NTP
National Toxicology Program

The DrugMatrix® (DM) Database

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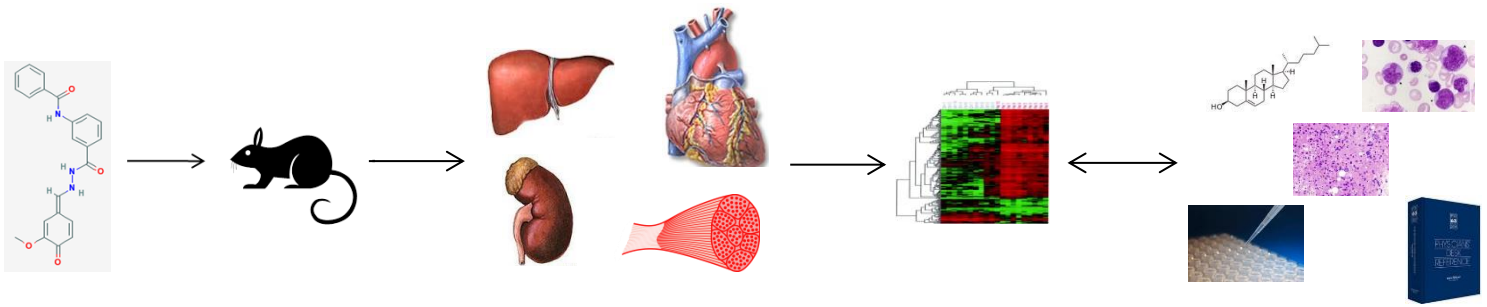

Disclaimer

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Photo courtesy of Steve McCaw

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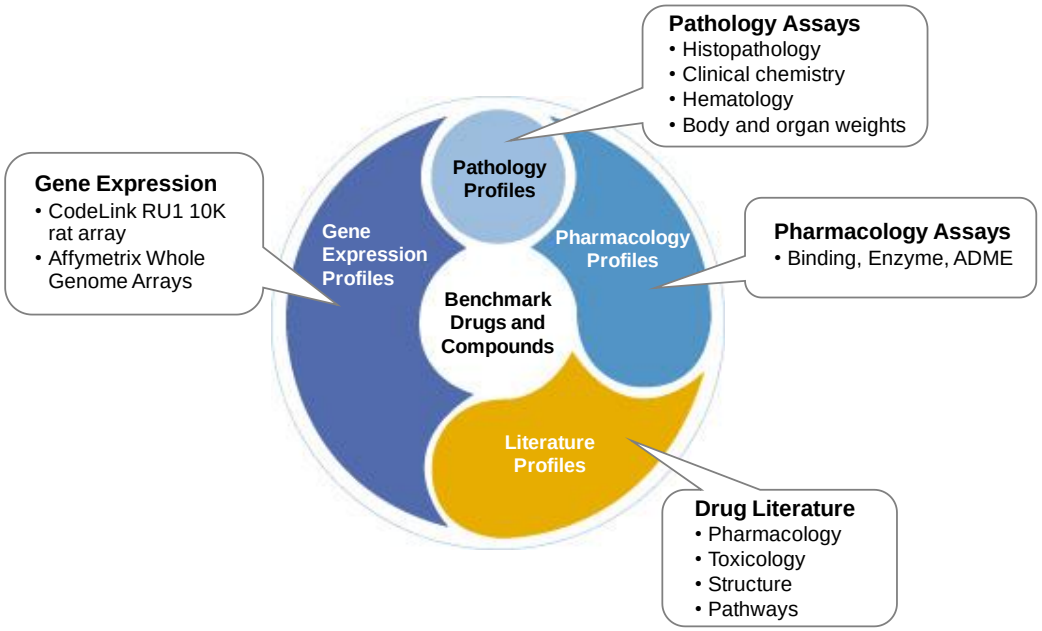
Outline

- Data
- Interface
- Example Application

DrugMatrix Data



DrugMatrix

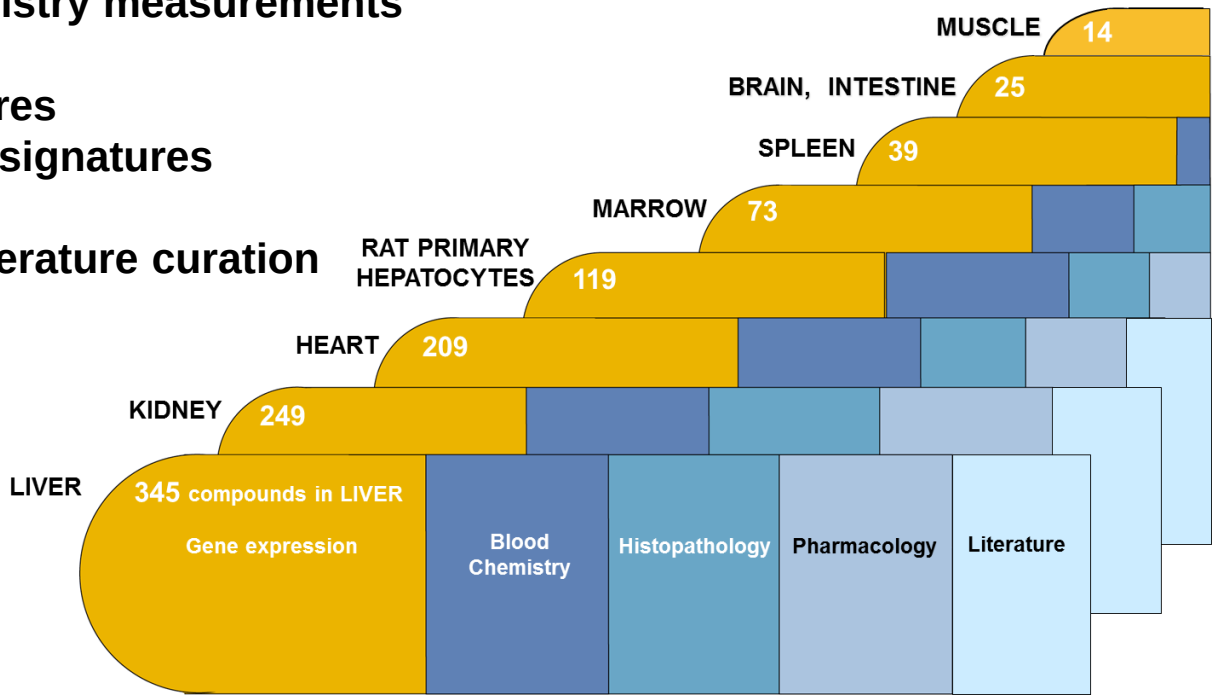
- DrugMatrix
  - Large-scale Rat Toxicogenomics Database and Analysis Tool
  - https://ntp.niehs.nih.gov/drugmatrix/index.html
- Originally owned by Iconix Pharmaceuticals and Entelos, Inc.
  - No data for these resources were generated by NTP
- Acquired by NTP in late 2010

Goals of Acquisition

- Make the computational and data resources **open to the public (no fee)**
- Facilitate the **integration** of toxicogenomics into hazard characterization
- **Build a bridge** between traditional toxicology and Tox21

