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Phthalates: Toxicogenomics and inferred human diseases

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ABSTRACT

Phthalates are widely used as plasticizers to soften and increase the flexibility in polyvinyl chloride plastics, but they can leach into the surrounding environment. There is sufficient evidence in rodents that phthalate exposure causes developmental and reproductive toxicity.

The curated interactions between 16 phthalates and genes/proteins were obtained from Comparative Toxicogenomics Database (CTD), and a total of 445 interactions between the five most frequently curated phthalates (DEHP/MEHP and DBP/BBP/MBP) and 249 unique genes/proteins were found. The GeneOntology, pathways and networks of these 249 unique genes/proteins were fully analyzed. The pathways and networks of top 34 genes/proteins were found to be very similar to those of the 249 unique genes/proteins. Thus, the top 34 genes/proteins may serve as molecular biomarkers of phthalate toxicity.

The top three phthalate toxicity categories were found to be cardiotoxicity, hepatotoxicity and nephrotoxicity, and the top 20 diseases included cardiovascular, liver, urologic, endocrine and genital diseases.

1. Introduction

Phthalates are a group of similar diesters of phthalic acid used as plasticizers to soften and increase the flexibility in polyvinyl chloride (PVC) plastics. Since phthalates are not covalently bound to plastics, they can leach into the surrounding environment [1,2]. Human exposure to phthalates mainly occurs through foods, because of the use of PVC in wrapping materials and food processing [3]. Phthalates are also found in meat, fish, milk products, and other foods with a high fat content. Diethylhexyl phthalate (DEHP) is produced by reacting 2-ethylhexanol with phthalic anhydride, and it is the highest production volume chemical. When ingested through food contamination, DEHP is converted by intestinal lipases to mono-(2-ethylhexyl) phthalate (MEHP), which is then preferentially absorbed. DEHP is currently the only phthalate plasticizer used in PVC medical devices such as intravenous tubing and blood transfusion bags [4]. Other sources of DEHP are indoor air and work exposure in manufacturing factories.

Table 1
Curated interactions between 16 phthalates and genes/proteins.

Phthalates		Phthalate–gene interactions
DEHP/MEHP[a]		256[c]
DBP/BBP/MBP[b]		189[c]
Phthalic acid		17
Di-n-pentyl phthalate		15
Dicyclohexyl phthalate		12
Diallyl phthalate		10
Diethyl phthalate		10
Diisodecyl phthalate		7
Di-n-hexyl phthalate		6
Diisobutyl phthalate		6
Di-n-octyl phthalate		5
Diisononyl phthalate		4
Diheptyl phthalate		2
16 phthalates	Sum	539[c]

[a] DEHP, diethylhexyl phthalate 157
MEHP, mono-(2-ethylhexyl)phthalate 99

[b] DBP, dibutyl phthalate 150
BBP, butylbenzyl phthalate 33
MBP, monobutyl phthalate 6

[c] (256 + 189) / 539 = 445 / 539 = 83%.

Abbreviations: BBP, Butylbenzyl phthalate; CTD, Comparative Toxicogenomics Database; DBP, Dibutyl phthalate; DEHP, Diethylhexyl phthalate; GO, GeneOntology; IPA, Ingenuity Pathways Analysis; MBP, Monobutyl phthalate; MEHP, Mono-(2-ethylhexyl) phthalate; OMIM, Online Mendelian Inheritance in Man; PVC, Polyvinyl chloride.

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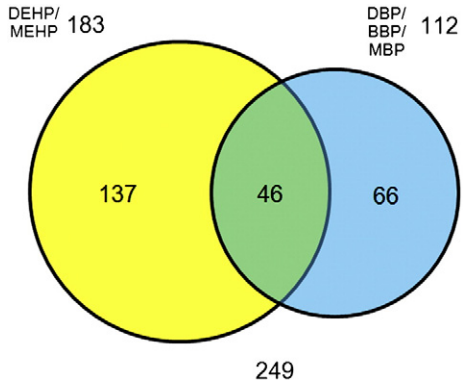


Fig. 1. Venn diagram of phthalate-interacting genes/proteins. DEHP/MEHP and DBP/BBP/MBP were found to interact with 183 and 112 unique genes/proteins, and 46 of them were in common. Thus, there were 249 unique genes/protein.

Dibutyl phthalate (DBP) is produced by reacting n-butanol with phthalic anhydride. Unlike many phthalates, DBP is not currently used as a plasticizer in PVC plastics. Typically, DBP is used as a component of latex adhesives. It is also used in cosmetics and other personal care products, as a plasticizer in cellulose plastics, and as a solvent for dyes [5]. Monobutyl phthalate (MBP) is the embryotoxic metabolite of butylbenzyl phthalate (BBP) and DBP.

Environmental chemicals have been shown to play a critical role in the etiology of many human diseases. The impact of phthalate exposure on human health has been extensively reviewed and reported by the National Toxicology Program—Center for the Evaluation of Risks to Human Reproduction [6]. There is sufficient evidence in rodents, but not yet in humans, that phthalate exposure causes developmental and reproductive toxicity. The Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org) has been established to analyze the impact of environmental chemicals on human health [7]. Biocurators at CTD manually curate toxicogenomic data, including over 116,000 interactions between 3900 chemicals and 13,300 genes/proteins from 270 species, 5900 gene/protein–disease direct relationships, and 2500 chemical–disease direct relationships. In order to understand the potential adverse health effects of the most abundantly used DEHP/MEHP and DBP/BBP/MBP, the curated interactions between these five phthalates and genes/proteins were downloaded from CTD, and 249 phthalate-interacting genes/proteins were fully analyzed for their GeneOntology (GO), pathways, networks, and human diseases inferred by the phthalate–gene/protein–disease relationships in this investigation.

2. Material and methods

2.1. Curated interaction analysis

All curated interactions of phthalate–genes/proteins, phthalate–diseases, and gene/protein–diseases in CTD are publicly available [7], and this analysis reported here was based on data downloaded on July 2, 2009. Prior to analysis, the data set was first manually processed: (1) all curated interactions with phthalates were first summarized and then only the interactions with DEHP/MEHP and DBP/BBP/MBP were analyzed in details, (2) "no effect" interactions were removed, and (3) binary interactions were identified [8].

