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Comparative Analysis of Transgenic Rodent Assays and In Vitro Models: Insights into Mutagenicity and Carcinogenicity Predictions

Kate Favre, Mounika Girireddy, Roustem Saiakhov
MultiCASE, Inc. | Mayfield Heights, OH | USA

Introduction

Transgenic rodent (TGR) assays measure gene mutation *in vivo*, extending genotoxicity assessment beyond what traditional *in vitro* systems can capture. Transgenic mutagenicity models are the only OECD approved *in vivo* models that measure gene mutations. Transgenic carcinogenicity models are accepted on a case-by-case basis under ICH S1. This study compares curated TGR datasets against the corresponding QSAR models to determine how well *in vitro* and *in vivo* genotoxicity QSAR predictions translate to experimental *in vivo* outcomes.

Dataset

	TGR Mutagenicity	TGR Carcinogenicity
Number of Compounds (positive/negative)	151 (66/85)	138 (13/125)
Key Strains	Big Blue, Muta Mouse	p53 +/-, Tg.rasH2
Data Sources	ECHA, AmesConcord, Gorelick, Honma, Kielhorn	FDA Labels

Methods

Because both datasets are small, standalone QSAR models were not developed for use for the TGR endpoints, however alerts were mined from the model created from the TGR mutagenicity data. A Bayesian conditional-probability analysis was also used to measure agreement between experimental TGR outcomes and the predictions of existing bacterial mutagenicity, genotoxicity, and carcinogenicity QSAR models.

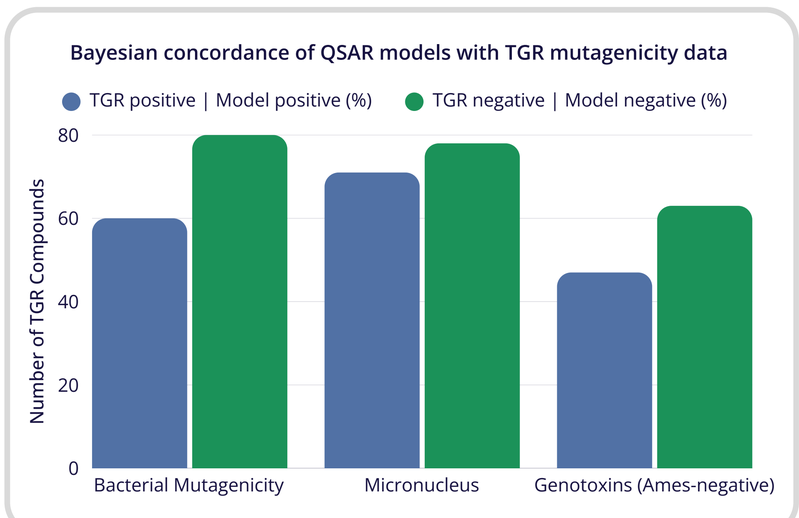
Software

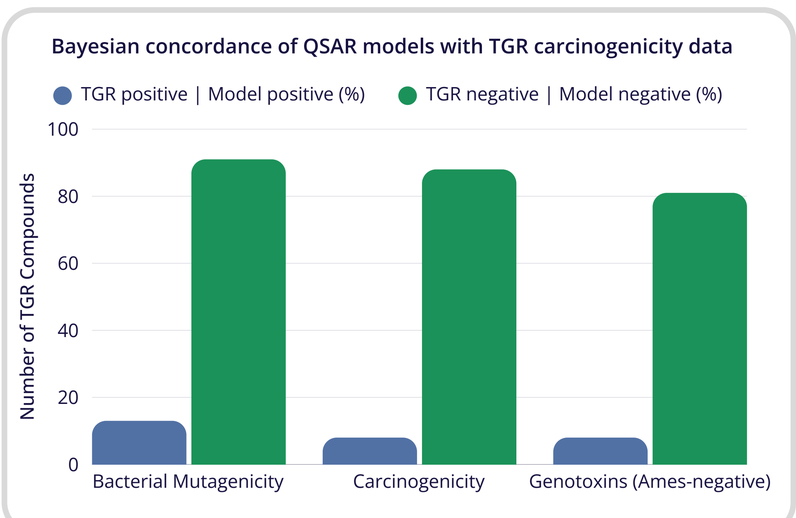
CASE Ultra (v1.7.0.5) was used for QSAR prediction and structural-alert analysis. Meta Ultra software for predicting metabolites of compounds.

Bayesian Concordance Analysis

Bayesian conditional-probability analysis was applied to the mutagenicity and carcinogenicity datasets to assess how well in vitro QSAR predictions translate to in vivo outcomes. The analysis estimates the probability of a positive or negative TGR result given a model's prediction. Concordance was compared across four models: bacterial mutagenicity, carcinogenicity, micronucleus, and Ames-negative genotoxicity for both the TGR mutagenicity and TGR carcinogenicity endpoints.