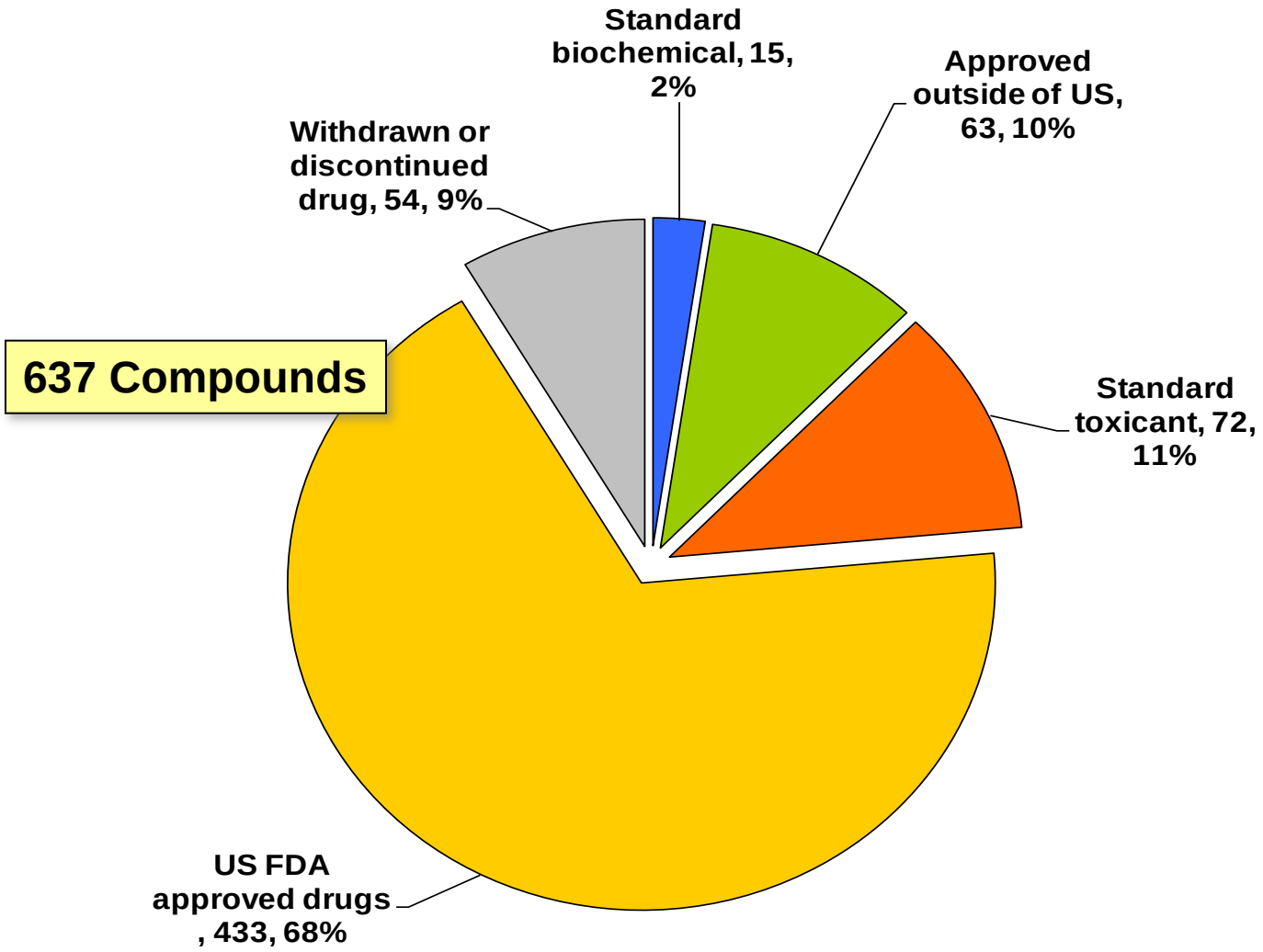
DrugMatrix Database Content

~ 700 Short-term toxicity studies (0.25 to 5 days) in male SD rats
~ 637 compounds studied at multiple doses, time points and tissues
~ 5600 drug-treatment transcript profiles
~ 13,000 Codelink RU1 Microarrays
~ 5,000 Affymetrix RG230-2 Arrays
~ 127,000 histopathology measurements
~ 150 histopathology diagnoses over 7 tissues
~ 100,000 hematology and chemistry measurements
~ 138 hand annotated pathways
~ 290 scorable genomic signatures
~ 2500 pathway-based scorable signatures
~ 130 in vitro assays
~ 900 chemicals with detailed literature curation
~ 8000 chemical structures
~ 60,000 literature facts
~ 123,000 frozen samples

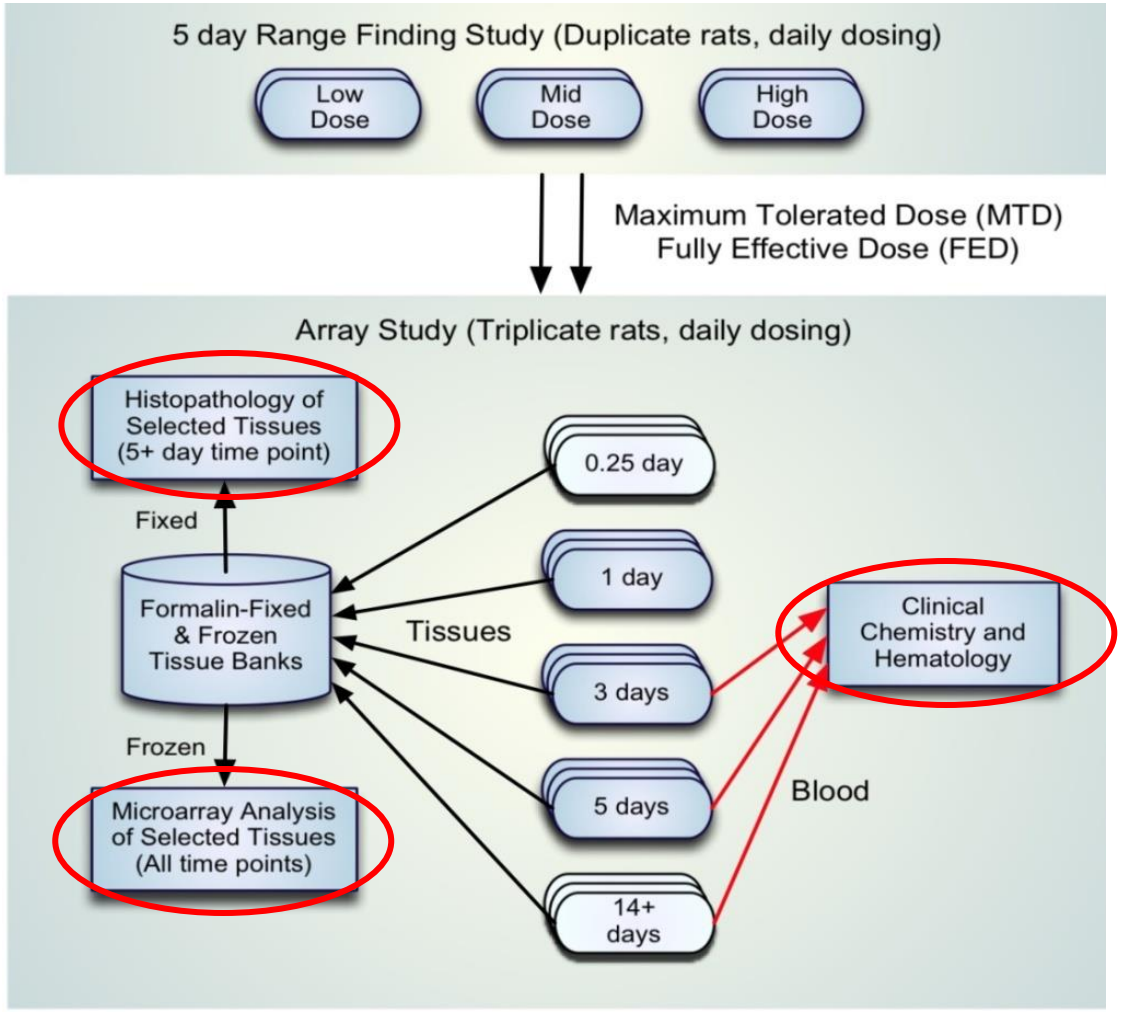


In vivo Studies

DrugMatrix Chemical Diversity



Standardized DrugMatrix *in vivo* Experimental Protocol



- Two doses
  - MTD
  - FED
- Four time points
  - 0.25
  - 1
  - 3
  - 5, 7, or 14
  - 3 rats/time point
- Daily dosing every morning at ~ same time
- Sacrifice in morning at ~ same time (except 0.25d)
- Tissues collected
  - Punches flash frozen
  - Part fixed in formalin

Frozen Samples from In vivo Studies

Tissue	Compounds	Treatments	Organism	Treatments/ Compound
Liver	661	5301	Sprague Dawley Male	8.0
Whole Blood	152	778	Sprague Dawley Male	5.1
Plasma	661	4665	Sprague Dawley Male	7.1
Heart	661	4510	Sprague Dawley Male	6.8
Kidney	661	5358	Sprague Dawley Male	8.1
Thigh Muscle	653	2892	Sprague Dawley Male	4.4
Total	**661**	**5622 unique**		

- Treatments could include: 2 dose levels (MTD/FED), 4 time points (1, 3 and 5 days + 4th (7, 14, 30 or 90 day)) = up to 8 treatments
- Gene arrays run on liver, heart, kidney, thigh muscle, bone marrow, spleen, brain, intestine, and primary hepatocytes.
- Snap frozen tissues retained for organs/fractions in table above.
- Histolopathology (100 endpoints) read on all tissues for which gene arrays were run, and for most of the remaining tissues. (Rat Altas of Histopathology). Histology slides and FFPE sections no longer available.
- RNA likely available for most tissues for which gene arrays were run, for most of the remaining tissues, and for most rat primary hepatocyte cultures.
- Clinical chemistry and hematology available on many samples

Dose Justification

DOSE JUSTIFICATION

Note: The final low array dose choice was based on a rat model for gastric ulcer formation and motor co-ordination.

High Array Dose Recommendation
At the high RF dose (54mg/kg/day), weight gain was 21.3%. No clinical signs were displayed by any animals at any of the doses. Animals gained 21.4% body weight at the mid (32mg/kg/day) and 19.2% body weight at the low (16mg/kg/day) RF doses. It has been decided to use the high RF dose as the high array dose.

