

NOTOX



# Hands-on training on ToxWiz software for the analysis of high-throughput “omics” data

*SEURAT-1 Summer School  
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# Objective of this training session

During this training you will learn

- The basic commands in ToxWiz
- How to perform text and chemical structure search
- How to import -omics data (microarray data)
- How to elucidate possible toxic endpoints and mechanisms of action both, chemistry and microarray data



# Method

- Perform text search for "Liver steatosis induction"
- Perform Chemistry search for Phthalic Acid and structurally related Phthalates
- Combine search results as to elucidate the connection between "Liver steatosis induction AND Phthalic Acid" searches
- Identify toxic endpoint clusters and molecular mechanisms for molecules structurally related to phthalates
- Import microarray data for mice treated with phthalates
- Identify toxic endpoint clusters for up- and down-regulated genes
- Study relationships between these genes and other molecules in pathway/cluster
- Identify genes from the microarray data that are directly or indirectly associated with liver steatosis and phthalates



# Chemical Structure Searches in ToxWiz



# ToxWiz Chemistry Search features and Benefits

- Allows input of "well-known" and novel chemical structures
- Compares novel structures to structures of compounds available in database
- Displays target molecules and toxic endpoints for compounds in database
- Allows predictions to be made for mechanisms of action of novel chemicals



# Logging into ToxWiz

Using the left mouse function, double click on ToxWiz icon on your desktop

A ToxWiz window will appear, requesting you to log in with your username and password

Type in username and password and click on “Log in”.

Login...

Cambridge Cell Networks

Login Proxy Server Advanced Connectivity ToxWiz Servers

Welcome to ToxWiz.  
Please login:

ToxWiz Server: Default

Username/Email: seurat1

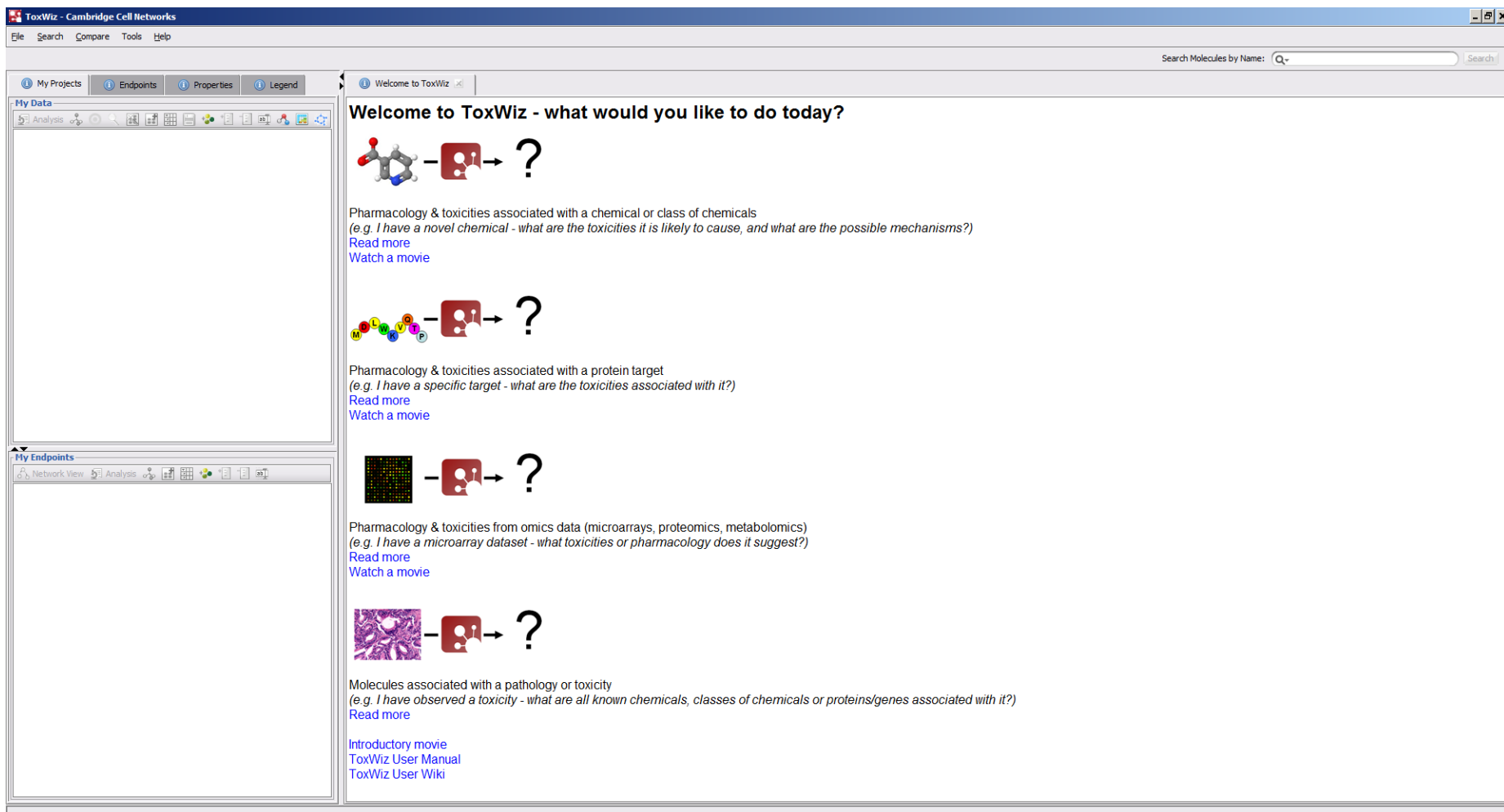
Password: .....

seurat1  
seurat2014



# Launching ToxWiz

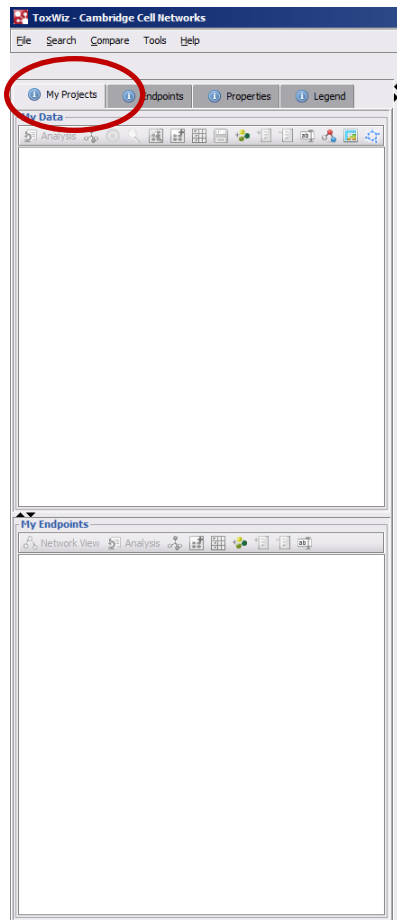
Double click on the ToxWiz icon in your screen and shortly after ToxWiz interface will appear



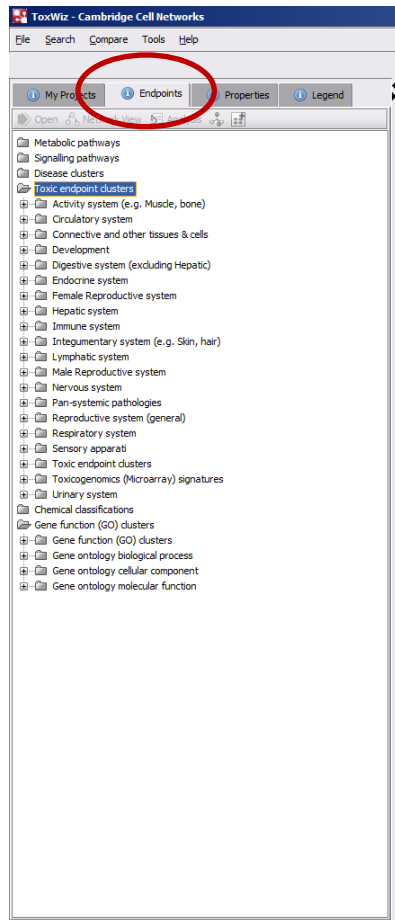
# Understanding the Top Left Window

Scrolling through the different tabs will provide information about your projects, endpoints, pathways, properties and what the different shapes and symbols mean.

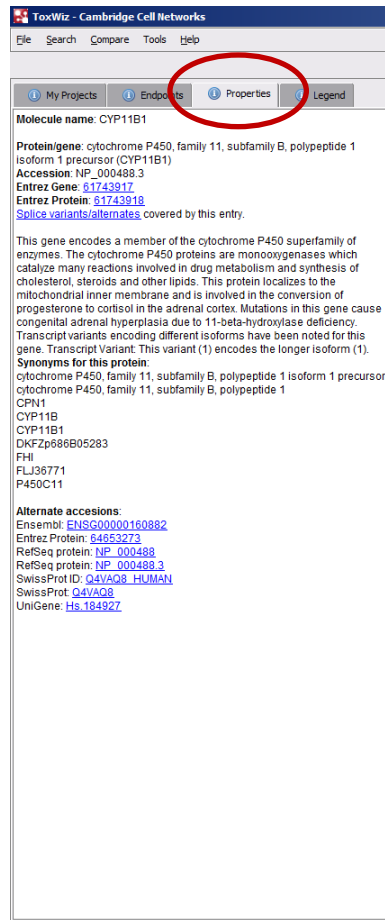
## My Projects



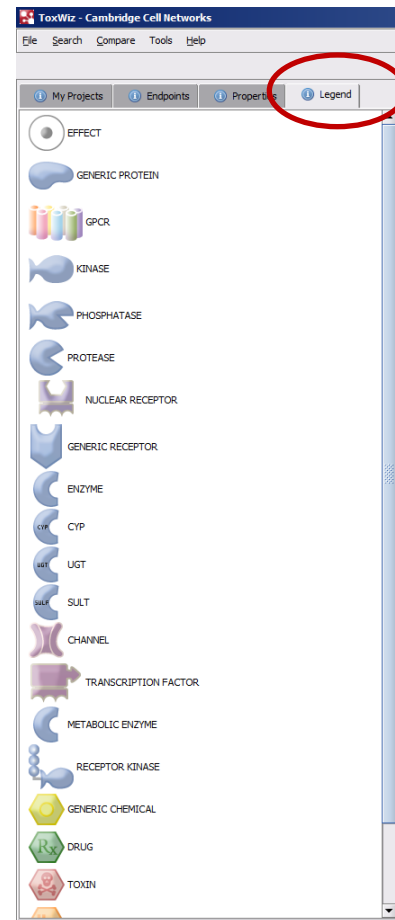
## Endpoints



## Properties



## Legends





# Text search option: Avoiding PubMed Overload

NCBI Resources How To Sign in to NCBI

PubMed.gov US National Library of Medicine National Institutes of Health

PubMed **liver steatosis induction** Search

RSS Save search Advanced Help

Show additional filters

Display Settings: Summary, 20 per page, Sorted by Recently Added

Send to: Manage Filters

Results: 1 to 20 of 2372

Article types: Clinical Trial, Review, More ...

Text availability: Abstract, Free full text, Full text

Publication dates: 5 years, 10 years, Custom range...

Species: Humans, Other Animals

Clear all

Show additional filters

1. [Hypoxia inducible lipid droplet associated \(HILPDA\) is a novel peroxisome proliferator-activated receptor \(PPAR\) target involved in hepatic triglyceride secretion.](#)  
Mattijssen F, Georgiadi A, Andasari T, Szalowska E, Zota A, Krones-Herzig A, Heier C, Ratman D, De Bosscher K, Qi L, Zechner R, Herzig S, Kersten S.  
J Biol Chem. 2014 May 29; pii: jbc.M114.570044. [Epub ahead of print]  
PMID: 24876382 [PubMed - as supplied by publisher] **Free Article**  
[Related citations](#)

2. [Activating transcription factor 6 is necessary and sufficient for alcoholic Fatty liver disease in zebrafish.](#)  
Howarth DL, Lindtner C, Vacaru AM, Sachidanandam R, Tsendensodnom O, Vasilkova T, Buettner C, Sadler KC.  
PLoS Genet. 2014 May 29;10(5):e1004335. doi: 10.1371/journal.pgen.1004335. eCollection 2014 May.  
PMID: 24874946 [PubMed - in process] **Free Article**  
[Related citations](#)

3. [Ribosomal protein-Mdm2-p53 pathway coordinates nutrient stress with lipid metabolism by regulating MCD and promoting fatty acid oxidation.](#)  
Liu Y, He Y, Jin A, Tikunov AP, Zhou L, Tollini LA, Leslie P, Kim TH, Li LO, Coleman RA, Gu Z, Chen YQ, Macdonald JM, Graves LM, Zhang Y.  
Proc Natl Acad Sci U S A. 2014 May 28; pii: 201315605. [Epub ahead of print]  
PMID: 24872453 [PubMed - as supplied by publisher]  
[Related citations](#)

4. [PPAR \$\delta\$  activation attenuates hepatic steatosis in Ldlr \$^{-/-}\$  mice by enhanced fat oxidation, reduced lipogenesis and improved insulin sensitivity.](#)  
Bojic LA, Telford DE, Fullerton MD, Ford RJ, Sutherland BG, Edwards JY, Sawyez CG, Gros R, Kemp BE, Steinberg GR, Huff MV.  
J Lipid Res. 2014 May 26; pii: jlr.M046037. [Epub ahead of print]  
PMID: 24864274 [PubMed - as supplied by publisher] **Free Article**  
[Related citations](#)

5. [Additive Effects of Nicotine and High-Fat Diet on Hepatocellular Apoptosis in Mice: Involvement of Caspase 2 and Inducible Nitric Oxide Synthase-Mediated Intrinsic Pathway Signaling.](#)  
Ivey R, Desai M, Green K, Sinha-Hikim I, Friedman TC, Sinha-Hikim AP.  
Harm Metab Res. 2014 May 15. [Epub ahead of print]

New feature: Try the new Display Settings option - Sort by Relevance

Results by year: Download CSV

PMC Images search for liver steatosis induction

See more (68)...

Titles with your search terms

Induction of liver steatosis and lipid droplet formation in ATF6alpha-knockout [Mol Biol Cell. 2010]

Role of adenosine monophosphate-activated protein kinase-p70 ribosomal S6 [Hepatology. 2009]

Combined metadoxine and garlic oil treatment efficaciously abrogates [Chem Biol Interact. 2007]

The ToxWiz database saves you trawling through what can be a very dense literature with high false positive rate in a conventional literature search: "Liver Steatosis induction" search gives you 2372 articles.



# Text Search Query for "Liver Steatosis induction" in ToxWiz

Step 1: In the "Search" pull-down menu select "PubMed" or simply select the type of search that you would like to perform in the Search Window selecting "Search PubMed Abstract"

The screenshot displays the ToxWiz web application interface. On the left, a sidebar menu is visible with a red circle highlighting the 'Search PubMed' option. The main content area shows a search results page for 'liver steatosis induction' (389 results). The search bar at the top right contains the query 'Q= liver steatosis induction' and a 'Search' button. The main content area is titled 'Welcome to ToxWiz - what would you like to do today?' and features four interactive cards, each with a red circle highlighting the 'Search PubMed Abstract' button:

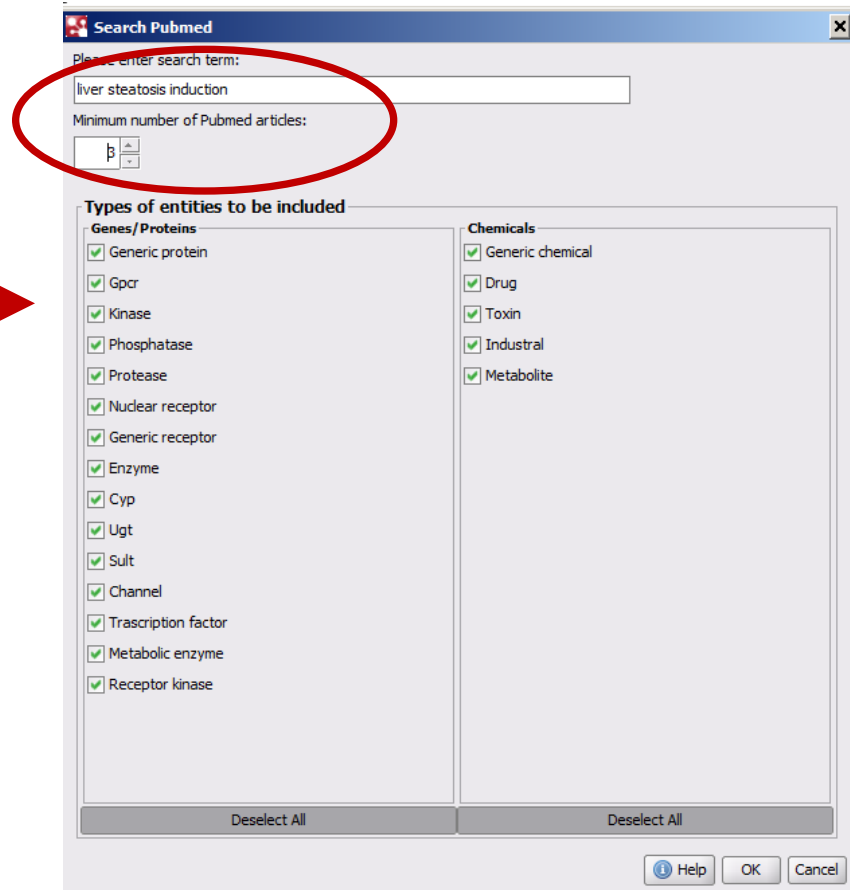
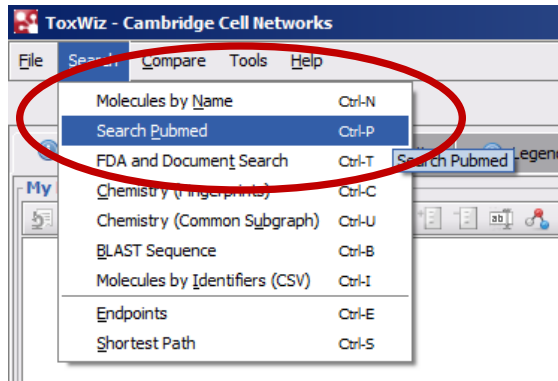
- Chemical Structure Card:** Shows a chemical structure icon and a question mark. Text: "Pharmacology & toxicities associated with a chemical or class of chemicals (e.g. I have a novel chemical - what are the toxicities it is likely to cause, and what are the possible mechanisms?)". Links: [Read more](#), [Watch a movie](#).
- Protein Target Card:** Shows a protein structure icon and a question mark. Text: "Pharmacology & toxicities associated with a protein target (e.g. I have a specific target - what are the toxicities associated with it?)". Links: [Read more](#), [Watch a movie](#).
- Omics Data Card:** Shows a heatmap icon and a question mark. Text: "Pharmacology & toxicities from omics data (microarrays, proteomics, metabolomics) (e.g. I have a microarray dataset - what toxicities or pharmacology does it suggest?)". Links: [Read more](#), [Watch a movie](#).
- Pathology/Toxicity Card:** Shows a histology image icon and a question mark. Text: "Molecules associated with a pathology or toxicity (e.g. I have observed a toxicity - what are all known chemicals, classes of chemicals or proteins/genes associated with it?)". Links: [Read more](#), [Introductory movie](#), [ToxWiz User Manual](#), [ToxWiz User Wiki](#).