Table 2
34 most frequently curated phthalate-interacting genes/proteins.

Gene symbols	DEHP/MEHP	DBP/BBP/MBP	Sum	Human	Rat	Mouse	Others	Gene names
PPARA	15	1	16	5	4	6	1	Peroxisome proliferator-activated receptor alpha
PPARG	9	1	10	3	5	1	1	Peroxisome proliferator-activated receptor gamma
PPARD	5	1	6	2	4	–	–	Peroxisome proliferator-activated receptor delta
NR1I2	5	4	9	4	2	–	3	Nuclear receptor subfamily 1, group I, member 2
STAR	4	8	12	–	6	2	4	Steroidogenic acute regulatory protein
HSD3B1	4	2	6	–	3	3	–	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1
INSL3	3	6	9	–	6	–	3	Insulin-like 3 (Leydig cell)
NR0B1	3	3	6	–	6	–	–	Nuclear receptor subfamily 0, group B, member 1
ESR1	2	11	13	9	–	–	4	Estrogen receptor 1
CYP11A1	2	7	9	1	4	–	4	Cytochrome P450, family 11, subfamily A, polypeptide 1
CYP17A1	2	5	7	–	4	–	3	Cytochrome P450, family 17, subfamily A, polypeptide 1
CYP1B1	2	2	4	4	–	–	–	Cytochrome P450, family 1, subfamily B, polypeptide 1
CYP4A1	2	2	4	–	3	1	–	Cytochrome P450, family 4, subfamily a, polypeptide 1
TSPO	2	2	4	–	4	–	–	Translocator protein (18 kDa)
EGR1	2	1	3	–	3	–	–	Early growth response 1
MYC	2	1	3	1	1	1	–	V-myc myelocytomatosis viral oncogene homolog (avian)
SCARB1	1	5	6	–	3	–	3	Scavenger receptor class B, member 1
VEGFA	1	5	6	6	–	–	–	Vascular endothelial growth factor A
ESR2	1	4	5	4	–	–	1	Estrogen receptor 2 (ER beta)
GRB14	1	3	4	–	4	–	–	Growth factor receptor-bound protein 14
LHCGR	1	3	4	–	3	–	1	Luteinizing hormone/choriogonadotropin receptor
AHR	1	2	3	–	–	3	–	Aryl hydrocarbon receptor
HNF1A	1	2	3	–	3	–	–	HNF1 homeobox A
INSIG1	1	2	3	–	3	–	–	Insulin induced gene 1
LDLR	1	2	3	–	3	–	–	Low density lipoprotein receptor
SVS5	1	2	3	–	3	–	–	Seminal vesicle secretory protein 5
FABP3	4	–	4	–	4	–	–	Fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)
SLC27A1	4	–	4	–	4	–	–	Solute carrier family 27 (fatty acid transporter), member 1
CELSR2	3	–	3	1	–	2	–	Cadherin, EGF LAG seven-pass G-type receptor 2 (flamingo homolog, Drosophila)
CYP19A1	3	–	3	–	3	–	–	Cytochrome P450, family 19, subfamily A, polypeptide 1
MT1	3	–	3	–	–	3	–	Metallothionein 1
MT2	3	–	3	–	–	3	–	Metallothionein 2
AR	–	9	9	3	3	1	2	Androgen receptor
SLC5A5	–	3	3	2	1	–	–	Solute carrier family 5 (sodium iodide symporter), member 5
34 SUM	94	99	193	45	92	26	30	
249 SUM	256	189	445	55	301	40	49	

2.2. GeneOntology, pathway and network analyses

GeneOntology (GO), pathways and networks of phthalate-interacting genes/proteins were analyzed using the software programs from MetaCore Analytical Suite (GeneGO, Inc.), Ingenuity Pathways Analysis (IPA, Ingenuity Systems), and/or Pathway Studio (Ariadne, Inc.). The MetaCore can analyze GO molecular functions and GeneGo maps (pathways). The Pathway Studio is better to analyze pathways and networks among chemicals, genes/proteins and diseases in addition to GO molecular functions. The IPA is best suitable for analysis of Tox Functions besides pathways and networks.

2.3. Disease analysis

There are two sources for direct disease relationship in CTD: curation of chemical–disease and gene/protein–disease relationships from the literature, and integration of gene–disease relationships from the Online Mendelian Inheritance in Man (OMIM; http://www.ncbi.nlm.nih.gov/omim) database. The potential human diseases inferred by the phthalate–gene/protein–disease relationships from CTD and OMIM were analyzed using IPA Tox Functions.

3. Results

3.1. Phthalate–gene/protein interactions

A total of 539 phthalate–gene/protein interactions (excluding no effects) were found for 16 phthalates from the CTD as of July 2, 2009. Since DEHP is *in vivo* metabolized quantitatively to its monoester MEHP, the DEHP and MEHP were thus combined as a group. Likewise, DBP, BBP and MBP were pooled together, because MBP is the embryotoxic metabolite of DBP and BBP. The five most frequently curated chemicals DEHP/MEHP and DBP/BBP/MBP exhibited 256 and 189 interactions, respectively, and their sum of 445 interactions accounted for 83% of all 539 interactions (Table 1). Only DEHP/MEHP and DBP/BBP/MBP groups were further analyzed in details. DEHP/MEHP and DBP/BBP/MBP interacted with 183 and 112 unique genes/proteins, respectively, and 46 of them were in common (Fig. 1). Thus, there were 249 unique genes/proteins exhibiting a total of 445 interactions with DEHP/MEHP and DBP/BBP/MBP (Supplementary Table S1), and the top 34 unique genes/proteins with three and more interactions are summarized in Table 2. It is of interest that DEHP/MEHP was found to have at least five interactions with all three PPAR isotypes (PPARA, PPARG and PPARD), while DBP/BBP/MBP had only one interaction with each of these three genes/proteins. DBP/BBP/MBP was found to have eleven and nine interactions with ESR1 and AR, but DEHP/MEHP had only two and none, respectively. It may also be noted that ESR1 and VEGFA genes/proteins had nine and six interactions, respectively, with DEHP/MEHP and DBP/BBP/MBP in humans, but none in rodents.

