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Research Article

Chemical-Induced Phenotypes at CTD Help Inform the Predisease State and Construct Adverse Outcome Pathways

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ABSTRACT

The Comparative Toxicogenomics Database (CTD; http://ctdbase.org) is a public resource that manually curates the scientific literature to provide content that illuminates the molecular mechanisms by which environmental exposures affect human health. We introduce our new chemical-phenotype module that describes how chemicals can affect molecular, cellular, and physiological phenotypes. At CTD, we operationally distinguish between phenotypes and diseases, wherein a phenotype refers to a nondisease biological event: eg, decreased cell cycle arrest (phenotype) versus liver cancer (disease), increased fat cell proliferation (phenotype) versus morbid obesity (disease), etc. Chemical-phenotype interactions are expressed in a formal structured notation using controlled terms for chemicals, phenotypes, taxon, and anatomical descriptors. Combining this information with CTD's chemical-disease module allows inferences to be made between phenotypes and diseases, yielding potential insight into the predisease state. Integration of all 4 CTD modules furnishes unique opportunities for toxicologists to generate computationally predictive adverse outcome pathways, linking chemical-gene molecular initiating events with phenotypic key events, adverse diseases, and population-level health outcomes. As examples, we present 3 diverse case studies discerning the effect of vehicle emissions on altered leukocyte migration, the role of cadmium in influencing phenotypes preceding Alzheimer disease, and the connection of arsenic-induced glucose metabolic phenotypes with diabetes. To date, CTD contains over 165 000 interactions that connect more than 6400 chemicals to 3900 phenotypes for 760 anatomical terms in 215 species, from over 19 000 scientific articles. To our knowledge, this is the first comprehensive set of manually curated, literature-based, contextualized, chemical-induced, nondisease phenotype data provided to the public.

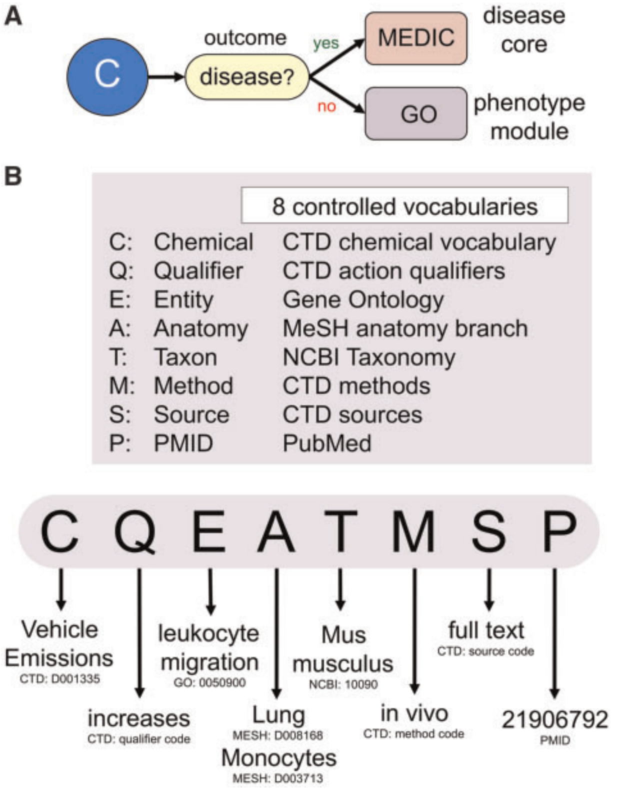
Key words: phenotype; database; curation; chemical; disease; adverse outcome pathway.

Since 2004, the freely available public Comparative Toxicogenomics Database (CTD; http://ctdbase.org/) has pioneered novel biocuration paradigms (Davis *et al.*, 2011b, 2015) to code, harmonize, and integrate a diverse compendium of toxicological (Davis *et al.*, 2009, 2011a, 2013a, 2017; Mattingly *et al.*, 2006), environmental (Davis *et al.*, 2008; Gohlke *et al.*, 2009; Planchart *et al.*, 2018), pharmaceutical (Davis *et al.*, 2013c; Pelletier *et al.*, 2016), and exposure data (Grondin *et al.*, 2016, 2018) from the scientific literature to help advance understanding about chemical effects on human health. CTD scientists read and manually curate information from peer-reviewed articles to transform authors' text, tables, figures, and supplemental files into annotated knowledge. This process, using a suite of controlled vocabularies and ontologies with accession identifiers, standardizes and organizes disparate data into a structured format, making it cohesive, manageable, and computable for discovery. These interactions also include species information, allowing data to be analyzed across taxa, including model laboratory organisms and humans. Content is derived from 3 integrated modules: CTD's *toxicogenomic core* details more than 1.7 million chemical-gene/protein interactions, *disease core* has over 245 000 chemical-disease and gene-disease associations, and *exposure module* contains over 111 000 curated statements relating environmental stressors, receptors, biomarker measurements, and outcomes.

Many toxicology articles, however, do not report a disease as an endpoint; rather, nonpathological cellular and physiological events are described as being influenced by chemicals. To capture this critical data, we designed a fourth module to curate such nondisease events in a controlled manner.

Here, we describe our latest project that curates chemical-induced phenotypes. Operationally, CTD distinguishes between the concepts of "disease" and "phenotype" wherein a phenotype refers to a biological outcome that is not inherently a disease. For example, smooth muscle cell migration is a phenotype while distal myopathy is a disease, decreased parathyroid hormone secretion is a phenotype while Barakat syndrome is a disease, and abnormal blood pressure is a phenotype while idiopathic orthostatic hypotension is a disease, etc. This operational distinction between phenotypes and diseases allows us to harness 2 unique controlled vocabularies (one for phenotypes and another for diseases) to capture both types of chemical-induced outcomes reported in the literature, with phenotype curation often providing greater molecular and biological specificity (eg, "cell migration involved in sprouting angiogenesis" and "regulation of cardiac muscle contraction by calcium ion signaling") compared with disease names, which often on their own provide no intuitive biological insight (eg, Fanconi syndrome, Arnold-Chiari malformation, Gilbert disease). For disease curation, CTD uses the MEDIC disease vocabulary (Davis *et al.*, 2012b); consequently, any chemical-induced outcome that does not appear in MEDIC (and thus cannot be curated via our disease core module) is considered de facto to be a phenotype and instead is captured using the phenotype paradigm when possible. These phenotypes potentially provide insight to the presymptomatic biological milieu before the overt manifestation of a disease, and understanding the predisease states may help elucidate the molecular and cellular backdrops to aid potentially in earlier clinical diagnoses and better therapeutic interventions (Celato *et al.*, 2013; McHale *et al.*, 2010; Sakai and Otomo, 2016; Yu *et al.*, 2017).

Capturing chemical-induced phenotypes in a structured format with controlled terms and integrating data with CTD's extensive environmental content helps address the community need to link phenotypes to the environment and make the information computable (Deans *et al.*, 2015). Combining CTD's chemical-gene interactions with key phenotype events, chemical/gene-disease associations, and exposure content can also generate predictive adverse outcome pathways (AOPs) and empower toxicologists to develop testable hypotheses about environmental diseases (Mortensen *et al.*, 2018; Nymark *et al.*, 2018; Oki *et al.*, 2016; Taboureau and Audouze, 2017; Villeneuve *et al.*, 2014a, 2014b; Wittwehr *et al.*, 2017). Furthermore, our unique chemical-phenotype content complements the myriad of well-established gene-phenotype systems for model organisms, such as the Monarch Initiative (Mungall *et al.*, 2017), Mouse Phenome Database (Bogue *et al.*, 2018), RGD PhenoMiner (Laulederkind *et al.*, 2013), and ZFIN (Howe *et al.*, 2017). Because many of these external databases use the same controlled vocabularies and accession identifiers for gene symbols, gene annotations, and diseases, the information can be cross-integrated to make novel computational discoveries, allowing gene-phenotype information to be brought into the chemical environment provided by CTD.

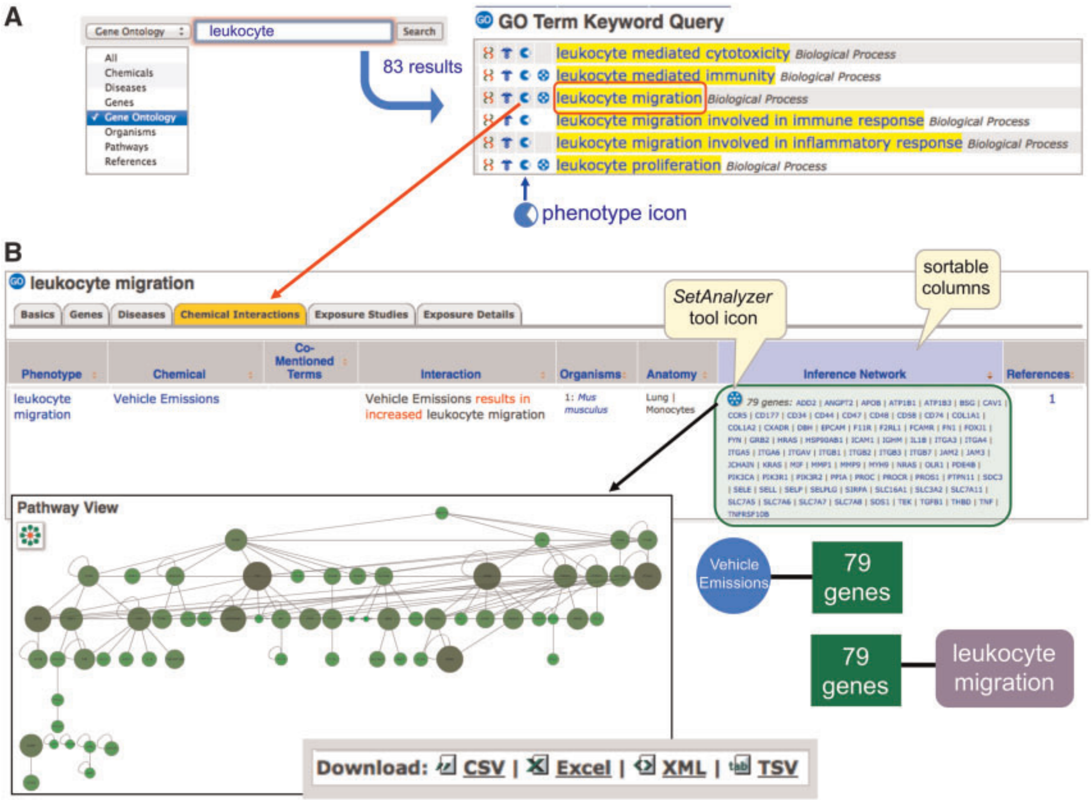


8 controlled vocabularies		
C:	Chemical	CTD chemical vocabulary
Q:	Qualifier	CTD action qualifiers
E:	Entity	Gene Ontology
A:	Anatomy	MeSH anatomy branch
T:	Taxon	NCBI Taxonomy
M:	Method	CTD methods
S:	Source	CTD sources
P:	PMID	PubMed

