United States Environmental Protection Agency

Ontology for Modeling Adverse Outcome Pathways: *Semantic tools for Systems Tox*

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EPA-NIEHS Advancing Environmental Health Data Sharing and Analysis: Finding a Common Language
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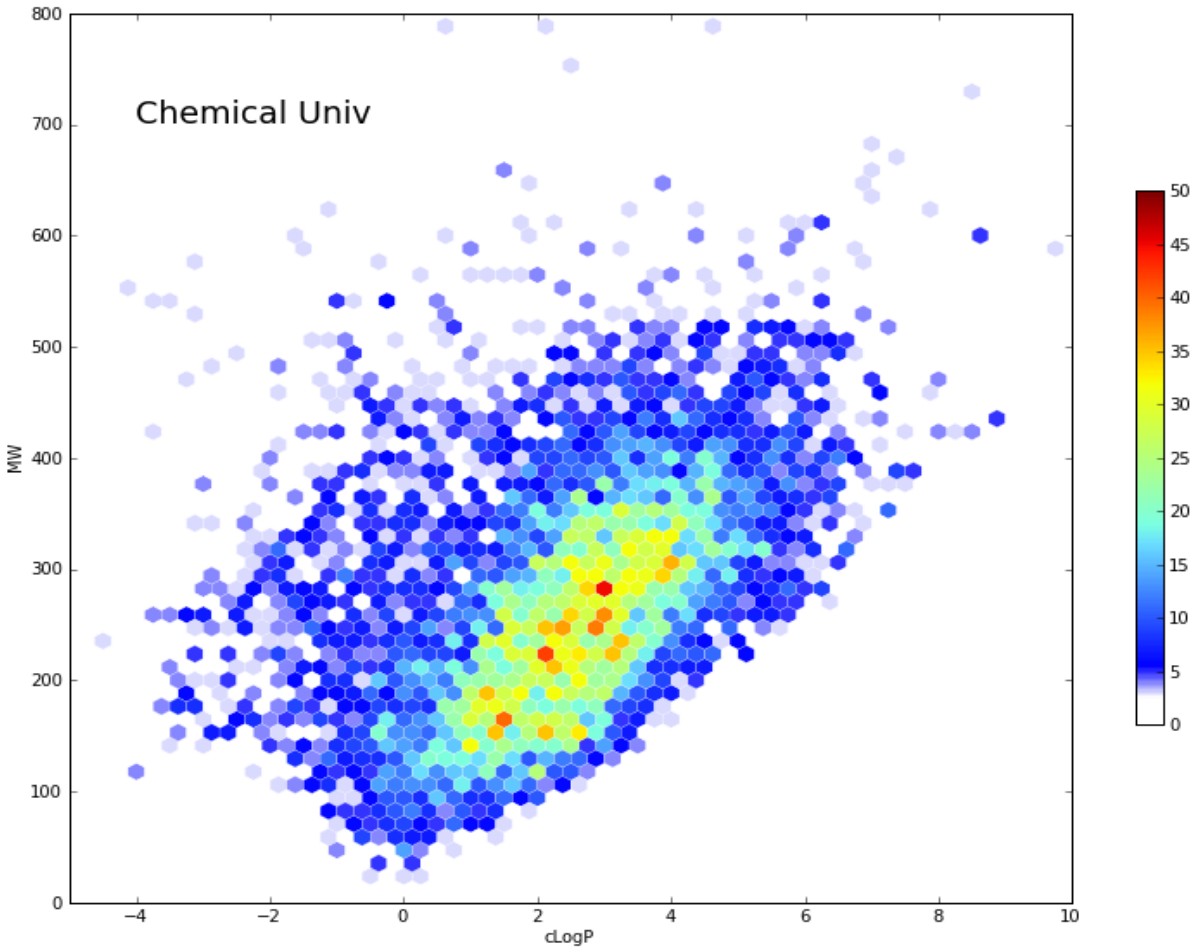
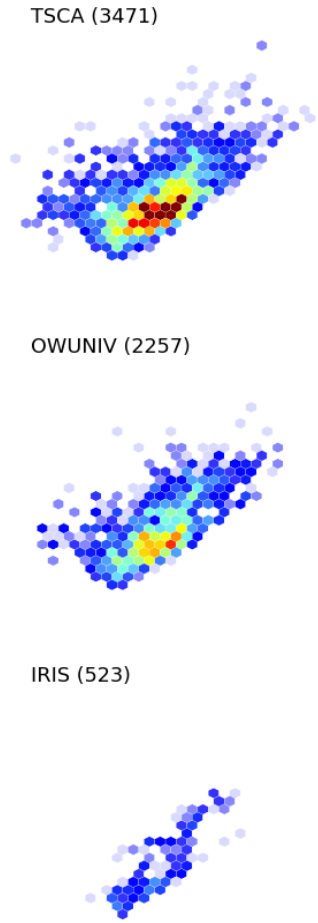
Office of Research and Development
National Center for Computational Toxicology
www.epa.gov/ncct

Outline

- Challenge: Chemical Evaluation
- Problem: Linking chemical to potential health effect
- Approach: Adverse outcome pathway
- Solution: Semantic / knowledge-based tools
- Case-study

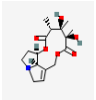
Challenge: Evaluating Chemicals

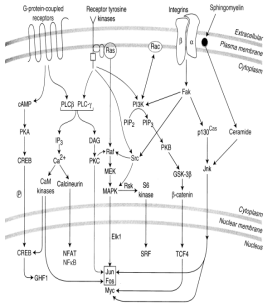


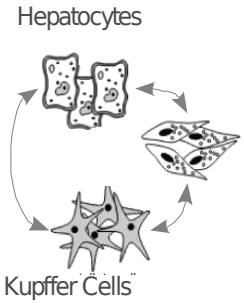


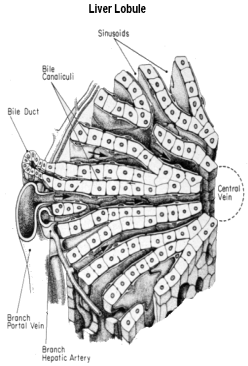
13,781/84,000 Chemicals on TSCA Inventory
(so far)