3.2. GeneOntology, pathway and network analyses

The GO molecular functions of 249 unique phthalate-interacting genes/proteins were analyzed by both MetaCore and Pathway Studio, and their top 20 functions are listed in Table 3. 13 and 10 out of their top 20 functions obtained by MetaCore and Pathway Studio, respectively, were involved with different receptor activities, and six of their top 20 functions identified by both methods were further found to be in common. It should be noted that many of the GeneGo pathways and GeneGo process networks of 249 unique phthalate-interacting genes/proteins were found to be very similar to those of the top 34 unique genes/proteins with three and more curated phthalate interactions (Fig. 2), suggesting that this subset of 34 unique genes/proteins may represent the overall biology of 249 genes/proteins responding to phthalate exposure. It is of interest that the top four pathways were involved in the regulation of lipid metabolism, and PPAR pathway. It may be further noted that three PPARs complexing with retinoid X receptor (RXRA) played important roles in many normal and pathological biological processes such as atherosclerosis and hepatocarcinogenesis (Fig. 3).

Table 3
Top 20 GO molecular functions analyzed by MetaCore and Pathway Studio.

GO molecular functions	p-value	
	MetaCore	Pathway Studio
G-protein coupled receptor activity	3.61E−28	1.49E−20
Receptor activity	2.25E−23	1.71E−23
Signal transducer activity	4.48E−19	2.31E−20
Cadmium ion binding	2.46E−14	3.64E−17
Steroid hormone receptor activity	8.76E−14	2.87E−10
Ligand-dependent nuclear receptor activity	1.39E−11	3.33E−14
Transmembrane receptor activity	5.08E−21	
Molecular transducer activity	4.48E−19	
Peptide receptor activity	1.23E−18	
Peptide binding	4.12E−18	
Peptide receptor activity, G-protein coupled	5.18E−17	
Neurotransmitter binding	1.50E−13	
Neurotransmitter receptor activity	4.41E−13	
Icosanoid receptor activity	1.10E−12	
Prostanoid receptor activity	1.10E−12	
Hormone binding	1.60E−12	
Neuropeptide receptor activity	6.76E−12	
Neuropeptide binding	8.67E−12	
Prostaglandin receptor activity	4.78E−11	
Amine receptor activity	1.34E−10	
Rhodopsin-like receptor activity		1.71E−42
Adrenoceptor activity		1.26E−09
Protein binding		2.54E−09
Neuropeptide Y receptor activity		2.66E−09
Monooxygenase activity		3.46E−09
Metabotropic glutamate, GABA-B-like receptor activity		7.27E−09
Sequence-specific DNA binding		1.33E−08
Copper ion binding		1.55E−08
Tachykinin receptor activity		1.84E−08
Protein heterodimerization activity		2.07E−08
Transcription factor activity		5.59E−08
Xenobiotic-transporting ATPase activity		9.14E−08
Transcription activator activity		1.20E−07
Double-stranded DNA binding		1.59E−07

3.3. Toxicity and disease associations

The toxicity of 249 unique genes/proteins interacting with DEHP/MEHP and DBP/BBP/MBP was analyzed using IPA Tox Functions, and their top three categories were cardiotoxicity, hepatotoxicity and nephrotoxicity (Table 4). The pathways and networks of the top three toxicities of MEHP and DBP were further analyzed by Pathway Studio (Fig. 4). The curated interactions of DEHP/MEHP and DBP/BBP/MBP with 249 unique genes/proteins in CTD have inferred relationships with 1437 GeneGo diseases identified using MetaCore by biomarkers, and the top 20 diseases are listed in Table 5. The top 20 diseases included cardiovascular diseases, liver diseases, urologic diseases, endocrine diseases, and genital diseases in addition to the number 1 disease-disorder of environmental origin.

4. Discussion

In this investigation, the flow chart for the different steps of our analyses is shown in Fig. 5. DEHP/MEHP, as well as DBP/BBP/MBP, was found to interact with all three PPAR isotypes PPARA, PPARD and PPARG (Table 2), and these three PPARs were reported to be functionally activated by DEHP/MEHP [9]. PPARs play broad roles in lipid and carbohydrate metabolism (Fig. 3), and they are involved in metabolic disorders such as atherosclerosis, hyperlipidemia, and

