Food for Thought

Guidance from EU imminent for PK-Pgx
Requirements and "important" qualifiers

Peculiarities of the European System
EMEA doesn't approve tests
Comparative data are expected and promoted

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Flexibility

- Retrospective may be an acceptable approach for decision making under certain situations
- More dialogue needs to happen because real concerns exist and rt-learning is necessary
- Roadmap can be developed for biomarker development in context of clinical trials
  - Methodology and communication
- Dialogue has to happen early and often, transparency
- While ideal approaches to co-development are desired, case x case evaluations are necessary for different development situations

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Straw Man Next steps

- SC to disseminate information from workshop
- Labeling: broader dialogue on how information→label→guidelines and rapid clinical adoption by communities
- Do we need industry/academic/government sampling consortium to deal with sampling issues
- Need a retrospective/prospective roadmap codified in guidance
- Public forum to discuss guiding principles for co-development
  - Acknowledge the dynamic nature of co-development situations
  - Communication between ≥ 4 entities (CDER/CBER/CDRH/Dx/Rx) is complex

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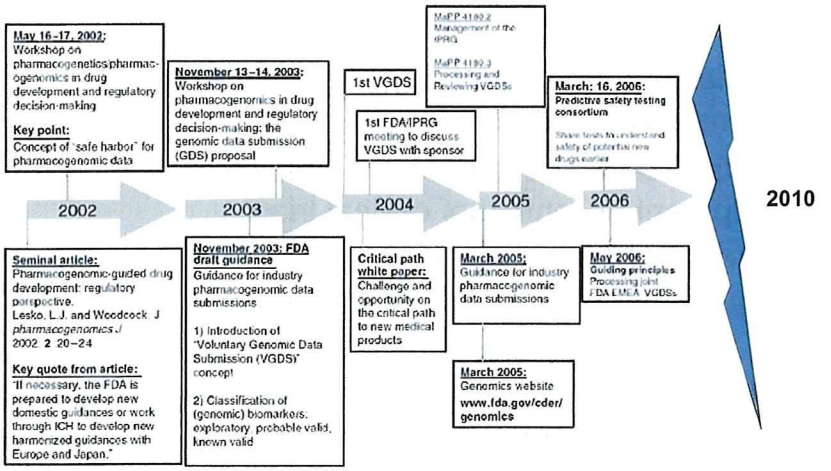
Early History of Pgx at FDA



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An Approach to Using Toxicogenomic Data In U.S. EPA Human Health Risk Assessments: A Dibutyl Phthalate Case Study

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PREFACE

The U.S. Environmental Protection Agency (EPA) is interested in developing methods to use genomic data most effectively in risk assessments performed at EPA. The National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD) prepared this document for the purpose of describing and illustrating an approach for using toxicogenomic data in risk assessment. The approach and dibutyl phthalate (DBP) case study described in this document were developed by a team of scientists at EPA laboratories and centers, and outside organizations including The Hamner Institutes for Health Sciences, the National Institute of Environmental Health Sciences (NIEHS), and the EPA National Center for Environmental Research (NCER) Science to Achieve Results (STAR) Environmental Bioinformatics and Computational Toxicology (Comp Tox) Center at the University of Medicine and Dentistry of New Jersey (UMDNJ) and Rutgers University. The intended audience for this document includes risk assessors as well as scientists with expertise in genomics, bioinformatics, toxicology, and statistics. The approach outlined in this document is expected to be useful to EPA risk assessors in the Integrated Risk Information System (IRIS) Program and other program offices and regions, as well as the scientific community at large. The review of the literature on the use of genomic data in risk assessment, as well as discussions of issues, recommendations, and methods for evaluating and analyzing toxicogenomic data, could be useful to scientists and risk assessors within and outside of EPA. The research needs identified in this document will be useful to scientists performing toxicology and toxicogenomic research studies for application to risk assessment. The DBP case study presented in this document is a separate activity from the IRIS DBP health assessment. The review of the literature included in this document was last updated in July 2007.

