Conclusion

The model's high-risk predictions for SR-MMP mitochondrial toxicity are driven by a multi-feature signature dominated by surface electronic heterogeneity (LEA_Var $\rho = +0.79$, LEA_Ave $\rho = -0.86$, $p < 0.001$) and molecular size/complexity (Complexity $\rho = -0.75$, $p < 0.001$), not membrane partitioning properties (XLogP $\rho = +0.16$, $p = 0.48$), explaining why debrominated metabolites are consistently flagged due to their increased surface electronic heterogeneity and reduced complexity.

Methods

This analysis employed Spearman rank correlation to identify molecular descriptors associated with SR-MMP mitochondrial toxicity predictions. The primary datasets (smiles2_toxicity_results.csv and prediction_results_with_smiles.csv) were merged using SMILES identifiers, yielding 21 TBBPA metabolites with 51 molecular descriptors and 31 toxicity endpoints. SR-MMP probability values were extracted and correlated against all 52 descriptors using scipy.stats.spearmanr. Non-parametric Mann-Whitney U tests were performed to compare descriptor distributions between debrominated (Br < 4, n=10) and fully brominated (Br = 4, n=10) metabolites. For pharmacophore comparison, the same correlation analysis was conducted for NR-PPARg (NR_PPAR_gamma) predictions. Bromine counts were calculated from SMILES strings using string matching. Inter-correlations among top descriptors were computed to assess multicollinearity. Statistical significance thresholds were set at $\alpha = 0.05$, 0.01, and 0.001.

Results

Top 5 Positively Correlated Descriptors with SR-MMP Probability:

1. LEA_Var (Local Electron Affinity Variance): $\rho = +0.7909$, $p = 0.000020$
2. ESPmin (Most negative electrostatic potential): $\rho = +0.7013$, $p = 0.000397$
3. Neg_Average (Average negative ESP): $\rho = +0.6714$, $p = 0.000860$
4. HOMO (Highest occupied molecular orbital energy): $\rho = +0.5714$, $p = 0.006810$
5. ALIE_Var (Average Local Ionization Energy Variance): $\rho = +0.5013$, $p = 0.020608$

Top 5 Negatively Correlated Descriptors with SR-MMP Probability:

1. LEA_Ave (Local Electron Affinity Average): $\rho = -0.8571$, $p = 0.000001$
2. Quadrupole_Moment: $\rho = -0.7571$, $p = 0.000071$
3. TPSA (Topological Polar Surface Area): $\rho = -0.7519$, $p = 0.000085$
4. Overall_Surface_Area: $\rho = -0.7494$, $p = 0.000092$
5. Complexity: $\rho = -0.7481$, $p = 0.000097$

SR-MMP Risk Distribution: 6 HIGH (29%), 2 MEDIUM (10%), 13 LOW (62%); mean probability = 0.385 ± 0.389

Debromination Effect: Debrominated metabolites (Br < 4) showed significantly higher SR-MMP risk (mean = 0.500) compared to fully brominated (mean = 0.208; Mann-Whitney $U = 80.0$, $p = 0.026$). Key descriptor differences: LEA_Var (+9.8%, $U = 79.0$, $p = 0.031$), Complexity (-45.8%, $U = 11.0$, $p = 0.004$).

Pharmacophore Comparison: SR-MMP and NR-PPARg showed nearly identical correlation patterns (LEA_Ave: -0.86 vs -0.89; LEA_Var: +0.79 vs +0.85), but NR-PPARg had zero HIGH risk predictions (all 21 LOW), indicating threshold differences rather than distinct pharmacophores.

Membrane Partitioning: XLogP showed no significant correlation with SR-MMP ($\rho = +0.16$, $p = 0.48$).

Inter-correlations: LEA_Ave and LEA_Var were strongly inversely correlated ($\rho = -0.92$, $p < 0.001$); top negative descriptors (Complexity, TPSA, Surface Area) were highly inter-correlated ($\rho >$

0.96).

Challenges

The primary analytical challenge was the small sample size (n=21), which limits statistical power for detecting differences and increases susceptibility to overfitting. The high inter-correlation among molecular size/complexity descriptors ($\rho > 0.96$) creates multicollinearity, making it difficult to isolate independent effects of individual descriptors. Bromine count showed only marginal correlation with SR-MMP ($\rho = -0.26$, $p = 0.25$), despite clear differences in mean probabilities between debrominated and fully brominated groups, suggesting that bromination effects are mediated through changes in electronic properties rather than bromine count per se. The hypothesis regarding membrane partitioning (XLogP) was not supported, requiring re-interpretation of the mechanism. The NR-PPARg endpoint showed zero discriminatory power (all LOW risk), preventing meaningful comparison of differential toxicity profiles. The underlying ToxD4C model is not accessible for validation or new predictions.

Discussion

The analysis reveals that the SR-MMP mitochondrial toxicity model employs a multi-feature pharmacophore centered on **surface electronic heterogeneity** rather than traditional membrane partitioning properties. The dominant signature combines: (1) high LEA variance (heterogeneous electron affinity distribution), (2) low average LEA, (3) less negative electrostatic potential, and (4) reduced molecular complexity. This is mechanistically plausible for mitochondrial toxicity: heterogeneous electronic surfaces may disrupt electron transport chain function, while higher HOMO energies indicate susceptibility to oxidation in the mitochondrial redox environment.

The consistent flagging of debrominated metabolites is explained by structural changes induced by bromine loss: debromination creates more electronically heterogeneous surfaces (increased LEA_Var) while reducing molecular size and complexity—all changes that align with the SR-MMP risk signature. This is counterintuitive, as traditional toxicology often associates larger, more complex halogenated compounds with greater hazard.

The near-identical correlation patterns between SR-MMP and NR-PPARg (differences < 0.08 in ρ) suggest both models use the same implicit feature space but differ in classification thresholds. SR-MMP's lower threshold results in 29% HIGH risk predictions, while NR-PPARg's conservative threshold yields zero HIGH predictions for this chemical space. This indicates that the ToxD4C model's multi-modal architecture may share underlying representations across endpoints.

Contrary to the hypothesis, membrane partitioning (XLogP) plays no significant role in SR-MMP predictions, suggesting the model does not explicitly encode bioavailability or cellular uptake. Instead, the strong negative correlation with TPSA and positive correlation with HOMO suggest the model focuses on intrinsic reactivity and electronic properties that would manifest after cellular uptake.

Proposed Next Hypotheses

1. The inverse relationship between molecular complexity and SR-MMP toxicity ($\rho = -0.75$) is specific to brominated metabolites; testing this hypothesis on structurally diverse mitochondrial toxicants would reveal whether this is a generalizable feature of the SR-MMP model or an artifact of the TBBPA chemical space.
2. The shared electronic pharmacophore between SR-MMP and NR-PPARg with different thresholds suggests that surface electronic heterogeneity (LEA_Var/LEA_Ave ratio) is a universal toxicophore across multiple stress response endpoints, and systematically

analyzing this ratio across all 26 classification endpoints would reveal a hierarchy of threshold sensitivities.

Artifacts

Artifact 1:

File name: SR_MMP_descriptor_correlations.png

Artifact description: Horizontal bar plot showing the top 10 molecular descriptors (5 positive, 5 negative) most strongly correlated with SR-MMP mitochondrial toxicity probability across 21 TBBPA metabolites. Generated using matplotlib with Spearman correlation coefficients and significance markers (*, **, ***). Color-coded by correlation direction (red = positive, blue = negative).