ToxMet: a web tool for toxicogenomic data analysis using genome-scale metabolic modeling

Archana Hari, Zhen Xu, Zachary Smith, John I Hendry, Scott S Auerbach, Mohamed Diwan M AbdulHameed, Valmik Desai, Anders Wallqvist, Venkat R Pannala

OXFORD
SOT | Society of Toxicology
academic.oup.com/toxsci

Toxicological Sciences, 2026, **209(7)**, kfag079
https://doi.org/10.1093/toxsci/kfag079
Advance Access Publication Date: June 29, 2026
Research article

ToxMet: a web tool for toxicogenomic data analysis using genome-scale metabolic modeling

Archana Hari[1,2], Zhen Xu[1,2], Zachary Smith[1,2], John I. Hendry[1,2], Scott S. Auerbach [3], Mohamed Diwan M. AbdulHameed[1,2], Valmik Desai[1,2], Anders Wallqvist[1,*], Venkat R. Pannala [1,2,*]

[1]Department of Defense Biotechnology High Performance Computing Software Applications Institute, Defense Health Agency Research & Development, Medical Research and Development Command, Fort Detrick, MD 21702, United States
[2]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, MD 20817, United States
[3]Division of Translational Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, United States

*Corresponding authors: Anders Wallqvist, DoD Biotechnology High Performance Computing Software Applications Institute, Defense Health Agency Research & Development, Medical Research and Development Command, FCMR-TT, 504 Scott Street, Fort Detrick, MD 21702-5012, United States. Email: sven.a.wallqvist.civ@health.mil; Venkat Pannala, The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., in support of the Biotechnology High Performance Computing Software Applications Institute, Frederick, MD 21702, United States. Email: vpannala@bhsai.org

Abstract

Chemical toxicity assessment commonly includes *in vivo* rat exposure experiments, with transcriptomic measurements collected at various exposure times and chemical doses. The mechanisms underlying chemical-induced toxicity are then inferred by analyzing changes in gene expression. Recently, genome-scale metabolic models (GSMs), which represent the metabolic network of a cell/organism and contain metabolites, reactions, genes, and the relationship between the genes and reactions, have been used to provide a systems-level understanding of gene expression. However, most of the algorithms that integrate gene expression with GSMs require familiarity with MATLAB or Python programming, making them less accessible for users without computational experience. Here, we introduce ToxMet (https://toxmet.bhsai.org), an open-access, user-friendly web application that provides tabular and graph-based network views to visualize the latest rat GSM (iRno v4.2) and predicts chemical-induced metabolic perturbations in rat tissues by integrating toxicogenomic measurements with the rat GSM. ToxMet uses two well-validated computational algorithms, TIMBR and Pheflux, to predict metabolic perturbations and provides the prediction results as interactive and downloadable tables, scatter plots, and network visualizations. As such, the web tool can process a maximum of 10 conditions for a single job, and the results can be used for dose–response studies to monitor organ metabolism at the subsystem level. We evaluated ToxMet's ability to predict toxicity mechanisms by applying it to publicly available toxicogenomic data for two exemplar toxicants: Gentamicin and thioacetamide, which are known to induce kidney and liver injury, respectively. ToxMet predicted known toxicity mechanisms for both chemicals, thus demonstrating its ability to provide novel insights into the metabolic mechanisms of chemical-induced toxicity and aid in the discovery of biomarkers and therapeutics using gene expression data.

Keywords: metabolism; genome-scale metabolic modeling; web application; computational toxicology; biomarker prediction

Exposure to environmental toxicants, including endocrine-disrupting chemicals such as per- and polyfluoroalkyl substances (PFAS), heavy metals such as lead, and emerging contaminants such as microplastics, represents a serious public health concern due to their ability to induce a wide range of adverse effects, including cancer, metabolic disorders, and cognitive impairments (Macedo et al. 2023; Shetty et al. 2023; Sokan-Adeaga et al. 2023; Meier et al. 2025; Mišľanová and Valachovičová 2025). Advances in high-throughput transcriptomic technologies have enabled comprehensive characterization of cellular responses to such toxic compounds, generating large toxicogenomic datasets across diverse biological systems (Igarashi et al. 2015; Cong et al. 2024; Organisation for Economic Co-operation and Development 2025a, 2025b). However, translating these gene-level changes into mechanistic insights at the metabolic and phenotypic levels remains a major challenge. Genome-scale metabolic models (GSMs) provide a powerful framework for integrating transcriptomic data with biochemical knowledge, enabling systematic analysis of how toxic exposures perturb cellular metabolism (Blais et al. 2017; Cortese et al. 2024). The genome-scale modeling approach has been widely applied to predict and analyze the metabolic effects of diverse toxicant exposures and disease mechanisms (Mardinoglu et al. 2014, 2017; Blais et al. 2017; Pannala et al. 2018, 2019, 2020; Baloni et al. 2019; Rawls et al. 2019; Moore et al. 2023). GSMs represent a comprehensive network of known metabolic reactions catalyzed by enzymes encoded in the genome of the modeled cell or organism (Gu et al. 2019; Passi et al. 2021). Depending on the organism, these models vary substantially in size, ranging from 554 reactions in bacteria (e.g., *Helicobacter pylori* 26695, iIT341) to approximately 10,600 reactions in humans (*Homo sapiens*, Recon3D) (King et al. 2016).

A variety of computational algorithms have been developed to simulate GSMs and predict flux-based metabolic phenotypes. Notably, the transcriptionally inferred metabolic biomarker

response prediction (TIMBR) (Blais et al. 2017) and Pheflux (González-Arrué et al. 2023) algorithms integrate gene expression data to predict changes in metabolite secretion profiles and metabolic reaction fluxes, respectively. These algorithms have been successfully used to characterize metabolic alterations induced by *in vivo* or *in vitro* exposure to chemicals and drugs, including dose-dependent studies on PFAS (Hari et al. 2025), gentamicin (Pannala et al. 2020), hepatotoxicants such as fenofibrate and pulegone (Pannala et al. 2025), and acetaminophen (Rawls et al. 2019). However, applying these algorithms typically requires proficiency in Python or MATLAB programming. To date, no open-access, web-based tools enable users to perform holistic metabolic network analyses and visualize results using metabolic maps, particularly in the context of rat metabolism. Although existing tools, such as Escher (King et al. 2015), Fluxer (Hari and Lobo 2020), and MetExplore (Chazalviel et al. 2018), offer network visualization capabilities, they are limited to selected metabolic pathways, rely on manual curation for specific organisms, or lack constraint-based predictive frameworks that integrate gene expression data with GSMs—making them unsuitable for holistic analysis of rat metabolism.

Here, we developed ToxMet, a user-friendly, open-access web application that enables prediction of toxicant-induced metabolic alterations and potential candidate biomarkers directly from gene expression data. ToxMet provides access to the latest rat GSM (iRno v4.2), allowing users to explore reactions, metabolites, genes, and subsystems through both searchable tables and interactive, graph-based visualizations of the full metabolic network. We implemented two open-source algorithms TIMBR (Blais et al. 2017) and Pheflux (González-Arrué et al. 2023) to predict candidate biomarkers and metabolic flux distributions, respectively. Each algorithm is implemented as an independent, streamlined workflow with dedicated job submission and results pages and provides interactive scatter plots and network-based visualizations that highlight metabolic perturbations induced by chemical exposures relative to control conditions. Users may submit their prediction jobs either by registering with an email address or by logging in as a guest. When gene expression data include multiple dose levels, ToxMet also enables straightforward visualization of dose-dependent metabolic trends.

To ensure accessibility for users without programming expertise, the application includes detailed documentation, example input files, precomputed demonstration results, and a comprehensive tutorial page (see Supplementary File S1). We also provide examples of ToxMet's utility for elucidating mechanisms of metabolic toxicity using two well-characterized toxicants with distinct target organs: Gentamicin, a nephrotoxicant, and thioacetamide, a hepatotoxicant that induces liver fibrosis. Using previously published gene expression datasets for each chemical exposure (Schyman et al. 2018; Pannala et al. 2020), ToxMet successfully recapitulated established toxicity mechanisms by identifying disrupted metabolites, reactions, and metabolic pathways as well as the directionality of their changes relative to control conditions. These case studies illustrate ToxMet's potential to translate toxicogenomic data into mechanistic, metabolism-centered hypotheses that can inform early toxicity prediction, biomarker discovery, and experimental design. Collectively, this work introduces ToxMet as the first user-friendly, open-access web platform to integrate gene expression data with a rat-specific genome-scale metabolic model and establishes it as a community resource for systems-level toxicology, enabling broader adoption of metabolic modeling approaches in preclinical safety assessment and environmental health research. By focusing on rat-based models and datasets, ToxMet supports mechanistic interpretation of toxicogenomic responses in a widely used preclinical species and can be extended to include other species.

