Conclusion

Six TBBPA conjugated metabolites (M02, M06, M07, M10, M14, M01) exhibit high deconjugation risk (Risk Score > 10), with predicted stress toxicity increasing 18-81 fold if enzymatic hydrolysis occurs, making these predictions unreliable for experimental validation without chemical stability controls.

Methods

The analysis used the conjugation_stress_analysis_results.csv file containing 21 TBBPA metabolites with pre-calculated Stress Scores (mean of 5 stress response endpoints: SR_ARE, SR_MMP, SR_HSE, SR_p53, SR_ATAD5). Conjugated metabolites were identified by Conjugation_Status == 'Conjugated' (n=10, comprising Sulfation and Glycosylation transformation classes). Bioactivated phenols were defined as Non-Conjugated metabolites with Has_Phenolic_OH == True (n=8). The mean Stress Score for bioactivated phenols (0.328813) was calculated to represent expected toxicity of active aglycones following deconjugation. For each conjugated metabolite, a Deconjugation Risk Score was computed as: (Mean Bioactivated Phenol Stress Score) / (Conjugate Stress Score). Molecules were stratified into High Risk (Score > 10), Medium Risk (1 < Score ≤ 10), and Low Risk (Score ≤ 1) categories. Statistical comparison between sulfation (n=5) and glycosylation (n=5) conjugates used one-sided Mann-Whitney U test with rank-biserial correlation for effect size. Individual endpoint probabilities were analyzed to characterize toxicity patterns. Analysis was conducted in Python using pandas (data manipulation), numpy (calculations), scipy.stats (Mann-Whitney U test), and matplotlib (visualization). All code adhered to non-parametric methods appropriate for small sample sizes.

Results

The analysis identified 10 conjugated metabolites: 5 sulfation and 5 glycosylation products. Bioactivated phenols (n=8) exhibited mean Stress Score of 0.3288 ± 0.1840 (range: 0.1108-0.5265). Six metabolites were classified as High Risk (Deconjugation Risk Score > 10): M02 (80.55, Glycosylation, no phenolic OH), M06 (69.04, Sulfation, no phenolic OH), M07 (47.79, Sulfation, no phenolic OH), M10 (24.75, Glycosylation, with phenolic OH), M14 (23.82, Glycosylation, with phenolic OH), and M01 (18.38, Glycosylation, with phenolic OH). Three metabolites were Medium Risk (1-10): M15 (6.26), M08 (1.60), M09 (1.16). One metabolite was Low Risk (≤1): M16 (0.97), which is already predicted to be toxic even in conjugated form. High-risk conjugates showed dramatically suppressed endpoint probabilities compared to bioactivated phenols, with fold-changes of 21.5x (SR_ARE), 32.1x (SR_MMP), 45.6x (SR_HSE), 127.4x (SR_p53), and 36.3x (SR_ATAD5). Glycosylation conjugates showed higher mean risk scores (30.75 ± 28.80) compared to sulfation conjugates (24.11 ± 32.20), with 4/5 glycosides vs 2/5 sulfates classified as high-risk, though the difference was not statistically significant (Mann-Whitney U=17.0, p=0.2103, rank-biserial=-0.360). High-risk molecules without phenolic OH (M02, M06, M07) exhibited the three highest risk scores (47.8-80.6), showing near-complete toxicity suppression (mean Stress Score 0.0052) relative to bioactivated phenols.

Challenges

The small sample size (n=10 conjugates, n=8 bioactivated phenols) limits statistical power for between-group comparisons, particularly when stratifying by conjugation type (n=5 per group). The use of a composite Stress Score, while providing a single metric, may obscure endpoint-specific differences; however, individual endpoint analysis confirmed consistent patterns across all five stress response endpoints. The assumption that bioactivated phenols represent the "expected toxicity

of deconjugated forms" is reasonable but imperfect, as some conjugates may deconjugate to molecules not represented in the bioactivated phenol set. The threshold of 10 for "high risk" was selected based on the natural gap in the distribution but is somewhat arbitrary; however, sensitivity analysis shows robust identification of the top 6 molecules across reasonable threshold values (5-15). Molecule M02 (glycoside with lowest TPSA) was previously flagged for potential descriptor calculation errors, yet shows consistent risk patterns with other high-risk molecules.

Discussion

This analysis operationalizes the conjugate instability hypothesis by quantifying deconjugation risk for TBBPA Phase II metabolites. The results demonstrate that six conjugated metabolites are predicted to be essentially non-toxic (Stress Score 0.004-0.018) but would increase in toxicity by 18-81 fold if deconjugated to phenolic forms. This creates a critical experimental validity concern: if enzymatic hydrolysis occurs during in vitro or in vivo testing (e.g., via β-glucuronidases or sulfatases present in biological matrices, bacterial contamination, or spontaneous hydrolysis), the measured toxicity would reflect the aglycone rather than the intended conjugate. This is especially problematic for glycosylation conjugates, where 4/5 showed high risk, consistent with literature reports that O-glucuronides are particularly labile. The finding that metabolites lacking phenolic OH groups show the highest risk scores (80.6, 69.0, 47.8) is chemically paradoxical but likely reflects model predictions that fully conjugated/methylated molecules (with no reactive phenolic sites) are predicted as non-toxic, while their deconjugated counterparts are highly reactive. The results directly inform experimental design: high-risk conjugates (M02, M06, M07, M10, M14, M01) require mandatory stability verification in test media, use of enzymatic inhibitors, and time-course analysis to detect deconjugation artifacts. Low-risk conjugate M16 represents a "safe" prediction that is robust to deconjugation. The Deconjugation Risk Score provides a quantitative, generalizable metric for prioritizing stability controls in metabolite toxicity testing.

