Informatics Approach Using Metabolic Reactivity Classifiers In Application To The ToxCast™ Phase I Data

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A. S9 Mediated Structure Data Mining

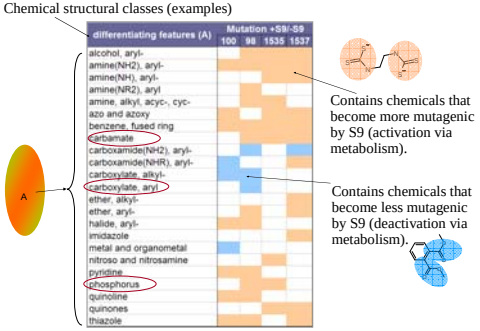
Hypothesis: Structural classes that are modulated by liver enzymes in S9 can be interpreted in a broad sense as the "activating/deactivating" groups in biotransformations.

Test: Can we apply a structure mining approach using a larger genetox database to find structural classes that are activated or deactivated by S9?

S9 Mediated Structure Mining based on Salmonella Reverse Mutations

- 1915 chemicals from the NTP salmonella data were used.
- Tests with rat S9 (no hamster) were included for analysis.
- Equivocal tests were not included.
- Weakly positives were considered positive. In general, Errol Zeiger's calls were used.
- Structural classes
  - Activating: classes that contain more mutagenic structures by S9
  - Deactivating: classes that contain less mutagenic structures by S9

Chemical structural classes (examples)

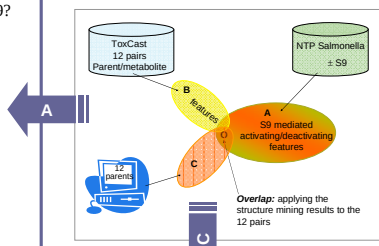


- This dataset was a donation from Errol Zeiger to DSSTox in 2006.
- For the poster, full probabilistic treatment was not conducted. For activating (salmon) features, the normalized mean activity ratios with and without S9 were > 2 times the average. For deactivating (blue) features, the normalized mean activity ratios were lower than ½ the average .
- Only data from male SD rats with liver S9 screening were used to evaluate metabolic activation/deactivation.
- Leadscope hierarchy features were used for the analysis.

Results: Structural Mining of S9 Mediation

- Most structural features were consistent with the current knowledge
  - Aromatic amines, fused benzene rings (poly aromatic hydrocarbons), azo/azoxy, phenol groups are well known for their dependency on metabolic pathways.
- In general, fewer groups were found to be deactivated by S9.
  - Carboxylate (alkyl and aryl), carboxamide (alkyl and aryl)
- Many groups are not significantly affected by S9.
  - Pyrimidine and triazine groups were not affected by S9.

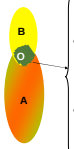
Background: This lack of metabolic capability presents one of the most significant and difficult challenges to relating *in vitro* assay profiles to *in vivo* toxicity outcomes. A chemoinformatics approach is presented for considering metabolism in *in vitro* assessment.



B. Applying the S9 Data Mining Results to the 12 Parent-Metabolite Pairs in ToxCast™ Phase I Chemicals

The structural features extracted from the data mining results based on S9 mediation are then applied to interpret the salmonella reverse mutation outcomes of the 12 parent/metabolite pairs. These metabolites were tested in ToxCast™ due to their known activities.

Results: The overlap of the Features in A and B



- Carbamates are more mutagenic with S9 for TA98, 1535, 1537 strains
  - Pairs 2-4 are carbamates.
- Organophosphates are more mutagenic with S9 for TA 98 and 1535 strains
  - Pairs 5-7 are organophosphates.
- Aryl carboxylates (such as phthalates) are less mutagenic with S9 for TA98.

