Compare gene signatures in common with treatments of different chemical stressors impinging on common biological processes.

Objective: Identify chemical classes from the Johnson & Johnson library of reference agents that are predicted to have a common mechanism of action. Retrieve microarray data for the selected rats and generate gene signature lists.

Further details of the study can be found here:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15135310&query_hl=2&itool=pubmed_docsum

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Inverse gene expression patterns for macrophage activating hepatotoxicants and peroxisome proliferators in rat liver.

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Macrophage activation contributes to adverse effects produced by a number of hepatotoxic compounds. Transcriptional profiles elicited by two macrophage activators, LPS and zymosan A, were compared to those produced by 100 paradigm compounds (mostly hepatotoxicants) using cDNA microarrays. Several hepatotoxicants previously reported to activate liver macrophages produced transcriptional responses similar to LPS and zymosan, and these were used to construct a gene signature profile for macrophage activators in the liver. Measurement of cytokine mRNAs in the same liver samples by RT-PCR independently confirmed that these compounds are associated with macrophage activation. In addition to expected effects on acute phase proteins and metabolic pathways that are regulated by LPS and inflammation, a strong induction was observed for many endoplasmic reticulum-associated stress/chaperone proteins. Additionally, many genes in our macrophage activator signature profile were well-characterized PPARalpha-induced genes which were repressed by macrophage activators. A shared gene signature profile for peroxisome proliferators was determined using a training set of clofibrate, WY 14643, diethylhexylphthalate, diisononylphthalate, perfluorodecanoic acid, perfluoroheptanoic acid, and perfluorooctanoic acid. The signature profile included macrophage activator-induced genes that were repressed by peroxisome proliferators.

NSAIDs comprised an interesting pharmacological class in that some compounds, notably diflunisal, co-clustered with peroxisome proliferators whereas several others co-clustered with macrophage activators, possibly due to endotoxin exposure secondary to their adverse effects on the gastrointestinal system. While much of these data confirmed findings from the literature, the transcriptional patterns detected using this toxicogenomics approach showed relationships between genes and biological pathways requiring complex analysis to be discerned.

Detailed work flow:
Go to the CEBS home page (www.cebs.niehs.nih.gov/prototype/)
Select the "Search by Study Characteristics" link.

Scroll down to the "Chemical Stressor" section. Select compounds from either one of the families mentioned in the abstract.

Family 1: LPS and zymosan

Family 2: WY 14643, diethylhexylphthalate, diisononylphthalate, perfluorodecanoic acid, perfluoroheptanoic acid, and perfluorooctanoic acid.

Use the Ctrl key to multi-select. Execute the search using the "Submit" button at the bottom of the page.

The search returns the Johnson & Johnson investigation, with one Study (J&J Hepatotoxicant Library). Click on the investigation title to see the Study. Under "Design Information, a check of the timeline reveals the procedures and timing of the study. Details of the protocols, for example the dose and route for each chemical protocol, can be obtained by clicking on the "protocol" links.

The Study Design shows the different dose groups deposited for the Study, and the comparator groups. Some agents were used at a single concentration; others at two or more. For clarity of presentation, if a compound was applied at only one dose within the study the treated group has the suffix "treated"; if a compound was applied at two doses, then the treated groups are suffixed "high" and "low". Some compounds were also applied at intermediate doses, and suffixed "mid1" and / or "mid2".

Returning to the main page, un-check the Study if it is checked, and then expand to see the groups. At the top of the page, select "Show Comparators".