Figure 1. CTD phenotype curation. A, When an article reports that a chemical (C) influences a biological outcome, the biocurator first determines if that outcome exists as a term in the MEDIC disease vocabulary; if yes, then the interaction is curated via disease core; if no, the interaction is curated using the GO vocabulary in the phenotype module. B, CTD biocurators leverage 8 controlled vocabularies to construct a chemical-phenotype statement that must include, at a minimum, a Chemical (C), Qualifier (Q), Entity phenotype (E), Anatomy (A), Taxon (T), Method (M), Source (S), and PubMed identification number (P). An example of terms for a chemical-phenotype statement is shown at the bottom (and its web display is shown in Figure 2B). Currently, source (S) and method (M) data are stored internally at CTD and not displayed on the public website.

MATERIALS AND METHODS

Training, triaging, and curating the phenotype literature. For training, CTD biocurators were provided with a curation manual (containing instructions, documentation, rules, tips, and policy issues) and test articles for practice curation. Extensive feedback was given during early project management to ensure all biocurators were adequately prepared and curating in a consistent manner. CTD's literature triaging process has been described in detail (Davis *et al.*, 2011b). All articles curated in CTD are identified using PubMed (Agarwala *et al.*, 2018), with chemical-centric queries to improve data completeness (Davis *et al.*, 2013b), targeted journal queries to enhance data currency (Davis *et al.*, 2012a), or exposure-themed queries for the exposure module (Grondin *et al.*, 2016). CTD-associated data are related to an article via its PubMed identification number (PMID). Articles slated for the toxicogenomic core, disease core, and exposure modules


A
Gene Ontology
leukocyte
Search
All
Chemicals
Diseases
Genes
✓ Gene Ontology
Organisms
Pathways
References
83 results
GO Term Keyword Query
leukocyte mediated cytotoxicity Biological Process
leukocyte mediated immunity Biological Process
leukocyte migration Biological Process
leukocyte migration involved in immune response Biological Process
leukocyte migration involved in inflammatory response Biological Process
leukocyte proliferation Biological Process
phenotype icon
B
leukocyte migration
Basics
Genes
Diseases
Chemical Interactions
Exposure Studies
Exposure Details
SetAnalyzer tool icon
sortable columns
Pathway View
Vehicle Emissions
79 genes
79 genes
leukocyte migration
Download: CSV | Excel | XML | TSV

Phenotype	Chemical	Co-Mentioned Terms	Interaction	Organisms	Anatomy	Inference Network	References
leukocyte migration	Vehicle Emissions		Vehicle Emissions results in increased leukocyte migration	1: *Mus musculus*	Lung | Monocytes	*79 genes:* ADD2 | ANGPT2 | APOB | ATP1B1 | ATP1B3 | BSG | CAV1 | CCR5 | CD177 | CD34 | CD44 | CD47 | CD48 | CD58 | CD74 | COL1A1 | COL1A2 | CXADR | DBH | EPCAM | F11R | F2RL1 | FCAMR | FN1 | FOXJ1 | FYN | GRB2 | HRAS | HSP90AB1 | ICAM1 | IGHM | IL1B | ITGA3 | ITGA4 | ITGA5 | ITGA6 | ITGAV | ITGB1 | ITGB2 | ITGB3 | ITGB7 | JAM2 | JAM3 | JCHAIN | KRAS | MIF | MMP1 | MMP9 | MYH9 | NRAS | OLR1 | PDE4B | PIK3CA | PIK3R1 | PIK3R2 | PPIA | PROC | PROCR | PROS1 | PTPN11 | SDC3 | SELE | SELL | SELP | SELPLG | SIRPA | SLC16A1 | SLC3A2 | SLC7A11 | SLC7A5 | SLC7A6 | SLC7A7 | SLC7A8 | SOS1 | TEK | TGFB1 | THBD | TNF | TNFRSF10B	1

Figure 2. Accessing chemical-phenotype interactions. A, A "Gene Ontology" (GO) Keyword Search Box query with "leukocyte" retrieves 83 results. Clicking the phenotype icon next to "leukocyte migration" opens up the chemical-phenotype interactions. B, The "Chemical Interactions" display is similar to other CTD pages, with data in a table format with sortable columns and hyperlinked terms that go to their respective pages in CTD. Shown is the interaction of how vehicle emissions affect the phenotype of leukocyte migration in mouse lung monocytes. The "Inference Network" lists 79 genes that have both a CTD curated interaction to vehicle emissions and, independently, have a GO-gene annotation to leukocyte migration, creating a chemical-gene-phenotype inference. Clicking the SetAnalyzer tool icon in front of the gene list generates a Pathway View map. A "Download" feature, found at the bottom of all CTD web pages, allows users to export data to their desktop in a variety of formats. For simplicity, all screenshots show an edited subset of actual web display.

are assigned to CTD biocurators on a routine basis. As articles are read for assigned projects, any reported phenotype data are curated concurrently in CTD's chemical-phenotype module.

Software engineering. CTD's web-based Curation Tool (CURAPP, curation application) has been previously described (Davis *et al.*, 2011b). CURAPP was modified to accommodate new syntax for the structured notation expressing phenotype-based chemical and gene relationships. The phenotype and anatomy vocabularies were integrated into CURAPP; revised and new schematic data structures were implemented, along with new reporting and quality control-related processes and correction mechanisms. Existing CTD load processes were modified and new ones developed to incorporate phenotype display mechanisms. Inference engines were developed to generate gene- and chemical-based inference networks. Anatomical terms from the Medical Subject Headings (MeSH) "Anatomy [A]" controlled vocabulary (Coletti and Bleich, 2001) were loaded to our public database in a requisite format sufficient to support hierarchical-based anatomy searches [similar to our existing chemical-, Gene Ontology- (GO), and organism-based search features]. Significant changes to CTD's database schemas, including the creation of new tables, and the modification of existing tables, were necessary to accommodate the new data storage requirements. CTD's Public Web Application (PWA) was modified to implement new display, search, and associated functionality requirements. All CTD data, including phenotype data, are stored in PostgreSQL database management systems. Load processes and inference engines are Java-based and run in a Linux environment. The CURAPP and PWA utilize a J2EE-based Model-View-Controller architecture within the context of Apache/Tomcat.

Data version. CTD is updated with new content on a monthly basis (http://ctdbase.org/about/dataStatus). All analyses and metrics reported here are derived from version 15351M (February 13, 2018).

GO-slim distribution analysis. A generic GO-slim list maintained by the GO Consortium was downloaded (http://www.geneontology.org/page/go-slim-and-subset-guide) and filtered for the 69 terms in "biological_process" category. Each phenotype term was looked up in CTD to record the number of associated chemical interactions. Because GO is a hierarchy, counts are subsumed; thus, the number of chemical interactions represents the CTD data associated with the term directly and all child/descendant terms.

Data analyses: leukocyte migration case study. Leukocyte data were collected from the relevant data-tabs on CTD's page for the GO term "leukocyte migration" (http://ctdbase.org/detail.go?type=go&acc=GO:0050900). A *Pathway View* map for genes associated with "vehicle emissions" and "leukocyte migration" was retrieved using the CTD web tool icon *SetAnalyzer* (http://

Table 1. CTD Chemical Interactions for Phenotype Terms, as Distributed Across GO-Slim

No. CTD Chemical Interactions	CTD Phenotype/GO-Slim Term	GO Term ID
29 640	Cell death	GO: 0008219
14 734	Biosynthetic process	GO: 0009058
12 638	Cellular nitrogen compound metabolic process	GO: 0034641
12 526	Lipid metabolic process	GO: 0006629
12 396	Cell proliferation	GO: 0008283
10 031	Catabolic process	GO: 0009056
9619	Response to stress	GO: 0006950
9111	Circulatory system process	GO: 0003013
7233	Transport	GO: 0006810
5783	Signal transduction	GO: 0007165
5391	Small molecule metabolic process	GO: 0044281
5375	Cofactor metabolic process	GO: 0051186
4816	Cellular protein modification process	GO: 0006464
4688	Homeostatic process	GO: 0042592
4356	Immune system process	GO: 0002376
4288	Sulfur compound metabolic process	GO: 0006790
3948	Anatomical structure development	GO: 0048856
3394	DNA metabolic process	GO: 0006259
3136	Cell differentiation	GO: 0030154
3115	Locomotion	GO: 0040011
2907	Cell motility	GO: 0048870
2901	Cell cycle	GO: 0007049
2345	Nucleobase-containing compound catabolic process	GO: 0034655
2181	Cell-cell signaling	GO: 0007267
1730	Transmembrane transport	GO: 0055085
1460	Mitotic cell cycle	GO: 0000278
1448	Autophagy	GO: 0006914
1366	Generation of precursor metabolites and energy	GO: 0006091
1125	Carbohydrate metabolic process	GO: 0005975
1038	Mitochondrion organization	GO: 0007005
1019	Chromosome organization	GO: 0051276
987	Reproduction	GO: 0000003
858	Cellular component assembly	GO: 0022607
761	Growth	GO: 0040007
758	Nucleocytoplasmic transport	GO: 0006913
651	Cell adhesion	GO: 0007155
585	Vesicle-mediated transport	GO: 0016192
559	Membrane organization	GO: 0061024
465	Nervous system process	GO: 0050877
348	Cellular amino acid metabolic process	GO: 0006520
309	Anatomical structure formation involved in morphogenesis	GO: 0048646
302	Cytoskeleton organization	GO: 0007010
295	Protein-containing complex assembly	GO: 0065003
291	Embryo development	GO: 0009790
234	Symbiont process	GO: 0044403
192	Cell morphogenesis	GO: 0000902
178	Plasma membrane organization	GO: 0007009
172	Secondary metabolic process	GO: 0019748
146	Developmental maturation	GO: 0021700
136	Protein maturation	GO: 0051604
125	Translation	GO: 0006412
59	Cytoskeleton-dependent intracellular transport	GO: 0030705
54	Extracellular matrix organization	GO: 0030198
52	Protein targeting	GO: 0006605
47	Cell division	GO: 0051301
38	Chromosome segregation	GO: 0007059
35	Mitotic nuclear division	GO: 0140014
34	Pigmentation	GO: 0043473
34	Cell junction organization	GO: 0034330
33	Ribonucleoprotein complex assembly	GO: 0022618
30	Nitrogen cycle metabolic process	GO: 0071941
16	Vacuolar transport	GO: 0007034
10	mRNA processing	GO: 0006397
9	Protein folding	GO: 0006457
5	tRNA metabolic process	GO: 0006399
1	Ribosome biogenesis	GO: 0042254
0	Cell wall organization or biogenesis	GO: 0071554
0	Photosynthesis	GO: 0015979
0	Transposition	GO: 0032196