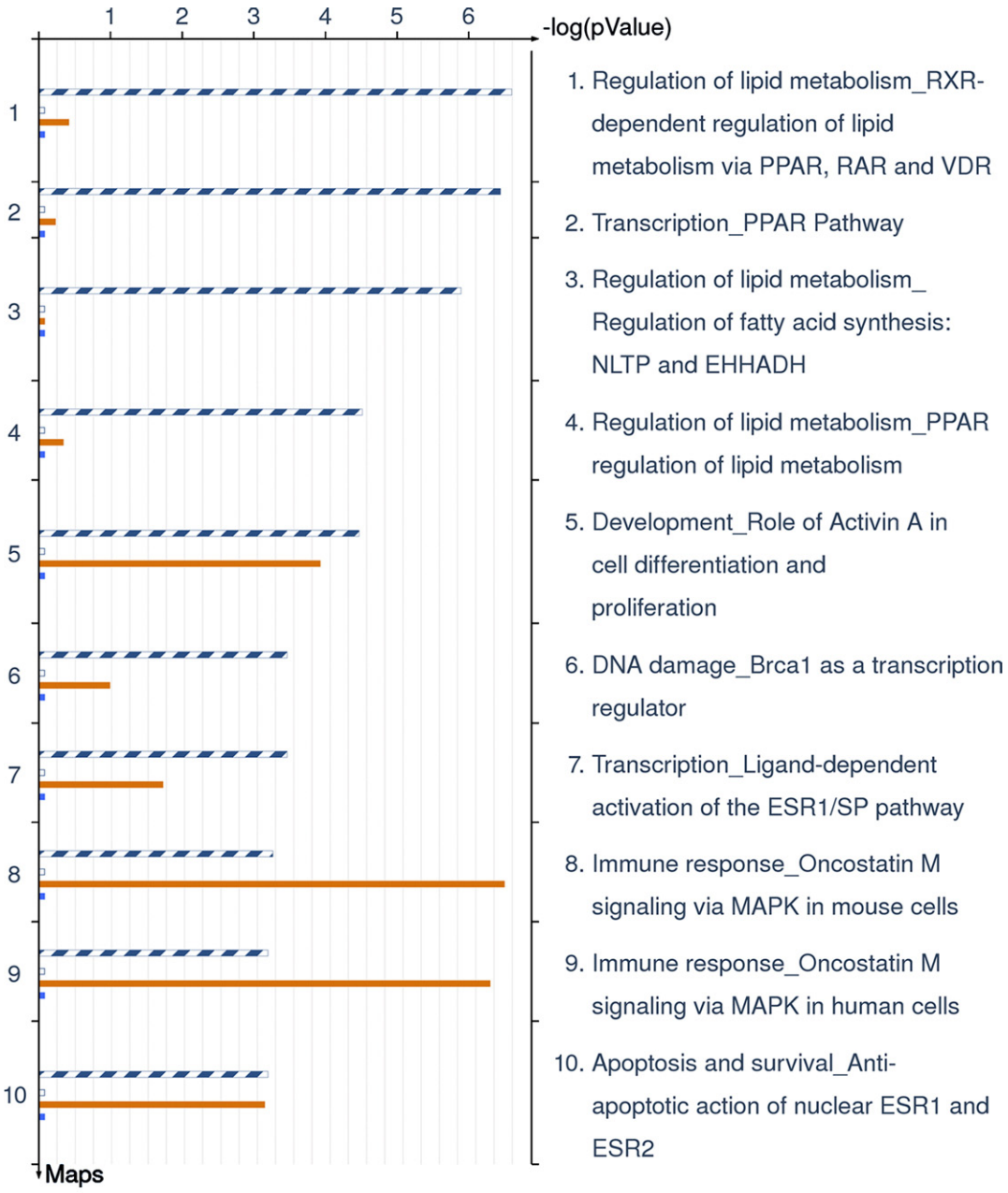


Fig. 2. Comparison of GeneGo pathway maps and process networks of 249 and top 34 unique genes/proteins. The top 10 GeneGo canonical pathway maps of top 34 genes/proteins (blue/while strips) and the other 215 genes/proteins (brown band). The degree of "relevance" to different GeneGo ontology categories is defined by p-value, so that the lower random p-value gets higher priority. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

obesity, which in general develop slowly over many years [10]. These disorders are consistent with the finding of the cardiotoxicity, including cardiovascular diseases, among top disorders caused by phthalate exposure (Table 4). The developmental effects of DBP/BBP/MBP depend on the embryonic age at the time of exposure and exposure duration. These effects are mainly on mesodermal structures, including cardiovascular and urogenital malformation. Recently, the cardiomyocyte differentiation of murine embryonic stem cells was reported to be inhibited by MBP [11]. Several urinary phthalate metabolites were also reported to be associated with key features of the metabolic syndrome (body mass index, abdominal adiposity and insulin resistance) [12,13].

Peroxisome proliferation is often seen in the liver and kidney, and sustained peroxisome proliferation phenomenon can lead to carcinogenesis. The activation of PPARA, which was the most frequently curated gene/protein, was responsible for the DEHP/MEHP toxicity, especially, liver tumors in rodents [14]. This mechanism may be less relevant for humans because of lower expression of PPARA in the human liver compared to that seen in the rodent liver. However, PPARA-deficient mice did develop hepatocarcinogenesis under DEHP exposure [15], and this was due to activation of constitutive androstane receptor [16,17]. DEHP/MEHP was also reported to have marked nephrotoxic effects on cultured kidney epithelial cells [18].

DEHP/MEHP causes animal toxicity in many organ systems [19,20]. Studies at low doses of exposure, which appear to be of most concern for humans, have demonstrated subtle reproductive toxicity in male rodents [21]. Since fetal and neonatal stages seem to be the critical periods for exposure to DEHP/MEHP, a similar toxicity (for example, impaired fertility) in humans might be dormant and might not become manifest for many years, until adulthood is reached. In

humans, population studies have established that dysmorphic disorders of the genital tract, observed in male infants, are significantly associated with prenatal exposure to phthalates. These effects likely result from the antiandrogenic and estrogeno-mimetic activities of DEHP/MEHP [22].

DBP/BBP/MBP, but not DEHP/MEHP, was found to have nine curated interactions with AR gene/protein (Table 2). However, these phthalates do not interact directly with the androgen receptor, but rather disrupt testosterone synthesis in the rat fetal testis [23]. DBP/BBP/MBP is known to affect male fertility and cause testicular atrophy in young adult rodents. DBP/BBP/MBP also has profound and permanent effects on the male reproductive development if exposure occurs during the critical periods of sexual differentiation (i.e. late in the gestation) [24]. The phenotypic alterations observed in male offspring rats exposed during the perinatal period have remarkable similarities with common human reproductive disorders, including cryptorchidism, hypospadias and low sperm counts [25].