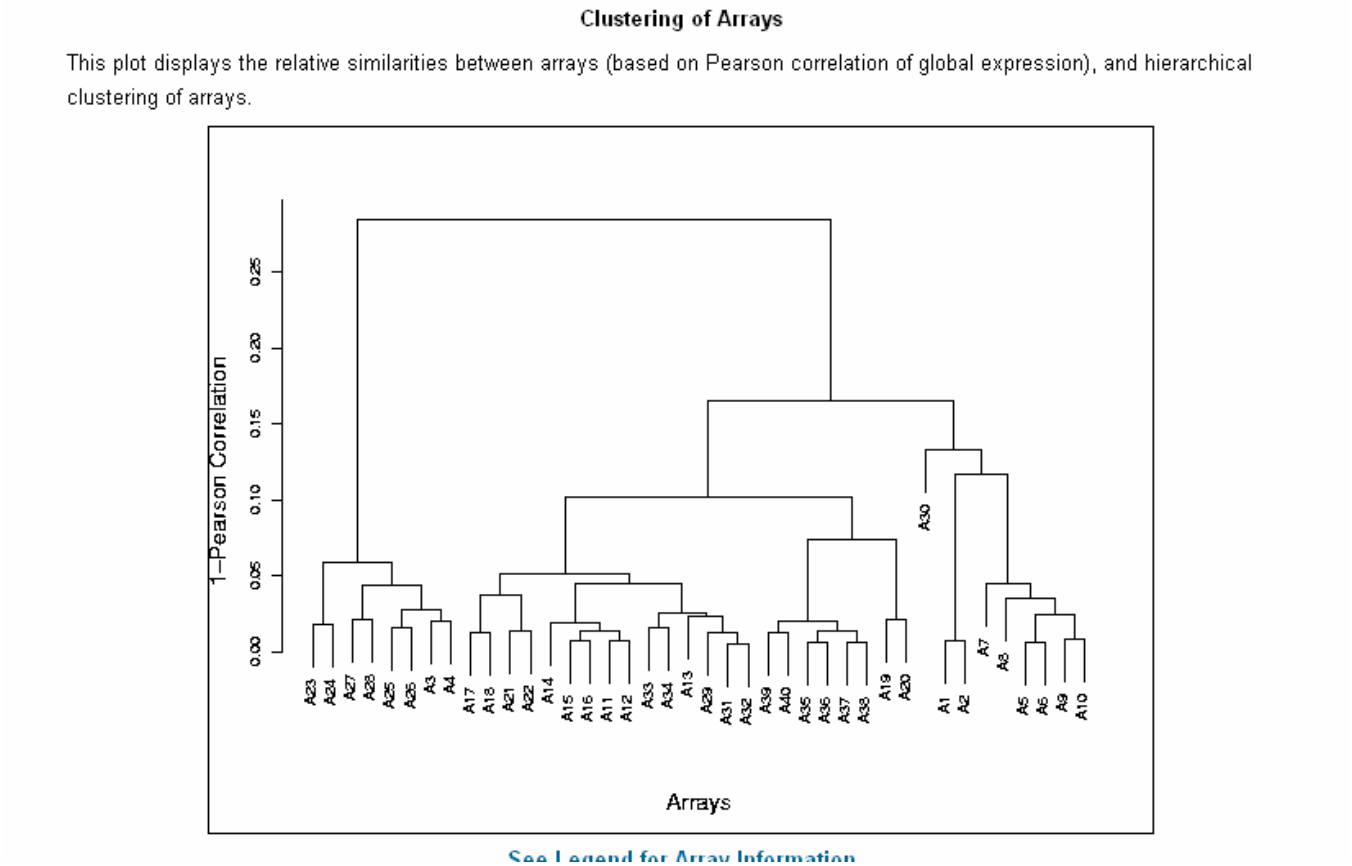
Select groups treated with either Family 1 or Family 2 of agents. Start with Family 1 (high dose LPS plus zymosan plus comparator). Be sure to allow the screen to refresh between every check. Once selected, scroll to the bottom of the page and select Individual Hybridizations.

Microarray analysis:

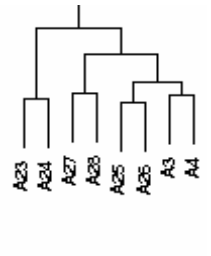
The microarray platform used in this study is a single-channel high-quality proprietary microarray printed in-house. (This information is available if the microarray domain is entered via the "All Microarray" page). Next you will be brought to the "Select Arrays to Retrieve" page, where all the arrays of interest are already pre-selected. Scroll to the bottom of the page and select "Retrieve Data".

On the next page, "Data Preprocessing Options", select the "Use Normalized Data" toggle and then select "Continue" at the bottom of the page. Once the data have been preprocessed, select the "Visualize Data" link.

This link returns the dendrogram shown below:



The most-distant branch corresponds to these arrays:



A3	839180001	Saline_Vehicle_HybGrpID_Rat14603L_RatMegaA7_14942_60759	Rat14603L_14942_LabelRNA
A4	839180001	Saline_Vehicle_HybGrpID_Rat14603L_RatMegaA7_14942_60739	Rat14603L_14942_LabelRNA
A23	839180001	Saline_Vehicle_HybGrpID_Rat14603L_RatMegaA7_14460_58051	Rat14603L_14460_LabelRNA
A24	839180001	Saline_Vehicle_HybGrpID_Rat14603L_RatMegaA7_14460_58050	Rat14603L_14460_LabelRNA
A25	839180001	Saline_Vehicle_HybGrpID_Rat14602L_RatMegaA7_14941_60758	Rat14602L_14941_LabelRNA
A26	839180001	Saline_Vehicle_HybGrpID_Rat14602L_RatMegaA7_14941_60740	Rat14602L_14941_LabelRNA
A27	839180001	Saline_Vehicle_HybGrpID_Rat14601L_RatMegaA7_14940_60741	Rat14601L_14940_LabelRNA
A28	839180001	Saline_Vehicle_HybGrpID_Rat14601L_RatMegaA7_14940_60726	Rat14601L_14940_LabelRNA

Continue to “Analyze Microarray Data” and choose “Compare two groups of arrays”. Select “Continue”.

On the next page, assign the treated arrays to Group A, and the Saline arrays to Group B. Omit the eight arrays in the list above. (This corresponds to omitting rows 3, 4, and 22 through 27). Select “Continue”.

On the next page, “Define Criteria for Differentially Expressed Gene(s)”, select a fold change of 2 and default p-value threshold (or a threshold of your choice). Using the default values will return 403 genes (found in the “View Expression Report” toggle). This list can be sorted by clicking on the column headers. Clicking on individual gene names brings up a gene report with links to annotation. Some probe sequences on the array are proprietary and cannot be accessed.

The gene list can be analyzed using BioCarta as described in Example 1. Workflow for the KEGG and GO analyses are similar.

Analysis of Family 2 compounds is left to the reader!