At the bottom left, there is a section for 'My Endpoints' with a 'Network View' button. At the bottom right, there is a 'Searches PubMed database' section.









# Text Search Query for "Liver Steatosis induction" in ToxWiz

Step 2: Type the pathology of interest "Liver steatosis induction" and tickle genes/proteins and chemicals box (one can tickle off individual boxes as to limit search results); one can constrain the search with minimum number of PubMed articles



# PubMed Abstract Search Results

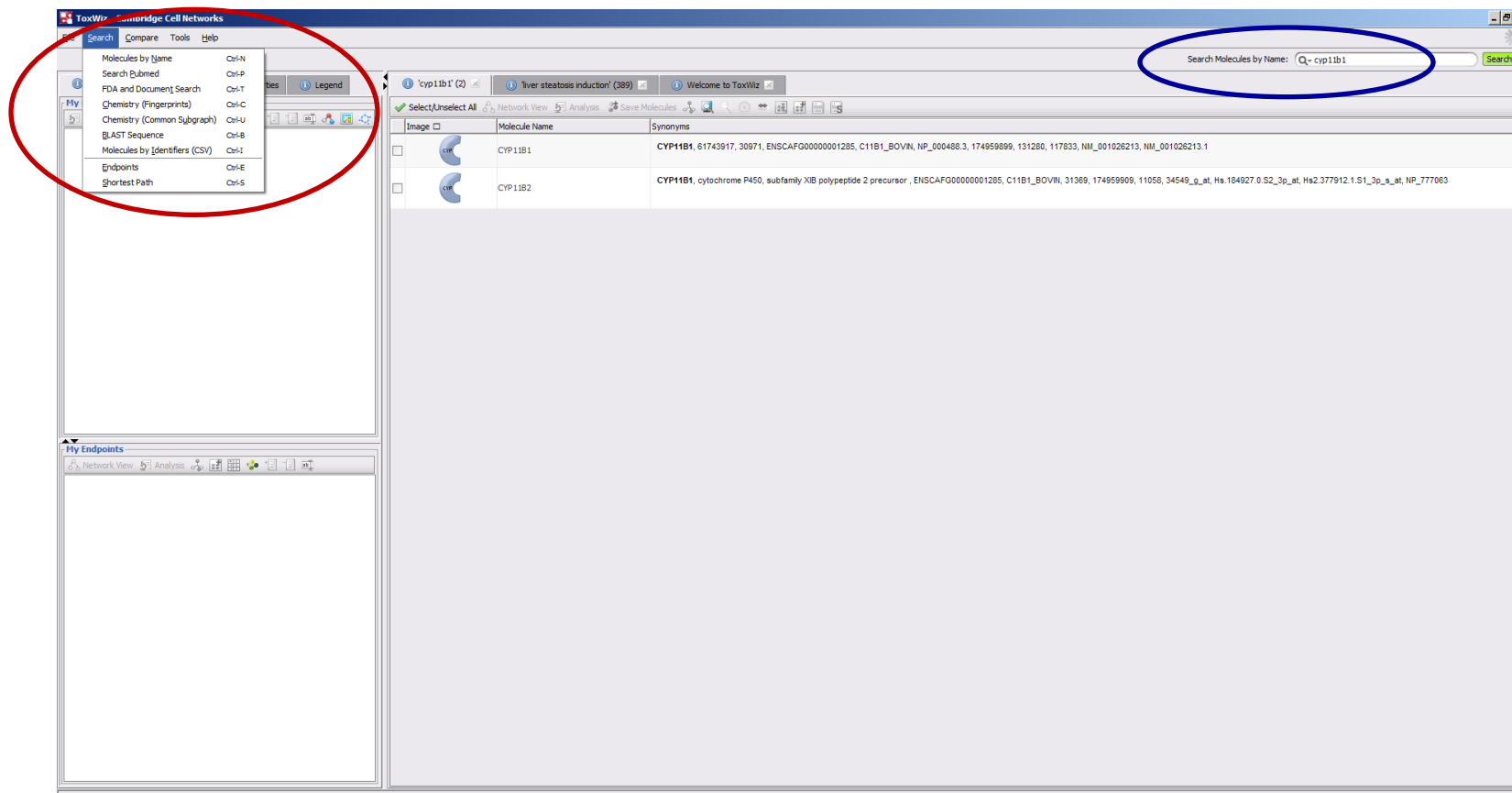
What is displayed in the Result window is a list of molecules which are associated with a query, in this case Liver steatosis induction. These molecules are directly linked to referenced PubMed journal articles which support their association.

Search PubMed Abstracts: Q- liver steatosis induction Search			
liver steatosis induction (389) Welcome to ToxWiz			
Select/Unselect All Network View Analysis Save Molecules			
Rank	Image	Molecule Name	Evidence/Pubmed Abstracts
<input type="checkbox"/>		triacylglycerol (260)	<p>PMID: 23576797, Exp Biol Med (Maywood) 2013 Feb;238(2):151-62, <b>Medium chain triglycerides dose-dependently prevent liver pathology in a rat model of non-alcoholic fatty liver disease.</b></p> <p>PMID: 23489367, Biochem J 2013 Apr 1;451(1):1-12, <b>Hepatic triacylglycerol synthesis and secretion: DGAT2 as the link between glycaemia and triglyceridaemia.</b></p> <p>PMID: 23326918, J Nutr 2013 Mar;143(3):253-9, <b>Lipid emulsion administered intravenously or orally attenuates triglyceride accumulation and expression of inflammatory markers in the liver of nonobese mice fed parenteral nutrition formula.</b></p> <p><a href="#">Click to see all of 260 abstracts</a></p>
<input type="checkbox"/>		ethanol (152)	<p>PMID: 23104821, Drug Metabol Drug Interact 2012;27(3):125-44, <b>CYP2E1 potentiates toxicity in obesity and after chronic ethanol treatment.</b></p> <p>PMID: 22749809, Free Radic Biol Med 2012 Sep 1;53(5):1170-80, <b>Cytochrome P4502E1, oxidative stress, JNK, and autophagy in acute alcohol-induced fatty liver.</b></p> <p>PMID: 22669179, Inflamm Res 2012 Oct;61(10):1053-61, <b>Estrogen suppresses hepatic IκB expression during short-term alcohol exposure.</b></p> <p><a href="#">Click to see all of 152 abstracts</a></p>
<input type="checkbox"/>		clofibrate (107)	<p>PMID: 23466978, Gene 2013 May 10;520(1):14-21, <b>Tissue-specific transcriptional modulation of fatty acid-binding protein genes, fabp2, fabp3 and fabp6, by fatty acids and the peroxisome proliferator, clofibrate, in zebrafish (Danio rerio).</b></p> <p>PMID: 22776158, BMC Evol Biol 2012 Jul 9;12:112, <b>Tissue-specific differential induction of duplicated fatty acid-binding protein genes by the peroxisome proliferator, clofibrate, in zebrafish (Danio rerio).</b></p> <p>PMID: 20132223, Cancer Sci 2010 Apr;101(4):869-75, <b>Histone acetylation and steroid receptor coactivator expression during clofibrate-induced rat hepatocarcinogenesis.</b></p> <p><a href="#">Click to see all of 107 abstracts</a></p>
<input type="checkbox"/>		beta-D-glucose (95)	<p>PMID: 23326918, J Nutr 2013 Mar;143(3):253-9, <b>Lipid emulsion administered intravenously or orally attenuates triglyceride accumulation and expression of inflammatory markers in the liver of nonobese mice fed parenteral nutrition formula.</b></p> <p>PMID: 23319015, Toxicol Appl Pharmacol 2013 Mar 1;267(2):174-83, <b>Saponins, especially platycodin D, from Platycodon grandiflorum modulate hepatic lipogenesis in high-fat diet-fed rats and high glucose-exposed HepG2 cells.</b></p> <p>PMID: 23238934, Am J Physiol Gastrointest Liver Physiol 2013 Feb 1;304(3):G241-56, <b>Impact of L-FABP and glucose on polyunsaturated fatty acid induction of PPARα-regulated β-oxidative enzymes.</b></p> <p><a href="#">Click to see all of 95 abstracts</a></p>
<input type="checkbox"/>		dextrose (95)	<p>PMID: 23326918, J Nutr 2013 Mar;143(3):253-9, <b>Lipid emulsion administered intravenously or orally attenuates triglyceride accumulation and expression of inflammatory markers in the liver of nonobese mice fed parenteral nutrition formula.</b></p> <p>PMID: 23319015, Toxicol Appl Pharmacol 2013 Mar 1;267(2):174-83, <b>Saponins, especially platycodin D, from Platycodon grandiflorum modulate hepatic lipogenesis in high-fat diet-fed rats and high glucose-exposed HepG2 cells.</b></p> <p>PMID: 23238934, Am J Physiol Gastrointest Liver Physiol 2013 Feb 1;304(3):G241-56, <b>Impact of L-FABP and glucose on polyunsaturated fatty acid induction of PPARα-regulated β-oxidative enzymes.</b></p> <p><a href="#">Click to see all of 95 abstracts</a></p>
<input type="checkbox"/>		glucose (95)	<p>PMID: 23326918, J Nutr 2013 Mar;143(3):253-9, <b>Lipid emulsion administered intravenously or orally attenuates triglyceride accumulation and expression of inflammatory markers in the liver of nonobese mice fed parenteral nutrition formula.</b></p> <p>PMID: 23319015, Toxicol Appl Pharmacol 2013 Mar 1;267(2):174-83, <b>Saponins, especially platycodin D, from Platycodon grandiflorum modulate hepatic lipogenesis in high-fat diet-fed rats and high glucose-exposed HepG2 cells.</b></p> <p><a href="#">Click to see all of 95 abstracts</a></p>





# Searching the Database

There are several ways to search the database, all of which are accessible using the search pull-down menu. Essentially, one always searches for words associated with elements, pathways and pathologies or queries with particular protein/gene sequence or chemical structure.



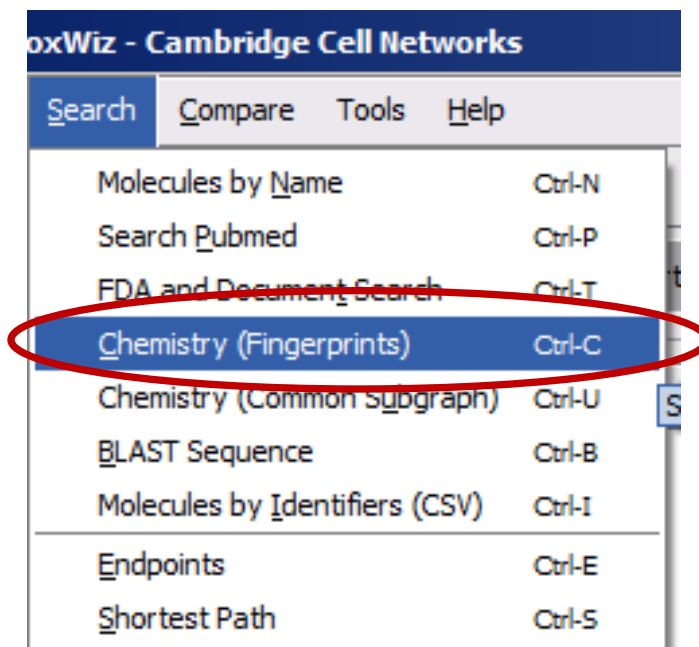
The screenshot displays the ToxWiz Cambridge Cell Networks application. The 'Search' menu is open, showing options such as 'Molecules by Name', 'Search PubMed', 'FDA and Document Search', 'Chemistry (Fingerprints)', 'Chemistry (Common Subgraph)', 'BLAST Sequence', 'Molecules by Identifiers (CSV)', 'Endpoints', and 'Shortest Path'. A search bar at the top right contains the query 'cyp 11b1'.

Image	Molecule Name	Synonyms
	CYP11B1	CYP11B1, 61743917, 30971, ENSCAFG000000001285, C11B1_BOVIN, NP_000488.3, 174959899, 131280, 117833, NM_001026213, NM_001026213.1
	CYP11B2	CYP11B1, cytochrome P450, subfamily XIB polypeptide 2 precursor, ENSCAFG000000001285, C11B1_BOVIN, 31369, 174959909, 11058, 34549_a_at, Hs.184927.0.S2_3p_at, Hs2.377912.1.S1_3p_s_at, NP_777063



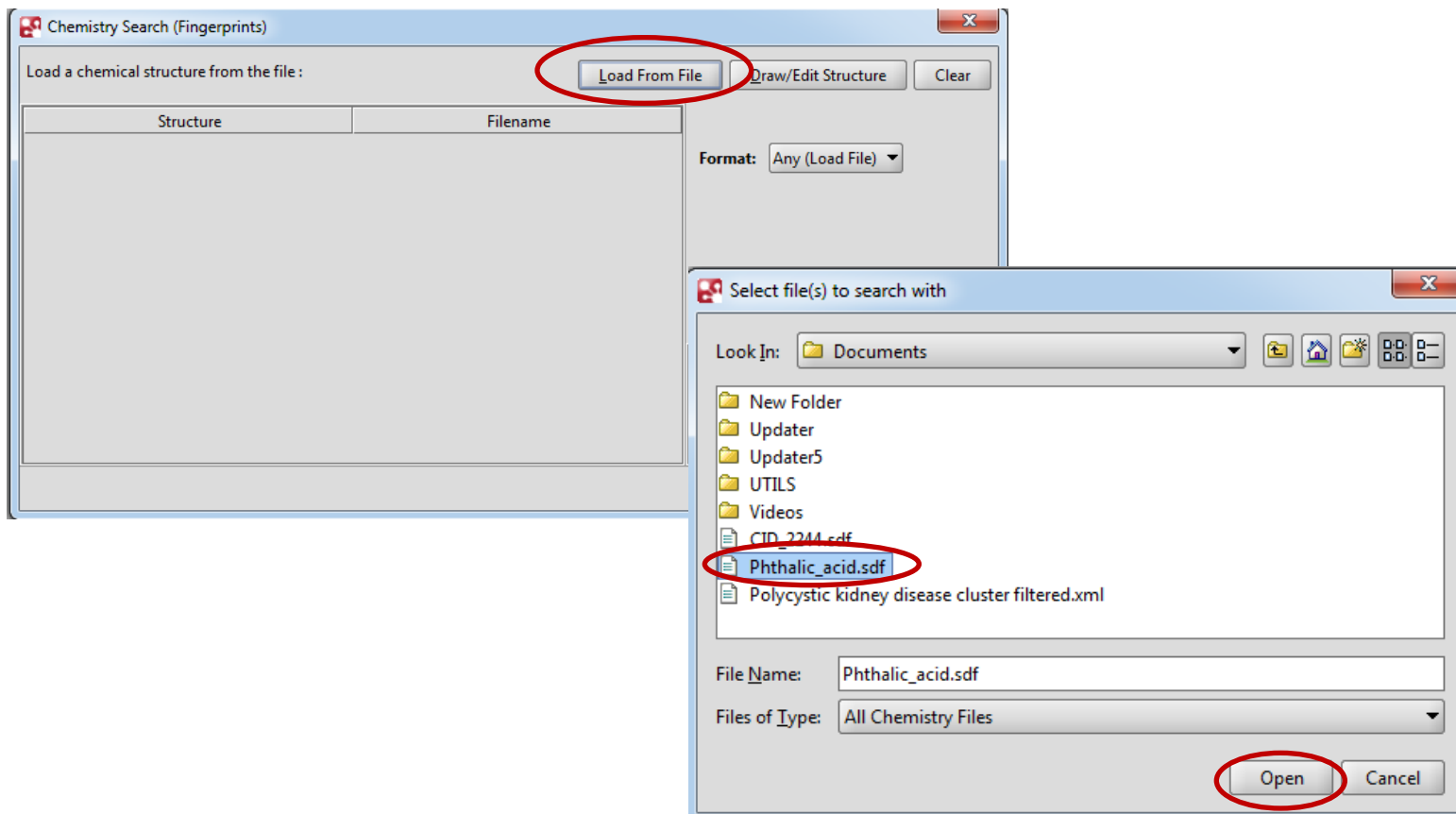
# Searching With Chemical Structure For Structurally Similar Molecules in ToxWiz