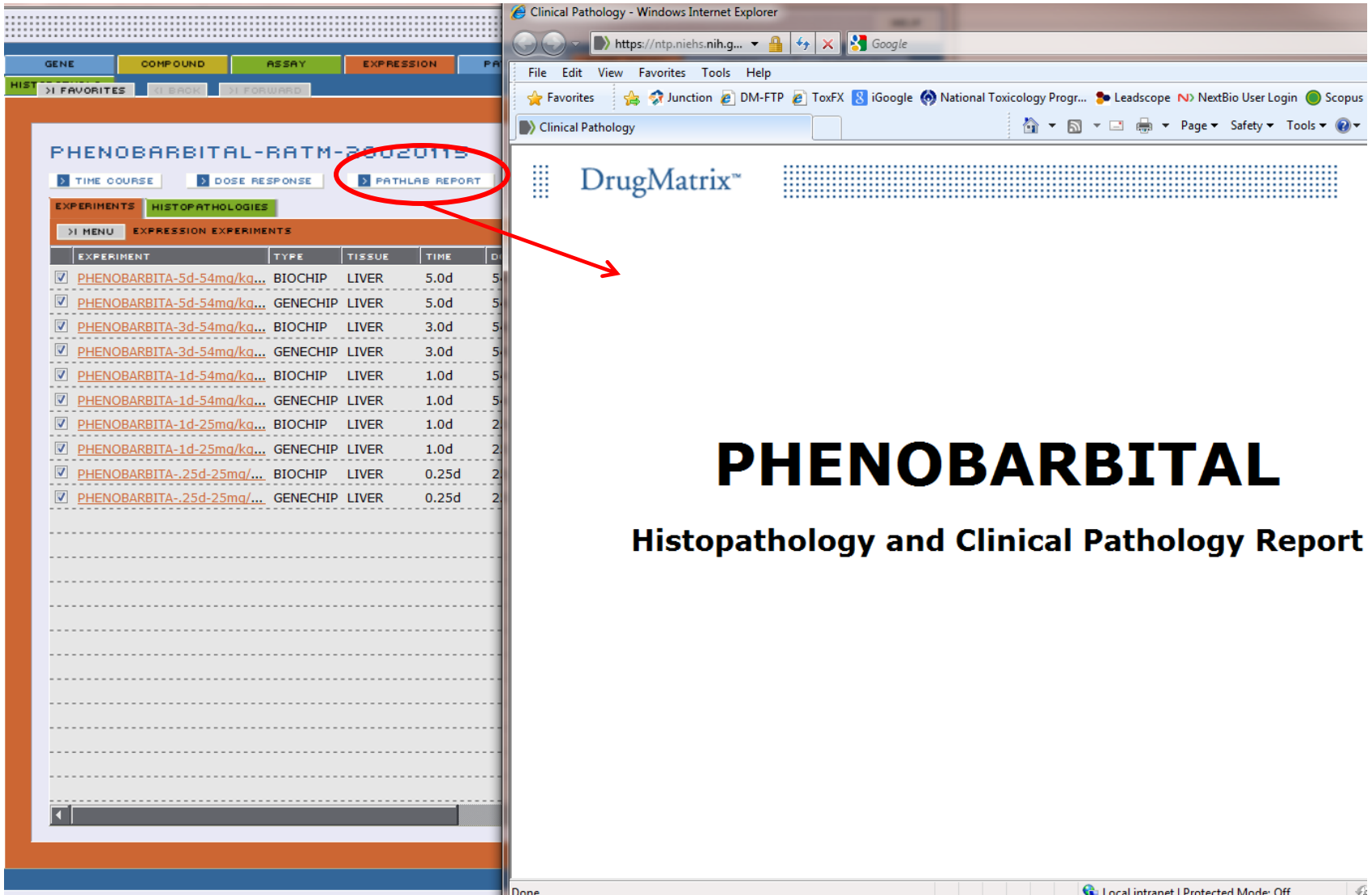
Low Array Dose Recommendation
Phenobarbital is indicated for gastrointestinal disturbances such as cramps, spasms, diarrhea, nausea, vomiting and peptic ulcer. It is also indicated as a treatment for irritable bowel syndrome and is a potent anesthetic/anti-convulsant. Human dose is 125mg/day (PDR). This scales to 7mg/kg/day in the rat. 50mg/kg/day for 10 days was administered to rats with chronic gastric ulcer. This dose significantly increases cytochrome P-450 in the gastric mucosa, which results in the stimulation of mucosal barrier protective glycoproteins (PMID 9206565 - abstract only).

Gastric ulcers were induced in rat glandular stomach by the cold-restraint method. The fore and hind limbs are tied together with metallic wire and the rats are left in a 4oC incubator for 2 hours. An oral dose of 50mg/kg gave 78% protection against ulcer formation under these conditions. 20mg/kg resulted in 33% protection and 10mg/kg resulted in 11% protection. Rats are also trained to walk on a rotating rod. The reduction in locomotor activity caused by Phenobarbital administration is reflected in the % of animals that fall off the rod in 2 minutes. 100mg/kg Phenobarbital will cause 100% of the rats to fall off the rod; 50mg/kg cause 70% to fall; 25mg/kg causes 40% to fall (PMID 2860988). 25mg/kg/day administered in the rat diet for 2 weeks resulted in significant induction of hepatic cytochrome P450 (PMID 1554380 - article retrieved).

I recommend that we use 25mg/kg/day as the low array dose, which would protect against gastric ulcer formation (50mg/kg/day) is too close to the high array dose. Floater tissue should be the brain.

All recommendations were accepted at the dose-setting meeting 12-17-01.

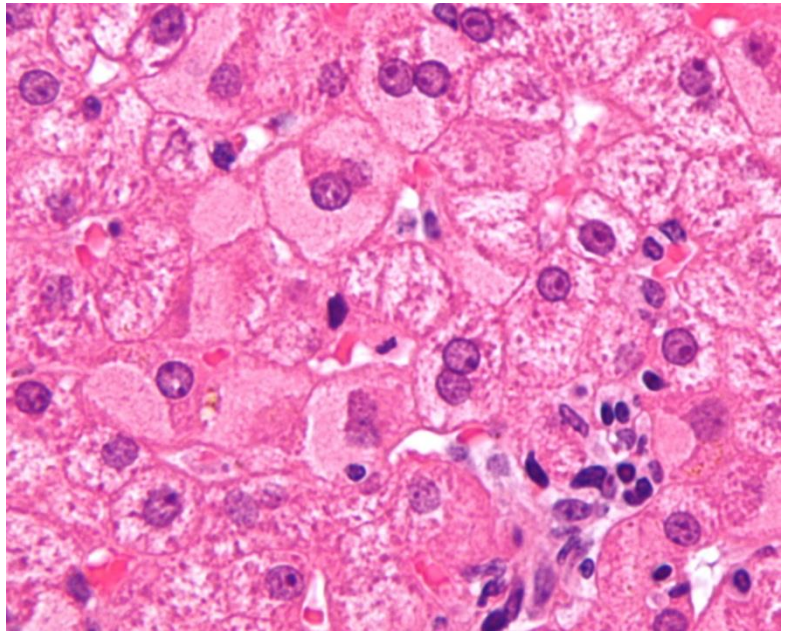
Pathology Lab Report



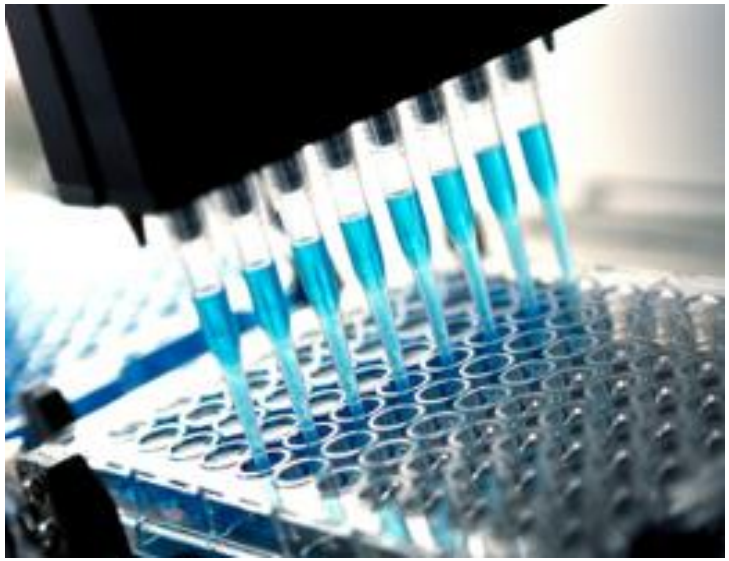
In vitro Studies

Rat Hepatocyte Genomics

- Male Sprague Dawley Rats
- 16 and 24 hr treatments
- Codelink
  - 119 chemicals
  - 244 treatments
- Affymetrix
  - 126 chemicals
  - 279 treatments

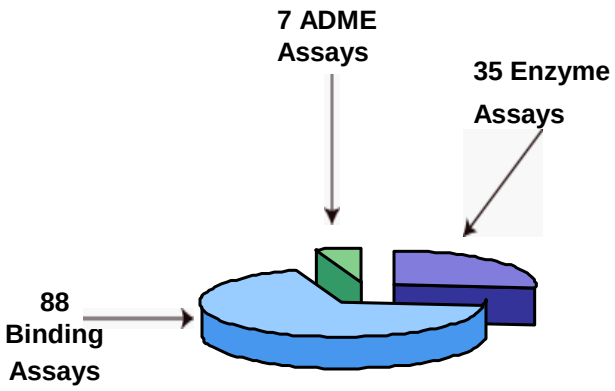


Pharmacology Assays



Molecular Pharmacology Profiles

- Over 870 compounds profiled across 130 *in vitro* pharmacology assays



DOXORUBICIN

SMILES | SYNONYMS | IDENTIFIERS | PHYSICAL PROPS.

