P519 ACT 2026

Identifying Undetected Genotoxic Potential in Ames-Negative Compounds: A Statistical QSAR Model Coupled with Mechanism-Based Expert Rules

Kate Favre[+], Roustem Saiakhov[+]

MultiCASE, Inc. | Mayfield Heights, OH | USA

Introduction

An Ames-negative result is not always evidence of non-genotoxicity. The bacterial reverse mutation assay (OECD 471) detects gene mutation in bacteria, however compounds acting through topoisomerase poisoning or tubulin binding can be clastogenic or aneugenic while testing negative. This matters most where the Ames test stands alone: under REACH Annex VII, substances registered at 1-10 tonnes per registrant per year require no further mutagenicity testing unless the Ames result is positive. Comparing Danish EPA advisory self-classifications with low-tonnage registrations, Hjorth et al. found an experimental Ames positive for only about a quarter of substances flagged as potential mutagens.[1] We developed two complementary *in silico* models to flag Ames-negative compounds with genotoxic potential and identify mechanism-based structural alerts.

Statistical Methodology

A statistical quantitative structure-activity relationship (QSAR) model was developed using a curated dataset of 1,236 Ames-negative compounds (negative across all 5 strains, OECD 471). Positive class assignment (618 compounds) required a positive result in at least one mammalian genotoxicity assay (see Table 1): *in vitro* gene mutation (OECD 476 or 490), *in vitro* chromosome aberration (OECD 473), or *in vivo* micronucleus (OECD 474). Negative class assignment (618 compounds) required at least one negative experimental result with no conflicting or out-of-domain prediction from all relevant genotoxicity QSAR models.

Training set criteria:

GT POSITIVE
- Ames Negative (5 strains)
- At least one experimental positive in a genotoxicity assay

GT NEGATIVE
- Ames Negative (5 strains)
- at least 1 experimental negative in a genotoxicity assay
- no predicted positive, inconclusive, or OOD in a genotoxicity assay*

*25 compounds with experimentally negative data in all 6 assays and were set aside for test set. No training set compounds had experimental results for every assay, therefore compounds with negative predictions were used

1. Combine compounds from GT models and test with GT1_BMUT. Filter compounds with "KNOWN NEGATIVE" outcome. Result: 2625 (673 GT positive/1952 GT negative).

2. Test 1952 GT negative compounds with CASE Ultra genotoxicity models. Remove any compounds that yield a positive, inconclusive or an out-of-domain call in any assay (659).

Table 1: MultiCASE genotoxicity (GT) models

CASE Ultra Model	Genotoxicity Endpoint
GT_1_BMUT	bacterial mutagenicity ±S9, tested in all 5 strains[†]
GT_2_CHROM_CHL	mammalian chromosomal aberrations, *in vitro*
GT_2_CHROM_CHO	mammalian chromosomal aberrations, *in vitro*
GT_3_MN_MOUSE	mammalian erythrocyte micronucleus, *in vivo*
GT_4_ML_ACT	mammalian cell gene mutation with S9, *in vitro*
GT_4_ML_UNACT	mammalian cell gene mutation, *in vitro*

[†] Negative classification = negative in all 5 strains

3. Randomize 659 GT positive compounds and 629 GT negative compounds separately. Remove 55 positives and 30 negatives + 25 experimentally negative holdouts (110 total) to use as test set. Load remaining 1236 compounds (618 GT positive/618 GT negative) into CASE Ultra software with activity listed as 0 or 1. Create new QSAR model and test with holdout set.

[+]*Both authors are employed by MultiCASE, Inc.*

Expert Rule-Based Methodology

An expert rule-based model consisting of 146 structural alerts representing 41 general mechanisms was created from a collection of various literature sources to detect genotoxic compounds that may not test positive in the Ames test. All alert mechanisms relate the molecular initiating events listed in the most recently published draft AOP network leading to permanent DNA damage (see Figure 1 below).[2] The model was benchmarked based on the 1236 chemicals contained in the QSAR model. The top 5 occurring alerts can be seen in Figure 2.

Figure 1: Draft AOP network leading to permanent DNA damage (aneugenicity, clastogenicity, mutagenicity).

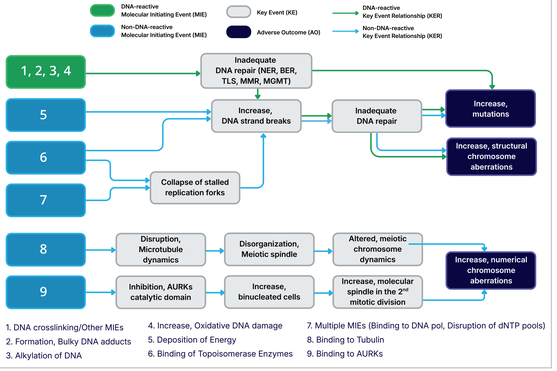
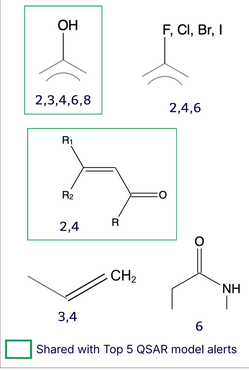


