

AN APPROACH TO USING TOXICOGENOMICS DATA IN RISK ASSESSMENT: A DIBUTYL PHTHALATE CASE STUDY

Susan Y. Euling¹, Susan Makris¹, Banalata Sen², Lori White², Bob Benson³, Kevin W. Gaido⁴, Andrea S. Kim¹, Susan Hester⁵, Vickie S. Wilson⁵, Channa Keshava¹, Nagu Keshava¹, Paul M. Foster⁶, Ioannis P. Androulakis⁷, Meric Ovacki⁷, Marianthi G. Ierapetritou⁷, L.E. Gray Jr.⁵, Chad Thompson¹, Weihsueh Chiu¹, William Welsh⁷, Panos Georgopoulos⁷ ¹NCEA, EPA, Washington, DC; ²NCEA, EPA, RTP, NC; ³Region 8, EPA, Denver, CO; ⁴The Hamner Institute, RTP, NC; ⁵NHEERL, EPA, RTP, NC; ⁶NIEHS, RTP, NC; ⁷ebCTC, Rutgers/UMDNJ, Piscataway, NJ

ABSTRACT

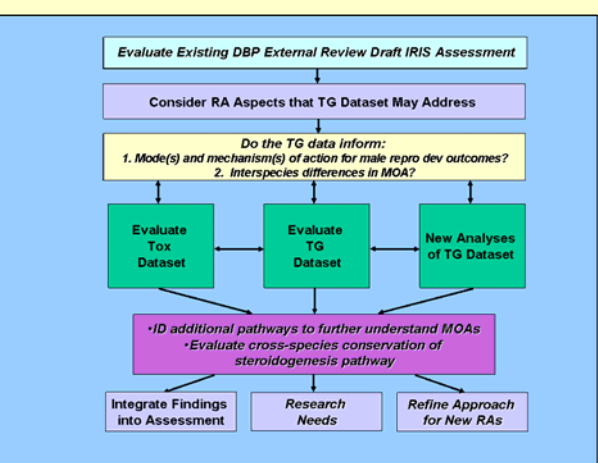
A case study to incorporate toxicogenomics data qualitatively into a U.S. Environmental Protection Agency (U.S. EPA) health assessment has been performed for dibutyl phthalate (DBP), focusing on the male reproductive developmental effects. Using U.S. EPA's Integrated Risk Information System (IRIS) external peer review draft DBP assessment as the starting point, we asked whether toxicogenomics data could further define the mode(s) or mechanism(s) of action and inform interspecies extrapolation. The modes of action that explain some of the male reproductive developmental effects observed after *in utero* DBP exposure in rodents are reduced fetal testicular testosterone production and *insl3* gene expression. The male reproductive developmental toxicology dataset was assessed for low incidence findings and endpoints with unexplained modes of action that may indicate additional pathways. The toxicogenomics dataset is composed of eight published microarray or real-time reverse transcriptase-polymerase chain reaction (RT-PCR) rat studies. To identify additional pathways affected besides steroidogenesis and *insl3*, pathway level analysis of the microarray data was performed. Results from two different analytical methods indicate that biological processes such as apoptosis, cell adhesion, cell growth, and differentiation may be affected in the testis by *in utero* exposure to DBP. Research needs for designing gene expression studies for use in risk assessment were also identified. The approach for utilizing toxicogenomics data in risk assessment defined in this study may be applied to other chemical assessments.

PROJECT GOALS

- 1) Develop an approach for using toxicogenomics data most effectively in risk assessment.
- 2) Perform a case study using this approach.

Dibutyl phthalate was selected for the case study because it has a relatively large toxicogenomics dataset with consistent findings and an ongoing risk assessment.