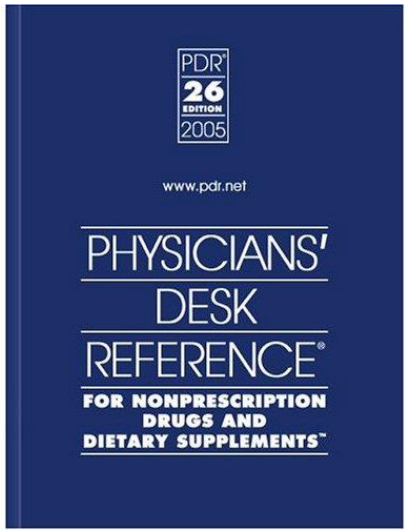
SIMILAR | INDUCED | REPRESSED | EXPERIMENTS | ACTIVITIES | LITER. | TARGET | MOTI

>I MENU BIO-ACTIVITIES

ASSAY		INHIBITION	IC50	KI
Protein Tyrosine Kinase, HER2 Receptor	☑	100.0%	2.485uM	
Protein Tyrosine Kinase, Fyn	☑	98.0%	5.147uM	
Serotonin 5-HT4	☑	82.0%	12.468uM	2.078uM
Sodium Channel, Site 2	☑	75.0%	15.245uM	13.668uM
Protease, Caspase 1	☑	73.0%	12.192uM	
Lipoxygenase 15-LO	☑	73.0%	13.59uM	
Protein Tyrosine Kinase, Lck	☑	61.0%	31.913uM	
Muscarinic M1	☑	53.0%	15.336uM	3.693uM
Serotonin 5-HT1B	☑	52.0%	34.464uM	15.666uM