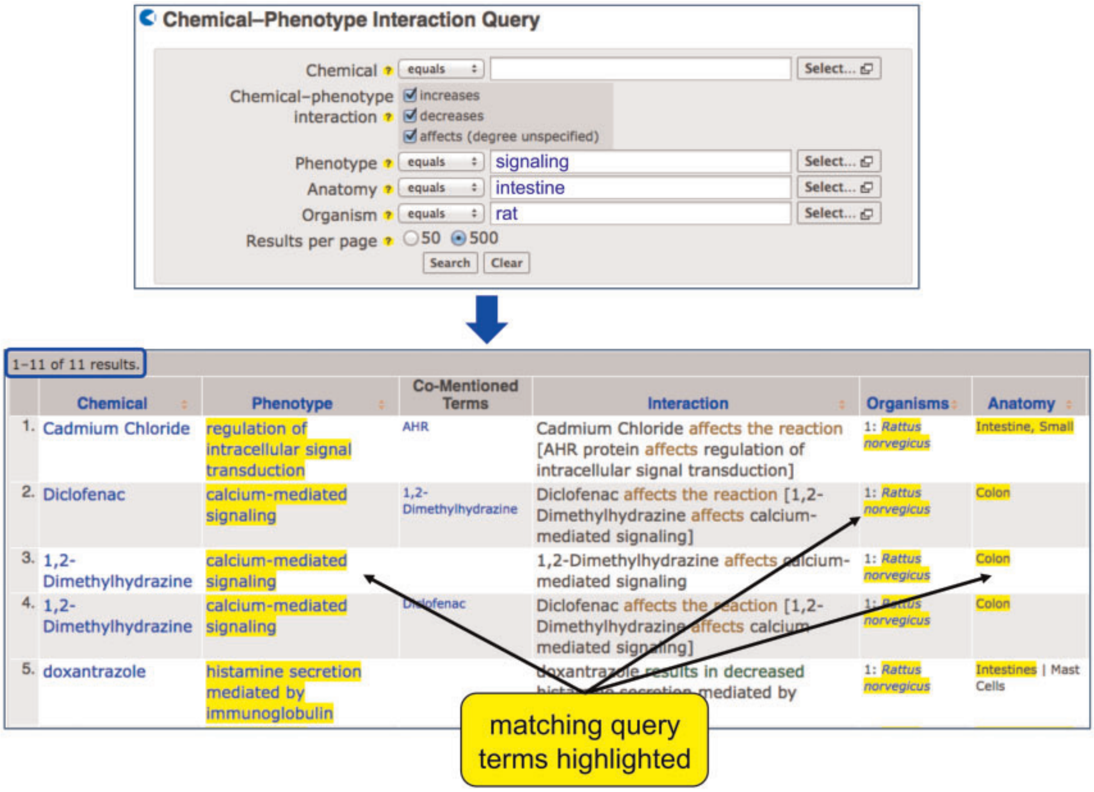
ctdbase.org/tools/analyzer.go) to display the gene-gene and protein-protein interactions derived from imported BioGRID data (Chatr-Aryamontri *et al.*, 2017; Davis *et al.*, 2015). The default map was manually configured (merged edges and tree view layout) and exported in PNG format. Genes were collected under the "Genes" data-tab and chemicals from the "Chemical Interactions" data-tab; all results were downloaded, sorted, and filtered to unique sets for manual inspection.

Data analyses: cadmium-Alzheimer's disease case study. Information was collected from the appropriate data-tabs on CTD's "Cadmium" page (http://ctdbase.org/detail.go?type=chem&acc=D002104). The 131 cadmium-associated phenotypes were derived from downloading the interactions under the "Phenotypes" data-tab and filtering the results to a set of unique terms. The 44 genes forming an Inference Network between cadmium and Alzheimer's disease (AD) were submitted to CTD's *Batch Query* (http://ctdbase.org/tools/batchQuery.go) to retrieve a list of 1, 641 GO biological process annotations. CTD's *MyVenn* tool (http://ctdbase.org/tools/myVenn.go) was used for all Venn analyses to prioritize phenotypes. Terms were manually inspected for GO ancestry using the CTD PWA (http://ctdbase.org/detail.go?type=go&acc=GO%3a0008150) and sorted to levels depending upon mappings. Subcellular level included terms mapping to "response to stimulus" (GO: 0006954), "signaling" (GO: 0023052), or "metabolic process" (GO: 0006006); cellular level included terms mapping to "cellular process" (GO: 0009987) or "cell proliferation" (GO: 0008283); and system level included ancestry terms "system process" (GO: 003008), "developmental process" (GO: 0001525), "behavior" (GO: 0007610), "reproduction" (GO: 0007283), or other. A few terms mapped to more than one level and were assigned based upon either the level that had a preponderance of mappings or the official definition provided for the term, whichever made the most biological sense.

RESULTS AND DISCUSSION

CTD Phenotype Curation Phases

In 2011, CTD initiated the manual curation of chemical-induced phenotypes as part of a collaborative project with Pfizer, Inc.



Chemical–Phenotype Interaction Query

Chemical equals [ ] Select...

Chemical–phenotype interaction ☑ increases ☑ decreases ☑ affects (degree unspecified)

Phenotype equals signaling Select...

Anatomy equals intestine Select...

Organism equals rat Select...

Results per page ○ 50 ● 500

1–11 of 11 results.

	Chemical	Phenotype	Co-Mentioned Terms	Interaction	Organisms	Anatomy
1.	Cadmium Chloride	regulation of intracellular signal transduction	AHR	Cadmium Chloride affects the reaction [AHR protein affects regulation of intracellular signal transduction]	1: *Rattus norvegicus*	Intestine, Small
2.	Diclofenac	calcium-mediated signaling	1,2-Dimethylhydrazine	Diclofenac affects the reaction [1,2-Dimethylhydrazine affects calcium-mediated signaling]	1: *Rattus norvegicus*	Colon
3.	1,2-Dimethylhydrazine	calcium-mediated signaling		1,2-Dimethylhydrazine affects calcium-mediated signaling	1: *Rattus norvegicus*	Colon
4.	1,2-Dimethylhydrazine	calcium-mediated signaling	Diclofenac	Diclofenac affects the reaction [1,2-Dimethylhydrazine affects calcium-mediated signaling]	1: *Rattus norvegicus*	Colon
5.	doxantrazole	histamine secretion mediated by immunoglobulin		doxantrazole results in decreased histamine secretion mediated by	1: *Rattus norvegicus*	Intestines | Mast Cells

matching query terms highlighted

Figure 3. Chemical-Phenotype Interaction Query page. CTD's Chemical-Phenotype Interaction Query page allows users to perform advanced searches, combining different parameters. Searching for a high-level phenotype (signaling) in an anatomical category (intestine) in rat retrieves 11 hits. Because many CTD curation vocabularies are hierarchical, the query returns child terms as well, and all matching terms are highlighted to alert the user.

(Davis *et al.*, 2013c). For this initial project, CTD was restricted to using 143 phenotype terms preselected by Pfizer from the "Phenomena and Process [G]" branch of MeSH (Coletti and Bleich, 2001). In 3 months, CTD biocurators manually curated 9488 articles and generated 38 083 interactions for 2849 chemicals and 121 MeSH phenotypes (Davis *et al.*, 2013c).

After 2011, CTD continued with phenotype curation as a new in-house module, but expanded the available phenotype terms by switching to the GO as a source of vocabulary terms for biological outcomes. Leveraging the GO affords several advantages (Ashburner *et al.*, 2000). It is a robust vocabulary that provides a greater depth and broader range of terms that better reflect the multitude of outcomes reported in the toxicology literature. It is a well-known resource that is widely used by other databases in the scientific community for gene annotations. All GO terms have comprehensive definitions and stable accession identifiers, the latter of which allows GO annotations to act as a nexus to connect, integrate, and harmonize knowledge from domains curated across a variety of databases. For example, CTD collects and stores all the GO-gene annotations from NCBI Gene (Maglott *et al.*, 2011); integration of these external GO-gene annotations with CTD's gene-disease content provides a novel way to explore disease mechanisms based upon shared GO terms as opposed to shared gene symbols (Davis *et al.*, 2016). Finally, the GO vocabulary is structured as a multi-level hierarchy, enabling users to view, navigate, and meta-analyze the associated content at different levels of granularity. This hierarchy also allows GO terms to be computationally sorted into categories that differentiate subcellular events (eg, "JNK cascade") from larger developmental and system level processes (eg, "learning and memory"). Because of these numerous advantages, we transitioned to using GO as the source of phenotype terms.