Materials and methods

Development of the ToxMet web application

We built ToxMet on a modular architecture consisting of a: (i) web-accessible graphical user interface, (ii) backend database, and (iii) Java-based controller. The front end, primarily implemented using Flutter (https://docs.flutter.dev/), uses the Cytoscape.js JavaScript library to render graphical visualizations of the GSM, which are generated using Graphviz (Gansner et al. 1993; Gansner and North 2000). The backend database implemented using PostgreSQL (https://www.postgresql.org/) allows users to input their datasets and keep their model prediction results for up to 2 wk after submission. All input datasets and corresponding results are deleted from the ToxMet database after 14 d for guest users and 30 d for registered users. None of the users' data is used for model training or any other research. At the heart of the ToxMet web application is the controller, implemented using Spring Boot (Davis 2020), that ties the browser-based graphical front end with the database and prediction algorithms. Given user input, the controller orchestrates execution of the TIMBR and Pheflux prediction algorithms implemented as Python scripts using the following tools: Pandas (The Pandas Development Team 2023), SciPy (Virtanen et al. 2020), COBRApy (Ebrahim et al. 2013), and CasADi (Andersson et al. 2019). The ToxMet web application is hosted on an Apache HTTP Server, and all communication between the application and the user's web browser is secured using HTTPS encryption.

Rat genome-scale metabolic model

The first rat GSM, iRno, was published by Blais et al. (2017) and has since been updated in multiple studies over the years (Pannala et al. 2018, 2019, 2020, 2025; Rawls et al. 2019; Vinnakota et al. 2019). The latest iRno model [iRno v4 (Pannala et al. 2025)] comprehensively covers known rat metabolic information by merging information from iRno and RAT-GEM (Wang et al. 2021), including metabolite, reaction, and gene cross-references to other databases. In this work, we modified iRno v4 to improve metabolic subsystem annotations and used this model as the core of the ToxMet web application. Table 1 compares the iRno model underlying ToxMet (iRno v4.2) with other rat models. As shown in Table 1, the model used by ToxMet contains the highest number of reactions (13,043), metabolites (8,414), and genes (3,102) compared with the other models. All the reactions in the model are classified into one of 58 metabolic subsystems (e.g., fatty acid oxidation, tryptophan metabolism, and pentose phosphate pathway) belonging to a major metabolic pathway (e.g., lipid metabolism, amino acid metabolism, and carbohydrate metabolism). Figure 1 summarizes the number of reactions and metabolites within each subsystem of the ToxMet model, with xenobiotic metabolism containing the highest number of reactions followed by fatty acid oxidation. Figure 1 also shows the number of genes within each subsystem beside each subsystem label. For predictions using the constraint-based algorithms, ToxMet simulates the rat GSM with all exchange reaction constraints set to either relaxed (−1,000, 1,000) or literature-derived specific media uptake rates for liver fasting metabolism as defined in iRno v4 (Pannala et al. 2025).

Table 1. Comparison of ToxMet's rat GSM with other models.

Rat GSM	No. of metabolites	No. of reactions	No. of genes
iRno (Blais et al. 2017; Pannala et al. 2018)	5,620	8,268	2,324
iRno v2 (Rawls et al. 2019; Vinnakota et al. 2019)	5,620	8,463	2,324
iRno v3 (Pannala et al. 2020)	5,756	8,564	2,326
RAT-GEM (Wang et al. 2021)	8,377	13,028	2,953
iRno v4 (Pannala et al. 2025)	8,425	13,043	3,102
iRno v4.2 (ToxMet)	8,414	13,043	3,102

GSM, genome-scale metabolic model.

Constraint-based algorithms

Prediction of metabolite biomarkers

ToxMet uses the TIMBR algorithm previously reported in Blais et al. (2017) to predict potential metabolite biomarkers in response to chemical exposures. Briefly, the algorithm uses gene fold-change data to calculate the change in metabolite secretions with respect to controls. To this end, TIMBR first uses the model gene–protein associations to map the gene fold-change value to the metabolic network to calculate weights for each reaction in the network in the treatment and control conditions. Next, it performs a flux balance optimization to predict the flux on the exchange reactions, which represents the secretion and uptake of metabolites in the model, while minimizing the weighted flux in the entire metabolic network. The optimization problem is defined as follows:

$$\min(w \cdot |v|)$$

where w represents the vector of reaction weights for all the reactions in the model and v denotes the vector of all the reaction fluxes, such that the steady-state assumption $S \cdot v = 0$ is true and the reaction flux is constrained between the lower bound (lb) and upper bounds (ub) ($v_{lb} \leq v \leq v_{ub}$).

Assuming that each input condition is a chemical dose, for each dose, the algorithm performs the flux optimization once using the treatment weights and then using the control weights. The difference between the two values is used to estimate the change in secretion of that exchangeable metabolite (m), i.e., the raw production score (P_m):

$$P_m = \left(\frac{\text{Control} - \text{Treatment}}{\text{Control} + \text{Treatment}}\right)$$

The raw production score is then transformed into a z-score, as follows:

$$Z_{m,\ dose} = \left(\frac{P_m - \mu_{dose}}{\sigma_{dose}}\right)$$

where $Z_{m,\ dose}$ denotes the z-score of the metabolite m in the given treatment condition (dose) and μ_{dose} and σ_{dose} represent the average and standard deviation, respectively, of all metabolite production scores for that dose. Negative and positive z-scores imply a decrease and an increase, respectively, in the secretion of metabolite compared with controls. The resulting scores help identify the metabolites that respond to each experimental condition and represent potential metabolites that can be strongly associated with alterations in gene expression.

For the scatter plot visualization of the TIMBR results, the application calculates an average z-score $\bar{Z}$ for each subsystem, as follows:

$$\bar{Z} = \left(\frac{\sum_{i=1}^{k} z_i}{k}\right)$$

where k denotes the number of exchangeable metabolites in the subsystem and z_i represents the z-score of the ith metabolite within the subsystem.

Prediction of change in metabolic flux activity

ToxMet uses the Pheflux algorithm previously published by González-Arrué et al. (2023) to predict the flux on each metabolic reaction using gene expression data for the control and chemical-treated conditions and then estimates the change in flux-based activity of the chemical-exposed conditions relative to the control condition. Briefly, the algorithm performs a nonlinear optimization to select the fluxome that produces the highest Shannon entropy from the input transcriptome. The optimization problem is thus defined as follows:

$$\text{Max}(H_g(v))$$

such that v is the selected fluxome from the fluxome space given by

$$\{R^N \ | S \cdot v = 0,\ \ lb \leq v \leq ub\}$$

and the Shannon entropy $H_g(v)$ is given by

$$H_g(v) = -\sum_{i=1}^{N}\left(\frac{v_i}{V}\log\frac{{}^{v_i}/_{g_i}}{V}\right)$$

where v_i represents the flux on reaction i, g_i denotes the gene expression value for reaction i, N represents the number of reactions in the model, V represents sum of all reaction fluxes, $S \cdot v = 0$ denotes the steady-state assumption for fluxome prediction, and lb and ub represent the lower and upper flux bounds for the reaction, respectively.