Chemical Evaluation:
A Complex Systems Problem







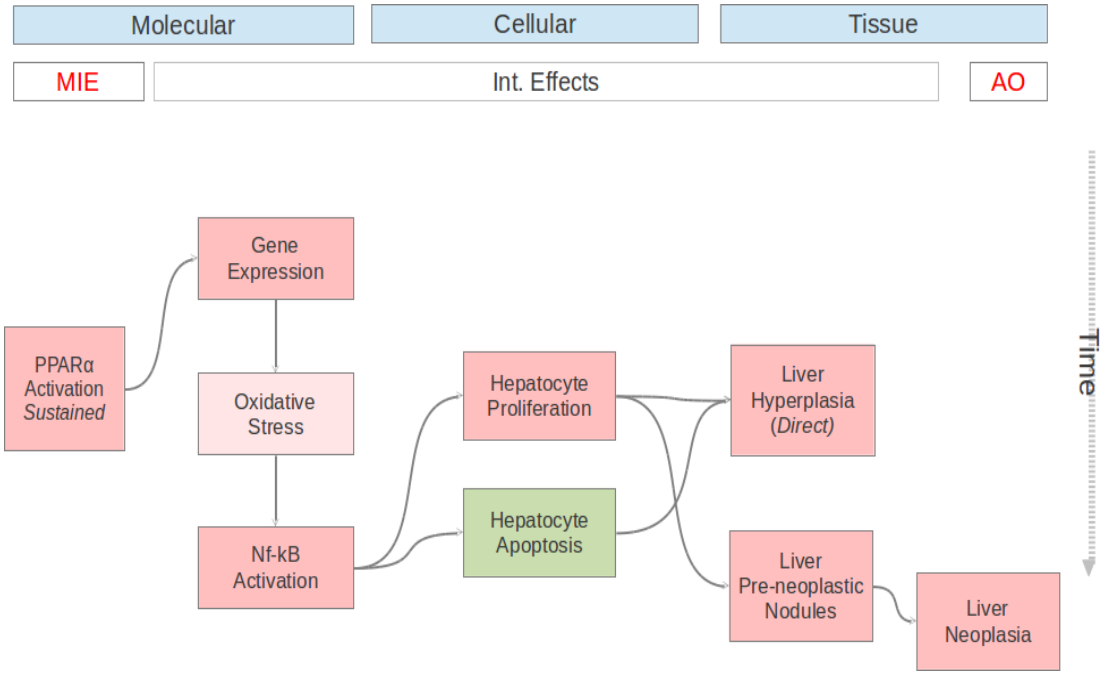


Chemical ↔ Molecular Pathways ↔ Cellular Responses ↔ Tissue Changes ↔ Adverse Effects

- **Problems:**
  - Exposure
  - Adverse Outcome Pathways – Describe key components of the system from molecular to injury
  - Dose-dependent responses – Simulate dynamic behaviour of system following chemical exposure
- **Semantic solution**
  - Domain-specific ontology – Toxicology
  - Describe normal biology & chemical perturbation
  - Enable automated reasoning
  - Useful for quantitative modeling

Adverse Outcome Pathways

- Some history
  - Mode of Action (MoA) framework
  - 21st Century Tox – *"Toxicity Pathways"*
  - Conceptual framework for evaluating ecological outcomes
- On-going efforts:
  - OECD: Molecular Screening. Define "template" to standardize development and submission of AOPs for regulatory application
  - Effectopaedia: EU effort to store and organize AOPs
  - EPA AOP Wiki: EPA/CSS-OECD collaboration to curate AOPs
  - …. many other efforts ….
- <u>Standardization is important!</u>



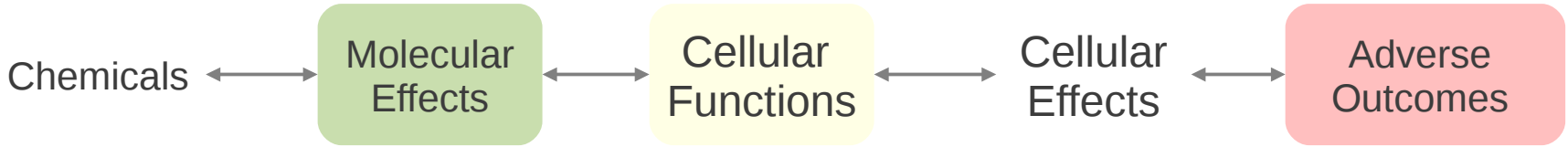
AOP: PPARα → Rodent Liver Cancer

Many possible pathways

Internal Dose ↔ Molecular Effects ↔ Cellular Functions ↔ Cellular Effects ↔ Adverse Outcomes

Internal Dose	Molecular Effects	Cellular Functions	Cellular Effects	Adverse Outcomes
Absorption	Receptor Activation	Endoplasmic Reticulum	Hepatocyte Necrosis Apoptosis Proliferation	Hypertrophy Injury Inflammation Regeneration
Distribution	Signal-transduction	Mitochondrial Function	Kupffer cell Activation Phagocytosis	Steatosis Cholestasis Fibrosis Cirrhosis
Metabolism	Gene expression	Peroxisomal Function	.	Hyperplasia Pre-neoplasia Neoplasia
Excretion	Metabolism	Cytoskeletal Architecture	.	
	.	Stress Pathways	.	
	.	.	.	
	.	.	.	
	.	.	.	

Pathway Inference / Reconstruction



2. Mol. Init. Events: Nuclear Receptors

3. Intermediate Effects

1.Define Hepatic Adverse Outcomes

- Top-down – i.e. Hypothesis-driven – Evaluate hypothesis using weight of evidence. Resource intensive.
- Bottom-up – i.e. Data-driven – Computational tools organize evidence and use heuristics to generate hypotheses. Results not always relevant.

Data → Semantics → Knowledge

- Semantic model / Ontology
  - Referential vocabulary – standardize entities (things)
  - Relational vocabulary – standardize linkages
- Ontology for modeling Toxicity Pathways (OnToP):
  - Referential vocab (Table 1)
  - Relational vocab (BFO)
- Concretely expressed:
  - OWL/RDF
  - N3,Turtle, LISP, etc.
- SPARQL endpoint (Intranet)

Entity Class	Source(s)	Count
chemical	KEGG	13681
	DrugBank	7080
	MeSH	2607
	NHANES	458
	ToxCast	1658
	ToxRefDB	307
	NTP	586
gene	NCBI Entrez	143916
	MeSH	227
protein	UniProt	43960
	MeSH	4030
cell	OBO: CellTypeOntology	983
cell-location	GO CC	2110
	MeSH	204
anatomic-location	OBO:FMA	75144
	MeSH	15
organism	NCBI Taxonomy	289
	MeSH	32
molecular-event	OBO: GO molecular function	8360
	MeSH	83
cell-event	OBO: GO biological process	17008
tissue-event	OBO: MPO	639
	ToxRefDB	16
	MeSH	239

Table 1. Named entities: classes, sources and instances

Shah et. al., PLOS Computational Biology (*in revision)*

Domain knowledge → Literature

Natural Language

DEHP and DCB were both able to suppress rat hepatocyte apoptosis

Disturbances of the mitochondrial membrane, induced by CCl4 treatment, were also evidenced as increased mitochondrial swelling

In DENA-initiated C3H and C3B6F1 mice, phenobarbital increased the labeling index in eosinophilic foci, while decreasing the labeling index in normal/non-involved hepatocytes with/without DENA initiation.

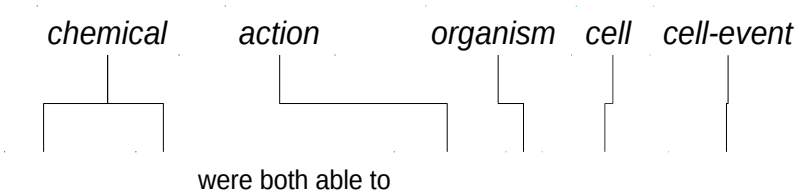
In contrast, PB, a non-genotoxic rodent hepatocarcinogen, enhances the growth of hepatic focal lesions in mice and rats by increasing cell proliferation and inhibiting apoptosis.

However, humans give a therapeutic response to the fibrate PPs via an alteration in lipid metabolism mediated by PPARalpha.

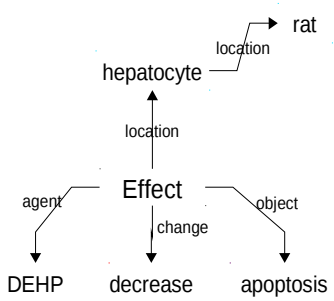
Acetaminophen treatment increased the plasma levels of aspartate transaminase, alanine aminotransferase, and alkaline phosphatase and caused hepatic DNA fragmentation and hepatocyte necrosis.

