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A Tissue- and Species-Specific Meta-Analysis of Transcriptomic Effects of Endocrine Disrupting Chemicals and Association with Cardiometabolic Disorders

Author

Zamora, Zacary Orrantia

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A Tissue- and Species-Specific Meta-Analysis of Transcriptomic Effects of Endocrine Disrupting Chemicals and Association with Cardiometabolic Disorders

A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Physiological Science

by

Zacary Orrantia Zamora

2021

ABSTRACT OF THE THESIS

A Tissue- and Species-Specific Meta-Analysis of Transcriptomic Effects of Endocrine Disrupting Chemicals and Association with Cardiometabolic Disorders

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Zacary Orrantia Zamora

Master of Science in Physiological Science

University of California, Los Angeles, 2021

Professor Xia Yang, Chair

Cardiometabolic disorders such as metabolic syndrome, obesity, diabetes, cardiovascular disease, and non-alcoholic fatty liver disease are growing public health problems across the world. Among known cardiometabolic risk factors are obesogens such as endocrine disrupting chemicals (EDCs), exogenous chemical compounds that induce endocrine and metabolic dysfunctions. To date, the species- and tissue-specific influence of EDCs on molecular programs and cardiometabolic risks has not been fully elucidated. We performed a comprehensive data-driven transcriptome-wide analysis of 44 publicly available datasets for 4 EDCs, namely Bisphenol(BPA), Bis(2-ethylhexyl) phthalate (DEHP), Tributyltin (TBT), and Perfluorooctanoic acid (PFOA), to elucidate the perturbation in genes and pathways induced by these chemicals in a species- and tissue-specific manner. Our study identified various metabolic pathways including lipid metabolism, cholesterol biosynthesis and fatty acid metabolism to be up-regulated across all chemicals and species, whereas down-regulated pathways were largely species- and tissue-specific, including immune response, cell cycle, and metabolism terms. Taken together, the genes and pathways identified highlight differential responses of the transcriptome between

tissue and species and help infer the mechanisms underlying the connections between EDCs and cardiometabolic diseases.

The thesis of Zacary Orrantia Zamora is approved.

Claudio Villanueva

Patrick Allard

Xia Yang, Committee Chair

University of California, Los Angeles

2021

Dedication

This thesis is dedicated to my wonderful family and friends who have supported, encouraged, and guided me throughout my academic journey.

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Introduction

Cardiometabolic diseases (CMDs) such as metabolic syndrome, obesity, diabetes, cardiovascular disease, and non-alcoholic fatty liver disease, contribute to a fast-growing health epidemic worldwide, imposing high mortality and morbidity (Hurt et al., 2012). CMDs are characterized by a myriad of interrelated conditions such as hypertension, hyperlipidemia, high blood sugar levels, insulin resistance, increased adiposity, and elevated triglycerides (Merianos et al., 2020; Cannon, 2012). Since 2000, cardiovascular diseases and diabetes have been among the top 10 leading causes of death worldwide and their prevalence continues to grow (World Health Organization, 2020). Cardiovascular disease, a blanket term for various heart-related diseases such as coronary heart disease, cerebrovascular disease, etc., have accounted for approximately 18 million deaths in 2016, or about 31% of the global total for that year (Stewart et al., 2017). In addition, diabetes, a disease characterized by dysregulation of blood glucose levels, has more than doubled in prevalence around the world since the 1980's and affects more than 8.5% of the global population (Xu et al., 2018). Consequently, current predictions show that diabetes will afflict 1 in 10 people globally by 2035 (Aguirre et al., 2013). In 2014, more than 2.1 billion people, nearly 30% of the global population, were overweight or obese and 5% of the deaths worldwide were attributable to obesity (Tremmel et al., 2017). At its current pace, obesity is estimated to affect almost half of the world's adult population by 2030, with global health care costs surpassing 2 trillion USD annually (González-Muniesa and Mártinez-González, 2017). Therefore, a better understanding of the causes and mechanisms of cardiometabolic diseases is critical for the development of improved preventative and therapeutic strategies.

Both genetic and environmental factors, as well as the interactions between the two, contribute strongly to CMD predisposition and development. Numerous studies have been conducted to demonstrate the heritability of CMDs, yet environmental factors remain highly understudied. In particular, environmental risks such as industrial chemicals pose a major problem for public health, yet a comprehensive understanding of the cardiometabolic effects and biosignatures of chemicals that remain ubiquitous in the environment and in humans is currently lacking. Therefore, leveraging and comparing large-scale transcriptome datasets of chemical exposures may lead to greater understanding of cardiometabolic disturbances and allow for comparisons of implicated genes and biological pathways perturbed by environmental and industrial chemicals.

Endocrine disrupting chemicals (EDCs) are a class of mostly man-made exogenous chemicals substances that are used in industrial products that have the potential to affect CMD susceptibility. EDCs affect the endocrine system and have been linked to abnormal development and increased susceptibility to disease in adulthood or even across multiple generations (Schug et al., 2011). As human exposure to EDCs is universal and the endocrine system is an important component of CMDs, it is vital to investigate the risks that EDCs impose on the cardiometabolic systems and CMD pathologies.

Among the numerous EDCs identified to date, Bisphenol A (BPA), Di(2-ethylhexyl) phthalate (DEHP), Tributyltin (TBT), and Perfluorooctanoic acid (PFOA) are of particular interest. BPA is a pro-estrogenic chemical that is used in synthetic polymer goods and for lining of various food items (Shecter et al., 2012). BPA exposure is ubiquitous, such that biomonitoring studies found detectable levels of BPA in 93% of urine samples from people six years and older (Calafat et al.,

2008). BPA has been linked with various cardiometabolic risks such as hypertension, dyslipidemia, and abdominal obesity (Plourde et al., 2002; Benmohhamed et al., 2011; Li et al., 2015).

Di(2-ethylhexyl) phthalate (DEHP) is an anti-androgenic chemical that is used in the production of flexible plastics and is found in building materials, toys, food containers and notably, is present in some medical devices (Jarfelt et al., 2005, Shea, 2003). Due to their widespread applications and usage, phthalates are known as "everywhere chemicals" and are pervasive in the environment (Huang et al., 2019). Similar to bisphenols, human DEHP exposure has been shown to increase body weight, increase levels of triglycerides, and elevate blood pressure (Mohammad et al., 2019).

Marine biocides such as Tributyltin (TBT), are used for disinfection and anti-fouling properties in paints applied to ships and fishnets (Ximenes et al., 2017). Despite limited epidemiological studies in human exposure, organotins such as TBT are classified as EDCs because of their toxicity and ability to bioaccumulate in higher order organisms such as fish, large mammals, and humans (Ronconi et al., 2018; Cuenca et al., 2020). TBT as a ligand activates peroxisome proliferator activated receptor gamma (PPARγ), as such TBT and its metabolites have been linked to increased adipogenesis and lipid accumulation in mammals and have been found in the blood and liver of humans (Heindel, et al., 2019, Jia et al., 2016). Compared to traditional obesogenic EDCs such as BPA and DEHP, TBT is understudied in CMDs.

Perfluorooctanoic acid (PFOA) is a chemical used in commercial household products and other products that resist heat, oil, stains, and grease (Begley et al., 2005). Notably, PFOA does not readily break down in the environment, allowing for it to bioaccumulate easily (Steenland et al., 2010). As one of the many chemicals in the per- and polyfluoroalkyl substances (PFAS) family, PFOA has risen to prominence as an EDC of public health interest and was observed in the blood of virtually all Americans (Street et al., 2018; Lau et al., 2006). Additionally, PFOA has been shown to be a PPARγ agonist like archetypal obesogenic EDCs (Yamamoto et al., 2015). Moreover, PFOA exposure in mouse and human studies have been linked to increased weight, waist circumference, cholesterol levels and prevalence of diabetes (Halldorsson et al., 2012; Fei et al., 2007; Hines et al., 2009; He et al., 2017). Due in part to it being a part of a larger known group of EDCs, PFOA-exposure remains poorly characterized among EDCs.

Since the launch of programs to characterize the effects of EDCs, such as Toxicology in the 21st Century (Tox21) and Toxicity Forecaster (Toxcast), transcriptomics studies of EDCs have helped reveal molecular insights into the perturbed genes and pathways. However, EDCs have shown to exhibit species-, tissue-, and dose-dependent effects, but systematic investigation of these effects within and across EDCs has not been conducted (Vandenberg et al., 2012). In addition, despite the large volume of existing transcriptome data, harmonization of data from different study designs and technology platforms remains challenging. As such, processing these studies in a consistent manner will allow for greater translation potential, where uncovering the chemical-, species-, and tissue-specific effects may yield greater understanding of the underlying molecular perturbations, expression alterations, and regulation of biological pathways.

This study aims to systematically meta-analyze existing, publicly available transcriptomic datasets on EDCs deposited in Gene Expression Omnibus (GEO) to understand species- and tissue-specific effects of EDCs on transcriptome perturbances and cardiometabolic risks. To this end, we 1) streamline microarray and RNA sequencing data acquisition and processing from GEO, 2) extract differentially expressed genes (DEGs) as gene signatures of the four EDCs described above from individual studies varying in species, tissue, and dosage, and 3) link the genes and pathways to cardiometabolic risks.

Materials and Methods

Dataset Curation and Identification

The National Center for Biotechnology Information's (NCBI) Gene Expression Omnibus (GEO) is an open access international genomics repository. GEO archives various high-throughput data, including microarray, RNAseq and other omics datatypes (Edgar et al., 2002). GEO was queried with various chemical nomenclatures for the four EDCs of interest in order to capture all potential expression profiles. This search encompassed both in vitro and in vivo datasets to ensure data coverage. BPA terms queried included: "Bisphenol A","4,4'-propane-2,2-diyldiphenol", "BPA"; DEHP terms queried included: "Bis(2)ethylhexyl phthalate", "Di-sec octyl phthalate", "Octyl Phthalate" ,"DEHP"; TBT queried terms included: "bis(tributyltin)oxide", "bis(tri)*n*-butyltinoxide", "TBTO", "Tributyltin", "TBT"; PFOA queried terms included: **"**perfluorooctanoic acid", "perfluorooctanoate", "PFOA". Each search was then filtered to the level "series". The resulting datasets were curated to meet the following criteria for inclusion: 1. Publicly available in GEO; 2. Transcriptome data (both RNASeq and Microarray data types); 3. If microarray dataset, single channel arrays, 4. Appropriate sample sizing

(n>=3/group); 5. Direct exposure (excluding transgenerational studies); 6. Not a duplicate (subseries/superseries). Datasets identified for inclusion were then filtered to include studies most relevant to cardiometabolic disorders based on tissue annotations (adipose, cardiovascular system, liver, and pancreas) (Figure 1). Additionally, only datasets that were derived from humans, mice, and rats as model organisms were included, as other species lacked either sufficient numbers of expression profiles or were non-mammalian organisms with less biological translational capacity. Lastly, all GEO expression profiles accessible at the end of December, 2020 were considered.

Downloading and Processing Transcriptome Expression Profiles

Microarray datasets were directly downloaded from GEO via the R package "GEOquery" (Davis and Meltzer, 2007; R Core Team, 2019). Expression profile meta-data containing descriptive information of the overall experiments and individual samples was processed, and organ systems/tissues were reannotated by Brenda Tissue Ontology to consolidate tissue terms. Datasets were then segregated by species/organism and organ system/tissue (Gremse et al., 2011). Obtained expression matrices were then checked for log2 transformation and correct GEO sample accession annotation for downstream analysis (Figure 2).

For RNAseq data, a different procedure was used to download data. Despite GEO's capacity to store various types of high-throughput data, GEO relies on NCBI's Sequence Read Archive (SRA), another genomics repository, to store raw RNAseq reads data and alignment information (Leinonen et al, 2011). We chose to use the raw sequencing reads from SRA instead of the processed data in GEO because different studies used different data processing pipelines and data harmonization across datasets was challenging using the processed data. The identified raw

RNAseq datasets were downloaded from SRA, quality checked, and processed using the following packages in the anaconda environment (Anaconda Software Distribution, 2020). As SRA's native download package "SRA toolkit" was slow, we used the Parallel FastQ Dump wrapper to retrieve FASTQ files from SRA (Valieris, 2020). We then conducted quality checks on the FASTQ files and trimmed adapters with packages Trim_galore and Cutadapt (Krueger, 2020; Marcel, 2011; Andrews, 2010). The sequence mapping and expression quantifier package Salmon was used to map the sequencing reads to the appropriate reference genomes (human genome build GRCh38.p13 , mouse genome build GRCm39, and rat genome build Rnor_6.0) and to quantify the mapped reads (version 1.3, Patro et al., 2017). The R package Tximport was then used to import and summarize Salmon quantification results for downstream differential expression analysis (Soneson et al., 2015).

Differential Gene Expression Analysis

Differential expression analysis was performed by high performing, gold-standard differential expression analysis tools appropriate for different data types (Schurch et al., 2015), namely Linear Models for Microarray Data (LIMMA) for microarrays and DESeq2 for RNAseq data to identify differentially expressed genes (DEGs)(Ritchie et al., 2015; Love et al., 2014). As multiple microarray datasets can be available for the same species and tissue type exposed to the same EDC, we further conducted meta-analysis to derive consensus DEGs of individual EDCs stratified by species and tissue. The Robust Rank Aggregation package in R (Kolde et al., 2012) was performed on DEGs identified by LIMMA and ordered by the effect size of differential expression, log fold change (logFC). The DEG lists from individual microarray datasets were segregated by organ system/tissue, then rank aggregation was used to identify differentially

expressed genes (DEGs) at a false discovery rate (FDR) < 0.05 across all studies of the same EDC in the same species and tissue. Rank aggregation was performed for both up-regulated and down-regulated genes. Due to limited numbers of RNAseq studies, up- and down-regulated gene sets from individual datasets identified with DESeq2 (FDR<0.05), and pathway annotations were directly compared to those identified by the microarray datasets.

Pathway Enrichment Analysis of DEGs to Identify Over-represented Pathways

To capture alterations to biological processes represented by the DEGs, pathway enrichment analysis of DEGs was performed. Both Kyoto Encyclopedia of Genes and Genomes Pathway (KEGG) and Gene Ontology Biological Processes (GO BP) were used to functionally annotate the DEGs (Kanehisa and Goto, 2000). First, to ensure consistency in pathway annotation across species, the gene symbols of the mouse and rat DEGs identified were converted to human symbols based on Ensemble homology using BiomaRt (Durinck et al., 2009, Howe et al., 2021). Then enrichment analysis was then performed on the significant up and down-regulated DEGs separately utilizing the "enrichR" package, with a FDR < 0.05 as the cutoff (Chen et al., 2013; Kuleshov et al., 2016; Xie et al., 2021). Significant pathway terms were then compared across species and tissues for overlap using Upset plots (Conway et al., 2017).

Association of DEGs to Cardiometabolic Diseases

To assess the association of the DEGs with various cardiometabolic diseases, we took the top 100 up- and down-regulated DEGs for each EDC from each species and each tissue and overlapped them with various cardiometabolic disease candidate genes identified from human genome-wide association studies (GWAS). GWAS candidate genes were downloaded from the

National Human Genome Research Institute (NHGRI)-European Bioinformatics Institute (EBI) catalog of GWAS studies (Buniello et al., 2019). We then imported the catalog to R and applied the merge function to return DEGs overlapping with candidate genes for CMDs in GWAS catalog, including type II diabetes (993 genes), metabolic syndrome (200 genes), obesity (100 genes), and coronary heart disease (726 genes).