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1. EXECUTIVE SUMMARY

We developed a systematic approach for evaluating and utilizing toxicogenomic data in health assessment. This report describes this approach and a case study conducted for dibutyl phthalate (DBP) to illustrate the approach. As a result of the case-study exercise, we refined the initial case-study approach for general use in new chemical assessments. In this report, we reviewed some of the recent and ongoing activities regarding the use of genomic data in risk assessment, inside and outside of the U.S. Environmental Protection Agency (EPA). We also identified research needs, recommendations, and issues for future consideration when using genomic data in risk assessments.

Toxicogenomics is the application of genomic technologies (e.g., transcriptomics, proteomics, genome sequence analysis) to study the effects of environmental chemicals on human health and the environment. The EPA Interim Genomics Policy (U.S. EPA, 2002) encourages the use of genomic data, on a case-by-case basis, in a weight-of-evidence (WOE) approach. Currently, EPA provides no guidance for incorporating genomic data into risk assessments of environmental agents. However, EPA's Science Policy Council (SPC) has developed interim guidance regarding other aspects of the use of microarray data at EPA, entitled *Interim Guidance for Microarray-Based Assays: Data Submission, Quality, Analysis, Management, and Training Considerations* (U.S. EPA, 2006b).

DBP was selected for the case study because it has a relatively large genomic data set and phenotypic anchoring of certain gene expression data to some male reproductive developmental outcomes. The scope of the case study was limited to the male reproductive developmental outcomes of DBP, and this effort was limited to evaluating the available published toxicity and toxicogenomic data for the DBP case study. The DBP case study is a separate endeavor with distinct goals from EPA's Integrated Risk Information System (IRIS) assessment of DBP.

1.1. APPROACH

Genomic data have the potential to inform the mechanism of action, inter- and intraspecies toxicodynamic differences, exposure assessment, toxicokinetics, and dose-response assessment. Our strategy was to design an approach for evaluating genomic data for risk assessment that is both systematic and flexible enough to accommodate different health and risk

assessment practices. The first step of the approach is to evaluate the available genomic data set for its application to a broad range of information types (e.g., mode of action [MOA], toxicokinetics [TK], interspecies variability) that are useful to risk assessment as well as the steps of health assessment (e.g., hazard characterization, dose-response assessment). Through this iterative process, the potential use of the available genomic data is determined. As part of the scoping step, the available human, toxicology, and genomics studies are reviewed to determine their use to the genomic data set evaluation. For instance, the toxicity, human, and toxicogenomic data sets are considered together to determine the relationship (i.e., degree of phenotypic anchoring) between gene and pathway changes to health or toxicity outcomes. As a result of the scoping step, questions are posed to direct and focus the evaluation of the genomic data set.

The next steps include detailed evaluations directed by the formulated questions of the toxicity and/or epidemiological data sets and the toxicogenomic data set. For example, when genomic data are available to inform mechanisms of action or MOAs, the toxicogenomic and toxicity data sets can be evaluated together, relating the affected endpoints (identified in the toxicity data set evaluation) to the genes and/or pathways (identified in the toxicogenomic data set evaluation) to establish or formulate hypotheses about an MOA. In addition to informing the mechanisms of action and the MOAs, genomic data also have the potential to inform inter- and intraspecies toxicodynamic differences, toxicokinetics, and dose-response assessment, depending on the genomic study design (e.g., species, organ, single dose vs. multiple doses, genomic method) of the available data. The approach also includes new analyses of the genomic data for the purpose of risk assessment when data are available and such new analyses may address questions that are relevant to the risk assessment.

1.2. DBP CASE STUDY

For the DBP case-study example, consideration of risk assessment information and steps was accomplished in two parallel processes. We utilized the data set summaries and data gaps identified in the external review draft IRIS Tox Review for DBP (U.S. EPA, 2006a) and asked whether the genomic data set could inform any of these data gaps. In parallel, the DBP genomic data set was considered, in light of all risk assessment aspects that these data might inform. As a

result of following these two processes, we posed two specific case-study questions that the available genomic data for DBP had the potential to inform:

- *Do the toxicogenomic data inform the mechanisms of action and/or MOAs for DBP?*
- *Do the toxicogenomic data inform interspecies toxicodynamic differences?*

The team considered it highly likely that the DBP toxicogenomic data set could inform the modes or mechanisms of action. The team considered it possible, but less certain, that the cross-species differences in one or more DBP MOAs could be informed by evaluating genomic data (e.g., DNA sequence data).