Natural Language Processing

Evidence



Semantics



Assertion

$$Effect(d_{20}) \wedge hasAgent(d_{20}, c_{101}) \wedge hasObject(d_{20}, e_{20}) \wedge hasChange(d_{20}, +) \wedge$$
$$Chemical(c_{101}) \wedge MolEvent(e_{20}) \wedge hasEvidence(d_{20}, f_{1020}) \wedge Literature(f_{1020}) \wedge$$
$$hasName(c_{101}, \text{DEHP}) \wedge hasExtId(c_{101}, \text{CAS:117-81-7}) \wedge$$
$$hasName(e_{20}, \text{apoptosis}) \wedge hasExtId(e_{20}, \text{GO:0006915})$$

Shah et. al., PLOS Computational Biology (*in revision)*

Ontology for Toxicity Pathways: *OnToP*

- Substances
  - Measurable
  - Biological molecules, cell, anatomic locations, tissues
  - Organisms and their attributes
- Phenomena
  - Events
  - Pathways: chain of events
- Effects
  - Changes in events
  - Chemical-effects
  - Latent-effects

CAR, TCPOBOP, c-Myc

Liver, rat

CAR-activation, c-Myc-activation, cell proliferation, hyperplasia

TCPOBOP induced CAR-activation

FoxM1-activation increases cell prolferation

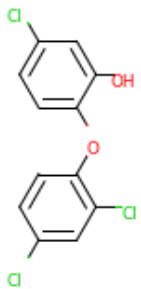
Evidence from Literature

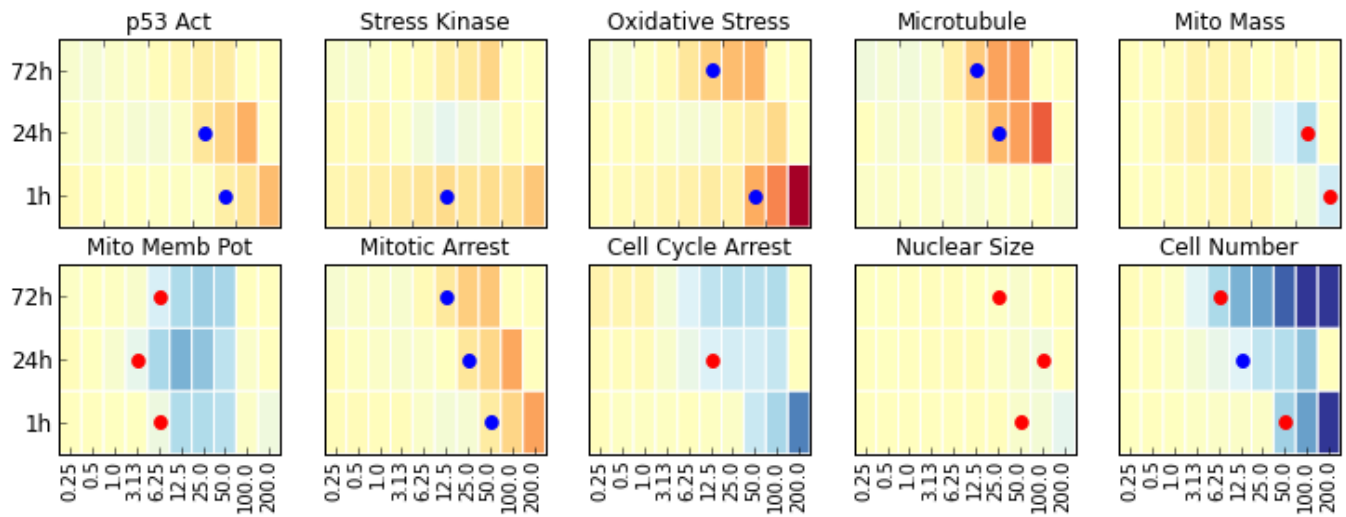
Effect			PubMed Abstract			Agent		Object	
Agent		Object	PMID	S	Phrase	Ext-ID	Class	Ext ID	Class
Perfluoro-decanoic acid	▲	Nr1i2	15826607	1	*PXR* was markedly increased in rats treated with clofibrate and *perfluorodecanoic acid*	335-76-2	chemical	EG:18171	gene
4-Nonylphenol	▲	Nr1i2	16013040	2	*4-Nonylphenol* (4-NP) is an environmental estrogen that also can activate the pregnane-X receptor (PXR)	104-40-5	chemical	EG:18171	gene
Diclofop-methyl	▲	Ppara	17084873	7	*diclofop-methyl* and pyrethrins induce *PPARalpha*	51338-27-3	chemical	EG:19013	gene
Perfluorooctanoic acid	▶	Ppara	19162173	5	*PFOA* and PFOS elicited transcript profile signatures that included many known *PPAR alpha* target genes	335-67-1	chemical	EG:19013	gene
Diisodecyl phthalate	▶	Ahr	18294747	3	*DIDP* and DBP affected only the *AhR*	26761-40-0	chemical	EG:11622	gene
Perfluorooctanoic acid	▲	apoptosis	17374408	8	*PFOA* are able to produce oxidative stress and induce *apoptosis*	335-67-1	chemical	GO:0006915	cell-event
Phenobarbital	▶	hepatic hyperplasia	1236193	7	*Phenobarbital* treatment resulted in *hyperplasia*	50-60-6	chemical	VL:1098	tissue-event
Cyproterone acetate	▲	hepatic hyperplasia	2139818	3	hepatomitogen *cyproterone acetate* (CPA) to induce liver *hyperplasia*	427-51-0	chemical	VL:1098	tissue-event

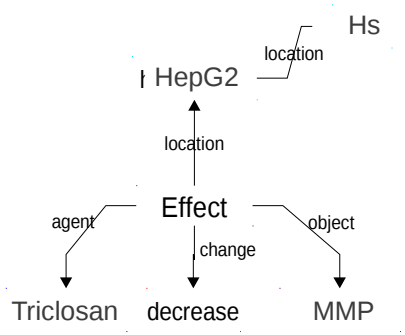
Shah et. al., PLOS Computational Biology (*in revision)*