ToxMet uses reaction fluxes to predict the change in metabolic subsystem activity between the treatment and control conditions. To visualize the treatment-induced changes per reaction, ToxMet calculates the log-normalized fold change of each reaction's flux, as follows:

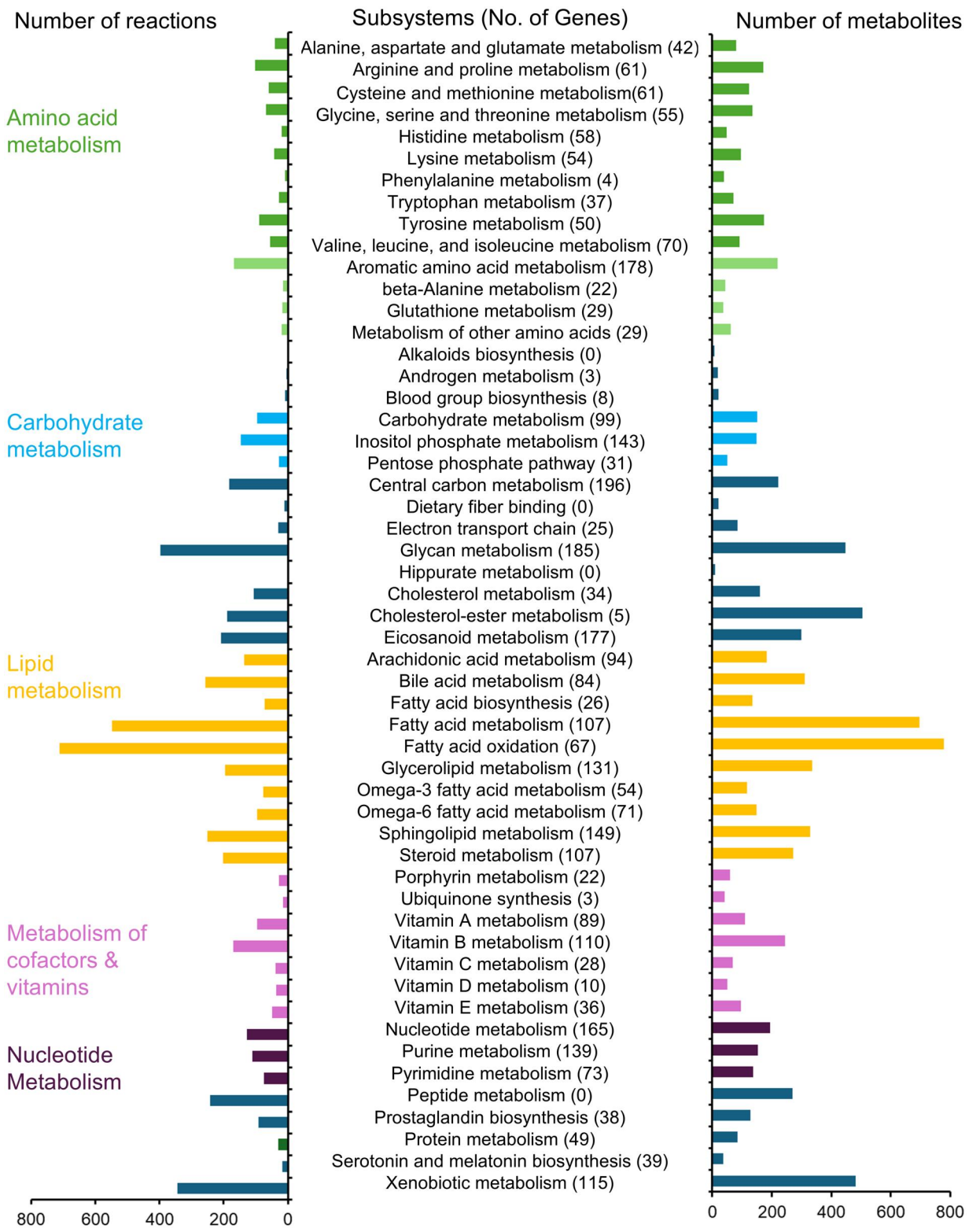

Fig. 1. Number of reactions and metabolites within each metabolic subsystem of iRno in ToxMet. The number of genes in each subsystem is shown beside the subsystem name in parentheses.

$$FC_R = \ln\left(\frac{|R_T|}{|R_C|}\right)$$

where FC_R represents the fold change of reaction R and $|R_T|$ and $|R_C|$ denote the absolute predicted flux of R in the treatment and control case, respectively.

ToxMet further summarizes the flux-based change in metabolic subsystems as an average of the log-normalized reaction fluxes within the subsystem, as follows:

$$\overline{FC} = \left(\frac{\sum_{i=1}^{n} FC_{R_i}}{n}\right)$$

where n represents the number of reactions in the subsystem and FC_{R_i} denotes the fold change of reaction i in the subsystem.

Network visualization

ToxMet displays graph-based network visualizations for each subsystem in the rat GSM. To this end, the application represents the metabolic network as a bipartite graph with nodes denoting metabolites and reactions. Edges connect reaction and metabolite nodes if the metabolite participates in the reaction as a reactant or a product. Large subsystems (such as fatty acid oxidation) are further compartmentalized into their functional parts (beta-oxidation, etc.) and cellular compartments (cytoplasm, peroxisome, etc.), which reduces the graph density and allows the Graphviz graph layout generator (Gansner et al. 1993; Gansner and North 2000) to produce visualizations with minimal overlap between the nodes and edges. The highly connected cofactor metabolites, such as NADPH, H_2O, and ATP, are hidden from the network to reduce network density. We obtained the list of cofactor metabolites from Ranganathan and Maranas (2010). We manually selected other high-degree metabolites, which were not classified as cofactors in the previous list, for duplication (multiple node copies in a graph) based on their number of connections in the subsystem. For example, because mitochondrial acetyl-CoA has 73 edge connections in fatty acid oxidation and only five in eicosanoid metabolism, we provided multiple node copies in the former subsystem but not in the latter.

Results

Web application

We designed the ToxMet web application to: (i) explore and visualize the contents of the latest rat GSM and (ii) predict chemical-induced alterations in metabolic activity and metabolite secretions from gene expression data using constraint-based algorithms. Figure 2 summarizes the inputs, outputs, and key features of ToxMet. Users can explore the contents of the rat GSM as a searchable database for metabolites, reactions, and genes and can visualize interactions between them in different model subsystems as graphical layouts. More importantly, the ToxMet web application provides users with the ability to analyze their own gene expression data from various tissues to understand the metabolic effects under a given perturbation using two different analysis pipelines implemented using the constraint-based algorithms. Users can perform a flux activity-based analysis or a metabolite production analysis by providing either absolute gene expression counts for control and treated conditions or gene fold-change values as inputs for a single condition or multiple treatment conditions with the same control samples. ToxMet provides a summary of the results from the predictive algorithms both as scatter plots and overlaid on the subsystem-based graphical layouts. All network visualizations and result plots can be downloaded as high-resolution PNG images, and

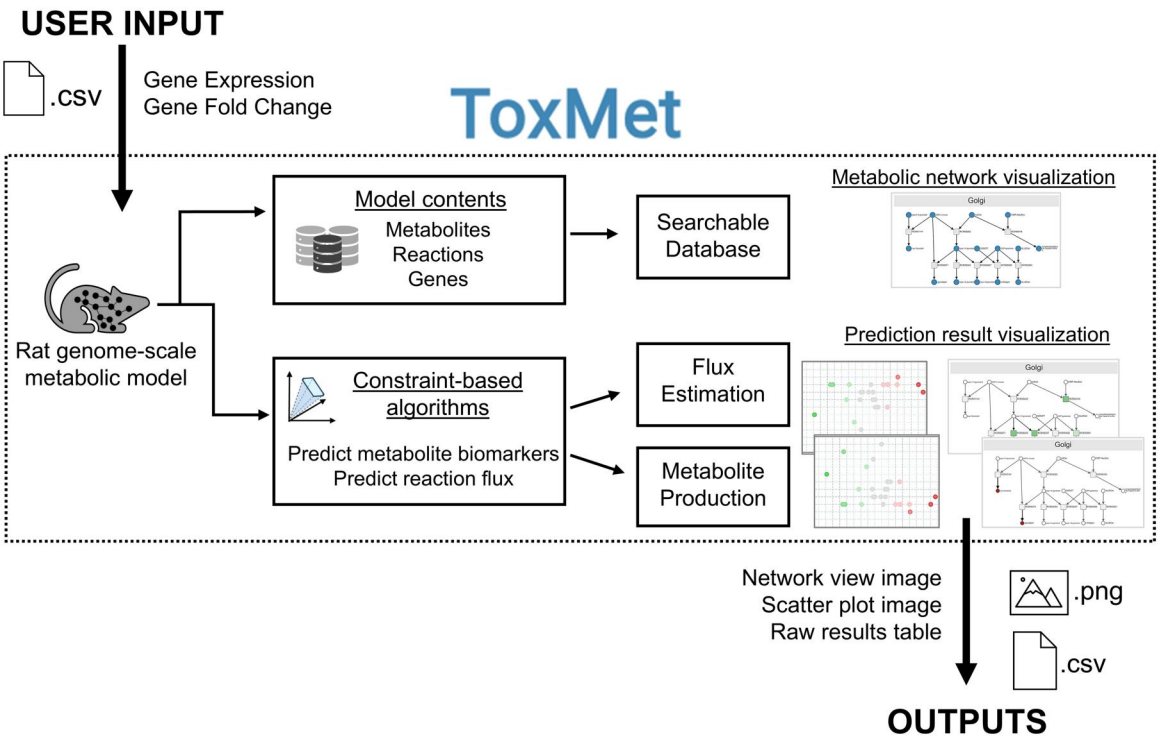

Fig. 2. ToxMet design and features. Users can explore the contents of the rat genome-scale metabolic model as tables or graph-based visualizations of the metabolic subsystems. Users can provide gene expression or logarithmic fold-change data as input to predict changes in metabolic activity or metabolite production, visualize the prediction results as graph-based networks, and download the prediction results as PNG images or CSV files.