To reconcile our legacy MeSH-based phenotype data-set with the new GO-based phenotype content, a CTD biocurator manually mapped the initial MeSH terms to their best equivalent terms in GO. This mapping and translation process retained 35 538 interactions (93%) to seed the new GO-based chemical-phenotype module. Going forward, all CTD chemical-phenotype interactions use only GO as the source of nondisease term events.

CTD Phenotype Curation Process

When an article reports a chemical's effect upon a disease, the information is curated via CTD's disease core using the MEDIC disease vocabulary (Davis *et al.*, 2012b); any reported outcome that is not in MEDIC is considered de facto to be a phenotype and is captured using GO terms in the chemical-phenotype module where possible (Figure 1A).

A CTD chemical-phenotype interaction statement includes 8 types of data (C-Q-E-A-T-M-S-P) annotated using 8 controlled vocabularies (Figure 1B), including, at a minimum: C, a chemical from the CTD Chemical Vocabulary (Davis *et al.*, 2009); Q, a CTD action qualifier that reflects the direction of the interaction ("increases," "decreases," or "affects," when not specified by the authors); E, the entity phenotype from GO; A, an anatomical term from the MeSH "Anatomy [A]" branch (Coletti and Bleich, 2001); T, an organism from NCBI Taxonomy (Federhen, 2012); M, a CTD method code (*in vivo*, *in vitro*); S, the CTD information source code (abstract, full text); and P, the article identifier

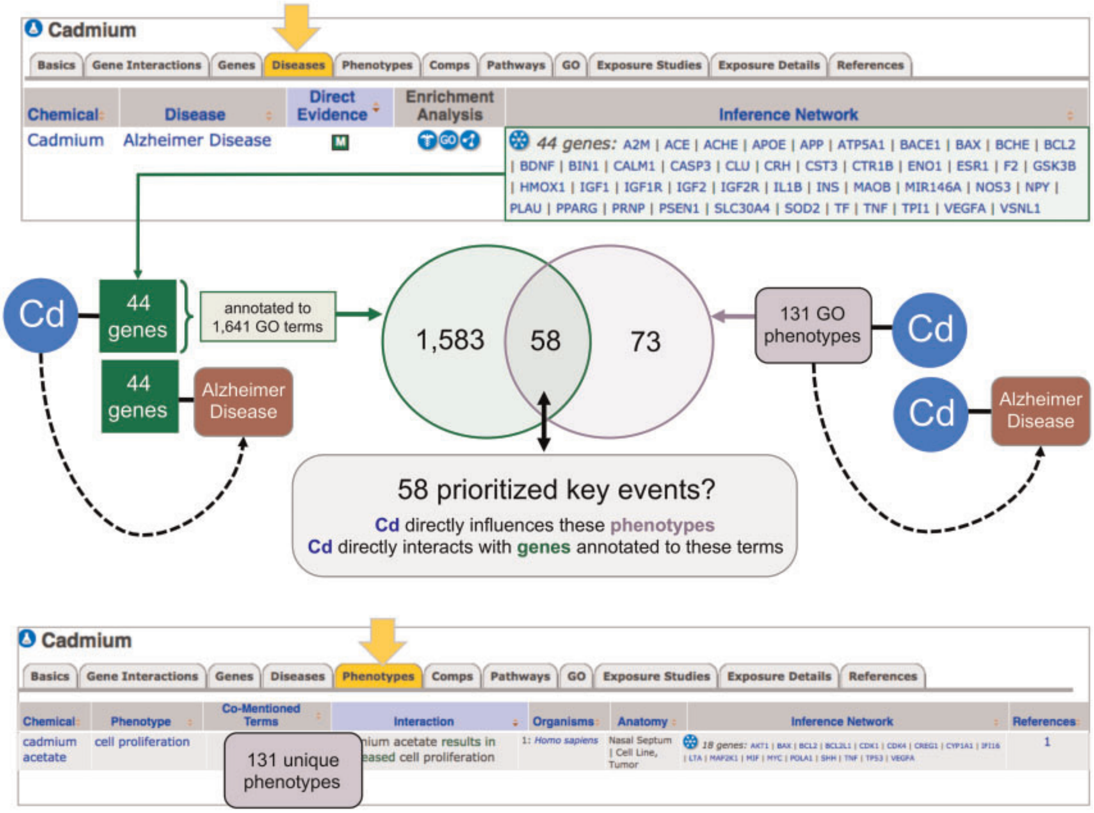

Cadmium
Basics | Gene Interactions | Genes | Diseases | Phenotypes | Comps | Pathways | GO | Exposure Studies | Exposure Details | References
Chemical | Disease | Direct Evidence | Enrichment Analysis | Inference Network
Cadmium | Alzheimer Disease | M
44 genes: A2M | ACE | ACHE | APOE | APP | ATP5A1 | BACE1 | BAX | BCHE | BCL2 | BDNF | BIN1 | CALM1 | CASP3 | CLU | CRH | CST3 | CTR1B | ENO1 | ESR1 | F2 | GSK3B | HMOX1 | IGF1 | IGF1R | IGF2 | IGF2R | IL1B | INS | MAOB | MIR146A | NOS3 | NPY | PLAU | PPARG | PRNP | PSEN1 | SLC30A4 | SOD2 | TF | TNF | TPI1 | VEGFA | VSNL1
Cd
44 genes
annotated to 1,641 GO terms
44 genes
Alzheimer Disease
1,583
58
73
131 GO phenotypes
Cd
Cd
Alzheimer Disease
58 prioritized key events?
Cd directly influences these phenotypes
Cd directly interacts with genes annotated to these terms
Cadmium
Basics | Gene Interactions | Genes | Diseases | Phenotypes | Comps | Pathways | GO | Exposure Studies | Exposure Details | References
Chemical | Phenotype | Co-Mentioned Terms | Interaction | Organisms | Anatomy | Inference Network | References
cadmium acetate | cell proliferation | nium acetate results in eased cell proliferation | 1: Homo sapiens | Nasal Septum | Cell Line, Tumor | 18 genes: AKT1 | BAX | BCL2 | BCL2L1 | CDK1 | CDK4 | CREG1 | CYP1A1 | IFI16 | LTA | MAP2K1 | MIF | MYC | POLA1 | SHH | TNF | TP53 | VEGFA | 1
131 unique phenotypes

Figure 4. Prioritizing phenotypes that connect cadmium to Alzheimer's disease (AD). The "Diseases" (top arrow) and "Phenotypes" (bottom arrow) data-tabs for the chemical Cadmium (Cd) are depicted from CTD (edited view). In the top panel, the Inference Network computes a list of 44 genes that directly interact with cadmium and also have a direct association with AD, allowing an inferred relationship (dotted arrow) to be made between Cd and AD: these 44 inference genes are annotated to 1641 GO terms (by external databases). As well, there are 131 unique phenotypes directly associated with Cadmium (bottom panel) that can also be inferred to the disease. A Venn analysis (middle panel) reveals 58 GO terms/phenotypes that are shared between these 2 independently derived data-sets; consequently, these 58 phenotypes could be prioritized as key events (KEs) connecting cadmium exposure to AD.

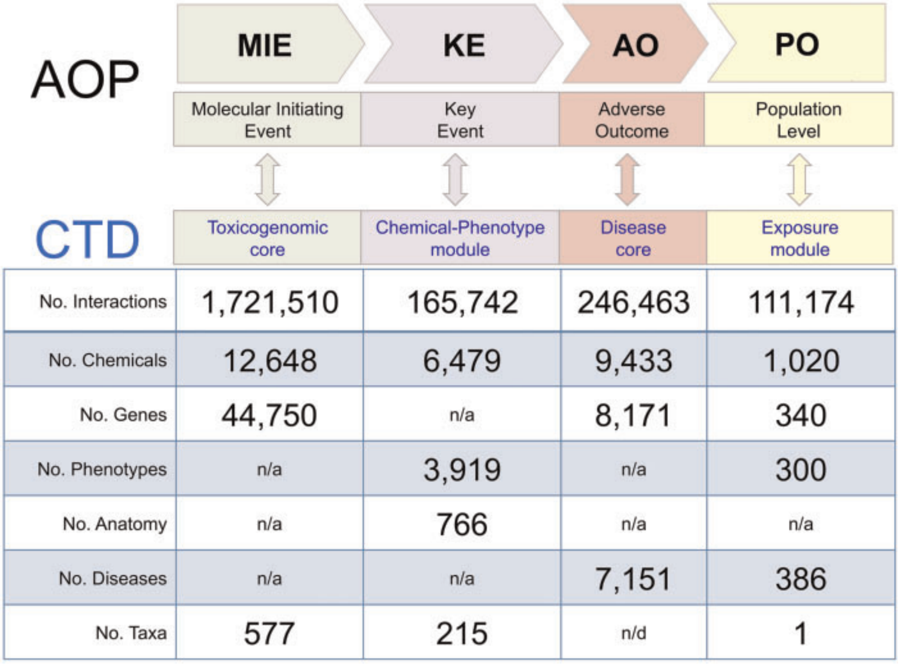
(PMID) from NCBI PubMed (Agarwala *et al.*, 2018). "Not reported" is allowed for both taxon and anatomy fields if the authors do not provide this information. CTD biocurators are careful to focus on collecting chemical-induced phenotypes, and not gene-associated phenotypes; this helps keep our resources focused on chemical-centric data and avoids duplicating gene-phenotype information that is more appropriately curated at other databases; however, we have found that sometimes a gene needs to be included as part of a complex chemical-phenotype interaction to best reflect the authors' intentions; when necessary, official gene symbols from NCBI Gene (Maglott *et al.*, 2011) are used in conjunction with the chemical and the phenotype.

CTD's practice of implementing well-established, community-accepted controlled vocabularies (with definitions and accession identifiers) and having all manually curated interactions traced directly to the source article ensures that the information conforms with the FAIR principle, allowing the data to be Findable, Accessible, Interoperable, and Reusable (Wilkinson *et al.*, 2016).