HTS data – molecular & cellular effects

Triclosan
PhI HepG2

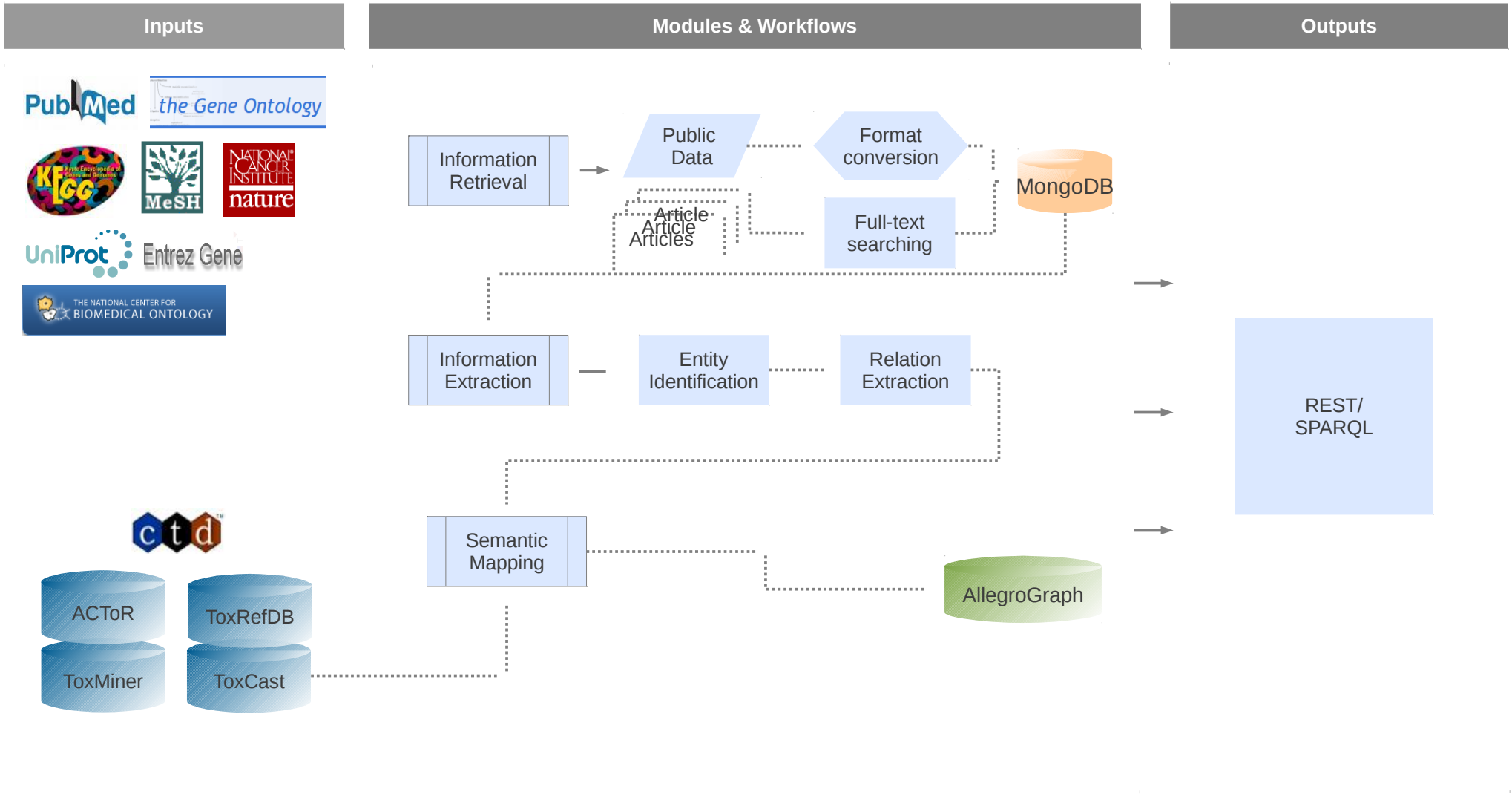






Shah et. al., in preparation

Workflow


Inputs
Modules & Workflows
Outputs
PubMed
the Gene Ontology
KEGG
MeSH
NATIONAL CANCER INSTITUTE
nature
UniProt
Entrez Gene
THE NATIONAL CENTER FOR BIOMEDICAL ONTOLOGY
ctd
ACToR
ToxRefDB
ToxMiner
ToxCast
Information Retrieval
Public Data
Format conversion
MongoDB
Article
Article
Articles
Full-text searching
Information Extraction
Entity Identification
Relation Extraction
Semantic Mapping
AllegroGraph
REST/ SPARQL

vLiver Knowledge-base

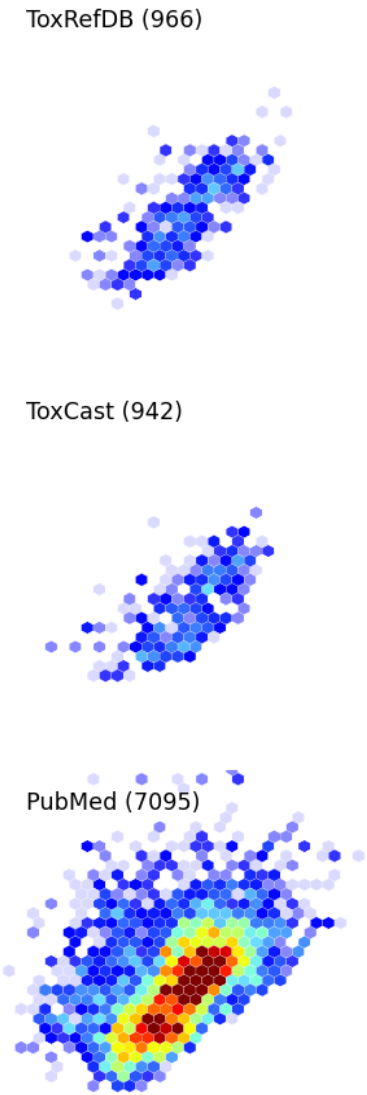
United States Environmental Protection Agency

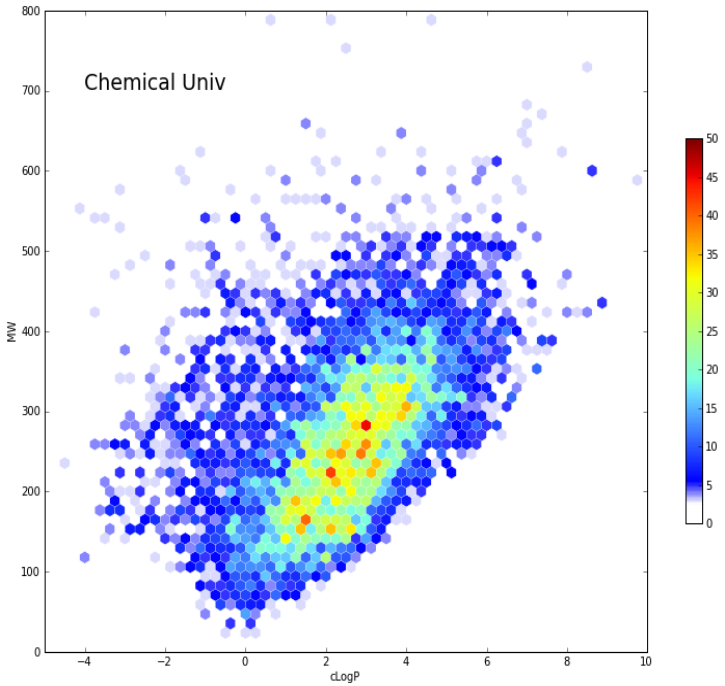
<u>Formal Ontology</u>
OWL/RDF; n3

<u>Entities (with external links)</u>
Chemicals > 13,781
Effects > 1,225,869
Assays > 38,294
Targets > 48,743

<u>Sources:</u>
PubMed >1e6
ToxCast 1008242
ToxRef 135512
PubMed/CTD: 82,020
PubMed/v-Liver: 2,302

<u>Accessible:</u>
SPARQL endpoint
REST





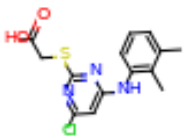
Literature – largest source of evidence ..