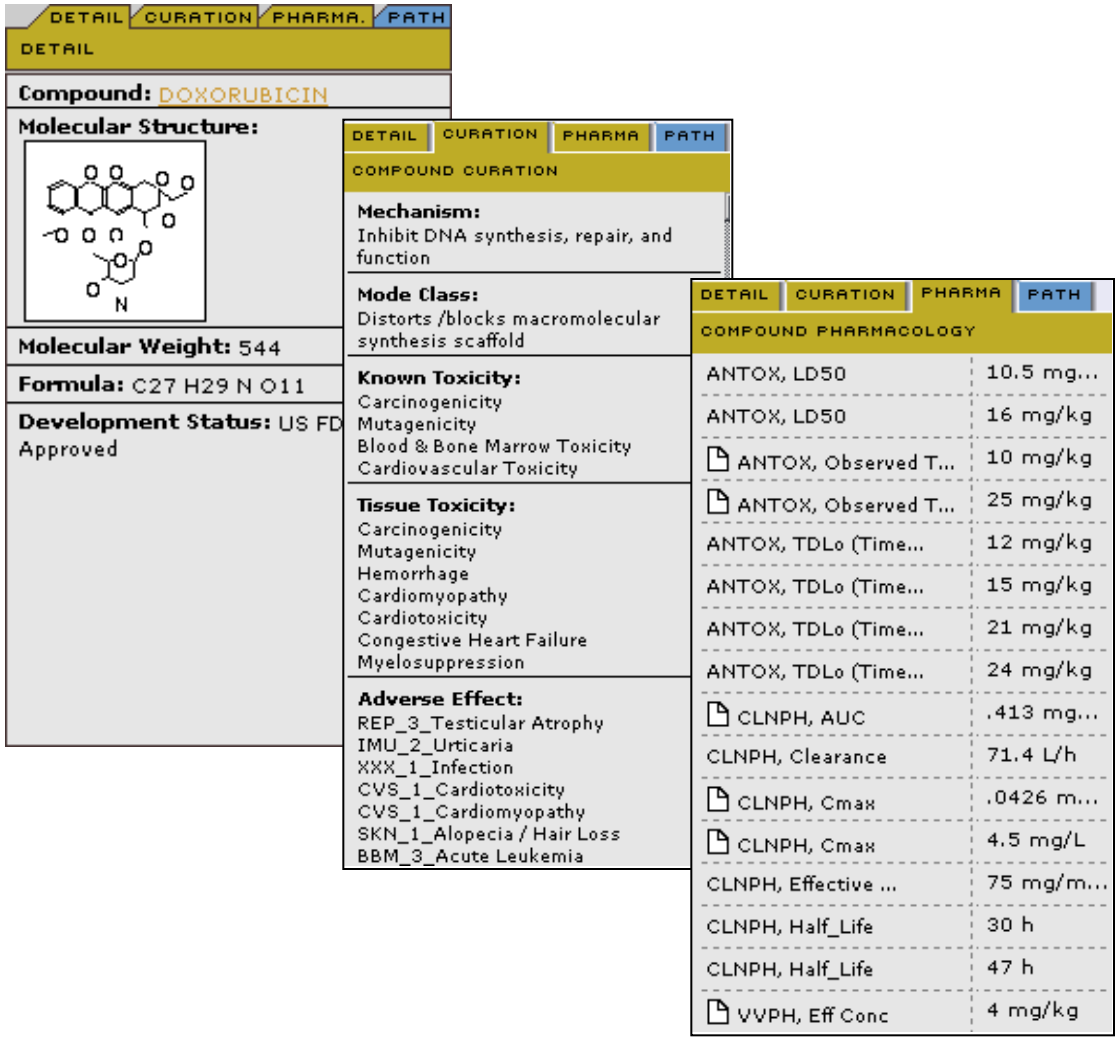
Literature and Structure Curation

PubMed



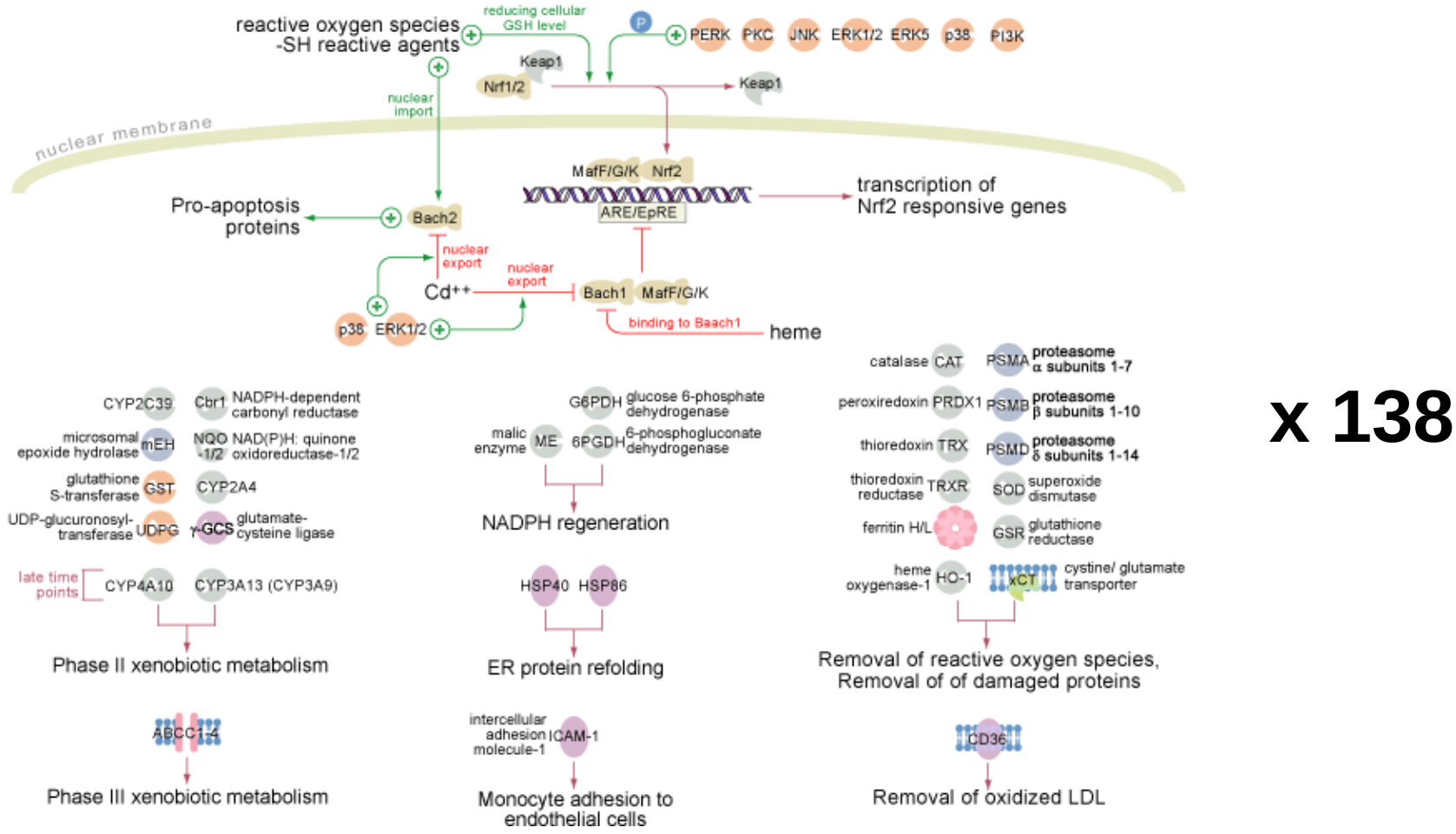
Literature and Structure Curation

- Clinical literature
  - indication, mechanism, toxicity
- Pharmacology literature
  - ED50, LD50, IC50
- Pharmacokinetic literature
  - CMax, half-life, AUC, Clearance
- Physical properties
  - MW, logP, logS, pKa
- Structure files
  - .mol files



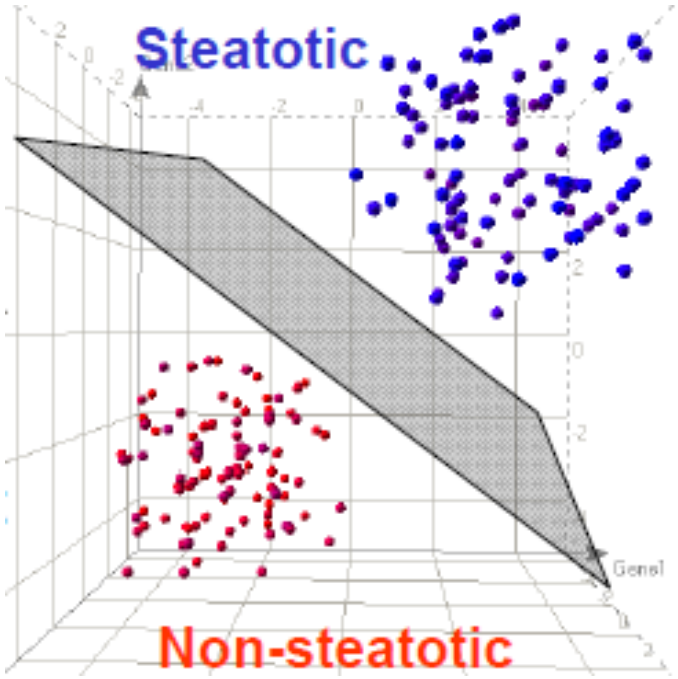
Pathways

Oxidative Stress Response Mediated by Nrf2



x 138

Signatures



Signature Types	Example Signatures*
Organ Pathology	Hepatic Necrosis, Bile Duct Hyperplasia, Renal Tubular Necrosis, Nephromegaly, Cardiac Myocyte Degeneration, Heart Weight Increase
Mechanistic Class	DNA Alkylator, PXR Activation, Peroxisome Proliferator

*Thousands of signatures

Where you can get the data???