Results

Tissue and Species Coverage of Endocrine Disrupting Chemicals

The total number of transcriptome datasets that passed selection criteria (Figure 1) for inclusion and of cardiometabolic tissue types was 44 (Table 1), including 16 for BPA, 17 for DEHP, 6 for TBT, and 5 for PFOA. Liver tissue was examined across all four EDCs (n = 38), whereas pancreas (n = 1) and adipose (n = 3) coverage was observed only in BPA exposure studies, and cardiovascular system coverage was observed only in DEHP studies (n = 2). Both TBT and PFOA were composed solely of liver studies.

Human, mouse, and rat species coverage was observed for both BPA and DEHP, albeit in mostly liver studies. BPA liver species coverage was composed of 2 human studies, 6 rat studies, and 4 mouse studies. BPA adipose coverage consisted of only human and mouse studies (human = 2, mouse = 1) while BPA pancreas coverage was subjected to only a single mouse study. DEHP liver studies were composed of 1 human study, 5 rat studies, and 9 mouse studies, whereas DEHP cardiovascular system species coverage was comprised solely of rat datasets (n = 2). TBT species coverage consisted of human and mouse studies only (human = 2, mouse = 4), while PFOA was limited to rat and mouse studies (rat = 1, mouse = 4).

Overall, the species and tissue coverage for the EDCs examined was uneven, with better coverage for rodents and liver tissue. BPA exposure studies have the best coverage of species and tissues compared to DEHP, TBT, and PFOA, highlighting the need for additional data collection efforts for the understudied EDCs.

Biosignatures of BPA-exposed Cardiometabolic Tissues Vary

For BPA exposure , in total we identified 9,160 significant DEGs across 3 cardiometabolic tissues (liver, adipose, and pancreas) based on microarray datasets (n = 13) and 1,471 DEGs across liver and adipose tissues based on RNAseq datasets (n = 3).

Across both microarray and RNAseq liver studies, we identified approximately 949 down- and 870 up-regulated DEGs on average per species (min = 750, max = 1,099; n = 13 studies, Figure 3). Overlap of total DEGs in all 3 species was 0.3% and overlap between any two species was 2.1-4.6%. Shared down-regulated pathway terms between human and mouse studies consisted of cell cycle and apoptosis terms. Across all three species, shared up-regulated pathways consisted of metabolism-related terms, including regulation of cholesterol/steroid/alcohol biosynthetic processes (Figure 4).

Adipose studies (n = 3 studies) revealed 1,110 down and 920 up-regulated DEGs. Two microarray data sets contributed 1,067 down-regulated DEGs, and 885 up-regulated DEGs in humans, whereas a single RNAseq mouse dataset yielded only 43 down- and 35 up-regulated DEGs, with 0.5% and 0.4% down and up-regulated genes overlapping between microarray and

RNAseq DEGs, respectively (Figure 5) . Given the difference in the transcriptome platform and large discrepancy in DEG number identified in human and mouse studies, functional annotation was performed separately for the two species. In humans, the top down-regulated biological pathways enriched among DEGs included immune and inflammatory response pathways; top up-regulated pathways were related to metabolic processes (cholesterol/fatty acid biosynthesis), neuronal development, and cell organization (Figure 6). For the mouse RNAseq dataset, the top down-regulated pathways consisted mainly of various mitotic cell cycle terms, and up-regulated pathways contained several lipid/cholesterol transport terms (Figure 7). There was no direct overlap of biological pathway terms between human and mouse adipose studies, except that lipid/cholesterol related processes were up-regulated in both, with biosynthesis terms over-represented in human and transport terms represented in mouse.

Coverage of BPA pancreas exposure studies was relegated to a single mouse study, therefore no comparisons could be made across species. DEG analysis yielded a total of 1,730 (865 down- and 865 up-regulated) significant DEGs. Enrichment analysis revealed up-regulation of transcriptional dysregulation in cancer, mineral absorption, and fat digestion (Figure 8); down-regulated pathways highlighted terms involved in cytosolic transport, golgi to endosome transport, and cholesterol efflux.

Across all BPA-exposed tissues, shared up-regulation of metabolism pathways is noted with lipid/cholesterol/fatty acid metabolism represented. However down-regulation pathways for each tissue diverged. In liver, pathways including metabolism pathways and cell cycle terms such as transition of mitotic cell cycle, apoptosis regulation, and regulation of programmed death were

highlighted. In adipose, downregulation pathways of immune response terms, cytokine signaling, inflammatory response, and leukocyte migration was noted. In pancreas, down-regulated terms contained mainly transport pathways.

DEHP-Exposed Tissues Reveal Tissue-Specific Expression Profiles

For DEHP exposure, in total we identified 7,355 significant DEGs for DEHP across 15 liver studies and two studies of the cardiovascular system.

Based on the 15 liver studies, we identified 938 down- and 1001 up-regulated DEGs on average per species (min = 741, max = 1360; n = 15, Figure 9). In the single human study 823 down- and 824 up-regulated genes were identified; from 9 mouse studies 1278 down- and 1360 up-regulated genes were identified ; from 5 rat studies 795 down- and 741 up-regulated DEGs were found. The percentage of shared DEGs across all 3 species was 0.4% and overlap between any two species (in either up or down direction) ranged from 1.5-9.1%. Despite the minimal overlap in DEGs, functional annotation for shared down-regulated pathways across the three species retrieved acylglycerol metabolism as the only shared term. Between human and rat, regulation of lipoprotein was enriched in down-regulated genes, whereas down-regulated human-mouse overlap pathways were composed of metabolism terms (sterol, secondary alcohol and cholesterol biosynthesis). Between mouse and rat models, 29 down-regulated terms associated with metabolism and immune response were shared. Functional annotation for shared up-regulated pathways across the three species retrieved regulation of lipid and primary metabolic processes. Between mouse and human studies, 15 up-regulated terms associated with cell organization, apoptosis, and immune response were shared. Between mouse and rat studies, 32 up-regulated

terms associated with metabolism were revealed (fatty acid catabolism, cholesterol metabolism, lipid biosynthesis). There were no shared up-regulated pathways between human and rat studies (Figure 10).

DEHP cardiovascular system exposure studies were composed of two rat datasets, revealing 715 down- and 819 up-regulated DEGs respectively. The top down-regulated pathway terms are involved with immune responses, the cell cycle, and extracellular matrix organization, whereas the top up-regulated biological pathways centered around regulation of ion homeostasis (cadmium, iron, and zinc) **(**Figure 11**)**.

Between liver and cardiovascular system, tissue specific effects were observed. Liver response to DEHP exposure resulted in various metabolism terms being represented in both down- and up-regulated pathways (lipid metabolism, fatty acid biosynthesis, acylglycerol homeostasis, sterol biosynthesis). Cardiovascular system yielded divergent terms, with none of the top down- and up-regulated terms being shared with liver, downregulated pathways pertained to cell proliferation and regulation of cell cycle, while upregulated pathways centered solely on cell ion homeostasis, including zinc, cadmium, copper regulation.

TBT-Exposure in Liver Acts in a Species-Specific Manner

TBT analysis was comprised entirely of liver datasets (5 microarray and one RNAseq). Microarray studies revealed 743 down-regulated DEGs in 2 human studies, 727 down-regulated DEGs in three mouse studies, and 46 shared between the two species. Similarly, there were 814 human up-regulated genes, and 692 mouse up-regulated genes, of which 36 were shared (Figure

12). Compared to three mouse microarray datasets, the single mouse RNAseq study yielded very few significant DEGs, 27 down- and 12 up-regulated respectively.

Functional annotation revealed 66 up-regulated pathways for humans and 50 pathways for the mice, none of which were shared between the two species. Human up-regulated pathways consisted of cell cycle and organization terms including DNA replication/metabolism and centromere assembly/mitotic spindle organization. Upregulated pathways in mouse studies consisted of various metabolism processes, such as regulation of cholesterol and alcohol biosynthetic processes. Despite a lack of shared up-regulated annotation terms between species, 3 down-regulated pathways involved in cell proliferation and transcription regulation were shared between the 47 down-regulated pathways in human studies and 18 in mouse studies (Figure 13).

PFOA Liver Studies Reveal Mixed Expression Profiles in Murine Models

PFOA coverage consisted of 5 liver datasets only (4 mouse microarray datasets, 1 rat RNAseq). Mouse microarray liver studies yielded 1,133 down- and 1,118 up-regulated significant DEGs, and the single rat RNAseq liver study yielded 1,111 down- and 1,085 up-regulated DEGs (Figure 14). Despite the high number of identified DEGs in the two different species, there were only 31 shared down- and 24 shared up-regulated DEGs (1.3% overlap).

Functional annotation overlaps revealed 32 common pathways in the down-regulated DEGs and 28 shared terms for the up-regulated DEGs between species (Figure 15). Shared down-regulated pathways included fatty acid oxidation and regulation of lipid metabolism, while shared up-

regulated pathways included immune system response and injury repair terms. Top mouse up-regulated pathways were also involved with metabolism, including fatty acid metabolism, fatty acid oxidation, cholesterol regulation, and PPAR signaling, whereas down-regulated pathways consisted of electron transport chain and neutrophil degranulation. Rat responses indicated down-regulation of long chain fatty acid import, arachidonic metabolism and mitosis, while up-regulated pathways centralized around endoplasmic reticulum distress (ER) stress, IRE1 protein response and golgi-related transport.

Lack of Concordance between Microarray and RNAseq DEGs

We further compared the identified DEGs between microarray and RNAseq datasets, revealing limited concordance. For example, the BPA liver studies showed overlaps between microarray and RNseq mouse studies at 2.9% for down-regulated DEGs and 2.0% concordance for up-regulated DEGs. Across studies that contained same species and tissue expression profiles, overlap of the identified DEGs between microarray and RNAseq datasets revealed a low average concordance of 1.52% (range of 0.14%-4.62%; Table 2).

DEGs of EDCs Show Association with CMDs based on Overlap between DEGs and Human GWAS Genes for CMDs

We compared the DEGs with candidate genes identified for CMDs from human GWAS studies to assess the disease relevance of the DEGs. Across species and tissues for each EDC, the DEGs showed numerous overlaps with human risk genes for CMDs, providing molecular support for the risks of the EDCs pose on CMDs (Tables 3-6). For example, BPA signatures across species and tissues showed many overlapping genes with type 2 diabetes and coronary artery disease.

Discussion

Overview of Key Findings

Our current analysis across 44 publicly available transcriptome studies within GEO demonstrated the various alterations of EDC exposures across chemicals, species, and tissues, revealing species- and tissue-specific molecular signatures with CMD associations (Figure 16). For chemicals with less data coverage such as PFOA and TBT inference for species and tissue specificity was limited, whereas in the case of BPA, sufficient study numbers allowed for greater cross-species comparisons. Furthermore, differences between EDCs could only be directly compared in liver tissue studies.

Across chemicals, our findings suggest that up-regulated pathways altered in liver-exposure studies react similarly, where lipid, fatty acid, and primary metabolic processes, were consistently identified. Despite these similarities, top downregulated terms across chemicals varied greatly. BPA downregulated terms included cell cycle, apoptosis, and DNA metabolism/replication; DEHP affected metabolism (acylglycerol homeostasis, sterol biosynthesis) and immune response terms. TBT downregulated terms included regulation of apoptosis, ERK1/2 cascade, and cell proliferation; PFOA down-regulated terms included steroid/lipid/fatty acid metabolism. These results indicate both shared and chemical-specific liver perturbations by EDCs.

BPA Findings

It is previously known that BPA can bind to estrogen receptors and influence bodily processes like cell proliferation and apoptosis due to its estrogen-like properties (Gao et al., 2015). In our

meta-analysis, liver exposure studies revealed down-regulated pathways that included cell cycle terms like apoptotic processes and DNA replication, thereby supporting previous literature (Can et al., 2005); up-regulated biological pathways largely remained uniform with lipid, cholesterol, and steroid biosynthesis being observed across all three species.

Previous studies in our lab have demonstrated that the effects of BPA exposure in mice are highly variable according to tissue, sex, age, and dose level, and that the liver is most susceptible to disruption according to both transcriptome and DNA methylome analyses across liver, adipose, and hypothalamus (Shu et al., 2019). The relative importance of liver to adipose tissues is confirmed in the current study, as supported by larger number of liver DEGs than adipose DEGs. According to our disease association GWAS analysis, liver perturbances yielded more DEGS than adipose tissue. However, our current analysis that additionally included pancreas tissue demonstrated that pancreas is also highly perturbed by BPA exposure, and GWAS/DEG overlap analysis yielded more CMD associations for mouse pancreas DEGs than the mouse liver counterpart. Of interest, our analysis of the pancreas study did not yield any terms involving insulin production and/or resistance but identified up-regulation of pathways were involved with cholesterol and lipid transport which were shared with adipose tissue.

Across species comparison showed that the species-specific effect is small in up-regulated liver pathways, capturing similar biosignatures across species, including regulation of alcohol/steroid/cholesterol biosynthesis processes. For BPA-exposed adipose studies, human and mouse studies shared no pathways within their top 10 down- and up-regulated pathways, with few or no pathways identified outside of these pathways. Among all EDCs, BPA contained the

greatest number of DEGs with CMD association across species (e.g., HMGCR, PPAR), indicating species-specific effects were least prominent in BPA-exposed tissues.

DEHP Findings

DEHP as a carcinogen has been well documented, in support of this we observed tissue-specific differences as alterations in cell cycling pathways such as MAPK cascade, cell proliferation, and mitosis regulation in cardiovascular system studies, supporting the cardiotoxic effects of DEHP as described in previous literature (Jaimes et al., 2017). However, our analysis of the DEHP-exposed liver revealed several metabolism perturbance with few hepatoxicity terms yielded. In contrast to cell cycle terms, DEHP-exposed liver revealed immune system perturbations through terms such as cytokine signaling and neutrophil activation. Notably, liver studies showed that metabolic pathways, are enriched in both up-regulated and down-regulated DEGs, suggesting a complex regulation of metabolic sub-pathways.

Cross-species comparison for DEHP exposure revealed limited (10.8%) shared biological pathways between any 2 species. However, some DEGs from CMD association were noted to be shared across species (e.g., APOA4, PFKF3B), suggesting some similarity across species compared to other EDCs.

TBT Findings

We were only able to investigate the liver tissue studies for TBT due to the lack of data for other tissues. The current study has implicated many biological pathways involved with the cell cycle and confirms the hepatotoxic capacity of TBT on various species, including cell death apoptotic

processes, and cell proliferation. However, TBT is a chemical of interest not only for its known carcinogenic properties, but also for its role as an agonist for classic lipogenic pathways such as PPAR and RXAR (Antizar-Ladislao, 2008). Our DEG and functional annotation analysis minimally supports previous literature, as only one PPARGC1B gene, a PPAR co-activator gene, and one annotation term, PPAR signaling pathway were revealed. Importantly, the GWAS catalog did not associate the identified PPAR co-activator DEG to select CMDs.

Our study suggests that TBT effects on the liver are species-specific, as both pathway level and CMD disease association overlap yielded minimal overlaps between human and mouse studies. Interestingly, human exposure studies yielded more DEGs, biological pathway terms, and CMD association overlaps.