Step 3: In the tool bar go to "Search" – "Chemistry (Fingerprints)"



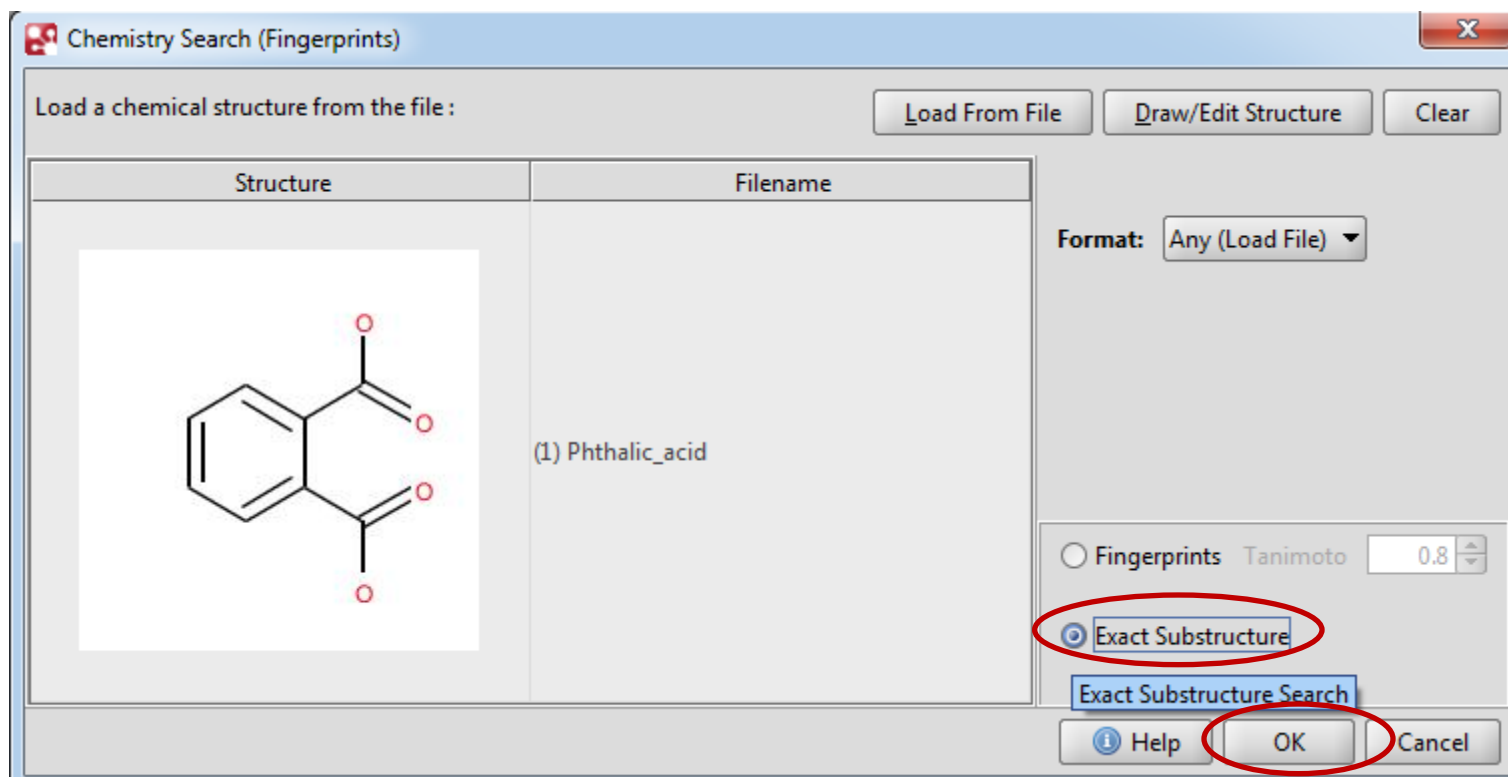
# Importing Chemical Structures (sdf/mol file formats) into ToxWiz

Step 4: Select the sdf file, in this case Phthalic acid.sdf and import it



# Chemistry Search for Structurally Related Compounds to Phthalic acid

Step 5: You can select what type of chemical search and sensitivity you would like to perform depending on how close match to the original structure you want to get. In this case we will search for all the compounds in the database containing "Phthalic acid" substructure, therefore we tickle "Exact Substructure"





# ToxWiz displays 45 Compounds Structurally Related to Phthalic Acid

ToxWiz - Cambridge Cell Networks

File Search Compare Tools Help









Search Molecules by Name:

My Projects Endpoints Properties Legend (1) CID\_1017 substructure (45) 'cyp11b1' (2) 'liver steatosis induction' (389) Welcome to ToxWiz

My Data

Analysis

Structure Molecule Name Score Matched Query

<input type="checkbox"/>		phthalic acid	1	(1) CID_1017
<input type="checkbox"/>		phthalate	1	(1) CID_1017
<input type="checkbox"/>		HEMIMELLITIC ACID	0.86	(1) CID_1017
<input type="checkbox"/>		TRIMELLITIC ACID	0.82	(1) CID_1017
<input type="checkbox"/>		PVAP	0.82	(1) CID_1017
<input type="checkbox"/>		Dimethyl phthalate	0.79	(1) CID_1017
<input type="checkbox"/>		Monoperoxyphthalate	0.74	(1) CID_1017
<input type="checkbox"/>		Magnesium monoperoxyphthalate	0.74	(1) CID_1017

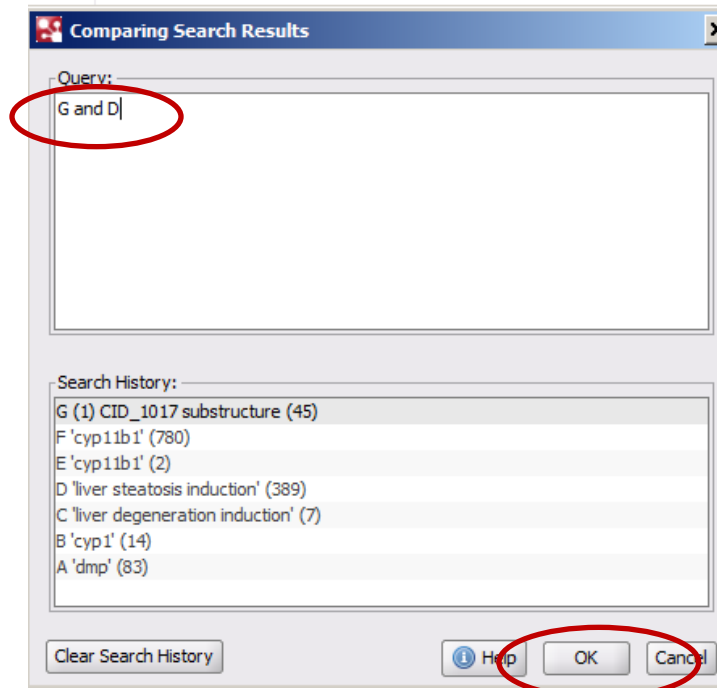
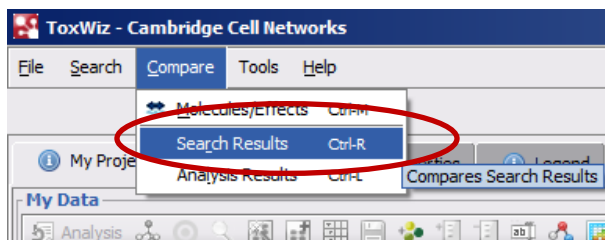
My Endpoints

Network View Analysis



# Combining Search Results in ToxWiz

Step 6: As to elucidate the connection between text ("Liver steatosis induction") and chemistry ("Phthalic Acid") searches that we have performed, we can combine the results by selecting "Compare Search Results" from the "Search" pull-down menu and selecting the search history results that we want to consider.



# Results of "Combined Search" in ToxWiz – 4 phthalates that are directly associated to Liver steatosis induction

ToxWiz - Cambridge Cell Networks

File Search Compare Tools Help




Search Molecules by Name:

My Projects Endpoints Properties Legend **G and D' (4)** (1) CID\_1017 substructure (45) 'cyp11b1' (2) Liver steatosis induction (389) Welcome to ToxWiz

My Data

Analysis

Network View Analysis Save Molecules

Image	Molecule Name	Properties
	Diethyl phthalate	<p><b>Chemical:</b> Diethyl phthalate PubChem: <a href="#">8346</a></p> <p><b>Synonyms (max. 50 shown):</b> 1,2-Benzenedicarboxylic acid, diethyl ester; 1,2-Benzenedicarboxylic acid, bis(1-methylethyl) ester; 1,2-Benzenedicarboxylic acid, bis(1-methylethyl) ester; 1,2-Benzenedicarboxylic acid, di-C8-11-branched and linear alkyl esters; 1,2-Benzenedicarboxylic acid, diethyl ester; 131-15-7; 36938_REDEL; 376655_ALDRICH; 4-69-00-03180 (Beilstein Handbook Reference); 4-09-00-03181 (Beilstein Handbook Reference); 48560U_SUPELCO; 68515-43-5; 80153_FLUKA; 8031-28-6; A8-15071 (USDA); Benzenedicarboxylic acid di-n-octyl ester; Bis(1-methylethyl) phthalate; Bis(2-octyl)phthalate; BIS(2-OCTYL) PHTHALATE; Bis-(2-octyl)ester kyseliny ftalove; Bis-(2-octyl)ester kyseliny ftalove [Czech]; Bis(n-octyl) phthalate; BRN 1915994; BRN 2005093; C010715; C14227; Capryl o-phthalate; CCRIS 6196; Celluflex DOP; DCP; Di-(2-octyl)phthalate; Dicapryl 1,2-benzenedicarboxylate; Dicapryl phthalate; Diisooctyl phthalate; di-n-octyl phthalate; Di-N-OCTYL PHTHALATE; Dinopol NOP; diocan-2-yl benzene-1,2-dicarboxylate; Diocan-2-phthalate; Diocetyl 1,2-benzenedicarboxylate; Diocetyl 1,2-benzenedicarboxylate; diocetyl benzene-1,2-dicarboxylate; Diocetyl o-benzenedicarboxylate; Diocetyl o-phthalate; Diocetyl phthalate; Diokylester kyseliny ftalove; Diokylester kyseliny ftalove [Czech]; Di-sec-octyl phthalate; DNOP;</p>
	Mehp	<p><b>Chemical:</b> Mehp PubChem: <a href="#">20393</a></p> <p><b>Synonyms (max. 50 shown):</b> 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (9CI); 2-(2-ethylhexyloxy)benzoic acid; 2-(2-ethylhexyloxy)benzoic acid; 2-[(2-ethylhexyloxy)carbonyl]benzoic acid; 2-[(2-ethylhexyloxy)carbonyl]benzoic acid; 25425-73-4; (2-Ethylhexyl) hydrogen phthalate; 2-Ethylhexyl hydrogen phthalate; 2-Ethylhexyl phthalate; 4376-20-9; 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (9CI); 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester; 2-(2-ethylhexyloxy)benzoic acid; 2-[(2-ethylhexyloxy)carbonyl]benzoic acid; 2-[(2-ethylhexyloxy)carbonyl]benzoic acid; (2-Ethylhexyl) hydrogen phthalate; 2-Ethylhexyl hydrogen phthalate; 2-Ethylhexyl phthalate; 25425-73-4; 4376-20-9; BAR 1; C016599; C03343; CCRIS 1742; CHEBI:17243; CID20393; EINECS 224-477-1; LS-1260; Mehp; Mono(2-ethylhexyl) phthalate; Mono(2-ethylhexyl)phthalate; mono-(2-ethylhexyl) phthalate; MONO-2-ETHYLHEXYL PHTHALATE; Mono-(2-ethylhexyl)phthalate; Mono-(2-ethylhexyl)phthalate, sodium salt; Monoethylhexyl phthalate; NCGC00090773-01; NCGC00090773-02; NCGC00090773-03; Phthalate, mono(2-ethylhexyl); Phthalic acid, 2-ethylhexyl ester (6CI,7CI); phthalic acid, 2-ethylhexyl ester; Phthalic acid, mono-(2-ethylhexyl) ester; Phthalic acid, mono-2-ethylhexyl ester; BAR 1; C016599; C03343; CCRIS 1742;</p>
	phthalic acid	<p><b>Chemical:</b> phthalic acid PubChem: <a href="#">1017</a></p> <p><b>Synonyms:</b> Acide phthalique [French]; A8-02409; benzene-1,2-dicarboxylic acid; BENZENEDICARBOXYLIC ACID; BRN 0608199; C01606; CCRIS 1446; CHEBI:29069; EINECS 201-873-2; HSDB 1339; InChI:1/C8H6O4/c6-7(10)5-3-1-2-4-8(11)12/h1-4H,(H,9,10)(H,11,12); Kyselina ftalova; Kyselina ftalova [Czech]; LS-1890; Magnesium phthalate; Magnesium phthalate, MgO4C8H4; Magnesium phthalate, MgO4C8H6; .ILSD01056215; NCGC00090689-01; Nickel phthalate (NCB404); NISTC88993; NSC273933; NSC30114; NSC 5348; NSC5348; NSC83565; o-benzenedicarboxylic acid; o-dicarboxybenzene; o-phthalic acid; Orthophthalic acid; P39303_SIAL; PHT; phthalate; Phthalate standard for IC; phthalic acid; phthalic acid; Phthalic acid-ring-UL-14C; p-Phthalic acid; SMR001224528; ST5192167; Sunhal 20; WLN: QVR BVQ; ZINC00090750;</p>

My Endpoints

Network View Analysis

# Predicting Toxic Endpoints and Searching for Toxic Endpoint Clusters

Step 7: To see how interaction of 45 chemicals (structurally related Phthalates) with proteins is affecting biology, one can select all the entities and analyze them for pathways or disease/toxic clusters. In this case, we will analyze them for toxic endpoint cluster.

The screenshot shows a software interface with a table of chemicals. The table has columns for 'Structure', 'Molecule Name', 'Score', and 'Matched Query'. Three chemicals are listed: phthalic acid, phthalate, and HEMIMELLITIC ACID. The 'Analysis' button in the top toolbar is circled in red.

Structure	Molecule Name	Score	Matched Query
	phthalic acid	1	(1) CID_1017
	phthalate	1	(1) CID_1017
	HEMIMELLITIC ACID	0.86	(1) CID_1017

Always give a name to the analysis you are performing

The screenshot shows the 'Analysis Preferences' dialog box. The 'Analysis Name' field is filled with 'Endpoint Liver\_phthalic a...,phthalate,HEMIMELLIT...,TRIMELLITI...'. The 'Result Size' section shows 'Maximum number of endpoints displayed for each endpoint type' set to 30 and 'Minimum significance p-value' set to 0.2. The 'Type of Endpoint' section has 'Toxic endpoint clusters' selected. The 'Filters' section has 'Include molecules next to endpoints' and 'Keywords to require' checked. The 'Keywords to require' field contains 'liver hepato'. The 'Keywords to ignore' field contains 'toxicogenomics'. The 'OK' button is circled in red.

One can chose the term of interest (target organ, pathology etc.)



# Analysis report

The screenshot shows a software window titled 'Analysis Parameters'. At the top, there are four tabs: 'Endpoint Liver\_phthalic a...,phthalate,HEMIMELLIT...,TRIMELLITI.....' (Analysis), 'G and D' (4), '(1) CID\_1017 substructure (45)', and 'cyp11b1' (2). Below the tabs, the 'Analysis Parameters' section contains the following text:

The maximum number of endpoints to show in table views was set to 30.  
The maximum number of endpoints to show on graphical view was set to 30.  
Minimum significance p-value was set to 0.2. There were a total of 17 endpoints where at least one molecule showed an association (whether significant or not).

The following types of endpoints were selected for this analysis:

- Toxic endpoint clusters

At the bottom of the window, there is a navigation bar with four tabs: 'Summary', 'Tabular view', 'Charts view', and 'Molecules'.

This window contains four tabs and shows the results of running an Analysis (previously called Map).

Analysis is a way of analyzing or predicting how a set of molecules impact or are related to various pathways, diseases, pathologies and other things stored in the database.

The **summary tab** shows you the settings you used to run the Analysis and gives some additional instructions.

