A Reliable Workflow for In Silico Assessment of Genetic Toxicity and Application to Pharmaceutical Genotoxic Impurities

CH Schwab², JF Rathman¹,³, J Marusczyk², A Mostrag¹, B Bienfait², V Gombar¹, C Yang¹,²*

¹Altamira LLC, Columbus, OH, USA; ²Molecular Networks GmbH, Erlangen, Germany; ³Chemical and Biomolecular Engineering. The Ohio State University, Columbus, OH, USA

Molecular Networks GmbH
Henkestr. 91
91052 Erlangen, Germany
mn-am.com

Altamira LLC
1455 Candlewood Drive
Columbus, Ohio 43235, USA
Genotoxic Impurities

- Drug products generally contain more than just the active pharmaceutical ingredient (API)
  - Collectively called "impurities"
  - Impurities during synthesis, storage, etc., no medical benefit
  - Genotoxic impurities - induce genetic mutations, chromosomal breaks, and/or chromosomal rearrangements
  - For patient safety, identification and control of impurities needed

ICH M7 Guidance
ICH M7 Compliance – *In Silico* Tools

**Predictive tools to identify "structure alerts" for mutagenicity**

- **Rich databases with experimental toxicity data from variety of sources**

  - No Mutagenicity data
    - No Structure alerts
      - Class 5: Non-carcinogenic / non-mutagenic
    - Yes Structure alerts
      - Class 4: Same alerts in DS or rel. chemical / non-mutagenic
      - Class 3: Alerts unrelated to DS / no data on mutagenicity
      - Class 2: Carcinogenicity unknown
      - Class 1: Known carniogen
  - Yes Known mutagen

**Application of 2 complementing (Q)SAR prediction methodologies**

- **Expert rule-based**
- **Statistical-based**
ChemTunes/ToxGPS – ICH M7 Tool

Genotoxic Impurities (GTI workflow)

Read-across

TTC

Predict toxicity

Export structures and data

Compare compounds

Liver BioPath

TTC export

Read-across

Genotoxic Impurities Workflow

Find analogs (in next release)

TTC workflow
ToxGPS GTI Workflow – Search Database

Chemistry searches
- Names
- IDs
- CAS
- Structure
- Inventory

Toxicity search
- Species
- Strain
- Metabolic activation
- Study call
- Study source

exact – partial – similar
ToxGPS GTI Workflow – Find Data or "Predict Toxicity"

- Find "nitroaniline"
- NTP original studies
- Dose-level data
- Data carefully cleaned and curated with study quality assignment
Bacterial Reverse Mutagenesis (Ames Mutagenicity) Model Information

**Biology**
- *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and *E. coli* (WP2, WP2 uvrA), with and without rat S-9

**Data source**
- US FDA CFSAN, US FDA Drugs@FDA, US NTP, EU SCCS, REACH (ECHA) database
- Published literature papers when reviewed by our experts

**Training sets**
- QSAR training set: 2,814 compounds (33% positive)
- Knowledgebase for alerts: over 8,000 structures
- OECD 471 or equivalent data quality preferred
Model Descriptions – Selection of Predictors

- **CORINA Symphony properties**
  - *Global molecular descriptors*
  - *Shape and size descriptors*
  - *Semi-empirical MO parameters*

- **ToxPrint chemotypes**
  - *Public library of chemotypes*
  - *Toxicity-related features relevant to human & environment safety*
  - *Generic compound classes*

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ToxGPS Modeling Approaches

Method
- QSAR development
  - Partial Logistic Regression
  - KNN
  - Random Forest
- Structural rules development
  - Mechanistic design
  - Chemoinformatics-assisted

Training strategy
- QSAR
  - Mechanistic MoA neighbors
  - Chemotype class
  - Global
- Structural rules
  - Mechanistic MoA neighbors
  - Chemotypes/fragments

Weight of evidence
- Final prediction
  - Decision theory (Dempster-Shafer) approach
  - Rigorous approach to handling uncertainty
  - Final outcome: Systematic and quantitative method of combining evidences (QSAR and structural rules)

Models have been validated at customer sites, FDA CFSAN, and NIHS Japan
ToxGPS GTI Workflow – Overall Prediction-based Weight of Evidence

- Details
  - Global and MoA models
  - Chemotype alerts
  - Nearest neighbors

![Diagram showing predictions for bacterial reverse mutagenicity.]

- Traffic light for final outcome
- 1 global model, 3 MoA models
- Probability bar(s)
- Likelihood (odds ratio)
ToxGPS Ames Model Performance

- Ames Mutagenicity prediction challenge by NIHS Japan
  - Phase 1: Test set with 3,950 compounds 16 participants
  - Phase 2: Test set with 3,840 compounds 18 participants
  - Phase 1 results were provided to participants and could be incorporated into models developed for Phase 2

- ToxGPS Ames Model
  - Excellent performance in both phases

<table>
<thead>
<tr>
<th>Phase</th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td></td>
<td>ToxGPS</td>
<td>Range*</td>
</tr>
<tr>
<td>1</td>
<td>66%</td>
<td>39 to 70%</td>
</tr>
<tr>
<td>2</td>
<td>57%</td>
<td>42 to 68%</td>
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*Range of values reported by the participants in each phase
Assay Load and Risk Rank

- **Assay load**: if an impurity is not predicted to be negative, then it must be tested experimentally
  - *False positives unnecessarily increase the assay load*
- **Risk rank**: impurities that are genotoxic but predicted to be negative present a product risk

- ToxGPS Ames model performs well with respect to these two important metrics
  - *Assay Load (e.g., ranked 4th out of 18 in phase 2)*
  - *Risk Rank (e.g., ranked 3rd out of 16 in phase 1 in false negatives rate)*
ChemTunes/ToxGPS

- Comprehensive knowledgebase – experimental toxicity data and predictive models
- QSAR modeling based on biologically meaningful grouping using mechanistically selected chemotypes and molecular descriptors
- Final outcome combines the evidences of QSAR models and chemotype rule-based predictions to provide good prediction performance
- Robust risk assessment system providing rigorous method for quantitative weight-of-evidence

In two open challenges involving over 8,000 compounds, ToxGPS Ames mutagenicity model ranked highly for GTI relevant statistics
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- NIHS Japan

- Eurotox 2016 organizers and audience