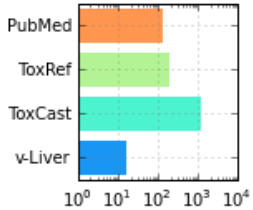
Case Study

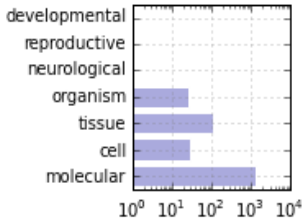
- Browse evidence for chemicals
- Identify nuclear receptor activators
- Visualize evidence
- Making inferences about mechanisms
- Automated inference – pathways
- Hypothetical pathways to adverse outcomes

WY-14,643 Effects → Structured

WY-14643







> WY-14643 molecular-effect ID: 5153011eb9743f479d00096f
Effect: dec HBA-A1 mRNA expression in mouse
Evid: Qu, A et al,(2010). "PPARalpha-dependent activation of cell cycle control and DNA repair genes in hepatic nonparenchymal cells." Toxicol. Sci. 1096-0929

> WY-14643 molecular-effect ID: 5153011db9743f479d00096e
Effect: dec PRP19 mRNA expression in mouse
Evid: Han, ES et al,(2008). "The in vivo gene expression signature of oxidative stress." Physiol. Genomics 1531-2267

> WY-14643 molecular-effect ID: 5153011cb9743f479d00096b
Effect: dec ARF2 mRNA expression in mouse
Evid: Iida, M et al,(2003). "Changes in global gene and protein expression during early mouse liver carcinogenesis induced by non-genotoxic model carcinogens oxazepam and Wyeth-14,643." Carcinogenesis 0143-3334

> WY-14643 molecular-effect ID: 5153011cb9743f479d000969
Effect: inc COPB1 mRNA expression in mouse
Evid: Sanderson, LM et al,(2008). "Effect of synthetic dietary triglycerides: a novel research paradigm for nutrigenomics." PLoS ONE 1932-6203

> WY-14643 molecular-effect ID: 5153011bb9743f479d000968
Effect: inc HNRNPF mRNA expression in rat
Evid: Ren, H et al,(2009). "Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional effects upon perfluoroalkyl acid exposure in diverse species." Reprod. Toxicol. 1873-1708

> WY-14643 molecular-effect ID: 5153011ab9743f479d000967
Effect: dec ATP11C mRNA expression in mouse
Evid: Han, ES et al,(2008). "The in vivo gene expression signature of oxidative stress." Physiol. Genomics 1531-2267

> WY-14643 molecular-effect ID: 5153011ab9743f479d000964
Effect: inc PTPN4 mRNA expression in mouse
Evid: Sanderson, LM et al,(2008). "Effect of synthetic dietary triglycerides: a novel research paradigm for nutrigenomics." PLoS ONE 1932-6203

> WY-14643 molecular-effect ID: 51530119b9743f479d000963
Effect: inc FABP3 mRNA expression in mouse
Evid: Ren, H et al,(2009). "Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional effects upon perfluoroalkyl acid exposure in diverse species." Reprod. Toxicol. 1873-1708

> WY-14643 molecular-effect ID: 51530119b9743f479d000962
Effect: inc HES3 mRNA expression in mouse
Evid: Qu, A et al,(2010). "PPARalpha-dependent activation of cell cycle control and DNA repair genes in hepatic nonparenchymal cells." Toxicol. Sci. 1096-0929

> WY-14643 molecular-effect ID: 51530118b9743f479d00095f
Effect: inc TOMM20 mRNA expression in mouse
Evid: Han, ES et al,(2008). "The in vivo gene expression signature of oxidative stress." Physiol. Genomics 1531-2267

> WY-14643 tissue-effect ID: 5152102fb9743f56860006bd
Effect: inc Atrophy in Testes hamster
Trt: 42.0 mg/kg/day 13.0 week
Evid: (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid (WY-14643) Subchronic oral toxicity in rodents in hamster

> WY-14643 tissue-effect ID: 5152102fb9743f563a000703
Effect: inc Mitotic Alteration in Liver mouse
Trt: 12.0 mg/kg/day 13.0 week
Evid: (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid (WY-14643) Subchronic oral toxicity in rodents in mouse

> WY-14643 tissue-effect ID: 5152102fb9743f563a000704
Effect: inc Apoptosis in Liver mouse
Trt: 22.0 mg/kg/day 13.0 week
Evid: (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid (WY-14643) Subchronic oral toxicity in rodents in mouse

> WY-14643 tissue-effect ID: 5152102fb9743f563a000706
Effect: inc Mitotic Alteration in Liver mouse
Trt: 22.0 mg/kg/day 13.0 week
Evid: (4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio) acetic acid (WY-14643) Subchronic oral toxicity in rodents in mouse

> WY-14643 molecular-effect ID: 51625b9fb9743f53ef0000df
Effect: inc NF-kB activation in Liver rat
Evid: Fischer, JG et al,(2002). "Moderate iron overload enhances lipid peroxidation in livers of rats, but does not affect NF-kappaB activation induced by the peroxisome proliferator, Wy-14,643." J. Nutr. 0022-3166

> WY-14643 molecular-effect ID: 51625b9fb9743f53ef0000e1
Effect: alter Gap Junctional communication in Liver rat
Evid: Mally, A et al,(2002). "Non-genotoxic carcinogens: early effects on gap junctions, cell proliferation and apoptosis in the rat." Toxicology 0300-483X

> WY-14643 cell-effect ID: 51625b9fb9743f53ef0000dd
Effect: inc Oxidative Stress in Liver rat
Evid: Conway, JG et al,(1989). "Relationship of oxidative damage to the hepatocarcinogenicity of the peroxisome proliferators di(2-ethylhexyl)phthalate and Wy-14,643." Carcinogenesis 0143-3334

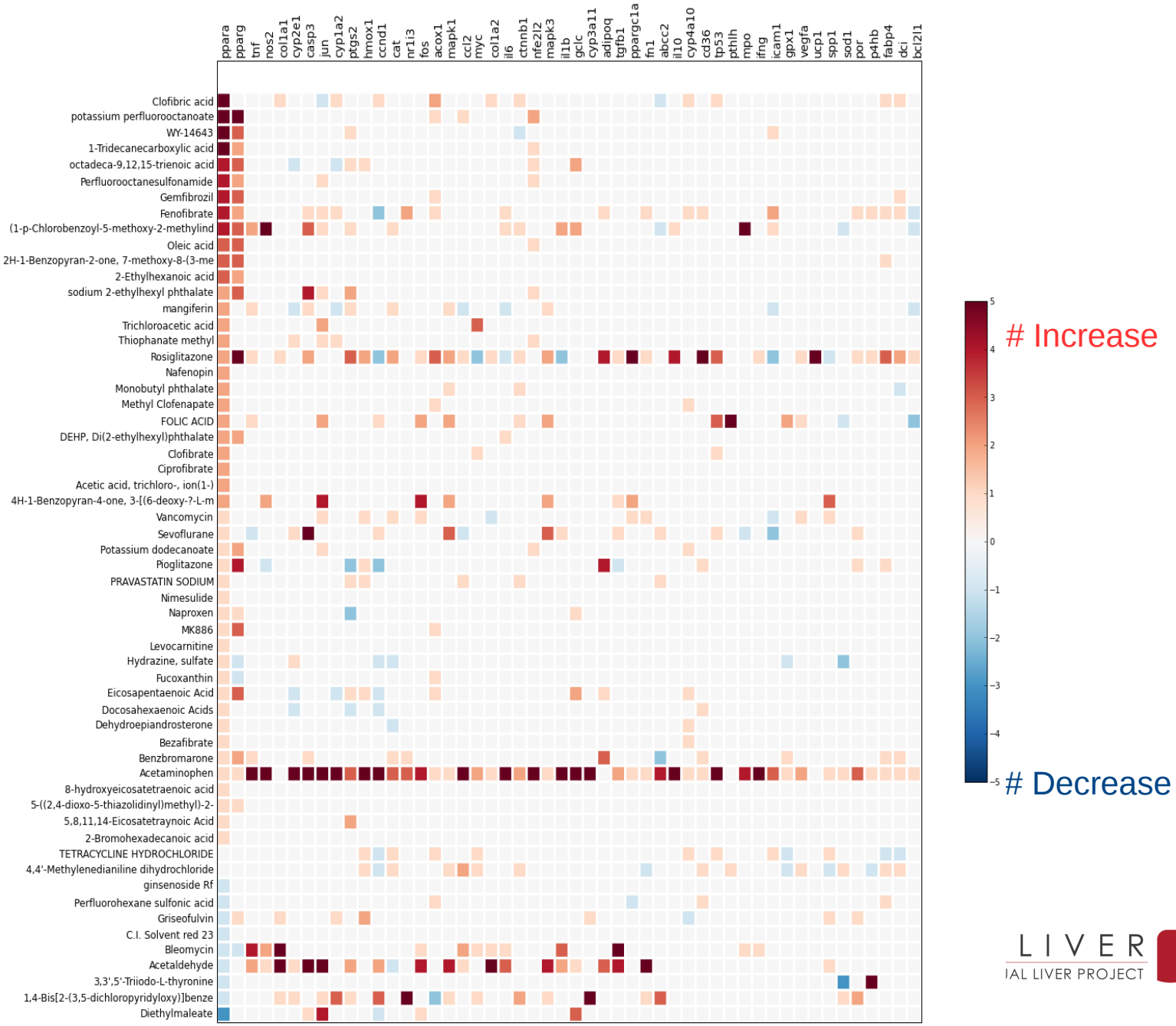
> WY-14643 cell-effect ID: 51625c48b9743f53ef00013a
Effect: inc Oxidative Stress in Liver mouse
Evid: Woods, CG et al,(2007). "WY-14,643 induced cell proliferation and oxidative stress in mouse liver are independent of NADPH oxidase." Toxicol. Sci. 1096-6080