The data captured in this new module are wide-ranging: over 6400 chemicals and 3900 phenotypes reported in 215 species using 760 anatomy terms, manually curated from more than 19 000 articles published in 1200 scientific journals. To explore the distribution of CTD phenotypes and provide a perspective of the knowledge landscape covered by this module, we analyzed our curated content against the generic GO-slim list developed and maintained by the GO Consortium (Davis *et al.*, 2010). The GO-slim list is a summarized subset of the entire biological process ontology and provides a broad, top-level view of the available information, without the detailed granular terminology that provides the depth and specificity to GO. The top phenotype terms curated with the most number of CTD chemical interactions include cell death, general and specific metabolic processes (including biosynthesis and catabolism), cell proliferation, response to stress, circulatory system processes, transport, and signal transduction (Table 1). Currently, CTD has curated interactions for every term in this GO-slim, except 3 (2 of which are processes found in plants and fungi which are taxa that CTD does not include). This extensive coverage of GO-slim illustrates the breadth of CTD's phenotype content from the toxicology literature.

Accessing CTD Phenotype Data

Chemical-phenotype content can be accessed using the *Keyword Search Box* in the upper right hand corner of any CTD page by querying either the "Chemical" or "GO" field (from the drop-down pick-list) with a term-of-interest (Figure 2A). A new data icon ("eye," for phenotype) identifies the retrieved matching terms that have chemical-phenotype associated data. Clicking the icon, or going to the "Chemical Interactions" tab on a respective GO page, shows all the curated chemical-phenotype interactions in a tabular web-display. Users can sort the information by clicking on any column header, and discover, for



	Toxicogenomic core	Chemical-Phenotype module	Disease core	Exposure module
No. Interactions	1,721,510	165,742	246,463	111,174
No. Chemicals	12,648	6,479	9,433	1,020
No. Genes	44,750	n/a	8,171	340
No. Phenotypes	n/a	3,919	n/a	300
No. Anatomy	n/a	766	n/a	n/a
No. Diseases	n/a	n/a	7,151	386
No. Taxa	577	215	n/d	1

Figure 5. Leveraging CTD curation modules for AOP components. An adverse outcome pathway (AOP) is composed of ordered components (top rows): the molecular initiating event (MIE) between a chemical and gene product launches a series of KEs resulting in an adverse outcome (AO), which can have effects at the population level (PO). These components mirror curation modules at CTD (double arrows and middle row): toxicogenomic core, chemical-phenotype module, disease core, and exposure module. The numbers of manually curated interactions, chemicals, genes, phenotypes, anatomy terms, diseases, and taxa (organisms) in each CTD module are listed (note: the exposure module only curates data for humans). n/a, not applicable; n/d, not determined/displayed (data are collected but not yet publicly released).

example, a report that exposure to vehicle emissions affects leukocyte migration of monocytes in the lungs of mice (Figure 2B). Any comentioned terms (eg, chemicals, genes, and other phenotypes) are hyperlinked to their respective CTD pages, allowing users to easily traverse the database.

In addition to the directly curated interaction between vehicle emissions and leukocyte migration (Figure 2B), CTD also provides inference networks (Davis *et al.*, 2011a, 2013a, 2015). In this example, there are 3 pieces of independently derived information: (1) the chemical vehicle emissions directly affects the phenotype leukocyte migration (from chemical-phenotype module), (2) vehicle emissions have effects on 79 unique genes or proteins (from toxicogenomic core), and (3) independently, these same 79 genes have been (externally) annotated to the GO term leukocyte migration (integrated into CTD from the NCBI Gene database). Thus, these 79 genes provide a potential (inferred) mechanistic link connecting vehicle emissions to leukocyte migration. Clicking on the *SetAnalyzer* tool icon diagrams an extensive gene/protein interaction module (Figure 2B), quickly providing a starting point for testable molecular-genetic hypotheses for vehicle emission-induced leukocyte migration in mouse lungs.

Furthermore, users can leverage additional curated content in CTD to expand the analysis. Although vehicle emissions and leukocyte migration share 79 genes, there are currently 205 genes in CTD annotated to the GO term “leukocyte migration”, providing an additional 126 genes to test for influence by exposure to vehicle emissions. As well, there are 471 additional chemicals (other than vehicle emissions) that influence leukocyte migration, including 68 that specifically increase this phenotype in lung compartments of mice, including such additional traffic-related air pollutants as soot, ozone, and particulate matter. Having diverse data manually curated from a variety of articles, but harmonized using controlled vocabularies in one database, helps discover information to broaden hypotheses about traffic-related exposure and immune system processes.

Content also can be accessed using the Chemical-Phenotype Interaction Query page (http://ctdbase.org/query.go?type=phenotype). Parameters such as chemical, phenotype, anatomy, or organism can be specified and combined to perform an advanced query, enabling scientists to find data specific for certain taxa or anatomical sites (Figure 3). Because many CTD curation vocabularies (chemicals, phenotypes, anatomy, and organisms) are hierarchical, the query returns associations for the entered term as well as all child/descendant terms, and highlights the matches in the results. Thus, a neurotoxicologist can search with just the word “brain” and immediately retrieve over 12 500 interactions for 1330 chemicals and 710 phenotypes occurring in 115 anatomical parts (from “amygdala” to “white matter”) for 35 organisms, whereas a user searching with “heart” will discover 6100 cardiotoxic events for 910 chemicals, 420 phenotypes, and 18 structures from 21 species.

Illuminating the Predisease State

Using CTD’s transitive process, the curated set of chemical-phenotype interactions can be integrated with CTD’s chemical-disease associations from our disease core: if phenotype A interacts with chemical B, and, independently, chemical B is associated with disease C, then phenotype A can be inferred to disease C (via chemical B). These inferences generate potential biological phenotypes that may be modulated by the chemicals during the predisease/presymptomatic state. The heavy metal environmental pollutant cadmium has evidence suggesting a connection to AD; as well, cadmium modulates 131 unique phenotypes in CTD (Figure 4, top and bottom panels). These 131 phenotypes can be inferred to AD using the transitive process to explore potential biological reactions happening in a predisease

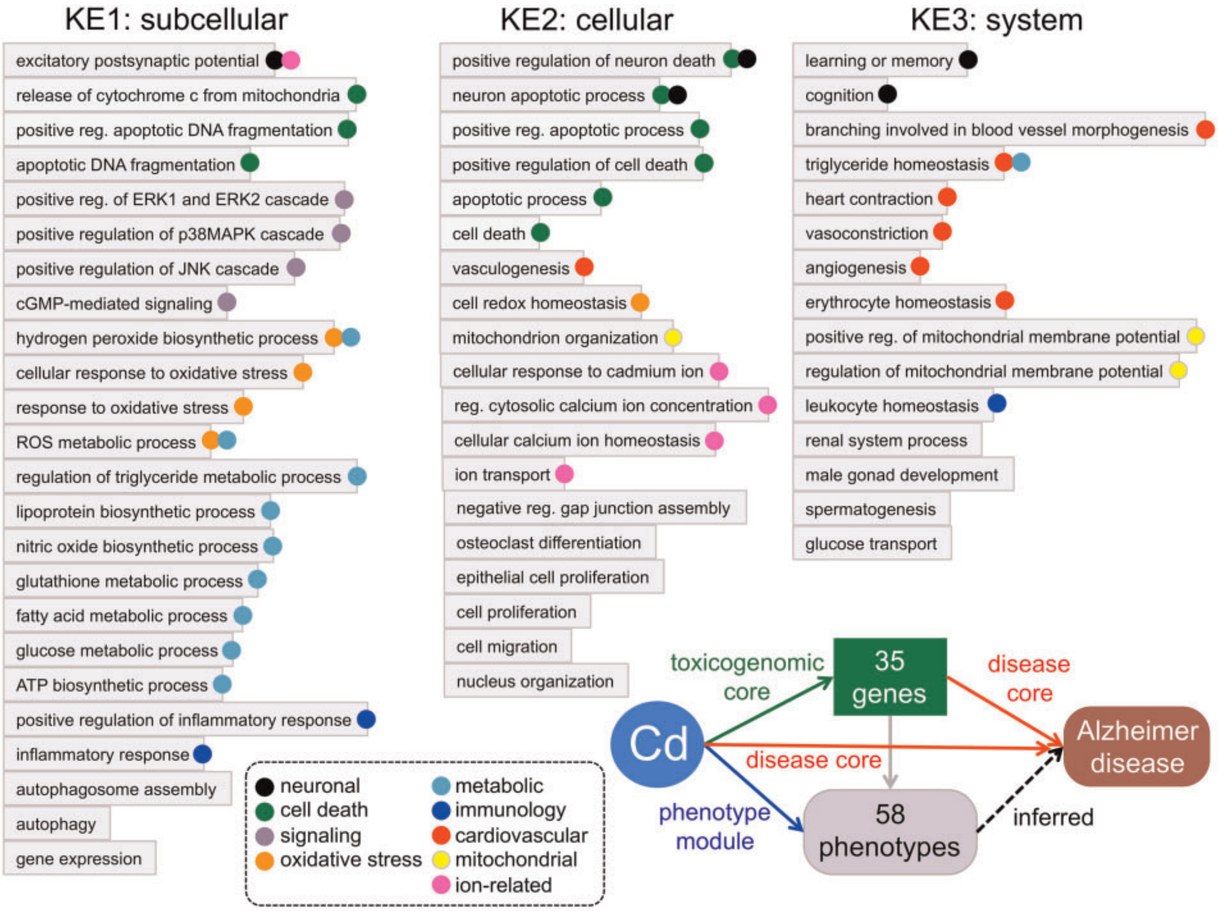

KE1: subcellular
excitatory postsynaptic potential
release of cytochrome c from mitochondria
positive reg. apoptotic DNA fragmentation
apoptotic DNA fragmentation
positive reg. of ERK1 and ERK2 cascade
positive regulation of p38MAPK cascade
positive regulation of JNK cascade
cGMP-mediated signaling
hydrogen peroxide biosynthetic process
cellular response to oxidative stress
response to oxidative stress
ROS metabolic process
regulation of triglyceride metabolic process
lipoprotein biosynthetic process
nitric oxide biosynthetic process
glutathione metabolic process
fatty acid metabolic process
glucose metabolic process
ATP biosynthetic process
positive regulation of inflammatory response
inflammatory response
autophagosome assembly
autophagy
gene expression
KE2: cellular
positive regulation of neuron death
neuron apoptotic process
positive reg. apoptotic process
positive regulation of cell death
apoptotic process
cell death
vasculogenesis
cell redox homeostasis
mitochondrion organization
cellular response to cadmium ion
reg. cytosolic calcium ion concentration
cellular calcium ion homeostasis
ion transport
negative reg. gap junction assembly
osteoclast differentiation
epithelial cell proliferation
cell proliferation
cell migration
nucleus organization
KE3: system
learning or memory
cognition
branching involved in blood vessel morphogenesis
triglyceride homeostasis
heart contraction
vasoconstriction
angiogenesis
erythrocyte homeostasis
positive reg. of mitochondrial membrane potential
regulation of mitochondrial membrane potential
leukocyte homeostasis
renal system process
male gonad development
spermatogenesis
glucose transport
neuronal
cell death
signaling
oxidative stress
metabolic
immunology
cardiovascular
mitochondrial
ion-related
toxicogenomic core
35 genes
disease core
Cd
disease core
Alzheimer disease
phenotype module
58 phenotypes
inferred