The **tabular view** shows how the various selected processes (pathways, diseases, pathologies, etc.) are related to your molecules. Processes are ranked by statistical significance of the match to the molecules you used. The table shows a summary for each group of processes, a sub-categorization if available, and details of Molecules that are Inside (i.e. directly connected to the process) or Next To (i.e. connected via a molecule connected to the process). There are also buttons to show a statistical significance 'heatmap' (i.e. blue = not significant; red = significant), to generate a report for the Analysis (PDF) and to export data in CSV format for importing into other applications such as Excel. Clicking on the Molecular Mechanism links allows one to have a view of how the molecules are linked to the pathway/process shown.

The **Charts view tab** shows the above details in a graphical format, and the **Molecules view** shows a summary of the molecules considered. If expression or molecular level information (e.g. transcriptomics, metabolomics) is available, then a summary of the data is also given in this tab.



# Analysis report

Endpoint Liver\_phthalic a...,phthalate,HEMIMELLIT...,TRIMELLITI... (Analysis)

G and D' (4)

(1) CID\_1017 substructure (45)

cyp11b1' (2)

Analysis Parameters

The maximum number of endpoints to show in table views was set to 30.  
The maximum number of endpoints to show on graphical view was set to 30.  
Minimum significance p-value was set to 0.2. There were a total of 17 endpoints where at least one molecule showed an association (whether significant or not).  
  
The following types of endpoints were selected for this analysis:  

- Toxic endpoint clusters

## Summary of the analysis

Endpoint Liver\_phthalic a...,phthalate,HEMIMELLIT...,TRIMELLITI... (Analysis)

G and D' (4)

(1) CID\_1017 substructure (45)

cyp11b1' (2)

Liver steatosis induction' (389)

Welcome to ToxWiz

Toggle Heatmap

Generate Report

Export (CSV)

Toxic endpoint clusters

Endpoint Category	P-val	Molecular Mechanism	P-val	Molecules Inside	Molecules Next To
Hepatic system	0.0005	<a href="#">Liver steatosis inhibition cluster</a>	0.0005		4: Mehp, dibutyl phthalate, Diethyl phthalate, phthalic acid
		<a href="#">Hepatocyte hyperplasia association cluster</a>	0.0005	Diethyl phthalate	3: Mehp, dibutyl phthalate, phthalic acid
		<a href="#">Hepatocyte hyperplasia induction cluster</a>	0.0005	Diethyl phthalate	3: Mehp, dibutyl phthalate, phthalic acid
		<a href="#">Liver steatosis association cluster</a>	0.005		6: dibutyl phthalate, Mehp, Diethyl phthalate, phthalic a...
		<a href="#">Liver inflammation inhibition cluster</a>	0.005		5: Mehp, dibutyl phthalate, Diethyl phthalate, phthalic a...
		<a href="#">Liver steatosis induction cluster</a>	0.005		5: Mehp, dibutyl phthalate, Diethyl phthalate, phthalic a...
		<a href="#">Liver toxicities cluster</a>	0.05	Dimethyl phthalate, Mehp	17: Diethyl phthalate, dibutyl phthalate, Benzyl butyl pht...
		<a href="#">Liver steatosis cluster</a>	0.05		13: Diethyl phthalate, dibutyl phthalate, Mehp, Benzyl b...
		<a href="#">Hepatocyte hypertrophy cluster</a>	0.05		11: dibutyl phthalate, Mehp, Diethyl phthalate, Mono-n...
		<a href="#">Liver hypertrophy cluster</a>	0.05		10: Diethyl phthalate, Mehp, Mono-n-butyl phthalate, C...
		<a href="#">Liver ischaemia association cluster</a>	0.05		4: Diethyl phthalate, Benzyl butyl phthalate, dibutyl pht...
		<a href="#">Liver fibrosis marker cluster</a>	0.05		3: Diethyl phthalate, dibutyl phthalate, Mehp
		<a href="#">Liver carcinoma cluster</a>	0.1	Mehp	13: Diethyl phthalate, dibutyl phthalate, Benzyl butyl pht...
		<a href="#">Hepatocyte apoptosis induction cluster</a>	0.1		4: Mehp, dibutyl phthalate, Diethyl phthalate, phthalic acid
		<a href="#">Liver hyperplasia cluster</a>	0.2		7: Diethyl phthalate, dibutyl phthalate, Mehp, Mono-n-b...
		<a href="#">Hepatocyte neoplasia cluster</a>	0.2		6: Diethyl phthalate, dibutyl phthalate, Mehp, Palatinol N...
		<a href="#">Liver steatosis marker cluster</a>	0.2		dibutyl phthalate, phthalic acid

Summary

Tabular view

Charts view

Molecules

Summary

Tabular view

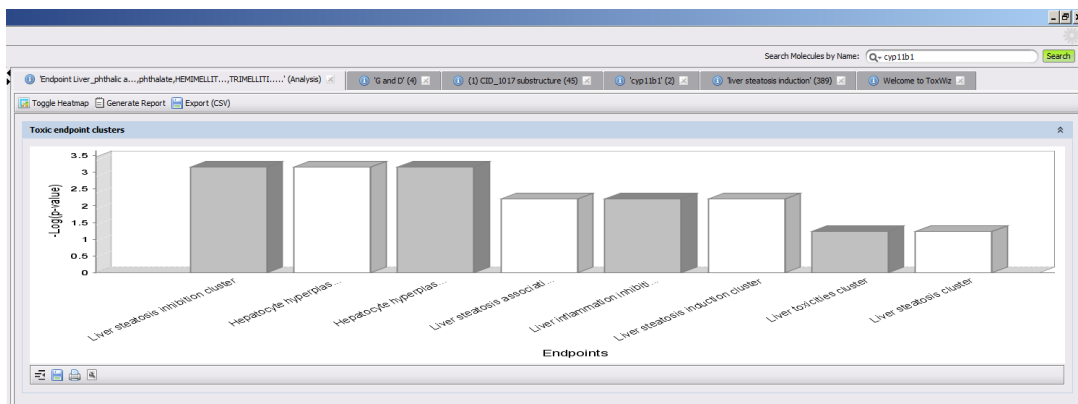
Charts view

Molecules

Tabular view of the analysis, with categorized endpoints and p-values of prediction, total and individual ones

Processes are ranked by statistical significance of the match to the molecules you used.

You get the list of toxicities according to your preferences.  
The Liver steatosis induction cluster is shown on the list of predicted pathologies.







## Charts view of the analysis


Summary / **Charts view** / Tabular view / Molecules

1 Endpoint Liver\_phthalic a...,phthalate,HEMIMELLIT...,TRIMELLIT... (Analysis)

Select/Unselect All

Order	Image	Molecule Name	GI/CAS Number	Occurrences in Endpoints	Analysis Chart
<input type="checkbox"/> 1		phthalic acid	1017	<a href="#">14</a>	
<input type="checkbox"/> 2		phthalate	181977	<a href="#">2</a>	
<input type="checkbox"/> 3		HEMIMELLITIC ACID	11288	<a href="#">0</a>	
<input type="checkbox"/> 4		TRIMELLITIC ACID	10708	<a href="#">2</a>	

List of analyzed molecules sorted according to the frequency they occur in endpoints

6  Dimethyl phthalate 8554

Summary / Tabular view / **Charts view** / Molecules



# Clusters Show Relationships between compounds and other Molecules Involved in Disease

Step 8: Click to view graphically Molecular mechanism hypothesis for Liver steatosis induction

The screenshot displays the ToxWiz software interface. The main window shows a table of 'Toxic endpoint clusters' under the 'Hepatic system' category. The table has columns for Endpoint Category, P-val, Molecular Mechanism, P-val, Molecules Inside, and Molecules Next To. The 'Liver steatosis induction cluster' is highlighted with a red circle. A text box on the left explains that clicking on this cluster leads to a filter molecular mechanism step, specifically the 'Advanced preferences' dialog.

When you click on Liver steatosis induction cluster, you will be asked to filter molecular mechanism first. Click on [Advanced preferences]

The 'Filter Molecular Mechanism - Advanced Preferences' dialog box is shown with the following sections:

- Genes/Proteins:** ☒ Generic protein, ☒ Gpcr, ☒ Kinase, ☒ Phosphatase, ☒ Protease, ☒ Nuclear receptor, ☒ Generic receptor, ☒ Enzyme, ☒ Cyp, ☒ Ugt, ☒ Sult, ☒ Channel, ☒ Transcription factor, ☒ Metabolic enzyme, ☒ Receptor kinase.
- Text/Effect:** ☒ Effect, ☒ Chemicals, ☒ Generic chemical, ☒ Drug, ☒ Toxin, ☒ Industrial, ☒ Metabolite.
- Protein-Protein Interactions:** ☒ Manual, ☒ Three-dimensional structures, ☒ Yeast two-hybrid (human), ☒ Yeast two-hybrid (inferred by orthology), ☒ Abstract co-occurrence (high), ☒ Abstract co-occurrence (medium), ☒ Abstract co-occurrence (low).
- Protein-Chemical Interactions:** ☒ Manual (drug), ☒ Manual (metabolic), ☒ Manual (other), ☒ Three-dimensional structures, ☒ Pubchem, ☒ Abstract co-occurrence (high), ☒ Abstract co-occurrence (medium), ☒ Abstract co-occurrence (low).

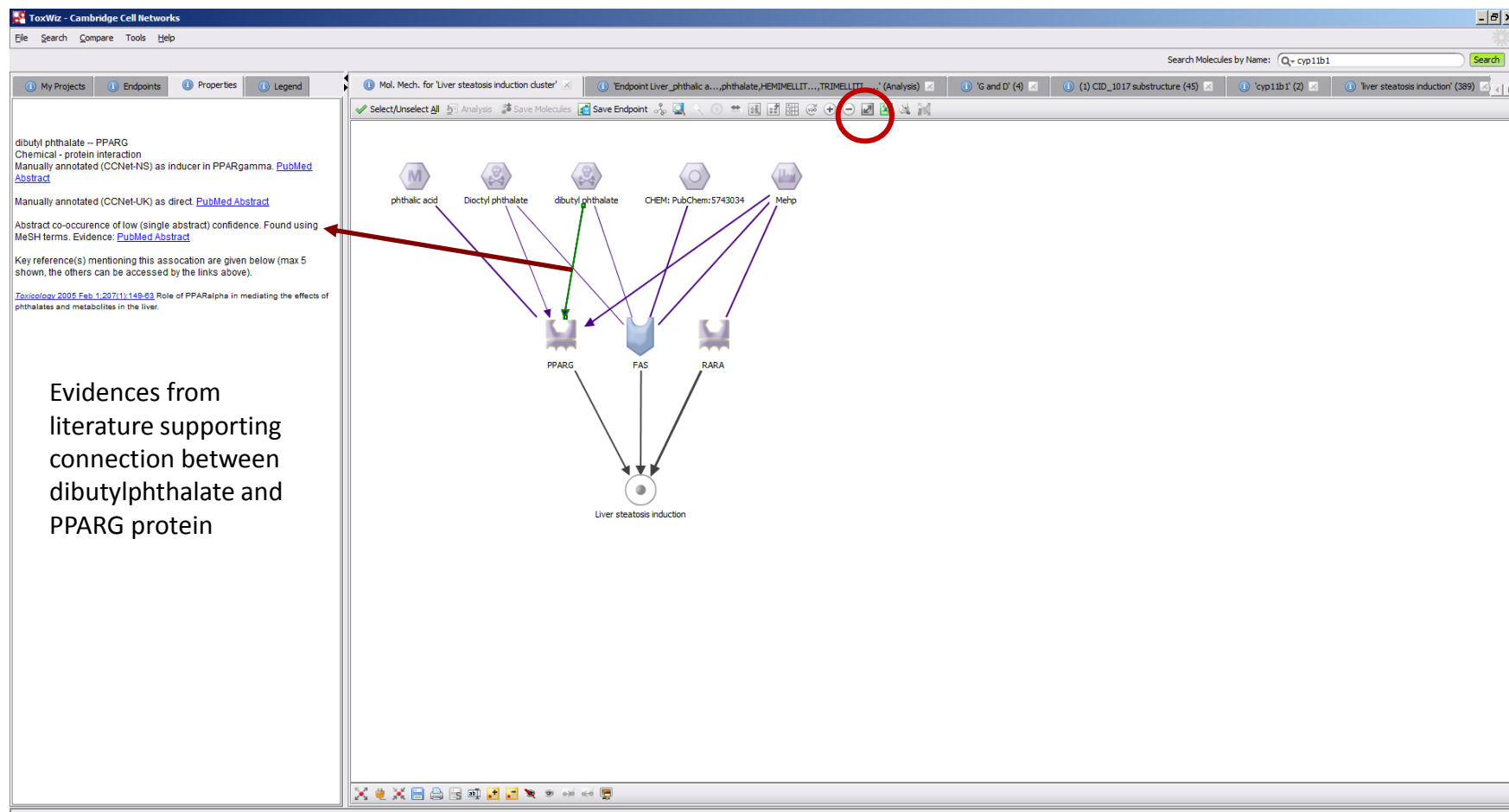
Buttons at the bottom include 'Deselect All', 'OK', and 'Cancel'. A 'Filters' section at the bottom left shows ☒ Include molecules next to pathway/cluster.

In the pop-up window you could choose which molecules you want to see and the source of their interaction. After selection, click [OK].



# Hypothesized Molecular Mechanism for Liver steatosis induction Cluster

Step 9: Cluster may be enlarged for convenient viewing



Indirect association observed in case of 5 different phthalates and Liver steatosis induction Cluster



# Microarray data and ToxWiz



# ToxWiz Features and Benefits in Omics Data Analysis

- Allows input of Microarray data and selection of genes for further study
- Provides pathways and clusters (disease, toxic) for selected genes
- Displays status of genes (up- or down-regulation) and their relationships to other molecules in pathway or cluster
- Allows predictions to be made for mechanisms of action and biological effects of tested chemicals



# Importing Microarray Data into ToxWiz

Step 1: On the menu bar on the top click to "File" icon => Import Microarray Data


**ToxWiz - Cambridge Cell Networks**


File Search Compare Tools Help


Import Endpoint/Network (SBML)  
**Import Microarray Data (Excel/CSV)**  
Import Metabolomics Data (Excel/CSV)  
Import Microarray Data (Excel/CSV)  
Create New Empty Network View Ctrl+N  
Logout & Exit Ctrl+X


Search Molecules by Name:

Welcome to ToxWiz - what would you like to do today?

 ?  
Pharmacology & toxicities associated with a chemical or class of chemicals  
(e.g. I have a novel chemical - what are the toxicities it is likely to cause, and what are the possible mechanisms?)  
[Read more](#)  
[Watch a movie](#)

 ?  
Pharmacology & toxicities associated with a protein target  
(e.g. I have a specific target - what are the toxicities associated with it?)  
[Read more](#)  
[Watch a movie](#)

 ?  
Pharmacology & toxicities from omics data (microarrays, proteomics, metabolomics)  
(e.g. I have a microarray dataset - what toxicities or pharmacology does it suggest?)  
[Read more](#)  
[Watch a movie](#)

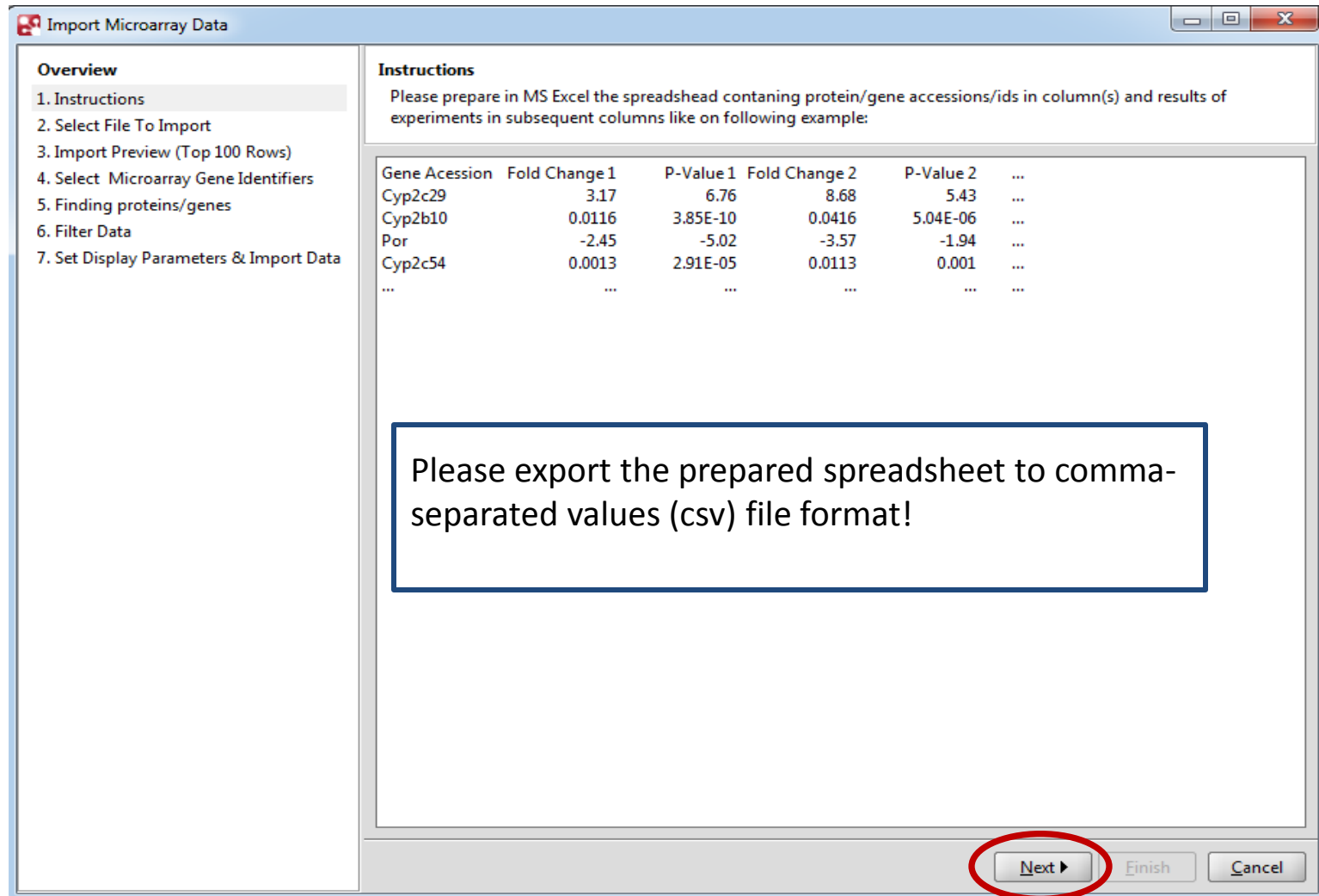
 ?  
Molecules associated with a pathology or toxicity  
(e.g. I have observed a toxicity - what are all known chemicals, classes of chemicals or proteins/genes associated with it?)  
[Read more](#)  
[Introductory movie](#)  
[ToxWiz User Manual](#)  
[ToxWiz User Wiki](#)