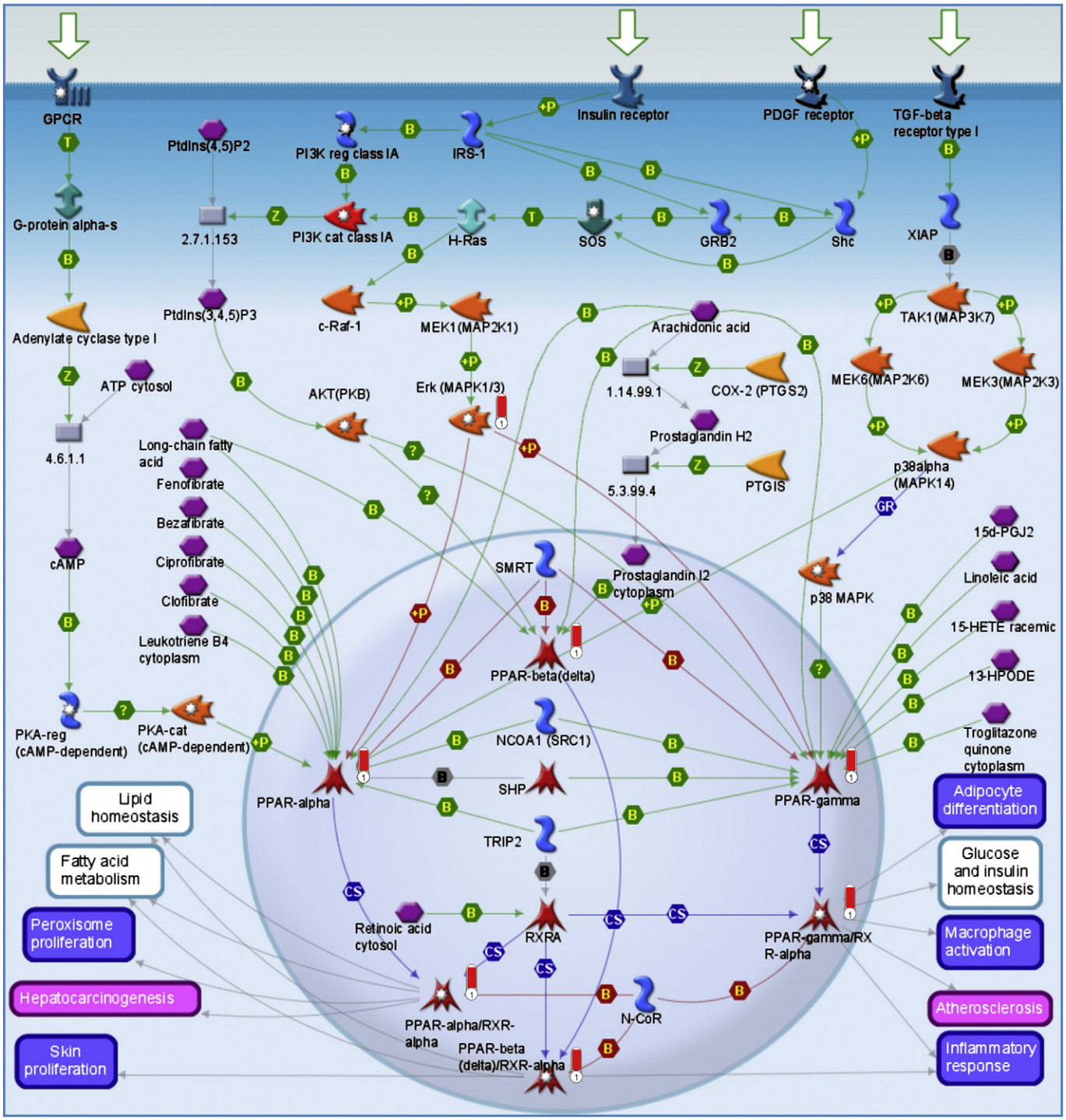


Fig. 3. PPAR pathway — MetaCore. The 249 unique genes/proteins were used as input for MetaCore pathway analysis. A. Three PPAR isotypes PPARA (alpha), PPARD (delta/beta) and PPARG (gamma) complexed with retinoid X receptor RXRA (alpha) were involved in normal and pathological processes. B. The explanation of the symbols used in GeneGo pathway.