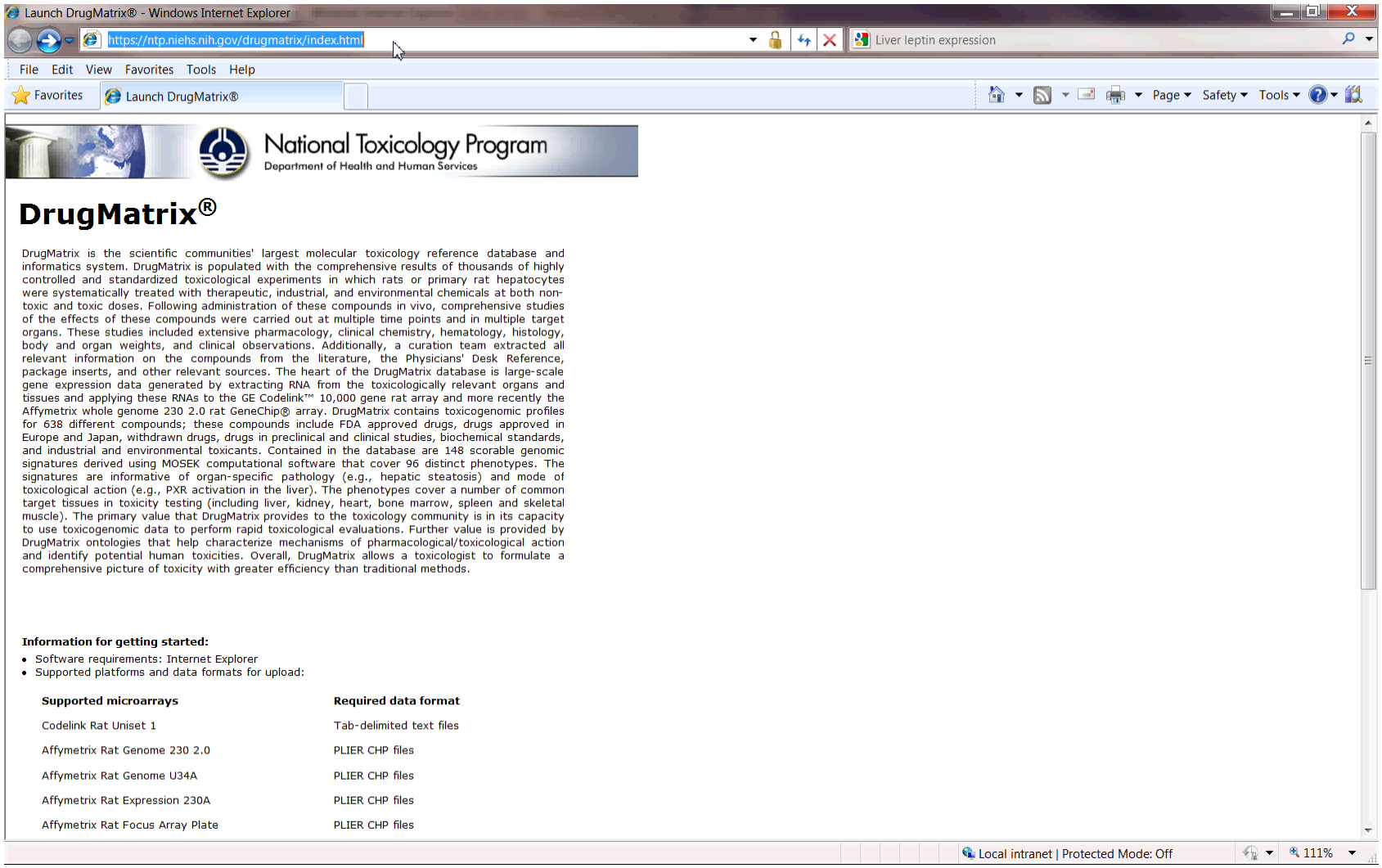
DrugMatrix Data

Download DrugMatrix Array Data 

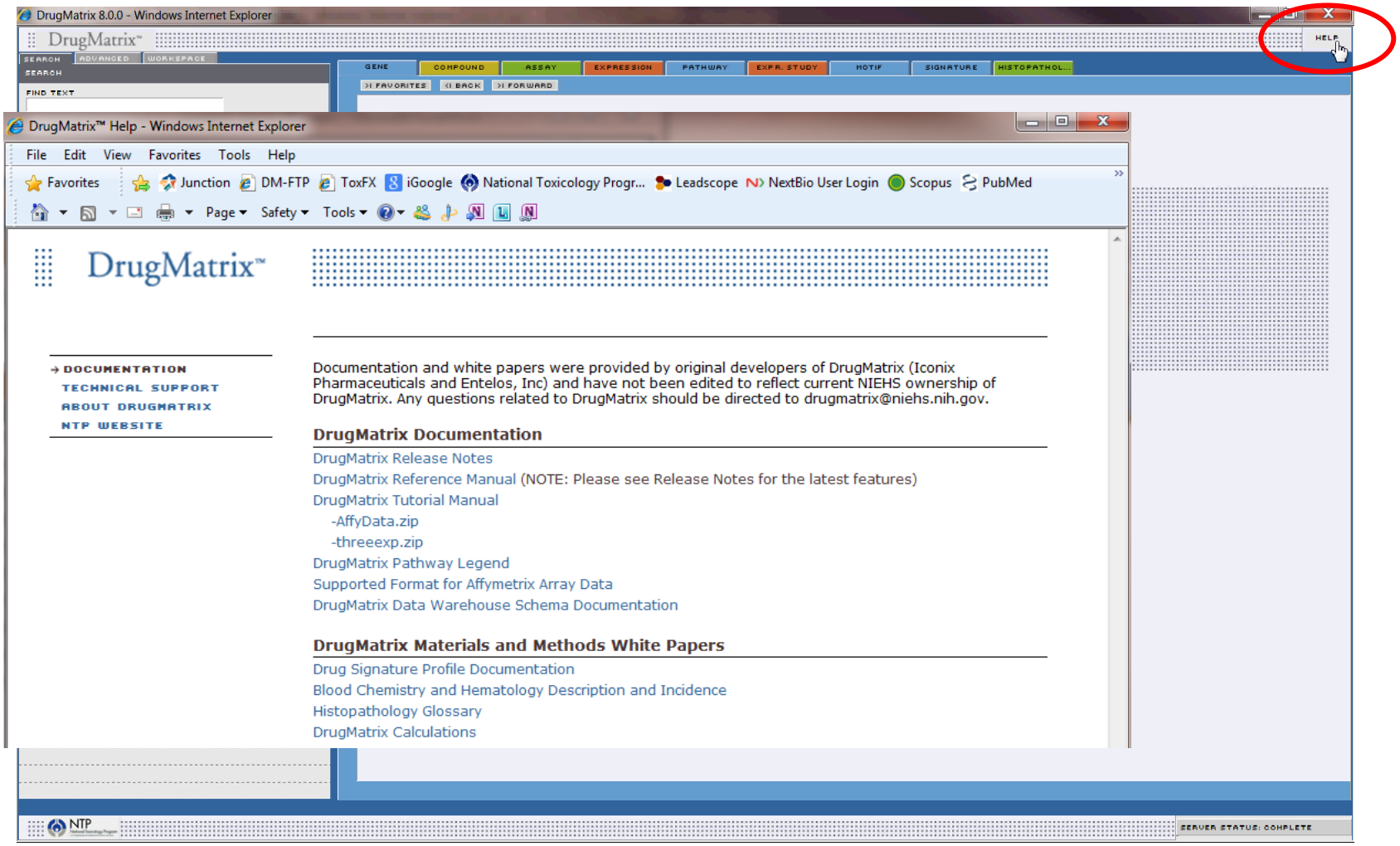
- ftp://anonftp.niehs.nih.gov/drugmatrix
- Unprocessed microarray data
- Microarray data normalized by organ
- Individual animal toxicology data
- In vitro screening data
- Chemical Annotations