> WY-14643 molecular-effect ID: 51625b9fb9743f53ef0000d3
Effect: inc PPARalpha activation in Liver rat
Evid: Corton, JC et al,(2005). "Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract?" Toxicol. Sci. 1096-6080

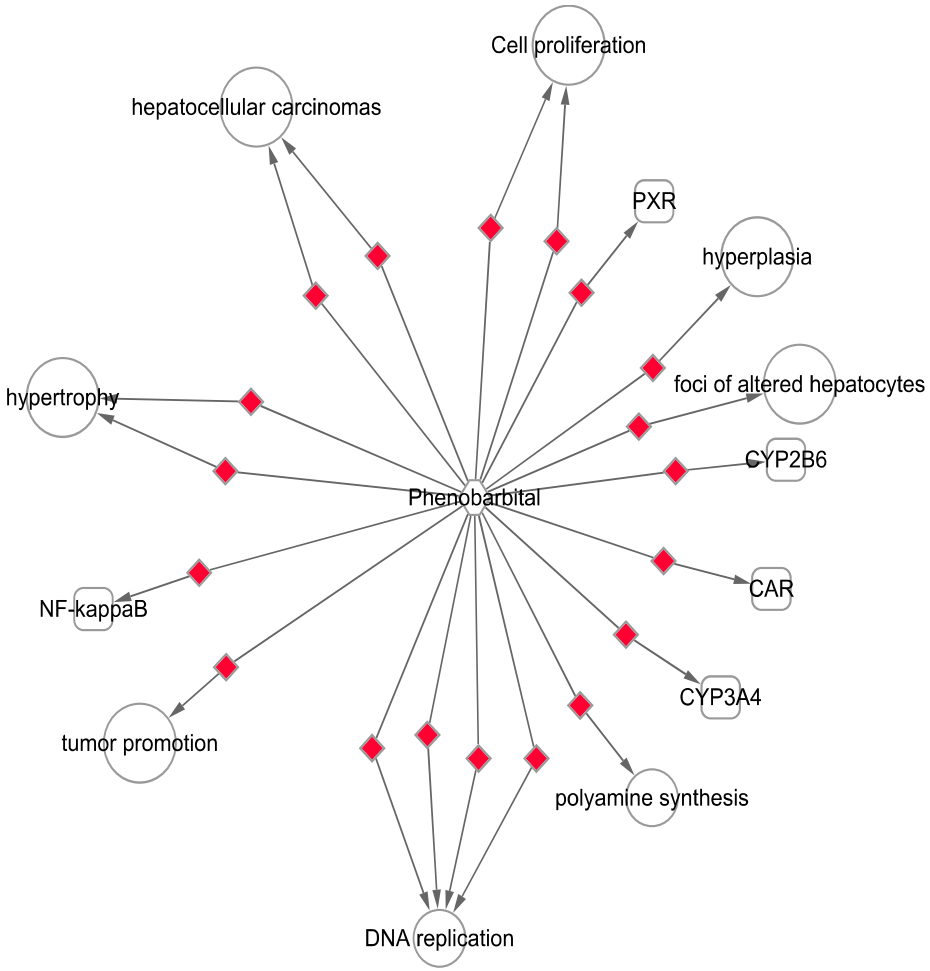
> WY-14643 molecular-effect ID: 51625c47b9743f53ef000132
Effect: inc PPARalpha activation in Liver mouse
Evid: Bility, MT et al,(2004). "Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters." Toxicol. Sci. 1096-6080