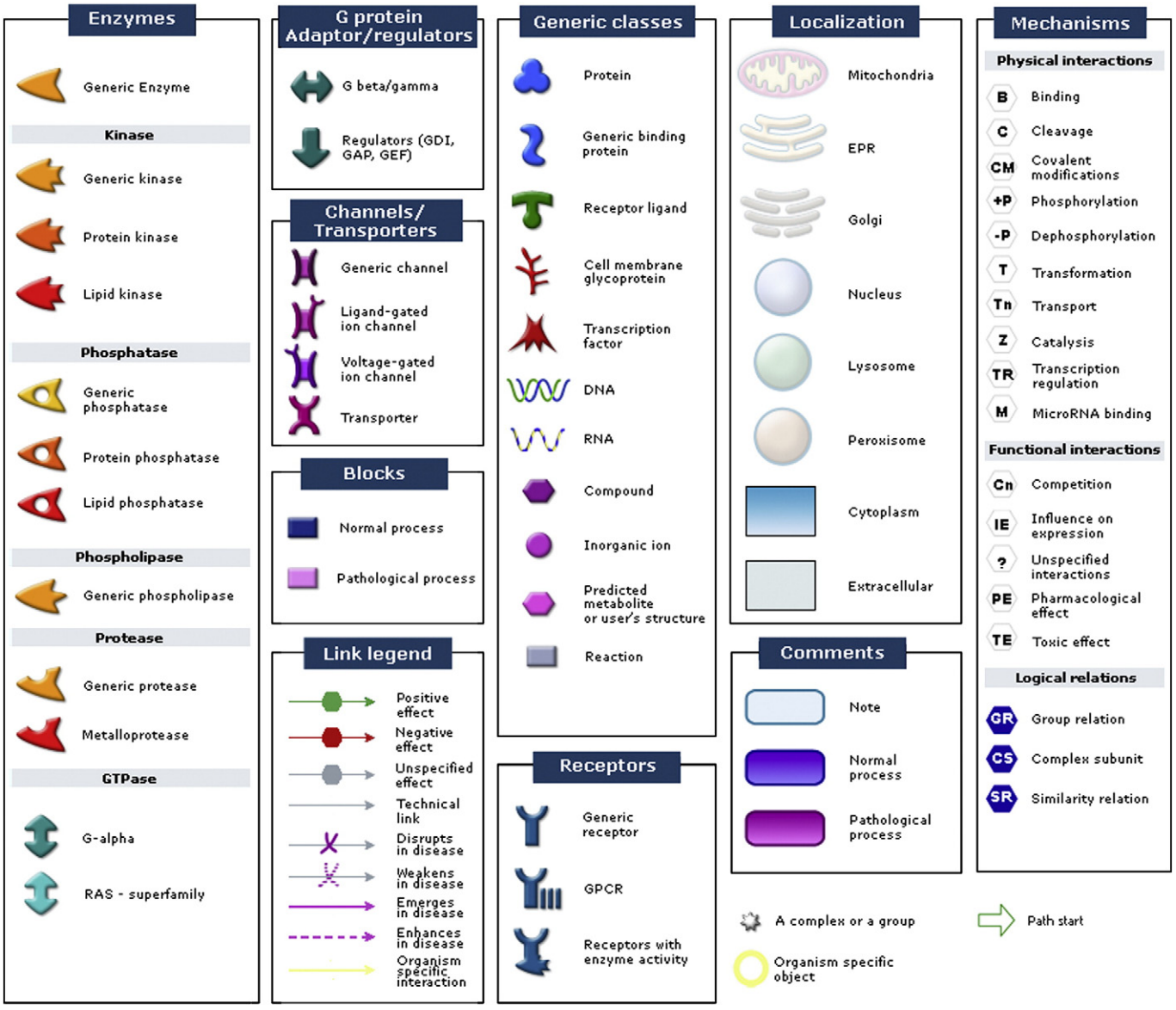


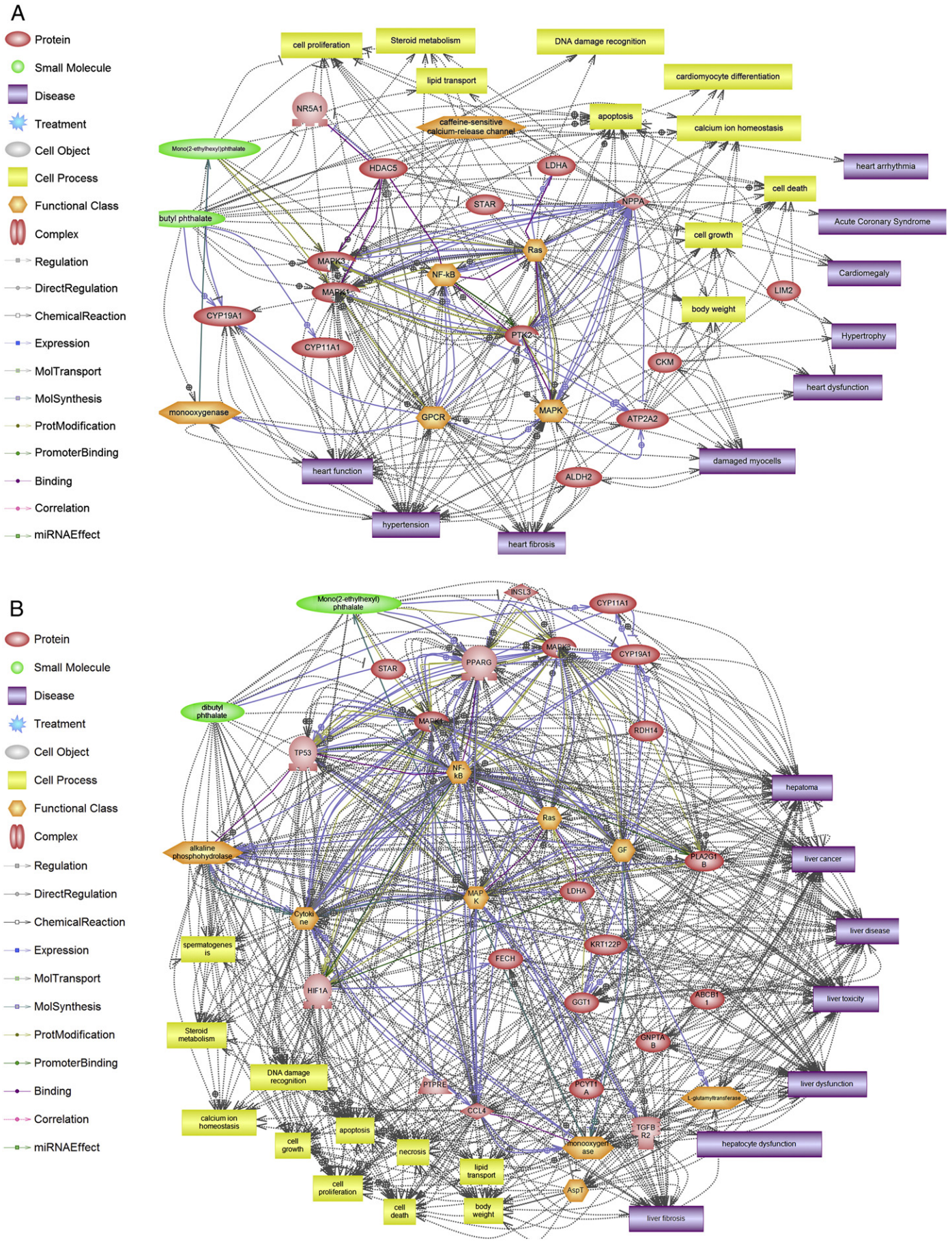
Fig. 3 (*continued*).

Table 4
Top three toxicity categories analyzed by IPA Tox Functions.

Name	p-value	# Molecules
Cardiotoxicity		
Heart failure	8.16E−14 to 2.06E−04	21
Tachycardia	1.32E−12 to 1.72E−07	13
Cardiac infarction	1.48E−08 to 2.40E−04	12
Cardiac congestive cardiac failure	4.20E−08 to 4.20E−08	10
Cardiac arrhythmia	5.64E−08 to 1.78E−07	9
Hepatotoxicity		
Liver necrosis/cell death	3.54E−09 to 5.89E−02	14
Liver steatosis	1.28E−07 to 4.67E−02	9
Liver hepatomegaly	3.35E−07 to 1.58E−02	6
Liver hepatitis	4.51E−07 to 1.06E−01	9
Liver hyperplasia/hyperproliferation	6.86E−07 to 6.18E−02	6
Nephrotoxicity		
Kidney failure	3.31E−10 to 3.14E−02	13
Renal nephritis	6.14E−05 to 2.00E−01	9
Renal proliferation	1.15E−04 to 3.14E−02	5
Renal hydronephrosis	2.42E−04 to 2.00E−02	4
Renal necrosis/cell death	3.52E−04 to 1.46E−03	5

The widespread exposure of the general human population has been demonstrated in several recent biomonitoring studies in the USA and Europe [2,26,27]. The potential public health risks associated with phthalate exposure include not only metabolic disorders, carcinogenesis, and reproductive toxicity but also endocrine disruption [28–30]. Estrogen receptor 1 (ESR1) was found to interact with both DEHP/MEHP and DBP/BBP/MBP in humans, but not in rodents (Table 2). The widely used phthalates are known to interfere with endocrine systems, including the estrogen and thyroid hormones [31–33]. Endocrine diseases and thyroid diseases were among the top 20 inferred diseases (Table 5) in this investigation. The vascular endothelial growth factor A (VEGFA) was also found to interact with DEHP/MEHP and DBP/BBP/MBP in humans, but not in rodents. DEHP has recently shown to exert a species-specific metabolic consequence of PPARA activation [34].