PFOA Findings

Similar to TBT, we were only able to assess the effects of PFOA on the liver due to data availability issues. Our study demonstrates that among the shared liver pathways between rat and mouse studies, metabolism alteration is common, with regulation of lipid and primary metabolism being represented in down-regulated pathways, and fatty acid/cholesterol biosynthesis observed in up-regulated pathways. Our study confirms previous human studies that have demonstrated PFOA's ability to alter genes involved in cholesterol metabolism (Fletcher et al, 2013). Despite the shared metabolic pathways, the majority (90.2%) of the identified pathways are not shared between species, suggesting that PFOA effects were species specific. Mouse biological pathways consisted of electron transport chain and neutrophil degranulation responses, whereas rat pathways involved ER stress and transport responses. Additionally, in

support of disparate species effects, within DEGs that were identified to have CMD associations, none were shared between mouse and rat species.

Cross-chemical Comparison and Discussion: Similarity and Differences Between EDCs

Across all four chemicals, direct comparison could only be made in liver studies. Notably, top up-regulated pathways of each chemical, regardless of species and tissue-specific effects, involved metabolism processes, such as primary/lipid metabolic processes or fatty acid metabolism. Despite this similarity, down-regulated pathways demonstrated varied responses. BPA and TBT livers most closely resembled each other, with down-regulated pathways consisting of cell cycle terms such as apoptosis pathways, cell proliferation, replication or ERK1/2 cascade. Both DEHP and PFOA resulted in down-regulation of more metabolism pathways, for example, acylglycerol homeostasis, steroid/lipid metabolism, and cholesterol biosynthesis. DEHP differed from the other DEGs in that immune response pathways were affected, such as regulation of neutrophil activation and degranulation.

Disease Association of DEGs to CMDs

The DEGs of each EDC from each tissue and species showed numerous overlaps with four CMDs, supporting their relevance to disease risks. Due to the larger number of candidate genes collected for type 2 diabetes and coronary heart disease, more DEGs showed overlap with these diseases than with metabolic syndrome and obesity. Future analysis normalizing the number of disease genes is needed. It is worth noting that the DEGs overlapping with disease risks genes are seldom consistent across species/tissues likely, except that 5 BPA DEGs (HMGCR, PPAR, INHBB, APOE4, APOE), 4 DEHP DEGs (PFKFB3, JAG1, ABCA1, APOA4), and one PFOA

DEG (RRBP1) across species/tissues which overlapped with GWAS genes for CMDs. These genes are likely the robust gene targets of EDCs that confer CMD risks. Notably, HMGCR, PFKFB3, PPAR, APOA4 and APOE/APOE4 genes are involved in glucose/cholesterol/lipid metabolism; PPAR and PFKFB3 are also involved in inflammation; APOA4 is involved in appetite and satiety; ABCA1 is involved with cholesterol and phospholipid transport (Stelzer et al., 2016).

Lessons Learned from Analyzing Publicly Available Datasets

One of the goals of our study was to leverage publicly available transcriptome data and re-process in a manner that allows for comprehensive interpretation of the various effects these chemicals confer. Previous studies reporting these individual datasets utilized different statistics and differential expression analysis tools, making it challenging to compare the results across studies. Our systematic curation and processing of data in a uniform manner help better compare the studies to infer the potential outcomes of these chemical perturbances.

In the current study the divergence of DEGs represented in the Microarray and RNAseq concordance comparisons was extreme, with the maximum overlap being only 4.62%. Few studies have assessed RNAseq and microarray DEG concordance, largely owing to the fact that the technologies are completely different in how they measure expression (relative signal intensity vs read counts). The limited previous comparative studies revealed a concordance between 20-85% (Guo et al., 2006, Wang et al, 2014). Our study leveraged transcriptome data sets from different species, tissues, brand/type of microarrays, and thus, our Microarray-RNAseq concordance (1.52%) was much lower than previously reported. For this reason, we focused less

on overlaps in DEGs but their functional annotation to allow for more biologically relevant and direct comparisons.

Strengths of the Study

Current interpretations of transcriptome perturbance rely on single dataset analyses where a focus on a singular tissue or species is most common. Here we present a meta-analysis including a large number of studies, multiple EDCs, species, tissues, revealing unique insights into species and tissue-specific effects and the translational potential of rodent studies of environmental chemicals to human CMD risk. In addition, we established a computational pipeline to analyze and document molecular effects where uniformly processing raw data have been cumbersome and challenging, which will facilitate future systems toxicogenomic studies.

Limitations of the Study

In the current study, we did not stratify gene sets by exposure dose and exposure window to simplify analysis design and focus on tissue and species effects. However, both exposure dose and window have great impact on the magnitude of transcriptome alteration (Golestanzadeh et al., 2019). Indeed, various EDC studies have indicated exposure window to be the main predictor for health outcomes in addition to exposure dose (Belzunce et al., 2004). Many of the tissue and species differences could be a result of exposure differences between studies, which will be addressed in our future studies. In addition, due to technology and platform differences, novel methods to robustly compare concordance across RNAseq and microarray technologies are needed to fully identify overlapping perturbed genes and biological pathways.

Conclusion and Future Directions

Through a meta-analysis of 44 transcriptomic studies across 4 EDCs in multiple cardiometabolic tissues in three mammalian species, our study offers unique insights into the species and tissues similarities and differences within and between EDCs and whether the genes and pathways are relevant to CMDs. Despite the differences in study designs across the datasets, metabolic genes pathways were found to be altered across tissues and species, supporting the CMD risks conferred by diverse types of EDCs. It is apparent after conducting a study reviewing the transcriptome changes of various chemicals, that more studies are needed to fill in the gaps between lesser characterized chemicals, particularly chemicals such as TBT and PFOA. Even for the best studied chemical, BPA, important tissues such as adipose and pancreas were poorly covered. Lastly, investigation of the effects of exposure dose and exposure window on transcriptome alterations is needed.

Figure 1. Study identification and filtering

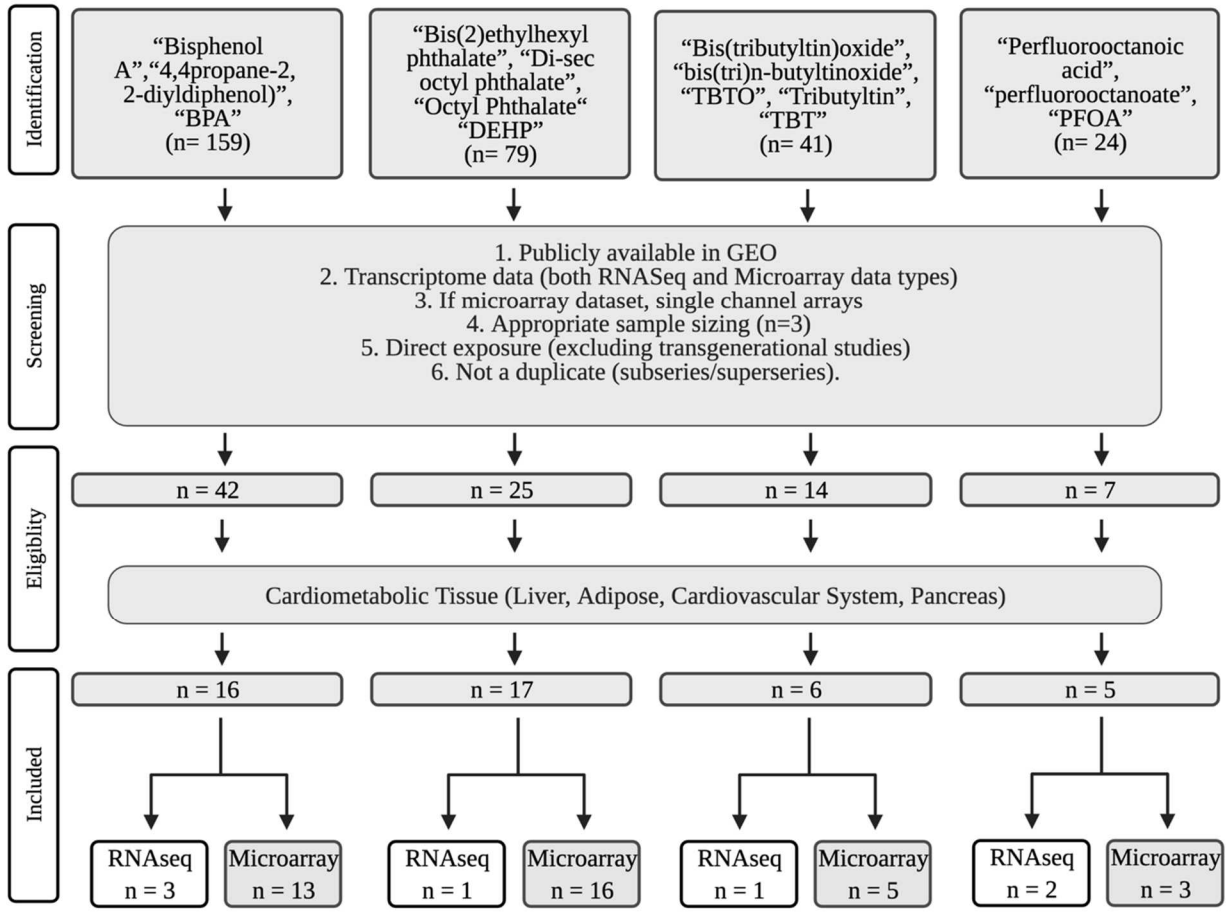

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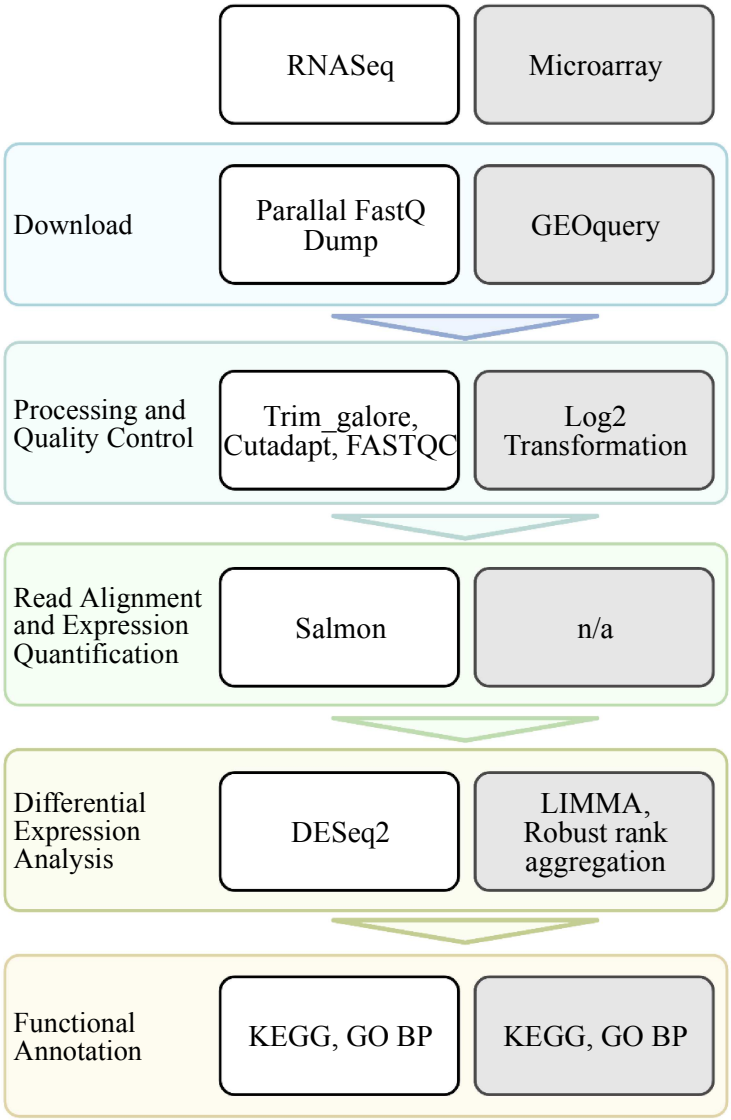
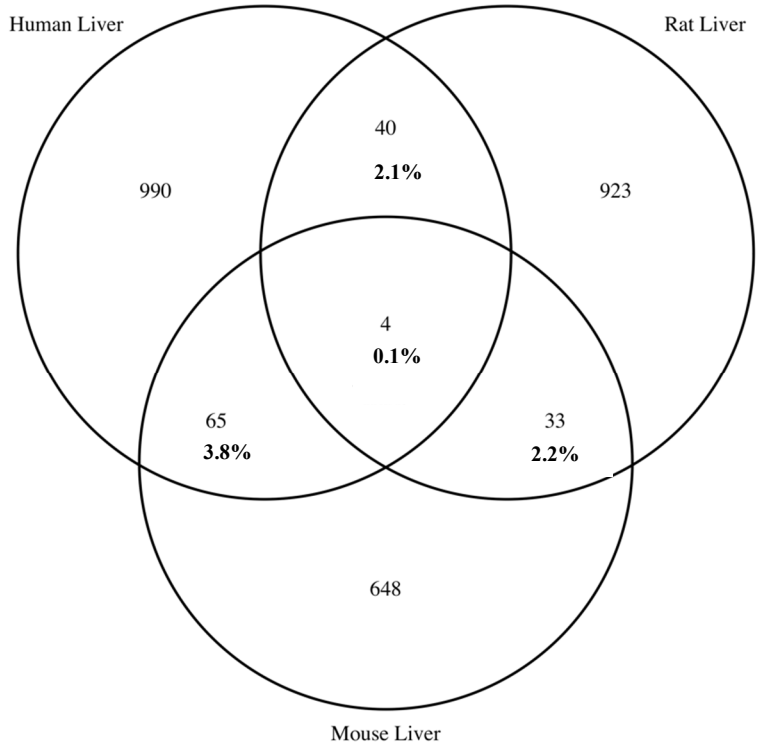
Figure 2. Data processing workflow for different data types



Created with BioRender.com

Figure 3. BPA: Number of identified differentially expressed genes A) Down-regulated DEGs B) Up-regulated DEGs.

A.



B.

