Building and Evaluating the Utility of an Adverse Outcome Pathway Network for Arsenic-Induced Diabetes

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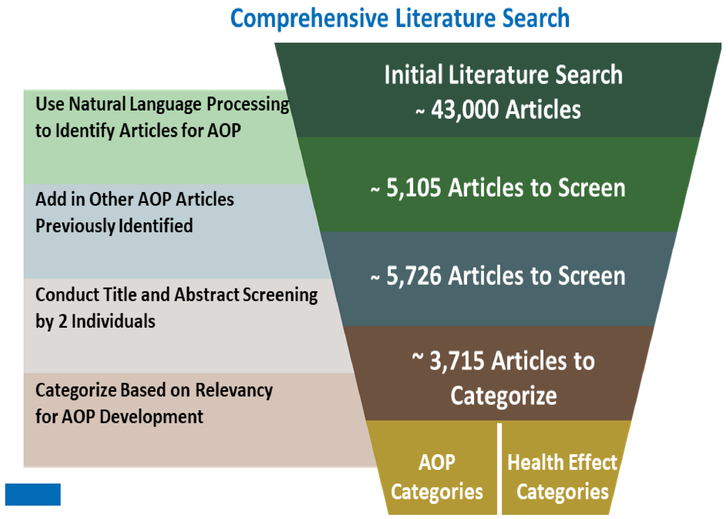
Identifying Arsenic-Specific Evidence

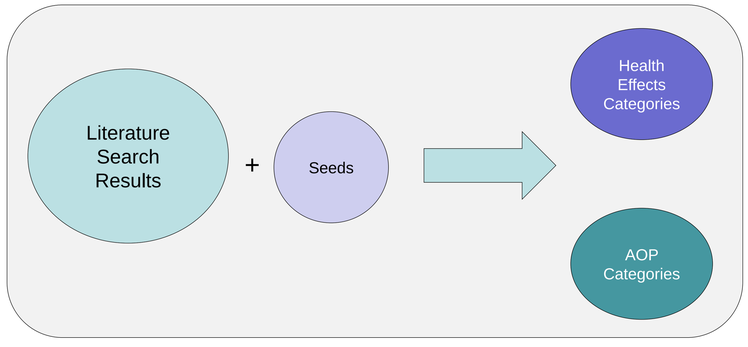
During the 2015 Arsenic Development Plan meeting with the National Academy of Science the National Research Council (NRC) recommended that EPA conduct mode of action (MOA) analyses to facilitate understanding of exposure-response relationships to below the observed range. The MOA approach was thought worthwhile to pursue as a complement to the analysis of epidemiological evidence (+1000 studies on a multitude of health outcomes). Importantly, the NRC was not clear whether an MOA analysis would be feasible for the stated purpose of understanding dose-response relationships below the observable range. As a case example, an MOA analysis of diabetes was pursued. Diabetes was one outcome the NRC asked EPA to prioritize for consideration in the assessment.

Our goal was to perform MOA analyses to aid in informing low dose extrapolation. Knowing that the arsenic database is very large we performed a targeted literature screening for MOA coupled with a clustering approach. We used machine learning to prioritize studies for screening. References were clustered into groups based on language similarity (i.e., natural language processing) using OmniViz reference visualization software (Instem, Staffordshire, United Kingdom).

- The initial literature search was designed to be comprehensive and not miss potentially relevant studies; clustering helped more efficiently identify those references most likely to contain data relevant to hazard identification.
- Approximately 900 additional references were used as “seeds;”
- The “seeds” and literature search results were combined, and, using natural language processing, the titles and abstracts of the references were grouped based on similarity.
- Reference clusters containing one or more of the “seed” references were selected to create the health effects cluster of 3,715 references that were then manually screened for relevance. Approximately 196 studies clustered to the diabetes outcome tag.
- No particular individual study evaluation tool was used due to the large number of studies, diversity in the type of mechanistic considered in the AOP analysis, and lack of accepted study evaluation tool at the time of the analysis

We decided to use the AOP framework to: organize and synthesize the refences & examine mechanistic commonalities among diseases and response modifiers, including susceptibility factors; help inform quantitative data. Conceptual models built and arsenic data overlay on top.





~ 3,715 Papers into 2 Broad Categories

Health Effect Specific		AOP Specific	
102	Bladder	218	ADME
116	Cardiovascular	426	Cell viability, Proliferation, Cycle Changes
81	Developmental	86	Cellular Differentiation, Malignant Transformation
21	Digestive System	354	Gene Expression Changes
70	Endocrine System / Diabetes	96	Immune Mechanisms
88	Hematopoietic System	75	Endocrine Mechanisms
152	Immune System/Lymphatic	100	Epigenetic Mechanisms
266	Liver	241	Non-Oxidative DNA /Chromosomal Damage
122	Nervous System	29	Specific Proteotoxicity
93	Renal	653	Oxidative Stress Effects
84	Reproductive & Pregnancy	17	Regenerative Proliferation
110	Respiratory	95	Vascular Mechanisms
171	Skin	240	Other AOP
504	Cancer		
	Categories not mutually exclusive		

Step 1 Natural Language processing combined literature search results and 900 seeds and grouped the tiles and abstracts based on similarity; (Categories were not mutually exclusive).