The pathways and networks of top 34 genes/proteins were found to be very similar to those of 249 unique genes/proteins interacting with phthalates DEHP/MEHP and DBP/BBP/MBP. Thus, these top 34 genes/proteins may serve as molecular biomarkers for assaying the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PromoterBinding
Binding
Correlation
miRNAEffect
cell proliferation
Steroid metabolism
DNA damage recognition
lipid transport
cardiomyocyte differentiation
NR5A1
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apoptosis
calcium ion homeostasis
Mono(2-ethylhexyl)phthalate
HDAC5
LDHA
heart arrhythmia
cell death
STAR
NPPA
butyl phthalate
Acute Coronary Syndrome
cell growth
Ras
MAPK3
NF-kB
Cardiomegaly
MAPK1
LIM2
CYP19A1
body weight
PTK2
Hypertrophy
CYP11A1
CKM
heart dysfunction
monooxygenase
GPCR
MAPK
ATP2A2
damaged myocells
heart function
ALDH2
hypertension
heart fibrosis
B
Protein
Small Molecule
Disease
Treatment
Cell Object
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CCL4
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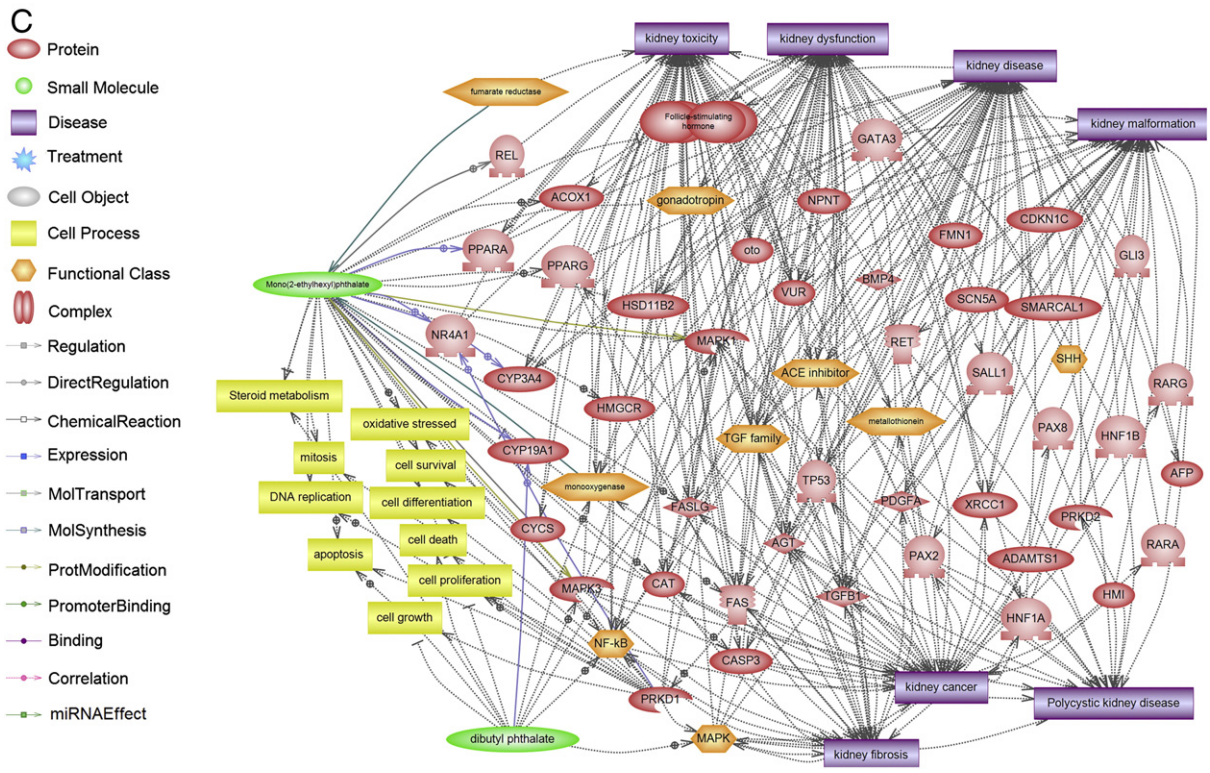

Fig. 4. The pathways and networks of top three toxicities of MEHP and DBP analyzed by Pathway Studio. The 249 unique genes/proteins were used as input for pathway and network analyses by Pathway Studio. A, cardiotoxicities; B, heptotoxicites; and C, nephrotoxicities.

toxicity of phthalates. Advances in the measurement of molecular biomarkers hold much promise in facilitating the study design and improving the epidemiological data. In view of limited human experimental evidence, human embryonic stem cells may be developed using systems biology with the top 34 genes/proteins as molecular biomarkers to assay the adverse health effects of phthalate toxicity [35,36]. Human embryonic stem cells may also be differentiated into beating cardiomyocytes as a new tool for testing cardiotoxicity [37]. Gene expression profiles of these differentiated cardiomyocytes have been found to be reminiscent of developing contractile cells in the normal human heart.

Table 5
Top 20 GeneGo diseases (by Biomarkers).