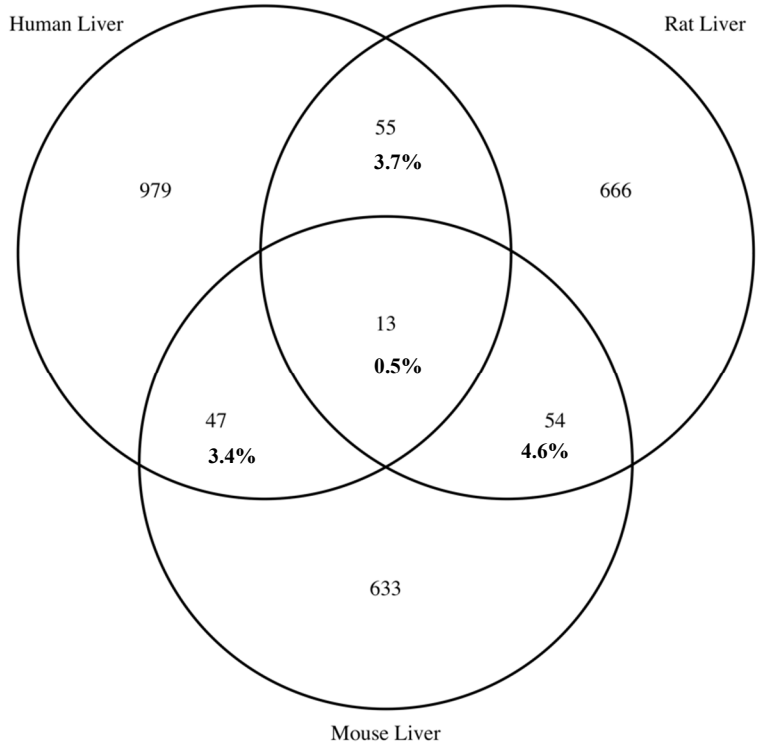
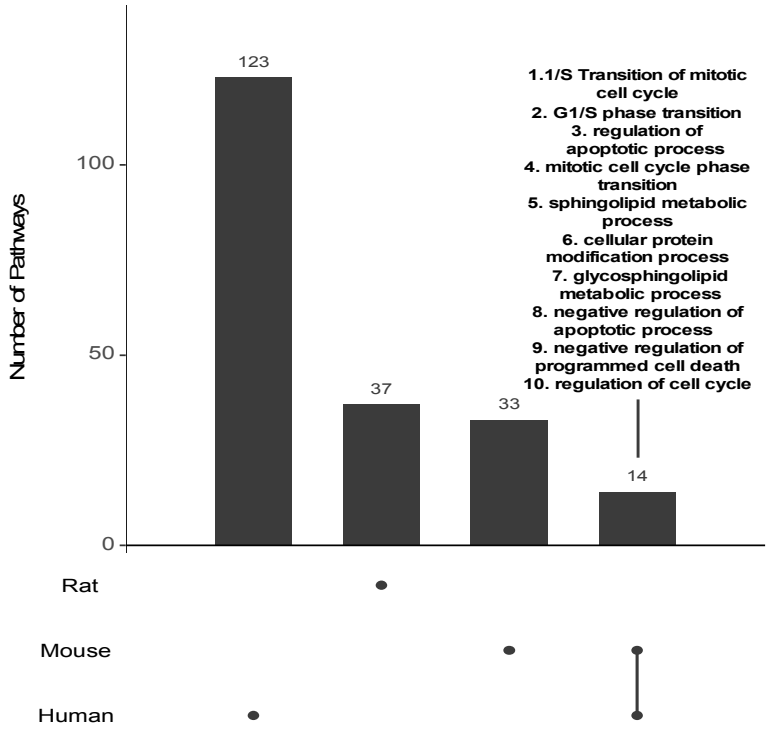


Figure 4. BPA: Combined microarray and RNAseq comparison of pathways for differentially expressed genes. A) Down-regulated pathways. B) Up-regulated pathways.

A.



B.

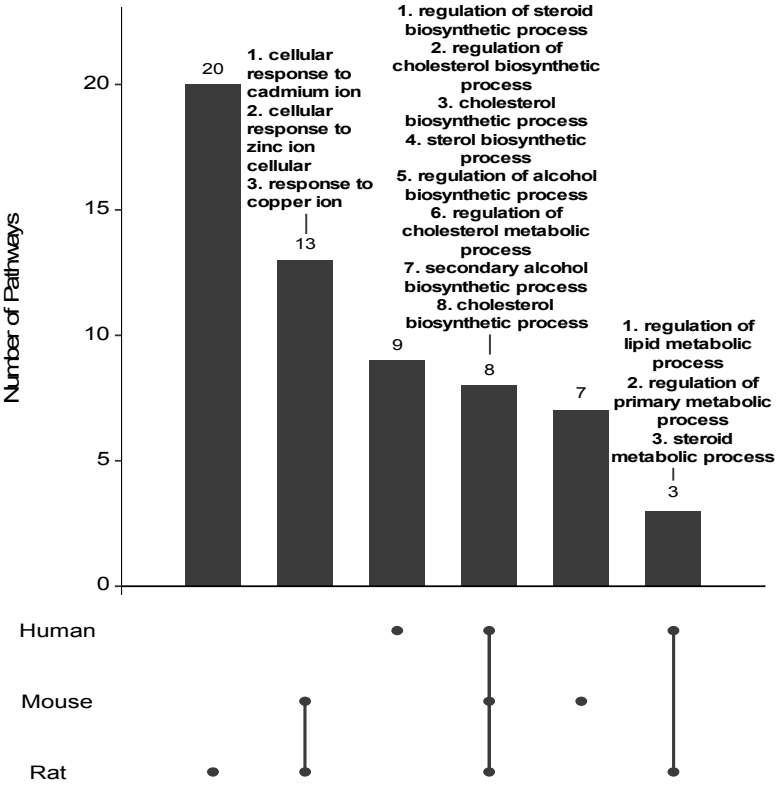


Figure 5. BPA: Number of identified DEGs in human microarray compared to mouse RNAseq in adipose tissue A) Down-regulated DEGs B) Up-regulated DEGs.

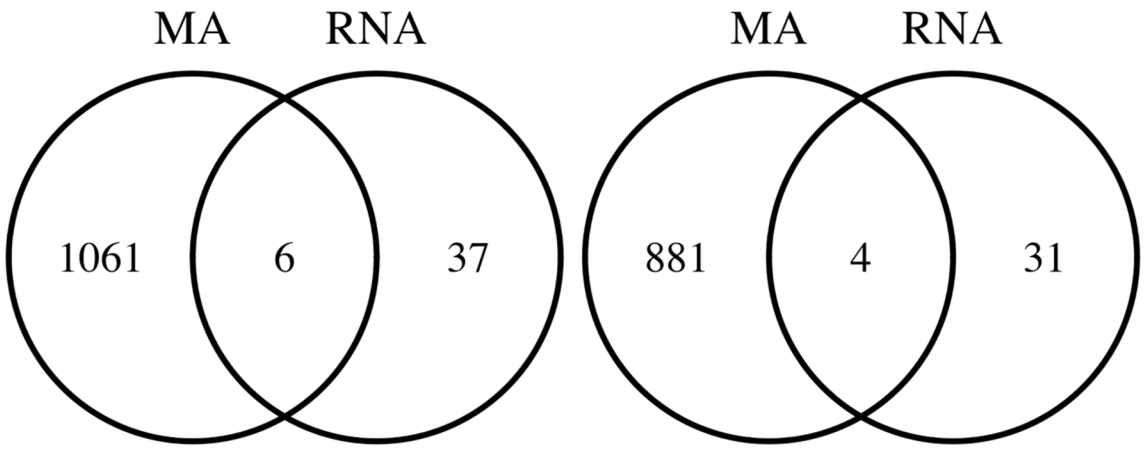
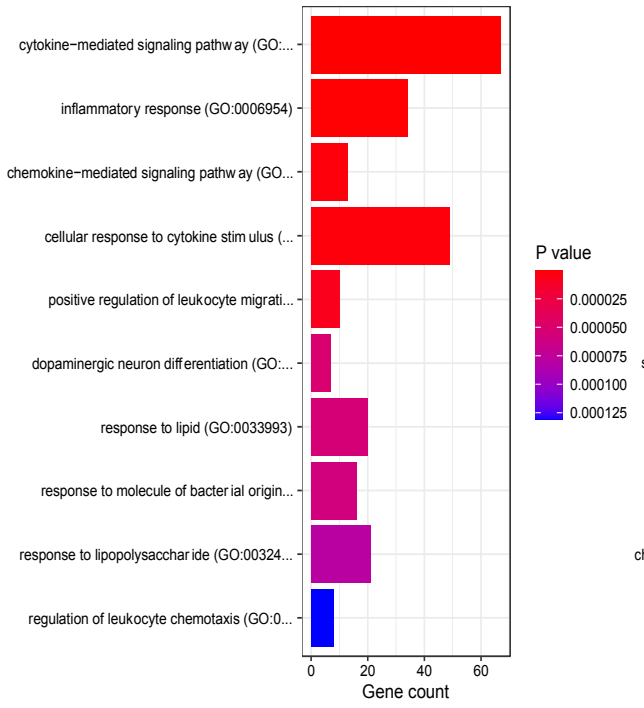


Figure 6. BPA: Functional annotation of human adipose studies A) Top 10 Down-regulated Pathways B) Top 10 Up-regulated Pathways.

A.



B.

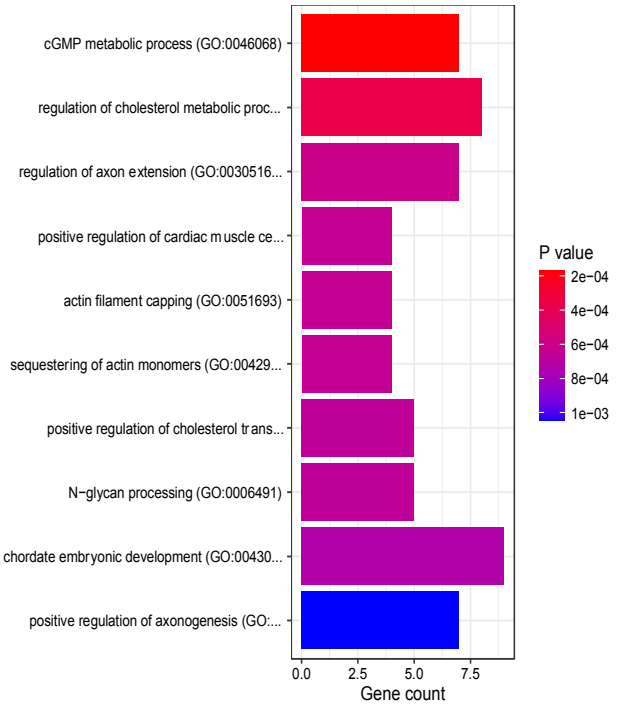
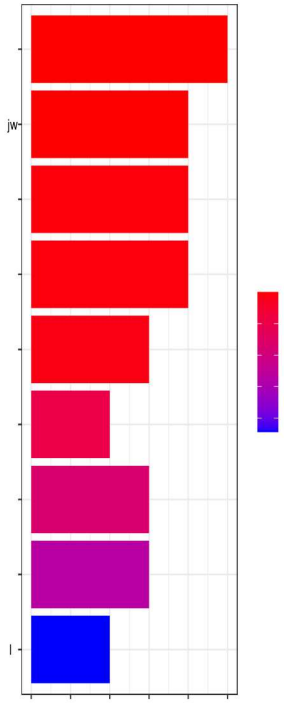


Figure 7. BPA: Functional annotation of mouse adipose study A) Top 10 Down-regulated Pathways B) Top 10 Up-regulated Pathways.

A.



B.

Figure 8. BPA: Functional annotation of mouse pancreas study A) Top 10 Down-regulated Pathways B) Top 10 Up-regulated Pathways.

A.

B.

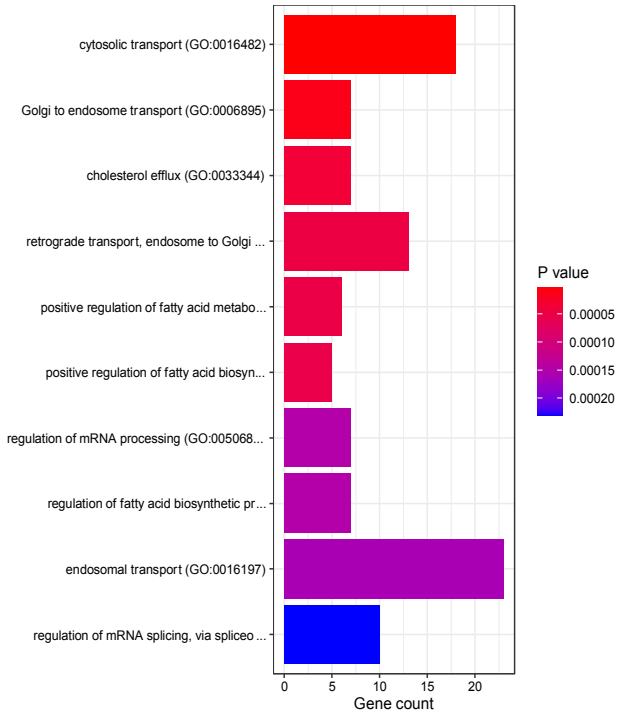
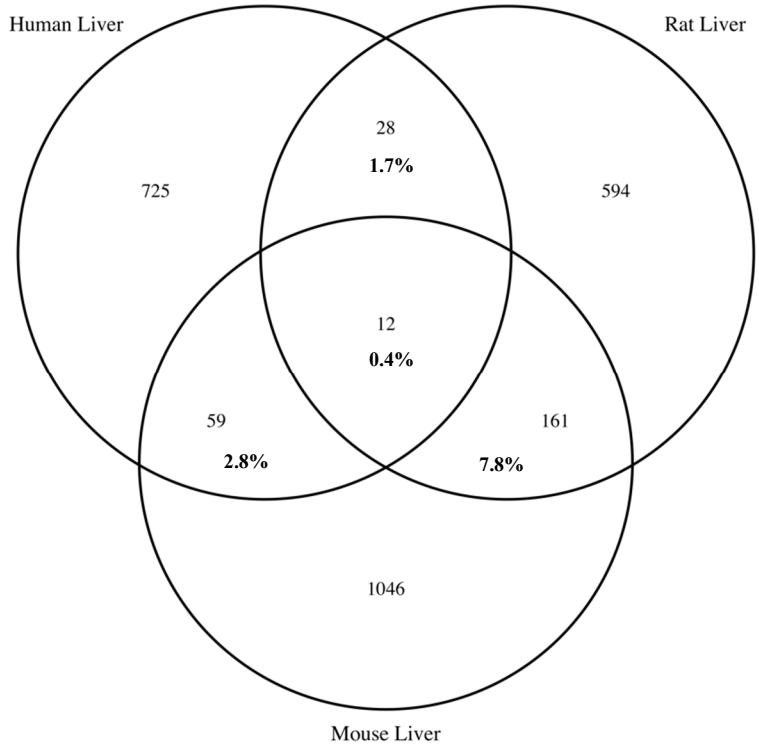


Figure 9. DEHP: Number of identified differentially expressed genes in microarrays. A) Down-regulated DEGs B) Up-regulated DEGs.

A.



B.

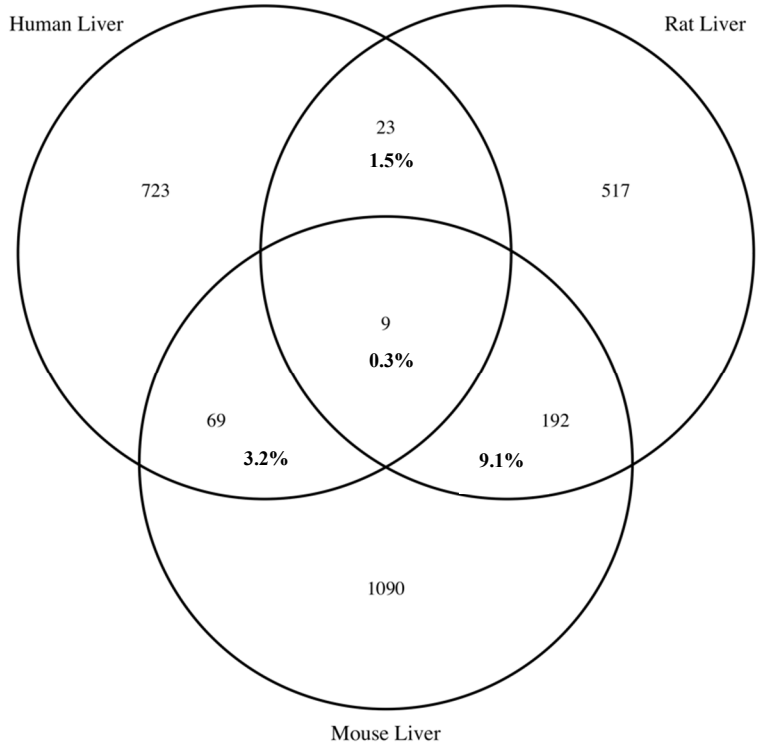
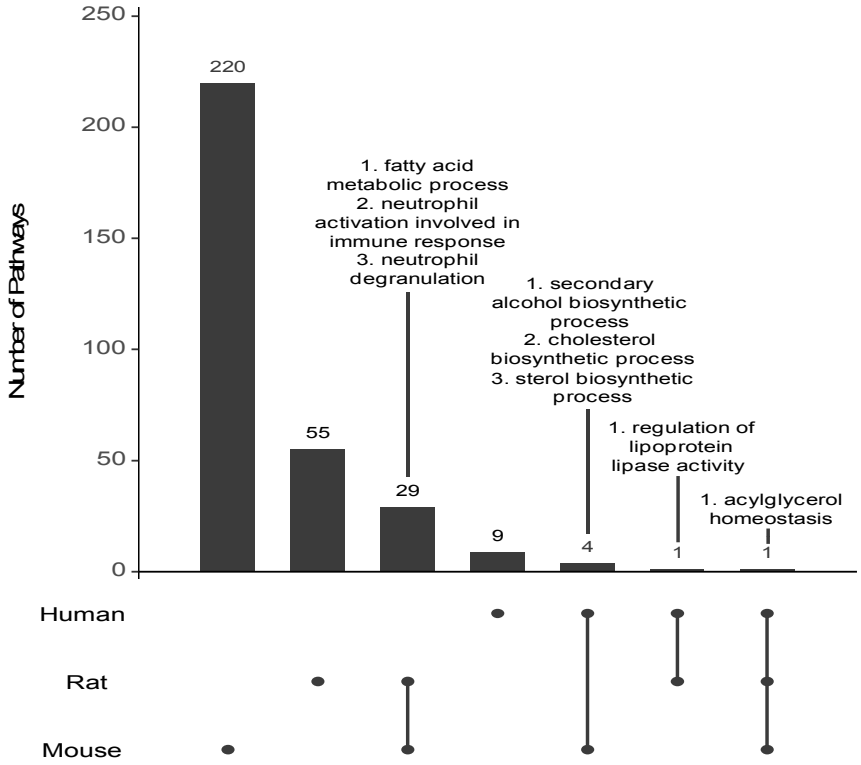


Figure 10. DEHP: Comparison of pathways for differentially expressed genes. A) Down-regulated pathways. B) Up-regulated pathways.