Chemicals Effects – Relational

Summary:
58 Chemicals
50 Targets
988 Effects



Chemical Effects → Semantic View



Chemical

Molecular Event

Cellular Function

Histological Outcome

Increase

Decrease

No change

Chemical Effects

Subset of experimental evidence about Phenobarbital (PB) from KB

Shah et. al., Bionformatics (*in revision)*

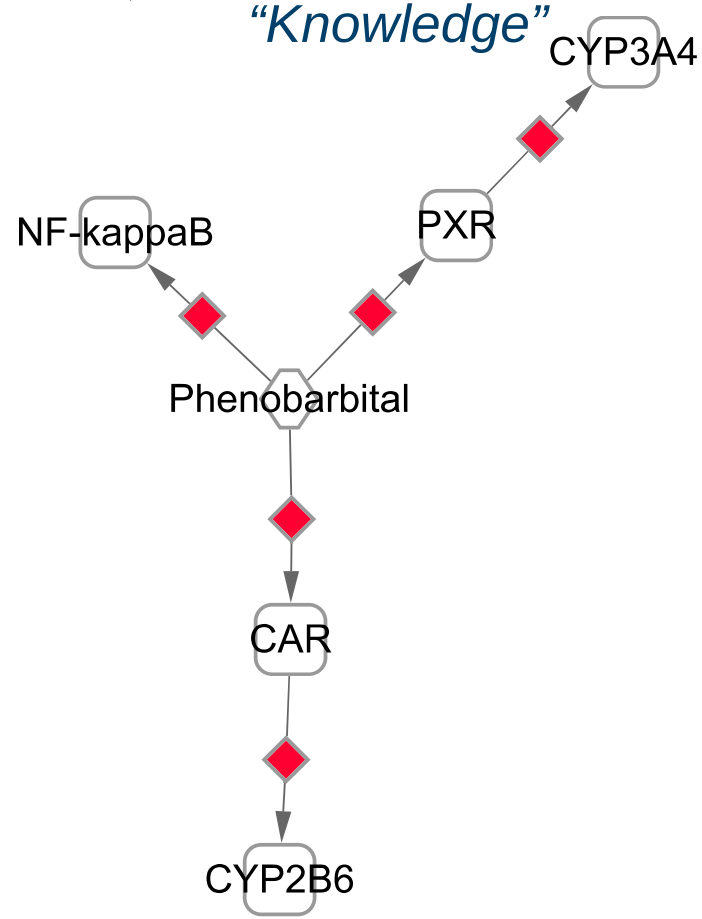
Semantics → Computational Inference

Prior Knowledge → *Inference Algorithm* → *New "Knowledge"*

PB → CYP3A4

PB → PXR

PXR → CYP3A4



→ Chemical Effect

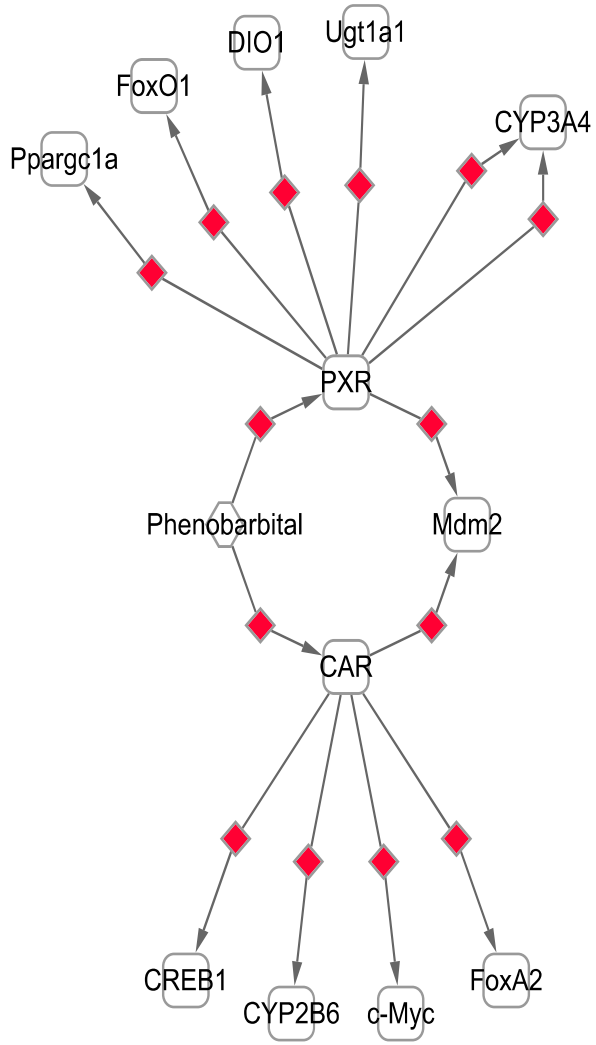
Latent Effects

Shah et. al., PLOS Comp Bio (*in revision)*

Computational Inference → Hypotheses

Plausible explanation of putative PB-mediated molecular changes

Evidence for Mdm2 activation by PB was not in the KB but has been shown experimentally



Chemical Effect

Latent Effects

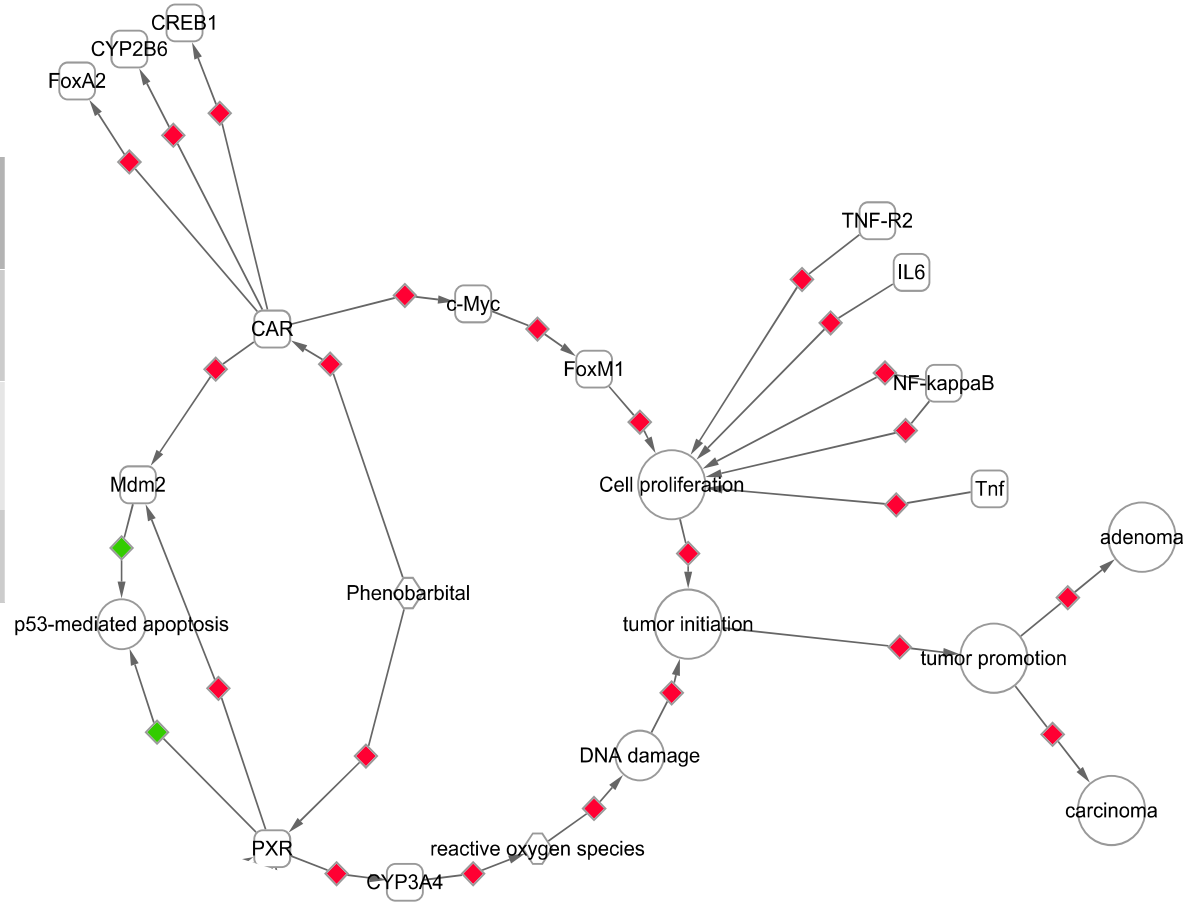
Hypotheses:

PB activates Mdm2 via CAR
PB activates FoxO1 via PXR

Shah et. al., PLOS Comp Bio (*in revision)*

Computational Inference → “AOPs”

Chemicals	Molecular	Cellular	Tissue
Phebobarb/ TCPOBOP	CAR > FoxM1	Initiation > promotion	Hyperplasia> Neoplasia
Phebobarb/ TCPOBOP	PXR > ROS	DNA Damage > Initiation > promotion	Neoplasia
Phebobarb/ TCPOBOP	CAR > Mdm2	Apoptosis	??