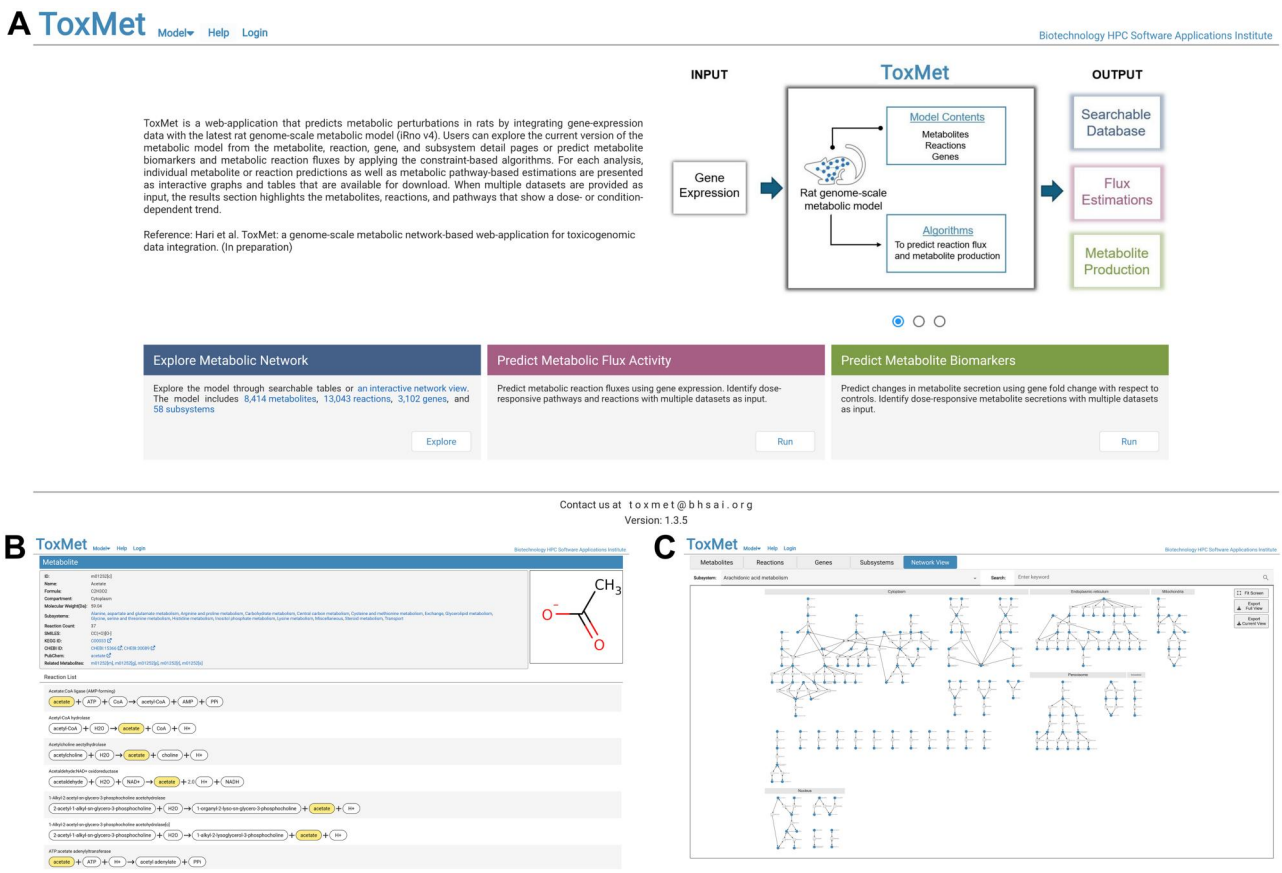

Fig. 3. Pages on ToxMet. (A) Home page. (B) Metabolite detail page for acetyl-CoA. (C) Network visualization for arachidonic acid metabolism subsystem.

users can also download the raw prediction results for other downstream analyses.

ToxMet can be accessed using the following link (https://toxmet.bhsai.org/), which takes users to the home page (Fig. 3A). The home page contains the information buttons for the three main functions of the application: Exploring the metabolic network, predicting metabolic flux activity, and predicting metabolite biomarkers. The model exploration pages (Explore Metabolic Network) summarize the contents of the rat GSM, with hyperlinks to their respective individual pages for detailed tabular information related to metabolites, reactions, genes, subsystems, or their interactions as network view diagrams (Fig. 3B and C). The Explore button automatically takes users to the network view tab as the default option but also provides access to tabs for other components of the model, which users can freely use without logging into the website. As part of model exploration, the detail pages for each model component (metabolite, reaction, gene, or subsystems) contain metadata (model ID, name, and molecular weight if metabolite), annotation (genes and E.C. number for reactions), and database cross-references (e.g., KEGG, NCBI, and GeneCards). Each component of the model can be searched by name or annotation from the model exploration pages.

The interactions between the contents of the rat GSM are visualized based on the model subsystem's annotation, and within each subsystem, they are further subdivided into smaller subgraphs based on cellular compartments, such as the cytosol, mitochondria, peroxisome, and endoplasmic reticulum. Figure 4A shows the full network view for Eicosanoid metabolism, and Fig. 4B shows an enlarged view of the network visualization for a section of the eicosanoid metabolism subsystem containing reactions occurring in the endoplasmic reticulum. The blue circle nodes represent metabolites, and the gray square nodes represent reactions. Edges connect metabolites to the reactions they participate in as reactants or products. Figure 4B also shows the metabolite node leukotriene B_4 selected for further details (highlighted in yellow), and the panel on the right shows information about this selected node, including its name, molecular weight, chemical structure (SMILES), and cross-reference links to KEGG and CHEBI. All network visualizations have cofactor nodes hidden, and some high-degree connections are duplicated. The search bar on top of the network visualization can be used to search for metabolites and reactions using the name or model identifiers. When a duplicated node is searched, all copies of the node are highlighted in the graph.

Job submission and workflow management

The ToxMet web application contains two prediction algorithms: Prediction of metabolite biomarkers and prediction of metabolic reaction fluxes. Users need to register or log in as a guest to run these algorithms to analyze their data. ToxMet uses a queue-based system to manage user-submitted jobs on a first come, first served basis. Guest users can submit one job into the queue, while registered users can submit up to five jobs. A queue position opens up each time a user's job completes; thus, guest users need to wait until their first job completes before submitting another. Each job is restricted to a maximum of 10 conditions (columns in the input file) together with a common control