Figure 6. Classifying prioritized phenotypes. The 58 prioritized phenotypes were classified to 3 key event levels: KE1 (subcellular), KE2 (cellular), or KE3 (system) based on their mapping in the GO hierarchy. Similar biological themes are coded (legend at bottom). A schematic summary of the meta-data is shown in lower right corner: direct interactions between cadmium (Cd) and 35 genes are reported in CTD's toxicogenomic core, and CTD's disease core reports that these 35 genes are also directly associated with Alzheimer disease, as well as independently annotated by external databases to 58 GO terms; simultaneously, CTD's phenotype module reports manually curated interactions between cadmium and these same 58 prioritized phenotypes; and CTD disease core also describes a connection between cadmium and Alzheimer disease, allowing the 58 prioritized phenotypes to be inferred (dotted arrow).

state. A diversity of phenotypes is seen, including the disruption of subcellular metabolic pathways (eg, fatty acids, triglycerides, vitamin E, phospholipids, amino acids), cellular processes (eg, cell cycle, mitochondrial depolarization, lymphocyte activation, testosterone secretion), cell death phenotypes (eg, apoptosis, cell killing, necrosis, DNA fragmentation), and system level events (eg, renal filtration, olfactory behavior, bone resorption). These phenotypes allow toxicologists, risk assessors, regulatory decision makers, and pharmaceutical drug scientists to explore biological events potentially preceding the clinical manifestation of cadmium-induced AD.

The 131 phenotypes can be prioritized by comparing them with the GO terms annotated to genes in the inference network. Currently, 44 genes form an inference network between cadmium and AD (Figure 4, top panel). All 44 genes have curated interactions with cadmium (from CTD's toxicogenomic core), and, independently, have curated associations with AD (from CTD's disease core). These 44 genes are also annotated to 1641 unique GO biological process terms (annotated by external GO annotators and imported into CTD via a monthly upload from NCBI Gene). A Venn analysis of the 1641 GO biological process terms (derived from external, independent GO curation) with the 131 GO-based phenotypes (from internal CTD chemical-phenotype curation), reveals 58 shared terms, which, because of their derivation from 2 separate, independent data analyses, could potentially prioritize them as key events (KEs) connecting cadmium to AD (Figure 4, middle panel). These prioritized 58 phenotypes are annotated to 35 of the original 44 genes.

Generating Predictive AOPs

Integrating content from all 4 CTD curation modules (toxicogenomic, chemical-phenotype, disease, and exposure) provides an opportunity to generate predictive AOPs. An AOP is a framework that organizes information to construct a biologically plausible explanation for how a toxicant can result in an adverse outcome (AO), such as a disease (Villeneuve *et al.*, 2014a, b). AOPs are useful constructs to organize data across species, identify information gaps, and inform bioassay development (Mortensen *et al.*, 2018; Nymark *et al.*, 2018; Oki *et al.*, 2016; Taboureau and Audouze, 2017; Wittwehr *et al.*, 2017). A generic AOP framework can include 4 components: a molecular initiating event (MIE) that links a toxicant to a gene product, followed by a series of KEs, resulting in an AO that enhances an understanding at the population level (PO). CTD's 4 curation modules reflect these 4 AOP components (Figure 5).

For cadmium and AD, the prioritized 58 phenotypes first can be sorted into arbitrary KE levels by their mapping in the GO hierarchy. All 58 terms ultimately map to the GO root term

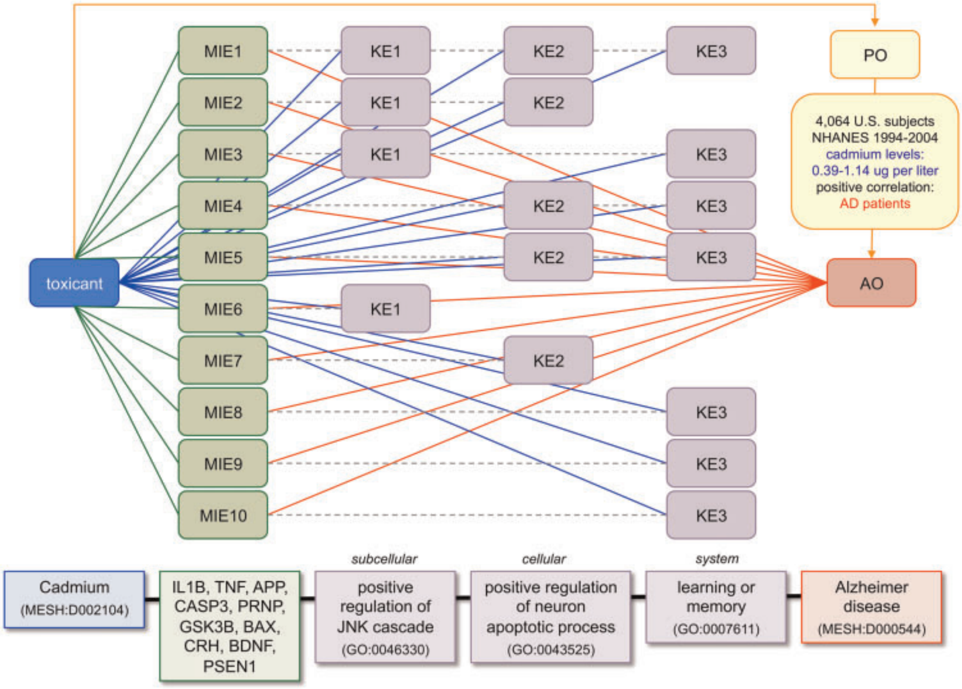

MIE1
MIE2
MIE3
MIE4
MIE5
MIE6
MIE7
MIE8
MIE9
MIE10
KE1
KE2
KE3
PO
4,064 U.S. subjects
NHANES 1994-2004
cadmium levels:
0.39-1.14 ug per liter
positive correlation:
AD patients
toxicant
AO
subcellular
cellular
system
Cadmium (MESH:D002104)
IL1B, TNF, APP, CASP3, PRNP, GSK3B, BAX, CRH, BDNF, PSEN1
positive regulation of JNK cascade (GO:0046330)
positive regulation of neuron apoptotic process (GO:0043525)
learning or memory (GO:0007611)
Alzheimer disease (MESH:D000544)

Figure 7. Integrating CTD content to generate predictive AOPs. Curated content from CTD toxicogenomic core is used to identify 10 molecular initiating events (MIE1-10) relating the toxicant cadmium to 10 genes that are also directly associated with the AO of AD from disease core. CTD's chemical-phenotype curation module fills in the intermediate KEs. Here, just 3 of the 58 prioritized phenotypes (KE1-KE3) are depicted that are directly influenced by cadmium in addition to being independently annotated to the same 10 genes by external GO databases (dotted lines). Phenotypes are organized into 3 KE levels (KE1 subcellular, KE2 cellular, and KE3 system) to generate a predicted AOP (bottom) connecting cadmium to AD. Furthermore, the association of cadmium exposure to AD is confirmed at the PO by CTD's exposure module (top arrow), with a U.S. study correlating cadmium blood levels (0.39-1.14 micrograms per liter) with patients.

"biological process" (GO: 0008150); however, by mapping to their penultimate or antepenultimate root terms, the phenotypes start to segregate into different levels of biological specificity. Using this strategy, the 58 prioritized phenotypes were divided into subcellular, cellular, and system KE levels (Figure 6), and similar biological themes emerged for many GO terms (even in different KE levels), such as neuron-related, cell death, oxidative stress, metabolism, cardiovascular, and mitochondrial. Many of these themes can be corroborated in the literature for AD. To wit, individually querying PubMed for abstracts that contain "AD" plus these words retrieves extensive literature-based co-occurrences for "cell death" (2107 articles), "oxidative stress" (3669 articles), "cardiovascular" (3327 articles), and "mitochondrion" (1432 articles), whereas other phenotypes may be simply coincidental (or not yet realized), such as "spermatogenesis" (only 3 articles).

Three phenotypes were selected from the prioritized 58 (one from each KE level), and used to construct a predictive AOP connecting cadmium to AD (Figure 7). Ten independent MIEs represent a curated interaction between cadmium and 10 genes (IL1B, TNF, APP, CASP3, PRNP, GSK3B, BAX, CRH, BDNF, and PSEN1) from CTD's toxicogenomic core. Independently, these 10 genes are directly annotated to AD from CTD's disease core. As well, these 10 genes are annotated to at least 1 of the 3 KEs as a GO-gene annotation (from NCBI Gene), and all 3 KEs have been curated as interacting with cadmium from CTD's chemical-phenotype module. Although an AOP describes a single toxicant-gene MIE, the fact that CTD's curated content provides 10 independent MIEs converging upon repeated occurrence of the same 3 KE phenotypes evinces multiple possible lines of support for the model. It should not be construed that all 10 independent MIEs must occur for the AOP to be valid; rather, the model simply provides at least 10 different independent ways for the AOP to be validated. Finally, an article leveraging data from the National Health and Nutrition Examination Survey (NHANES), and curated in CTD's exposure module, confirms a relationship between cadmium exposure and AD (Min and Min, 2016). This is just one of the several predictive AOPs that can be generated using all 35 genes and 58 prioritized phenotypes connecting cadmium to AD. Recall that cadmium influences 131 phenotypes (Figure 4); we only focused on the 58 that were prioritized based upon them also being annotated to genes associated with AD. This leaves an additional 73 novel phenotypes for constructing additional KEs in cadmium-induced AOPs.