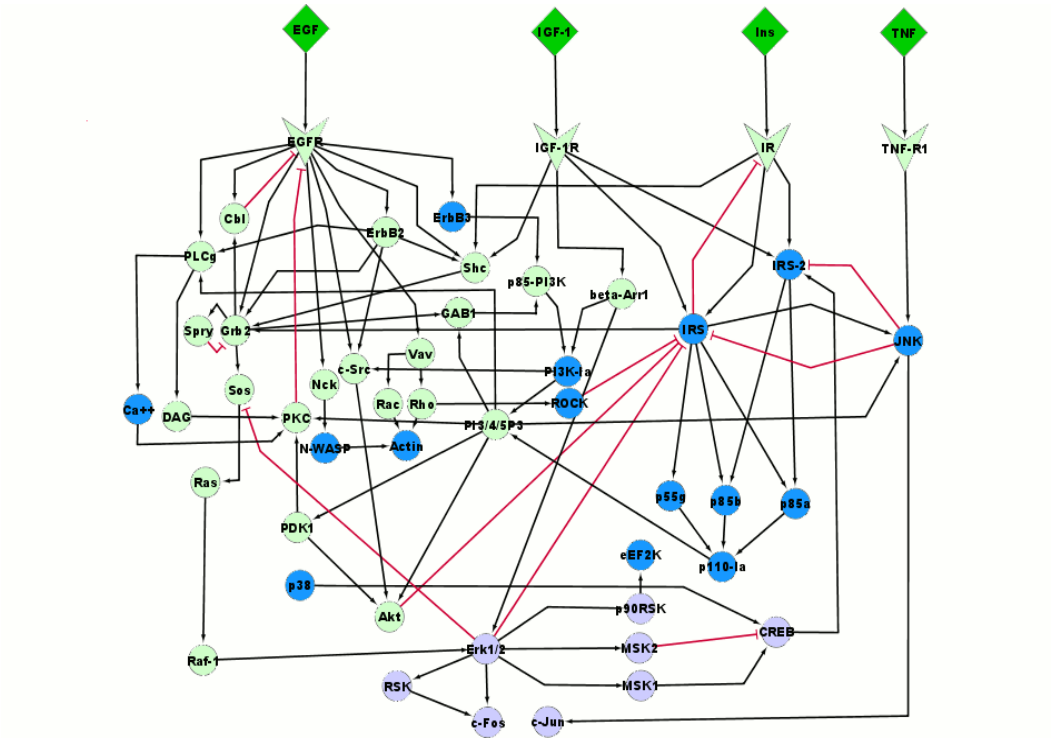


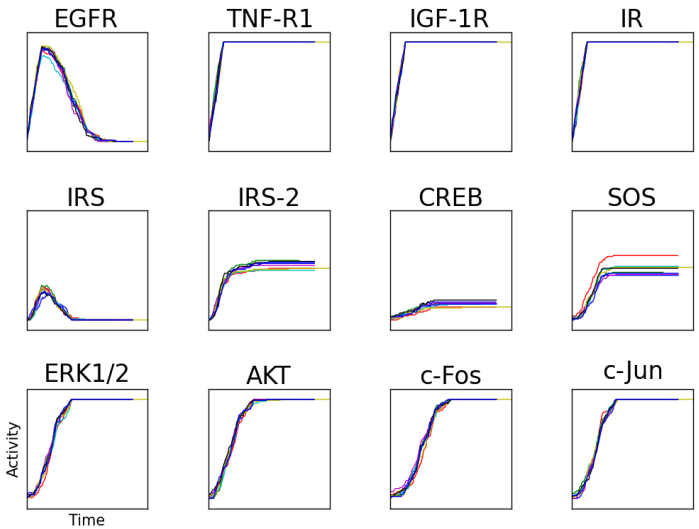
Hypothetical “AOPs” – require further curation

Shah et. al., PLOS Comp Bio (*in revision)*

“AOPs” → Dose Response

Hepatocyte





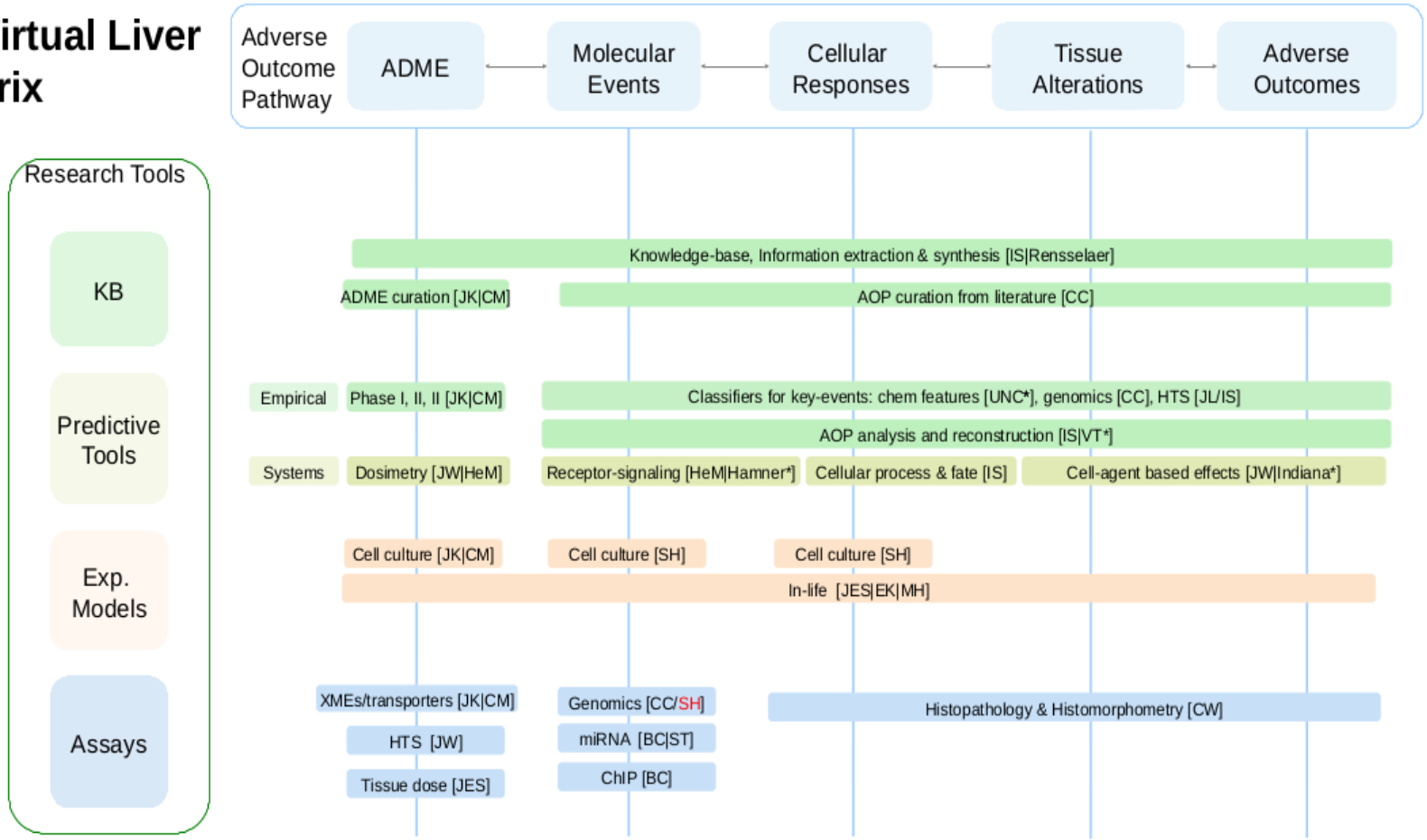
Quantitative systems modeling – simulating changes in early cell-cycle progression using *in vitro* data

Jack, et al. BMC Systems Biology (2011)

Summary

- Pathways – key to chemical evaluation
  - Interpreting HTS data for new chemicals
  - Quantitative dose-response modeling
- Approach: Data → Ontology → Knowledge → Inference → Hypotheses
- Implementation
  - Ontology for describing toxicity pathways (OWL/RDF)
  - Knowledge-base (KB) for capturing assertions (SPARQL)
  - KB Visualization tool (Cytoscape/c-Mantic)
  - Custom pathway-inference engine
- Broadly applicable to toxicology & AOPs
- Utility of ontology dependent on linkage with:-
  - Public referential vocabularies
  - Public relational vocabularies

CSS 2.2.1 Virtual Liver Project Matrix



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Not for distribution