Additional questions were excluded because appropriate data were lacking. For example, one question of great interest is, *Do the toxicogenomic data inform dose-response?* However, this question could not be addressed in this case study because there were no dose-response genomic data for DBP. Few chemicals have available dose-response genomic data and DBP is not unusual in this respect. The evaluation of the one available DBP dose-response gene expression study, although not global, is discussed in the report. As a result of the DBP genomic data set limitations, the case study focuses on the qualitative application of genomic data to risk assessment. In addition, exposure assessment was not considered in this approach because the case study was performed using the IRIS chemical assessment model, which only includes hazard identification and dose-response steps of the risk assessment paradigm.

We found that the DBP toxicogenomic data did inform the mechanism of action, and generated hypotheses about possible additional MOAs, for DBP and male reproductive developmental outcomes. There is substantial evidence in the published literature that a number of the gene expression changes observed in genomic studies are phenotypically anchored for a number of the male reproductive developmental outcomes observed after *in utero* DBP exposure in the rat. The available genomic and other gene expression data, hormone level data, and toxicity data for DBP are instrumental in the establishment of two MOAs: (1) a decrease in fetal testicular testosterone (T); and (2) a decrease in Insulin-like 3 (*Insl3*) expression. The well-established MOA for a number of the male reproductive developmental effects observed in the male rat after *in utero* DBP exposure, is a decrease in fetal testicular T. The genomic and single gene expression data, after *in utero* DBP exposure, identified changes in genes involved in

steroidogenesis and cholesterol transport, consistent with the observed decrease in fetal testicular T. Decreased *Insl3* expression is a second well-established MOA responsible, in conjunction with reduced T, for the undescended testis descent effect observed following *in utero* DBP exposure. *Insl3* is required for one of the two steps of testis descent which is supported by reverse transcription-polymerase chain reaction (RT-PCR) and *in vivo* toxicology study results.

Evaluating genomic and toxicity data together also provides information on putative novel MOAs. A number of the DBP toxicity and toxicogenomic studies were performed in the same strain of rat using similar doses and exposure intervals that allowed for comparisons across studies. In this case study, rodent reproductive developmental toxicity studies were evaluated for low incidence and low-dose findings and for the male reproductive developmental effects that currently do not have an explained MOA (termed "unexplained endpoints"). In the case study, we focused on the outcomes in the testes because all, but one, of the DBP toxicogenomic studies were performed on testes. We identified five testiscular endpoints without a known MOA that were pursued further in the evaluation of the toxicogenomic data set.

The nine published RT-PCR and microarray studies in the rat were evaluated as part of the toxicogenomic and associated gene expression data set to identify genes and pathways affected after *in utero* DBP exposure. Both the microarray data set alone and the entire gene expression data set (including all gene expression studies including microarray studies) were evaluated for consistency of findings. At the gene level, the findings from the DBP genomic studies (i.e., microarray, RT-PCR, and protein expression) were relatively highly correlated with one another in both the identification of differentially expressed genes (DEGs) and their direction of effect. The evaluation of the published toxicity and toxicogenomic studies corroborates the two known MOAs for DBP.

The published microarray studies for DBP focused primarily on pathways related to the reduced fetal testicular T MOA, such as the steroidogenesis pathway. We performed new analyses of the data from one rat testes microarray study in order to identify all possible pathways significantly affected by *in utero* DBP exposure. Using two different analytical methods, pathways associated with the two well-established MOAs (decreased *Insl3* and fetal testicular T), as well as new processes (e.g., growth and differentiation, transcription, cell adhesion) and pathways (e.g., *Wnt* signaling, cytoskeleton remodeling) not associated with either *Insl3* or steroidogenesis pathways, were identified. The newly identified putative pathways may

play a role in the regulation of steroidogenesis (i.e., related to a known MOA for DBP) or, alternatively, may inform additional MOAs for one or more unexplained outcomes in the testes. The new analyses and the approach allowed us to develop hypotheses about possible DBP MOAs for some male reproductive developmental outcomes.