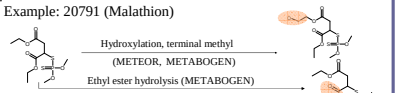
C. Applying the S9 Data Mining Results to the Predicted Metabolites of the 12 Parents in ToxCast™ Phase I Chemicals

Metabolite Predictions

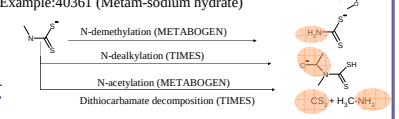
- Combine the predictions of METABOGEN, METEOR, and TIMES to collect possible metabolites.
- Strategies were designed for each product. Mainly considered were Phase I reactions or the first two levels.
  - METABOGEN (Molecular Networks) – calculated reaction types based on human metabolism knowledge
  - METEOR (Lhasa) – reaction types based on expert knowledge of human metabolism
  - TIMES (LMC) – ***t****issue* ***me****tabolite* ***s****imulator uses heuristic rules based on S9 mediation data*

Metabolic Transformation Rules

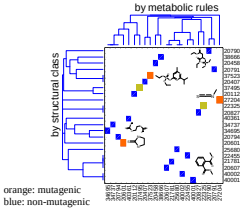
- Example: 20791 (Malathion)



- Example:40361 (Metam-sodium hydrate)

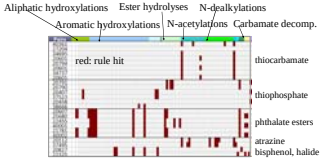


2-way clustering of 12 pairs



The compounds are clustered by pooled metabolic transformation rules and cross grouped by structural classes. The color of the map represents salmonella mutation. A set of metabolic rules and compound structural features are used to map compound mutagenic potential.

Fingerprint map of pooled metabolic rules



Summary

- Structural classes mining for S9 mediation
- Predicted metabolites on 12 parents in addition to the pairs
- Link S9 mediated structural classes with structural features of parents/metabolites

Next Steps

- Construct the metabolic activity profile (reaction type map) for all 309 chemicals using possible metabolites generated by 3 software programs.
- Associate genotoxic potential with metabolic maps
  - Prediction of salmonella mutations will be used when no data are available.
- Extend the method to group *in vitro* assay results in ToxCast™ Phase I
- Apply reaction rules to parents and develop alternative classification method that can be used when interpreting in vitro results with proper statistics.

Reverse Mutation Calls for Parent (P)/Metabolite (M) Pairs.

SAL: salmonella outcome — Negative — Positive — Intermediate

12 Parent/Metabolite Pairs	P/M	Structure Classes (B)	100 -S9	100 +S9	1535 -S9	1535 +S9	98 -S9	98 +S9	1537 -S9	1537 +S9	SAL
40361 (Metam-sodium hydrate)	P1	thiocarbamate									Pred. only
27204 (Methyl isothiocyanate)	M1	isothiocyanate									
20794 (Maneb), 34695 (Mancozeb) 34737 (Metiram)	P2, P3, P4	thiocarbamate									
20601 (Ethylenethiourea)	M2,M3, M4	imidazolidine									
20791 (Malathion)	P5	organo (thio)phosphate									
20790 (Malaoxon)	M5	organo (thio)phosphate									
20407 (Diazinon)	P6	organo (thio)phosphate, pyrimidine									
37523 (Diazoxon)	M6	organo (thio)phosphate, pyrimidine									
20458 (Chlorpyrifos)	P7	organo (thio)phosphate, pyridine									
38666 (Chlorpyrifos oxon)	M7	organo (thio)phosphate, pyridine									
20607 (Diethylhexyl phthalate)	P8	aromatic carboxylate ester/acid									
25680 (Mono(2-ethylhexyl) phthalate)	M8	aromatic carboxylate ester/acid									
22455 (Dimethyl phthalate)	P9	aromatic carboxylate ester/acid									
40001 (Methyl hydrogen phthalate)	M9	aromatic carboxylate ester/acid									Pred. only
21781 (dibutyl phthalate)	P10	aromatic carboxylate ester/acid									
40002 (Monobutyl phthalate)	M10	aromatic carboxylate ester/acid									Pred. only
20112 (Atrazine)	P11	triazine, aromatic halide, sec-amine									
37495 (Deisopropylatrazine)	M11	triazine, aromatic halide, sec-amine									Pred. only
20827 (Methoxychlor)	P12	alkyl halide, aromatic alkoxy									
22325 (HPTE)	M12	alkyl halide, aromatic alcohol									Pred. only

- In both cases where the parent and metabolite showed different outcomes, the tests also employed different assay techniques (standard plate vs. preincubation). Only rat S9 data were used in considering metabolic activation.
- Salmonella predictions were made using the FDA CERES knowledgebase prototype (Poster # 429).

Disclaimer: This study does not reflect the policies of either US FDA or US EPA nor does it endorse products used in the analysis.