CASE STUDY APPROACH



Proposed DBP Mechanism of Action

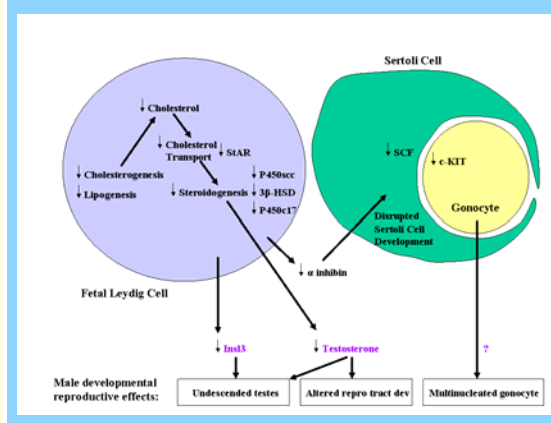


Figure adapted from Barlow et al. (2003), Liu et al. (2005), Shultz et al. (2001), Thompson et al. (2004), and Wilson et al. (2004). Based on male reproductive developmental toxicity and toxicogenomics studies. Some genes and pathways found to be altered are included. Purple lettering, proposed modes of action.

Male Reproductive Developmental Toxicity Endpoints and MOAs: Testes

Effect	MOA	
	Reduced fetal testicular T	Reduced <i>insl3</i> signaling
Multinucleated gonocytes; increased number of gonocytes in fetal testes	?	?
Altered proliferation of Sertoli and peritubular cells; fewer Sertoli cells	?	?
Gonocyte apoptosis increase; early postnatal decrease in gonocyte number	?	?
Abnormal Sertoli cell-gonocyte interaction	?	?
Small incidence of Leydig cell adenomas, aggregates, and hyperplasia	+	?
Decreased number spermatocytes or cauda epididymal sperm conc.	+	?
Small or flaccid; other abnormalities; decreased wt	+	?
Increased weight due to edema	?	?
Decreased number or degeneration of seminiferous cords/tubules; altered morphology; degeneration of the epithelium; enlarged cords/tubules	?	?
Cryptorchidism (no testes descent); delayed testes descent	+	+

? = Current data indicate that it is unlikely the MOA (i.e., key event).
 ⊕ = MOA for outcome supported by the weight of evidence of studies.

Published DBP Toxicogenomics Dataset

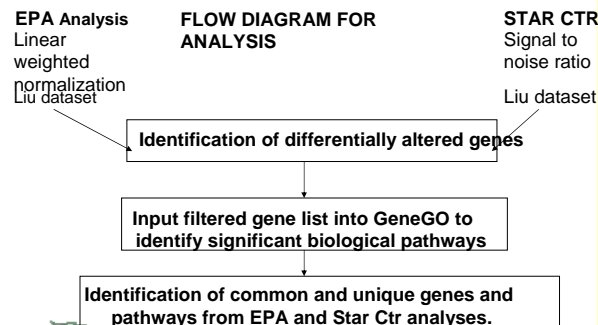
STUDY ^a	DBP DOSE	TREATMENT INTERVAL (gestation days)	TOXICOGENOMIC METHOD		TISSUE COLLECTED
			MICROARRAYS (Platform)	RT-PCR	
Barlow et al., 2003	500 mg/kg/day	GD 12-19	No	Yes	Testis
Bowman et al., 2005	500 mg/kg/day	GD 12-19 or 19-21	Yes (Clontech cDNA arrays)	Yes	Wolffian ducts
Lehmann et al., 2004	0.1, 1.0, 10, 50, 100, or 500 mg/kg/day	GD 12-19	No	Yes	Testis
Liu et al., 2005 ^b	500 mg/kg/day	GD 12-19	Yes (Affymetrix GeneChip oligo arrays)	Yes	Testis
Shultz et al., 2001	500 mg/kg/day	GD 12-16, 12-19 or 12-21	Yes (Clontech cDNA arrays)	Yes	Testis
Thompson et al., 2004	500 mg/kg/day	GD 12-17, 18, or 19; 13-19, 14-19, 15-19, 16-19, 17-19, 18-19 or 19	No	Yes	Testis
Wilson et al., 2004	1000 mg/kg/day	GD 13-17	No	Yes	Testis
Thompson et al., 2005	500 mg/kg/day	0.5–24 hr beg. GD 18 or 19 (all saced on GD 19)	Yes (Affymetrix GeneChip oligo arrays)	Yes	Testis

WOE: Testis Microarray Studies



New Analyses of Toxicogenomics Data

Are Additional Pathways affected after *in utero* DBP exposure?



