Overall Approach

Proposed Approach	• Implement filtering • Perform benchmark dose modeling • Define gene sets • Report potency
Recommendations	**Scope:** Clarify the scope of the objectives to include use of BMD approaches to: • Model the dose-response behavior of genes and gene sets • Identify a dose below which biological and toxicological effects are unlikely to occur • The design is sufficient at this time to consider its future application to RA **Out of scope:** • Limit the toxicological interpretation of effects
	Context of use: • Screening and prioritization • Interim POD
	Time points: • Specify how the approach will consider changes in dose-response relationships across different time points and how it will accommodate bioaccumulative substances
	Add examples to document to illustrate the method and test approach on existing datasets
	Include more details about objectives to discern objectives of *in vivo* and *in vitro* studies in approach

Filtering Measure Features

Proposed Approach	Empirical approach maximizing permissiveness, noise reduction, and reproducibility Details: • ANOVA p-value < 0.05 • Fold change > 1.5 • No multiple testing correction
Recommendations	Do not use proposed approach. Instead, customize specific filter parameters and tests for different platforms or experiments, with the goal to enhance reproducibility of results
	Begin to introduce nonparametric tests

Fitting Features to Dose-Response Curves

Proposed Approach	• Features are fit to 9 parametric continuous models • BMR = 1.349 x SD of controls • 2 step process for best model selection (nested chi square and AIC) • From the best fitting model a BMD, BMD_L and BMD_U is determined
Recommendations	Use the parametric models proposed; consider additional parametric models when available
	Introduce nonparametric models into BMDExpress to build confidence and experience
	Constrain parameters of polynomial models to eliminate multiple direction changes
	Specify explicitly whether the model-fitting approach uses dose or log-dose and investigate the effects of each
	Consider using model averaging to take into account model uncertainty as approach moves toward risk assessment

Gene Set-Level Potencies

Proposed Approach	• Fit p-value threshold >0.0001 • BMD_U/BMD_L ratio threshold of <40 • Threshold for "active" gene sets ○ 3 genes, 5% populated, and Fisher Exact Test p-value <0.05 • Determining potency of a gene set: median and mean BMD
Recommendations	Eliminate use of Fisher Exact Test and investigate other methods such as resampling to perform enrichment testing
	When estimating gene set potency, use weighted average instead of median of individual gene BMDs to capture variability
	Consider higher curve fit p-value >0.0001 • Alternative: Use R^2 value instead of or in addition to a global goodness-of-fit p-value
	Investigate the use of bootstrapping to determine confidence intervals on gene set

Study Design

Proposed Approach	**BMD-centric design** ***in vivo* parameters** • Male S-D rats, 6-8 weeks of age • 5 day repeat dose • Liver and other expert-selected organs • Use of a 5-day MTD 10 to 12 dose levels, 3 replicates/dose group ***in vitro* parameters** • Humans, sex based on availability • Expert-determination of duration • Organotypic culture • Top dose selection: LC20
Recommendations	Consider study design as 1st phase of larger effort to inform genomic-based risk assessment
	Include an earlier time point to the 5-day study design as a pilot for application to risk assessment
	Use pharmacokinetic predictions to determine steady-state timescale for duration determination and time point selection
	Consider including additional replicates in the control group
	Use most sensitive sex in *in vivo* studies • Range-finding studies can be used to find differences between sexes
	Expand organ collection list beyond liver to top 3 endpoints [kidney toxicity, and lung (inhalation), neurotoxicity] for future testing
	Incorporate metabolic considerations in study design in both *in vivo* and *in vitro*

Biological Interpretation

Proposed Approach	**Expand and curate hallmark datasets to provide a toxicological and mechanistic interpretation that is species and organ/tissue specific** **Expand:** • Mine the GEO database to identify co-regulated gene sets not currently captured in the Hallmark Gene sets • Mine existing phenotypic-anchored signatures such as those that contained in the DrugMatrix database and those from the published literature • Remine MSigDB and CPDB in manner similar to what was done to create the Hallmark gene sets to identify additional sets that may have been overlooked
Recommendations	Do not use the proposed approach at this time
	Use an existing curated data set to produce a functioning pipeline
	Focus proposal on identifying biologically responsive dose and not hazards
	With release of data, include a statement that this is a screening assessment
	Report the lowest gene set and its name; list the bottom 5-10 gene sets; do not interpret further • Release all data publically
	Consider proposed approach at a later time with evaluation and comparison with more traditional gene sets