A.



B.

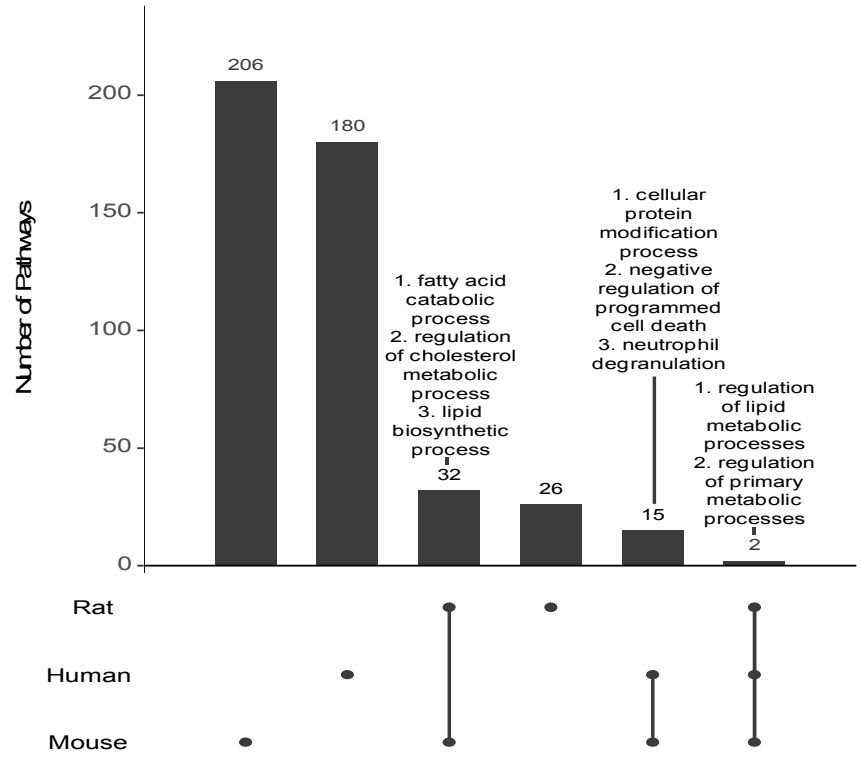


Figure 11. DEHP: Functional annotation of rat cardiovascular system studies A) Top 10 Down-regulated Pathways B) Top 10 Up-regulated Pathways.

A. B.

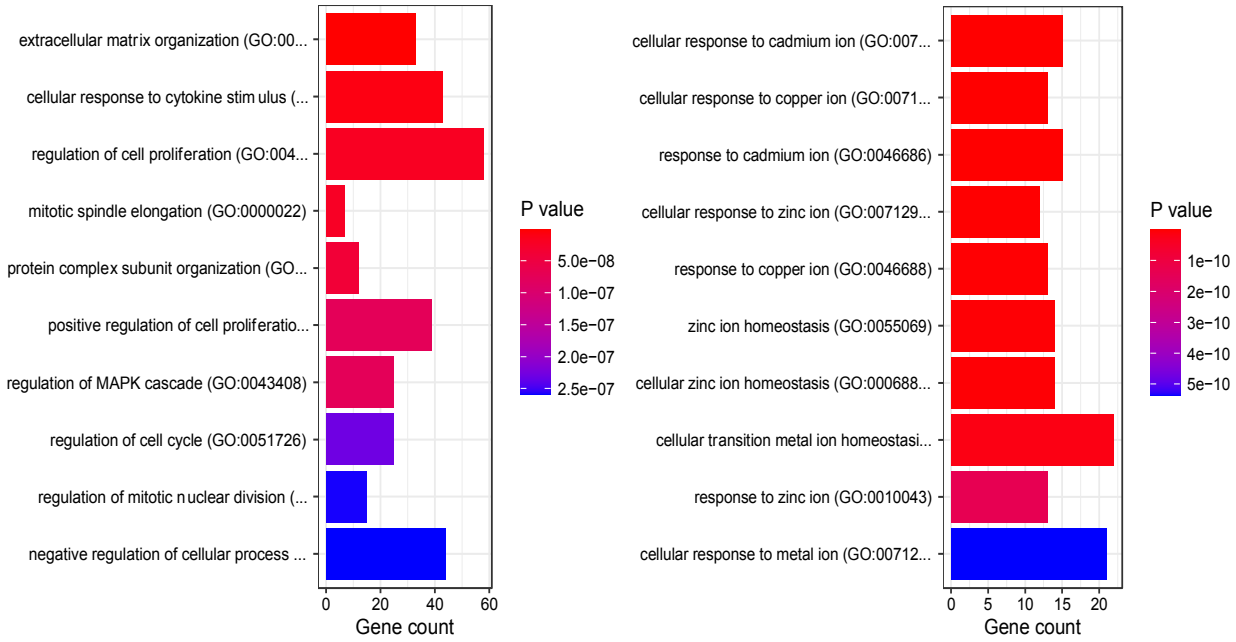


Figure 12. TBT: Number of identified differentially expressed genes A) Down-regulated DEGs B) Up-regulated DEGs.

A. B.

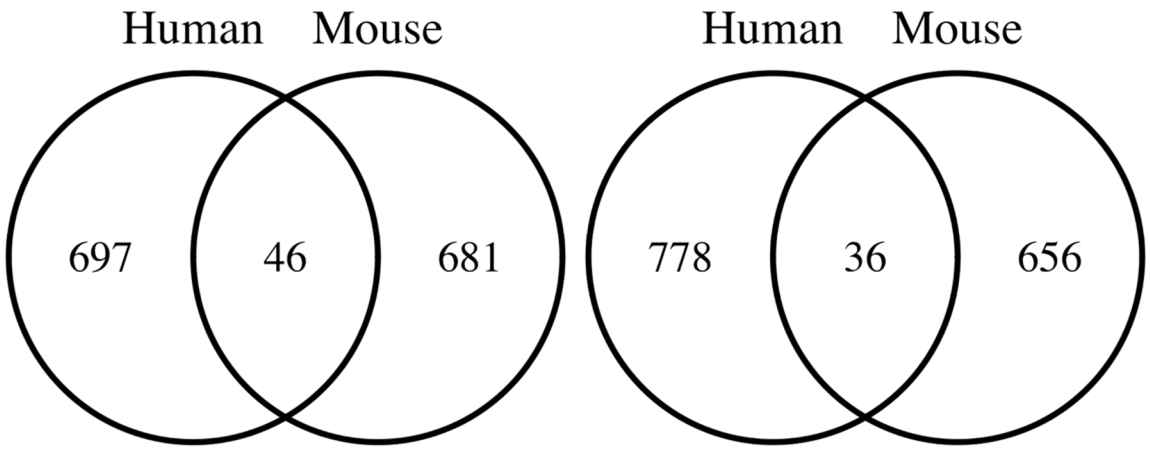
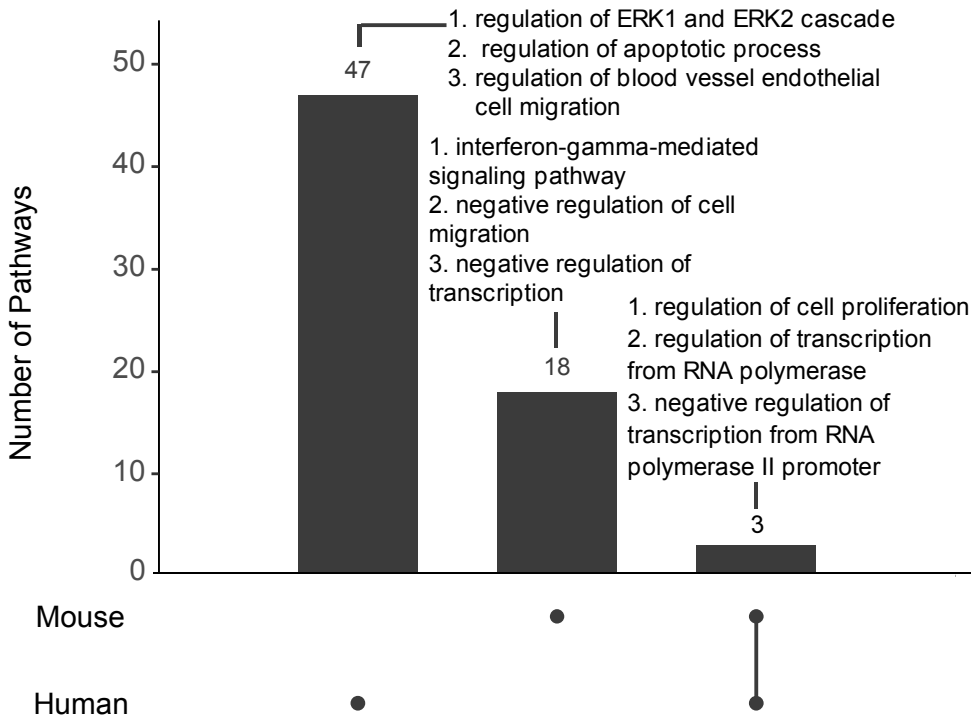


Figure 13. TBT: Comparison of pathways for differentially expressed genes. A)Down-regulated pathways. B) Up-regulated pathways.

A.



B.

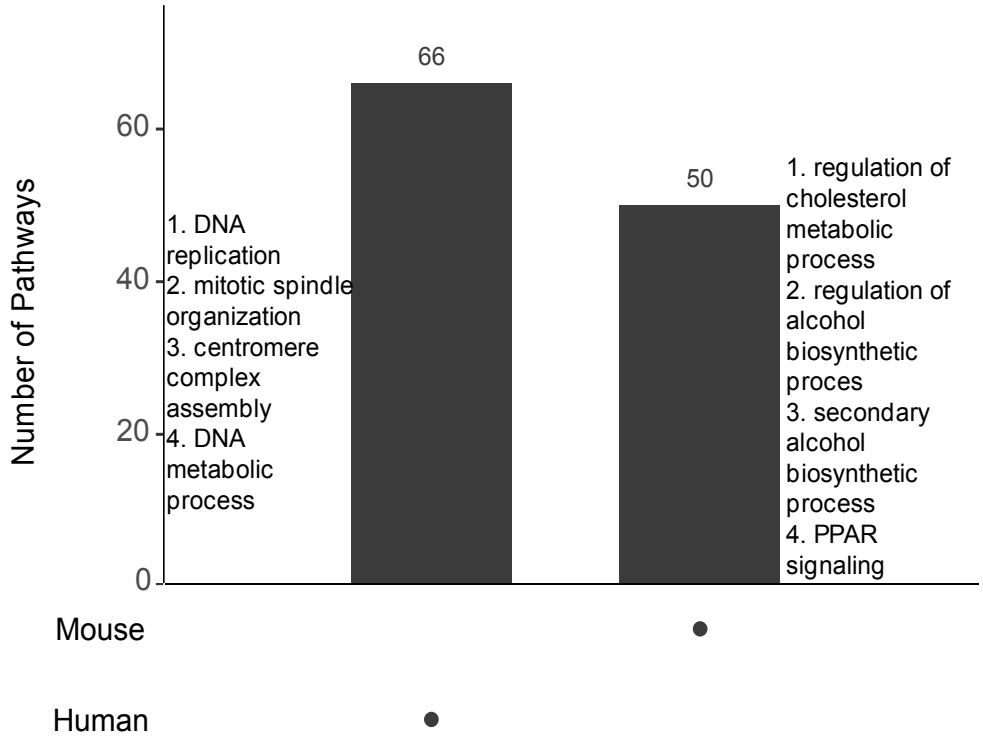
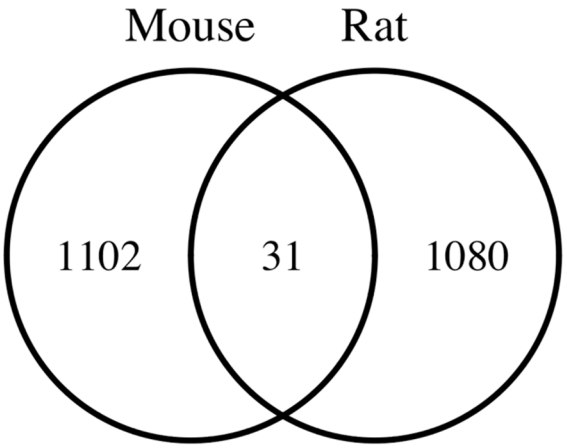


Figure 14. PFOA: Number of identified differentially expressed genes in liver. A) Down-regulated DEGs B) Up-regulated DEGs.

A.



B.