To address the question of whether the available genomic data for DBP could inform the interspecies toxicodynamic part of the MOA, genomic data were evaluated to inform interspecies differences in the steroidogenesis pathway, relevant to the decreased fetal testicular T MOA. We explored the development of new methods to evaluate interspecies toxicodynamic (TD) differences. To evaluate cross-species similarity metrics for the steroidogenesis gene and pathway for rats and humans, we explored three approaches: protein sequence similarity; pathway network similarities; and promoter region conservation. Preliminary results from all three methods suggest that steroidogenesis genes are relatively highly conserved between rats and humans. However, we do not recommend utilizing these data to inform interspecies uncertainty for DBP because it is difficult to make unequivocal conclusions regarding a "high" versus "low" degree of conservation for the genes in this pathway based on these data alone. With further refinement and improved data sources, these methods could potentially be applied to other chemical assessments.

New methods for evaluating microarray data for the purposes of risk assessment were explored and developed during the DBP case study. A new pathway analysis method, the pathway activity level method, was developed and tested with two DBP study data sets. The pathway activity level method determines pathway level changes as the initial step as opposed to standard pathway analysis methods in which DEGs are first identified, followed by mapping of the DEGs to pathways, as a second step. Further, the pathway activity level method was used to evaluate time-course microarray data. A preliminary gene network model for DBP, based on the results from one time-course study, identified a temporal sequence of gene expression and pathway interactions that occur over an 18-hour interval within the critical window of exposure for DBP and testicular development effects.

1.3. RECOMMENDATIONS

In addition to following the principles of the approach (i.e., systematically consider all types of information with respect to the steps of risk assessment, identify questions to direct the

evaluation, and evaluate genomic data and toxicity data together), several specific methodological recommendations arose from the DBP case-study experience. The first two recommendations are straightforward and could reasonably be performed by a risk assessor with basic training in genomics data evaluation and interpretation. The third recommendation requires expertise in genomic data analysis methods for implementation. The recommendations are presented below:

1. *Evaluate the genomic and other gene expression data for consistency of findings across studies to provide a WOE evaluation of the affected gene expression and pathways.* Some simple methods, such as using Venn diagrams and gene expression compilation approaches, can be applied to risk assessment. When evaluating the consistency of toxicogenomic data findings, it is advantageous to include all available gene expression data (single gene, global gene expression, protein, RNA) because single gene expression techniques have been traditionally used to confirm the results of global gene expression studies and because single gene expression data add to the database.

2. *Perform benchmark dose (BMD) modeling on high-quality RT-PCR dose-response studies of genes known to be in the causal pathway of an MOA or outcome of interest.* Obtaining a BMD and benchmark dose lower confidence limit (BMDL) is a useful starting point for both linear low-dose extrapolation and reference value approaches. We are not indicating which approach is appropriate to take for making predictions about the potential risk below the BMD or BMDL. "High quality" is defined in this context as a well-conducted study that assessed enough animals and litters for sufficient statistical power for characterizing the mean responses and the variability (interlitter and intralitter).

3. *Perform new analysis of toxicogenomic data in cases when the new analysis is likely to yield new information that would be useful to the risk assessment. Examples include:*

    - Perform a new pathway analysis in order to identify all affected pathways or other risk assessment applications. When the available published microarray studies have been conducted for purposes (e.g., basic science, pharmaceutical development) other than risk assessment, it may be useful to reanalyze the raw data for risk assessment purposes. Information about all affected pathways may contribute to an understanding of the mechanisms and MOAs.

    - Identify the genes and pathways affected over a critical window of exposure if global gene expression time-course data are available. Specifically, by developing a gene network over time, it may be possible to identify the earliest affected genes and/or pathways, which in turn may represent the earlier or initiating events for the outcome of interest.