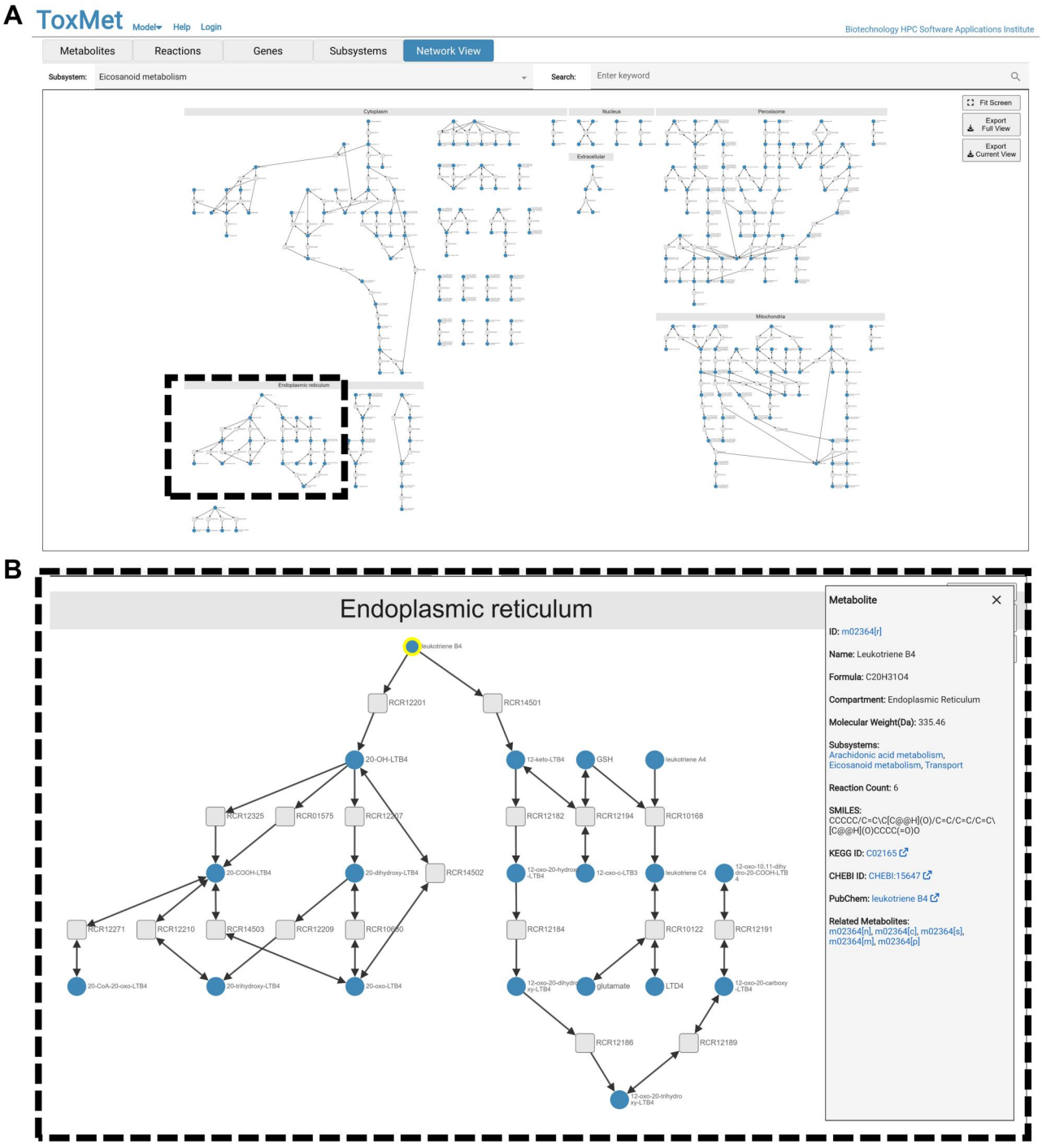

Fig. 4. Network view of eicosanoid metabolism (A) with a full network and (B) with a subset of reactions in the endoplasmic reticulum and the metabolite leukotriene B_4 selected (yellow border node). Blue circles and gray squares represent the metabolites and reactions within the subsystem, respectively. The arrows show the participation of the metabolites as a reactant or product in the connected reaction. The panel on the right in (B) contains additional information about the selected node.

sample. Each algorithm takes 5 to 20 min to predict results on a single condition. Users can download all the input and output data from their submitted jobs and have the option to delete them at any time. The users' input data and results from the submitted jobs will be automatically deleted from the system after 30 d for registered users and 14 d for guest users. ToxMet also provides a comprehensive tutorial page (https://toxmet.bhsai.org/#/help), detailed instructions, example job input files, and example prepopulated results to make the application more user-friendly for first-time users.

Predicting metabolite biomarkers for gentamicin

Gentamicin is an exemplar nephrotoxicant, and we aimed to assess ToxMet's ability to predict its metabolic effects in the kidney. We obtained gene fold-change values from a previously published gentamicin exposure study (Pannala et al. 2020). In the

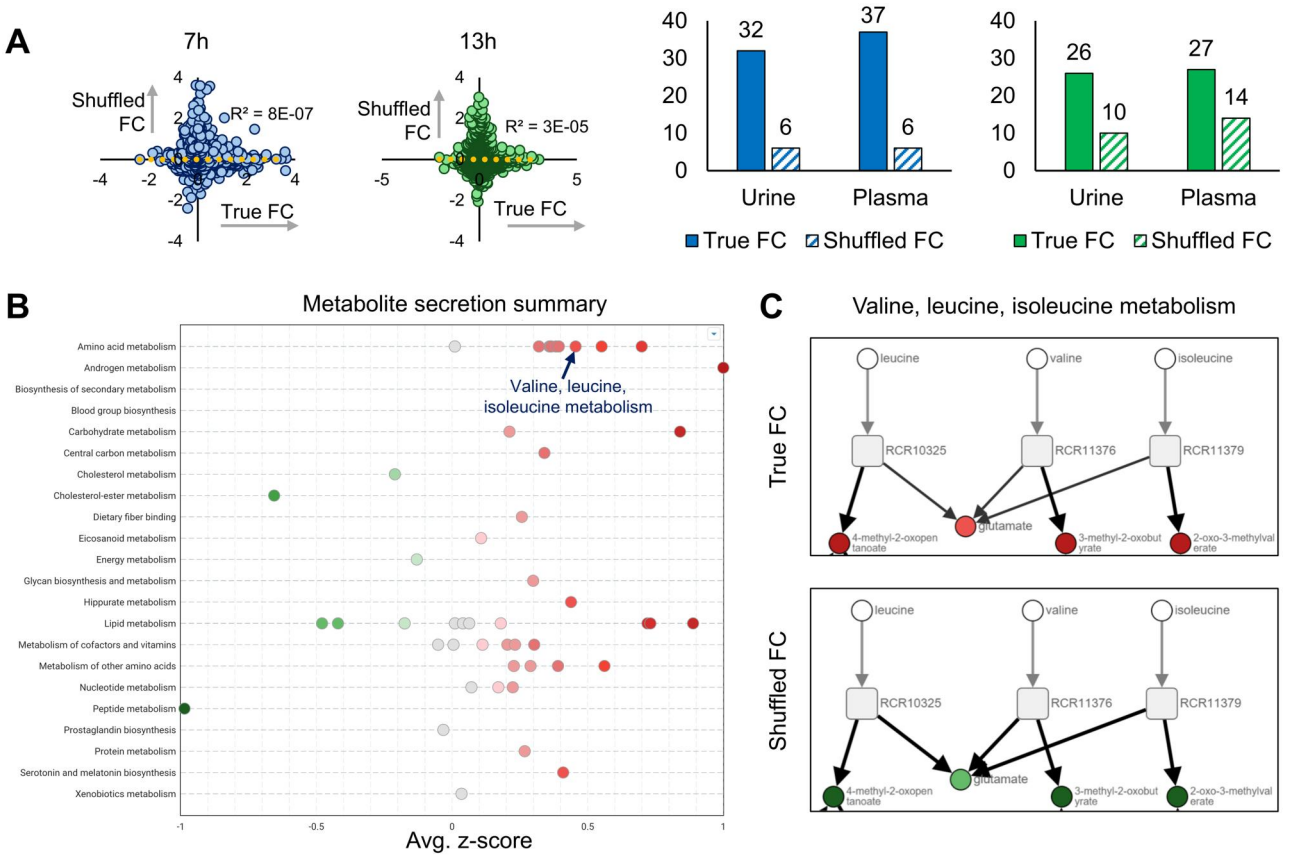

Fig. 5. Prediction of metabolite biomarkers for gentamicin exposure using gene fold-change data. (A) Scatter plots show the correlation between true and shuffled gene fold changes (FC), and bar plots show the number of metabolites with predictions that matched the metabolomic measurements for the 7-h (blue) and 13-h (green) timepoints. (B) Scatter plot shows the predictions for each subsystem using the true gene fold change. Red and green dots show subsystems with increased and decreased metabolite secretions, respectively, compared with controls. (C) ToxMet correctly predicted the change in valine, leucine, isoleucine in response to gentamicin with the original gene fold-change data but incorrectly predicted it with the shuffled data.

original study, rats were exposed to 0.5 g/kg gentamicin, and transcriptomic and metabolomic data were collected 7 h and 13 h after exposure. We provided the 7- and 13-h kidney gene fold-change data as input to the "Predict Metabolite Biomarkers" workflow on ToxMet and selected "Other" for the tissue. Furthermore, to determine to what extent the model predictions were driven by the original gene expression data, we tested the effect of random gene expression values by shuffling the gene IDs in the expression data. The scatter plots in Fig. 5A show the correlation between the original and shuffled datasets, with a poor correlation indicating that the shuffled and true fold-change datasets were different from each other. The bar graphs in Fig. 5A show that shuffling the gene fold changes reduced the number of metabolites that ToxMet correctly predicted to change compared with the original fold-change data for both the 7- and 13-h gentamicin exposure studies. Figure 5B shows a scatter plot of the subsystems and pathways from which the metabolite secretions were most altered at the 7-h time point. These results show an increase in secretion of metabolites from the valine, leucine, isoleucine pathway (marked with an arrow) among several other pathways. Figure 5C shows a specific region of the valine, leucine, isoleucine pathway where production of the downstream metabolites was predicted to increase based on the original fold-change data. Supplementary File S2 contains the full table of ToxMet's metabolite secretion predictions for this dataset. ToxMet's results are consistent with the experimentally measured increase in their accumulation in plasma in the original publication. However, when we used the shuffled gene expression data, we observed opposite predictions for these metabolites, implying that the algorithm truly predicts the changes that are dependent on the input gene expression data.