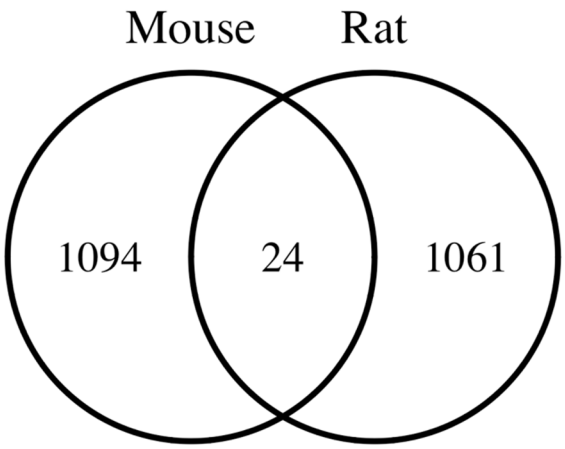
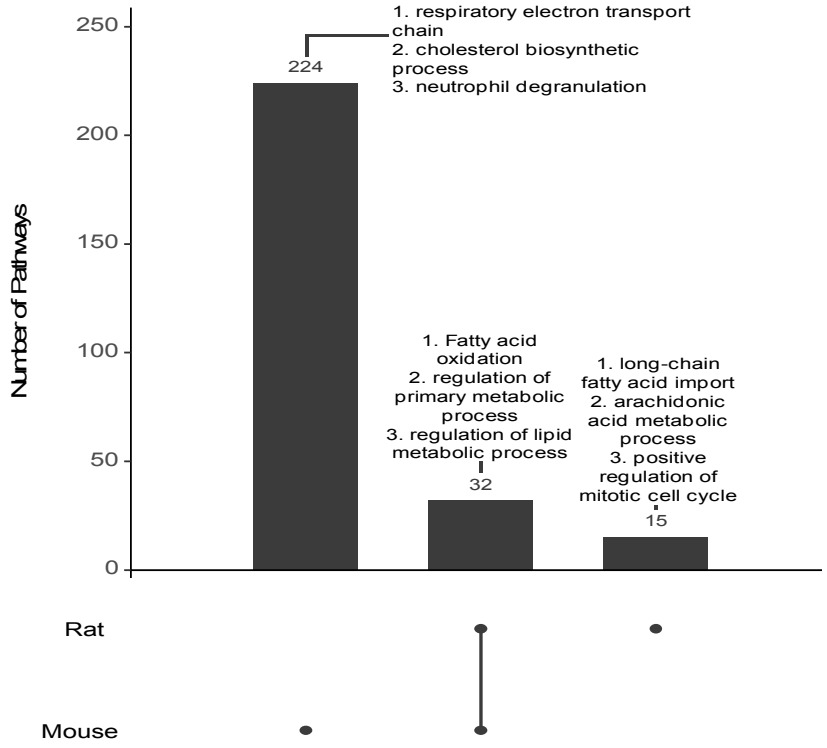


Figure 15. PFOA: Comparison of pathways for differentially expressed genes. A)Down-regulated pathways. B) Up-regulated pathways.

A.



B.

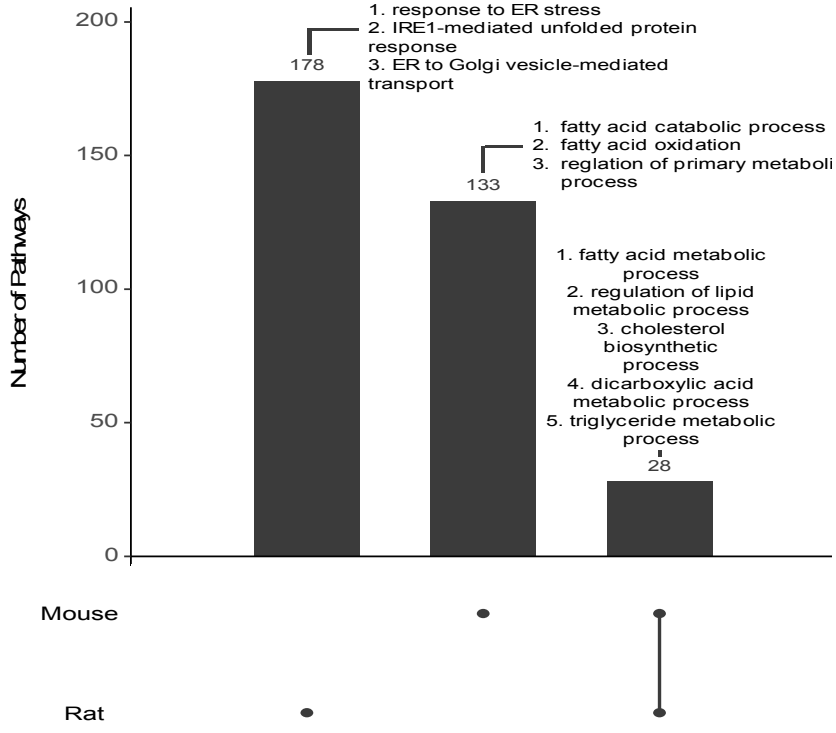


Figure 16. Top pathways summary. Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs.

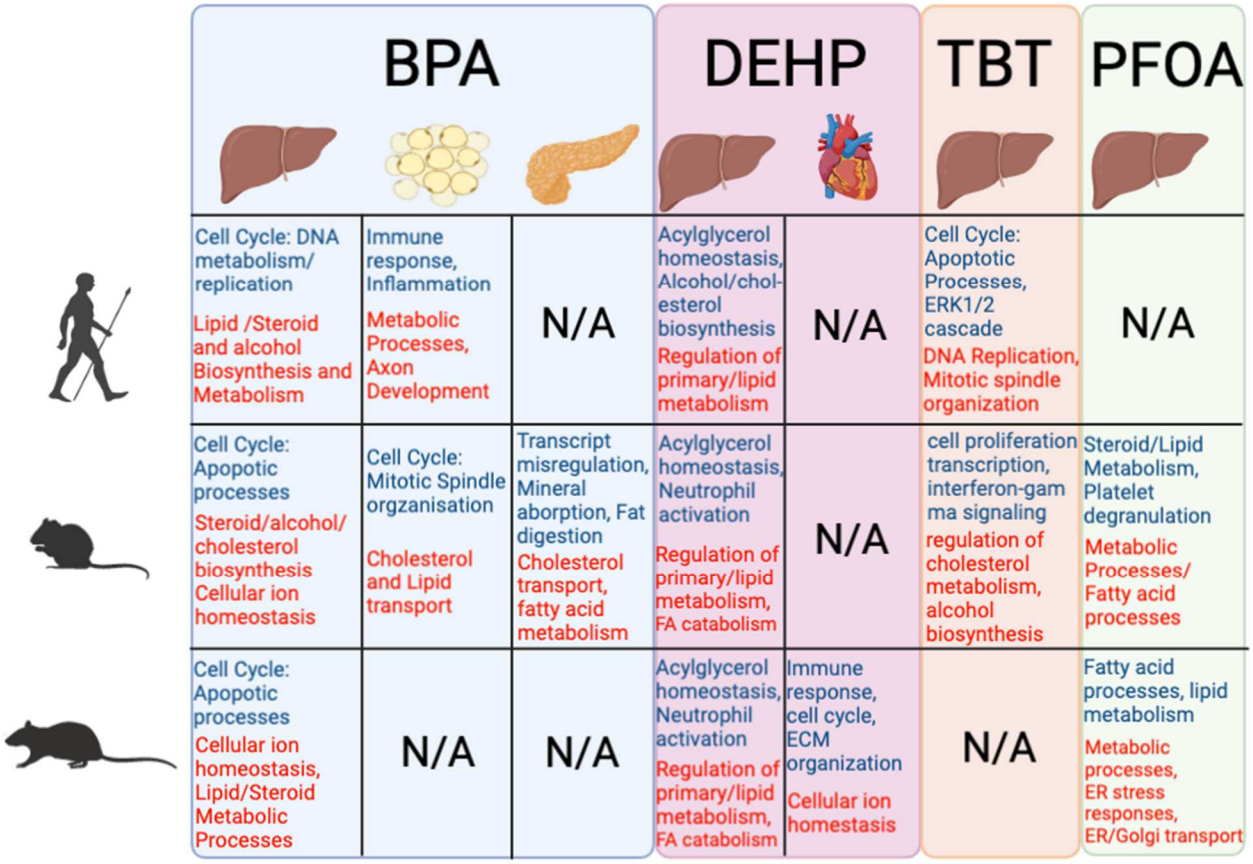


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Table 1. Datasets included in meta-analysis. Bold font identifies RNAseq datasets.

GSE #	Chemical	Species	Tissue	Exposure Route	Dosage	Citation
98680	BPA	Human	Adipose	Culture	10μM,10nM	Verbank et al., 2018
58516	BPA	Human	Adipose	Culture	10nM	Menale et al., 2015
69844	BPA	Human	Liver	Culture	1,10,100μM	De Abrew et al., 2016
69850	BPA	Human	Liver	Culture	1,10,100μM	De Abrew et al., 2016
59923	BPA	Rat	Liver	Oral Gavage	100,610mg	N/A
8858	BPA	Rat	Liver	Oral Gavage	100,610mg	Natsoulis et al., 2008
8251	BPA	Rat	Liver	Oral Gavage	610mg	Fielden et al., 2007
57815	BPA	Rat	Liver	Oral Gavage	100,610mg	Gusenleitner et al., 2014
130434	BPA	Rat	Liver	Chow	50μg	Treviño et al., 2020
19662	BPA	Rat	Liver	Culture	10ppm	Deng et al., 2010
121603	BPA	Mouse	Adipose	Oral Gavage	5mg	Shu et al., 2019
43977	BPA	Mouse	Liver	Chow	5,000 ppm	N/A
26728	BPA	Mouse	Liver	Chow	50,5000μg	Marmugi et al., 2012
121603	BPA	Mouse	Liver	Oral Gavage	5mg	Shu et al., 2019
44088	BPA	Mouse	Liver	Culture	10μM	Schaap et al., 2015
126297	BPA	Mouse	Pancreas	Subcutaneous Injection	100μg	Martinez-Pinna et al., 2019
28878	DEHP	Human	Liver	Culture	10mM	Magkoufopoulou et al., 2012
21641	DEHP	Rat	Cardiovascular System	Culture	1,10,50μg	Posnack et al., 2011
21640	DEHP	Rat	Cardiovascular System	Culture	50 μg	Posnack et al., 2011
2303	DEHP	Rat	Liver	Oral Gavage	20g	Jolly et al., 2005
8251	DEHP	Rat	Liver	Oral Gavage	1000mg	Fielden et al., 2007
57815	DEHP	Rat	Liver	Oral Gavage	1000mg	Gusenleitner et al., 2014
59923	DEHP	Rat	Liver	Oral Gavage	100,1000mg	N/A
40337	DEHP	Rat	Liver	Culture	250,1000μM	De Abrew et al., 2015
14629	DEHP	Mouse	Liver	Oral Gavage	30,180,1100mg	Eveillard et al., 2009
14920	DEHP	Mouse	Liver	Oral Gavage	20,200mg	Eveillard et al., 2009
43977	DEHP	Mouse	Liver	Chow	6,000ppm	N/A
121057	DEHP	Mouse	Liver	Oral Gavage	2500mg	N/A
64187	DEHP	Mouse	Liver	Chow	750,1500,3000,6000ppm	Lake et al., 2016
55733	DEHP	Mouse	Liver	Oral Gavage	1150mg	Currie et al., 2005
18564	DEHP	Mouse	Liver	Oral Gavage	1150mg	Ren et al., 2010
72081	DEHP	Mouse	Liver	Culture	2000μM	Rieswijk et al., 2016
53523	DEHP	Mouse	Liver	Chow	139, 845,3147mg	Wood et al., 2014
86259	TBT	Human	Liver	Culture	2,6,10μM	Tu et al., 2016
28878	TBT	Human	Liver	Culture	0.02 nM	Magkoufopoulou et al., 2012
43977	TBT	Mouse	Liver	Chow	200ppm	N/A
143304	TBT	Mouse	Liver	Water	0.5mg	Katz et al., 2020
44088	TBT	Mouse	Liver	Culture	0.3 μM	Schaap et al., 2015
47345	TBT	Mouse	Liver	Culture	250 nM	Schaap et al., 2015
147072	PFOA	Rat	Liver	Oral Gavage	.156,1.25,10mg	Gwinn et al., 2020
13044	PFOA	Mouse	Liver	Water	1,3,5,10mg	Rosen et al., 2007
9796	PFOA	Mouse	Liver	Oral Gavage	1,3mg	Rosen et al., 2008
9786	PFOA	Mouse	Liver	Oral Gavage	3mg	Rosen et al., 2008
119441	PFOA	Mouse	Liver	Oral Gavage	1mg	Li et al., 2019

Table 2. Microarray and RNAseq concordance. Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs

	Microarray	RNAseq	Overlap	%
BPA Mouse Liver	716	420	34	**2.91%**
BPA Rat Liver	973	188	27	**2.27%**
DEHP Mouse Liver	1258	1459	20	**0.73%**
TBT Mouse Liver	724	24	3	**0.40%**
PFOA Mouse Liver	1093	1439	40	**1.56%**
BPA Mouse Liver	723	468	24	**1.98%**
BPA Rat Liver	743	187	45	**4.62%**
DEHP Mouse Liver	1339	1077	21	**0.86%**
TBT Mouse Liver	691	11	1	**0.14%**
PFOA Mouse Liver	1091	1071	27	**1.23%**
TOTAL	9351	6344	242	**1.52%**

Table 3. BPA DEG disease association overlap (GWAS). Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs.

	BPA Liver Human	BPA Liver Mouse	BPA Liver Rat	BPA Adipose Human	BPA Adipose Mouse	BPA Pancreas Mouse
Type 2 Diabetes	CCND1, PROX1, TLE1 HMGCR, RBM6	BACH2, BCL6, HMGA2 C1QTNF6, CADM1 CCND2 PNPLA3, PPARA, TPT1	EML2, FCGR3A, PRC1 RHOBTB1 ANKH, ARL4A CDH2, CLEC14A, GCK HMGCR, KLF12, LPIN2 PALD1, PIM3, PPARA, ST18	BDNF, DNER, FAM167A PCSK1 FMO4, SGCG, ST6GAL1 ZNF713	ATP2A1, KIF11, PNPLA3 PRC1 APOE, TP53INP1	IRS2, JMJD1C, KLF5 MEG3, ROBO2 APOE, ARG1, CRYBA2 GP2, HP, SNF8
Metabolic Syndrome	RBM6	APOA4, CADM1, MLXIPL SNX10	ENDRA, ME1 GCK	BDNF	APOE	APOA4, JMJD1C, PTPRT APOE, CD68
Obesity	INHBB, PROX1	PNPLA3	CMKLR1, PRF1, RARB	BDNF, PRL INHBB	PNPLA3	ARG1
Coronary Heart Disease	RAB5C, SERPINH1 C6orf48, HMGCR TMEM106B	ABCG8, APOA4,ATXN2 CNPY4, COL4A1, NRP1 CYP17A1, RGS12	CLOCK, CST3, EDNRA SPC24, TBXAS1, TSPAN14 ATF3, GCK, HMGCR TAT	BDNF, CCDC68, CXCL8 TCF21 ARHGAP20, C8orf34 CASTOR1, FNDC1, ZNF32 GALNT13, SORBS2	APOC3, APOE, FKBP5 IBTK, WBP1L	APOA1, APOA4, ARL5B GEM, SLC5A3 APOE, FN1, HPR, PLA2G7 SNF8

Table 4. DEHP DEG disease association overlap (GWAS). Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs.

	DEHP Liver Human	DEHP Liver Mouse	DEHP Liver Rat	DEHP Cardiovascular System Rat
Type 2 Diabetes	C11orf74, DLEU1, FAM13A AKAP12, COBLL1, JAG1 PALLD, TRIB1	MEG3, SLC2A2, TTC39C ARL4A, CEBPB, HMGB1 PCK1, PIM3, TCF4 TP53INP1, TTC39C	ABCA1, ALDH1B1, ATP8A1 AVPR1A, BCL6, FGFR4 FMO1, NOTCH1, PTPRM ST6GAL1 CACNA2D3, SCD5 CPT1A, IMPA2, PNPLA3	ABCA1, ADARB1, JAG1 MYH10 ACSL1, ATP1B2, OASL SFRP2, TGFBR3
Metabolic Syndrome	BCDIN3D ASPH, COBLL1 F2RL1, TRIB1	APOA4 DYRK1A, PTPRT	ABCA1, APOA4 ANGPTL4, FADS2, ME1 SNX10	ABCA1 EDNRA
Obesity	PFKFB3 ITPR3	PFKFB3, TCF4	RARB PNPLA3	RAMP3
Coronary Heart Disease	APOC3 AKAP12, CXCL8, MAP1B NOS3, PALLD, RASD1 TRIB1	APOA4, HGFAC, NCOA6 SMAD2 SLC5A3	ABCA1, ABCG8, ABHD2 APOA4, C1S, CTSS, LOX PROCR ANGPTL4, CYP17A1 SLC22A4	ABCA1, AGT, CNNM2 EDN1, FNDC1, RAB23 EDNRA, PLA2G7, SEMA5A

Table 5. TBT DEG disease association overlap (GWAS). Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs.