#	Disease name	p-value	Network objects
1	Disorders of environmental origin	2.755E−34	144/1567
2	Cardiovascular diseases	5.884E−32	153/1833
3	Endocrine system diseases	4.198E−30	161/2080
4	Urologic diseases	5.400E−28	116/1210
5	Vascular diseases	7.040E−28	134/1572
6	Adenocarcinoma	8.261E−27	141/1760
7	Endocrine gland neoplasms	2.822E−26	121/1362
8	Liver diseases	5.730E−26	128/1515
9	Wounds and injuries	1.019E−25	97/928
10	Body weight	2.299E−25	70/504
11	Liver neoplasms	1.708E−24	103/1072
12	Urologic and male genital diseases	8.423E−24	170/2571
13	Autoimmune diseases	2.106E−23	111/1261
14	Pathological conditions	3.817E−23	216/3867
15	Mental disorders	4.066E−23	127/1601
16	Urogenital diseases	4.328E−23	151/2142
17	Prostatic diseases	5.636E−23	110/1256
18	Genital diseases, male	6.712E−23	119/1441
19	Urogenital neoplasms	6.728E−23	147/2057
20	Endometrial neoplasms	1.236E−22	53/322

6. Conclusions

A total of 445 interactions curated in CTD were obtained between the five most frequently curated phthalates (DEHP/MEHP and DBP/BBP/MBP) and 249 unique genes/proteins. The pathways and networks of the top 34 genes/proteins were found to be very similar to those of the 249 unique genes/proteins. Thus, the top 34 genes/proteins may serve as molecular biomarkers of phthalate toxicity. The top three phthalate toxicity categories were found to be cardiotoxicity, hepatotoxicity and nephrotoxicity. Further studies are warranted to demonstrate the significance of these associations of phthalates with respect to the newly discovered cardio and reno toxicities. The 1437 potential human diseases were inferred by the phthalate–gene/protein–disease relationships, and the top 20 diseases included cardiovascular diseases, liver diseases, urologic diseases, endocrine diseases, and genital diseases in addition to the number 1 disease-disorder of environmental origin.

7. Note added at revision

New CTD data dated September 8, 2010 were similarly analyzed, and the results described in supplementary Tables S2 and S3 are found to be very similar to those based on July 2, 2009.

Supplementary materials related to this article can be found online at doi:10.1016/j.ygeno.2010.11.008.

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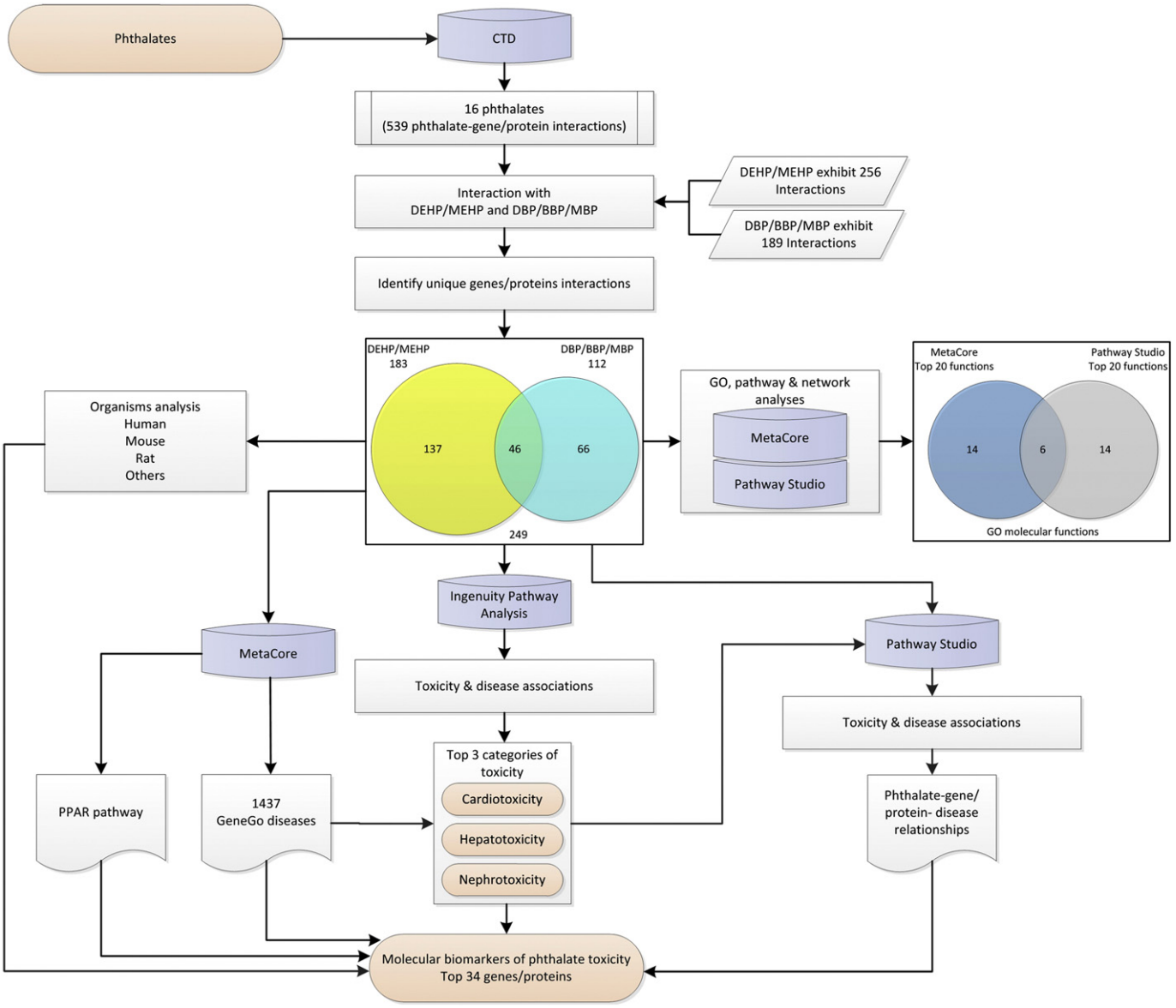

Fig. 5. Flow chart of the different steps in our analyses. The curated interactions between 16 phthalates and genes/proteins were obtained from CTD, and a total of 445 interactions between the five most frequently curated phthalates and 249 unique genes/proteins were found. The top 34 genes/proteins may serve as molecular biomarkers of phthalate toxicity. The top three phthalate toxicity categories were found to be cardiotoxicity, hepatotoxicity and nephrotoxicity.

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