Predicting thioacetamide-induced changes in metabolic activity

Thioacetamide is a liver toxicant reported to induce fibrosis at high doses and with repeated exposures, and we aimed to assess ToxMet's ability to predict its metabolic effects in the liver. We used data from a study where rats were exposed to either low-dose (25 mg/kg) or high-dose (100 mg/kg) thioacetamide and gene expression was measured 8 h and 24 h after chemical exposure (Schyman et al. 2018). We provided the log2-normalized gene expression counts as input for ToxMet to predict the change in metabolic subsystem activity in the liver. Figure 6 shows the scatter plots summarizing the metabolic changes after 24 h of low-dose or high-dose exposure to thioacetamide. Supplementary File S3 contains the full table of ToxMet's raw flux predictions for the low-dose and high-dose thioacetamide exposure conditions. ToxMet's results show that the metabolic changes are higher for the high-dose condition, suggesting dose-dependent severity, which matches the results in the original publication (Schyman et al. 2018). Next, we investigated the specific metabolic subsystems that were altered due to the chemical

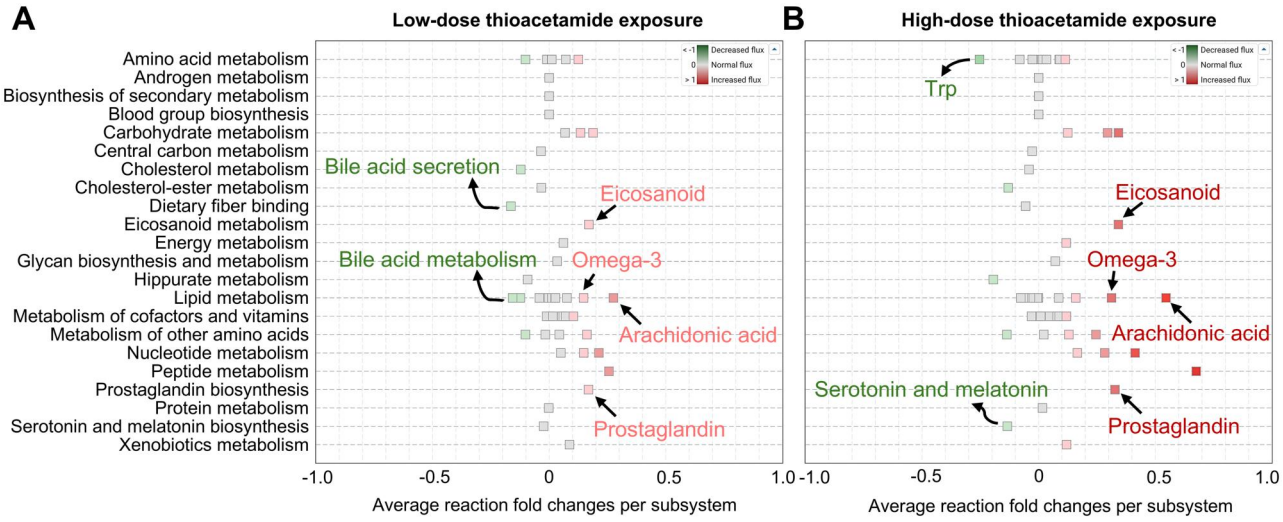

Fig. 6. Pheflux results for thioacetamide (A) low-dose (25 mg/kg) and (B) high-dose (100 mg/kg) exposures. Each red or green square represents a subsystem with increased or decreased flux-based activity, respectively, compared with controls. The x-axis shows the average reaction fold changes with respect to controls in each subsystem. The y-axis shows the major metabolic pathway to which each subsystem belongs.

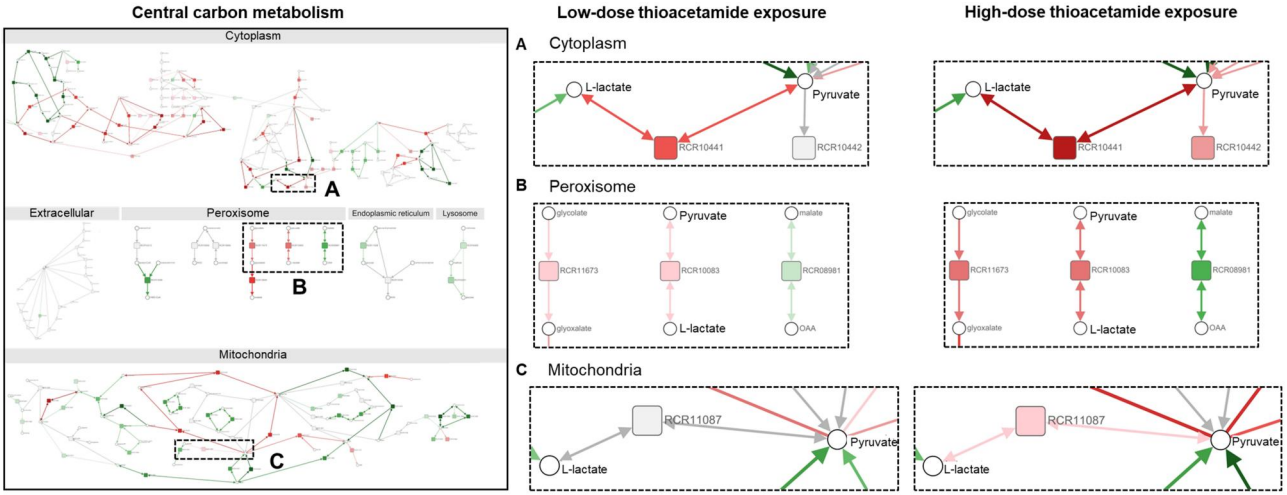

Fig. 7. Alterations in the production of L-lactate from pyruvate due to thioacetamide exposure in the (A) cytoplasm, (B) peroxisome, and (C) mitochondria. The left panel shows the predictions for the high-dose exposure overlaid on the central carbon metabolism map, and the right panels compare the predictions for exposure to low and high doses. Red and green squares indicate reactions with increased and decreased flux activity, respectively, due to chemical exposure.

exposure. At both doses, the subsystems related to inflammation, namely, eicosanoid metabolism, omega-3 metabolism, arachidonic acid metabolism, and prostaglandin metabolism, showed increased flux-based activity compared with controls, suggesting increased inflammation. Figure 6A and B shows these inflammation-associated subsystems with red labels. Similarly, the results showed an increased activity for the carbohydrate metabolism, peptide metabolism, and nucleotide metabolism subsystems. ToxMet predicted that the metabolic activity for bile acid secretion and metabolism decreased for the low-dose exposure but did not change for the high-dose exposure. In addition, predictions for the high-dose exposure showed a decrease in tryptophan as well as serotonin and melatonin metabolism. Figure 6A and B shows these subsystems with green labels.

To evaluate if the GSM predicts a change in lactate production caused by thioacetamide exposure, we analyzed the reactions within the central carbon metabolism subsystem and searched for the "lactate" metabolite. The search result showed "L-lactate" in three compartments: Cytoplasm, peroxisome, and mitochondria. The flux prediction results showed increased production of L-lactate from pyruvate in all three cellular compartments. In Fig. 7, the left panel shows the complete central carbon metabolism map with the L-lactate locations marked with squares for each compartment, and the right panel compares the flux predictions for low- and high-dose exposures for reactions producing L-lactate from pyruvate. All the results showed a dose-dependent trend, with lactate production increasing as the dose of thioacetamide increased. Interestingly, the results showed that low-dose exposure did not induce lactate production from pyruvate in the mitochondria.