Figure 2: Top 5 alerts from expert rule-based model and associated MIEs

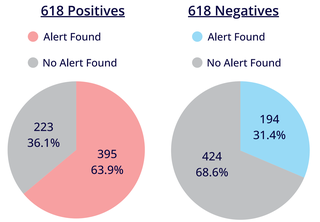


Results

Table 2: Model performance on holdout set of 110 compounds

	Statistical Model	Expert Rule-Based Model
True Positives	39	32
False Positives	10	11
True Negatives	41	42
False Negatives	5	13
Coverage	86.36%	89.09%
Sensitivity	88.64%	71.11%
Specificity	80.39%	79.25%

Figure 3: Expert alert attribution to 1236 QSAR training set compounds



References

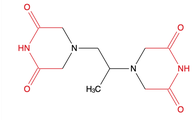
1. Hjorth, Rune et al. "How Many Mutagens Are Missed under REACH Due to Limited Low Tonnage Information Requirements?" Regulatory Toxicology and Pharmacology 164 (January 2026): 105946.
2. Demuynck, E., et al. "Advancing Genotoxicity Assessment by Building a Global AOP Network." Environmental and Molecular Mutagenesis 66, nos. 6–7 (2025): 341–66.
3. Jeremy P. Braybrooke, et al; A Phase II Study of Razoxane, an Antiangiogenic Topoisomerase II Inhibitor, in Renal Cell Cancer with Assessment of Potential Surrogate Markers of Angiogenesis." Clinical Cancer Research (December 2000); 6 (12): 4697–4704.

Discussion

Sensitivity and specificity is reported for each model for transparency, but they are not directly comparable. The detection layer (QSAR model) is optimised to minimize missed positives, and the attribution layer (expert rule-based model) to propose a mechanism when an alert is present. Between both models, 45 compounds were predicted positive; 38 were identified by both models. This complementary use of the 2 models supports mechanistic hypothesis generation and can help guide follow-up testing.

The example in Figure 4 shows that Razoxane has positive experimental data for assays that indicate all three types of DNA damage. The prediction results that would help eliminate mutagenicity are discordant with a positive and negative prediction. With only this information available, more experimental information would be needed to determine the compounds mechanism of action.

Figure 4: Hypothesis generation



Razoxane
CAS No. 21416-67-1

GT_1_BMUT	KNOWN NEGATIVE
GENETOX_EXPERT	KNOWN POSITIVE
GT_2_CHROM_CHL	Negative
GT_2_CHROM_CHO	Positive
GT_3_MN_MOUSE	KNOWN POSITIVE
GT_4_ML_ACT	KNOWN POSITIVE
GT_4_ML_UNACT	KNOWN POSITIVE

Looking to the expert rule-based model, the alert group highlighted is associated with topoisomerase enzyme binding, which could potentially explain the compounds experimental activity. After further investigation, Razoxane is confirmed to be a non-DNA-reactive clastogen through Topoisomerase II inhibition.[3] Stereochemistry should be taken into account with chiral compounds that have structural alerts related to protein binding (e.g. thalidomide).

Figure 5: QSAR training set data composition

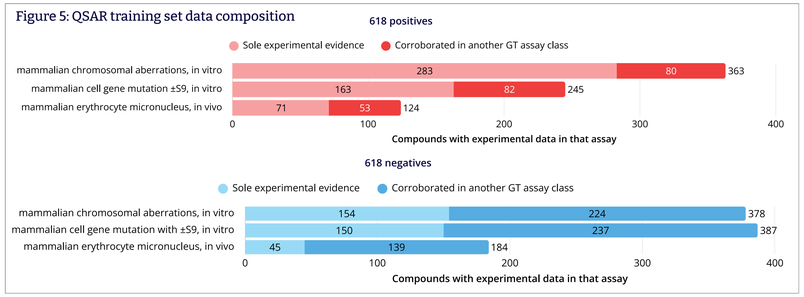


Figure 5 represents the QSAR model composition, which covers several endpoints and is composed of more *in vitro* data than *in vivo* data. The negative class contains more robust data per compound than the positive class.

Conclusion

A statistical QSAR model and a mechanism-based expert rule model identify complementary information about Ames-negative compounds. The statistical model indicates which compounds warrant further assessment (88.6% sensitivity, 86.4% coverage). The expert rules found alerts for 63.9% of training-set positives, proposing a mechanism-based structural feature that can be used as a testable hypothesis while also showing gaps in model coverage. Prediction confidence is limited by a positive class composed of predominantly single *in vitro* results, demonstrating an approach with potential to prioritize and review compounds for tier-2 testing, not to replace it.