	TBT Liver Human	TBT Liver Mouse
Type 2 Diabetes	CCND1, F3, ITGA2, LRI PALM2, SACS, SH2B3 SLC22A3, STAT3 CCDC77, TTC39C	ARID5B, BACH2, CCND2 UBE2E2
Metabolic Syndrome		ENG, SIPA1
Obesity	CCDC77, PFKFB3	RARB
Coronary Heart Disease	CDKN1A, RGS19, SEC2 SH2B3, SLC22A3, TSPAN14 KLF2, LOX, N4BP2L2 TTC32	BBS9, KLF4, SIPA1 RND2

Table 6. PFOA DEG disease association overlap (GWAS). Blue font identifies down-regulated DEGs, red font identifies up-regulated DEGs.

	PFOA Liver Mouse	PFOA Liver Rat
Type 2 Diabetes	AVPR1A	HUNK, MTAP, PCDH17 RNF6, SACS
	ARL4A, CEBPB, HMGB LPIN2, PIM3, TCF4	CAMK1D, HNF4A, SSR1 TLE1
Metabolic Syndrome	DYRK1A, ME1, PTPRT	ARNTL , HNF4A
Obesity	PFKFB3, TCF4	FARS2, NCAM2, PITPNB PTER
	C1S, CYP17A1	MTAP
Coronary Heart Disease	FKBP5, PLTP, RRBP1 SLC5A3	ARNTL, LDAH, MAD1L1 RRBP1

References

Aguiree F, Brown A, Cho NH, Dahlquist G, Dodd S, Dunning T, Hirst M, Hwang C, Magliano D, Patterson C. IDF Diabetes Atlas. 2013.

Anaconda Software Distribution. (2020). Anaconda Documentation. Anaconda Inc. Retrieved from https://docs.anaconda.com/

Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Antizar-Ladislao , B. "Environmental levels, toxicity and human exposure to tributyltin (TBT)-contaminated marine environment". *Environment International* vol. 34, 2. 2008. Pages 292-308. doi:10.1016/j.envint

Belzunce, Maria & Solaun, Oihana & González-Oreja, José & Millán, Esmeralda & Perez Landa, Víctor. (2004). Chapter 12 Contaminants in sediments. *Elsevier Oceanography Series*. 70. 283-315. 10.1016/S0422-9894(04)80050-3.

Benmohammed K, Nguyen M, Khensal S, Valensi P, Lezzar A. 2011. Arterial hypertension in overweight and obese Algerian adolescents: role of abdominal adiposity. *Diabetes Meta*b. 37:291–297.

Buniello A, MacArthur JAL, Cerezo M, Harris LW, Hayhurst J, Malangone C, McMahon A, Morales J, Mountjoy E, Sollis E, Suveges D, Vrousgou O, Whetzel PL, Amode R, Guillen JA, Riat HS, Trevanion SJ, Hall P, Junkins H, Flicek P, Burdett T, Hindorff LA, Cunningham F and Parkinson H.
The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019.
Nucleic Acids Research, 2019, Vol. 47 (Database issue): D1005-D1012.

Calafat AM, Ye X, Wong LY, Reidy JA, Needham LL. Exposure of the U.S. population to bisphenol A and 4-tertiary-octylphenol: 2003-2004. *Environ Health Perspect.* 2008;116(1):39-44. doi:10.1289/ehp.10753

Can, A., Semiz, O., & Cinar, O. (2005). Bisphenol-A induces cell cycle delay and alters centrosome and spindle microtubular organization in oocytes during meiosis. *Molecular Human Reproduction, 11*(6), 389-396. doi:10.1093/molehr/gah179.

Cannon CP. Cardiovascular disease and modifiable cardiometabolic risk factors. Clin Cornerstone. 2007;8(3):11-28. doi: 10.1016/s1098-3597(07)80025-1. PMID: 18452839.

Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*. 2013; 128(14).

Conway, J. R., Lex, A., & Gehlenborg, N. (2017). UpSetR: an R package for the visualization of intersecting sets and their properties. *Bioinformatics*, 33(18), 2938-2940. doi:10.1093/bioinformatics/btx364

Cuenca, Luciann et al. "Environmentally-relevant exposure to diethylhexyl phthalate (DEHP) alters regulation of double-strand break formation and crossover designation leading to germline dysfunction in Caenorhabditis elegans." *PLoS genetics* vol. 16,1 e1008529. 9 Jan. 2020, doi:10.1371/journal.pgen.1008529

Currie RA, Bombail V, Oliver JD, Moore DJ et al. Gene ontology mapping as an unbiased method for identifying molecular pathways and processes affected by toxicant exposure: application to acute effects caused by the rodent non-genotoxic carcinogen diethylhexylphthalate. *Toxicol Sci* 2005 Aug;86(2):453-69. PMID: 15901911

Davis S, Meltzer P (2007). "GEOquery: a bridge between the Gene Expression Omnibus (GEO) and BioConductor." *Bioinformatics*, 14, 1846–1847.

De Abrew KN, Kainkaryam RM, Shan YK, Overmann GJ et al. Grouping 34 Chemicals Based on Mode of Action Using Connectivity Mapping. *Toxicol Sci* 2016 Jun;151(2):447-61. PMID: 27026708

De Abrew KN, Overmann GJ, Adams RL, Tiesman JP et al. A novel transcriptomics based in vitro method to compare and predict hepatotoxicity based on mode of action. *Toxicology* 2015 Feb 3;328:29-39. PMID: 25475144

Deng Y, Johnson DR, Guan X, Ang CY et al. In vitro gene regulatory networks predict in vivo function of liver. *BMC Syst Biol* 2010 Nov 12;4:153. PMID: 21073692

Diamante G, Cely I, Zamora Z, Ding J, Blencowe M, Lang J, Bline A, Singh M, Lusis AJ, Yang X. Systems Toxicogenomics of Prenatal Low-dose BPA Exposure on Liver Metabolic Pathways, Gut Microbiota, and Metabolic Health in Mice. *Environment International*, advanced online, 2020.

Durinck S, Spellman P, Birney E, Huber W (2009). "Mapping identifiers for the integration of genomic datasets with the R/Bioconductor package biomaRt." *Nature Protocols*, 4, 1184–1191.

Eveillard A, Lasserre F, de Tayrac M, Polizzi A et al. Identification of potential mechanisms of toxicity after di-(2-ethylhexyl)-phthalate (DEHP) adult exposure in the liver using a systems biology approach. *Toxicol Appl Pharmacol* 2009 May 1;236(3):282-92. PMID: 19245819

Eveillard A, Mselli-Lakhal L, Mogha A, Lasserre F et al. Di-(2-ethylhexyl)-phthalate (DEHP) activates the constitutive androstane receptor (CAR): a novel signalling pathway sensitive to phthalates. *Biochem Pharmacol* 2009 Jun 1;77(11):1735-46. PMID: 19428328

Fei C, McLaughlin JK, Tarone RE, Olsen J. Perfluorinated chemicals and fetal growth: a study within the Danish National Birth Cohort. *Environ Health Perspect*. 2007 Nov;115(11):1677-82. doi: 10.1289/ehp.10506. PMID: 18008003; PMCID: PMC2072850.

Fielden MR, Brennan R, Gollub J. A gene expression biomarker provides early prediction and mechanistic assessment of hepatic tumor induction by nongenotoxic chemicals. *Toxicol Sci* 2007 Sep;99(1):90-100. PMID: 17557906

Gao H, Yang BJ, Li N, et al. Bisphenol A and hormone-associated cancers: current progress and perspectives. Medicine (Baltimore). 2015;94(1):e211. doi:10.1097/MD.0000000000000211

García-Arevalo M, Alonso-Magdalena P, Rebelo Dos Santos J, Quesada I, Carneiro EM, Nadal A (2014) Exposure to Bisphenol-A during Pregnancy Partially Mimics the Effects of a High-Fat Diet Altering Glucose Homeostasis and Gene Expression in Adult Male Mice. *PLoS ONE* 9(6): e100214. https://doi.org/10.1371/journal.pone.0100214

GEO Overview [Internet]. National Library of Medicine. 2016. Available from: www.ncbi.nlm.nih.gov/geo/info/overview.html.

Golestanzadeh, M., Riahi, R. & Kelishadi, R. Association of exposure to phthalates with cardiometabolic risk factors in children and adolescents: a systematic review and meta-analysis. *Environ Sci Pollut Res* 26, 35670–35686 (2019). https://doi.org/10.1007/s11356-019-06589-7.

González-Muniesa, P., Mártinez-González, MA., Hu, F. et al. Obesity. *Nat Rev Dis Primers* 3, 17034 (2017). https://doi.org/10.1038/nrdp.2017.34

Gremse M, Chang A, Schomburg I, Grote A, Scheer M, Ebeling C, Schomburg D. The BRENDA Tissue Ontology (BTO): the first all-integrating ontology of all organisms for enzymesources. *Nucleic Acids Res*. 2011;39(Database issue):D507-13. doi: 10.1093/nar/gkq968. PubMed PMID: 21030441; PMCID: PMC3013802.

Guo, L., Lobenhofer, E., Wang, C. et al. Rat toxicogenomic study reveals analytical consistency across microarray platforms. *Nat Biotechnol* 24, 1162–1169 (2006). https://doi.org/10.1038/nbt1238

Gusenleitner D, Auerbach SS, Melia T, Gómez HF et al. Genomic models of short-term exposure accurately predict long-term chemical carcinogenicity and identify putative mechanisms of action. *PLoS One* 2014;9(7):e102579. PMID: 25058030

Gwinn WM, Auerbach SS, Parham F, Stout MD et al. Evaluation of 5-day In Vivo Rat Liver and Kidney With High-throughput Transcriptomics for Estimating Benchmark Doses of Apical Outcomes. *Toxicol Sci* 2020 Aug 1;176(2):343-354. PMID: 32492150

Halldorsson, Thorhallur I et al. “Prenatal exposure to perfluorooctanoate and risk of overweight at 20 years of age: a prospective cohort study.” *Environmental health perspectives* vol. 120,5 (2012): 668-73. doi:10.1289/ehp.1104034

Heindel, J. J., & Blumberg, B. (2019). Environmental Obesogens: Mechanisms and Controversies. *Annual review of pharmacology and toxicology*, *59*, 89–106. https://doi.org/10.1146/annurev-pharmtox-010818-021304

Hines EP, White SS, Stanko JP, Gibbs-Flournoy EA, Lau C, Fenton SE. Phenotypic dichotomy following developmental exposure to perfluorooctanoic acid (PFOA) in female CD-1 mice: Low doses induce elevated serum leptin and insulin, and overweight in mid-life. *Mol Cell Endocrinol*. 2009 May 25;304(1-2):97-105. doi: 10.1016/j.mce.2009.02.021. Epub 2009 Mar 9. PMID: 19433254.

Huang Y, Wu C, Ye Y, Zeng J, Zhu J, Li Y, Wang W, Zhang W, Chen Y, Xie H, Zhang H, Liu J. The Increase of ROS Caused by the Interference of DEHP with JNK/p38/p53 Pathway as the Reason for Hepatotoxicity. *International Journal of Environmental*

Hurt, Ryan T et al. “The obesity epidemic: challenges, health initiatives, and implications for gastroenterologists.” *Gastroenterology & hepatology* vol. 6,12 (2010): 780-92.

Jaimes, R., 3rd, Swiercz, A., Sherman, M., Muselimyan, N., Marvar, P. J., & Posnack, N. G. (2017). Plastics and cardiovascular health: phthalates may disrupt heart rate variability and cardiovascular reactivity. *American journal of physiology. Heart and circulatory physiology*, 313(5), H1044–H1053. https://doi.org/10.1152/ajpheart.00364.2017

Jarfelt K, Dalgaard M, Hass U, et al. Antiandrogenic effects in male rats perinatally exposed to a mixture of di(2-ethylhexyl) phthalate and di(2-ethylhexyl) adipate. Reproductive Toxicology (Elmsford, N.Y.). 2005 Mar-Apr;19(4):505-515. DOI: 10.1016/j.reprotox.2004.11.005.

Jia, Yiyang et al. “Effects of Di-(2-ethylhexyl) Phthalate on Lipid Metabolism by the JAK/STAT Pathway in Rats.” *International journal of environmental research and public health* vol. 13,11 1085. 4 Nov. 2016, doi:10.3390/ijerph13111085

Jolly RA, Goldstein KM, Wei T, Gao H et al. Pooling samples within microarray studies: a comparative analysis of rat liver transcription response to prototypical toxicants. *Physiol Genomics* 2005 Aug 11;22(3):346-55. PMID: 15914576

Kanehisa, M; Toward understanding the origin and evolution of cellular organisms. *Protein Sci*. 28, 1947-1951 (2019) [pubmed] [doi]

Kanehisa, M. and Goto, S.; KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res*. 28, 27-30 (2000). [pubmed] [doi]

Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., and Tanabe, M.; KEGG: integrating viruses and cellular organisms. *Nucleic Acids Res*. 49, D545-D551 (2021). [pubmed] [doi]

Katz TA, Grimm SL, Kaushal A, Dong J et al. Hepatic Tumor Formation in Adult Mice Developmentally Exposed to Organotin. *Environ Health Perspect* 2020 Jan;128(1):17010. PMID: 31939706

Kevin L Howe, Premanand Achuthan, James Allen, Jamie Allen, Jorge Alvarez-Jarreta, M Ridwan Amode, Irina M Armean, Andrey G Azov, Ruth Bennett, Jyothish Bhai, Konstantinos Billis, Sanjay Boddu, Mehrnaz Charkhchi, Carla Cummins, Luca Da Rin Fioretto, Claire Davidson, Kamalkumar Dodiya, Bilal El Houdaigui, Reham Fatima, Astrid Gall, Carlos Garcia Giron, Tiago Grego, Cristina Guijarro-Clarke, Leanne Haggerty, Anmol Hemrom, Thibaut Hourlier, Osagie G Izuogu, Thomas Juettemann, Vinay Kaikala, Mike Kay, Ilias Lavidas, Tuan Le, Diana Lemos, Jose Gonzalez Martinez, José Carlos Marugán, Thomas Maurel, Aoife C McMahon, Shamika Mohanan, Benjamin Moore, Matthieu Muffato, Denye N Oheh, Dimitrios Paraschas, Anne Parker, Andrew Parton, Irina Prosovetskaia, Manoj P Sakthivel, Ahamed I Abdul Salam, Bianca M Schmitt, Helen Schuilenburg, Dan Sheppard, Emily Steed, Michal Szpak, Marek Szuba, Kieron Taylor, Anja Thormann, Glen Threadgold, Brandon Walts, Andrea Winterbottom, Marc Chakiachvili, Ameya Chaubal, Nishadi De Silva, Bethany Flint, Adam Frankish, Sarah E Hunt, Garth R IIsley, Nick Langridge, Jane E Loveland, Fergal J Martin, Jonathan M Mudge, Joanella Morales, Emily Perry, Magali Ruffier, John Tate, David Thybert, Stephen J Trevanion, Fiona Cunningham, Andrew D Yates, Daniel R Zerbino, Paul Flicek. Ensembl 2021. *Nucleic Acids Res*. 2021, vol. 49(1):884–89. PubMed PMID: 33137190.doi:10.1093/nar/gkaa942

Kolde R, Laur S, Adler P, Vilo J. Robust rank aggregation for gene list integration and meta-analysis. *Bioinformatics*. (2012) 28:573–80. doi: 10.1093/bioinformatics/btr709

Krueger F. Trim Galore!. GitHub. 2020. Available: https://github.com/FelixKrueger/TrimGalore.

Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW, Ma'ayan A. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Research*. 2016; gkw377 .

Lake AD, Wood CE, Bhat VS, Chorley BN et al. Dose and Effect Thresholds for Early Key Events in a PPARα-Mediated Mode of Action. *Toxicol Sci* 2016 Feb;149(2):312-25. PMID: 26519955

Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, Strynar MJ. Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci. 2006 Apr;90(2):510-8. doi: 10.1093/toxsci/kfj105. *Epub* 2006 Jan 16. PMID: 16415327.

le Maire, A., Grimaldi, M., Roecklin, D., Dagnino, S., Vivat-Hannah, V., Balaguer, P., & Bourguet, W. (2009). Activation of RXR-PPAR heterodimers by organotin environmental endocrine disruptors. *EMBO reports*, 10(4), 367–373. https://doi.org/10.1038/embor.2009.8

Leinonen, R., Sugawara, H., Shumway, M., & International Nucleotide Sequence Database Collaboration (2011). The sequence read archive. *Nucleic acids research*, 39(Database issue), D19–D21. https://doi.org/10.1093/nar/gkq1019

Li L, Wang Q, Zhang Y, Niu Y, Yao X, Liu H (2015) The Molecular Mechanism of Bisphenol A (BPA) as an Endocrine Disruptor by Interacting with Nuclear Receptors: Insights from Molecular Dynamics (MD) Simulations. *PLoS ONE* 10(3): e0120330. https://doi.org/10.1371/journal.pone.0120330

Li X, Wang Z, Klaunig JE. The effects of perfluorooctanoate on high fat diet induced non-alcoholic fatty liver disease in mice. *Toxicology* 2019 Mar 15;416:1-14. PMID: 30711707

Love MI, Huber W, Anders S (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." *Genome Biology*, 15, 550. doi: 10.1186/s13059-014-0550-8.

Magkoufopoulou C, Claessen SM, Tsamou M, Jennen DG et al. A transcriptomics-based in vitro assay for predicting chemical genotoxicity in vivo. *Carcinogenesis* 2012 Jul;33(7):1421-9. PMID: 22623647

Marcel Martin. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17(1):10-12, May 2011. DOI: http://dx.doi.org/10.14806/ej.17.1.200

Markey CM, Coombs MA, Sonnenschein C, Soto AM. 2003. Mammalian development in a changing environment: exposure to endocrine disruptors reveals the developmental plasticity of steroid□hormone target organs. *Evol Dev*. 5:67–75.

Marmugi A, Ducheix S, Lasserre F, Polizzi A et al. Low doses of bisphenol A induce gene expression related to lipid synthesis and trigger triglyceride accumulation in adult mouse liver. *Hepatology* 2012 Feb;55(2):395-407. PMID: 21932408

Martinez-Pinna J, Marroqui L, Hmadcha A, Lopez-Beas J et al. Oestrogen receptor β mediates the actions of bisphenol-A on ion channel expression in mouse pancreatic beta cells. *Diabetologia* 2019 Sep;62(9):1667-1680. PMID: 31250031

Menale C, Piccolo MT, Cirillo G, Calogero RA et al. Bisphenol A effects on gene expression in adipocytes from children: association with metabolic disorders. *J Mol Endocrinol* 2015 Jun;54(3):289-303. PMID: 25878060.

Merianos AL, Jacobs W, Oloruntoba O, Gittens OE, Smith ML. Perceived Severity of Interrelated Cardiometabolic Risk Factors among U.S. College Students. *Am J Health Educ.* 2020;51(4):234-243. doi:10.1080/19325037.2020.1765907

Mohammad Mehdi Amin, Karim Ebrahim, Majid Hashemi, Bahareh Shoshtari-Yeganeh, Nasim Rafiei, Marjan Mansourian & Roya Kelishadi (2019) Association of exposure to Bisphenol A with obesity and cardiometabolic risk factors in children and adolescents*, International Journal of Environmental Health Research*, 29:1, 94-106, DOI: 10.1080/09603123.2018.1515896

Mohammad Mehdi Amin, Karim Ebrahimpour, Saeed Parastar, Bahareh Shoshtari-Yeganeh, Majid Hashemi, Marjan Mansourian, Parinaz Poursafa, Zahra Fallah, Nasim Rafiei, Roya Kelishadi, Association of urinary concentrations of phthalate metabolites with cardiometabolic risk factors and obesity in children and adolescents, *Chemosphere*,Volume 211,2018,Pages 547-556.

Nagel, Susan C, and John J Bromfield. "Bisphenol a: a model endocrine disrupting chemical with a new potential mechanism of action." *Endocrinology* vol. 154,6 (2013): 1962-4. doi:10.1210/en.2013-1370

Natsoulis G, Pearson CI, Gollub J, P Eynon B et al. The liver pharmacological and xenobiotic gene response repertoire. *Mol Syst Biol* 2008;4:175. PMID: 18364709

Patro, R., Duggal, G., Love, M. I., Irizarry, R. A., & Kingsford, C. (2017). Salmon provides fast and bias-aware quantification of transcript expression. *Nature Methods*.

Plourde G. 2002. Impact of obesity on glucose and lipid profiles in adolescents at different age groups in relation to adulthood. *BMC* Fam Pract. 3:18.

Popkin, Barry M et al. "Global nutrition transition and the pandemic of obesity in developing countries." *Nutrition reviews* vol. 70,1 (2012): 3-21. doi:10.1111/j.1753-4887.2011.00456.x

Posnack NG, Lee NH, Brown R, Sarvazyan N. Gene expression profiling of DEHP-treated cardiomyocytes reveals potential causes of phthalate arrhythmogenicity. I 2011 Jan 11;279(1-3):54-64. PMID: 20920545

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ren H, Aleksunes LM, Wood C, Vallanat B et al. Characterization of peroxisome proliferator-activated receptor alpha--independent effects of PPARalpha activators in the rodent liver: di-(2-ethylhexyl) phthalate also activates the constitutive-activated receptor. *Toxicol Sci* 2010 Jan;113(1):45-59. PMID: 19850644. Research and Public Health. 2019; 16(3):356. https://doi.org/10.3390/ijerph16030356

Rieswijk L, Brauers KJ, Coonen ML, Jennen DG et al. Exploiting microRNA and mRNA profiles generated in vitro from carcinogen-exposed primary mouse hepatocytes for predicting in vivo genotoxicity and carcinogenicity. *Mutagenesis* 2016 Sep;31(5):603-15. PMID: 27338304

Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015). "limma powers differential expression analyses for RNA-sequencing and microarray studies." *Nucleic Acids Research*, 43(7), e47. doi: 10.1093/nar/gkv007.

Ronconi, K. d. S., Stefanon, I., & Ribeiro Junior, R. F. (2018). Tributyltin and Vascular Dysfunction: The Role of Oxidative Stress. *Frontiers in Endocrinology*, 9(354). doi:10.3389/fendo.2018.00354

Rosen MB, Abbott BD, Wolf DC, Corton JC et al. Gene profiling in the livers of wild-type and PPARalpha-null mice exposed to perfluorooctanoic acid. *Toxicol Pathol* 2008 Jun;36(4):592-607. PMID: 18467677

Rosen MB, Lee JS, Ren H, Vallanat B et al. Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. *Toxicol Sci* 2008 May;103(1):46-56. PMID: 18281256

Rosen MB, Thibodeaux JR, Wood CR, Zehr RD et al. Gene expression profiling in the lung and liver of PFOA-exposed mouse fetuses. *Toxicology* 2007 Sep 24;239(1-2):15-33. PMID: 17681415

Schaap MM, Wackers PF, Zwart EP, Huijskens I et al. A novel toxicogenomics-based approach to categorize (non-)genotoxic carcinogens. *Arch Toxicol* 2015 Dec;89(12):2413-27. PMID: 25270620

Schecter A, Malik N, Haffner D, Smith S, Harris TR, Paepke O, Birnbaum L. Bisphenol A (BPA) in U.S. food. *Environ Sci Technol*. 2010 Dec 15;44(24):9425-30. doi: 10.1021/es102785d. Epub 2010 Nov 1. PMID: 21038926.

Schug, Thaddeus T et al. "Endocrine disrupting chemicals and disease susceptibility." *The Journal of steroid biochemistry and molecular biology* vol. 127,3-5 (2011): 204-15. doi:10.1016/j.jsbmb.2011.08.007

Schurch, N. J., Schofield, P., Gierliński, M., Cole, C., Sherstnev, A., Singh, V., . . . Barton, G. J. (2015). Evaluation of tools for differential gene expression analysis by RNA-seq on a 48 biological replicate experiment. arXiv:1505.02017. Retrieved from https://ui.adsabs.harvard.edu/abs/2015arXiv150502017S

Shea, K. Pediatric Exposure and Potential Toxicity of Phthalate Plasticizers. Committee on Environmental Health Pediatrics. Jun 2003, 111 (6) 1467-1474; DOI: 10.1542/peds.111.6.1467

Shu L, Meng Q, Diamante G, Tsai B et al. Prenatal Bisphenol A Exposure in Mice Induces Multitissue Multiomics Disruptions Linking to Cardiometabolic Disorders. *Endocrinology* 2019 Feb 1;160(2):409-429. PMID: 30566610

Shu, Qingying Meng, Graciel Diamante, Brandon Tsai, Yen-Wei Chen, Andrew Mikhail, Helen Luk, Beate Ritz, Patrick Allard, Xia Yang, Prenatal Bisphenol A Exposure in Mice Induces Multitissue Multiomics Disruptions Linking to Cardiometabolic Disorders, *Endocrinology*, Volume 160, Issue 2, February 2019, Pages 409-429, https://doi.org/10.1210/en.2018-00817

Somm E, Schwitzgebel VM, Toulotte A, Cederroth CR, Combescure C, Nef S, Aubert ML, Hüppi PS. 2009. Perinatal exposure to bisphenol A alters early adipogenesis in the rat. *Environ Health Perspect.* 117:1549.

Soneson C, Love MI, Robinson MD (2015). "Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences." F1000Research, 4. doi: 10.12688/f1000research.7563.1.

Steenland, Kyle et al. "Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA)." *Environmental health perspectives* vol. 118,8 (2010): 1100-8. doi:10.1289/ehp.0901827

Stelzer G, Rosen R, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Iny Stein T, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan - Golan Y, Kohn A, Rappaport N, Safran M, and Lancet D. *The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analysis* , Current Protocols in Bioinformatics(2016), 54:1.30.1 - 1.30.33.doi: 10.1002 / cpbi.5.

Stewart, J., Manmathan, G., & Wilkinson, P. (2017). Primary prevention of cardiovascular disease: A review of contemporary guidance and literature. *JRSM cardiovascular disease*, 6, 2048004016687211. https://doi.org/10.1177/2048004016687211

Street, Maria Elisabeth et al. "Current Knowledge on Endocrine Disrupting Chemicals (EDCs) from Animal Biology to Humans, from Pregnancy to Adulthood: *International journal of molecular sciences* vol. 19,6 1647. 2 Jun. 2018, doi:10.3390/ijms19061647

T. H. Begley, K. White, P. Honigfort, M. L. Twaroski, R. Neches & R. A. Walker (2005) Perfluorochemicals: Potential sources of and migration from food packaging, Food Additives & Contaminants, 22:10, 1023-1031, DOI: 10.1080/02652030500183474

Tremmel M, Gerdtham U-G, Nilsson PM, Saha S. Economic Burden of Obesity: A Systematic Literature Review. *International Journal of Environmental Research and Public Health*. 2017; 14(4):435. https://doi.org/10.3390/ijerph14040435

Treviño LS, Dong J, Kaushal A, Katz TA et al. Epigenome environment interactions accelerate epigenomic aging and unlock metabolically restricted epigenetic reprogramming in adulthood. *Nat Commun* 2020 May 8;11(1):2316. PMID: 32385268

Tu WW, Ji LD, Qian HX, Zhou M et al. Tributyltin induces disruption of microfilament in HL7702 cells via MAPK-mediated hyperphosphorylation of VASP. *Environ Toxicol* 2016 Nov;31(11):1530-1538. PMID: 26018654

Valieris R. parallel-fastq-dump. *GitHub*. 2020. Available: https://github.com/rvalieris/parallel-fastq-dump.

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs, D. R., Jr, Lee, D. H., Shioda, T., Soto, A. M., vom Saal, F. S., Welshons, W. V., Zoeller, R. T., & Myers, J. P. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine reviews*, 33(3), 378–455. https://doi.org/10.1210/er.2011-1050

Verbanck M, Canouil M, Leloire A, Dhennin V et al. Low-dose exposure to bisphenols A, F and S of human primary adipocyte impacts coding and non-coding RNA profiles. *PLoS One* 2017;12(6):e0179583. PMID: 28628672

Wang T, Pehrsson EC, Purushotham D, Li D et al. The NIEHS TaRGET II Consortium and environmental epigenomics. *Nat Biotechnol* 2018 Mar 6;36(3):225-227. PMID: 29509741.

Wang, C., Gong, B., Bushel, P. et al. The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance. *Nat Biotechnol* 32, 926–932 (2014). https://doi.org/10.1038/nbt.3001

Wood CE, Jokinen MP, Johnson CL, Olson GR et al. Comparative time course profiles of phthalate stereoisomers in mice. *Toxicol Sci* 2014 May;139(1):21-34. PMID: 24496636

World Health Organization. WHO Mortality Database. Published online 2020. http://www.who.int/healthinfo/statistics/mortality_rawdata/en/

Xie Z, Bailey A, Kuleshov MV, Clarke DJB., Evangelista JE, Jenkins SL, Lachmann A, Wojciechowicz ML, Kropiwnicki E, Jagodnik KM, Jeon M, & Ma'ayan A. Gene set knowledge discovery with Enrichr. Current Protocols, 1, e90. 2021. doi: 10.1002/cpz1.90

Ximenes, C.F., Rodrigues, S.M.L., Podratz, P.L. et al. Tributyltin chloride disrupts aortic vascular reactivity and increases reactive oxygen species production in female rats. *Environ Sci Pollut Res* 24, 24509–24520 (2017). https://doi.org/10.1007/s11356-017-0061-8

Xu G, Liu B, Sun Y, Du Y, Snetselaar LG, Hu FB, Bao W. Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ*. 2018 Sep 4;362:k1497. doi: 10.1136/bmj.k1497. PMID: 30181166; PMCID: PMC6122253.

Yamamoto, J., Yamane, T., Oishi, Y., & Kobayashi-Hattori, K. (2015). Perfluorooctanoic acid binds to peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation in 3T3-L1 adipocytes. Bioscience, Biotechnology, and Biochemistry, 79(4), 636-639. doi:10.1080/09168451.2014.991683

Yusuf S, Hawken S, Ounpuu S, et al. INTERHEART Study Investigators Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): Case-control study. *Lancet*. 2004;364:937–52.