Imports microarray data from Excel or comma separated values file



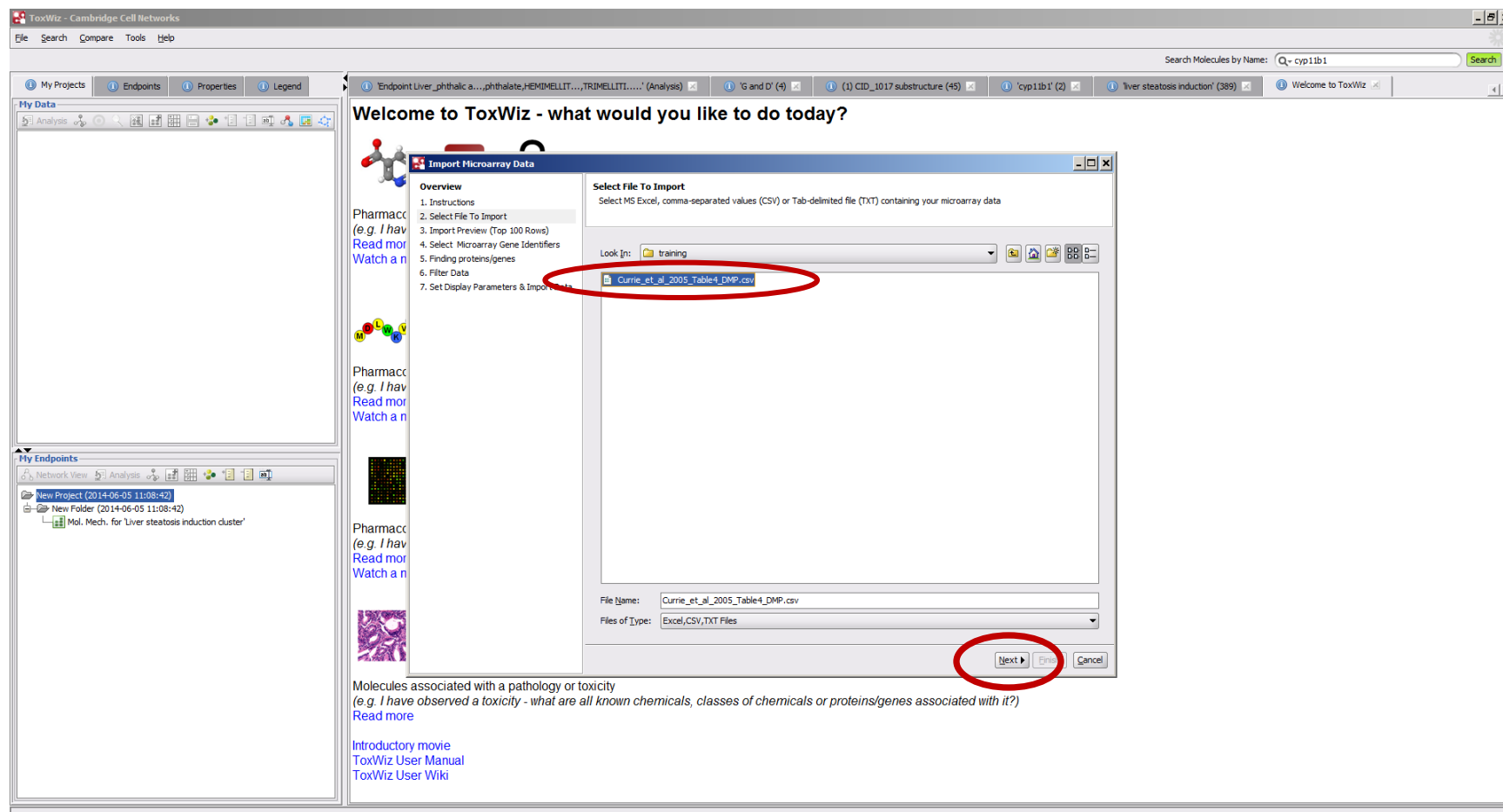
# Importing Microarray Data into ToxWiz

Step 2: A new window with instructions on how to import microarray data will appear. We will follow them by clicking [Next].



# Importing Microarray Data into ToxWiz

Step 3: Select the Currie\_et\_al\_2005\_Table4\_DMP.csv file to select the genes and click [Next].



# Importing Microarray Data into ToxWiz

Step 4: In imported preview, one could change the meaning of each column in the drop-down menu. If no problems are encountered then click [Next].

**Welcome to ToxWiz - what would you like to do today?**

**Import Microarray Data**

**Overview**

1. Instructions
2. Select File To Import
3. Import Preview (Top 100 Rows)
4. Select Microarray Gene Identifiers
5. Finding proteins/genes
6. Filter Data
7. Set Display Parameters & Import Data

**Import Preview (Top 100 Rows)**

You may change the meanings of the each column from the drop-down column header. At least one gene-id column is required. Fold change/ratio and p-value columns belonging to single experiment should be grouped together.

gene-id	fold-change 1	fold-change 2	fold-change 3	fold-change 4
Gene	2h	8h	24h	72h
F2	0.84	0.83	0.87	0.72
F3	1.04	1.19	0.34	1.51
F5	1	0.69	0.67	0.73
F7	0.83	0.76	0.86	0.76
F9	1.16	0.89	0.78	0.71
F10	0.89	0.89	0.83	0.69
Serpinc1	0.8	0.75	0.81	0.64
Serpind1	0.96	0.89	0.91	0.78
Klkb1	0.76	0.56	0.89	0.8
Fgb	0.93	0.79	0.83	0.8
Fgg	0.78	0.81	0.83	0.76
Ptg	0.88	0.79	0.8	0.7
Hrg	0.9	0.7	0.82	0.75
ProC	0.84	0.7	0.89	0.82
ProZ	0.89	0.6	0.69	0.65
Gadd45b	52.72	67.74	0.61	0.51
Ikbkg	1.22	2.25	0.96	0.76
Myd88	1.48	1.29	1.16	1
Txnip	2.24	1.78	1.04	0.82
Ilrak2	1.81	1.5	1.13	0.79
Map3k7ip2	1.52	0.7	0.85	0.7
Per2	0.34	0.86	0.77	0.62
Per3	0.57	1.05	1.45	2.02
Dbp	0.22	0.73	0.61	7.29
dock	1.38	1.33	1.15	0.79
cry1	2.11	1.02	1.25	0.47
THRA	1.26	1	1.06	0.83
G0s2	5.01	6.61	1.58	1.29

**Next** **Cancel**

Molecules associated with a pathology or toxicity  
(e.g. I have observed a toxicity - what are all known chemicals, classes of chemicals or proteins/genes associated with it?)  
[Read more](#)

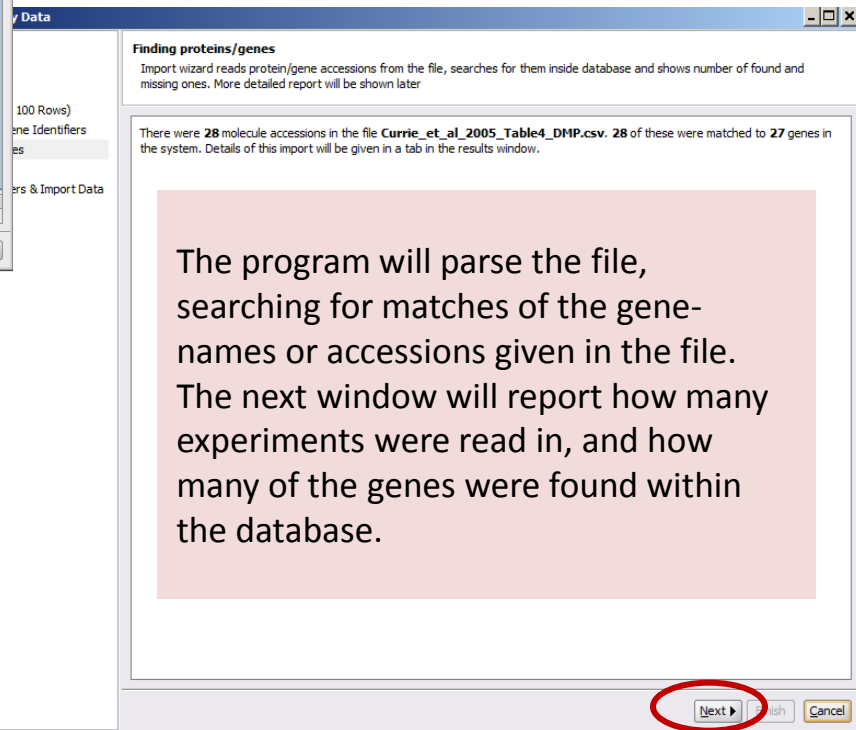
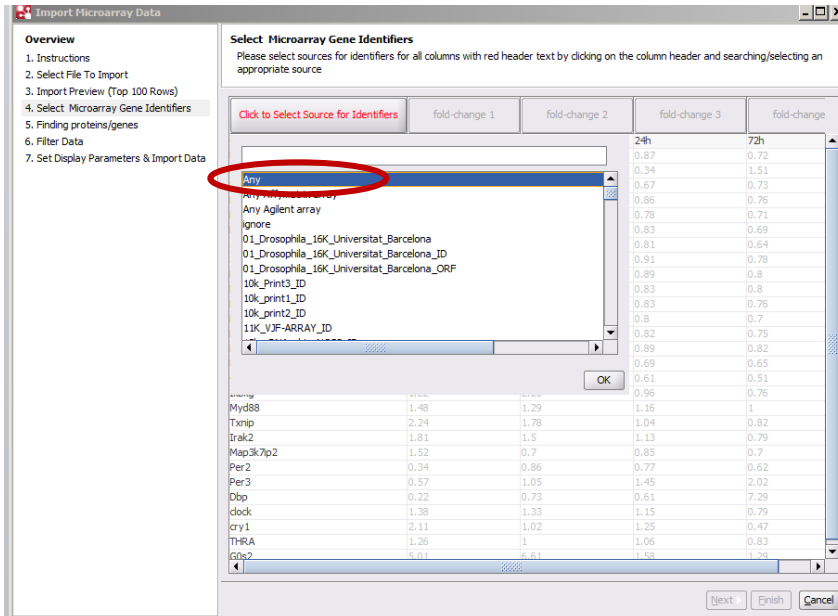
[Introductory movie](#)  
[ToxWiz User Manual](#)  
[ToxWiz User Wiki](#)

Time points columns represent fold changes of genes from the dataset



# Importing Microarray Data into ToxWiz

Step 5: The system will read the gene list from the csv file. Sometime some manual cleaning is required if genes/accession cannot be read due to name mismatch. If no problems are encountered, select [Next].



The program will parse the file, searching for matches of the gene-names or accessions given in the file. The next window will report how many experiments were read in, and how many of the genes were found within the database.





# Importing Microarray Data into ToxWiz

Step 6a: In this screen you can select the threshold values for the experiments that you are importing. You can also do this manually later on, after the genes have been imported into the system.

First round Select [Import All] and click [Next].

Second round, we will import just up-regulated genes (High range) which are having fold change 1.5 or higher.

**Import Microarray Data**

**Overview**

1. Instructions
2. Select File To Import
3. Import Preview (Top 100 Rows)
4. Select Microarray Gene Identifiers
5. Finding proteins/genes
6. Filter Data
7. Set Display Parameters & Import Data

**Filter Data**

You can rename dataset and whether to import all data or limit the import by specifying low and high ranges. All values outside the range(s) will be filtered out.

Name: ALL Currie\_et\_al\_2005\_Table4\_DMP

☒ Import All

**Low Range (Fold Change)**

☐ Enable Min : -∞ Max : 0

**High Range (Fold Change)**

☐ Enable Min : 0 Max : ∞

**P-Value**

☐ Enable Minimum significance p-value: 10.0

**Always give a name to the imported dataset**

Next Finish Cancel

This window asks you to tell the program ranges of the data you wish to consider. This is useful to define up or down-regulated genes in a specific way. One might wish to define down-regulated genes (i.e. 'Low range') as those genes having a fold-change of -1.5 or smaller, and up-regulated genes (i.e. 'High range') as those having 1.5 or higher.



# Importing Microarray Data into ToxWiz

Step 7: Here you could select display parameters of the data, when done, click [Finish].

**Import Microarray Data**

**Overview**

1. Instructions
2. Select File To Import
3. Import Preview (Top 100 Rows)
4. Select Microarray Gene Identifiers
5. Finding proteins/genes
6. Filter Data
7. Set Display Parameters & Import Data

**Set Display Parameters & Import Data**

Display parameters allow you to specify the values that will correspond to the highest red or green values when these data are displayed.

For example, setting the upper limit to 10 means that values of 10 or higher will all have the highest possible red bar.

**Low Range**

Min :  Max :

**High Range**

Min :  Max :



# Molecule lists in “My Projects” Window

Step 8: Imported data is automatically loaded in the My projects space, under My data.

The screenshot displays the ToxWiz - Cambridge Cell Networks software interface. On the left, the 'My Projects' window shows a tree view of projects, with 'ALL Currie\_et\_al\_2005\_Table4\_DMP' selected. A red circle highlights this project. The main window displays the 'Import Report' for this project, showing a table of molecule accessions and their corresponding gene/protein names. The table is organized by experiment (2h, 8h, 24h, 72h) and shows the number of molecules that were matched to genes in the system. The table includes columns for 'Gene/Protein Name (System)', 'Matched Gene-Id (File)', and 'f' (fold change) for each experiment. The table is sorted by the 'f' value for the 2h experiment. The table shows that 28 molecules were matched to 27 genes in the system. The table also includes a note that many molecules/rows in the file can match to single genes in the system.

Import report

Gene/Protein Name (System)	Matched Gene-Id (File)	2h	8h	24h	72h
F2	F2	f=0.84 (0.168)	f=0.83 (0.166)	f=0.87 (0.174)	f=0.72 (0.144)
F3	F3	f=1.04 (0.208)	f=1.19 (0.238)	f=0.34 (0.068)	f=1.51 (0.302)
F5	F5	f=1 (0.2)	f=0.69 (0.138)	f=0.67 (0.134)	f=0.73 (0.146)
F7	F7	f=0.83 (0.166)	f=0.76 (0.152)	f=0.86 (0.172)	f=0.76 (0.152)
F9	F9	f=1.16 (0.232)	f=0.89 (0.178)	f=0.78 (0.156)	f=0.71 (0.142)
F10	F10	f=0.89 (0.178)	f=0.89 (0.178)	f=0.83 (0.166)	f=0.69 (0.138)
SERPINC1	Serpinc1	f=0.8 (0.16)	f=0.75 (0.15)	f=0.81 (0.162)	f=0.64 (0.128)
SERPINC1	Serpinc1	f=0.96 (0.192)	f=0.89 (0.178)	f=0.91 (0.182)	f=0.78 (0.156)
KUB1	Kub1	f=0.76 (0.152)	f=0.56 (0.112)	f=0.89 (0.178)	f=0.8 (0.16)
Fgb	Fgb	f=0.53 (0.106)	f=0.79 (0.158)	f=0.83 (0.166)	f=0.8 (0.16)
Fgg	Fgg	f=0.78 (0.156)	f=0.81 (0.162)	f=0.83 (0.166)	f=0.76 (0.152)
PLG	Plg	f=0.88 (0.176)	f=0.79 (0.158)	f=0.8 (0.16)	f=0.7 (0.14)
HRG	Hrg	f=0.9 (0.18)	f=0.7 (0.14)	f=0.82 (0.164)	f=0.75 (0.15)
PROC	Proc	f=0.84 (0.168)	f=0.7 (0.14)	f=0.89 (0.178)	f=0.82 (0.164)
human_protein_2	Pro2	f=0.89 (0.178)	f=0.6 (0.12)	f=0.69 (0.138)	f=0.65 (0.13)
GADD45B	Gadd45b	f=52.72 (1)	f=67.74 (1)	f=61.12 (1.22)	f=51 (0.102)
30kqg	30kqg	f=1.22 (0.244)	f=2.25 (0.45)	f=0.96 (0.192)	f=0.76 (0.152)
MYD88	Myd88	f=1.48 (0.296)	f=1.29 (0.258)	f=1.16 (0.232)	f=1 (0.2)
TNFR1	Tnfr1	f=2.24 (0.448)	f=1.78 (0.356)	f=1.04 (0.208)	f=0.82 (0.164)
IRAK2	Irak2	f=1.81 (0.362)	f=1.5 (0.3)	f=1.13 (0.226)	f=0.79 (0.158)
MAP3K7P2	Map3k7p2	f=1.52 (0.304)	f=0.7 (0.14)	f=0.85 (0.17)	f=0.7 (0.14)
PER2	Per2	f=0.34 (0.068)	f=0.86 (0.172)	f=0.77 (0.154)	f=0.62 (0.124)
PER3	Per3	f=0.57 (0.114)	f=1.05 (0.21)	f=1.45 (0.29)	f=2.02 (0.404)
DBP	Dbp	f=2.22 (0.444)	f=1.71 (0.342)	f=0.61 (0.122)	f=7.29 (1)
CLOCK	Clock	f=1.38 (0.276)	f=1.33 (0.266)	f=1.15 (0.23)	f=0.79 (0.158)
CRY1	Cry1	f=2.11 (0.422)	f=1.02 (0.204)	f=1.25 (0.25)	f=0.47 (0.094)
THRA	Thra	f=1.36 (0.272)	f=1 (0.2)	f=1.06 (0.212)	f=0.83 (0.166)
GOS2	Gos2	f=5.01 (1)	f=6.61 (1)	f=1.58 (0.316)	f=1.29 (0.258)

Selecting finish will create a group in the right window, with the name specified in the left ‘My data’ window (by default, this is the file name without the extension ‘.csv’). This group will contain a list corresponding to each experiment in the file. List in ‘My data’ behave just like normal lists with the single difference that highlighted genes (those that are checked) will, in addition to being colored, show a bar with a red (up-regulated) or green (down-regulated) bar indicating the degree of expression.