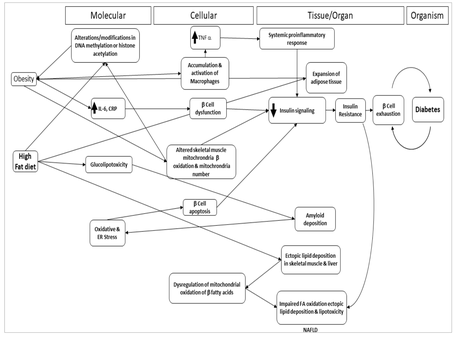


Figure 1: Postulated adverse outcome network for idiopathic diabetes in humans

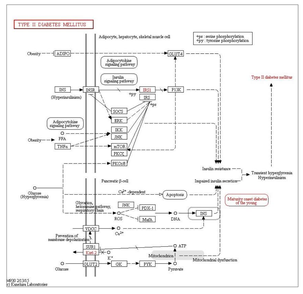


Figure 2: Kyoto Encyclopedia of Genes and Genomes (KEGG) database for diabetes mellitus in humans

Step 2: Establishing the Disease-Base AOP:

- In response to NRC’s tiering of health outcomes, MOA analyses was conducted for diabetes
- To delineate a postulated mode of action for arsenic-induced diabetes, the molecular basis for idiopathic diabetes disease, irrespective of a specific chemical insult, was first established.
- The AOP framework (Villenueve et al., 2014) was used to organize and identify important key events and data gaps in the arsenic-induced diabetes MOA.
- To identify the key events (KE) leading to the adverse outcome (AO), we performed a literature search in PubMed and identified peer reviewed medical reviews of idiopathic diabetes disease. We screened the results and included publications that described mechanisms and or molecular events in the onset of idiopathic diabetes mellitus disease. We assembled the AOP for idiopathic diabetes by binning the results into key events in the disease process. **(Figure 1)**
- The adverse outcome network was compared to the KEGG (Kyoto Encyclopedia of Genes and Genomes) database for diabetes mellitus in humans to ensure concordance **(see Figure 2).**

Lessons Learned

- We performed a targeted literature search for arsenic MOA and used clustering to identify and tag AOs using studies from the previous IRIS arsenic assessments as seeds. We took the information under the diabetes tag and overlaid the information onto the AOP for idiopathic diabetes disease. This allowed us to identify key events in the progression of iAs-induced diabetes. **(Figure 3; Table 1).**
- The clustering approach was useful in identifying mechanistic relevant literature from this very large database.
- The AOP approach was useful in organizing references for evaluating the MOA.
- The MOA evaluation provided additional support by identifying arsenic-specific mechanisms and risk modifier likely to increase risk of diabetes,
- The challenges presented in this analysis included the doses required to observe diabetogenic effects in rodent models were great and not physiologically relevant to humans.
- **This mode of action analysis was conducted for diabetes but ultimately determined to be insufficient to dictate dose-response shape, especially given the availability of epidemiological data that could be used for dose-response analysis. Therefore additional analyses were not pursued.**

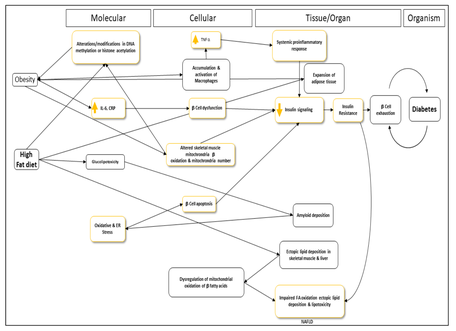


Figure 3: Postulated adverse outcome network for arsenic-induced

Key Event	Evidence	References
Molecular		
Alterations/modifications in DNA methylation or histone acetylation		Bailey KA et al, 2013 Hong et al., 2012, Xie Y et al., 2007
Increases in IL-6, CRP	Increased IL-6 transcription Increased CRP	Chakraborty et al 2012 Druwe et al, 2012 Wu et al 2003
Oxidative and ER Stress	ROS production in primary pancreatic β cells and hepatocytes derived from swiss albino mice; Increased pancreatic GSH, oxidative damage	Chakraborty et al 2012 Izquierdo-Vega et al, 2004
Cellular		
Increased TNF alpha	Increased TNF alpha transcription	Chakraborty et al, 2012 Yu et al, 2002
Altered skeletal muscle β oxidation and mitochondrial number		
Beta cell dysfunction	Decreased insulin secretion via decrease proteolysis of SNAP-25 Impaired insulin secretion & insulin mRNA expression	Diaz-Señor et al, 2008 Diaz-Señor et al, 2006
Beta cell apoptosis	Decreased β cell viability in response to 10 μM NaAsO3 in vivo (rat, oral)	Diaz-Señor et al, 2006
Tissue/Organ		
Systemic proinflammatory response	Elevated expression of IL-8 and TGF-β Increased miRNAs involved in diabetes and inflammation	Escobar-Garcia et al, 2012 Rager et al, 2014
Decreased insulin signaling	Inhibition of glucose-stimulated insulin secretion	Douillet et al, 2013
Insulin resistance	Inhibition of insulin-activated signal transduction in adipocytes Impaired glucose tolerance	Douillet et al, 2013 Wauson et al, 2002 Paul et al, 2008
Impaired fatty acid oxidation, ectopic lipid deposition and lipotoxicity	Increased pancreatic LPO	Izquierdo-Vega et al, 2004

Table 1: Representative evidence supporting adverse outcome pathway (AOP) network for arsenic-induced diabetes (Figure 3)

Step 3 Identifying Arsenic-specific Modification in the Diabetes Network

- After establishing a general disease-based network for diabetes, we performed a targeted literature search for arsenic MOA and used clustering to identify and tag AOs using studies from the previous IRIS arsenic assessments as seeds.
- We took the information under the diabetes tag and overlaid the information onto the AOP for idiopathic diabetes disease. This allowed us to identify key events in the progression of iAs-induced diabetes.

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