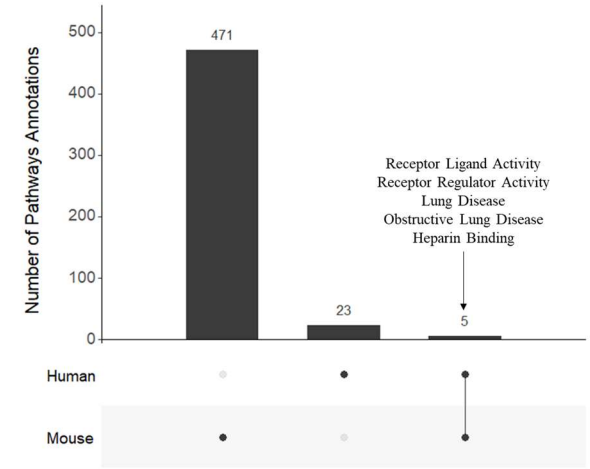
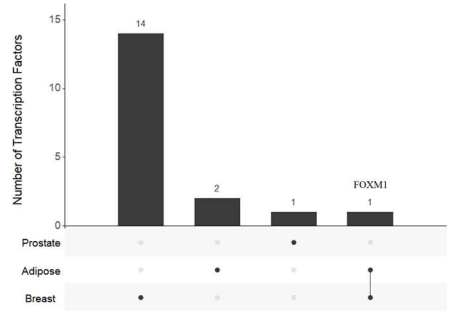
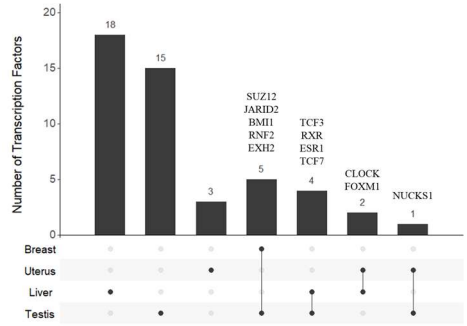


Figure 5. Predicted transcription factors whose downstream targets are enriched among the differentially expressed genes in individual species. A) Human. B) Mouse. C) Rat.

A



B



C

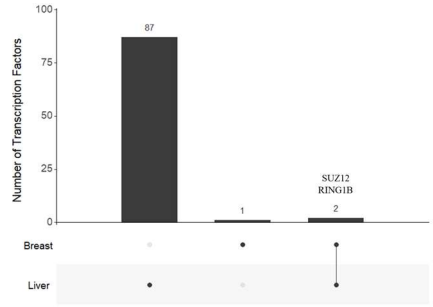
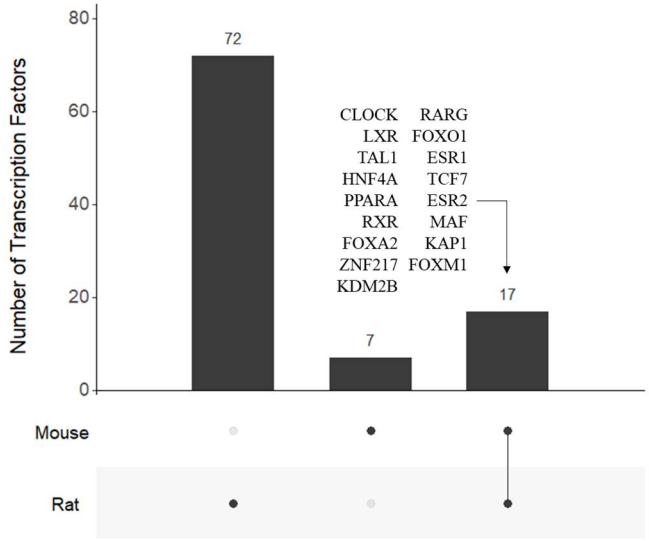


Figure 6. Between-species comparison of predicted transcription factors whose downstream targets are enriched among the differentially expressed genes in individual tissues A). Liver B) Breast.

A



B

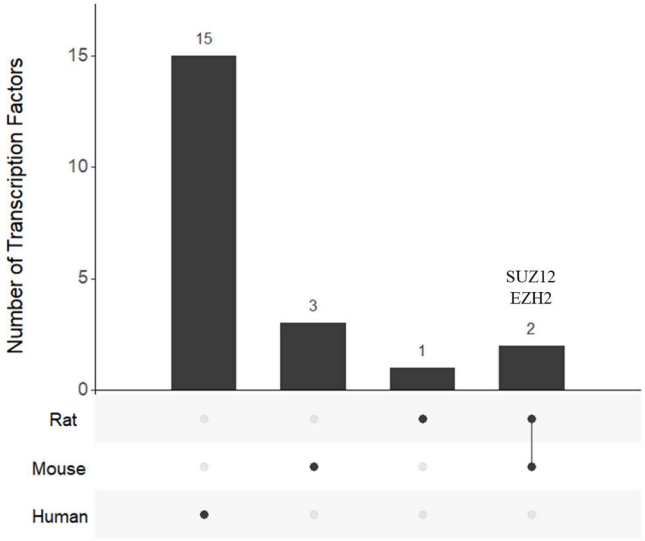


Figure 7. Summary of target molecular processes of BPA in individual tissues.

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