## Step 6b

In Second round, we will import just up-regulated genes (High range) which are having fold change 1.5 or higher.

**Import Microarray Data**

**Overview**

1. Instructions
2. Select File To Import
3. Import Preview (Top 100 Rows)
4. Select Microarray Gene Identifiers
5. Finding proteins/genes
6. Filter Data
7. Set Display Parameters & Import Data

**Filter Data**

You can rename dataset and whether to import all data or limit the import by specifying low and high ranges. All values outside the range(s) will be filtered out.

Name: FC 1.5 Currie\_et\_al\_2005\_Table4\_DMP

☐ Import All

**Low Range (Fold Change)**

☐ Enable Min: 0.22 Max: 1

**High Range (Fold Change)**

☒ Enable Min: 1.5 Max: 67.74

**P-Value**

☐ Enable Minimum significance p-value: 10.0

Next

Give another name to the imported dataset

**ToxWiz - Cambridge Cell Networks**

File Search Compare Tools Help

**My Projects** **Endpoints** **Properties** **Legend**

**My Data**

- ALL Currie\_et\_al\_2005\_Table4\_DMP
- FC 1.5 Currie\_et\_al\_2005\_Table4\_DMP

**Import Report for FC 1.5 Currie\_et\_al\_2005\_Table4\_DMP**

**Import Statistic**

There were 28 molecule accessions in the file Currie\_et\_al\_2005\_Table4\_DMP.csv. 28 of these were matched to 27 genes in the system. The remaining 27 genes were filtered for each experiment based on the parameters you gave: up-regulated (1.5 - 67.74). This left the following counts for each experiment:

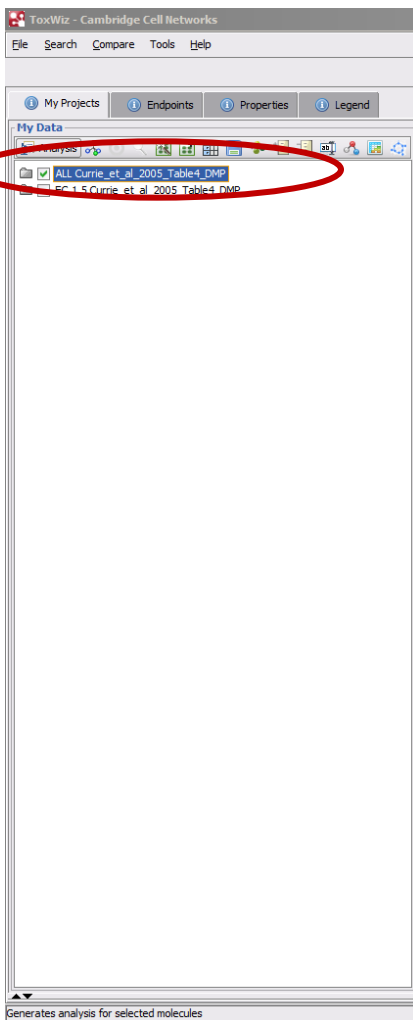
Experiment	Total	Kept	Kept-up	Kept-down	Filtered
2h	28	6(6)	6(6)	0(0)	22(21)
8h	28	5(5)	5(5)	0(0)	23(22)
24h	28	1(1)	1(1)	0(0)	27(26)
72h	28	3(3)	3(3)	0(0)	25(24)

The numbers in the table above refer to the molecule accession (i.e. each row) provided in the file, with those in parentheses denoting the molecules in the system.

Gene/Protein Name (System)	Matched Gene-Id (File)	2h	8h	24h	72h
F2	F2	fc=0.84 (Filtered Out)	fc=0.83 (Filtered Out)	fc=0.87 (Filtered Out)	fc=0.72 (Filtered Out)
F3	F3	fc=1.04 (Filtered Out)	fc=1.19 (Filtered Out)	fc=0.34 (Filtered Out)	fc=1.51 (0.002)
F5	F5	fc=1 (Filtered Out)	fc=0.69 (Filtered Out)	fc=0.67 (Filtered Out)	fc=0.73 (Filtered Out)
F7	F7	fc=0.83 (Filtered Out)	fc=0.76 (Filtered Out)	fc=0.86 (Filtered Out)	fc=0.76 (Filtered Out)
F9	F9	fc=1.16 (Filtered Out)	fc=0.89 (Filtered Out)	fc=0.76 (Filtered Out)	fc=0.71 (Filtered Out)
F10	F10	fc=0.89 (Filtered Out)	fc=0.89 (Filtered Out)	fc=0.83 (Filtered Out)	fc=0.69 (Filtered Out)
SERPINC1	Serpinc1	fc=0.8 (Filtered Out)	fc=0.75 (Filtered Out)	fc=0.81 (Filtered Out)	fc=0.64 (Filtered Out)
SERPIND1	Serpind1	fc=0.96 (Filtered Out)	fc=0.89 (Filtered Out)	fc=0.91 (Filtered Out)	fc=0.78 (Filtered Out)
KLKB1	Klkb1	fc=0.76 (Filtered Out)	fc=0.56 (Filtered Out)	fc=0.89 (Filtered Out)	fc=0.8 (Filtered Out)
FGB	Fgb	fc=0.93 (Filtered Out)	fc=0.79 (Filtered Out)	fc=0.83 (Filtered Out)	fc=0.8 (Filtered Out)
FGG	Fgg	fc=0.78 (Filtered Out)	fc=0.81 (Filtered Out)	fc=0.83 (Filtered Out)	fc=0.76 (Filtered Out)
PLG	Plg	fc=0.88 (Filtered Out)	fc=0.79 (Filtered Out)	fc=0.8 (Filtered Out)	fc=0.7 (Filtered Out)
Hrg	Hrg	fc=0.9 (Filtered Out)	fc=0.7 (Filtered Out)	fc=0.82 (Filtered Out)	fc=0.75 (Filtered Out)
PROC	ProC	fc=0.84 (Filtered Out)	fc=0.7 (Filtered Out)	fc=0.89 (Filtered Out)	fc=0.82 (Filtered Out)
human_protein_Z	ProZ	fc=0.89 (Filtered Out)	fc=0.6 (Filtered Out)	fc=0.69 (Filtered Out)	fc=0.65 (Filtered Out)
GADD45B	Gadd45b	fc=52.72 (1)	fc=67.74 (1)	fc=0.61 (Filtered Out)	fc=0.51 (Filtered Out)
IKBK	Ikbk	fc=1.22 (Filtered Out)	fc=2.25 (0.15)	fc=0.96 (Filtered Out)	fc=0.76 (Filtered Out)
MYD88	Myd88	fc=1.48 (Filtered Out)	fc=1.29 (Filtered Out)	fc=1.16 (Filtered Out)	fc=1 (Filtered Out)
TXNP	Txnp	fc=2.24 (0.148)	fc=1.78 (0.056)	fc=1.04 (Filtered Out)	fc=0.82 (Filtered Out)
IRAK2	Irak2	fc=1.81 (0.062)	fc=1.5 (0)	fc=1.13 (Filtered Out)	fc=0.79 (Filtered Out)
MAP3K7P2	Map3k7p2	fc=1.52 (0.004)	fc=0.7 (Filtered Out)	fc=0.85 (Filtered Out)	fc=0.7 (Filtered Out)
PER2	Per2	fc=0.34 (Filtered Out)	fc=0.86 (Filtered Out)	fc=0.77 (Filtered Out)	fc=0.62 (Filtered Out)
PER3	Per3	fc=0.57 (Filtered Out)	fc=1.05 (Filtered Out)	fc=1.45 (Filtered Out)	fc=2.02 (0.104)
DBP	Dbp	fc=0.22 (Filtered Out)	fc=0.73 (Filtered Out)	fc=0.61 (Filtered Out)	fc=7.29 (1)
CLOCK	dock	fc=1.38 (Filtered Out)	fc=1.33 (Filtered Out)	fc=1.15 (Filtered Out)	fc=0.79 (Filtered Out)
CRY1	cry1	fc=2.11 (0.122)	fc=1.02 (Filtered Out)	fc=1.25 (Filtered Out)	fc=0.47 (Filtered Out)
THRA	Thra	fc=1.26 (Filtered Out)	fc=1 (Filtered Out)	fc=1.06 (Filtered Out)	fc=0.83 (Filtered Out)
GOS2	Gos2	fc=5.01 (0.702)	fc=6.61 (1)	fc=1.58 (0.016)	fc=1.29 (Filtered Out)

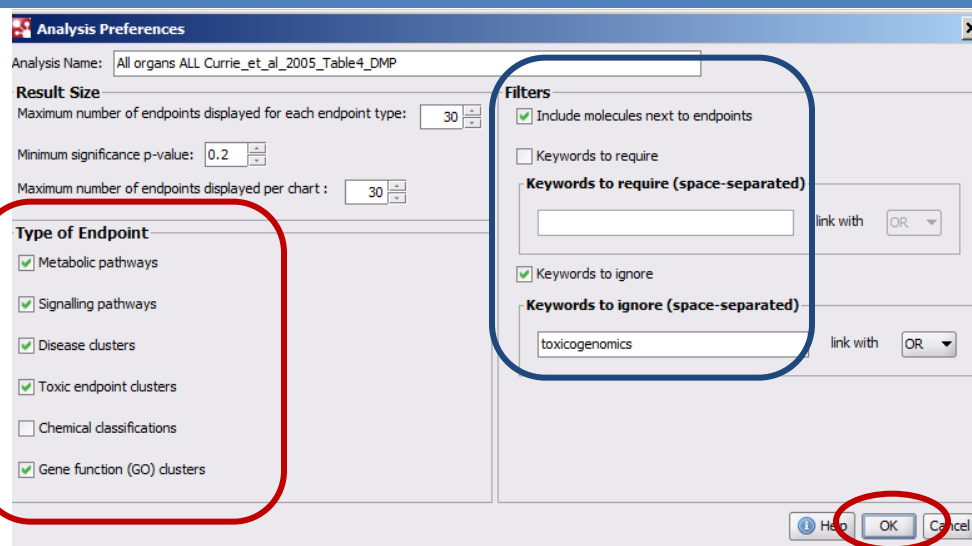
Import report, with shaded filtered-out genes

# Selecting Dysregulated Genes From the Molecule list



Step 9: Click on the ALL Currie\_et\_al\_2005\_Table4\_DMP folder to select genes.

Always give a name to the analysis you are performing



Step 11: With a mouse right click function select "Analysis". This will launch a "Preferences" window.

Predicting Toxic Endpoints with Pathways  
and Searching for Toxic Endpoint Clusters  
and involved Pathways



# Clusters Show Relationships between ALL disregulated genes and other molecules Involved in pathology

Tabular view of the analysis, with categorized endpoints and p-values of prediction, total and individual ones

Processes are ranked by statistical significance of the match to the molecules you used.

Analysis report for All genes,  
no organ specified

List of disregulated genes sorted according to the  
frequency they occur in endpoints

# Clusters Show Relationships between FC>1.5 dysregulated genes and other molecules Involved in pathology

**Analysis Preferences**

Analysis Name: All organs FC 1.5 Currie\_et\_al\_2005\_Table4\_DMP

**Result Size**

Maximum number of endpoints displayed for each endpoint type: 30

Minimum significance p-value: 0.2

Maximum number of endpoints displayed per chart: 30

**Type of Endpoint**

- ☒ Metabolic pathways
- ☒ Signalling pathways
- ☒ Disease clusters
- ☒ Toxic endpoint clusters
- ☐ Chemical classifications
- ☒ Gene function (GO) clusters

**Filters**

☒ Include molecules next to endpoints

☐ Keywords to require

**Keywords to require (space-separated)**

link with OR

☒ Keywords to ignore

**Keywords to ignore (space-separated)**

toxic

Analysis report for FC>1.5 genes,  
no organ specified

Tabular view of the analysis, with categorized endpoints and p-values of prediction, total and individual ones

Processes are ranked by statistical significance of the match to the molecules you used.

Endpoint Category	P-val	Molecular Mechanism	P-val	Molecules Inside	Molecules Next To
<input checked="" type="checkbox"/> Digestive system (excluding Hepatic)	0.0001	<a href="#">Gastrointestinal tract toxicities cluster</a>	0.0001		5: GADD45B, TNIP, PER3, CRY1, F3
		<a href="#">Gastrointestinal tract carcinoma cluster</a>	0.0001		5: GADD45B, TNIP, PER3, CRY1, F3
<input checked="" type="checkbox"/> Circulatory system	0.0001	<a href="#">Blood inflammation cluster</a>	0.0001		9: IKKKG, MAP3K7IP2, F3, PER3, DBP, GADD45B, ...
		<a href="#">Cardiac hypertrophy association cluster</a>	0.0001		8: DBP, TNIP, CRY1, F3, GADD45B, GOS2, PER3, ...
		<a href="#">Myocardial hypertrophy association cluster</a>	0.0001		7: TNIP, GADD45B, CRY1, F3, DBP, GOS2, PER3
		<a href="#">Myocardial fibrosis association cluster</a>	0.0001		6: DBP, TNIP, GADD45B, GOS2, CRY1, IKKKG
		<a href="#">Heart fibrosis inhibition cluster</a>	0.0001		GADD45B, TNIP
<input checked="" type="checkbox"/> Activity system (e.g. Muscle, bone)	0.0001	<a href="#">Tendon toxicities cluster</a>	0.0001		8: CRY1, PER3, DBP, IKKKG, F3, MAP3K7IP2, TNIP...
		<a href="#">Muscle atrophy inhibition cluster</a>	0.0001		5: MAP3K7IP2, TNIP, DBP, CRY1, GADD45B
		<a href="#">Tendon calcification cluster</a>	0.0001		4: PER3, CRY1, DBP, F3
<input checked="" type="checkbox"/> Immune system	0.0001	<a href="#">Eosinophil phagocytosis cluster</a>	0.0001		5: PER3, CRY1, DBP, TNIP, GADD45B
		<a href="#">B-cell hyperplasia cluster</a>	0.0001		5: IKKKG, MAP3K7IP2, CRY1, IRAK2, F3
		<a href="#">Macrophage toxicities cluster</a>	0.0005	TNIP	9: IKKKG, GADD45B, CRY1, F3, IRAK2, MAP3K7IP...
<input checked="" type="checkbox"/> Connective and other tissues & cells	0.0001	<a href="#">Adipose tissue vacuolation cluster</a>	0.0001	GADD45B	4: DBP, F3, TNIP, CRY1
<input checked="" type="checkbox"/> Urinary system	0.0001	<a href="#">Urinary tract inflammation cluster</a>	0.0001		6: IRAK2, GADD45B, TNIP, MAP3K7IP2, IKKKG, F3
<input checked="" type="checkbox"/> Male Reproductive system	0.0001	<a href="#">Leydig cell hyperplasia association cluster</a>	0.0001	DBP	4: CRY1, PER3, GADD45B, F3
		<a href="#">Leydig cell hyperplasia induction cluster</a>	0.0001	DBP	4: CRY1, PER3, GADD45B, F3
		<a href="#">Leydig cell toxicities association cluster</a>	0.0001	DBP	4: CRY1, PER3, GADD45B, F3
<input checked="" type="checkbox"/> Endocrine system	0.0001	<a href="#">Adrenal gland neoplasia association cluster</a>	0.0001		6: TNIP, PER3, DBP, CRY1, IKKKG, GADD45B
<input checked="" type="checkbox"/> Female Reproductive system	0.0001	<a href="#">Ovary neoplasia cluster</a>	0.0001		7: TNIP, CRY1, GADD45B, F3, GOS2, PER3, DBP
<input checked="" type="checkbox"/> Respiratory system	0.0001	<a href="#">Lung epithelium fibrosis cluster</a>	0.0001		6: IKKKG, CRY1, TNIP, GADD45B, MAP3K7IP2, F3
<input checked="" type="checkbox"/> Lymphatic system	0.0001	<a href="#">Lymphocyte apoptosis inhibition cluster</a>	0.0001		10: IKKKG, IRAK2, MAP3K7IP2, TNIP, CRY1, GA...
		<a href="#">Spleen atrophy cluster</a>	0.0001		6: GADD45B, PER3, TNIP, GOS2, DBP, CRY1
		<a href="#">Lymphocyte apoptosis cluster</a>	0.0005		10: IKKKG, F3, GADD45B, CRY1, MAP3K7IP2, TNIP...
<input checked="" type="checkbox"/> Hepatic system	0.0001	<a href="#">Liver degeneration cluster</a>	0.0001	MAP3K7IP2	9: IKKKG, GADD45B, CRY1, IRAK2, F3, GOS2, PER...
<input checked="" type="checkbox"/> Pan-systemic pathologies	0.0001	<a href="#">Mitochondrial toxicities association cluster</a>	0.0001		3: DBP, TNIP, IKKKG
		<a href="#">Apoptosis cluster</a>	0.0005	3: MAP3K7IP2, IKKKG, TNIP	7: F3, GADD45B, CRY1, DBP, GOS2, PER3, IRAK2
<input checked="" type="checkbox"/> Nervous system	0.0005	<a href="#">Nerve toxicities cluster</a>	0.0005		10: IKKKG, CRY1, F3, GADD45B, TNIP, DBP, MA...
<input checked="" type="checkbox"/> Toxic endpoint clusters	0.0005				



