Evaluating the Utility of Computational and Cheminformatic Analyses to Screen for Potential Toxicological Hazard of Flavor Compounds Relevant to Tobacco Products

CENTER FOR TOBACCO PRODUCTS

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BACKGROUND

- Chemicals in tobacco products as ingredients and as constituents in smoke and aerosol are numerous and varied. Hazard identification for every chemical using conventional *in vitro* and *in vivo* testing is time-consuming and resource-intensive.
- *In silico* predictive technologies are being explored by CTP for their utility in tobacco regulatory science as an integral part of FDA's Predictive Toxicology Roadmap.

OBJECTIVES

1. To evaluate the performance of (Q)SAR models for prediction of *in vitro* mutagenicity of chemicals in tobacco products.
2. To investigate the combination of (Q)SAR model(s) for the improvement of the overall predictability of computational tools for the mutagenicity endpoint.

OVERVIEW

Data Mining & Compilation
Experimental mutagenicity data
Chemical in tobacco data
Flavorant data

Curate Data
Valid chemical structure, CASRNs, Name
Resolve conflicting mutagenicity data & duplicates

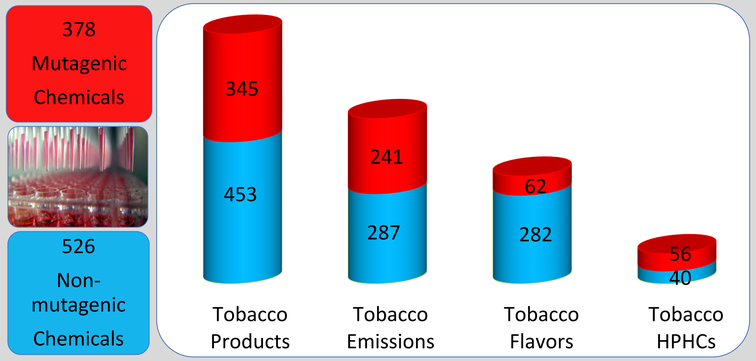
(Q)SAR Models
CASE Ultra
Sarah and Derek Nexus
Chemtunes ToxGPS
ADMET Predictor

Predictive Performance Metrics
Sensitivity
Specificity
Applicability domain

METHODS

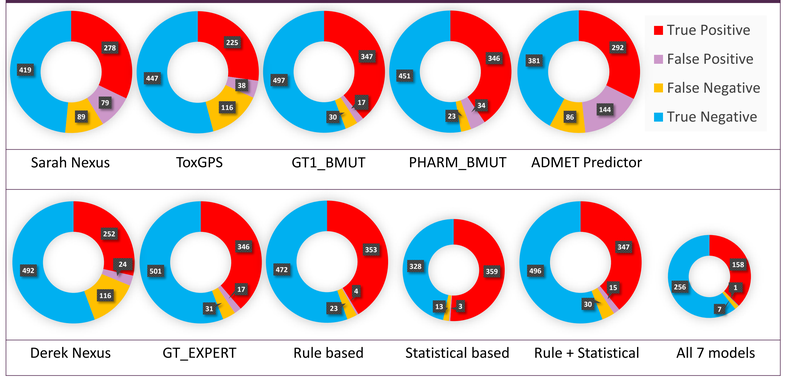
Quantitative structure-activity relationship (Q)SAR software applied for predicting in vitro bacterial mutagenicity of tobacco chemicals	
Statistical-based model	
Sarah Nexus (v.3.0.0)	positive=positive, negative=negative, equivocal=inconclusive, outside domain=out of domain
ChemTunes ToxGPS (v.3)	positive=positive, negative=negative, uncertain =inconclusive, blank=out of domain
GT1_BMUT CASE Ultra (v. 1.7.0.3.13514.500)	known positive/positive=positive, known negative/negative=negative, inconclusive=inconclusive, out of domain=out of domain The gray zone is between 40.0% to 60.0%
PHARM_BMUT CASE Ultra (v. 1.7.0.5.17696.350)	known positive/positive=positive, known negative/negative=negative, inconclusive=inconclusive, out of domain=out of domain The gray zone is between 25.0% to 45.0%
ADMET Predictor (v.9.5)	positive=positive, negative=negative
Expert rule-based model	
Derek Nexus (v.6.0.1)	plausible/probable/certain=positive, inactive/improbable=negative, equivocal=inconclusive
GT_EXPERT CASE Ultra (v. 1.7.0.3.13514.500)	known positive/positive=positive, known negative/negative=negative, inconclusive=inconclusive, out of domain=out of domain The gray zone is between 40.0% to 60.0%.
Combination of models	
Rule based	CASE Ultra GT_EXPERT + Derek Nexus positive in any model=positive, negative in all models=negative, inconclusive in all models=inconclusive, out of domain in all models=out of domain
Statistical based	CASE Ultra PHARM_BMUT+ Sarah Nexus + ChemTunes ToxGPS positive in any model=positive, negative in all models=negative, inconclusive in all models=inconclusive, out of domain in all models=out of domain
Rule + Statistical	CASE Ultra GT_EXPERT + CASE Ultra GT1_BMUT positive in any model=positive, negative in all models=negative, inconclusive in all models=inconclusive, out of domain in all models=out of domain
All 7 models	positive in any model=positive, negative in all models=negative, inconclusive in all models with no positives=inconclusive, out of domain in all models=out of domain