Discussion

We developed and made publicly available ToxMet, the first open-access web-based tool that can predict metabolic perturbations from gene expression data using genome-scale metabolic

modeling. We designed the application to allow users to explore the contents of the latest rat GSM and visualize the underlying metabolic network using graph-based maps of metabolic subsystems. A web-based GSM database for rat metabolism offers significant advantages for toxicology and systems biology research. The rat remains one of the most widely used and biologically relevant preclinical models for studying chemical toxicity, disease mechanisms, and therapeutic safety, yet access to comprehensive rat-specific metabolic models has traditionally required specialized computational expertise. A centralized, web-based platform lowers this barrier by enabling researchers from diverse backgrounds to explore, query, and analyze rat metabolic networks without the need for advanced programming skills or local software installation. Such accessibility promotes reproducibility, transparency, and standardization by providing a consistent, curated metabolic framework that can be readily integrated with transcriptomic and other omics datasets. Moreover, interactive access to GSMs facilitates rapid hypothesis generation, comparative analyses across experimental conditions, and efficient identification of perturbed pathways and metabolites. Overall, an accessible web-based genome-scale model database for rat metabolism accelerates systems-level interpretation of toxicological data, fosters collaboration across disciplines, and supports more informed preclinical decision-making in environmental health and safety assessment.

We implemented two complementary constraint-based predictive algorithms, TIMBR (Blais et al. 2017) and Pheflux (González-Arrué et al. 2023), which substantially enhances the practical utility of the platform and GSMs for experimental scientists. By integrating gene expression data with GSMs, TIMBR enables prediction of toxicant-induced changes in metabolite secretion and Pheflux provides insight into alterations in metabolic reaction fluxes, together offering a system-level view of the metabolic perturbations. Although both algorithms are openly available, their use traditionally requires computational expertise, limiting accessibility for nonmodelers. ToxMet overcomes this barrier by providing an intuitive, web-based interface that executes these algorithms seamlessly and predicts results through interactive scatter plots and metabolic network maps. The ability to overlay predictions directly over the metabolic maps, download the results in a standard format for downstream analysis, and analyze multiple dose conditions to identify dose-dependent trends makes the platform particularly valuable for experimental researchers. These features allow toxicology research scientists to move beyond gene-level observations toward mechanistic, testable hypotheses about metabolic dysfunction, without requiring programming or modeling expertise.

We validated the predictive capability of ToxMet by applying the platform to two well-characterized toxicological case studies involving distinct target organs: Gentamicin-induced kidney injury and thioacetamide-induced liver injury. Using transcriptomic data as input, ToxMet successfully recapitulated established metabolic toxicity mechanisms, demonstrating the robustness of the underlying constraint-based algorithms and the rat GSM framework. Consistent with prior findings (Pannala et al. 2020), ToxMet predicted increased renal secretion of branched-chain amino acids, including valine, leucine, and isoleucine, in response to gentamicin exposure, which are some of the metabolic alterations associated with nephrotoxicity. Similarly, we observed that thioacetamide exposure resulted in clear dose-dependent metabolic changes, including elevated lactate production and increased activity of inflammation-associated metabolic pathways, which are both hallmark features of metabolic phenomena known to occur during liver fibrosis (Horn and Tacke 2024). Beyond qualitative validation, ToxMet also supports quantitative downstream analyses. For example, while visualizations focus on subsystem-level and reaction- or metabolite-specific fold changes relative to controls, all the underlying prediction outputs are fully downloadable and can be readily leveraged for additional analyses, such as benchmark dose modeling as described in Hari et al. (2025).

Limitations

Despite their utility, GSMs have inherent limitations that should be considered when interpreting ToxMet's results. In particular, tissue-specific boundary constraints, such as precise definitions of nutrient availability, metabolite exchange, and physiological uptake or secretion rates for target organs, are often incompletely characterized, which can introduce uncertainty into model predictions. Furthermore, caution should be taken when interpreting the results for chemicals at the lower doses, where experimental data can be noisy. In addition, the sheer size and complexity of genome-scale networks substantially increase the computational burden of constraint-based simulations, resulting in longer run times for current algorithm implementations, especially when analyzing multiple conditions or dose levels. Ongoing efforts to refine tissue-specific constraints and optimize computational performance will be important for further improving the accuracy, scalability, and usability of genome-scale metabolic modeling platforms. GSMs are continuously being refined as new biochemical knowledge, improved genome annotations, and experimental evidence become available, resulting in ongoing updates to reaction content, gene–protein–reaction associations, and network structure. Recognizing the dynamic nature of these models, we are committed to maintaining ToxMet as a living, actively updated web platform that incorporates the latest versions of rat GSMs as they are released. Continuous model updates will ensure that predictions remain accurate, biologically relevant, and aligned with current knowledge of rat metabolism. By providing users with access to up-to-date models through a stable and accessible interface, we designed ToxMet to remain a reliable and future-proof community resource for toxicogenomic and systems-level metabolic analyses. Furthermore, in the future, we plan to expand the application to the human metabolic network (Brunk et al. 2018) so that human cell-based gene expression data can also be analyzed and visualized using the application.

Conclusions

In conclusion, ToxMet is the first open-access, web-based application to predict and visualize toxicant-induced metabolic perturbations directly from gene expression data using a systems biology-driven, genome-scale modeling framework. By enabling identification of metabolic signals that contribute to tissue injury and elucidating mechanistic links between transcriptional changes and toxicity outcomes, ToxMet bridges a critical gap between toxicogenomics and metabolic phenotypes. Through its rat-specific implementation, validated predictive algorithms, interactive visualizations, and commitment to continuous model updates, ToxMet provides an accessible and evolving resource for the toxicology community. Overall, the platform supports reproducible, mechanistic, and hypothesis-driven analyses, advancing preclinical safety assessment and systems-level understanding of toxicant-induced metabolic dysfunction. Users can access ToxMet, the freely available web tool for

toxicogenomic analysis, at the following web address: https://toxmet.bhsai.org.

Acknowledgments

We acknowledge Dr Warren Casey from NIEHS for his insight and suggestions for the web application.

Author contributions

Conceptualization, Archana Hari, Venkat R. Pannala, and Anders Wallqvist; methodology and analysis, Archana Hari; software, Archana Hari, Zhen Xu, Zachary Smith, Valmik Desai, John I. Hendry, Venkat R. Pannala; writing—original draft preparation, Archana Hari and Venkat R. Pannala; writing—review and editing, Archana Hari, Zhen Xu, Zachary Smith, Valmik Desai, John I. Hendry, Scott S. Auerbach, Mohamed Diwan M. AbdulHameed, Anders Wallqvist, and Venkat R. Pannala; supervision, Anders Wallqvist and Venkat R. Pannala; funding acquisition, Anders Wallqvist All authors have read and agreed to the published version of the manuscript.

Supplementary material

Supplementary material is available at *Toxicological Sciences* online.

Funding

National Institutes of Health's National Institute of Environmental Health Sciences (NIEHS) (ZIAES103385); Interagency Agreement between NIEHS and DoD Biotechnology High Performance Computing Software Applications Institute (AES22010-001-00001); U.S. Army Medical Research and Development Command (W81XWH20C0031 and HT942524F0189).

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Data availability

The datasets presented in this study are derived from the original study, which is openly available in the NCBI's GEO database gene repository for rats under accession numbers GSE120195 and GSE141628. The derived datasets supporting the conclusions of this article will be made available by the authors on request.

Disclaimer

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Defense Health Agency, the U.S. Department of Defense, the U.S. Government, or The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc. This paper was approved for public release with unlimited distribution.

References

Andersson JAE, Gillis J, Horn G, Rawlings JB, Diehl M. 2019. CasADi: a software framework for nonlinear optimization and optimal control. Math Prog Comp. 11:1–36.

Baloni P, Sangar V, Yurkovich JT, Robinson M, Taylor S, Karbowski CM, Hamadeh HK, He YD, Price ND. 2019. Genome-scale metabolic model of the rat liver predicts effects of diet restriction. Sci Rep. 9:9807.

Blais EM, Rawls KD, Dougherty BV, Li ZI, Kolling GL, Ye P, Wallqvist A, Papin JA. 2017. Reconciled rat and human metabolic networks for comparative toxicogenomics and biomarker predictions. Nat Commun. 8:14250.

Brunk E, Sahoo S, Zielinski DC, Altunkaya A, Drager A, Mih N, Gatto F, Nilsson A, Preciat Gonzalez GA, Aurich MK, *et al.* 2018. Recon3D enables a three-dimensional view of gene variation in human metabolism. Nat Biotechnol. 36:272–281.

Chazalviel M, Frainay C, Poupin N, Vinson F, Merlet B, Gloaguen Y, Cottret L, Jourdan F. 2018. MetExploreViz: web component for interactive metabolic network visualization. Bioinformatics. 34:312–313.