# Clusters Show Relationships between ALL disregulated genes and other molecules Involved in pathology

**Analysis Preferences**

Analysis Name:

**Result Size**

Maximum number of endpoints displayed for each endpoint type:

Minimum significance p-value:

Maximum number of endpoints displayed per chart:

**Type of Endpoint**

☐ Metabolic pathways

☐ Signalling pathways

☐ Disease clusters

☒ Toxic endpoint clusters

**Filters**

☒ Include molecules next to endpoints

☒ Keywords to require

Keywords to require (space-separated):  link with

☒ Keywords to ignore

Keywords to ignore (space-separated):  link with

Analysis report for All genes, specified for Liver toxicity (keywords: liver hepato bile)

**Toxic endpoint clusters**

Endpoint Category	P-val	Molecular Mechanism	P-val	Molecules Inside	Molecules Next To
Hepatic system	0.0001	<a href="#">Liver carcinoma cluster</a>	0.0001	GADD45B	26: MYD88, TXNIP, PER2, FGB, F2, CLOCK, THRA, PRO...
		<a href="#">Liver fibrosis cluster</a>	0.0001	MYD88	26: F2, IKBK, PER2, PROC, PLG, THRA, FGB, CRY1, CL...
		<a href="#">Liver toxicities cluster</a>	0.0001	6: MAP3K7IP2, GOS2, CLOCK, IKBK, GADD45B, MYD88	21: F2, PER2, THRA, F3, CRY1, FGB, PROC, PLG, TXNIP...
		<a href="#">Hepatocyte toxicities cluster</a>	0.0001	MYD88	26: IKBK, PER2, GADD45B, F2, PROC, FGB, TXNIP, F3...
		<a href="#">Hepatocyte apoptosis cluster</a>	0.0001	MYD88	26: IKBK, GADD45B, PER2, PROC, F2, FGB, CRY1, F3...
		<a href="#">Liver inflammation cluster</a>	0.0001	IKBK, MYD88	25: F3, PROC, GADD45B, TXNIP, PER2, CLOCK, FGB, F2...
		<a href="#">Liver apoptosis cluster</a>	0.0001	3: MAP3K7IP2, IKBK, MYD88	24: GADD45B, TXNIP, PER2, THRA, F2, CLOCK, CRY1, F...
		<a href="#">Liver steatosis cluster</a>	0.0001	3: GOS2, CLOCK, IKBK	23: MYD88, PER2, THRA, GADD45B, CRY1, TXNIP, PRO...
		<a href="#">Hepatotoxicity cluster</a>	0.0001	GADD45B	25: MYD88, FGB, PER2, THRA, TXNIP, F2, F7, CRY1, F3...
		<a href="#">Hepatotoxicity cluster</a>	0.0001		26: PER2, FGB, TXNIP, CLOCK, MYD88, THRA, FGG, CR...
		<a href="#">Liver necrosis cluster</a>	0.0001		26: MYD88, FGB, PER2, THRA, PROC, GADD45B, CLOCK...
		<a href="#">Hepatocyte degeneration cluster</a>	0.0001		26: FGB, PER2, CLOCK, THRA, MYD88, TXNIP, FGG, F2...
		<a href="#">Hepatocyte necrosis cluster</a>	0.0001	MYD88	25: FGB, GADD45B, F3, TXNIP, PROC, FGG, F2, IKBK...
		<a href="#">Hepatotoxicity induction cluster</a>	0.0001		25: FGB, THRA, FGG, PER2, TXNIP, MYD88, SERPINC1...
		<a href="#">Liver neoplasia cluster</a>	0.0001	GADD45B	24: MYD88, FGB, PLG, PER2, FGG, F2, CRY1, THRA, CL...
		<a href="#">Liver ischaemia cluster</a>	0.0001		24: MYD88, TXNIP, CRY1, PER2, CLOCK, F3, GADD45B...
		<a href="#">Hepatocyte degeneration induction cluster</a>	0.0001		23: FGB, THRA, DBP, F2, FGG, TXNIP, SERPINC1, PER2...
		<a href="#">Liver steatosis association cluster</a>	0.0001	FGG, FGB	21: F2, PLG, GADD45B, MYD88, THRA, CLOCK, TXNIP...
		<a href="#">Hepatocyte apoptosis association cluster</a>	0.0001		23: GADD45B, MYD88, THRA, CLOCK, TXNIP, FGG...
		<a href="#">Bile duct toxicities cluster</a>	0.0001	MYD88, FGG, F3	23: MYD88, FGG, F3
		<a href="#">Liver degeneration cluster</a>	0.0001	MAP3K7IP2, MYD88	21: IKBK, GADD45B, PER2, PROC, F2, FGB, CRY1, F3...
		<a href="#">Hepatocyte apoptosis induction cluster</a>	0.0001		20: DBP, FGG, FGB, F2, CRY1, THRA, CL...
		<a href="#">Hepatocyte hyperplasia cluster</a>	0.0001		18: FGB, SERPINC1, PER2, THRA, FGG, F2, CRY1, THRA, CL...
		<a href="#">Liver fibrosis association cluster</a>	0.0001		18: FGB, THRA, MYD88, TXNIP, FGG, F2, CRY1, THRA, CL...
		<a href="#">Liver hyperplasia cluster</a>	0.0001	GADD45B	16: FGB, PER2, CLOCK, MYD88, TXNIP, FGG, F2, CRY1, THRA, CL...
		<a href="#">Hepatocyte steatosis cluster</a>	0.0001		17: MYD88, F3, GOS2, CLOCK, IKBK, GADD45B, MYD88...

**Filter Molecular Mechanism**

Preferences

☒ Genes/Proteins

☐ Chemicals

**NO CHEMICALS**

Advanced Preferences OK Cancel

Click to view graphically Molecular mechanism hypothesis for Liver steatosis



# Clusters Show Relationships between FC>1.5 dysregulated genes and other molecules Involved in pathology

**Analysis Preferences**

Analysis Name: Liver FC 1.5 Currie\_et\_al\_2005\_Table4\_DMP

**Result Size**

Maximum number of endpoints displayed for each endpoint type: 30

Minimum significance p-value: 0.2

Maximum number of endpoints displayed per chart: 30

**Type of Endpoint**

☐ Metabolic pathways

☐ Signalling pathways

☐ Disease clusters

☒ Toxic endpoint clusters

**Filters**

☒ Include molecules next to endpoints

☒ Keywords to require

**Keywords to require (space-separated)**

liver hepato bile link with OR

☒ Keywords to ignore

**Keywords to ignore (space-separated)**

toxicogenomics link with OR

Analysis report for FC>1.5 genes, specified for Liver toxicity (keywords: liver hepato bile)

**Toxic endpoint clusters**

Endpoint Category	P-val	Molecular Mechanism	P-val	Molecules Inside	Molecules Next To
Hepatic system	0.0001	<a href="#">Liver degeneration cluster</a>	0.0001	MAP3K7IP2	9: IKBKG, GADD45B, CRY1, IRAK2, F3, GOS2, PER3, DB...
		<a href="#">Liver ischaemia cluster</a>	0.0005		10: TXNIP, CRY1, F3, GADD45B, IKBKG, PER3, DBP, IRA...
		<a href="#">Liver fibrosis cluster</a>	0.0005		10: IKBKG, CRY1, F3, GADD45B, GOS2, TXNIP, DBP, IRA...
		<a href="#">Liver steatosis cluster</a>	0.0005	GOS2, IKBKG	8: GADD45B, CRY1, TXNIP, F3, DBP, PER3, IRAK2, MAP...
		<a href="#">Liver toxicities cluster</a>	0.0005	4: MAP3K7IP2, GOS2, IKBKG, GADD45B	6: F3, CRY1, TXNIP, DBP, PER3, IRAK2
		<a href="#">Hepatocyte toxicities cluster</a>	0.0005		10: IKBKG, GADD45B, TXNIP, F3, CRY1, MAP3K7IP2, DB...
		<a href="#">Hepatocyte apoptosis cluster</a>	0.0005		10: IKBKG, GADD45B, CRY1, F3, TXNIP, MAP3K7IP2, DB...
		<a href="#">Liver inflammation cluster</a>	0.0005	IKBKG	9: F3, GADD45B, TXNIP, CRY1, MAP3K7IP2, IRAK2, DBP...
		<a href="#">Liver apoptosis cluster</a>	0.0005	MAP3K7IP2, IKBKG	8: GADD45B, TXNIP, CRY1, F3, PER3, GOS2, DBP, IRAK2
		<a href="#">Hepatocyte necrosis cluster</a>	0.0005		10: GADD45B, F3, TXNIP, IKBKG, CRY1, DBP, IRAK2, PE...
		<a href="#">Liver necrosis cluster</a>	0.0005		9: GADD45B, TXNIP, F3, CRY1, IKBKG, DBP, GOS2, PER...
		<a href="#">Hepatoma toxicities cluster</a>	0.0005		9: GADD45B, PER3, F3, TXNIP, DBP, CRY1, GOS2, IKBK...
		<a href="#">Hepatoma carcinoma cluster</a>	0.0005		8: GADD45B, F3, GOS2, PER3, DBP, TXNIP, CRY1, IKBKG
		<a href="#">Hepatocyte apoptosis induction cluster</a>	0.0005		8: DBP, GADD45B, F3, TXNIP, GOS2, PER3, CRY1, IKBKG
		<a href="#">Hepatocyte apoptosis association cluster</a>	0.0005		8: GADD45B, DBP, IKBKG, F3, TXNIP, CRY1, PER3, GOS2
		<a href="#">Hepatoma necrosis cluster</a>	0.0005		8: GADD45B, PER3, TXNIP, F3, DBP, CRY1, MAP3K7IP2,...
		<a href="#">Liver hyperplasia cluster</a>	0.0005	GADD45B	6: TXNIP, DBP, CRY1, IKBKG, F3, GOS2
		<a href="#">Hepatocyte apoptosis inhibition cluster</a>	0.0005		7: GADD45B
		<a href="#">Liver ischaemia inhibition cluster</a>	0.0005		5: IKBKG, TX
		<a href="#">Liver steatosis inhibition cluster</a>	0.0005		4: GADD45B
		<a href="#">Hepatocyte vacuolation cluster</a>	0.0005		GADD45B, G
		<a href="#">Hepatocyte steatosis cluster</a>	0.001		6: F3, GOS2
		<a href="#">Liver inflammation inhibition cluster</a>	0.001		4: GADD45B
		<a href="#">Hepatotoxicity cluster</a>	0.005	GADD45B	8: TXNIP, CR
		<a href="#">Hepatotoxicity cluster</a>	0.005		9: TXNIP, CR
		<a href="#">Bile duct toxicities cluster</a>	0.005		9: GADD45B

**Click to view graphically Molecular mechanism hypothesis for Liver steatosis**

**Filter Molecular Mechanism**

**Preferences**

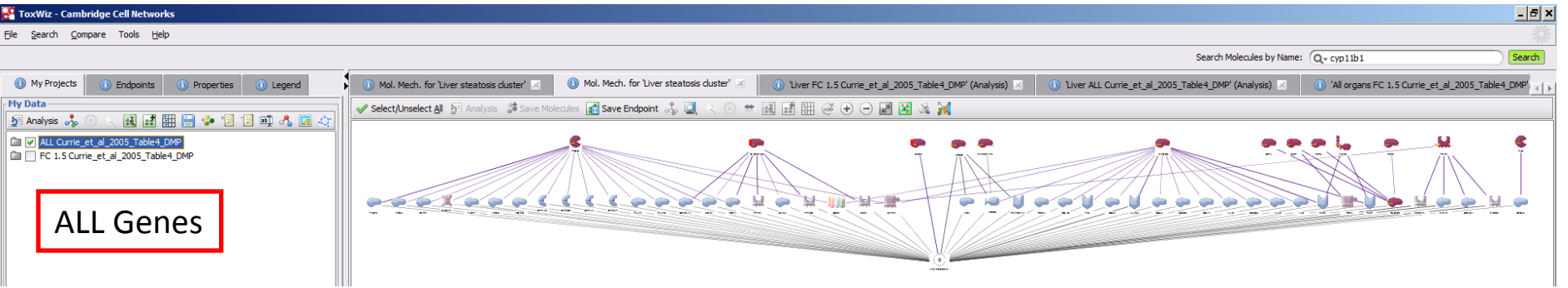
☒ Genes/Proteins

☐ Chemicals

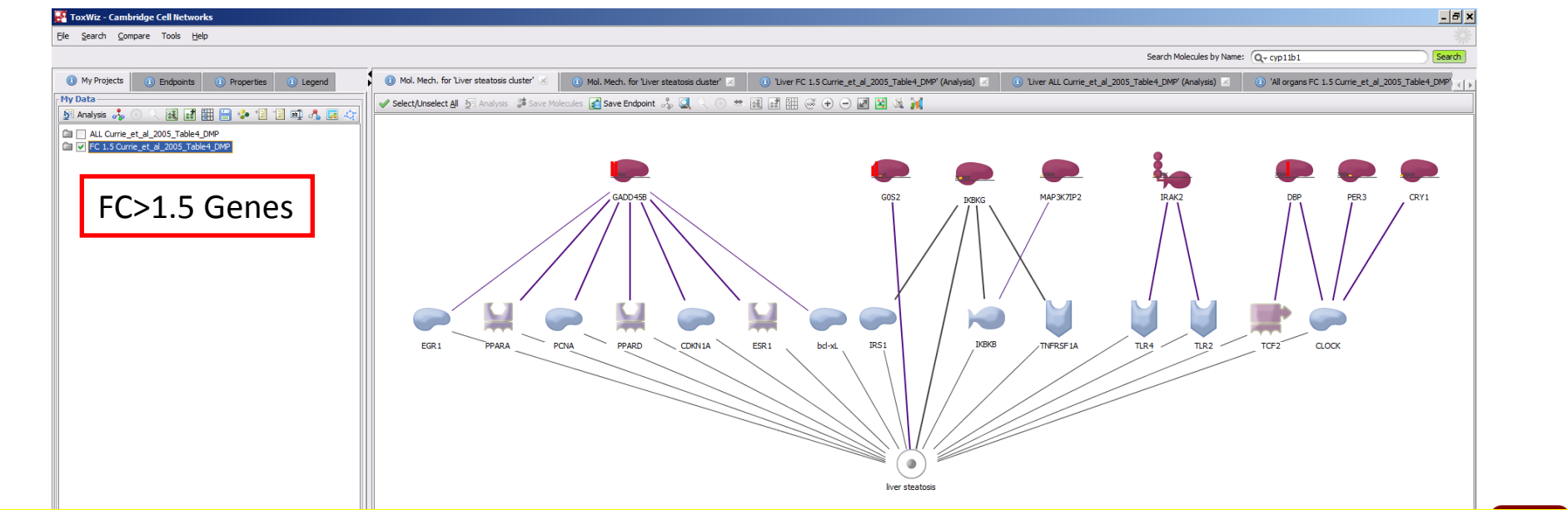
**NO CHEMICALS**

Advanced Preferences OK Cancel

# Step 10: Hypothesized Molecular Mechanism for Liver steatosis



Dark molecules are from the list of dysregulated genes. Next to each gene there is a bar indicating the level of dysregulation.



Notice the difference in number of molecules used for predictive analysis in the opened mechanism

# What genes in Liver steatosis are affected directly by Phthalates?

Step 11: Tickle off all 45 chemicals that share structural similarity to Phthalic acid. Select "Add to opened network view" icon from the tool bar.