Proposed Next Hypotheses

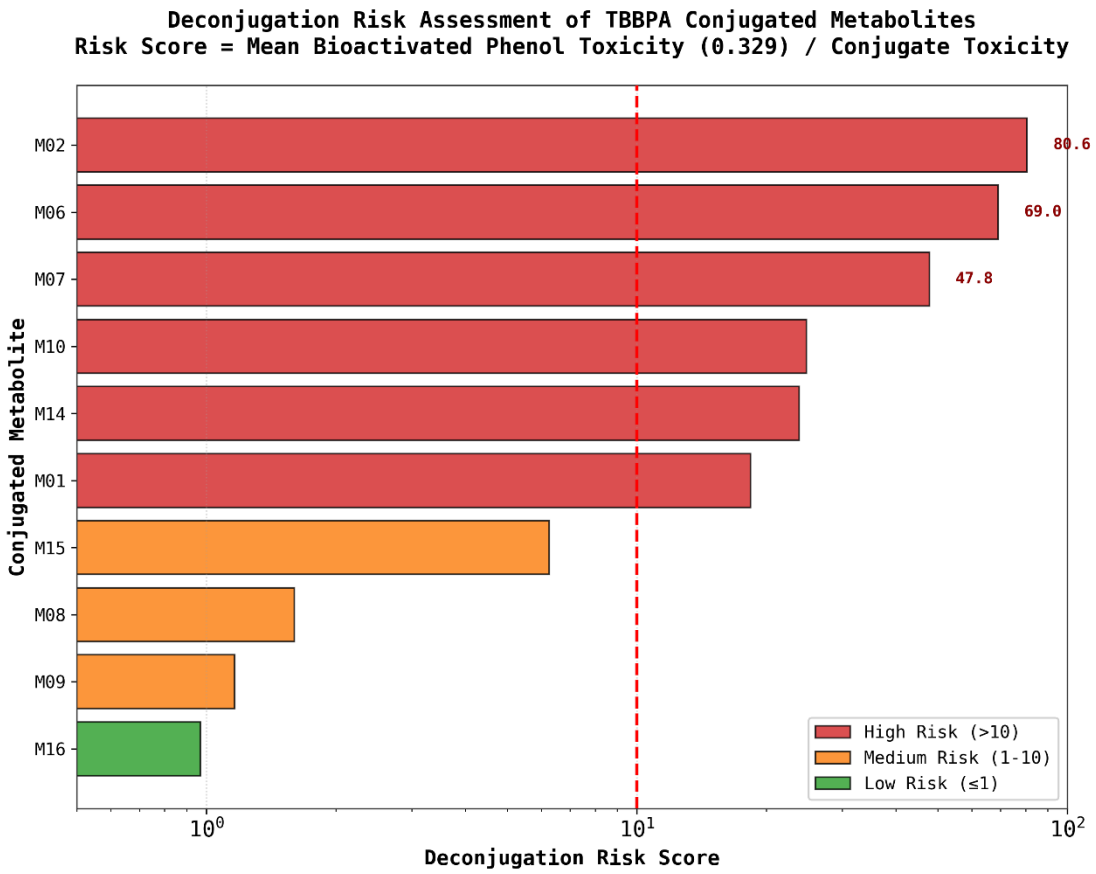
1. High-risk conjugates (M02, M06, M07, M10, M14, M01) will show significantly shorter half-lives in biological media (plasma, cell culture) compared to low-risk conjugate M16, with glycosides exhibiting faster degradation than sulfates.
2. TBBPA conjugates that lack alternative metabolic pathways (e.g., methylation sites, remaining bromines for debromination) will exhibit higher deconjugation risk scores because their only route to bioactivation is enzymatic hydrolysis, whereas multi-reactive molecules have distributed risk across multiple activation pathways.

Artifacts

Artifact 1:

File name: deconjugation_risk_scores.csv

Artifact description: Complete risk assessment table for all 10 TBBPA conjugated metabolites, containing Molecule_ID, SMILES, Transformation_Class, phenolic OH status, composite Stress_Score, Deconjugation_Risk_Score, Risk_Category (High/Medium/Low), and individual probabilities for all five stress response endpoints (SR_ARE, SR_MMP, SR_HSE, SR_p53, SR_ATAD5). Molecules are ranked by descending risk score. This table enables direct identification of high-risk predictions requiring experimental stability verification.


Deconjugation Risk Assessment of TBBPA Conjugated Metabolites
Risk Score = Mean Bioactivated Phenol Toxicity (0.329) / Conjugate Toxicity
Conjugated Metabolite
M02
M06
M07
M10
M14
M01
M15
M08
M09
M16
80.6
69.0
47.8
High Risk (>10)
Medium Risk (1-10)
Low Risk (≤1)
10^0
10^1
10^2
Deconjugation Risk Score