Similar analyses can be performed easily with any chemical in CTD. Arsenicals, for example, currently modulate over 600 distinct phenotypes (http://ctdbase.org/detail.go?type=chem-acc=D001152&view=phenotype), including 10 related to glucose metabolism (Table 2). The genes that form the inference networks between arsenical compounds and these 10 phenotypes can be compared with known diabetic genes in CTD. In total, of the 171 unique inference genes that relate arsenicals to a glucose phenotype, 39 (23%) are also independently associated with diabetes (Table 2), allowing researchers to rapidly design predictive AOPs wherein arsenic interacts with the genes (MIEs) to affect glucose metabolism (KE) as potential presymptomatic phenotypes before the onset of diabetes (AO).

The seamless integration of CTD content allows users to explore additional information, such as identifying other chemicals (besides cadmium) that influence "learning or memory" by viewing the "Chemical Interactions" tab for this phenotype term

Table 2. Glucose-Related Phenotypes Modulated by Arsenicals in CTD

Arsenical Compound	Phenotype (ID)	Inference Network Genes	Subset of Inference Network Genes Also Associated With Diabetes
Arsenic trioxide; arsenite; methylarsonite; sodium arsenate; sodium arsenite	Glucose homeostasis (GO: 0042593)	95 genes: ADGRF5; ADIPOQ; ADRA1B; ADRA2A; AKT1; AKT2; ATG7; BAD; C5; CACNA1A; CACNA1C; CACNA1E; CAV3; CD36; CDKN2A; CEBPA; CNR1; CRH; CRY2; DBH; ERO1B; FABP5; FFAR2; FGFR4; FOXA1; FOXO1; FOXO3; G6PC; GCK; GLIS3; GPI; GPR39; GPRC5B; HIF1A; HNF1A; HNF1BA; HNF4A; IGFBP5; IL6; INS; INSR; IRS1; KCNB1; KLHL42; LEP; LEPR; MAP2K1; MCU; MET; NCOR2; NEUROD1; NMB; NOTCH2; NR1H4; NUCKS1; PARK2; PAX4; PAX6; PCK1; PDK2; PDK4; PDX1; PFK; PGD; PLA2G6; PPARG; PPP3CB; PRCP; PRKAA1; PRKAA2; PTCH1; PTPN11; PYGL; RAB13; RBM4; RPS6; SELENOT; SERPINE1; SESN2; SESN3; SIRT1; SLC16A1; SLC18A2; SLC2A4; SLC37A4; SOX4; SRI; SSTR5; STAT3; TCF7L2; TRPV4; UBA1; USF1; VSNL1; XBP1	28 genes: ADIPOQ; AKT2; CAV3; FOXO3; GCK; GLIS3; HIF1A; HNF1A; HNF4A; IL6; INS; INSR; IRS1; LEP; LEPR; NEUROD1; NOTCH2; PAX4; PAX6; PCK1; PDK4; PDX1; PPARG; SERPINE1; SIRT1; SLC2A4; STAT3; TCF7L2
Sodium arsenite	Glucose metabolic process (GO: 0006006)	43 genes: ADIPOQ; AKR1A1; AKT1; AKT2; CPT1A; CREM; FABP5; G6PD; G6PDX; GALM; GAPDH; GCK; GHRL; HK2; IGF2; IGFBP1; IGFBP5; INS; INS1; IRS2; LEP; MAPK14; MYC; NISCH; PCK1; PDHA2; PDHB; PDHK-2; PDK1; PDK2; PDK3; PDK4; PDX1; PGM1; PIK3CA; PIK3R1; PKM; PRKAA1; SERP1; SERPINA12; SORD; TNF; TPI1	13 genes: ADIPOQ; AKT2; CPT1A; CREM; GCK; INS; INS1; IRS2; LEP; PCK1; PDK4; PDX1; TNF
Arsenic trioxide	Gluconeogenesis (GO: 0006094)	21 genes: ALDOA; ALDOC; ATF3; ATF4; FBP1; G6PC3; GAPDH; GOT1; GOT2; GPD1; GPD2; GPI; MDH1; PCK2; PER2; PFKFB4; PGK1; PGM1; PPARGC1A; TAT; TPI1	3 genes: ATF3; GPD2; PPARGC1A
Sodium arsenite	Canonical glycolysis (GO: 0061621)	18 genes: ALDOA; ALDOB; ALDOC; BPGM; ENO1; ENO2; ENO3; GAPDH; GCK; GPI; HK2; HK3; PGAM1; PGAM2; PGK1; PKLR; PKM; TPI1	2 genes: GCK; PKLR
Sodium arsenite	Regulation of glycolytic process (GO: 0006110)	13 genes: ECD; GCK; HIF1A; NDC1; NUP155; NUP210; NUP62; NUP85; NUP88; NUP93; PGAM1; RAE1; TPR	2 genes: GCK; HIF1A
Arsenic trioxide	Positive regulation of insulin secretion involved in cellular response to glucose stimulus (GO: 0035774)	10 genes: ATG7; BAD; CRH; HIF1A; PARK2; PLA2G6; PPP3CB; SIRT1; SRI; VSNL1	2 genes: HIF1A; SIRT1
Sodium arsenite	Positive regulation of gluconeogenesis (GO: 0045722)	9 genes: DGAT2; FOXO1; HIF1A; HNF4A; PCK1; PPARGC1A; PPP4R3A; PRKAA1; SIRT1	5 genes: HIF1A; HNF4A; PCK1; PPARGC1A; SIRT1
Sodium arsenite	Positive regulation of glucose import in response to insulin stimulus (GO: 2001275)	9 genes: AGT; AKT2; INS; IRS1; MARCKS; PIK3R1; PIK3R2; PIK3R3; PTPN11	4 genes: AGT; AKT2; INS; IRS1
Arsenite; methylarsonite	Positive regulation of glycogen biosynthetic process (GO: 0045725)	6 genes: AKT1; IGF1; INS; INSR; IRS1; RACK1	4 genes: IGF1; INS; INSR; IRS1
Arsenic trioxide	Regulation of glycogen metabolic process (GO: 0070873)	2 genes: KHK; PHLDA2	0 genes

(http://ctdbase.org/detail.go?type=go&acc=GO%3a0007611&view=phenotype), or discovering other diseases inferred to this phenotype under the "Diseases" tab (http://ctdbase.org/detail.go?type=go&acc=GO%3a0007611&view=disease), or retrieving the details that describe this phenotype as an exposure outcome at the PO (http://ctdbase.org/detail.go?type=go&acc=GO%3a0007611&view=expConsol). Similar data extensions can be done for any of the other listed phenotypes in the cadmium-AD AOP or the 10 genes forming the MIEs. This helps discover and connect information to grow and extend the predictive AOPs, as well as interrelate other AOPs to generate testable hypotheses about chemical exposures and environmentally influenced diseases. The predictive AOPs easily generated from CTD curated content should help toxicologists review, test, and refine AOPs for official approval (https://aopwiki.org/).

SUMMARY

We present a new chemical-phenotype module at CTD that describes chemical-induced, nondisease biological outcomes that are manually curated from the peer-reviewed scientific literature. These interactions are embellished with taxon and anatomical descriptors, allowing the data to be analyzed comparatively across model organisms and humans. The use of well-known vocabularies (with accession identifiers) enhance database interoperability and provide an easy way for model organism databases to bring their gene-phenotype information into the chemical landscape provided by CTD. This freely available module will help toxicologists better understand the predisease state and generate predictive AOPs for environmental diseases. To our knowledge, this is the first comprehensive set of manually curated, literature-based, contextualized, chemical-induced, nondisease phenotype data provided to the public.

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REFERENCES

Agarwala, R., Barrett, T., Beck, J., Benson, D. A., Bollin, C., Bolton, E., Bourexis, D., Brister, J. R., Bryant, S. H., Canese, K., *et al.* (2018). Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*. **46**, D8–D13.

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., *et al.* (2000). Gene Ontology: Tool for the unification of biology. *Nat. Genet*. **25**, 25–29.

Bogue, M. A., Grubb, S. C., Walton, D. O., Philip, V. M., Kolishovski, G., Stearns, T., Dunn, M. H., Skelly, D. A., Kadakkuzha, B., TeHennepe, G., *et al.* (2018). Mouse Phenome Database: An integrative database and analysis suite for curated empirical phenotype data from laboratory mice. *Nucleic Acids Res*. **46**, D843–D850.

Celato, A., Mitola, C., Tolve, M., Giannini, M. T., De Leo, S., Carducci, C., Carducci, C., and Leuzzi, V. (2013). A new case of malonic aciduria with a presymptomatic diagnosis and an early treatment. *Brain Dev*. **35**, 675–680.

Chatr-Aryamontri, A., Oughtred, R., Boucher, L., Rust, J., Chang, C., Kolas, N. K., O'Donnell, L., Oster, S., Theesfeld, C., Sellam, A., *et al.* (2017). The BioGRID interaction database: 2017 update. *Nucleic Acids Res*. **45**, D369–D379.

Coletti, M. H., and Bleich, H. L. (2001). Medical subject headings used to search the biomedical literature. *J. Am. Med. Inform. Assoc*. **8**, 317–323.

Davis, A. P., Grondin, C. J., Johnson, R. J., Sciaky, D., King, B. L., McMorran, R., Wiegers, J., Wiegers, T. C., and Mattingly, C. J. (2017). The Comparative Toxicogenomics Database: Update 2017. *Nucleic Acids Res*. **45**, D972–D978.