The screenshot shows the ToxWiz - Cambridge Cell Networks interface. The top window displays a table of chemicals with their structures, names, scores, and matched queries. A red circle highlights the 'Add Selection to Opened Network View' icon in the toolbar. A dialog box is open, asking to select an opened network view, with 'Mol. Mech. for Liver steatosis cluster' selected and circled in red.

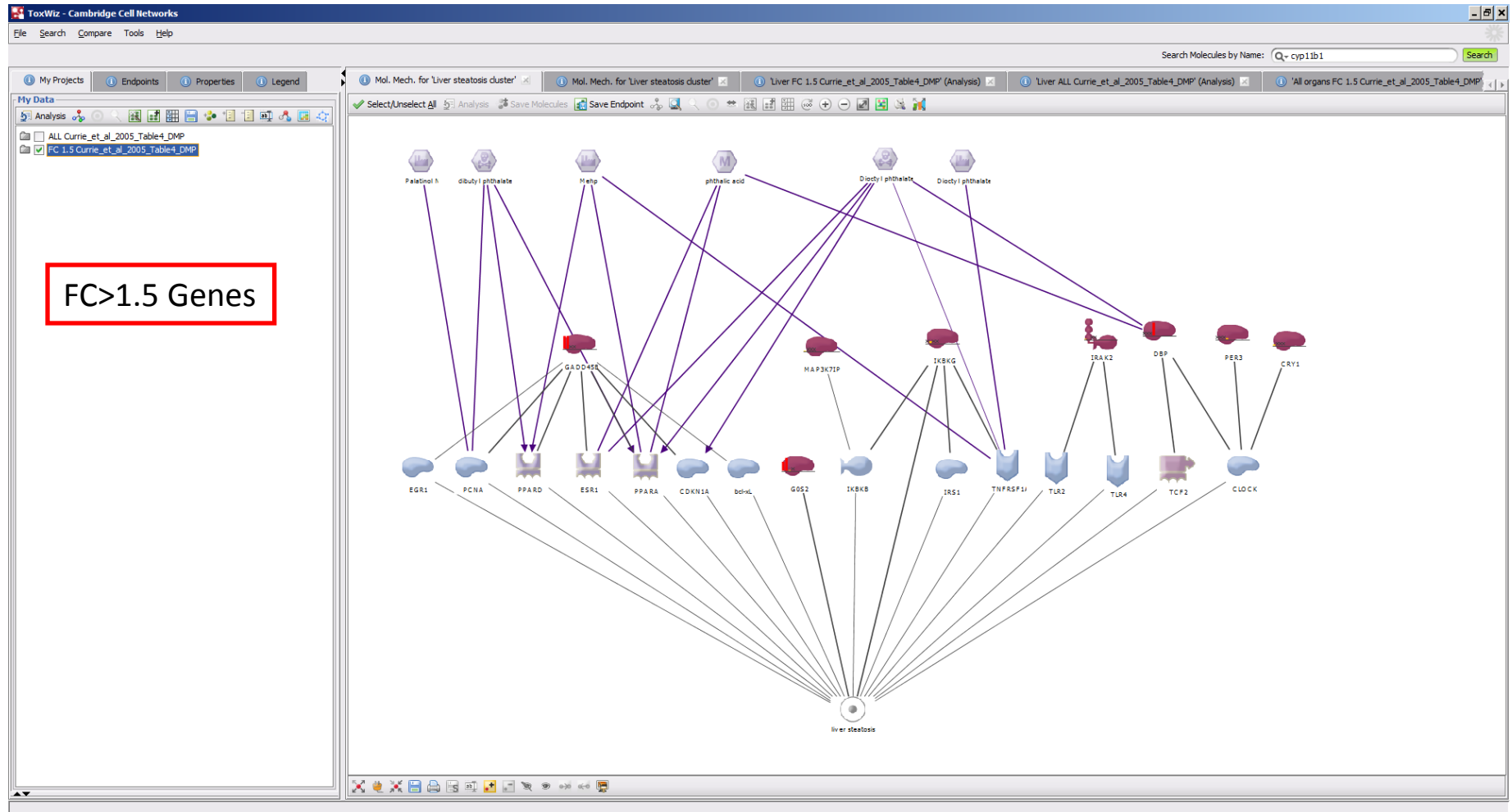
Structure	Molecule Name	Score	Matched Query
	phthalic acid	1	(1) CID_1017
	phthalate	1	(1) CID_1017
	HEMIMELLITIC ACID	0.86	(1) CID_1017
	TRIMELLITIC ACID	0.82	(1) CID_1017
	PIVAP	0.82	(1) CID_1017

The bottom window shows a network diagram with nodes representing genes and chemicals. The nodes are arranged in a hierarchical manner, with 'Liver steatosis' at the bottom and various chemicals at the top. The network is connected by lines representing interactions.

You can rearrange manually opened network



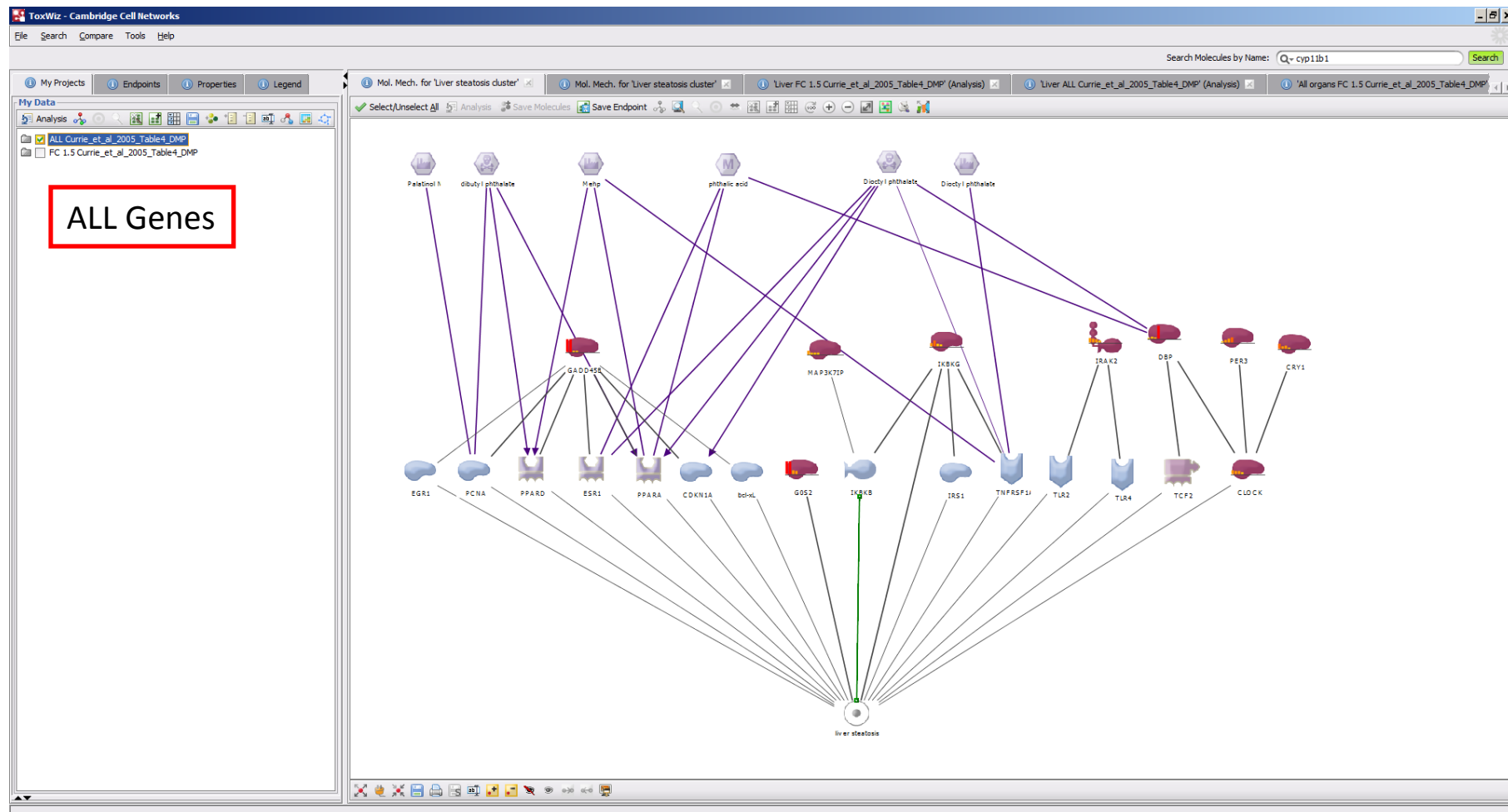
# Liver steatosis mechanistic hypothesis involving dysregulated genes from the dataset (FC>1.5)



Genes from Microarray data and other involved molecules directly affected by different phthalate derivatives

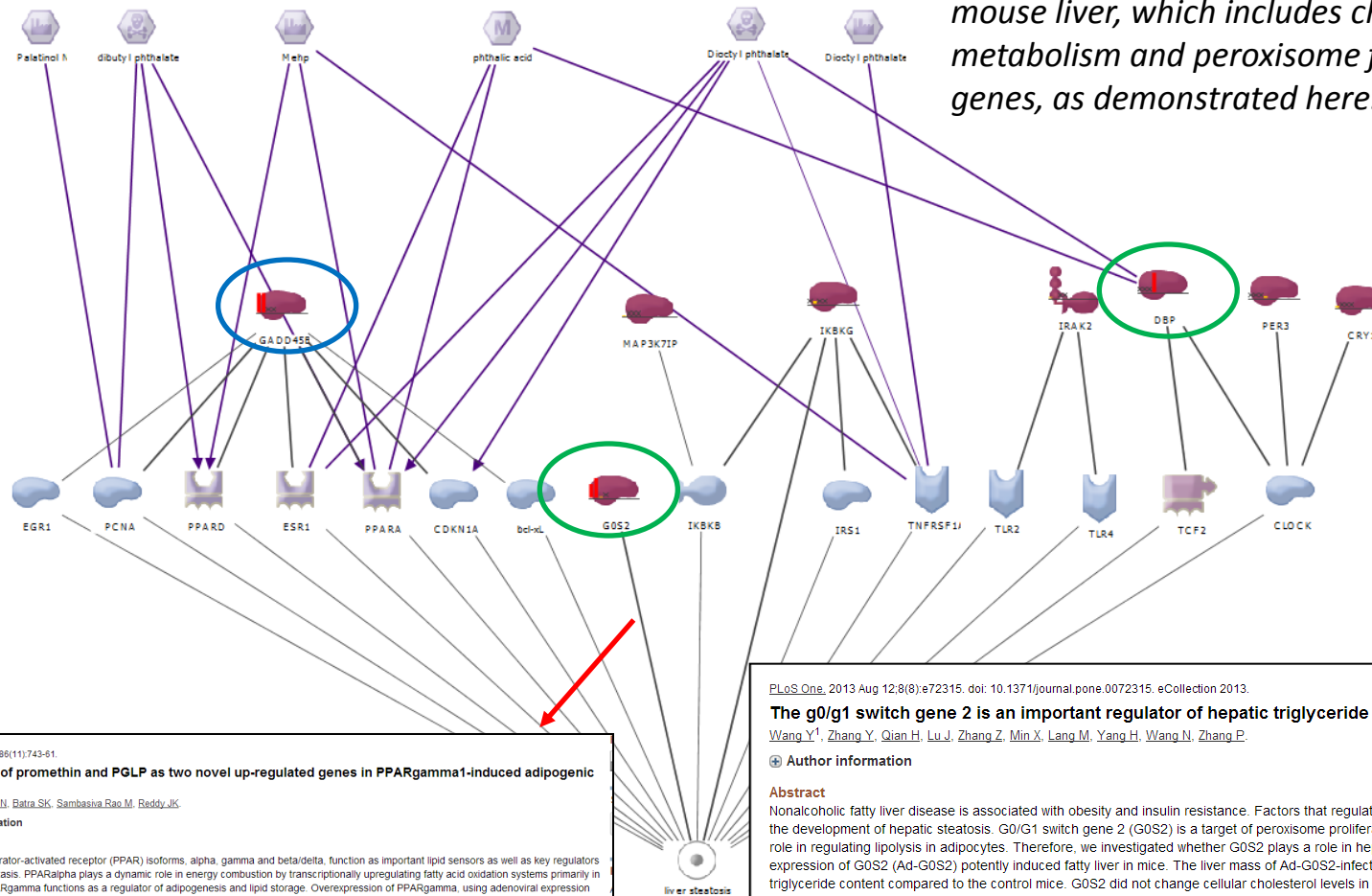


# Liver steatosis mechanistic hypothesis involving dysregulated genes from the dataset (ALL)



Genes that show the highest change in expression upon treatment with DEHP, are those involved in **Signaling response (Gadd45b)** and **Circadian genes (G0s2 and Dbp)** and they are predicted by ToxWiz to be involved in Liver steatosis.

*DEHP induces known molecular changes in the mouse liver, which includes changes in fatty acid metabolism and peroxisome formation-associated genes, as demonstrated here.*



Biochimica, 2004 Nov;86(11):743-61.

#### Identification of promethin and PGLP as two novel up-regulated genes in PPARgamma1-induced adipogenic mouse liver.

Yu S<sup>1</sup>, Viswakarma N, Batra SK, Sambasiva Rao M, Reddy JK

Author information

#### Abstract

Peroxisome proliferator-activated receptor (PPAR) isoforms, alpha, gamma and beta/delta, function as important lipid sensors as well as key regulators of energy homeostasis. PPARalpha plays a dynamic role in energy combustion by transcriptionally upregulating fatty acid oxidation systems primarily in liver, whereas PPARgamma functions as a regulator of adipogenesis and lipid storage. Overexpression of PPARgamma, using adenoviral expression approach, in PPARalpha deficient mouse liver results in hepatic steatosis with concurrent expression of adipocyte specific genes. In this study, to gain a global molecular understanding of PPARgamma1-induced gene expression in liver, we have analyzed gene expression profiles using the Affymetrix GeneChip mouse expression array set 430, that enables a comprehensive gene expression profiling with >39,000 transcripts. Microarray data analysis provided us with over 278 genes up-regulated fourfold or higher, and 121 genes down-regulated fourfold or higher in liver with PPARgamma-induced hepatic adiposis. We have found 101 uncharacterized genes out of 278 up-regulated and 29 uncharacterized among the down-regulated gene categories, respectively. Of 177 functionally characterized candidate genes in the up-regulated category many appear to be involved in adipogenesis, lipid metabolism and signal transduction. To focus a cDNAs of two novel candidates, which we designate but induced robustly in liver with hepatic adiposis as protein, is a low abundant transcript in normal liver, genes was not increased in fatty livers induced by it should provide a basis for understanding the molecular

PMID: 15589683 [PubMed - indexed for MEDLINE]

PLoS One. 2013 Aug 12;8(8):e72315. doi: 10.1371/journal.pone.0072315. eCollection 2013.

#### The g0/g1 switch gene 2 is an important regulator of hepatic triglyceride metabolism.

Wang Y<sup>1</sup>, Zhang Y, Qian H, Lu J, Zhang Z, Min X, Lang M, Yang H, Wang N, Zhang P.

Author information

#### Abstract

Nonalcoholic fatty liver disease is associated with obesity and insulin resistance. Factors that regulate the disposal of hepatic triglycerides contribute to the development of hepatic steatosis. G0/G1 switch gene 2 (G0S2) is a target of peroxisome proliferator-activated receptors and plays an important role in regulating lipolysis in adipocytes. Therefore, we investigated whether G0S2 plays a role in hepatic lipid metabolism. Adenovirus-mediated expression of G0S2 (Ad-G0S2) potentially induced fatty liver in mice. The liver mass of Ad-G0S2-infected mice was markedly increased with excess triglyceride content compared to the control mice. G0S2 did not change cellular cholesterol levels in hepatocytes. G0S2 was found to be co-localized with adipose triglyceride lipase at the surface of lipid droplets. Hepatic G0S2 overexpression resulted in an increase in plasma Low-density lipoprotein (LDL)/Very-Low-density (VLDL) lipoprotein cholesterol level. Plasma High-density lipoprotein (HDL) cholesterol and ketone body levels were slightly decreased in Ad-G0S2 injected mice. G0S2 also increased the accumulation of neutral lipids in cultured HepG2 and L02 cells. However, G0S2 overexpression in the liver significantly improved glucose tolerance in mice. Livers expressing G0S2 exhibited increased 6-(N-(7-nitrobenz-2-oxa-1-3-diazol-4-yl) amino)-6-deoxyglucose uptake compared with livers transfected with control adenovirus. Taken together, our results provide evidence of G0S2 as a potential molecular target for the

**Literature Evidence supporting direct association between G0s2 gene and Liver steatosis**

# Summary of ToxWiz Benefits

- Search using chemical, complicated text queries or molecule-to-toxic-endpoint associations to find out the right answers quickly.
- Import your microarray data to find which biological processes are affected in your toxicity studies. Quickly learn what role your up- and down- regulated genes play in a particular pathway.
- ToxWiz is the only toxicology-ready system exploiting the complex network of genes, proteins, chemicals and experimental outcomes to provide deep insights into molecular mechanisms.
- ToxWiz can prioritize new experiments using results from thousands of those previously done. Let our biological networks help you make better decisions.