Mutagenicity Validation Dataset of 904 Unique Chemicals



Chemicals were classified as mutagen based on positive Ames test in any strain, with or without metabolic activation according to study conclusions. Tobacco Products = chemicals in any tobacco product (cigarettes/Electronic Nicotine Delivery Systems (ENDS) e-liquids/cigars/smokeless/waterpipe). Tobacco Emissions = chemicals in tobacco smoke or ENDS aerosols. Tobacco Flavors = chemicals listed as flavor by FEMA/IOFI/public databases or reviewed as a flavor by JECFA, and also found in any tobacco product as an ingredient or smoke/aerosol constituent. Tobacco HPHCs = harmful and potentially harmful constituents (HPHCs) in FDA's established list and in FDA's PMTA Notice of Proposed Rulemaking "Premarket Tobacco Product Applications and Recordkeeping Requirements."
Tobacco Product, Tobacco Emission, Tobacco Flavor and Tobacco HPHC chemicals do not contain chemicals that are exclusive to these groups.

(Q)SAR Model Prediction Compared to Experimentally Observed Data

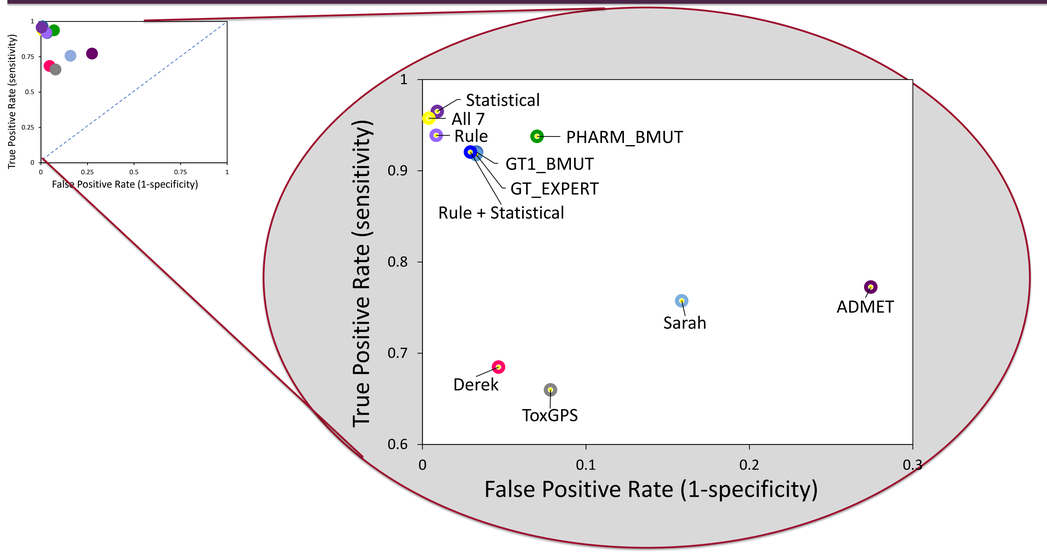


In silico (Q)SAR Model Predictive Performance

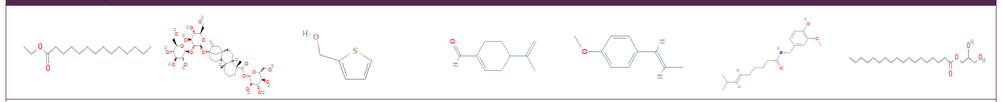
	Applicability Domain (%)	Coverage (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Statistical-based model					
Sarah Nexus	99.3	95.7	75.7	84.1	80.6
ChemTunes ToxGPS	94.9	91.4	66.0	92.2	81.4
CASE Ultra GT1_BMUT	99.0	98.6	92.0	96.7	94.7
CASE Ultra PHARM_BMUT	99.1	94.5	93.8	93.0	93.3
ADMET Predictor	99.9	99.9	77.2	72.6	74.5
Expert rule-based model					
Derek Nexus	100.0	97.8	68.5	95.3	84.2
CASE Ultra GT_EXPERT	99.0	99.0	91.8	96.7	94.6
Combination of models					
Rule based	100.0	94.3	93.9	99.2	96.8
Statistical based	100.0	77.8	96.5	99.1	97.7
Rule + Statistical	99.0	98.3	92.0	97.1	94.9
All 7 models	100.0	46.8	95.8	99.6	98.1

Applicability Domain = Percent of queried chemicals that are within the model's chemical space where a prediction can be made.
Coverage= (TP +TN + FP + FN) / all chemicals Evaluates the proportion of chemicals for which the model can make a positive or negative prediction
Sensitivity =TP/(TP+FN) Percent known positives that are correctly predicted,
Specificity =TN/(TN+FP) Percent known negatives that are correctly predicted
Accuracy =(TP+TN)/(TP+TN+FP+FN) Percent of chemicals in the training set which were correctly predicted by the model