Davis, A. P., Grondin, C. J., Lennon-Hopkins, K., Saraceni-Richards, C., Sciaky, D., King, B. L., Wiegers, T. C., and Mattingly, C. J. (2015). The Comparative Toxicogenomics Database's 10th year anniversary: Update 2015. *Nucleic Acids Res*. **43**, D914–D920.

Davis, A. P., Johnson, R. J., Lennon-Hopkins, K., Sciaky, D., Rosenstein, M. C., Wiegers, T. C., and Mattingly, C. J. (2012a). Targeted journal curation as a method to improve data currency at the Comparative Toxicogenomics Database. *Database (Oxford)* **2012**, bas051.

Davis, A. P., King, B. L., Mockus, S., Murphy, C. G., Saraceni-Richards, C., Rosenstein, M., Wiegers, T., and Mattingly, C. J. (2011a). The Comparative Toxicogenomics Database: Update 2011. *Nucleic Acids Res*. **39**, D1067–D1072.

Davis, A. P., Murphy, C. G., Johnson, R., Lay, J. M., Lennon-Hopkins, K., Saraceni-Richards, C., Sciaky, D., King, B. L., Rosenstein, M. C., Wiegers, T. C., *et al.* (2013a). The Comparative Toxicogenomics Database: Update 2013. *Nucleic Acids Res*. **41**, D1104–D1114.

Davis, A. P., Murphy, C. G., Rosenstein, M. C., Wiegers, T. C., and Mattingly, C. J. (2008). The Comparative Toxicogenomics Database facilitates identification and understanding of chemical-gene-disease associations: Arsenic as a case study. *BMC Med. Genom*. **1**, 48.

Davis, A. P., Murphy, C. G., Saraceni-Richards, C. A., Rosenstein, M. C., Wiegers, T. C., and Mattingly, C. J. (2009). Comparative Toxicogenomics Database: A knowledgebase and discovery tool for chemical-gene-disease networks. *Nucleic Acids Res*. **37**, D786–D792.

Davis, A. P., Wiegers, T. C., Johnson, R. J., Lay, J. M., Lennon-Hopkins, K., Saraceni-Richards, C., Sciaky, D., Murphy, C. G., and Mattingly, C. J. (2013b). Text mining effectively scores and ranks the literature for improving chemical-gene-disease curation at the comparative toxicogenomics database. *PLoS One* **8**, e58201.

Davis, A. P., Wiegers, T. C., King, B. L., Wiegers, J., Grondin, C. J., Sciaky, D., Johnson, R. J., and Mattingly, C. J. (2016). Generating gene ontology-disease inferences to explore mechanisms of human disease at the Comparative Toxicogenomics Database. *PLoS One* **11**, e0155530.

Davis, A. P., Wiegers, T. C., Roberts, P. M., King, B. L., Lay, J. M., Lennon-Hopkins, K., Sciaky, D., Johnson, R., Keating, H., Greene, N., *et al.* (2013c). A CTD-Pfizer collaboration: Manual curation of 88, 000 scientific articles text mined for drug-disease and drug-phenotype interactions. *Database (Oxford)* **2013**, bat080.

Davis, A. P., Wiegers, T. C., Rosenstein, M. C., and Mattingly, C. J. (2012b). MEDIC: A practical disease vocabulary used at the Comparative Toxicogenomics Database. *Database (Oxford)* **2012**, bar065.

Davis, A. P., Wiegers, T. C., Rosenstein, M. C., Murphy, C. G., and Mattingly, C. J. (2011b). The curation paradigm and application tool used for manual curation of the scientific literature at the Comparative Toxicogenomics Database. *Database (Oxford)* **2011**, bar034.

Davis, M. J., Sehgal, M. S., and Ragan, M. A. (2010). Automatic, context-specific generation of Gene Ontology slims. *BMC Bioinformatics* **11**, 498.

Deans, A. R., Lewis, S. E., Huala, E., Anzaldo, S. S., Ashburner, M., Balhoff, J. P., Blackburn, D. C., Blake, J. A., Burleigh, J. G., Chanet, B., *et al.* (2015). Finding our way through phenotypes. *PLoS Biol*. **13**, e1002033.

Federhen, S. (2012). The NCBI taxonomy database. *Nucleic Acids Res* **40**, D13–D143.

Gohlke, J. M., Thomas, R., Zhang, Y., Rosenstein, M. C., Davis, A. P., Murphy, C., Becker, K. G., Mattingly, C. J., and Portier, C. J. (2009). Genetic and environmental pathways to complex diseases. *BMC Syst. Biol*. **3**, 46.

Grondin, C. J., Davis, A. P., Wiegers, T. C., King, B. L., Wiegers, J. A., Reif, D. M., Hoppin, J. A., and Mattingly, C. J. (2016). Advancing exposure science through chemical data curation and integration in the Comparative Toxicogenomics Database. *Environ. Health Perspect*. **124**, 1592–1599.

Grondin, C. J., Davis, A. P., Wiegers, T. C., Wiegers, J. A., and Mattingly, C. J. (2018). Accessing an expanded exposure science module at the Comparative Toxicogenomics Database. *Environ. Health Perspect*. **126**, 014501.

Howe, D. G., Bradford, Y. M., Eagle, A., Fashena, D., Frazer, K., Kalita, P., Mani, P., Martin, R., Moxon, S. T., Paddock, H., *et al*. (2017). The Zebrafish Model Organism Database: New support for human disease models, mutation details, gene expression phenotypes and searching. *Nucleic Acids Res*. **45**, D758–D768.

Laulederkind, S. J., Liu, W., Smith, J. R., Hayman, G. T., Wang, S. J., Nigam, R., Petri, V., Lowry, T. F., de Pons, J., Dwinell, M. R., *et al*. (2013). PhenoMiner: Quantitative phenotype curation at the rat genome database. *Database (Oxford)* **2013**, bat015.

Maglott, D., Ostell, J., Pruitt, K. D., and Tatusova, T. (2011). Entrez Gene: Gene-centered information at NCBI. *Nucleic Acids Res*. **39**, D52–D57.

Mattingly, C. J., Rosenstein, M. C., Davis, A. P., Colby, G. T., Forrest, J. N., and Boyer, J. L. (2006). The Comparative Toxicogenomics Database: A cross-species resource for building chemical-gene interaction networks. *Toxicol. Sci*. **92**, 587–595.

McHale, C. M., Zhang, L., Hubbard, A. E., and Smith, M. T. (2010). Toxicogenomic profiling of chemically exposed humans in risk assessment. *Mutat. Res*. **705**, 172–183.

Min, J. Y., and Min, K. B. (2016). Blood cadmium levels and Alzheimer's disease mortality risk in older US adults. *Environ. Health* **15**, 69.

Mortensen, H. M., Chamberlin, J., Joubert, B., Angrish, M., Sipes, N., Lee, J. S., and Euling, S. Y. (2018). Leveraging human genetic and adverse outcome pathway (AOP) data to inform susceptibility in human health risk assessment. *Mamm. Genome* **29**, 190–204.

Mungall, C. J., McMurry, J. A., Köhler, S., Balhoff, J. P., Borromeo, C., Brush, M., Carbon, S., Conlin, T., Dunn, N., Engelstad, M., *et al*. (2017). The Monarch Initiative: An integrative data and analytic platform connecting phenotypes to genotypes across species. *Nucleic Acids Res*. **45**, D712–D722.

Nymark, P., Rieswijk, L., Ehrhart, F., Jeliazkova, N., Tsiliki, G., Sarimveis, H., Evelo, C. T., Hongisto, V., Kohonen, P., Willighagen, E., *et al*. (2018). A data fusion pipeline for generating and enriching adverse outcome pathway descriptions. *Toxicol. Sci*. **162**, 264–275.

Oki, N. O., Nelms, M. D., Bell, S. M., Mortensen, H. M., and Edwards, S. W. (2016). Accelerating adverse outcome pathway development using publicly available data sources. *Curr. Environ. Health Rep*. **3**, 53–63.

Pelletier, D., Wiegers, T. C., Enayetallah, A., Kibbey, C., Gosink, M., Koza-Taylor, P., Mattingly, C. J., and Lawton, M. (2016). ToxEvaluator: An integrated computational platform to aid the interpretation of toxicology study-related findings. *Database (Oxford)* **2016**, baw062.

Planchart, A., Green, A., Hoyo, C., and Mattingly, C. J. (2018). Heavy metal exposure and metabolic syndrome: Evidence from human and model system studies. *Curr. Environ. Health Rep*. **5**, 110–124.

Sakai, N., and Otomo, T. (2016). Challenge of phenotype estimation for optimal treatment of Krabbe disease. *J. Neurosci. Res*. **94**, 1025–1030.

Taboureau, O., and Audouze, K. (2017). Human Environmental Disease Network: A computational model to assess toxicology of contaminants. *ALTEX* **34**, 289–300.

Villeneuve, D. L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T. H., LaLone, C. A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., *et al*. (2014a). Adverse outcome pathway (AOP) development I: Strategies and principles. *Toxicol. Sci*. **142**, 312–320.

Villeneuve, D. L., Crump, D., Garcia-Reyero, N., Hecker, M., Hutchinson, T. H., LaLone, C. A., Landesmann, B., Lettieri, T., Munn, S., Nepelska, M., *et al*. (2014b). Adverse outcome pathway development II: Best practices. *Toxicol. Sci*. **142**, 321–330.

Wilkinson, M. D., Dumontier, M., Aalbersberg, I. J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J. W., da Silva Santos, L. B., Bourne, P. E., *et al*. (2016). The FAIR Guiding Principles for scientific data management and stewardship. *Sci. Data* **3**, 160018.

Wittwehr, C., Aladjov, H., Ankley, G., Byrne, H. J., de Knecht, J., Heinzle, E., Klambauer, G., Landesmann, B., Luijten, M., MacKay, C., *et al*. (2017). How adverse outcome pathways can aid the development and use of computational prediction models for regulatory toxicology. *Toxicol. Sci*. **155**, 326–336.

Yu, X., Zhang, J., Sun, S., Zhou, X., Zeng, T., and Chen, L. (2017). Individual-specific edge-network analysis for disease prediction. *Nucleic Acids Res*. **45**, e170.