DrugMatrix Interface

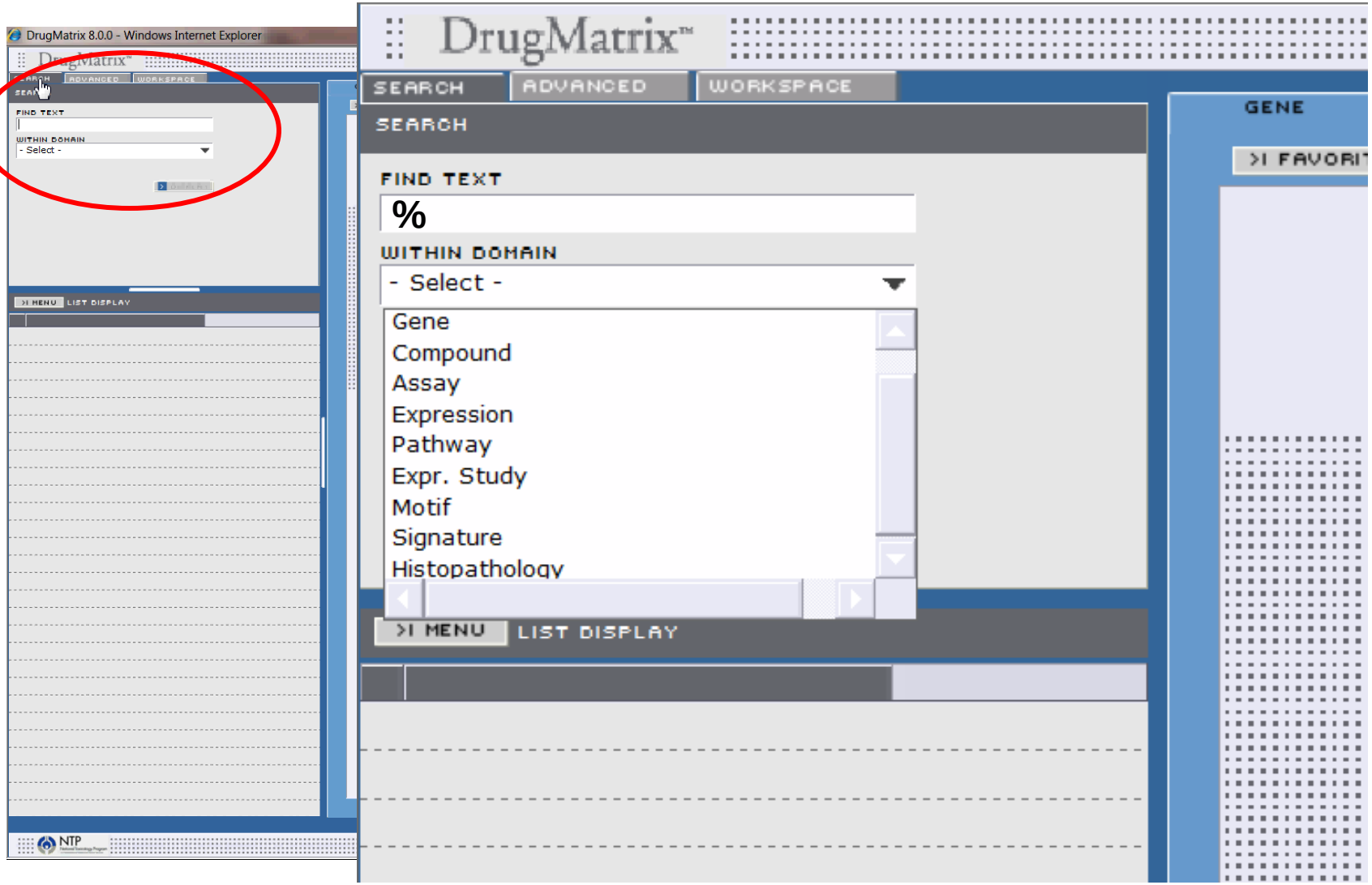
DrugMatrix Homepage



DrugMatrix Interface – Help Section



DrugMatrix – Search and List Management Tools



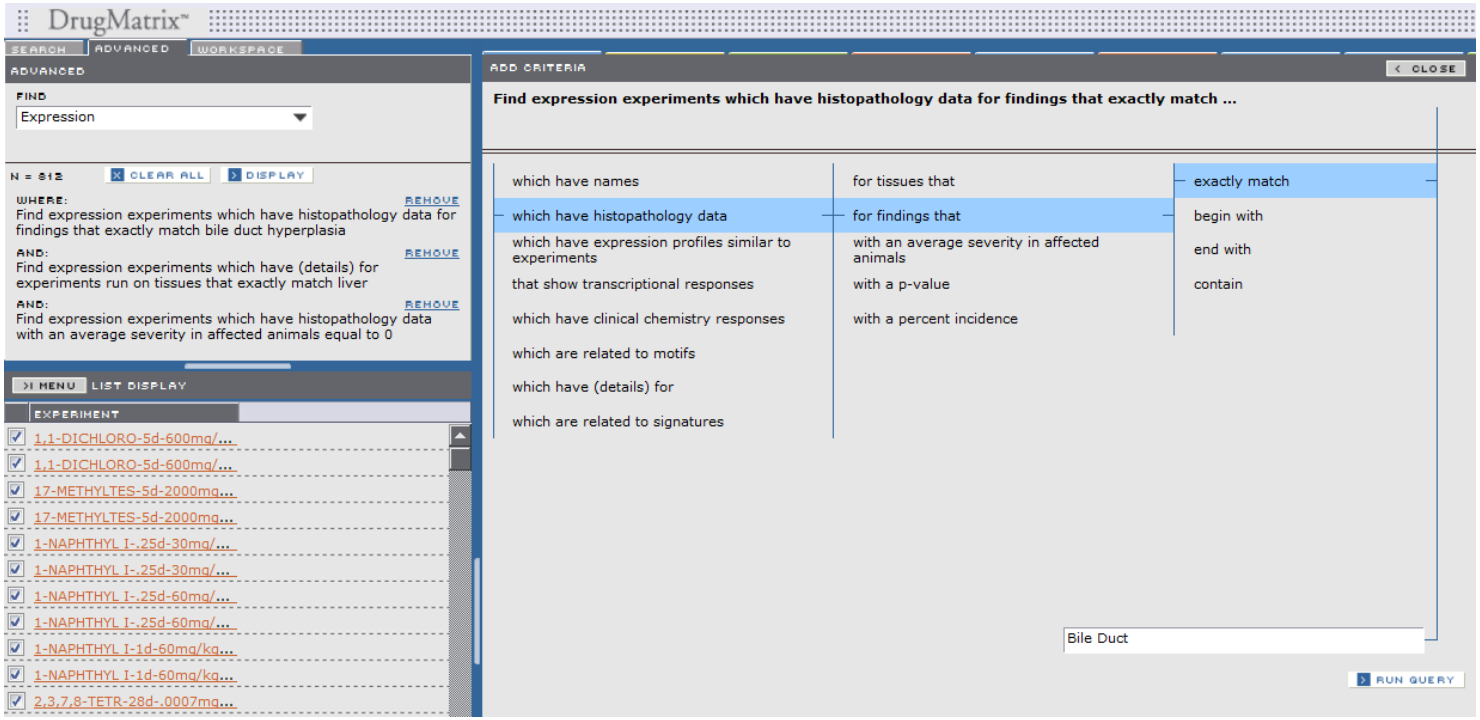
DrugMatrix Integrated Data Domains

- **Gene Domain** – search individual genes to find out how they behave with different treatments
- **Chemical Domain** – search individual chemicals to identify structurally similar chemicals, gene expression perturbations, assay hits, etc
- **Assay Domain** – search and display in vitro assay results and clinical chemistry/hematology results
- **Expression Domain** – view results for single expression studies
- **Pathway Domain** – explore over 130 different biological pathways
- **Expression Study Domain** – view composite results multi-day/dose toxicity/toxicogenomic studies
- **Signature Domain** – Search predefined genomic signatures in DrugMatrix
- **Histopathology Domain** – Find details on specific histopathologies annotated in DrugMatrix
- **Motif Domain** – Search DrugMatrix Motif Signatures that are based solely on genes annotated to biological pathways

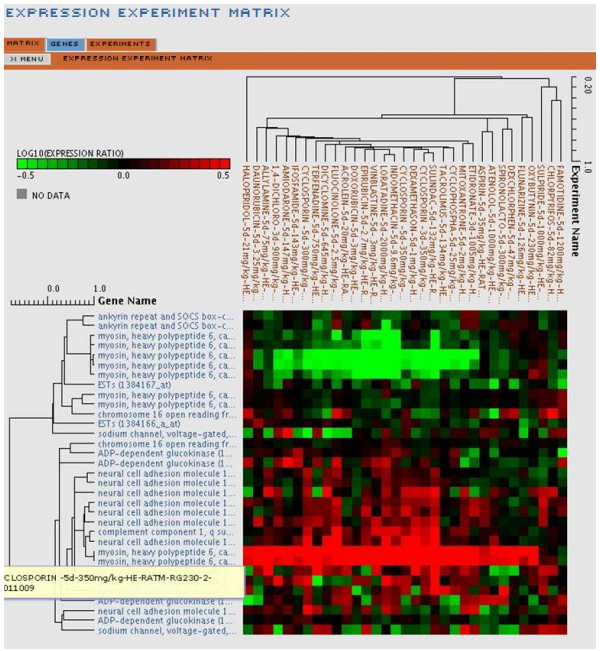
Advanced Search Tool

Advanced Search Tool

- Conduct a variety of sophisticated queries of the DrugMatrix data
- Filter for drug/target interactions
- Mine data relevant to your research



Analysis Tools



DrugMatrix Functionality and Analysis Tools

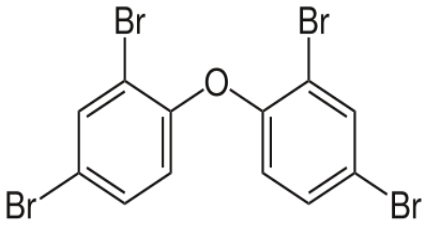
- Upload your own data for analysis or mine the DrugMatrix data
  - Data you upload is private – not shared with the government or other users
- Contextualize your data relative to over 4000 expression profiles elicited by >600 well characterized, phenotypically anchored prototype agents
- Find similar expression profiles
- Determine significantly up and down regulated genes
- Perform gene ontology analysis of perturbed genes
- Visualize expression profiles on pathways
- Score expression profiles for >50 phenotypes with genomic signatures
- Construct expression patterns for putative biomarker sets
- Test the performance of biomarker sets for detecting phenotypes
- Find consistently changed genes
- Identify enriched literature annotations in groups of expression profiles
- Mine the literature

Example Application of DrugMatrix

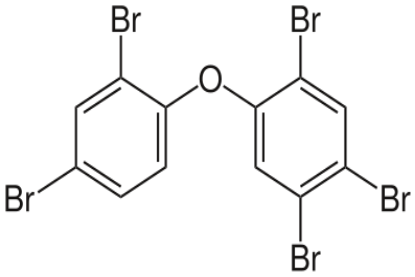
Toxicogenomic Assessment of DE-71

(Study Scientist: Dr. June Dunnick)

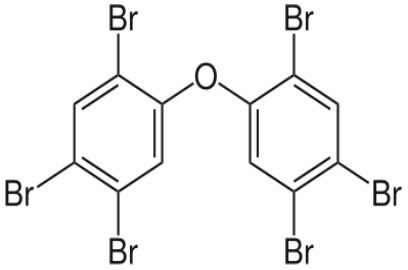
DE-71: A mixture of polybrominated diphenyl ethers



BDE 47



BDE 99



BDE 153

- PBDEs are flame retardant components that bioaccumulate; persistent organic pollutants

- Widespread human exposure

Gene Expression Study design

- Dose level: 0 or 50 mg/kg/day
- Route: Oral Gavage (corn oil)
- Model: Male Wistar Han rats
- Exposure period: gestational day (GD) 6 to postnatal day (PND) 21
- Euthanized: PND 22
- Tissue evaluated: Liver
- Question: What are the potential toxicological effects of DE-71 that can be identified by toxicogenomics?
- DE-71 expression studies are not included in DrugMatrix Database

DrugMatrix Analysis of DE-71- Top DEGs (Liver)

- Cyp1a1, Cyp2b, Cyp2c

- Fgf21, Cyp17a1, Abcg8

Induced

SIMILAR | INDUCED | REPRESSED | DENDROGRAM | CLIN. PATH. | MOTIF | SPLP | TRANK | HISTOPATHOLOGY

>| MENU TRANSCRIPTIONAL RESPONSES (INDUCED)

GENE	CONFIDENCE INTERVAL	P VALUE
urinary protein 2 (1370396_x_at,rc_AA945585_at)		2.68E-10
urinary protein 2 (1370349_a_at)		1.41E-10
estrogen sulfotransferase (1368733_at,M86758_at,NM_012883_PROBE1)		9.59E-8
cytochrome P450, family 2, subfamily A, polypeptide 3a (1369136_at)		1.07E-10
transmembrane protein 27 (1387013_at,NM_020976_PROBE1)		3.49E-9
CEA-related cell adhesion molecule 10 (Non-specific probe) (1370371_a_at)		1.78E-11
urinary protein 2 (1389270_x_at)		1.87E-5
cytochrome P450, family 2, subfamily c, polypeptide 29 (DBSS_moderate) (139615...		0.00E0
Kruppel-like factor 2 (lung) (DBSS) (1386040_at)		1.11E-12
CLIP associating protein 2 (1396604_at)		1.50E-7
Iroquois related homeobox 2 (Drosophila) (1391457_a_at)		1.30E-9
cytochrome P450, family 1, subfamily a, polypeptide 1 (1370269_at)		1.63E-6
cytochrome P450 2c13 (1370495_s_at,M82855cds_s_at)		7.54E-6
RT1 class I, CE10 (1388202_at)		1.14E-4
Cytochrome P450 2C24 (CYPIIC24) (P450-PROS2) (DBSS) (1370241_at,M18335_f...		1.14E-9
ESTs (1397343_at)		8.82E-8
Transcribed locus (1380543_at)		7.32E-10
cytochrome P450, family 2, subfamily b, polypeptide 13 (1387993_at)		2.69E-8
leptin (1387748_at)		9.76E-8
MGC14161 protein (DBSS) (1396720_at)		1.50E-7

Repressed

SIMILAR | INDUCED | REPRESSED | DENDROGRAM | CLIN. PATH. | MOTIF | SPLP | TRANK | HISTOPATHOLOGY

>| MENU TRANSCRIPTIONAL RESPONSES (REPRESSED)