TP = true positives, TN = true negatives, FN = false negatives, FP = false positives.

Receiver Operating Characteristics (ROC) showing the performance of (Q)SAR models



False Negative Flavors in all Seven (Q)SAR Models.



SUMMARY and CONCLUSIONS

- This is the first known study to examine the utility of computational toxicology tools focused on chemical ingredients in tobacco products including constituents in the emissions of tobacco products.
- The representation of the tobacco chemical space was >94% demonstrating a high domain of applicability of the computational models for screening tobacco chemicals.
- The average accuracy of the QSAR prediction was 0.90, ranging between 0.74 – 0.98 for the different models. This is higher than the laboratory to laboratory experimental variability of the Ames test (0.80-0.85) suggesting some potential overtraining of the models.
- The use of a combination of two complementary (Q)SAR models (expert and statistical) improved the confidence in the predictions without compromising performance metrics such as sensitivity, specificity and coverage.
- This research suggests that in silico structure-based analyses of chemicals relevant to tobacco products for the mutagenicity endpoint has utility as a predictive tool prior to performing conventional genetic toxicity testing. This research supports screening strategies in the risk management of tobacco products to potentially reduce the harm by:
  a. providing a hazard identification tool
  b. providing broad coverage of thousands of tobacco chemicals
  c. generating rapid and reliable screening to prioritize tobacco chemicals for further investigation
  d. reducing the potential use of in vivo mutagenicity testing
  e. generating robust prediction data with high confidence in prediction results
- In silico technologies for predicting other genetic toxicity endpoints and other health endpoints are also interesting for further investigation for chemicals relevant to tobacco products.

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***Disclaimer**: The findings and conclusions in this report are data dependent, and those of the authors and do not necessarily represent the official position of the Food and Drug Administration.*