Additional Pathways (excluding T and insl3) by Process Identified by STAR CTR and/or EPA

Process	Pathways
Cell adhesion	Chemokines and adhesion; ECM remodeling; Integrin-mediated cell adhesion; Cytoskeleton remodeling; Reverse signalling by ephrin B; Slit-Robo signaling; Gap junctions; Integrin outside-in signaling
Cell cycle control	Brcal as transcription regulator; Spindle assembly and chromosome separation
Apoptosis	BAD phosphorylation; Caspases cascade
Growth and differentiation	WNT signaling pathway; Leptin signaling via JAK/STAT and MAPK; ESR/Interaction with G-proteins signaling; Angiotensin signaling via beta-Arrestin; Angiotensin activation of ERK
Immune response	CCR3 signaling in eosinophils; MIF: neuroendocrine-macrophage connector; Signaling pathway mediated by IL-6 and IL-1; MIF-JAB1 signaling; NTS activation of IL-8 in colonocytes; IFN gamma signaling pathway; IL6 signaling pathway
Proteolysis	Akt in hypoxia induced HIF1 activation
Regulation of transcription	ChREBP regulation pathway; P53 signaling pathway; Insulin regulation of protein synthesis
G-proteins	G-Protein beta/gamma signaling cascades; Regulation of actin cytoskeleton; A3 receptor signaling
Transcription factors	Regulation of lipid metabolism via PPAR; VDR in regulation of genes involved in osteoporosis; PPAR Pathway
Chemokines	CXCR4 signaling pathway
Growth factors	Prolactin receptor signaling
Amino acid metabolism	Lysine metabolism; TCA; Urea cycle; Glycine, serine, cysteine and threonine metabolism; Histidine-glutamate-glutamine and proline metabolism; Leucine, isoleucine and valine metabolism; Arginine metabolism; Phenylalanine metabolism; Tryptophan metabolism; Aspartate and asparagine metabolism
Carbohydrates metabolism	Glycolysis and gluconeogenesis; Fructose metabolism; Pentose phosphate pathway
Energy metabolism	Oxidative phosphorylation; Peroxisomal branched chain fatty acid oxidation; Mitochondrial unsaturated fatty acid beta-oxidation; Arachidonic acid production; Triacylglycerol metabolism p.1
Metabolism of mediators	Gamma-aminobutyrate (GABA) biosynthesis and metabolism; Polyamine metabolism; Serotonin - melatonin biosynthesis and metabolism
Nucleotide metabolism	DMP biosynthesis; GTP metabolism; dGTP metabolism; dATP/dTTP metabolism; ATP metabolism
Vitamin and cofactor metabolism	Ubiquinone metabolism; Glutathione metabolism

CONCLUSIONS

- Developed an approach to evaluating toxicogenomics data for use in risk assessment.
- Identified additional functions and pathways affected by *in utero* DBP exposure which may provide a better understanding of DBP modes of action.
- Identified research needs for toxicity and toxicogenomics studies for use in risk assessment:
 - For Toxicity Studies:
 - Report individual animal data.
 - Report all endpoints that were evaluated (regardless of positive or negative finding).
 - Expose and assess animals at optimal developmental stage/time.
 - For Toxicogenomics Studies:
 - Time-course data over critical window of exposure for endpoint(s) of interest to develop a genetic regulatory network model.
 - Increased number of samples and replicates to improve power.
 - Multiple doses in microarray studies to address dose-response.
 - For Both:
 - Parallel study design characteristics (e.g., dose, timing of exposure, organ/tissue evaluated).
- Assessing toxicity and toxicogenomic datasets in conjunction is a useful approach for informing endpoints and pathways that in turn inform MOA(s).
- Reanalysis of TG raw data can be important for a complete mining of the data (in our case, for identifying additional pathways) for use in risk assessment.