GENE	CONFIDENCE INTERVAL	P VALUE
hypothetical protein FLJ32871 (DBSS) (1394309_at)		6.62E-11
ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminyltransfer...		3.68E-5
protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), beta is...		3.61E-6
CDNA clone IMAGE:7460165 (1371298_at)		5.89E-11
olfactory receptor 1696 (1370741_at)		8.72E-5
fibroblast growth factor 21 (1387643_at)		3.48E-6
N-terminal acetyltransferase 1 (DBSS) (1381204_at)		6.13E-5
ESTs (1392613_at)		9.46E-6
ESTs (1379156_at)		2.07E-5
nuclear factor, erythroid derived 2 (1375040_at,BF397726_PROBE1)		2.09E-6
low-density lipoprotein receptor-related protein 10 (Non-specific probe) (1...		1.45E-6
F-box protein FBL2 (DBSS) (1381961_at)		1.06E-2
Transcribed locus (1381317_at)		5.61E-3
cytochrome P450, family 17, subfamily a, polypeptide 1 (1387123_at,M21...		1.77E-7
sorting nexin associated golgi protein 1 (DBSS) (1390064_at)		2.18E-3
Transcribed locus (1380306_at)		3.14E-5
alpha-2-macroglobulin (1367794_at,J02635_PROBE1)		4.14E-6
ATP-binding cassette, sub-family G (WHITE), member 8 (1369440_at)		3.65E-5
hypothetical protein MGC35130 (DBSS) (1386132_at)		2.10E-4
ESTs (1374610_at,AI599365_PROBE1)		2.59E-4

DrugMatrix Analysis of DE-71- Signature Scoring

DE71_21.OD_50.OMG/KG_LIVER

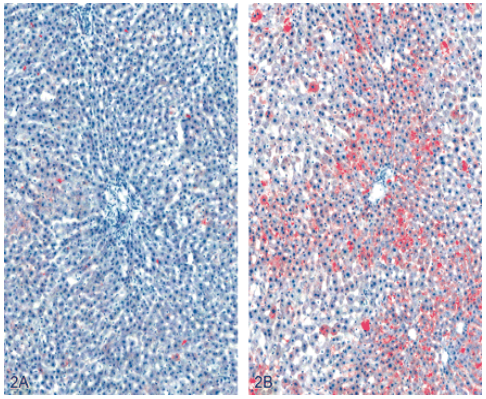
TRANSCR. RESP.

SIMILAR | INDUCED | REPRESSED | DENDROGRAM | CLIN. PATH. | MOTIF | SPLP | TRANK | HISTOPATHOLOGY

MENU | DRUG CLASSIFIER

SIGNATURE NAME	SP SCORE	POSTERIOR	LOGIT	DERIVATION
Hepatic hypertrophy, centrilobular LIVER RG230-2 ASPLP ToxFX.1.2.4	2.668	0.999835654...	6.9067547...	RG230-2
Hepatic lipid accumulation, centrilobular LIVER RG230-2 SPLP ToxFX.1.2.4	0.93	0.902890876...	2.2297663...	RG230-2
Hepatic lipid accumulation, macrovesicular LIVER RG230-2 ASPLP ToxFX.1.2.4	0.482	0.873777575...	1.9347802...	RG230-2
Hepatic lipid accumulation, periportal LIVER RG230-2 SPLP ToxFX.1.2.4	0.192	0.776136029...	1.2432892...	RG230-2
Hepatomegaly LIVER RG230-2 ASPLP ToxFX.1.2.4	0.292	0.775934833...	1.2421316...	RG230-2

Rat Liver - Oil Red O



Vehicle DE71

Dunnick, *et al*, Tox. Path., 2012

DrugMatrix Analysis of DE-71- Chemical Enrichment Analysis

- Chemical ontology enrichment analysis of the top 25 most similar expression studies (Hypergeometric Analysis)

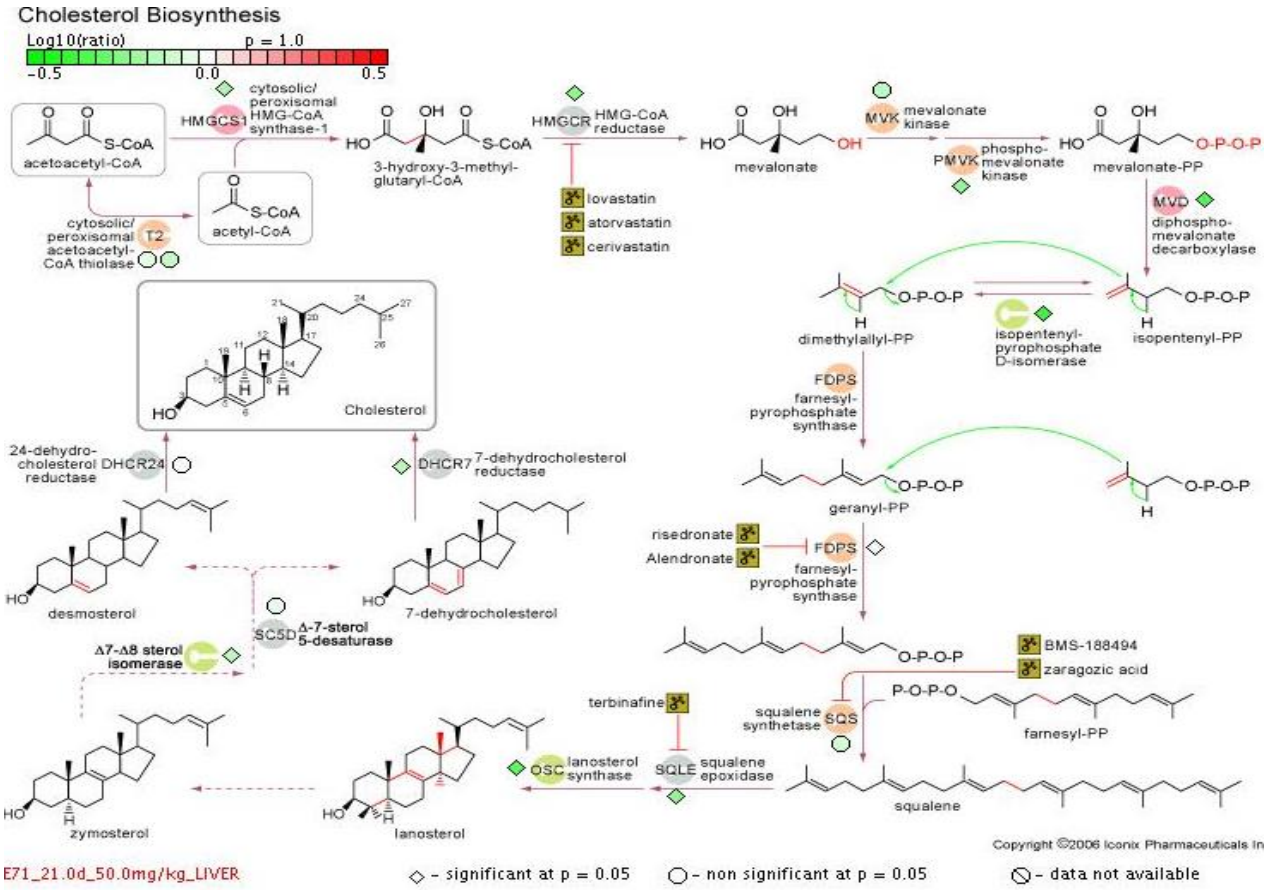
	A	B	C
1	CATEGORY	TERM	PVALUE
2	MECH_LEVEL_3	aromatase *	4.17E-06
3	MECH_LEVEL_2	Inhibit estrogen biosynthesis *	4.44E-06
4	SOLVENT	CMC .5 %	8.35E-06
5	ADVERSE_EFFECT	BBM_2_Bone Marrow Toxicity	1.07E-06
6	ADVERSE_EFFECT	NEU_1_Ataxia	3.35E-06
7	ADVERSE_EFFECT	END_2_Acute Intermittent Porphyria	1.07E-06
8	ADVERSE_EFFECT	KID_3_Acute Tubular Necrosis	1.07E-06
9	STRUCTURE_ACTIVITY	NSAID, COX-3, antipyrine like	1.07E-06
10	STRUCTURE_ACTIVITY	Estrogen antagonist, aromatase inhibitor *	6.99E-07

* DE-71 has been shown to alter aromatase activity in number of studies

DrugMatrix Analysis of DE-71- Pathway Analysis

Pathway	% Gene Changed in Pathway
Cholesterol Biosynthesis	75
Xenobiotic Metabolism	52
Bile Acid Synthesis	50

*Multiple subchronic studies have observed increases in serum cholesterol following DE71 exposure



Conclusions

- Identified 3 hepatic/non-hepatic toxicological effects of DE-71
  - Steatosis
  - Repro-related endocrine perturbations
  - Alterations in lipid homeostasis
  - Overall profile suggests DE-71 may exacerbate metabolic syndrome
- Suggestion of an AhR, CAR/PXR related MOA
- Helps focus future toxicological assessments

QUESTIONS???