Cong G, Patton RM, Chao F, Svoboda DL, Casey WM, Schmitt CP, Murphy C, Erickson JN, Combs PA, Auerbach SS. 2024. Completion of the DrugMatrix toxicogenomics database using ToxCompl. Chemical effects in biological systems. Research Triangle Park (NC): NIEHS Division of Translational Toxicology. https://doi.org/10.22427/NTP-DATA-500-010-001-000-7

Cortese N, Procopio A, Merola A, Zaffino P, Cosentino C. 2024. Applications of genome-scale metabolic models to the study of human diseases: a systematic review. Comput Methods Programs Biomed. 256:108397.

Davis AL. 2020. Spring boot. In: Spring Quick Reference Guide. Berkeley (CA): Apress. p. 185–197.

Ebrahim A, Lerman JA, Palsson BO, Hyduke DR. 2013. COBRApy: COnstraints-based reconstruction and analysis for python. BMC Syst Biol. 7:74.

Gansner ER, Koutsofios E, North SC, Vo K. 1993. A technique for drawing directed graphs. IEEE Trans Software Eng. 19:214–230.

Gansner ER, North SC. 2000. An open graph visualization system and its applications to software engineering. Softw Pract Exper. 30:1203–1233.

González-Arrué N, Inostroza I, Conejeros R, Rivas-Astroza M. 2023. Phenotype-specific estimation of metabolic fluxes using gene expression data. iScience. 26:106201.

Gu C, Kim GB, Kim WJ, Kim HU, Lee SY. 2019. Current status and applications of genome-scale metabolic models. Genome Biol. 20:121.

Hari A, Balik-Meisner MR, Mav D, Phadke DP, Scholl EH, Shah RR, Casey W, Auerbach SS, Wallqvist A, Pannala VR. 2025. Genome-scale metabolic modeling predicts per- and polyfluoroalkyl substance-mediated early perturbations in liver metabolism. Toxics. 13:684.

Hari A, Lobo D. 2020. Fluxer: a web application to compute, analyze and visualize genome-scale metabolic flux networks. Nucleic Acids Res. 48:W427–W435.

Horn P, Tacke F. 2024. Metabolic reprogramming in liver fibrosis. Cell Metab. 36:1439–1455.

Igarashi Y, Nakatsu N, Yamashita T, Ono A, Ohno Y, Urushidani T, Yamada H. 2015. Open TG-GATEs: a large-scale toxicogenomics database. Nucleic Acids Res. 43:D921–D927.

King ZA, Drager A, Ebrahim A, Sonnenschein N, Lewis NE, Palsson BO. 2015. Escher: a web application for building, sharing, and

embedding data-rich visualizations of biological pathways. PLoS Comput Biol. 11:e1004321.

King ZA, Lu J, Drager A, Miller P, Federowicz S, Lerman JA, Ebrahim A, Palsson BO, Lewis NE. 2016. BiGG models: a platform for integrating, standardizing and sharing genome-scale models. Nucleic Acids Res. 44:D515–D522.

Macedo S, Teixeira E, Gaspar TB, Boaventura P, Soares MA, Miranda-Alves L, Soares P. 2023. Endocrine-disrupting chemicals and endocrine neoplasia: a forty-year systematic review. Environ Res. 218:114869.

Mardinoglu A, Agren R, Kampf C, Asplund A, Uhlen M, Nielsen J. 2014. Genome-scale metabolic modelling of hepatocytes reveals serine deficiency in patients with non-alcoholic fatty liver disease. Nat Commun. 5:3083.

Mardinoglu A, Bjornson E, Zhang C, Klevstig M, Soderlund S, Stahlman M, Adiels M, Hakkarainen A, Lundbom N, Kilicarslan M, *et al.* 2017. Personal model-assisted identification of NAD^+ and glutathione metabolism as intervention target in NAFLD. Mol Syst Biol. 13:916.

Meier MJ, Harrill J, Johnson K, Thomas RS, Tong W, Rager JE, Yauk CL. 2025. Progress in toxicogenomics to protect human health. Nat Rev Genet. 26:105–122.

Mišľanová C, Valachovičová M. 2025. Health impacts of per- and polyfluoroalkyl substances (PFASs): a comprehensive review. Life. 15:573.

Moore CJ, Holstege CP, Papin JA. 2023. Metabolic modeling of sex-specific liver tissue suggests mechanism of differences in toxicological responses. PLoS Comput Biol. 19:e1010927.

Organisation for Economic Co-operation and Development (OECD). 2025a. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing.

Organisation for Economic Co-operation and Development (OECD). 2025b. Test No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents, OECD Guidelines for the Testing of Chemicals, Section 4. Paris: OECD Publishing.

Pannala VR, Hari A, AbdulHameed MD, Balik-Meisner MR, Mav D, Phadke DP, Scholl EH, Shah RR, Auerbach SS, Wallqvist A. 2025. Quantifying liver-toxic responses from dose-dependent chemical exposures using a rat genome-scale metabolic model. Toxicol Sci. 204:154–168.

Pannala VR, Vinnakota KC, Estes SK, Trenary I, O'Brien TP, Printz RL, Papin JA, Reifman J, Oyama T, Shiota M, *et al.* 2020. Genome-scale model-based identification of metabolite indicators for early detection of kidney toxicity. Toxicol Sci. 173:293–312.

Pannala VR, Vinnakota KC, Rawls KD, Estes SK, O'Brien TP, Printz RL, Papin JA, Reifman J, Shiota M, Young JD, *et al.* 2019. Mechanistic identification of biofluid metabolite changes as markers of acetaminophen-induced liver toxicity in rats. Toxicol Appl Pharmacol. 372:19–32.

Pannala VR, Wall ML, Estes SK, Trenary I, O'Brien TP, Printz RL, Vinnakota KC, Reifman J, Shiota M, Young JD, *et al.* 2018. Metabolic network-based predictions of toxicant-induced metabolite changes in the laboratory rat. Sci Rep. 8:11678.

Passi A, Tibocha-Bonilla JD, Kumar M, Tec-Campos D, Zengler K, Zuniga C. 2021. Genome-scale metabolic modeling enables in-depth understanding of big data. Metabolites. 12:14.

Ranganathan S, Maranas CD. 2010. Microbial 1-butanol production: identification of non-native production routes and *in silico* engineering interventions. Biotechnol J. 5:716–725.

Rawls KD, Blais EM, Dougherty BV, Vinnakota KC, Pannala VR, Wallqvist A, Kolling GL, Papin JA. 2019. Genome-scale characterization of toxicity induced metabolic alterations in primary hepatocytes. Toxicol Sci. 172:279–291.

Schyman P, Printz RL, Estes SK, Boyd KL, Shiota M, Wallqvist A. 2018. Identification of the toxicity pathways associated with thioacetamide-induced injuries in rat liver and kidney. Front Pharmacol. 9:1272.

Shetty SS, Deepthi D, Harshitha S, Sonkusare S, Naik PB, Kumari NS, Madhyastha H. 2023. Environmental pollutants and their effects on human health. Heliyon. 9:e19496.

Sokan-Adeaga AA, Sokan-Adeaga MA, Sokan-Adeaga ED, Oparaji AN, Edris H, Tella EO, Balogun FA, Aledeh M, Amubieya OE. 2023. Environmental toxicants and health adversities: a review on interventions of phytochemicals. J Public Health Res. 12:22799036231181226.

The Pandas Development Team. 2023. Pandas-dev/pandas: Pandas (v1.5.3). Geneva, Switzerland: Zenodo.

Vinnakota KC, Pannala VR, Wall ML, Rahim M, Estes SK, Trenary I, O'Brien TP, Printz RL, Reifman J, Shiota M, *et al.* 2019. Network modeling of liver metabolism to predict plasma metabolite changes during short-term fasting in the laboratory rat. Front Physiol. 10:161.

Virtanen P, Gommers R, Oliphant TE, Haberland M, Reddy T, Cournapeau D, Burovski E, Peterson P, Weckesser W, Bright J, *et al.*; SciPy 1.0 Contributors. 2020. SciPy 1.0: fundamental algorithms for scientific computing in Python. Nat Methods. 17:261–272.

Wang H, Robinson JL, Kocabas P, Gustafsson J, Anton M, Cholley PE, Huang S, Gobom J, Svensson T, Uhlen M, *et al.* 2021. Genome-scale metabolic network reconstruction of model animals as a platform for translational research. Proc Natl Acad Sci USA. 118: e2102344118.

Published by Oxford University Press on behalf of the Society of Toxicology 2026.

Toxicological Sciences, 2026, 209, 1–12
https://doi.org/10.1093/toxsci/kfag079
Research article