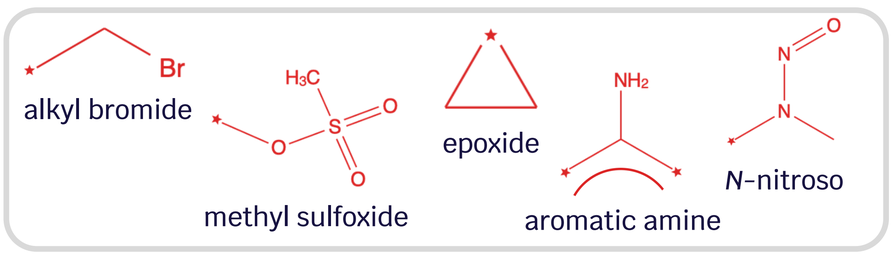
- Negative predictions showed strong and consistent concordance with negative TGR outcomes, at 63–80% for mutagenicity and 81–91% for carcinogenicity.
- Positive predictions were considerably weaker and more variable, at 47–71% for mutagenicity and 8–13% for carcinogenicity, reflecting the small number of confirmed *in vivo* positives available for comparison.





QSAR Model Structural Alerts: Bacterial Mutagenicity vs. TGR Mutagenicity

Substructures including alkyl bromide, methyl sulfoxide, epoxide, aromatic amine, and *N*-nitroso groups were statistically mined alerts in both the bacterial mutagenicity and TGR mutagenicity models, indicating partial mechanistic consistency between *in vitro* alerts and *in vivo* responses. A small subset of compounds confirmed positive in TGR assays triggered nitroso-related alerts and were predicted positive by every model, suggesting that highly reactive structural classes are strongly associated with DNA reactivity and with *in vivo* mutagenic and carcinogenic potential.

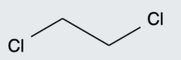
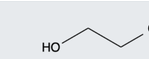
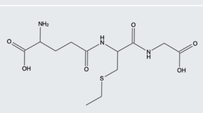
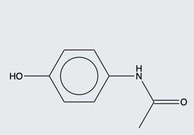
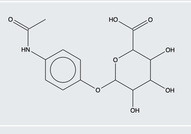
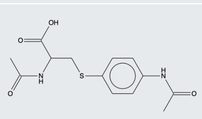
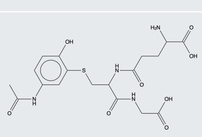


Role of Metabolism

To test whether *in vivo* metabolism explains the discordance between bacterial mutagenicity and TGR results, compounds predicted positive by the bacterial mutagenicity model but negative in TGR assays were processed through Meta Ultra, and their predicted metabolites were re-evaluated against the bacterial mutagenicity and *in vivo* mouse carcinogenicity QSAR models.

1. Identify false positive predictions of TGR mutagenicity data against bacterial mutagenicity QSAR models
2. Generate metabolites *in silico* for each false positive
3. Predict metabolite activity for ICH M7 and carcinogenicity QSAR models

QSAR models capture *in vitro* structural reactivity, but *in vivo* metabolism, particularly phase I oxidation and phase II conjugation, may neutralize that reactivity before a compound reaches TGR target tissues. Metabolism could explain some bacterial mutagenicity-positive / TGR-negative discordance, although the evidence remains inconclusive.

Parent Compound	TGR Mutagenicity	Metabolite	Name of Reaction	ICH M7	Mouse Carcinogenicity
 1,2-dichloroethane	Known Negative		Aliphatic C-Hydroxylation	Known Positive	Known Negative
			Glutathionation	Positive	Negative
 paracetamol	Known Negative		Glucuronidation	Positive	Negative
			Acetylcysteination	Positive	Negative
			Aromatic Cysteination	Positive	Negative

Note: **ICH M7:** Compound was tested with statistical and expert rule-based model for bacterial mutagenicity as per OECD 471 test guidance in CASE Ultra, then additional evidence was used to provide a final call; **Mouse Carcinogenicity:** Statistical models for rodent carcinogenicity in mice per ICH S1A, ICH S1B, and OECD TG-451 regulatory guidelines

Limitations

Small dataset sizes precluded the development of standalone QSAR models. Only 13 of 138 compounds were confirmed carcinogenicity positives, which limits confidence in the positive-prediction concordance estimates. The role of metabolism in Ames/TGR discordance is suggestive rather than proven, as similar S9 profiles were observed in both known positives and known negatives.

Conclusion

Translating *in vitro* mutagenicity predictions into *in vivo* TGR outcomes remains challenging. Agreement across multiple models, combined with attention to metabolism, exposure, and structural context, offers the most reliable path to confident genotoxicity and carcinogenicity risk assessment.

References

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Authors are employees of MultiCASE, Inc.

girireddy@multicase.com, saiakhov@multicase.com, kate@multicase.com